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Some Aspects of Family Living and the Need for Family-Oriented Psychiatry in Turkey

Orhan M. Öztürk, M. D.*

The importance of afamily-oriented, diagnostic and therapeutic approach is particularly great in a country like Turkey which has been in a process of rapid social change during the last 40 years. In the nineteen-twenties and thirties, radical steps were taken to modernize the whole social structure. Many religious, educational and judicial institutions which had prevailed in the country for centuries had to be either entirely abolished or substantially altered. Universities and schools were reorganized. The Roman alphabet was adopted in place of the Arabic. The state and its civil and penal codes were secularized. Polygamy was outlawed, and women were given equal legal rights, including the right to vote. However, in spite of these serious endeavors, it cannot be claimed that all segments of society have been equally affected. A considerable portion of the population, the rural in particular, is still unchanged in its value orientation system and pattern of living.

It is unnecessary to point out the implications of these changes for family life in Turkey. Although the state made substantial efforts toward social assistance and toward establishing new institutions to replace the

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old, the major responsibility in maintaining equilibrium, security, growth and health in the family still rests within the family itself.

In modern societies, there are institutions besides the family which are indispensible in rearing children, i.e. nurseries, kindergartens, Sunday, schools, youth centers, etc., and there are also therapeutic or preventive organizations to secure and maintain good health. In countries where adequate or superior socio-economic development has been achieved, these institutions have drawn the interest of the state and individuals or groups to the extent that responsibilities have shifted considerably from the family. In such societies where the individual is usually expected to move away from his nuclear family as soon as possible, and where he is encouraged to develop a high sense of self-reliance, autonomy and initiative, it is understandable that the main diagnostic and therapeutic focus would be more on the individual than on the family.

In Turkey, however, neither the child's independence of the family is encouraged as much as it is in the western world, nor are extra-familial (i.e. state or community) supports of personality development and health sufficiently established and distributed. Therefore, the structure and the dynamics of family life, rôle and responsibility patterns and the interdependence of family members must have a special significance for any health officer in this country.

A Turkish psychiatrist has to recognize the patterns of family life characteristic of various segments of society or socio-economic levels. Although Turkish society is not homogenous, it does not have any serious class or cast barrier systems between the various sections or levels. It is possible to distinguish traditional, transitional and modern types of families[1] which have distinctive as well as common characteristics. With a high migration rate from the rural areas to urban and metropolitan centers, and with an increase in the literate and urban population, the body of transitional and modern families is rapidly growing. While this does not necessarily mean a quantitative rise in medical or socio-psychological troubles, new problem areas do emerge as a result of the on-going changes.

Briefly, the traditional Turkish family is typically extended, patriarchal, patrilocal and patrilineal, and belongs mainly to an agrarian community. In Kluckhohn's terminology,[2] the man-nature orientation is in the form of subjugation to nature; time focus is more on the present and past than on the future; activity orientation is more in the form of "being" than "doing"; and relational orientation is more lineal and collateral than individualistic. In rearing children, there is a great emphasis on early sex rôle differentiation, and therefore, assimilation of sex rôles is thoroughly ac-

complished at very early stages of childhood. Autonomy and initiative in Erikson's sense[3] are restricted, while conformity and submission to authority are systematically reinforced. As the family changes in a modern direction, it becomes urban and nuclear, with the patriarchal characteristics diminishing. Most value orientations become more similar to those in western countries, but the basic tendency to maintain the lineal and collateral orientations still persists in varying degrees.

Many writers have implied or distinctly stated[4 5 6 7] that structural changes in the traditional family are usually associated with disorganization, and therefore give rise to serious conflicts and anxieties. It is difficult to find much support for this view in Turkish society, although it must be admitted that our knowledge is rather limited in this area. A study carried out by questionnaires answered by 60 psychiatrists from various parts of Turkey suggests that certain aspects of traditional family life and methods of child-rearing may contribute at least as much as some aspects of modern family life to the development of personality vulnerabilities and anxiety-producing conflicts.[9] Some important areas of conflict within a traditional family are precocious and intense differentiation of sex rôles with a high level of suppression of sexuality, low esteem of womanhood, unusual emphasis on masculinity and virility, loss of one's own identity in relation to the authority figure, frustration of such needs as autonomy, curiosity and initiative, and an ever increasing demand for powerful mastery over one's own accumulating aggression, not to mention those related to economic and other social conditions. I do not wish to go into the details of the problems of modern families, as these do not seem to be very different from those in western countries, except that individual autonomy is much less pronounced in Turkish families. From the clinical point of view, while we frequently see typical identity crises or negative identities in the adolescents of modern families, it is possible to evaluate the intense inhibitions or constrictions encountered in the adolescents of traditional families as being incipient counterparts of serious identity problems. Similarly, a child in a modern family may show symptoms of school phobias, whereas the children of traditional families may manifest classical conversion symptoms.

In traditional families, in spite of legal prohibition, folk beliefs and methods concerning the etiology and treatment of mental illness are common. Although severely psychotic patients are easily taken to medical doctors, common neurotic manifestations, like conversion hysterias, hypochondriacal conditions or anxiety reactions are often treated by folk healers. These healers mainly use methods of religious persuasion and rely on measures which reinforce the patients's defenses like projection, displacement and concretization.[8] As modern medical facilities and communications media

are becoming more readily available, the practice of prescientific medicine is gradually diminishing. Modern families are usually aware of the rôle of the psychiatrist and in general there is adequate cooperation with the therapist. Because of the strength of the lineal and collateral ties within the family, whether traditional or modern, all forms of psychiatric treatment have to deal with the family in one way or another. In general, the therapist needs to be actively guiding and probably authoritative in his approach toward members of the family. This does not preclude the necessity for a dynamic understanding of the individual patient and his family. Even authoritative and directive measures could be aimed at psycho-dynamically loaded rôle conflicts or traumatic experiences. For example, a yet uncompleted study of children with conversion reaction indicates that these children have a characteristic rôle pattern within the family in that they assume the rôle of the parent of the same sex, overburdening themselves with heavy responsibilities which would ordinarily pertain to the parent. Actually, this sort of rôle distribution is quite common in traditional families, but the burden of responsibilities assumed by the child or assigned by the parents seems to be unusually high in those children who show conversion reactions. In treatment, a brief separation of the child from the family and active guidance of the parents toward a change in this rôle pattern seem to produce good results.

Finally, I would like to comment briefly on future possibilities. With the great impact on Turkey of contemporary western medicine, which has already developed highly indivualized and hospital-based techniques, the realistic needs of the country were not adequately recognized until recently. Among current endeavors, one can mention the mother-child health centers that have been established around the country since 1950. In the early nineteen-sixties, attempts at organizing and applying the nationalization of medicine and family planning, particularly in the less developed sections of the country, were among the important contributions of the Ministry of Health and Social Welfare. Our new medical school, established in Ankara in 1963, organized its entire teaching program in an integrated system.[10] To insure departmental cooperation there are three main departments in the Medical School: The Department of Basic Medical Sciences, the Department of Clinical Sciences and the Department of Community Medicine. In the Department of Community Medicine, through the collaboration of psychiatrists, pediatricians, public health specialists and sociologists, a family oriented approach is one of the main goals of teaching and practice. By assigning each medical student to a family from a low socio-economic level from the first year, the student studies the social and psychological problems of the Turkish family. A course in child-develop-

ment is an integral part of the teaching of community medicine, as well as of psychiatry. During his clerkship the student also rotates in the specially designed rural health centers where he has ample opportunity to study the traditional family and its problems *in vivo*.

With an increasing awareness of the importance of family and community factors in health and in sickness, the current trend in nationalized health planning in Turkey is toward establishing integrated medical units which provide for both preventive mental hygiene and psychiatric care for the family.

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Age at Inception of Menstruation in Turkish Women

A Survey

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This investigation covers over 800 female students from the Hacettepe Hospital School of Nursing, Hacettepe University School of Nursing and Gevher Nesibe Institute of Health Education. Because of incomplete notification and unanswered questionnaires, only 692 of the original number of cards were fully taken into account.

The aim of this study was to examine any menstrual problems which the girls might have had, and may not apply to the population in a broader sense, as it does not rely on any sampling basis. However, since it covers a large number of individuals, a satisfactory amount of statistically significant information was obtained.

Not all the answers given in the questionnaires were evaluated; for example, the answers to those questions intended simply to verify the preciseness of replies to previous questions were not included. The information gathered from the questionnaires was coded, classified and put on punch cards in the School of Public Health and Hygiene (Hıfzıssıhha) of the Ministry of Health and Social. Welfare.

The Age at Menarche

The ages of the girls included in this study varied from 15 to 35, and the average age was 18. According to our findings, the age at which the girls started menstruating was between 9 and 19, the average being 13.98, and the mean deviation was 0.972 (13.98 ± 0.972). The median age at menarche was 14.27, and the mode age 14.

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There were some questions concerning the menses of the girls' mothers, but only a few knew at what age their mother's started to menstruate. However, the mean age was 14, the mean deviation 1.106 (14 ± 1.106) and the median age at menarche 14.24.

If the ages of menarche in the mothers and the girls questioned are compared, it will be noticed that it occurs earlier in this than in the previous generation. In Europe, Asia and various countries in South America, the age at menarche varies from 14 to 16. In a research study covering 2,590 English girls, the median age at menarche was found to be 13.49 and the standard deviation 1.149 years.[1]

Various factors, including variations in climate, nutrition, urbanization, chronic diseases, race, socio-economic background and living conditions affect the onset of menarche.

The Month Menarche Occurs

Fifteen point eight per cent of the girls included in our investigations had their first menses in May, 12.3 in June, and 11.6 in July, which indicates that menarche occurs in the spring and summer in the majority of cases. This closely resembles that of Japanese and Chinese girls, whereas in cities such as New York, Vienna and Berlin it more frequently occurs in the winter months.

The First Menstrual Cycle

It is known that the menstrual cycle during puberty differs from that of adults,[2] and it appeared from our study that its length sometimes changes constantly in the same individual. During the early years following the menarche, menstrual irregularities gradually lessened and the cycle began to last longer. Thus 53.4 per cent of 262 young girls who had had menstrual irregularities said that these cleared up within several years, and that the duration of their cycles grew longer; however, 46.6 per cent had experienced no improvement in their menstrual irregularities.

Menstrual Irregularities

Of a total of 692 young girls, 38.1 per cent complained of irregularity, and 61.9 per cent had normal menses. Fifty-six point five per cent of 262 girls with irregular menses complained of pain during menstruation, and the remaining 43.5 per cent were normal. Forty per cent of all the young

girls included in our study had menstrual irregularities of one sort or another, and more than half complained of menstrual pains. Thirty-eight point four per cent of 147 girls with dysmenorrhea said that their menstrual bleeding differed in quantity from that of their acquaintances.

Seventy-four point one per cent of the girls with menstrual problems wished to consult a doctor, but the remaining 25.9 per cent did not.

Duration of Menstrual Cycle

The average human menstrual cycle is 28 days according to our findings, and the mode is also 28 days. The duration of the cycle varied between 18 and 90 days. Menstrual irregularities differed from a minimum of one day to a maximum of 90 days. Some girls stated that these irregularities were not uniform, but that they changed every month. One said that she had menstruated three months later than the usual time during a period of grief.

Duration of Menstrual Bleeding

As can be seen from Table 4, in 27.9 per cent of the girls bleeding lasted five days, in 24.5 per cent for four days, and in 16.8 per cent for six days. The average duration of the bleeding period was 4.98, and the mode five days.

The Age of the Girls' Mothers at Menopause

Of all the girls questioned, 67 per cent of their mothers still menstruated, and 33 per cent had ceased. In 23.4 per cent the menopause occurred at 45 years of age, and in 20 per cent at 40 years. Examining these figures closely, it will be noticed that the girls often gave erroneous statements regarding their mothers' ages. Round figures such as 40 and 45 indicate clearly their unreliability.

The average age of the mothers' menopause as first seen was found to be 43.13 years.

Conclusion

The average age at which menarche first occurs is shown as the arithmetic mean 13.98 ± 0.972; the median is 14.27 years of age. The average age at the onset of menarche in the girls' mothers was 14.0 ± 1.106, and the median was calculated at 14.24.

TABLE I

AGE OF TURKISH GIRLS AT MENARCHE

Name of School	Total	No. of Cases	Age at Menarche 9	10	11	12	13	14	15	16	17	18	19	20	No. Response
Total	692	688	1	1	15	63	151	219	179	45	12	—	2	—	4
	%	100.0	0.2	0.2	2.2	9.2	21.9	31.8	26.0	6.5	1.7	—	0.3	—	—
Hacettepe University School of Nursing	66	66	—	1	6	8	19	14	13	5	—	—	—	—	—
Gevher Nesibe Health Education Institution	35	35	—	—	2	6	3	13	7	3	1	—	—	—	—
Cebeci Health College	189	188	1	—	—	15	43	58	56	12	3	—	—	—	1
Yenişehir Health College	145	145	—	—	2	10	27	59	40	7	—	—	—	—	—
Hacettepe Hospital School of Nursing	257	254	—	—	5	24	59	75	63	18	8	—	2	—	3

TABLE II

LENGTH OF MENSTRUAL CYCLE IN TURKISH GIRLS

Total	Number of Cases	DAYS																				No Response
		-18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35-44	45-59	60-90	
692	672	4	1	8	9	7	8	8	26	28	31	244	49	172	4	4	3	6	33	17	10	20
%	100.0	0.6	0.1	1.2	1.3	1.1	1.2	1.2	3.9	4.2	4.6	36.3	7.3	25.6	0.6	0.6	0.4	0.9	4.9	2.5	1.5	—

TABLE III

MONTH OF ONSET OF MENARCHE

Total	Number of Cases	MONTHS												No Response
		Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
692	595	43	50	45	62	94	73	69	55	40	38	15	11	97
%	100.0	7.2	8.4	7.6	10.4	15.8	12.3	11.6	9.2	6.7	6.4	2.5	1.9	—

TABLE IV

LENGTH OF GIRLS' MENSTRUAL CYCLES IN DAYS

Total	Number of Cases	Length of cycle in days										No Response
		1	2	3	4	5	6	7	8	9	10	
692	689	—	10	89	169	192	116	95	14	2	2	3
%	100.0	—	1.5	12.9	24.5	27.9	16.8	13.8	2.0	0.3	0.3	—

TABLE V

GIRLS' MENSTRUAL IRREGULARITIES IN DAYS

Total	Number of Cases	Differences in days									10-14	15-19	20-24	25-29	30+	No Response
		1	2	3	4	5	6	7	8	9						
262	231	2	12	15	11	26.0	8	21	9	1	36	29	11	1	49	31
%	100.0	0.9	5.2	6.5	4.7	11.3	3.5	9.1	3.9	0.4	15.6	12.6	4.7	0.4	21.2	—

TABLE VI

THE LENGTH OF MENSTRUAL CYCLES ACCORDING TO AGE OF GIRLS

Length of Cycle	Total	FULL AGE																					No Response
		14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35+	
Total	692	—	12	48	91	124	112	105	73	54	32	9	3	3	4	3	2	2	3	2	2	2	5	1
-20	13	—	—	1	4	3	2	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
21	9	—	—	—	1	4	1	2	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
22	7	—	—	2	—	—	1	1	—	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—
23	8	—	—	—	1	—	3	—	1	1	—	—	—	—	—	—	—	—	—	2	—	—	—	—
24	8	—	—	1	—	—	4	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
25	26	—	—	2	1	5	6	2	5	1	4	—	—	—	—	—	—	—	—	—	—	—	—	—
26	28	—	1	—	2	7	5	5	4	3	1	—	—	—	—	—	—	—	—	—	—	—	—	—
27	31	—	—	—	5	8	6	6	3	2	—	—	—	—	—	—	—	—	—	—	—	—	1	—
28	244	—	3	11	26	39	38	36	23	28	19	3	2	3	1	—	—	2	2	—	2	1	4	1
29	49	—	4	4	3	11	9	8	4	2	2	1	—	—	—	—	1	—	—	—	—	—	—	—
30	172	—	4	15	33	31	21	28	20	5	4	5	1	—	2	2	1	—	—	—	—	—	—	—
31-44	50	—	—	4	6	6	12	11	3	4	1	—	—	—	—	1	—	—	1	—	—	1	—	—
45-60	17	—	—	2	4	5	—	2	1	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
61-99	10	—	—	2	3	2	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No Response	20	—	—	4	2	3	1	2	5	2	—	—	—	—	1	—	—	—	—	—	—	—	—	—

TABLE VII

AGE OF MOTHERS AT MENARCHE

Total	Number of Cases	Age at menarche												No Response
		9	10	11	12	13	14	15	16	17	18	19	20	
692	407	1	1	10	47	93	117	85	33	9	10	—	1	285
%	100.0	0.2	0.2	2.5	11.5	22.9	28.8	20.9	8.1	2.2	2.5	—	0.2	—

TABLE VIII

AGE OF MOTHERS AT MENOPAUSE

Total	Number of Cases	Age at menopause																No Response
		35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50+	
218	175	5	4	2	17	2	35	3	10	5	3	41	8	5	12	3	20	43
%	100.0	2.9	2.3	1.1	9.7	1.1	20.0	1.7	5.7	2.9	1.7	23.4	4.6	2.9	6.9	1.7	11.4	—

Today's girls experience menarche earlier than their mothers, which is partly due to improved socio-economic conditions and better nutrition. The majority of the girls had their first menses during spring or summer, and the likelihood of the onset of menstruation decreases in winter. From this study, the duration of a typical menstrual cycle was found to be 28 days (mode).

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Chromosome Abnormalities in Bladder Tumors

Nevzat A. Duruman, M. D.*

Since 1901 attempts have been made to analyze the karyotypes of solid human tumors. Although sporadic and limited studies were performed on primary cancers as far back as 1947[1] it was not until 1965 that cytological techniques permitted exact chromosomal analysis of human cells. With the discovery of the Philadelphia chromosome (Ph. 1) in chronic myeloid leukemia there was renewed interest in chromosome analyses as possible adjuncts to the diagnosis and prognosis of epithelial tumors. In 1962 Spriggs et al[2] described hypertetraploid chromosomal counts (120-140) in ascitic fluid cells from a primary bladder carcinoma and demonstrated an abnormal elongated chromosome in the cells from a pleural effusion due to a metastatic bladder tumor. In 1963 Bush, Bauer and Mitra[3] described similar findings in an analysis of four papillary bladder carcinomas. In 1965 Shigematsu[4] reported on the karyotypes of 34 benign and malignant bladder tumors. In the study to be presented chromosome preparations were made from bladder tumors in 31 patients from the Memorial and James Ewing Hospitals and the results were correlated with the pathologic stage and grade (Table 1) and prior therapy of the tumor (Table II).

Materials and Methods

A total of 31 bladder tumors were examined. Only 23 of these were suitable for chromosome analysis. Thirteen specimens were obtained by transurethral resection, six by radical cystectomy and four by excision of retroperitoneal lymph nodes. Of these 13 specimens, one (Case No. 9) was also studied after three months in tissue culture.

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TABLE I

Case No.	Hospital Case No.	Stage	CHROMOSOMES NO. (Range) GRADES In Situ	O - I	II	III	IV	Marker Chromosomes
1	603700			44 - 46				
2	601508	Papilloma		43 - 46				
3	265650		46 - 55					+
4	265760	In Situ	64 - 91					+
5	266312				46 - 71			
6	602839				52 - 68			+
7	602877					65 - 81		
8	263526	Low Stage				45 - 52		
9	260792				53 - 92			+
10	603620				53 - 93			
11	266146				61 - 83			+
12	603403					59 - 65		
13	603588					81 - 104		
14	247847					82 - 100		
15	261600						51 - 132	+
16	250891	High Stage			60 - 85			+
17	603009					61 - 85		+
18	252235					59 - 78		
19	602719					53 - 74		
20	603798					58 - 73		
21	603157					56 - 71		
22	603031	Metastacic				57 - 71		+
23	603346 PR.					95 - 106		+
	Node					58 - 74		

PR. = Primary

TABLE II

Case No.	Hospital Case No.	Duration of disease	Primary or metastatic	Surgical procedure	Radiation therapy	Av. chromosome count
1	603700	3 years	Primary	T.U.R.	—	46
2	601508	2 years	Primary	T.U.R.	—	46
3	265650	4 years	Primary	T.U.R.	—	46 - 55
4	265760	8 years	Primary	T.U.R.	—	64 - 91
5	266312	2 years	Primary	Rad. cyst.	—	46 - 71
6	602839	2 years	Primary	Rad. cyst.	+	52 - 68
7	602877	1 year	Primary	T.U.R.	+	65 - 81
8	263526	2 years	Primary	T.U.R.	+	45 - 52
9	260792	8 months	Primary	Rad. cyst.	+	53 - 92
10	603620	6 years	Primary	Rad. cyst.	+	53 - 93
11	266146	2 years	Primary	T.U.R.	—	51 - 83
12	603403	2 months	Primary	Rad. cyst.	+	59 - 65
13	603588	5 years	Primary	T.U.R.	+	81 - 104
14	247847	1½ years	Primary	T.U.R.	+	82 - 100
15	261600	2 years	Primary	Exp. lap.	—	51 - 132
16	250891	18 years	Metastatic	Exp. lap.	+	60 - 85
17	603009	2 years	Primary	T.U.R.	—	61 - 85
18	252235	2 years	Primary	T.U.R.	+	49 - 78
19	602719	2 years	Primary	T.U.R.	—	53 - 74
20	603798	8 years	Primary	Rad. cyst.	—	58 - 73
21	603157	3 years	Metastatic	Exp. lap.	—	56 - 71
22	603031	4 years	Metastatic	Rad. cyst.	—	57 - 71
23	603346	2 years	Metastatic	Exp. lap.	+	58 - 74

Part of the fresh bladder tumor tissue was sent for histologic evaluation and part was kept for chromosome study. The chromosome preparation was made with the air-dry method using a modification of the Moorhead technique.[5] After drying, the smears are left at least 24 hours before staining with 1:10 Giemsa solution.

Results

1. Two papilloma cases were analyzed. In these the chromosomes were diploid and no abnormal chromosomes were encountered (Figure I).

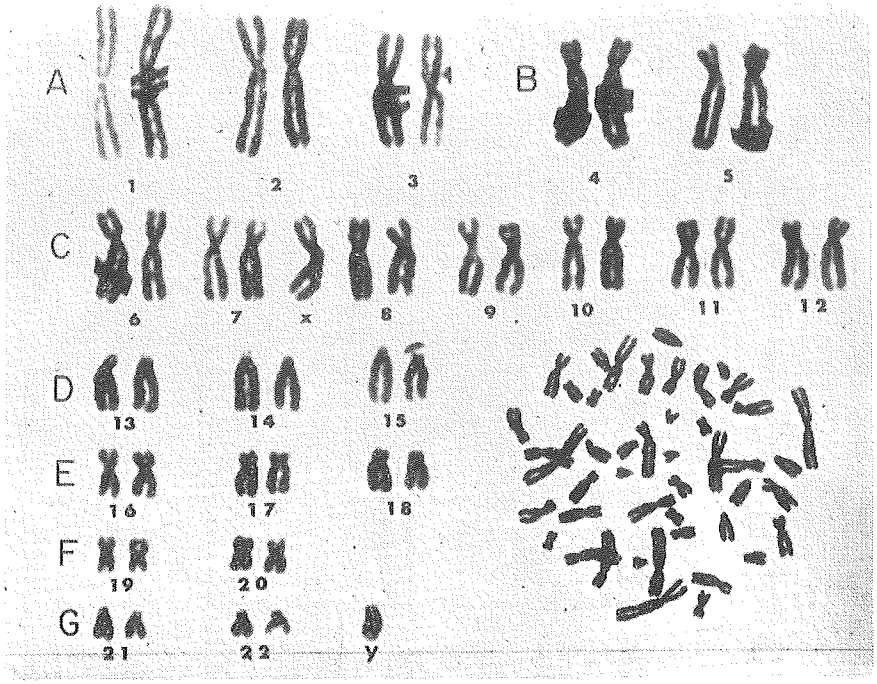

Figure 1. Normal Chromosome Karyotype

2. Two carcinoma *in situ* cases were analyzed. These revealed aneuploidy with gross chromosomal abnormalities (Figure II).

3. There were 15 cases of carcinoma in which the chromosome number varied from 45-132. Most of the tumors had a chromosome count in the range of 53-84. No correlation was evident between the chromosome number and the grade or stage of the tumor (Table III).

4. There were four cases of metastatic carcinoma in the lymph nodes. The best preparation for chromosome analysis was obtained in these

83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
8	11	8	10	1	2	1	1	2									
1	2					1			1								
		3	1			1	1	3	2	1							
11	14	7	5	1	1				4	3	1	3	1	1	1	4	3
2	5	3	2	3	6	2	5	1	2								2
1										1		1		1	4		9
11	16	10						1									
4	1	3															
												8	7	11	6	8	14

TABLE III *

58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	8
PAPILLOMA																									
IN SITU CA																									
						2	1	4	1		3	4	1	3						2	4		1	7	
						4	7	9	10	9	8	3	1												
LOW STAGE																									
9	7	9	10	3	1	1			2	1															
							3	7	8	10	1	5	1						4	3	1	3	3	
	8	5	10			3	2				1		2				1	2			4	3	1		
8	7	16	12	1	1							3	5	7	1	1	1	4	3	1	2				
HIGH STAGE																									
				8	15	1	1	10	1		2	3	5	1	3	1	1	2	1	4			4	2	
	3	7	11	13	8	1	1																		
																								3	8 1
																								3	
	1	5	1								1								1						
		1		1					1		1	1				4			1	2			5		1
			3	1	5	6	8	11	7	10	3	1	2	5						1	1	3	1		
	2	1	5	1	7	1	3	4	7	8	11	2	1		3	1	3								
3	1	5						2	3	1	3	8	1	2		3									
METASTATIC																									
3	8	10	1	5	7	9	3	1			2	3	1		5										
1			1				1	2		2	4	7	5												
5	1	3	5	1	8	8	11	3	1	3	1	2	1												
3	7	8	9	4	7	1	4					1	3	2											

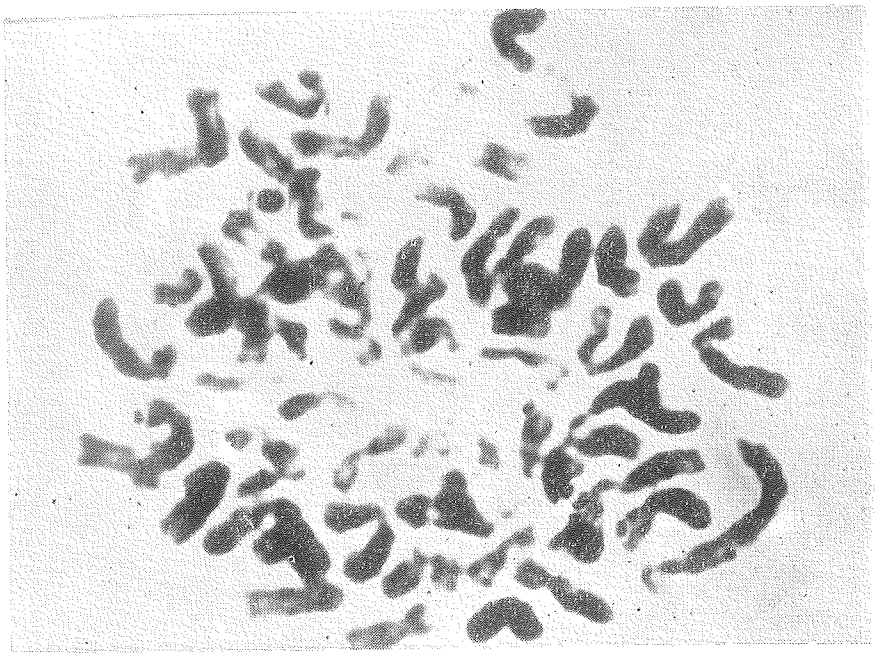

Figure 2. In-situ carcinoma shows abnormal chromosomes

four cases because dividing cells were abundant. There was minimal overlap of the chromosomes, and their outlines were clearly visible. The count ranged between 53 and 74. Case No. 23 had a count of 58 to 74. The suspected original tumor in the latter instance had a chromosomal count of 95-106.

5. In one tissue culture, after three months the cells had a normal chromosome count and were thought to represent fibroblasts.

6. In ten cases there were abnormal chromosomes (Marker) which were ring-shaped, elongated or of otherwise bizarre composition: these chromosomes were evident in four out of eight D lesions (grade II-IV), two out of six C lesions (grade II), one out of five B 1 and B 2 lesions (grade II) and in both *in situ* carcinomas. (Figure 3).

Discussion

Abnormal chromosomes can be caused by radiation, by several viruses and by a variety of chemicals, including chemotherapeutic agents. Chromosomal abnormalities are found in certain congenital diseases, such, as: Klinefelter's syndrome, in which there is one or more extra

Figure 3. Chromosome preparation from a primary bladder carcinoma, in addition to the abnormal distributions in various groups, there are four abnormal marker chromosomes. (Arrow indicates marker chromosomes).

X chromosome (XXY) (XXXY); Turner's syndrome, in which one X chromosome is missing (XO); and mongolism (Down's syndrome) in which there is an extra No. 21 chromosome. The chromosome abnormalities in such congenital anomalies differ from those seen in cancer in the following. respects:

1. They occur in most somatic cells in congenital diseases.
2. They are relatively slight compared with those seen in many cancers. Autosome abnormalities in congenital diseases are restricted to gains of single autosomes or parts of autosomes.
3. Although different chromosome patterns may occasionally occur in the same individual (Mosaicism) with a congenital syndrome, marked differences from cell to cell are much more common in cancer.

Several investigations of chromosome abnormalities in solid tumors have been made. In Hodgkins disease chromosome counts in the sub-tetraploid range were usually found, presumably representing the Reed-Sternberg cells.[5] In lymphosarcoma, karyotypes have been near diploid although

many show some variation in abnormality. In almost all cases of cancer of the lung, breast, stomach and ovary the number of chromosomes is increased, and a pooling of data from various reports indicates that although stemline chromosomes vary widely, the range of variation is remarkably similar among those four types of cancer. In 25 cases of dysplasia and *in situ* carcinoma of the uterine cervix,[6] analysis showed that both dysplasia and *in situ* carcinoma exhibit aneuploidy. Abnormal karyotypes were demonstrated in four primary gastrointestinal cancers and one potentially malignant colonic polyp.[7] Chromosome analysis of one primary medulloblastoma[8] revealed the consistent evidence of two to five extra chromosomes in groups 4-5. X and 6-12. Similar karyotypes were seen in certain cells from the peripheral blood and from the bone marrow, presumably representing metastatic medullablastoma.

Shigematsu[4] studied the chromosomes in 34 bladder tumors. In low grade papillary carcinoma (papilloma) he found normal chromosome numbers. In higher grade carcinomas, increased numbers of chromosomes, but with a limited numerical range, were obtained. In stage D tumors and grade IV tumors he emphasized that the number of chromosomes was reduced. He concluded that variation in the chromosomes and analysis of the chromosome types are important factors in distinguishing malignant from normal cells.

The normal chromosome number and karyotype observed in two papillomas in this study correlate with the clinically and pathologically benign behavior usually exhibited by these lesions. Further observations are necessary to determine whether chromosome abnormalities precede, or occur concomitantly with, the development of the histologic features of atypia and overt malignant change recognizable in atypical papillary carcinoma, which may ultimately develop in the patient with papilloma.

Chromosome abnormalities were found in *in situ* carcinomas in two patients with concurrently visible gross epidermoid cancers. Whether such abnormalities are equally prominent in *in situ* bladder lesions unassociated with overt cancers remains to be determined. Such an occurrence has already been recorded for the analogous situation of *in situ* carcinoma and dysplasia of the uterine cervix. The variable natural history of *in situ* bladder cancer is being increasingly documented and it would be of considerable interest to correlate the chromosome number and karyotypes of such lesions with their clinical behavior.

In 19 overt bladder carcinomas studied the chromosome number was increased, but no correlation between the pathologic grade and/or stage of the tumor and chromosome number was evident in this small series.

If the chromosome number or karyotype could be demonstrated to correlate with tumor growth potential in a manner not necessarily correlated with tumor grade and/or stage, this would be a clinically useful tool for the evaluation of bladder tumors, and treatment and prognosis would be available.

In one patient in whom a chromosome study of both primary bladder tumor and lymph node metastasis was possible, the chromosome number in the two preparations were different. This might be an indication of:

1. A metastasis of cells from a previously treated tumor of different chromosome composition than the primary tumor that was sampled.
2. Metastasis of a select population of cells from the primary tumor of mixed chromosome composition.
3. An environmental effect of the lymph node on the chromosome composition of the metastatic growth.

Though Marker chromosomes were demonstrated in the tumors we studied, they are not specific for bladder tumors, and no specific extra chromosome such as the Philadelphia (Ph. 1) chromosome was identified. Chromosome changes occur almost invariably in bladder cancer but these changes have not been shown to be specific.

Summary

Twenty-three bladder tumors were studied for chromosomal abnormalities. Papilloma of the bladder showed the diploid chromosome number to be similar to somatic cells. Carcinoma showed a marked increase in the number of chromosomes with abnormal karyotypes. In one observation metastatic carcinoma had fewer chromosomes than the presumed primary. In this study bladder tumors showed no specific chromosomal abnormalities.

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Malabsorption Syndrome in Portal Hypertension

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Years ago it was suggested that cirrhosis of the liver could cause malabsorption.[1 2 3] However, only recently has the improvement of methods of investigating the absorptive functions of the intestine revived interest in this subject.

In searching for the etiologic factors of malabsorption in cirrhotic patients, the authors attributed malabsorption to disorders of the pancreatic and hepatic functions.[4 5 6] The relation of portal pressure to malabsorption in cirrhosis of the liver has never been mentioned; this study was made to investigate such a relationship.

Materials and Methods

Four control cases and 24 patients with cirrhosis of the liver were included in the study. Two of the control cases were male and two female, while 16 male and eight female patients had cirrhosis. The age of the control group ranged from 22 to 55 years, and that of the cirrhotic patients from 35 to 65 years. In all of the 28 cases, a liver biopsy was performed and liver functions were investigated by means of BSP, SGOT, SGPT, thymol turbidity, CCF, alkaline phosphatase and serum albumin/globulin ratio. In order to exclude other factors leading to malabsorption, the following criteria were observed in the selection of patients:

1) Absence of diabetes mellitus.[7 8]

2) Absence of jaundice. Patients with serum bilirubin higher than 1.5 mg per 100 ml of serum were not included in the study.

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3) Absence of pancreatic insufficiency.[9] Secretin tests were done in every case to rule out pancreatic disease.[10 11]

4) Absence of large bowel or small bowel disease.[12]

5) Absence of gastric or bowel resection.[13 14]

6) Absence of pernicious anemia or intestinal parasites.

7) Absence of previous use of neomycin or related drugs.[15]

The tests performed to investigate the intestinal absorption were as grouped below:

A - Fat Absorption

1) Quantitative fat in the stools: Patients received a special diet for six days containing 100 gm of fat daily. During the last three days of this period, stool samples were collected for fat determination, which was done according to the method of Watson, considering values below 22 per cent as normal.[16]

2) Radioactive triolein and oleic acid: Patients were given Lugol's solution 10 drops t.i.d. three days prior to the test. After the oral administration of 0.5 gm of triolein or oleic acid, labeled with 25 microcurie of I^{131}, blood samples taken at the third, fourth and fifth hours were counted in a well-counter. The mean values above seven per cent were considered normal.[17 18]

B - Carbohydrate Absorption

This was investigated by D-xylose test. After an overnight fast, patients were given 25 gm of D-xylose orally, and D-xylose excretion in a five-hour urine specimen was measured by the Roc and Rice method. Excretion over five gm was considered normal.[19 20]

C - Schilling Test

Patients received an oral dose of 0.5 micrograms of vitamin B_{12} labeled with 0.5 microcurie of Co^{60}. Urine was collected for 24 hours and radioactivity was measured. If the result was abnormal the test was repeated with the intrinsic factor.[21]

In every case portal pressure was checked by percutaneous spleen puncture. Values below 150 mm of H_2O were considered normal.[22]

Results

All the control cases had normal absorption test results (Table 1). In only 15 of the 24 patients with cirrhosis were stool fat, radioactive triolein and oleic acid tests, the Schilling test and D-xylose test found to be abnormal (Table III).

In the remaining nine patients, the above tests were within normal limits (Table II). The 15 patients with absorption abnormalities had portal pressures between 240 and 425 mm of water, whereas in the nine patients with normal absorption portal pressures ranged from 184 to 200 mm of water.

Discussion

The reason for intestinal malabsorption observed in liver cirrhosis is not well understood. Summerskill et al [5] found obvious abnormalities in intestinal absorption in seven non-icteric cirrhotic patients. These authors thought first that bile played a role in the pathogenesis of malabsorption. In order to clarify this, they gave their patients bile after each meal obtained from a patient who had undergone cholecystectomy, through a duodenal tube. However, they observed no change in the absorption tests. On the other hand, when liver function tests showed some improvement with a high protein, high carbohydrate diet, intestinal absorption also improved. According to these observations, the authors suggested a relationship between liver function and derangements in intestinal absorption.

Friedman and McEwan[4] tried to relate the abnormalities in absorption tests in 10 cirrhotic patients to probable pancreatic disorder.

In the present study pancreatic disorder was excluded because the response to secretin injections was normal, therefore it is not believed that the abnormalities in intestinal absorption are related to the pancreas.

The importance of bile in fat absorption has been known for a long time.[5] Some authors have suggested that in cirrhosis the possible abnormalities in bile flow and bile composition might lead to intestinal malabsorption.[2,3] Nevertheless, studies made on non-icteric patients with cirrhosis of the liver have demonstrated that bile flow into the intestinal canal was not insufficient, that plenty of bile could be obtained by duodenal aspiration, and that daily stercobilin excretion was normal. The malabsorption seen in the present study cannot be explained by the bile factor alone, because even if bile could explain the abnormalities in fat absorption, it would be difficult to understand why the D-xylose and radioactive vitamin B_{12} tests

TABLE I
THE LABORATORY FINDINGS OF CONTROL CASES

Case No.	P. Pressure mm/H2O	Ascites	Fecal Fat %	R-Active Triolein %	R-Active Oleic A. %	D-Xylose Gm.	R-Active B12 %
1	125	—	6.2	15.9	14.5	9.6	21.8
2	116	—	8	13.8	14.1	9.1	24.2
3	130	—	4.6	16.3	15.7	8.7	22.3
4	104	—	7.3	14.9	13.8	10.3	22.8

TABLE II
THE LABORATORY FINDINGS OF PATIENTS WITHOUT MALABSORPTION

Case No.	P. Pressure mm/H2O	Ascites	Fecal Fat %	R-Active Triolein %	R-Active Oleic A. %	D-Xylose Gm.	R-Active B12 %
1	184	+	10.4	10.5	12.6	6.2	18.8
2	196	—	14	12.6	13.2	8.3	19.1
3	194	+	9.6	14.3	12.1	5.8	22.3
4	189	—	14.8	11.6	14.1	7.4	16
5	190	—	19.4	9.8	10.6	6.4	9.9
6	199	+	20.6	8.7	9.2	5.6	19.3
7	200	—	18.4	14.1	14.6	7.8	15.6
8	187	+	11.2	10.8	12.8	6.8	11.9
9	195	—	16.1	9.2	15.1	5.9	14.6

TABLE III

THE LABORATORY FINDINGS OF PATIENTS WITH MALABSORPTION

Case No.	P. Pressure mm/H2O	Ascites	Fecal Fat %	R-Active Triolein %	R-Active Oleic A. %	D-Xylose Gm.	R-Active B12 %	R-Active B+12 Intrin F. %
1	356	+	42.5	3.7	4	2.2	6.2	7.1
2	415	—	38.4	2.4	2.8	1.9	4.6	4.3
3	425	+	40	3.2	3.9	0.8	2.8	4.6
4	340	—	32.6	4.1	4.7	0.6	7.3	7.2
5	390	+	33.5	2.9	3.8	3.3	6.9	6.4
6	335	+	37.3	4.3	5.2	1.7	3.8	4.2
7	295	+	26.4	6.1	5.6	4.8	8.2	9.4
8	240	—	25.3	5.2	6.3	3.9	6.3	6.1
9	258	—	24.6	5.8	6.1	4.3	2.9	4.3
10	262	—	28.2	4.8	3.8	4	7.8	8.3
11	250	+	30.2	5.4	3.4	2.9	9.6	9.1
12	270	+	27.9	4.1	5.6	1.8	5.7	6.3
13	265	+	23.6	6.9	7.2	4.3	8.2	9.1
14	250	+	26.2	5.7	7.2	3.9	8.4	7.6
15	265	+	25.9	6.1	5.8	4.2	8.9	8.2

were abnormal, or why the results of all the tests, including fat absorption in some of our patients, were normal. Besides, the failure of orally administered bile to non-icteric cirrhotic patients to improve absorption shows that bile is not the sole factor. To the best of our knowledge, the malabsorption observed in some of the patients in the present study is probably unrelated to liver function abnormalities. In nine patients with cirrhosis of the liver, intestinal absorption was essentially normal, although liver function was not.

We believe that portal hypertension is one of the factors leading to intestinal malabsorption, because in all 15 patients with malabsorption portal pressure was high (Table III). On the other hand, in nine patients with minimal portal hypertension no abnormalities in intestinal absorption could be demonstrated (Table II).

The absorptive function of the intestine is a complex mechanism involving many factors and enzymes. At the present time it is impossible to attribute the disturbances of absorption in cirrhosis of the liver to one factor only. The results derived from this study suggest that portal hypertension is one of the multiple factors.

Summary

The relation of intestinal malabsorption to portal hypertension was investigated in patients with cirrhosis of the liver. In 15 cirrhotic patients with portal pressures between 150 and 525 mm of water, malabsorption was found. In the remaining nine patients with cirrhosis who had portal pressures ranging from 184 to 200 mm of water, intestinal absorption tests were within normal limits. These observations suggest that portal hypertension is one of the factors leading to intestinal malabsorption.

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Milestones of Research: Recent Developments in the Recognition and Treatment of Pulmonary Embolus

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The time when pulmonary embolism was considered a rarity is not far in the past. Despite this, a great deal of information has accumulated during the last 10 to 15 years on this subject. Some of the most interesting points established by these studies are as follows: each year an estimated 47,000 people die of pulmonary embolism.[1] This figure becomes alarming when it is remembered that only 20 per cent of all pulmonary emboli are fatal. In most series studied by routine autopsy methods it has been found that nine to 30 per cent of patients dying of a variety of causes also had pulmonary emboli.[2] This already high incidence climbs to 64 per cent in series where the arterial tree of the lungs is studied by special techniques,[2] for in less than half of this latter group are the emboli large enough to be clinically recognized. Another very interesting finding concerns post-operative deaths due to lung emboli. Different studies report that from 0.11 to five per cent of fatalities during convalescence from surgical interventions are due to pulmonary embolism.[3-5] It should, furthermore, be realized that 80 per cent of cases of pulmonary embolism are silent, and 90 per cent cannot be clinically diagnosed. These and other studies have proved how desperate a need there is to improve clinical methods of diagnosing this disease more accurately.

Pulmonary embolism presents itself with extremely variable clinical pictures, which are primarily related to the size of the embolus and to the

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impairment of hematosis. At least 40 per cent of patients reveal no preliminary symptoms before pulmonary embolization occurs.[5] Some cases reveal themselves through phlebitis, usually located in the lower extremities[4 6 8] or the pelvis. [8 10] However, in 40 per cent of pulmonary emboli neither clinical nor postmortem evidence of thrombophlebitis can be detected.[8 11] Apart from phlebitis, no particular element in the case history clearly favors the diagnosis of embolization. Sudden chest pain, dyspnea, cough and bloody sputum - once considered cardinal symptoms of pulmonary emboli - are only rarely encountered together. Most frequently the clinical picture suggests cardiac disease, or when there is dizziness and loss of consciousness cerebro-vascular accidents are stimulated. In some cases pulmonary symptoms are superimposed by predominant concurrent infections, leading the physician to overlook the real disease. Some cases present severe dyspnea and signs of bronchial asthma, while others give no symptoms at all. Despite this wide variation in presentation, there are no symptoms which distinguish those patients with emboli from those without.[8] Clinical findings also vary from sudden death, collapse or coma to simply slow respiratory rate.[7] Cyanosis, distended neck veins, dyspnea, systolic murmur and accentuation of the second sound at the pulmonic valve area, and even gallop rhythm and systemic hypotension, which may simulate a primary cardiac disease, are often seen. In other cases only right heart failure or signs of chronic pulmonary hypertension predominate. Some present pneumonia or pleural effusion, while others experience no symptoms at all.[12]

Findings from x-ray studies are hardly ever diagnostic in themselves; they are, however, indicative of hyperlucent areas distal to occlusion, elevation of the diaphragmatic leaf, blunting of the costophrenic angle, and pleural effusion, for example.

Electrocardiographic findings are also variable, and are rarely diagnostic;[11] Q_3 with elevated ST_3 and inverted T_3, deep S, low T, and depressed ST_2, all occur in 10 per cent of cases. Right BBB, high voltage of P in the precordia, atrial premature beats, or auricular fibrillation, may be important if they are present. Seventy-three per cent of Hildner's patients revealed no electrocardiographic abnormalities[6] and some investigators are even convinced that ECG is often misleading.[11]

Neither are the other laboratory findings consistent. For example, leucocytosis is often mild, and increased serum level of lactic dehydrogenase, SGOT, LDH and serum bilirubin triad are often absent.[8]

It is not surprising, therefore, that pulmonary emboli were very frequently unrecognized in the past. However, necopsy findings are clear and

impressive; 64 per cent of cases coming to the autopsy table proved to have pulmonary embolus when examined carefully.[2] This entity, then, deserves to be called one of the most frequently misdiagnosed diseases of the past. The situation has changed since the development of the now available diagnostic methods, two of which have been widely used and conclusively tested. These are selective pulmonary angiography[13] and lung scanning.[14]

Pulmonary angiography can be performed even on extremely ill patients by simply passing a cardiac catheter into the main pulmonary artery and into its branches. One of the radio opaque dyes (75 per cent diatrozate sodium-hypaque) is then injected, and a roentgenogram of the bronchial tree is taken.[15 16] Before or after this procedure, pulmonary arterial pressure is measured. The rise in pulmonary vascular pressure and its rôle in the genesis of the cor pulmonale have recently been subject to many studies.[18 19]

Lung scanning, even though less informative, can be performed easily by injecting radioactively marked human serum albumin (RIHSA). Because of adequate mixing scanning of the lungs by detecting equipment reveals even distribution throughout the pulmonary area under normal circumstances. If a branch of the pulmonary vascular tree is closed to circulation the corresponding area reveals no radiation. Causes other than emboli occluding parts of the vascular bed, however, would also give similar results.[12-14]

In the diagnosis of pulmonary embolism the newest development is the use of ultra-sound methods. Rushmer and his associates used a crystal to detect reflected ultra-sound waves which reveal a different pattern between obstructed and patent vessels.[19] This method promises to become a very important addition to the already efficient diagnostic armamentarium discussed above.

Because of these recent developments it is now possible to diagnose the disease more successfuly, which is important as it is now known that in most cases pulmonary emboli do not cause rapid death. Time is allowed for confirmation of the clinical impression and for surgical treatment. According to Grahagan, the majority of patients with massive pulmonary embolus have enough time to permit embolectomy.[11]

The most important point in the detection of pulmonary embolism no longer concerns the possibility of its presence alone, but its inclusion in differential diagnosis. This is even more important nowadays because embolectomy, first performed by Trendelemburg in 1908 as a master-

piece of surgical adventure, today is commonly done.[20 21] It can be performed quite easily by the use of the cardiopulmonary by-pass technique, or even by simple embolectomy when the lesion is one-sided. The majority of cases, however, can be managed by anticoagulation and by more conventional measures.[1 2]

Summary

Three major developments have occurred in pulmonary embolus:

1) It has been realized that the disease occurs very frequently;
2) It is possible to recognize the disease early in its course if modern procedures are resorted to;
3) Its treatment is greatly improved, because in addition to anticoagulation and conventional measures, surgery now has a great deal to offer. Ligation of the venous tree at local or caval levels, and even pulmonary embolectomy, are now frequently used and save many lives.

The most important development in recent years is the realization that the physician is not justified in excluding pulmonary emboli when clinical findings warrant this unless pulmonary angiography and lung scan have been performed.

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The Gingiva in Diabetes Mellitus

E. Ferzan Telatar, M. D.*

Diabetes, which ranks eighth in the mortality lists prepared in the United States, is known to cause death most frequently by vascular complications.[1] Large and medium-size arteries, as well as arterioles, capillaries and venules, are involved, [2 3] and the retina, bulbar conjunctiva, glomerulus, placenta, skin, muscles, gastro-intestinal tract, and the nutritious arterioles of the nerves and great vessels have been the subject of extensive investigation with regard to vascular changes. [4-15] Thickening of the basement membrane in non-vascular structures such as the renal tubule, Bowman's capsule and the sweat glands has also been described.[15]

The object of this study was to investigate the changes in the gingiva of diabetic and non-diabetic patients.

Materials and Methods

Eighteen patients with diabetes mellitus and four non-diabetic control subjects were included in the study. The ages of the diabetic group ranged from 14 ½ to 90 years, the average being 67 10/12 years. Fifteen of the patients were female and three were male. The control group consisted of two males and two females whose ages ranged from 50 to 84 years with a mean of 69 3/12 years. The known duration of the disease among the patients with diabetes mellitus varied from five days to 30 years, and averaged 6 9/12 years.

Gingival biopsies were performed under local anesthesia with one to 1.5 ml of one per cent xylocaine injected into the labio-alveolar sulcus.

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Gingival tissues 5 to 8 mm long, 1.5 to 2 mm wide, and 1 to 2 mm thick, were obtained by vertical, interdental incision. If necessary, one or two 0-0-0 silk sutures were left in place for 48-72 hours. The biopsy specimens were placed first in Bouin solution, and four to 24 hours later were transferred to 70 per cent alcohol. The sections were stained with periodic acid Schiff and hematoxylin eosin, and studied under a light microscope.

Results

Microscope studies of the biopsy specimens revealed that 17 diabetic patients out of 18 had various degrees of arteriolar and capillary changes. (Figures 1 and 2). Only one patient showed no suggestion of vascular lesion (Figure 3), and none of the control cases had any vascular changes in the gingiva (Figure 4), though two showed histologic evidence of gingivitis.

The biopsy findings of the 17 diabetic patients are summarized below:

1. Thickening of the arteriole and capillary walls;

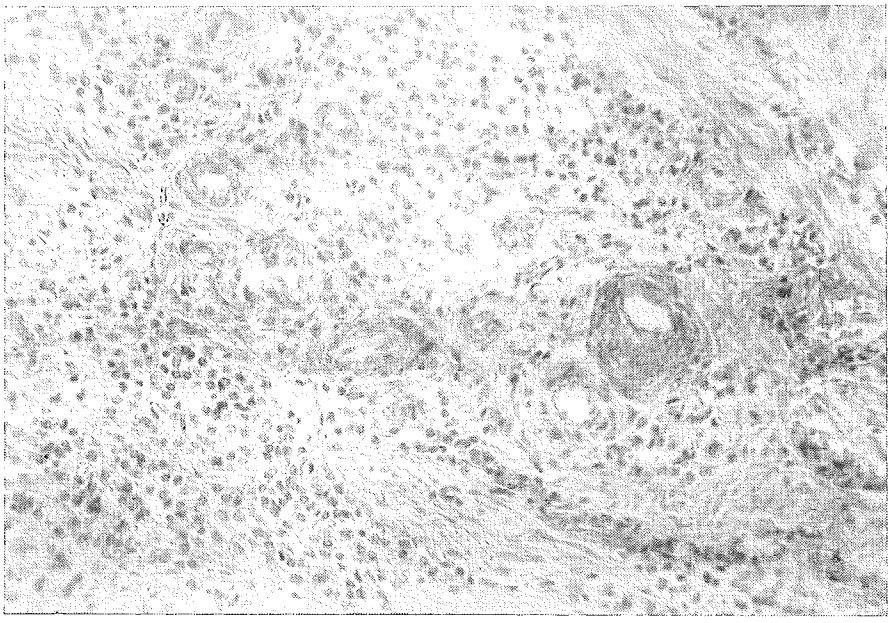

Figure 1. Histologic appearance of gingiva in a diabetic patient showing endothel proliferation and thickening of the basement membrane.

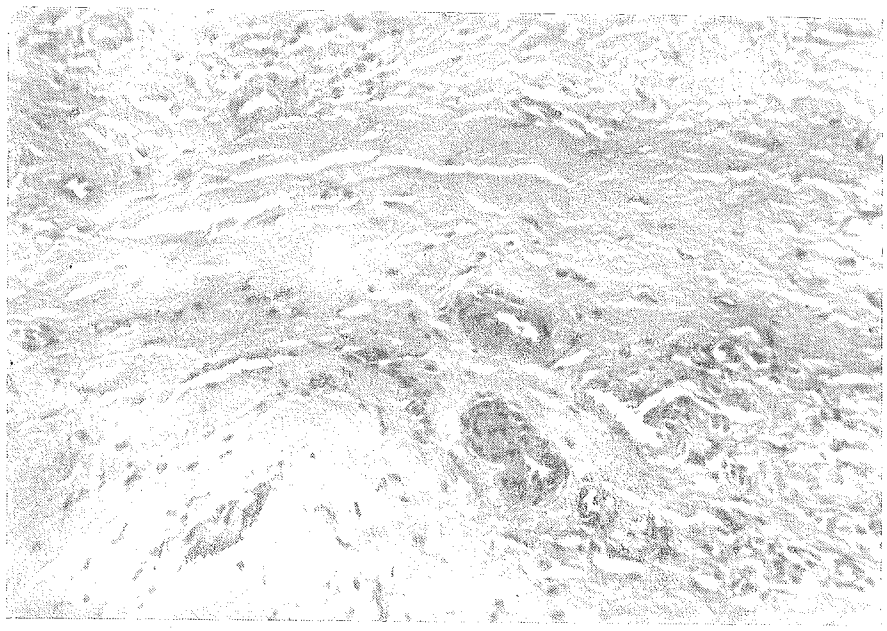

Figure 2. Histologic appearance of gingiva in a diabetic patient showing vascular lesion. One of the capillaries is almost obliterated.

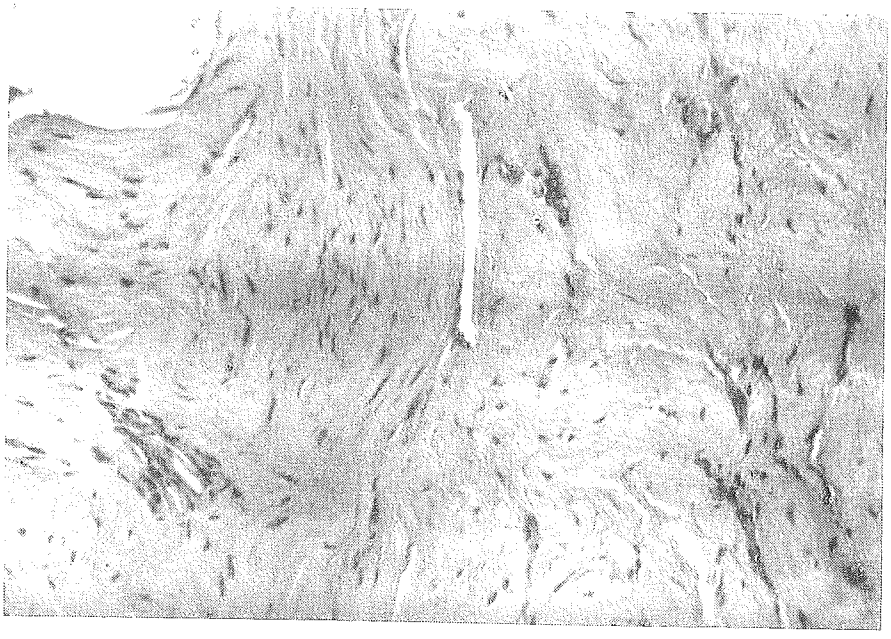

Figure 3. Gingiva from a diabetic patient with no vascular pathology.

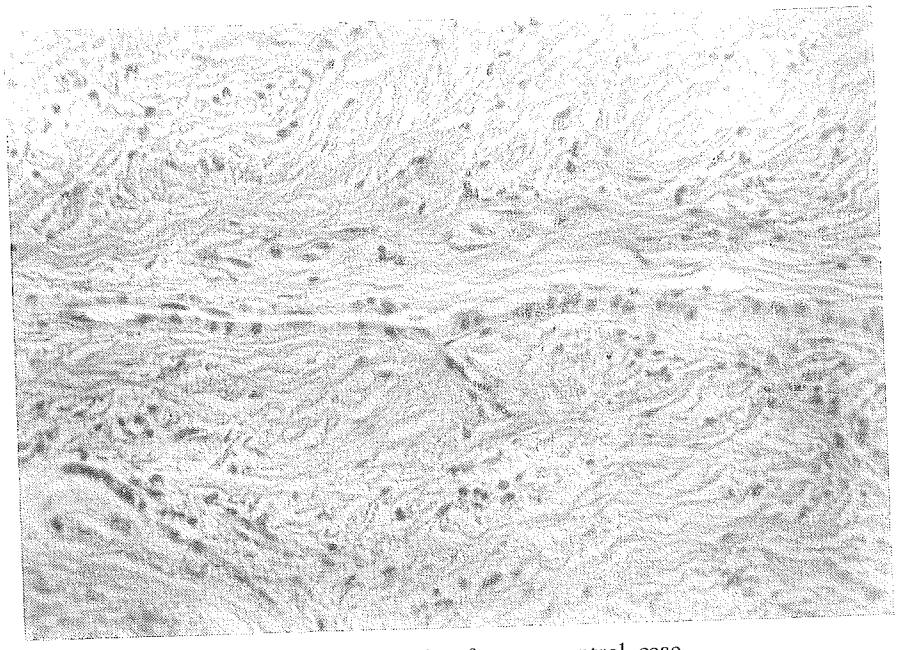

Figure 4. Gingiva from a control case.

2. Proliferation of the endothelium, almost to the point of obliterating some of the capillaries;
3. Accumulation of a PAS-positive material among the endothelial cells and in the basement membrane.

Besides these findings, plasma cell and polymorphonuclear leukocyte infiltration, suggesting gingivitis, was detected in some cases. Of 17 patients with vascular changes in the gingiva, four had ocular lesions such as cataract, micro-aneurysms, macular degeneration, and hemorrhages and exudates at or around the maculae.

Peripheral vascular lesions such as abcesses and gangrene, so advanced as to necessitate the amputation of the extremity, were present in four patients.

Discussion

Williams and Mahan noted the frequency of gingivitis among diabetics,[16] and Camerini-Davalos et al performed interdental gingival biopsies on three subjects with pre-diabetes, but failed to find any pathology.[17] Nishijima and Okada, comparing the histologic appearance of gingiva

in diabetic and non-diabetic patients, noticed evidence of chronic inflammation in patients with diabetes mellitus, but there was no evidence of difference in the severity of lesions between the two groups. The stainability of the gingival epithelia and mucosa with PAS, colloidal iron and toluidine blue did not show any significant difference between the diabetic and non-diabetic patients either, though the stainability of the vascular wall was higher in diabetics. These authors noted slight to moderate thickening of the capillary walls, but stated that no endothelial thickening was seen.[18]

In the present study the four control subjects showed no gingival vascular lesions, while 17 out of 18 diabetic patients had lesions of various degrees. The detection of vascular lesions in two patients with very short histories of diabetes mellitus (five days in one case and one week in the other) confirms the opinion that such lesions can occur in the very early stages of the disease, and even in pre-diabetes.

It is believed that gingivitis observed in some patients with diabetes does not influence the development of vascular lesions. The fact that not all the patients with diabetes showing vascular lesions had gingivitis, and the presence of marked polymorphonuclear leukocyte infiltration in two of the control subjects without vascular pathology, support this belief. There does not seem to be any relation between age and the thickness of the vessel walls, endothelial proliferation and accumulation of PAS-positive material. The patients in the control group had no vascular pathology, although they were 50 to 84 years old whereas a 14 ½-year-old girl with diabetes mellitus had such changes to a moderate degree, and another patient aged 39 years to a severe degree.

Though three patients with retinopathy and peripheral vascular disease also had lesions in the gingiva, such a direct relation cannot definitely be claimed for all patients. In five patients with gingival lesion no evidence of retinopathy was found, and in only one was there any suggestion of peripheral vascular disease.

This study suggested that gingival lesions appear prior to vascular changes in many of the other organs or tissues. Gingival biopsy is easy to perform and to repeat, so it offers a simple and convenient tool in the detection of vascular pathology in the course of diabetes mellitus.

Summary

Four control subjects and eighteen diabetic patients were studied by means of gingival biopsies. None of the control cases and only one diabetic

patient had any changes in the vascular structures, but seventeen patients with diabetes mellitus showed various degrees of lesions in the gingival vessels. No relation to age or coexistent gingivitis was found.

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Ultrastructural Observations on the Full-Term Human Chorda Umbilicalis

Amniotic epithelial covering and mucoid connective tissue with special reference to collagenogenesis

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Introduction

Studies of the amniotic epithelial lining under electron microscopy only date back five years. Italian authors paid most attention to this subject, considering the parietal amniotic epithelial lining. The morphological characteristics of the epithelial covering of the 50 cm umbilical cord between its initiation at the umbilical region and its termination at the placenta are very interesting to observe at light microscopy level. The structure of the cells, the number of layers they form on the basal lamina, and the interrelations between these cells in the direction of the placenta vary immensely. In the literature on this subject, it is stated that the amniotic epithelium is single-layered. In this study, the amniotic epithelial lining of the umbilical cord close to the placenta region was observed in an endeavor to compare and contrast it with the ultrastructure of the parietal amniotic epithelial lining.

Almost no electron microscopic study of the mucoid connective tissue filling the interior of the umbilical cord has thus far been encountered. There is no doubt, however, that this tissue, which keeps most of its embryological characteristics in all the connective tissues, presents interesting

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morphological features when the fibroblast and the collagen formation in it are studied at electron microscopy level. The interrelation between fibroblast and collagen formation has presented a problem to various outstanding authors for a century. It is hoped that a consideration of collagen formation in the mucoid connective tissue under electron microscopy may contribute towards the solution of this question.

Materials and Methods

Three mm thick bisections from the umbilical cord of a full-term fetus were taken from 10 cm away from the placenta. The Kerse method[1] was mostly employed in the electron microscopic procedures, but the amniotic epithelium and mucoid connective tissue portions from each of the bisections were taken separately in blocks before being placed in absolute alcohol. The amniotic epithelium and the mucoid connective tissue blocks were separated and numbered accordingly. The remaining routine procedures were applied to these two different blocks.

Observations

Amniotic epithelium covering the surface of the umbilical cord

It was observed that the epithelial lining was in three layers. In every region the amniotic epithelium was based on a highly developed basal lamina. The cells in the epithelial lining were identified as superficial, middle and basal. The superficial cells were rather flattened, with dense cytoplasm and partially superimposed on one another. There were short, rather frequent and irregular microvilli on the surfaces of the cells facing the amniotic lumen (Figures 1 and 2). It was observed at higher magnifications that the dense appearance of the superficial cells was due to the bundles of tonofilaments, 50-60 A thick. These bundles were densely arranged, and generally parallel to the cell surface (Figure 3). Few cell organelles could be seen in the abundance of tonofilaments. The cells were poor in endoplasmic reticulum elements and mitochondria. The tubuli of the endoplasmic reticulum that could be observed, were narrow and contained few ribosomes. The mitochondria were round in shape, and their cristae were not well-developed (Figures 3, 4, 5). The superficial layer cells had interrelations among themselves as well as with those in the middle layer through the membrane surfaces; they were deeply invaginated with one another and had numerous junctional complexes. All the units of the junctional complex, zonula occludens, zonula adherens and macula

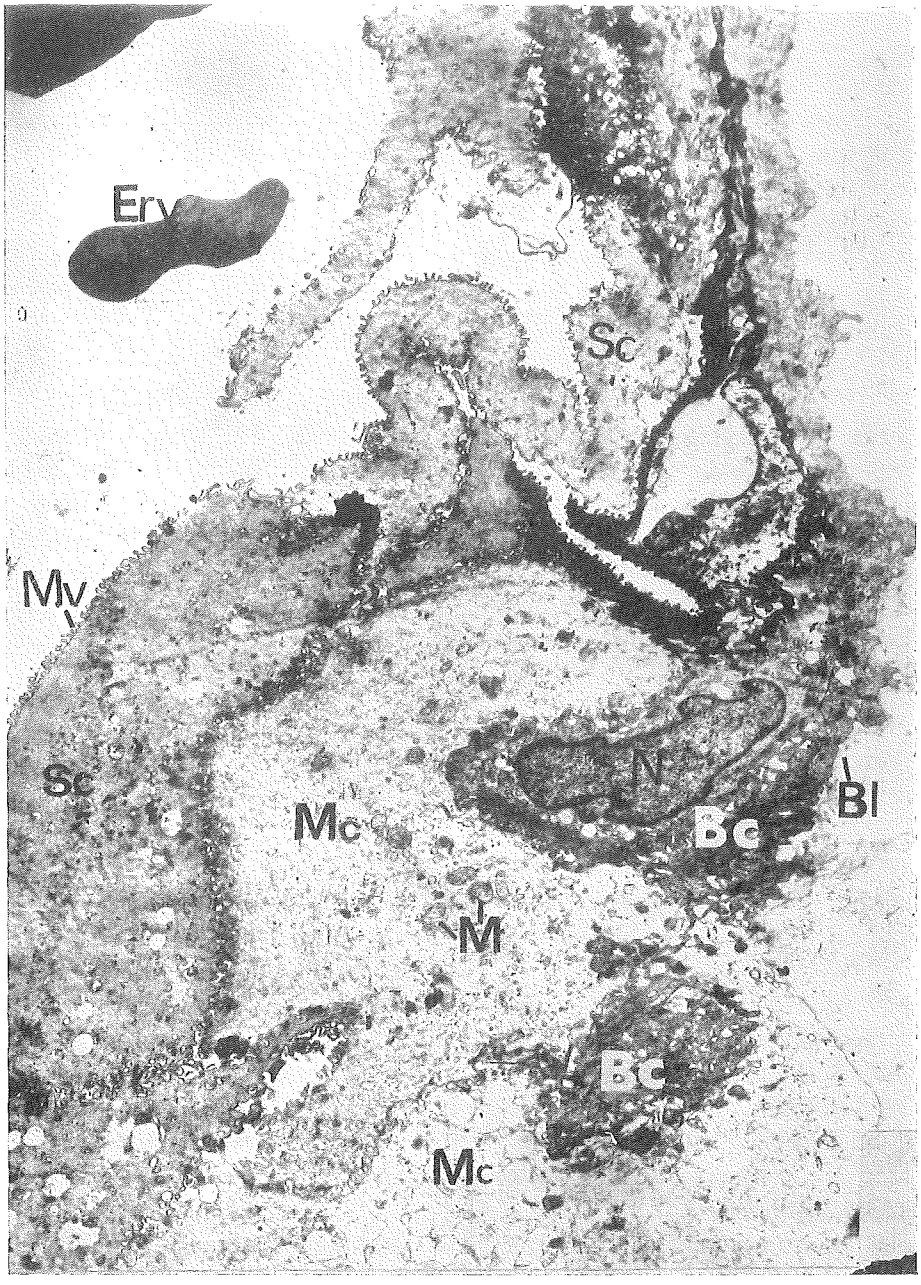

Figure 1

Ultrastructure of the amniotic epithelial lining covering the outer surface of the human umbilical cord. It is observed that the amniotic epithel is in three layers and made of epithelial cells of varying morphological characteristics. These cells with cytoplasms are generally denser and have numerous microvilli on their surfaces in the surface layer (Sc), light in the middle (Mc) and dense in the basal layer. The amnion epithelium is based on a highly developed basal lamina (Bl). N, Nucleus, Ery, Erythrocyte; M, Mitochondrium. X 6600.

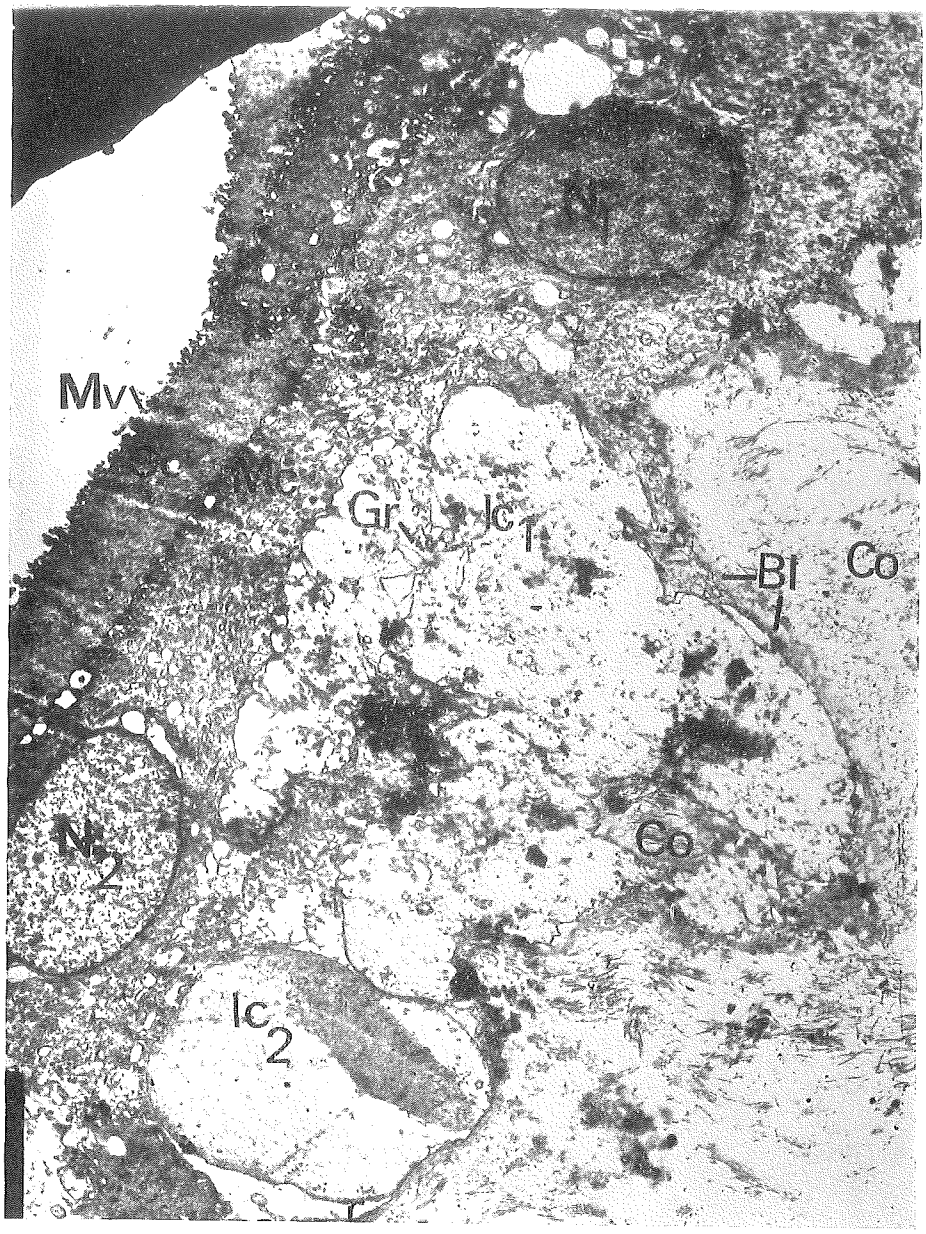

Figure 2

Amniotic epithelial lining covering the outer surface of the human umbilical cord as seen in another region. Surface cells (Sc) with their dense cytoplasms are outstanding. Short but numerous microvilli (Mv) are observed in their surfaces opposite the aminotic cavity. The cytoplasm of the cells in the middle layer (Mc) is also rather dense in this region. Furthermore the nuclei of two middle layer cells are quite different in density. Compare N1 and N2. In this micrograph, intermediary cells, namely Ic1 and Ic2, with very light cytoplasms could be very easily observed besides surface and middle layer cells. The intermediary cells are very poor in cytoplasmic organelles and contain rather enlarged granular type endoplasmic reticulum from place to place. No basal layer cell could be observed in this section. The middle and intermediary cells were directly based on a very highly developed basal lamina (Bl). In the lower part of the mucoid connective tissue numerous collagenous fibres (Co) are observed. X 6600.

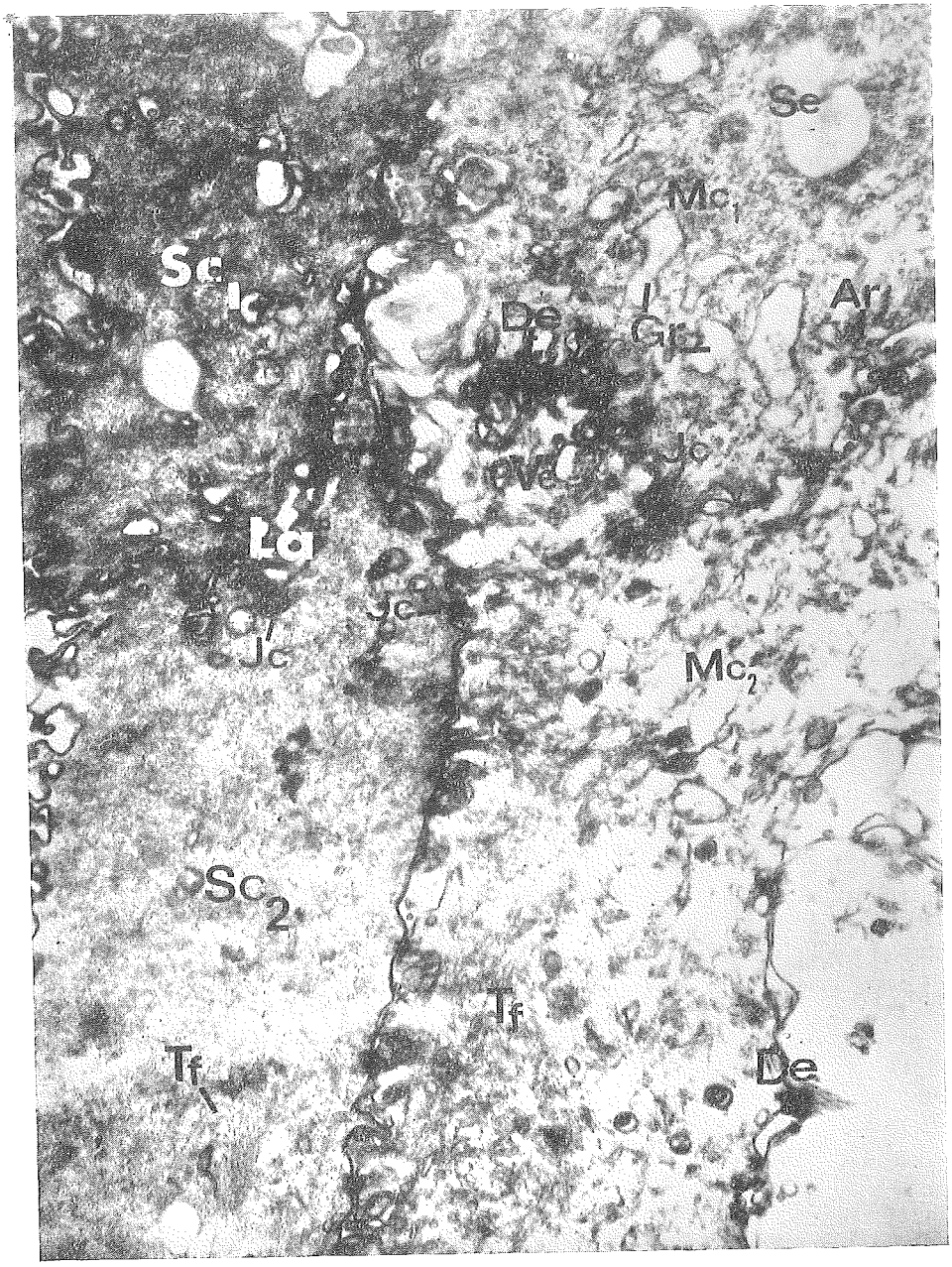

Figure 3

Detailed ultrastructure of the cells in the superficial and middle layers of the amniotic epithel. In the electronmicrograph mainly two superficial (Sc1 - Sc2) and two middle layer cells (Mc1 - Mc2) are observed. Cells in the same layer and two different layers are closely interrelated by way of their membrane surfaces. It is possible to observe the whole units of the junctional complex (Jc). It is also clearly observed that the density in the superficial cells filling up the cell cytoplasm completely is caused by extending tonofilaments (Tf). Other organelles in the cytoplasm are not very well observed. The tonofilaments in the cytoplasms of the middle layer cells are quite few. Enlarged granular-type (Gr) and agranular-type (Ar) endoplasmic reticulum elements and multivesicular aggregates (Ve) are observed. Se, secretion granules; De, desmosomes. X 24.500.

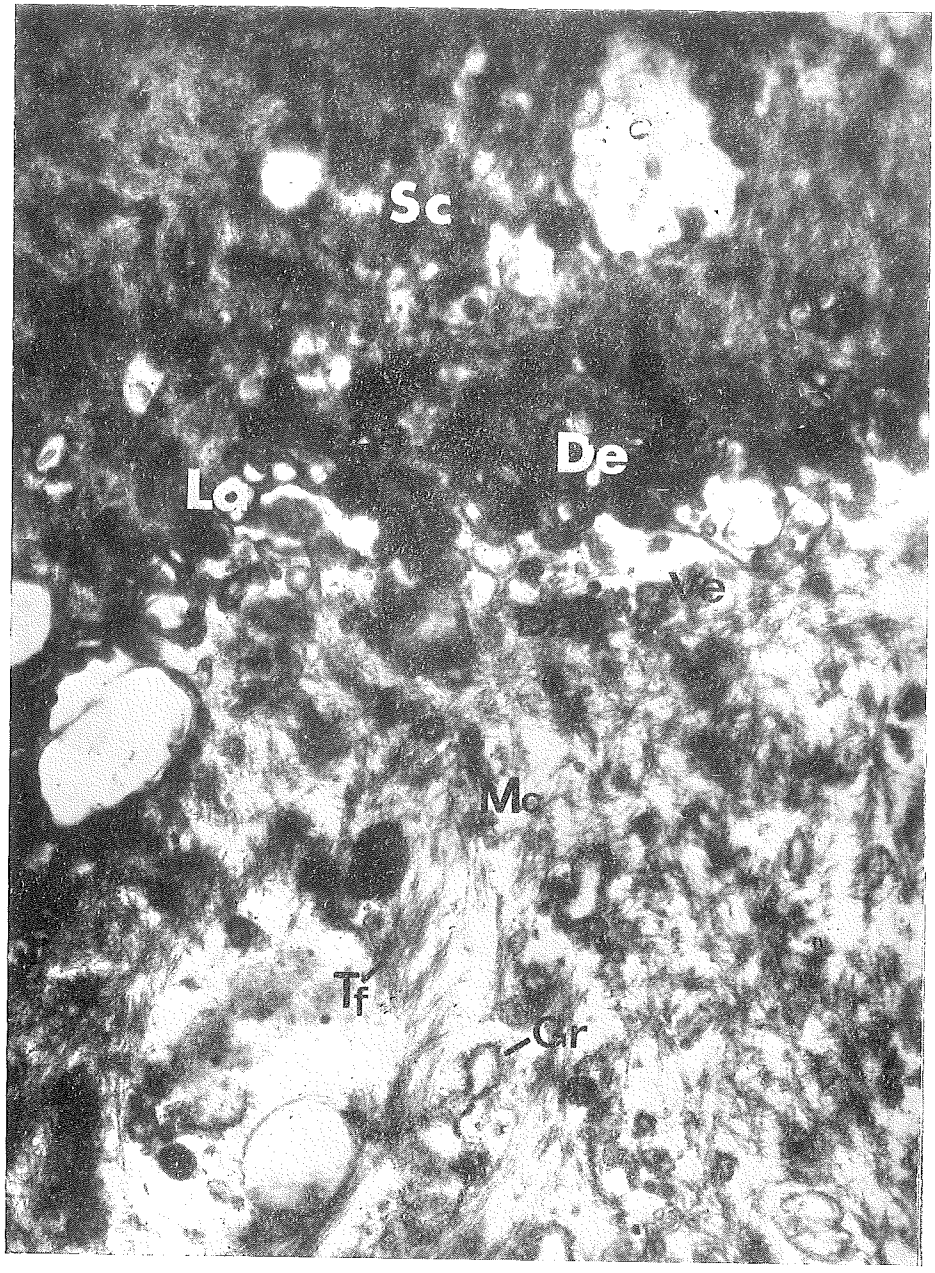

Figure 4

Detailed cytoplasmic ultrastructure of the superficial (Sc) and middle layer cells (Mc). All the units of the junctional complex between the two types of cells i.e. zonula occludens (Zo), zonula adherens (Za) and desmosomes (De) are clearly observed. The tonofilaments observed in the surface cells are very dense but rather dispersed in the middle cells. There is little granular-type endoplasmic reticulum (Gr) in the middle cells. In addition, multivesicular bodies (Ve) and dense bodies (Db) are observed. Tf, tonofilaments. X 56.000.

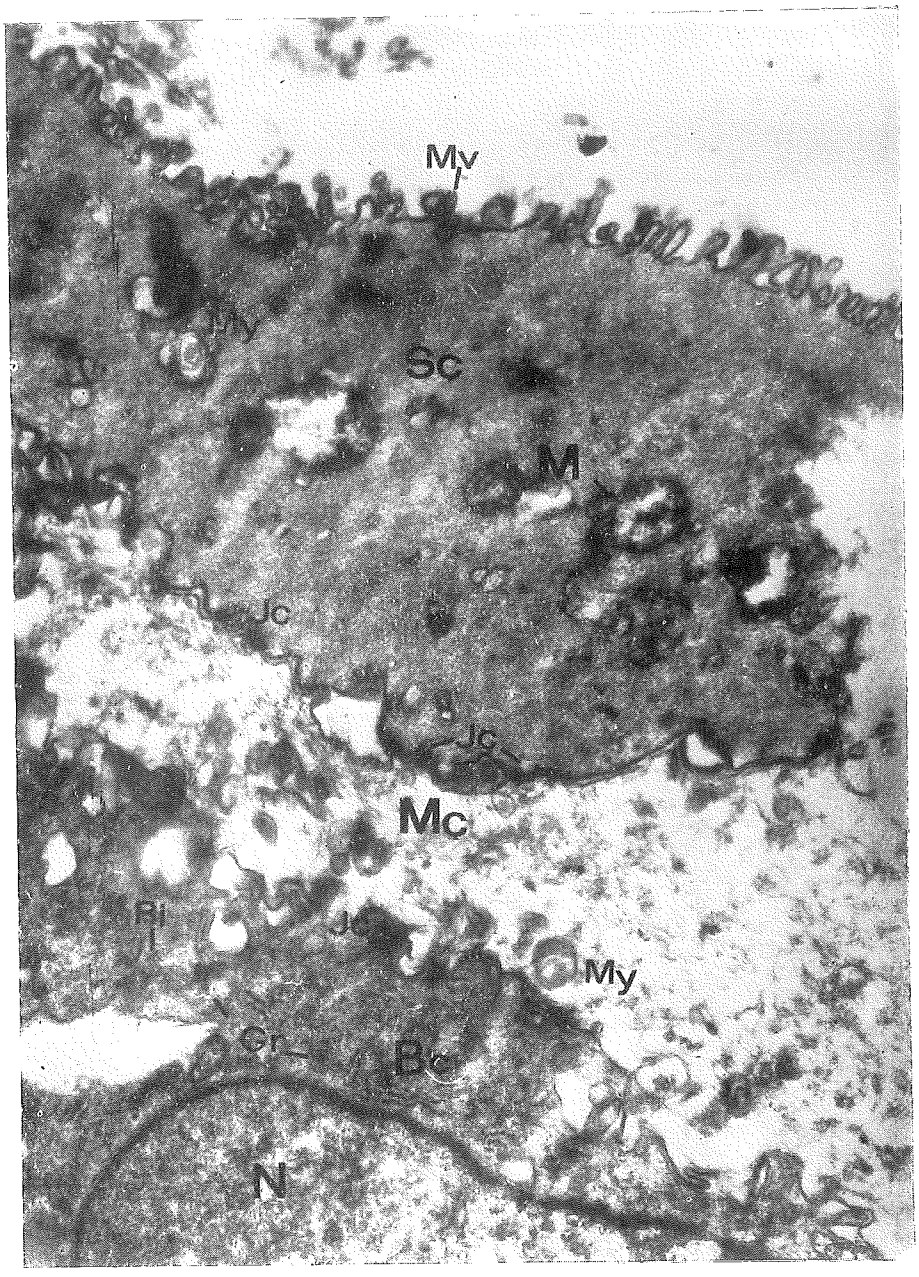

Figure 5

Detailed ultrastructure of the superficial (Sc) Middle (Mc) and Basal (Bc) layer cells of the amniotic epithelium .Short and dense but rather irregular microvilli (Mv) exist on the surface of the upper cells. In addition a few mitochondria (M) exist in-between the dense bundles of tonofilaments in the cell cytoplasms in this region. Myelin-like bodies (My) were observed in the surface and middle cells. It seemed that the density of the cytoplasm of the basal cells resulted from the bundles of tonofilaments as well as the abundant ribosomes and the granular-type of endoplasmic reticulum (Gr) elements. Jc, Junctional complex; N, nucleus. X 24.500.

adherens (desmosome) were highly developed (Figures 3, 4 and 5). As a result of the occasional enlargement of spaces between the interdigitated cells, a very complex lacunar or tubulo-lacunar system was noted (Figures 3 and 4).

The middle layer cells were higher than those in the superficial layer and their cytoplasmic appearance was less dense (Figures 1 and 2). They were interrelated and digitated among themselves as well as with those in the superficial and basal layers. Abundant junctional complexes existed where the cell surface interrelated. These were invested with a tubulolacunar system (Figures 3 and 4). The reason the middle layer cells appeared less dense was due to the fact that fewer bundles of tonofilaments were present in their cytoplasms than those in the superficial layer cells (Figures 3, 4, 5 and 6). Organelles were observed in the cell cytoplasms. Rather enlarged granular endoplasmic reticulum tubuli and mitochondria, round in shape with poorly developed cristae were noted (Figures 7, 8 and 9). While some of the middle layer cells had the morphological characteristics mentioned above, others were identified as having the typical nature of secretive cells and their cytoplasms displayed a granular endoplasmic reticulum which was highly developed and enlarged cisternal in form with a large Golgi complex near the nucleus among which big secretion granules were noted. The nuclei of both these middle layer cells were oval in shape, and the distribution of chromatin was homogenous (Figures 7, 8 and 9).

Among the secretive and non-secretive middle layer cells there were intermediary cells which seemed to have the morphological characteristics of undifferentiated mesenchymal cells. Their cytoplasms were very pale and poor in organelles and their endoplasmic reticulum was much enlarged and appeared in cisternal forms. Few free ribosomes and no tonofilaments were observed (Figure 2).

The morphological characteristics of the basal layer cells varied from place to place. Some were rather flattened, others rather high and irregular in shape. Their cytoplasms were dense, like those of the superficial layer cells. The cell surfaces were deeply interdigitated with the surfaces of the middle layer cells, and had numerous junctional complexes among themselves (Figures 5, 6, 10 and 11). The cytoplasms of the basal cells appeared very dense not only because there were abundant bundles of tonofilaments, but there were also highly developed granular endoplasmic reticula. Furthermore, numerous free ribosomes were observed (Figures 5 and 6). The basal layer cells were not continuous, and the middle layer cells rested on the basal lamina in the discontinued places (Figures 1 and 7). The basal layer had secretive and

Figure 6

Detailed cytoplasmic ultrastructure of the surface (Sc) middle (Mc) and basal (Bc) cell layers of the amniotic epithelium without nuclei. Generally bundles of tonofilaments (Tf) were observed in the surface cells. Dispersed tonofilaments at random, few granular endoplasmic reticulum (Gr) elements and ribosomes were noted in the middle cells. In the basal cells both the tubuli of the granular endoplasmic reticulum with free ribosomes and the tonofilaments filled up the cytoplasm in an outstanding manner. Numerous junctional complexes (Jc) between the membrane surfaces of the cells existing in all the layers were observed. These characteristics prove that the cells have the morphological features of the surface covering epithelium. Basal lamina (Bl) was highly developed. The collagenous fibers (Co) in the mucoid connective tissue, are dense and abundant. These were observed to be very close to the basal lamina (Bl). X 24.500

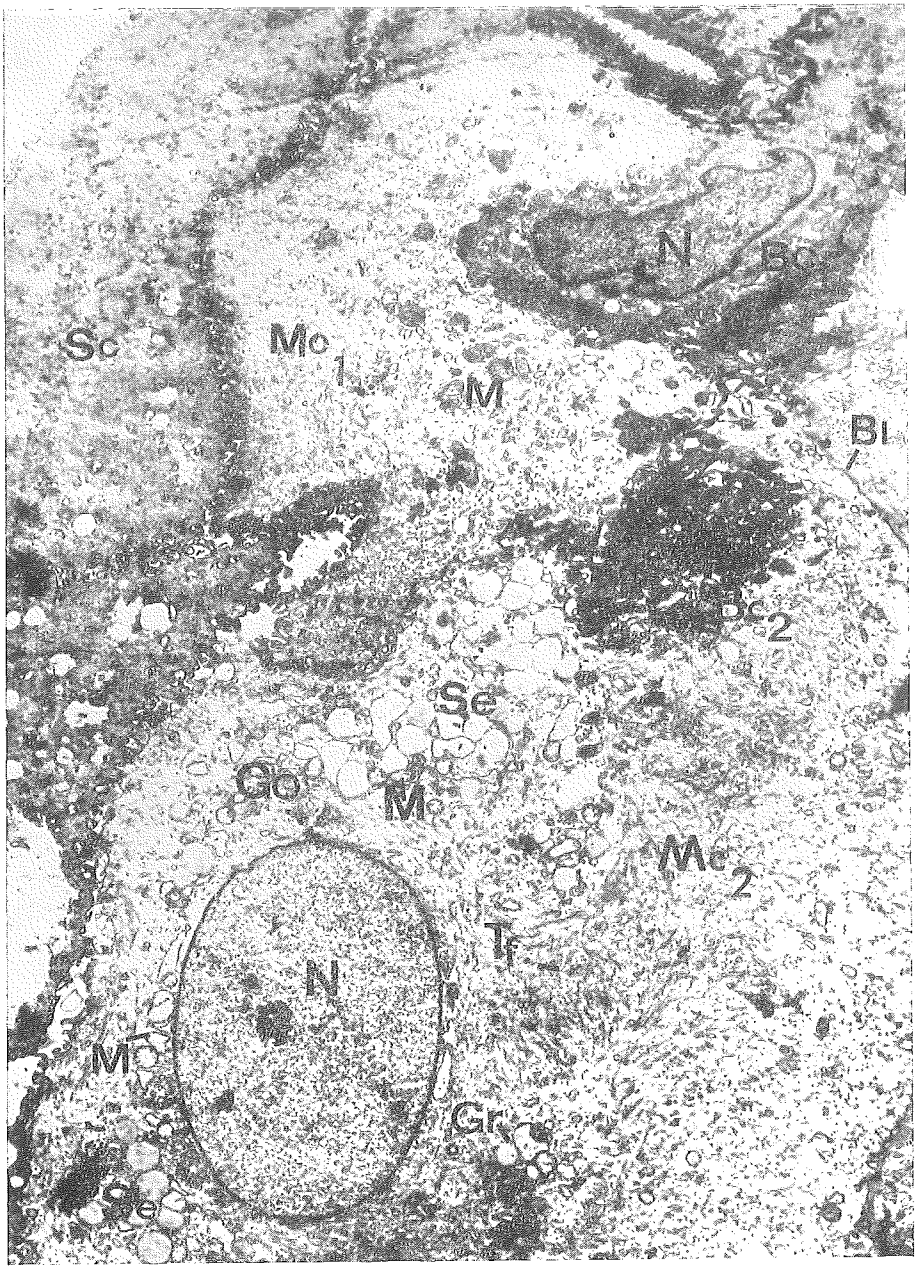

Figure 7

Superficial (Sc), middle (Mc1 - Mc2) and basal layer (BC1-BC2) cells in the amniotic epithelium. Morphologically it is observed that one of the middle layer cells (MC2) has secretive ability. The nucleus (N) of the cell is oval with smooth contours and the distribution of chromatin is homogenous. A large Golgi apparatus, (Go) a granular endoplasmic reticulum (Gr) and mitochondria (M) are also observed. In the cytoplasm secretion granules (Se) are observed in groups. Bundles of tonofilaments (Tf) are also observed. The cytoplasm of the non-secretive middle layer cell (MC1) does not contain any secretion. Bl, basal lamina. X 85.000

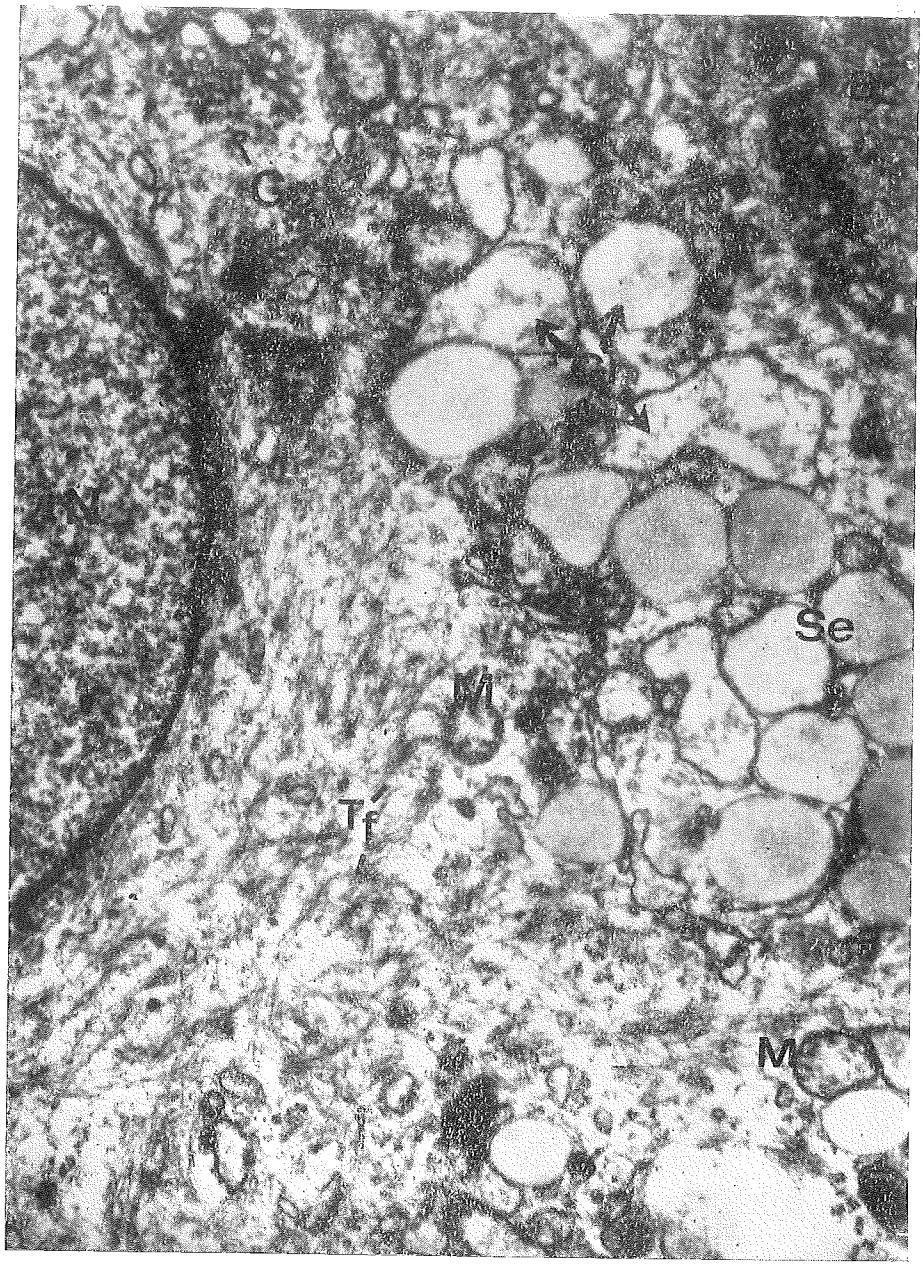

Figure 8

Detailed ultrastructure of the secretive middle layer cell of the amniotic epithelium. A highly developed Golgi apparatus (Go) is observed adjacent to the cell nucleus (N). Secretion granules (Se) among the expanded granular endoplasmic reticulum (Gr) elements are seen in the lower part of the micrograph. Abundant tonofilaments (Tf) in this cell show that it also has the nature of a surface covering epithelial cell. M, mitochondrium; Bc, Basal cell. X 24.500.

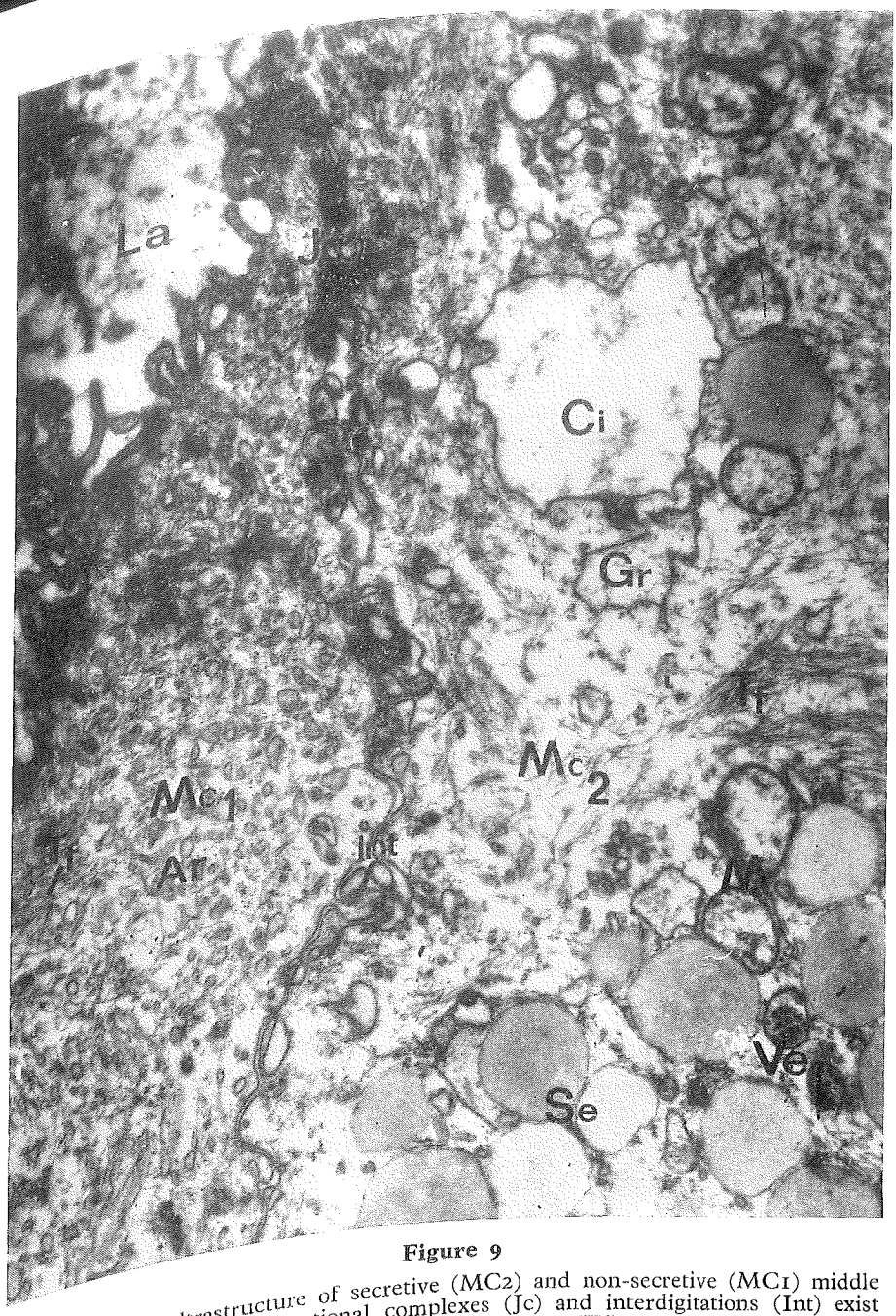

Figure 9

The detailed ultrastructure of secretive (MC2) and non-secretive (MC1) middle layer cells. Numerous junctional complexes (Jc) and interdigitations (Int) exist along the surfaces between the cells. Tonofilaments (Tf) are scattered in the cell. The granular endoplasmic reticulum (Gr) is much enlarged. A wide cisterna (Ci) is found in the central part. It is observed that this cisterna and the lumen of the enlarged granular endoplasmic reticulum are interrelated (X). In the right lower part of the micrograph large and homogenous secretion granules (Se) exist. The presence of small and large multivesicular bodies (Ve) is interesting. On the upper left, an enlarged lacuna (La) between the middle and superficial cells is observed with some contents in it. Numerous tubuli and vesicules of the agranular endoplasmic reticulum (Ar) and a loose network of tonofilaments are observed in the non-secretive middle layer cell (MC1) as characteristic features.
M, mitochondrium. X. 27.000.

non-secretive cells as in the case of the middle layer. In both types, the granular endoplasmic-reticulum was highly developed. The non-secretive types were more flattened, and rested on the basal lamina with a smooth membrane surface (Figure 10). The secretive cells contained large secretion granules and had a developed Golgi apparatus. They were based on the basal lamina along a very indented membrane surface. The end-feet of the basal cells touched the basal lamina, with lacunae occurring in the spaces between them (Figures 11, 12 and 13). The nuclei of the basal layer cells had indented contours (Figure 11).

The epithelial covering was based on a highly developed basal lamina which was homogenous, about 700 A thick, and had very close relations with the fibers in the mucoid connective tissue under it (Figures 10, 11, 12 and 13).

Fibroblasts in the mucoid connective tissue, collagenogenesis and collagenous fibers

Numerous fibroblasts were observed in the mucoid connective tissue. The bodies and extensions of fibroblasts among the rather dense bundles of collagenous fibers were noted to be abundant, and the morphological characteristics of the fibroblasts in this connective tissue were very interesting. The nuclei of the elongated and flattened fibroblasts were oval in shape and centric. The chromatin content of the nucleus was small and the distribution homogenous. The structures of the endoplasm investing the nucleus and the ectoplasm in the peripheral region differed from each other in a remarkable manner (Figures 14 and 15). The endoplasm was observed to be granular in structure due to a very rich ribosome content, the ectoplasm was full of thin bundles of filaments parallel to the cell surface. The filaments were about 60 A thick. A well-developed granular endoplasmic reticulum barrier occurred in between the two regions. These granular endoplasmic reticulum elements extended towards the equatorial region near the nucleus. Ribosomes were closely attached on the walls of the enlarged tubuli of the granular endoplasmic reticulum. A few mitochondria were observed, and they were round in shape, with short cristae occurring in the ectoplasm (Figure 16). In these fibroblasts the cell membranes were generally observed to be continuous.

In the other group of fibroblasts, the ribosomes were noted to increase greatly in the endoplasm and there were bundles of filaments in the ectoplasm. However, the granular endoplasmic reticulum barrier had disappeared, and the cell surface membrane was occasionally discontinued (Figures 17, 18 and 19). The ectoplasmic filaments were ob-

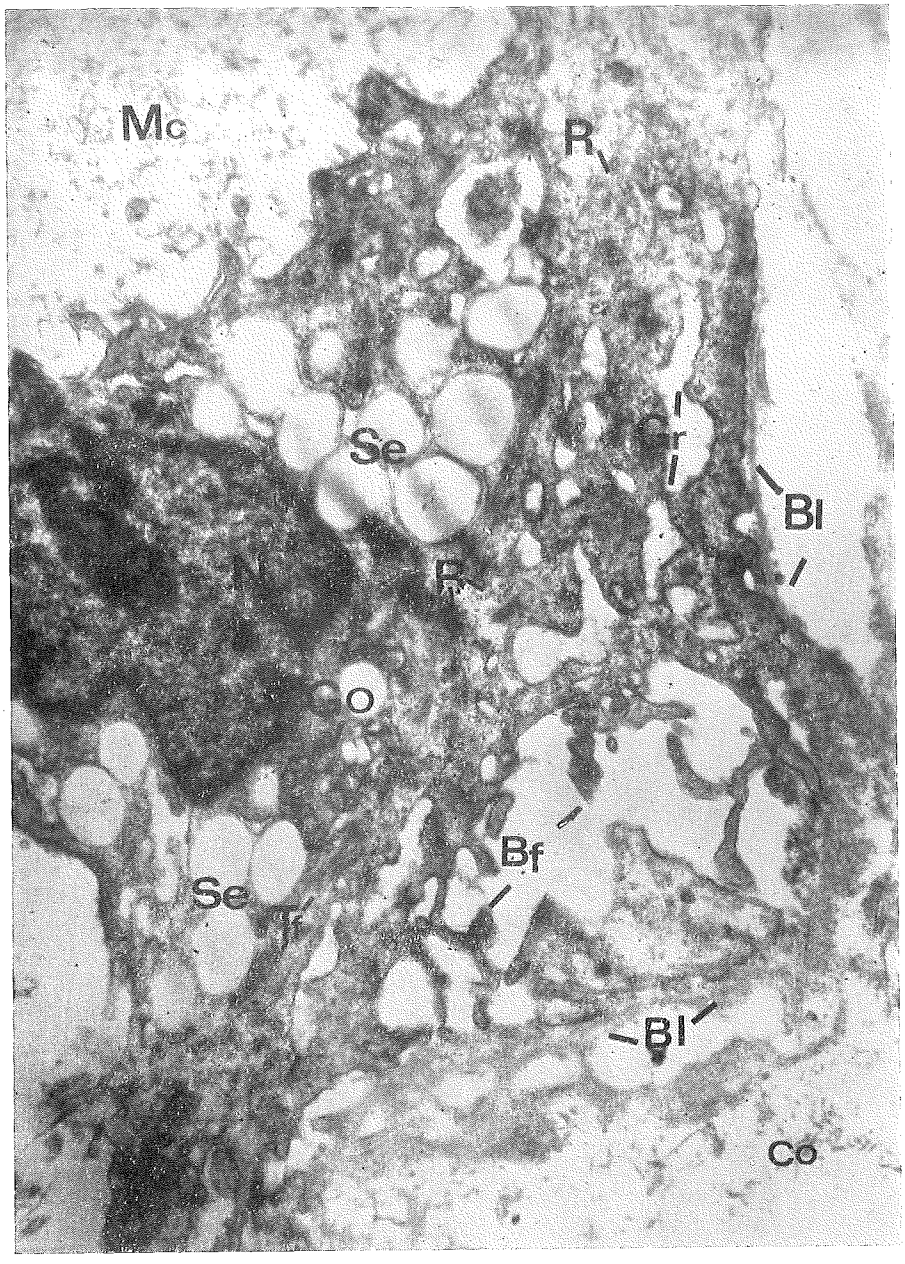

Figure 11

Detailed ultrastructure of a secretive basal layer cell of the amniotic epithelium. The Golgi complex (Go) of the cell is highly developed. Aggregates of pale secretion granules (Se) occur especially around the nucleus. The granular endoplasmic reticulum (Gr) is enlarged in cisternal forms. Ribosomes (R) are abundant with frequent rosette forms. Parallel bundles of tonofilaments are observed throughout the cytoplasm. The nucleus (N) has irregular contours and a heterogenous chromatin content. The nucleolus is well-developed. The basal part of the cell which is based on the basal lamina (Bl) is very much indented. It is attached to the basal lamina with numerous feet-like projections termed basal-feet (Bf). The basal lamina is highly developed. Co, collagen. X 24.500.

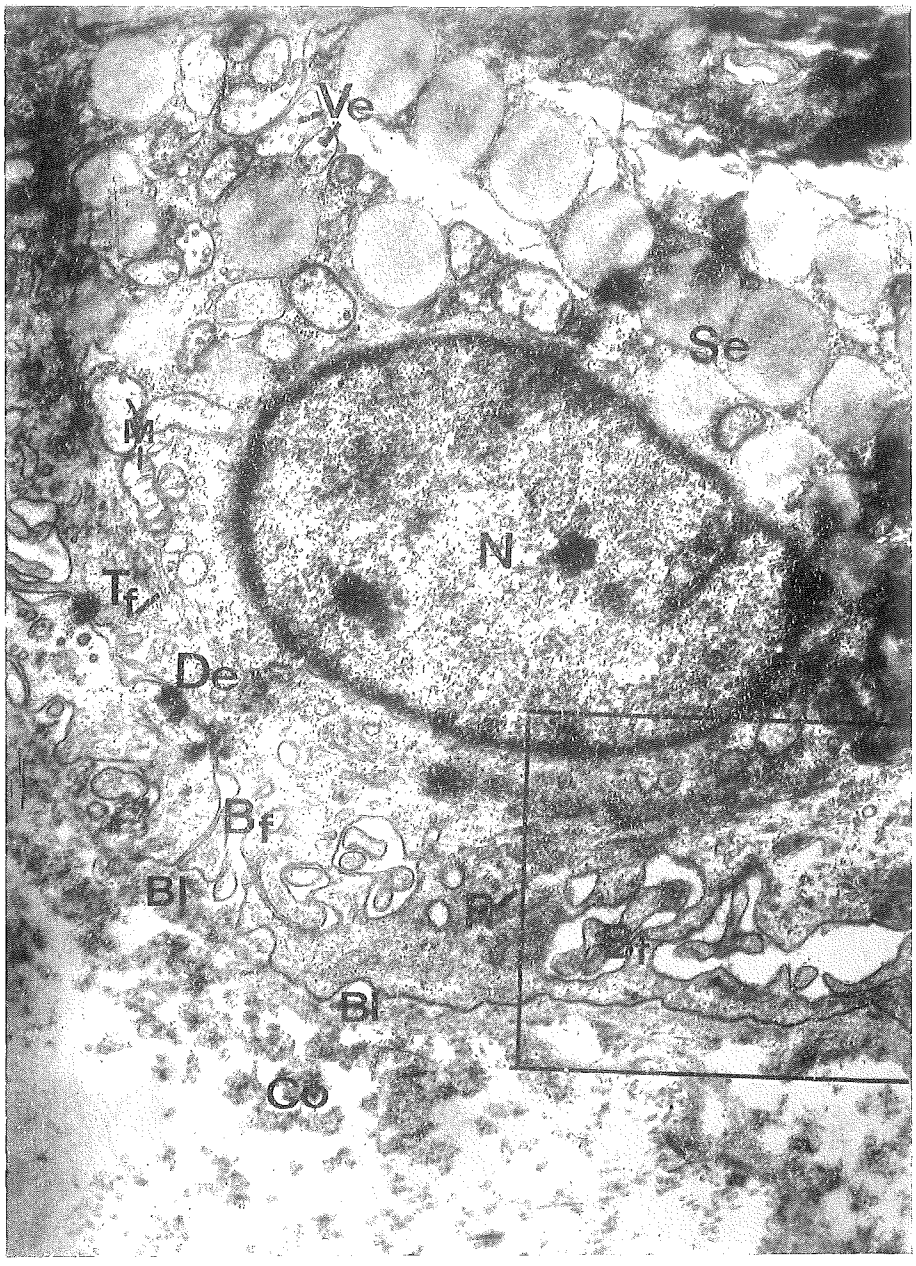

Figure 12

Secretive basal layer cell. The cell is based on a highly developed basal lamina (Bl) with deep folds all along its surface which we previously termed feet-like projections or basal feet (Bf). The secretion granules (Se) are large, pale and have a homogenous content. These are located particularly in the apical region of the nucleus (N). The cytoplasm is rich in multivesicular bodies (Ve), tonofilaments (Tf) and ribosomes (R). At the lower left desmosomes (De) are observed in between the two basal layer cells. M, mitochondrium; Co, collagenous fibers. X 27.000.

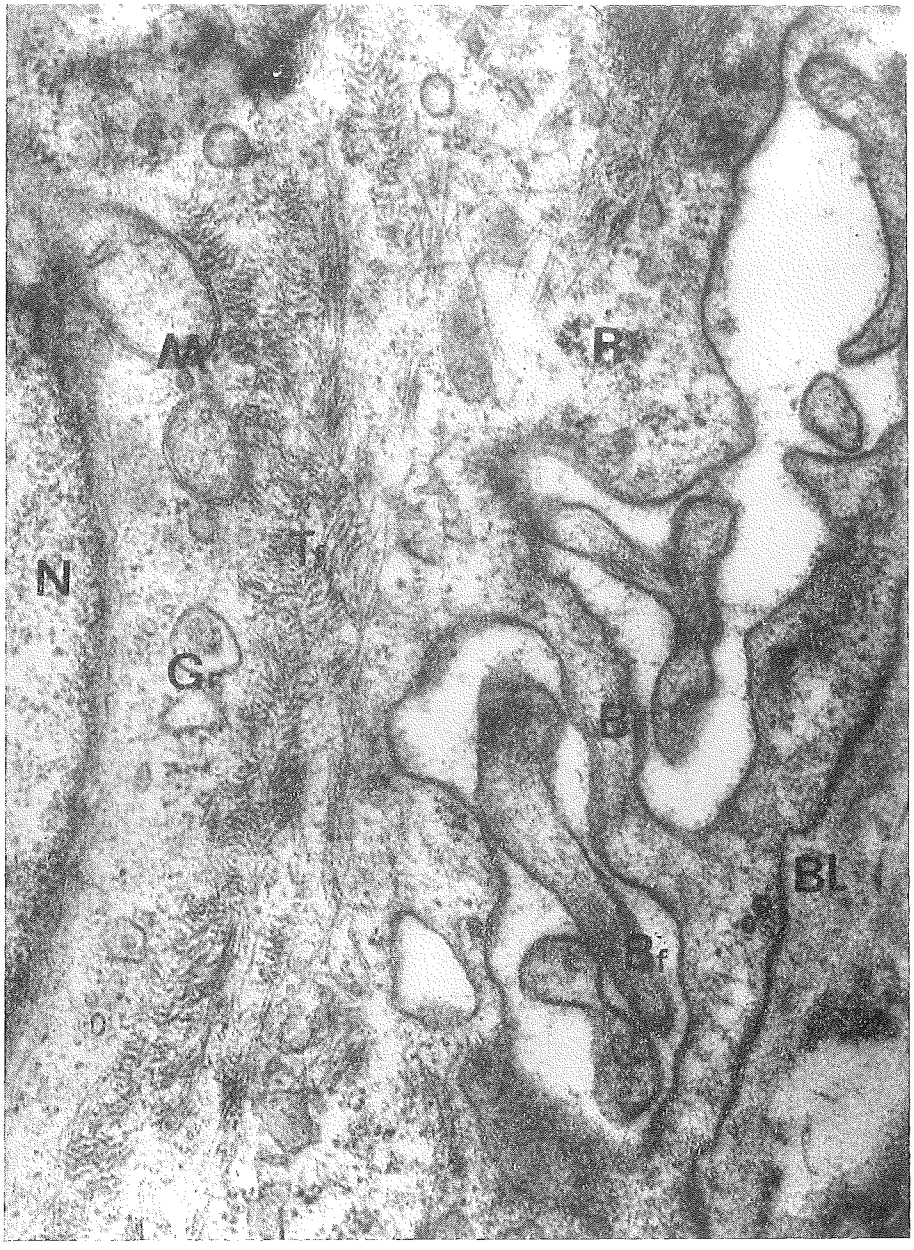

Figure 13

Greater magnification of the basal layer cell region marked in a square in Figure 12. Deep folds of the basal surface of the cell based on the basal lamina (Bl) are seen in further details. The feet-like projections or basal-feet (Bf) have a labrynth-like structure and do not seem to have open connections with the basal lamina (Bl) in this region. Tf, tonofilaments; Gr, granular endoplasmic reticulum; R, ribosomes; N, nucleus. X 81.000.

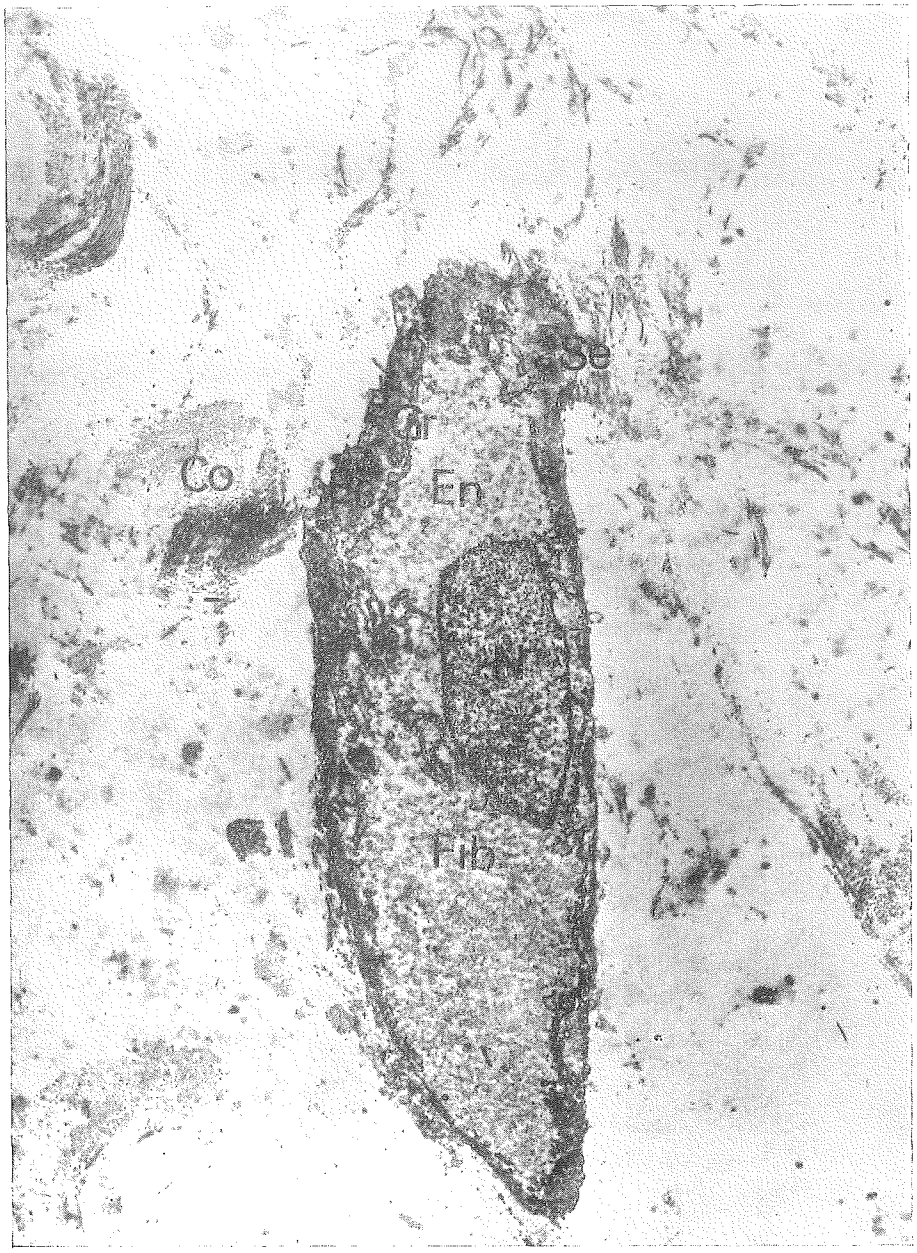

Figure 14

The ultrastructure of a fibroblast (Fib) existing in the mucoid connective tissue of the umbilical cord. The ecto-(Ec) end endoplasms (En) of the cell differ from each other in density and structure. The tubuli of the granular endoplasmic reticulum (Gr) are localized in between the ecto- and endoplasmic regions in a way that forms a barrier. They were also observed towards the nucleus. The endoplasm is very poor in cytoplasmic organelles. The nucleus (N) is centric, oval in shape and has smooth contours. The distribution of its chromatin is homogenous. At one end of the cell two large secretion granules (Se) are observed. Co, collagenous fibers. X 6.600.

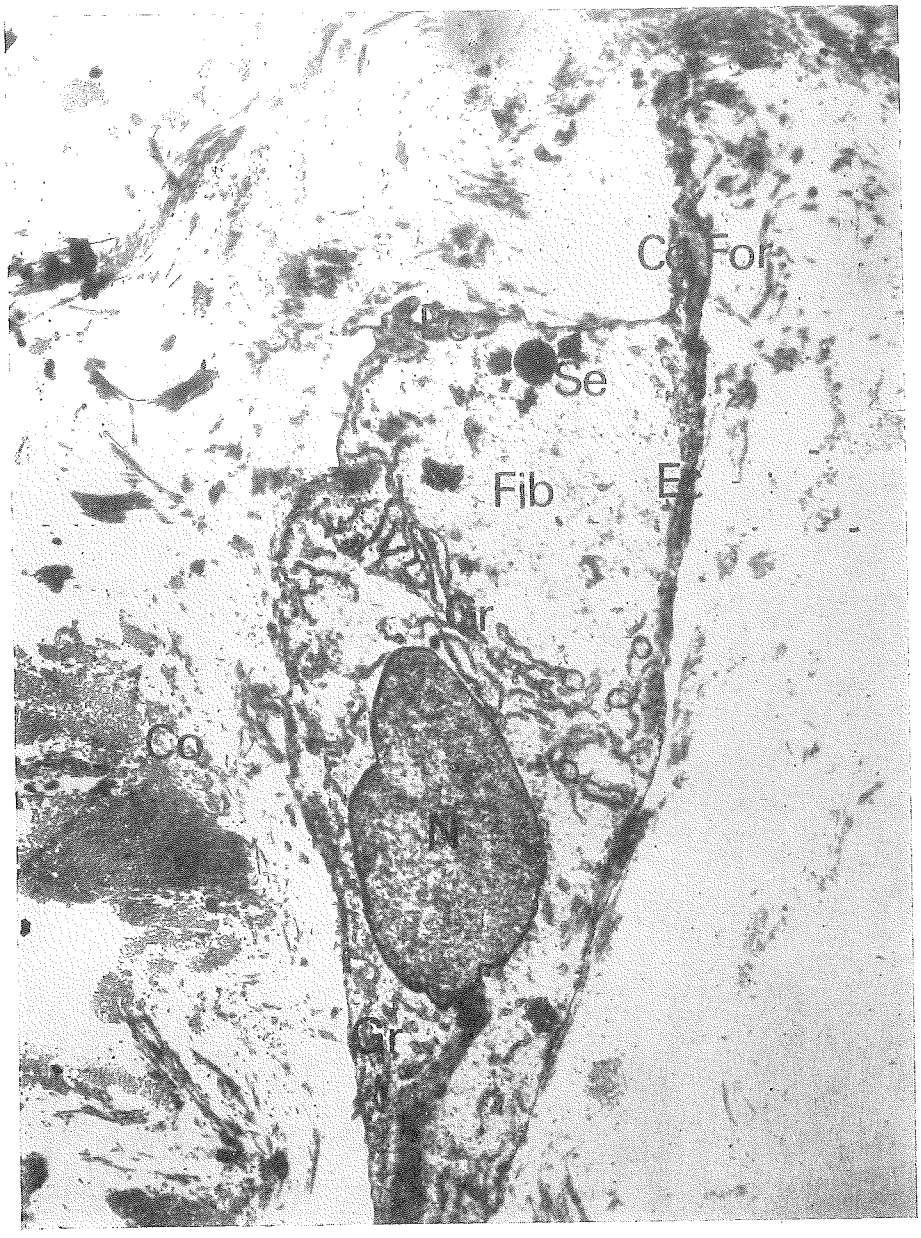

Figure 15

The ultrastructure of another fibroblast (Fib) in the mucoid connective tissue. The density of the ectoplasm (Ec) is not like the one in Fig. 14, i.e. it seems to exist in all the peripheral parts of the fibroblast but only from place to place. It is immediately noted that the granular endoplasmic reticulum (Gr) is again located peripherally in between the ecto- and endoplasms. The cytoplasm is very poor in other organelles. The cell membrane is discontinued at the upper right of the cell. The collagen fibrils (Co For) are excreted from that part of the cell to the intercellular space. Co, collagenous fibers; Se, secretion granules; N, nucleus. X 8.500.

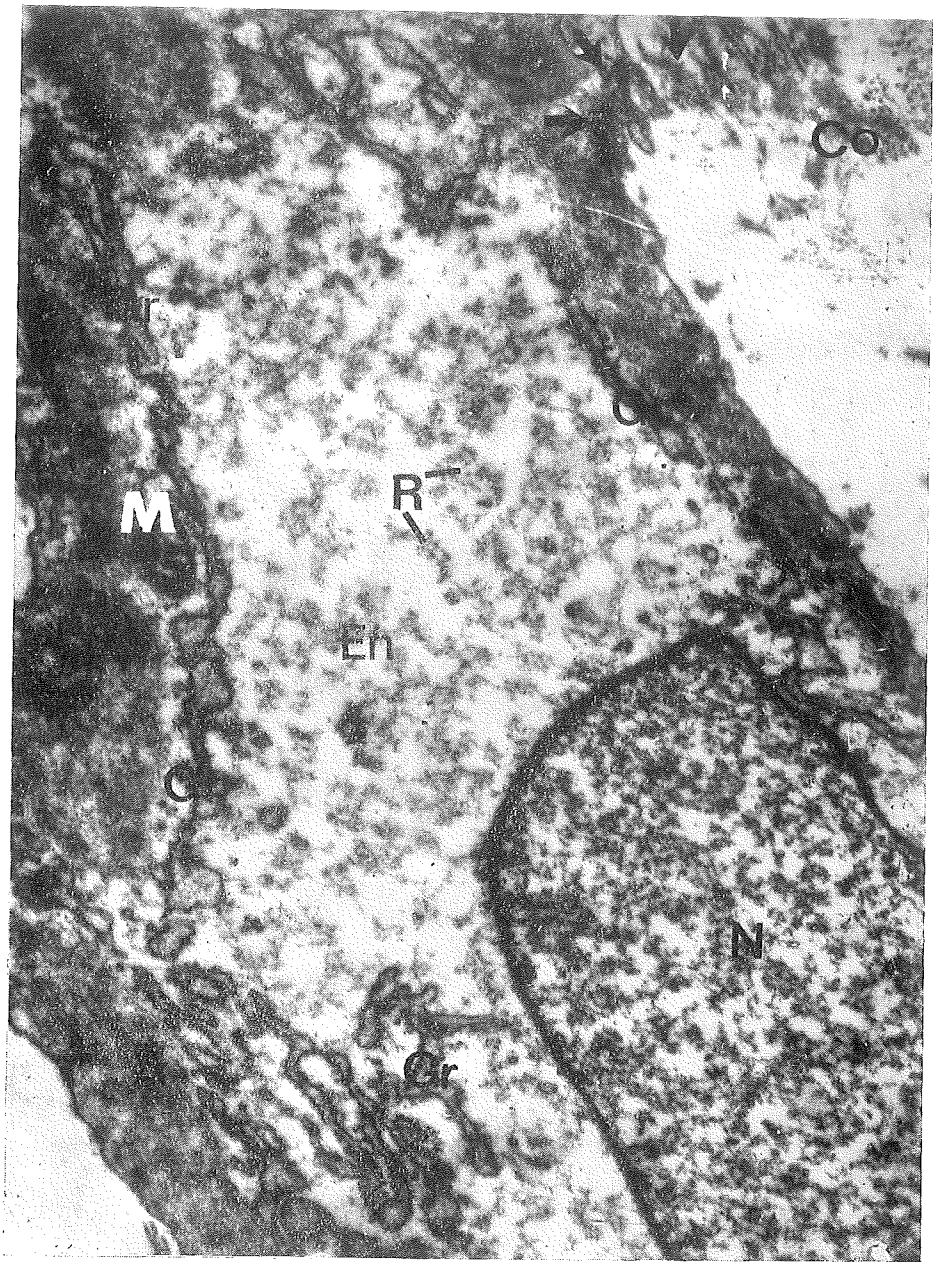

Figure 16

Detailed ultrastructures of the ecto-(Ec) and endoplasmic (En) regions of the fibroblast. Enlarged tubuli of the granular endoplasmic reticulum (Gr) in between the ecto- and endoplasms occur like a barrier, and furthermore similar tubuli can be observed in regions close to the nucleus. Mitochondria (M) are also found in the ectoplasm. In this magnification it can easily be noted that the density of the ectoplasm is due to the abundance of fine filaments. The endoplasm is very poor in cytoplasmic organelles. It contains numerous ribosomes (R) and polysomes. It is possible that these may be the initial formations of the filaments observed in the ectoplasm. The cell membrane which is discontinued at top right excretes filaments (arrows). Co, collagen fibrils; N, nucleus. X 24.500.

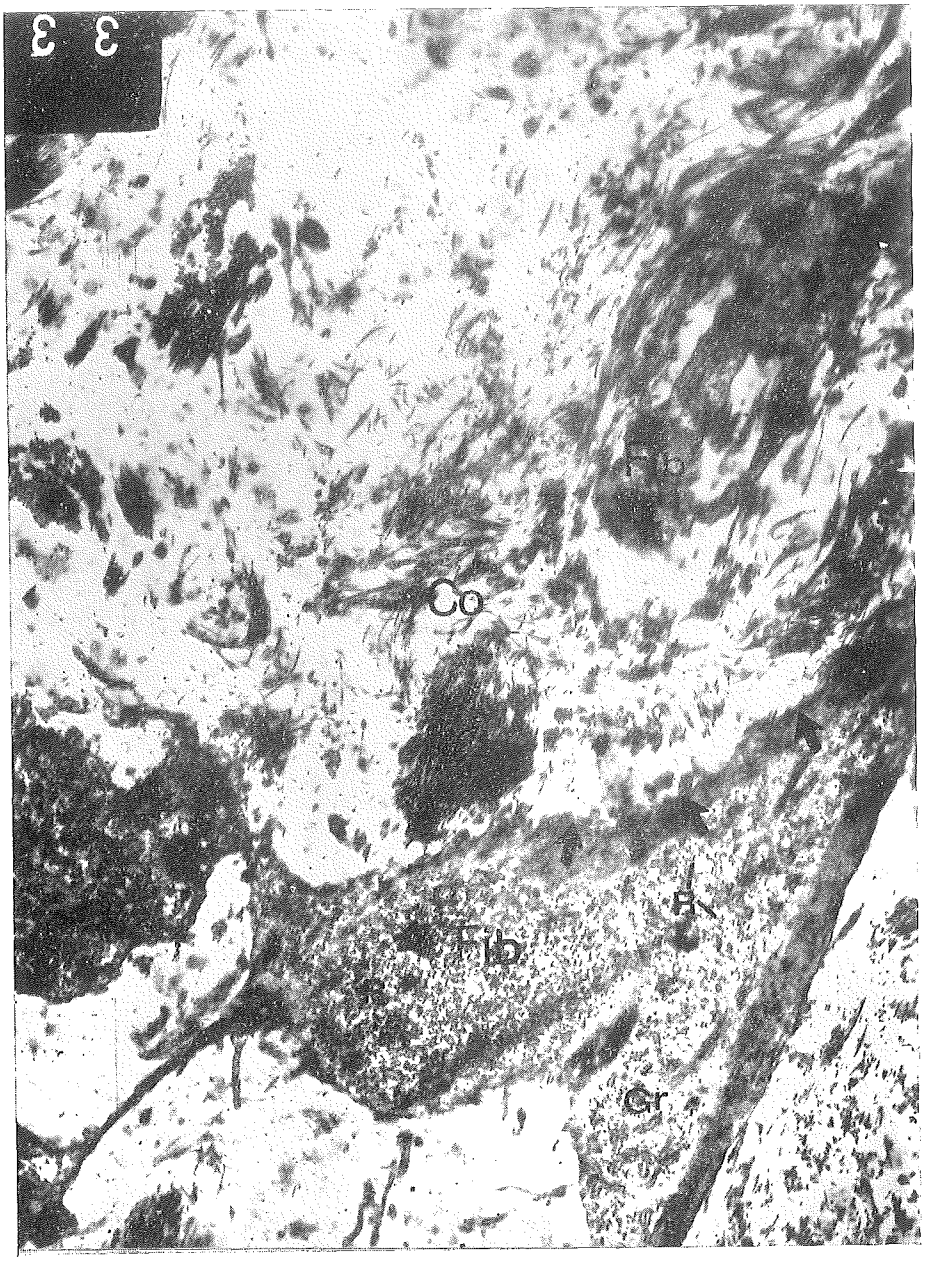

Figure 17

The ultrastructures of the fibroblast with its extensions (Fib) and the bundles of collagenous fibers (Co) in the intercellular space. It is observed that in the endoplasmic region of the fibroblast occasional granular type endoplasmic reticulum (Gr), abundant ribosomes (R) and polysomes exist. The ectoplasm has a dense filamentous structure. The barrier of the tubuli of the granular endoplasmic reticulum in between the ecto- and endoplasms is not seen in thsi fibroblast. It is also observed that the filaments seem to sprout into the intercellular space (arrows) all along the discontinued fibroblast membrane. X 8.500.

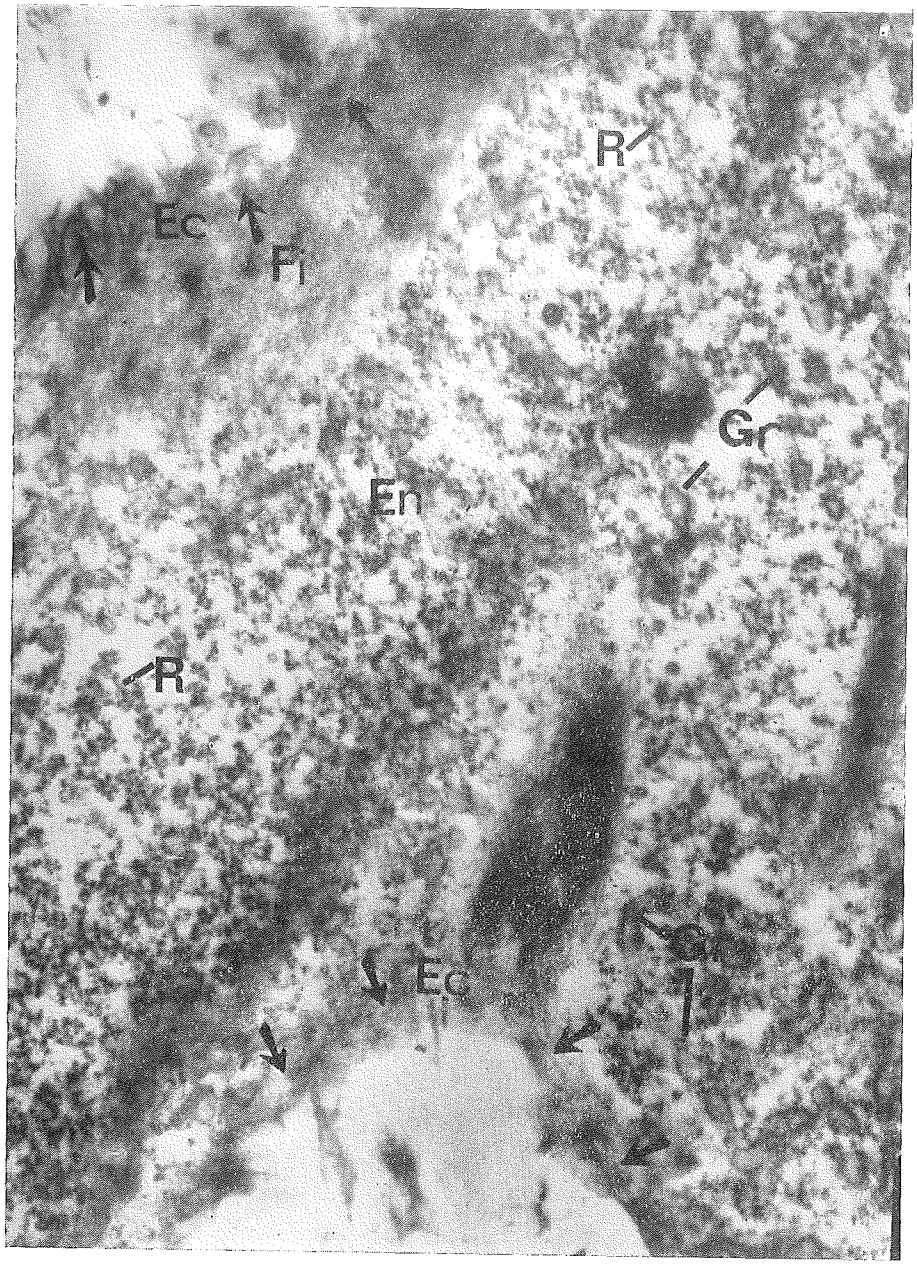

Figure 18

Detailed ultrastructure of one part of another fibroblast. Numerous ribosomes (R) exist in the endoplasm (En), but the elements of the granular endoplasmic reticulum (Gr) are scarce. The ectoplasm (Ec) is completely filled up by filaments (Fi). The cell membrane in all the regions of the micrograph that can be observed seems to have disappeared (arrows). The barrier of the tubuli of the granular endoplasmic reticulum observed in some other fibrocytes between ecto- and endoplasms cannot be seen in this fibroblast. X 72.000

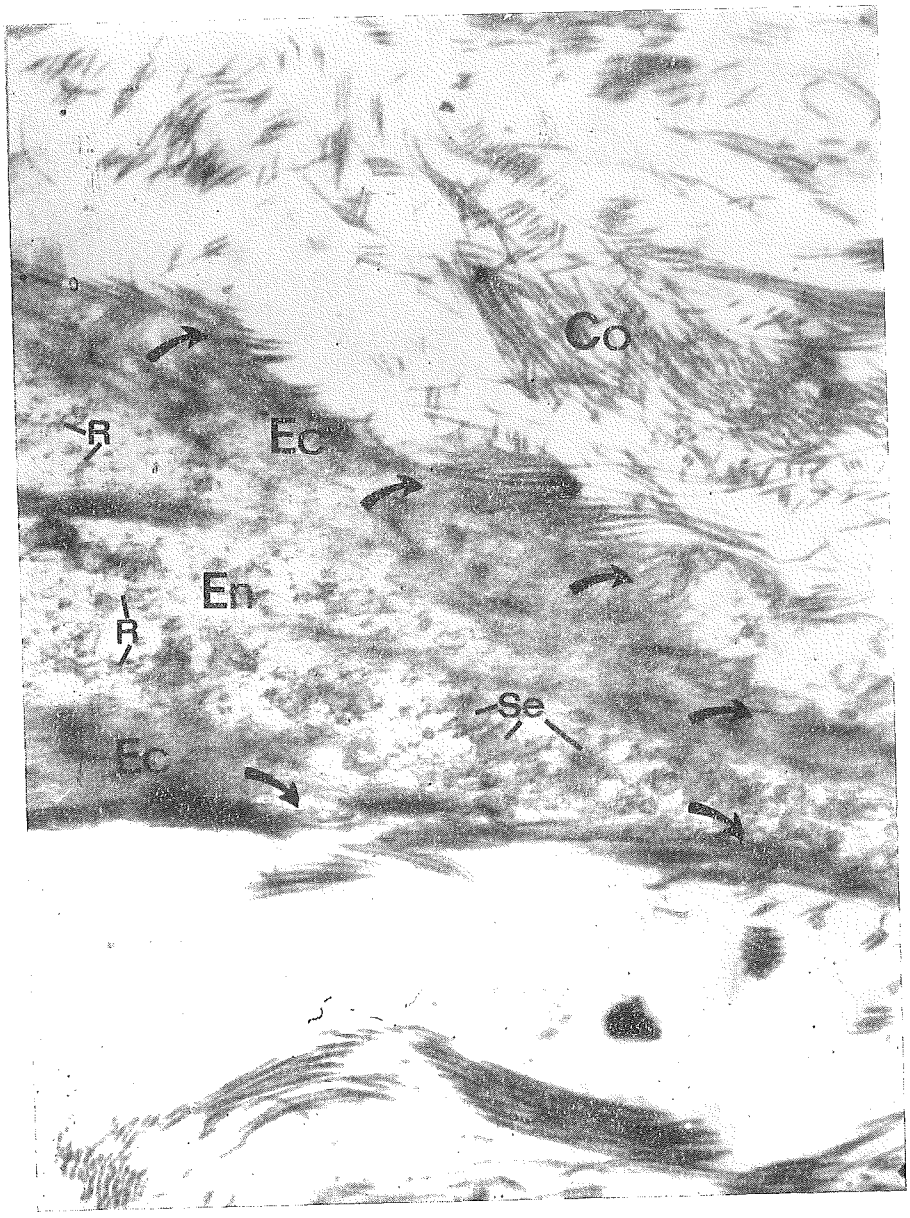

Figure 19

Detailed ultrastructure of the fibroblast extension. Ribosomes (R) and scattered pale secretion granules (Se) occur in the endoplasm (En). The ectoplasm (Ec) is completely full of parallel bundles of filaments. A granular endoplasmic reticulum barrier is not seen place in between the endo- and ectoplasms. In many places, it is observed that the cell membrane is discontinued (arrows). It is clearly observed that the transformation of the filaments into definitive collagen fibrils occurs just outside the surface of the cell membrane. X 72.000.

served to aggregate in thick units and to sprout out through these discontinued regions. The subunits of collagenous fibers were observed to initiate just inside and outside the region where the cell membrane had once existed. The periodicity of 640A long native collagenous fibres began to appear in these subunits as well (Figures 20 and 21).

Varying activities originating in different parts of the cell surfaces were noted in some of the fibroblasts. In one part of the cell the membrane was discontinued and the collagen subunits seemed to sprout out, whereas in another part of the same cell a continous membrane was observed with numerous micropinocytotic vesicules under it It was also noted that very tiny vesicules of material were excreted from the cell (Figure 22).

In some other fibroblasts, another secretive activity was observed in the cell cytoplasm besides collagenogenesis. Well-developed granular endoplasmic reticulum elements wholly invested one part of the fibroblast. Among these, irregular, large and homogenous secretion granules were characteristic in this activity. A few mitochondria were also noted. (Figures 23 and 24).

Discussion

Amniotic Epithelium

There are few studies of the amniotic epithelium, but those made during recent years may be considered in two groups:

In the first group the different regions of the parietal amniotic membrane have been considered. Authors have almost all expressed the same view on certain basic structural characteristics existing in these epithelia, observing that the parietal amniotic epithelium is single-layered and low-columnar in height. The nuclei of the epithelia are oval, rather dense and centric, and poor in cell organelles, i.e. the Golgi complex is under-developed, endoplasmic reticulum is scarce and there are few mitochondria. Many tonofilaments in the cells are observed as irregularly arranged bundles. The tonofilaments are 50-70 A thick. Numerous desmosomes take place in between the cells and some of the tonofilament bundles seem to originate from them. Irregular and short microvilli exist on the apical surfaces of the cells. Their basal surfaces are indented and rest on a highly developed basal lamia closely parallel to the basal indentations [1 2 3 4 5 6 7 8 9 10 11]. However, various authors have stressed certain particular details, pointing out that microvilli occurr on the epithelial surface only from place to place[9] and that abundant lipoid granules exist in the apical region of the cell [1 8 11].

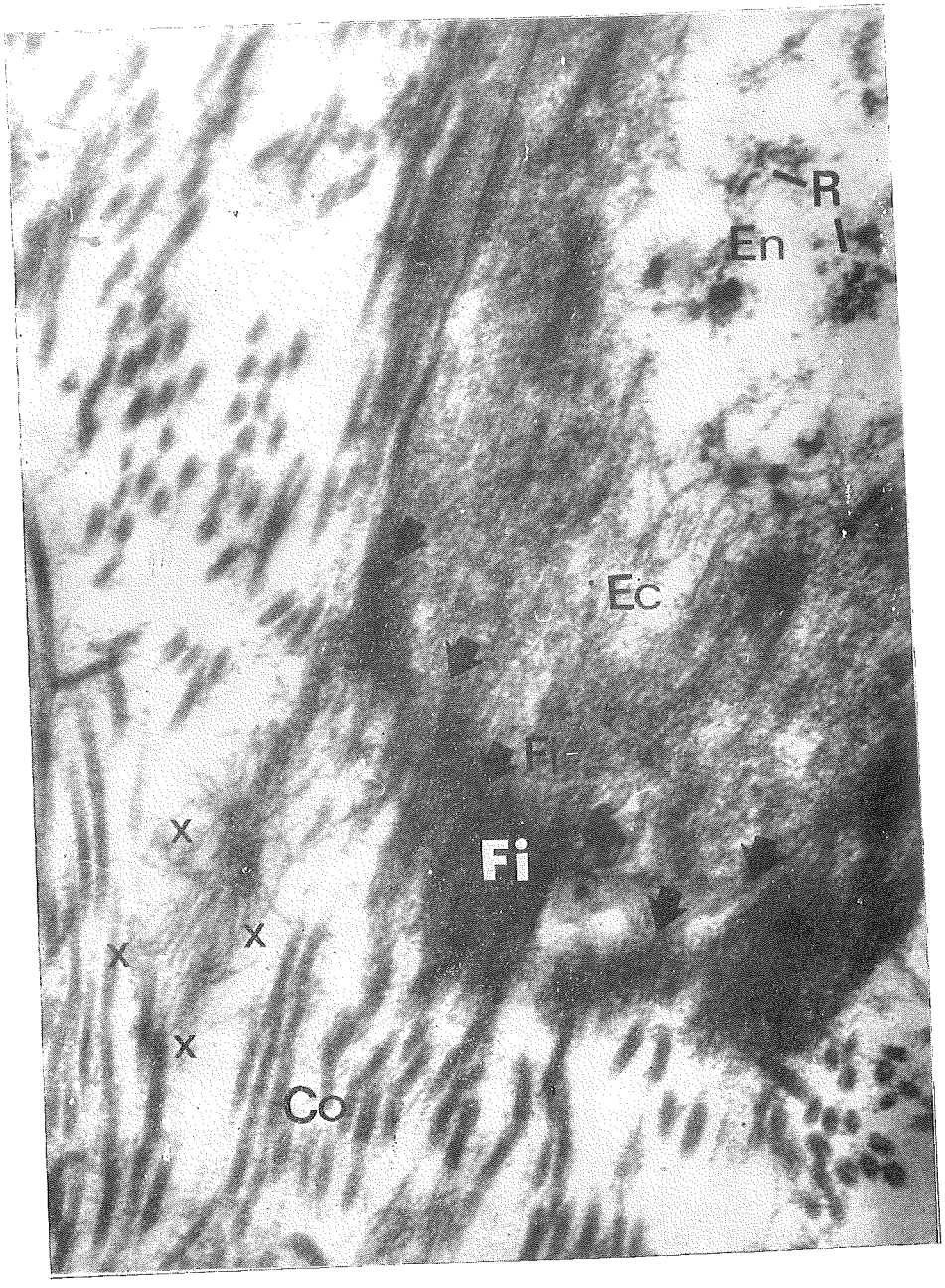

Figure 20

Detailed ultrastructure of the ectoplasmic (Ec) region of the fibroblast. The cell is in the most active phase of collagen formation. The ectoplasm is completely filled up by parallel bundles of filaments (Fi). The filaments seem to sprout out from the membrane (arrows). At top right ribosomes (R) and polysomes are seen in the endoplasm (En). Newly forming filaments are observed to pass from the endoplasm into the ectoplasm. The cell membrane is generally discontinued. It is noted that the filaments excreted all along the cell membrane region immediately tranform into the periodic collagen subunits. It can also be seen that the periodicity formation takes place in the finer filaments observed in the ectoplasm and just outside the cell (region between the 'X's). Co, collagen fibrils.
X 150.000

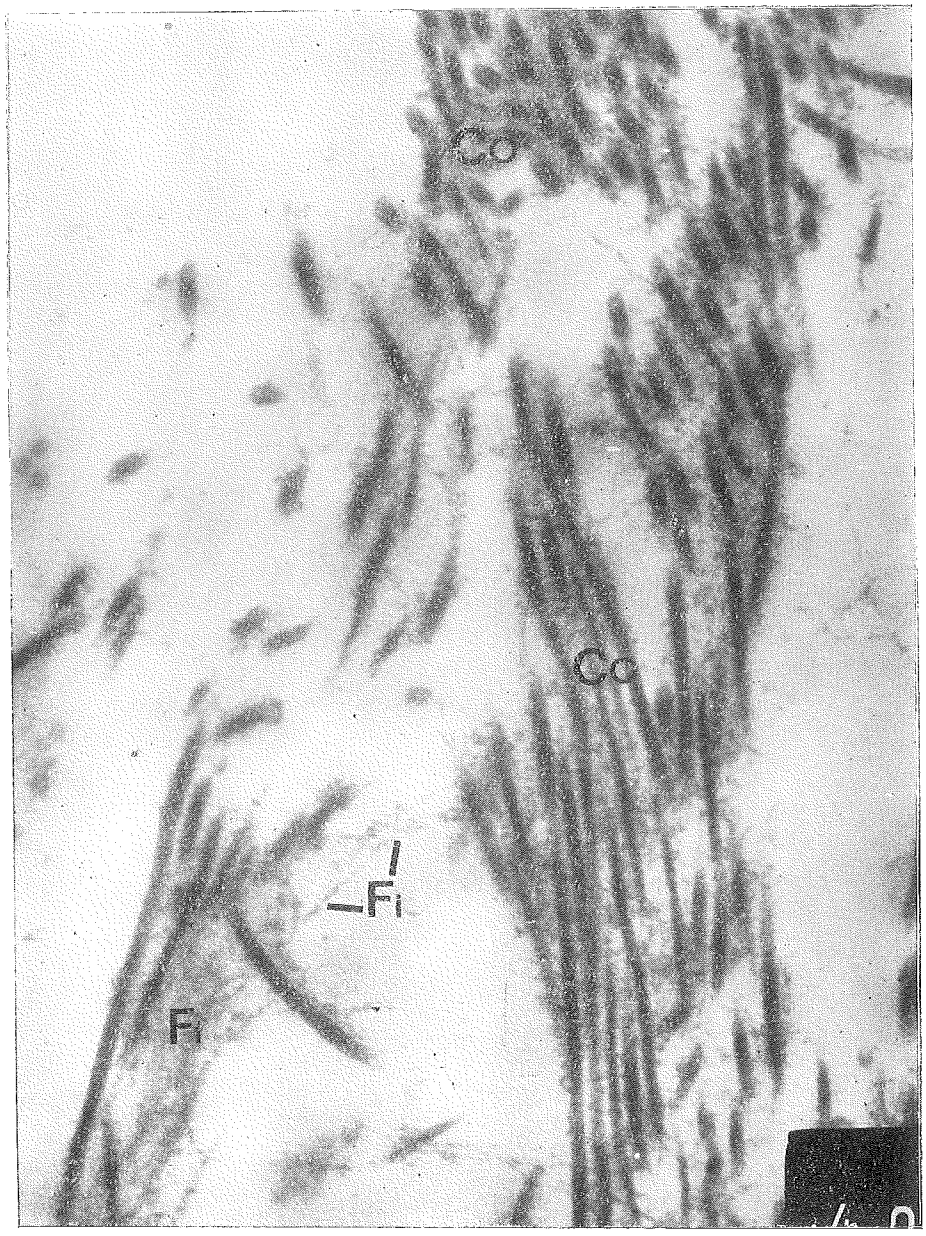

Figure 21

Horizontal, oblique and vertical sections of the collagenous fibers (Co) occurring in the intercellular space of the mucoid connective tissue are observed. Undeveloped scattered filaments (Fi) may be noted among the collagenous fibers of a periodic structure. X 150.000.

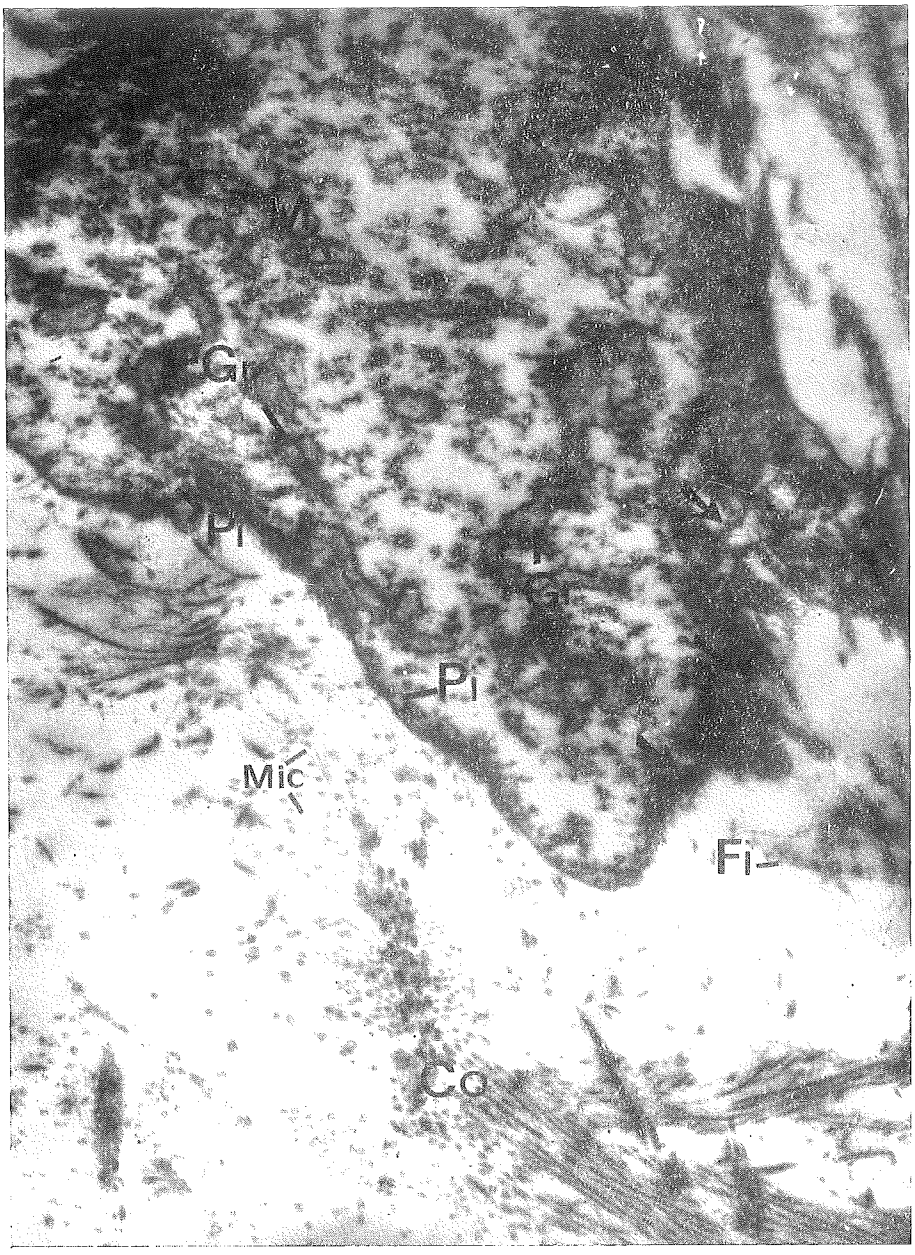

Figure 22

Ultrastructure of one part of another fibroblast. In various regions of the membrane surface the cell displays different activities. At the upper right and the minute upper left of the micrograph, the cell membrane is discontinued from place to place. Filaments are excreted outside the cell from the free regions of the membrane. At the lower left of the cell, numerous micropinocytotic vesicules (Pi) exist along the cell membrane (arrows). It is highly probable that the microvesicules (Mic) observed outside the cell are closely related to the micropinocytotic activity mentioned above. The granular endoplasmic reticulum (Gr) is highly developed and spread throughout the cell. M, mitochondrium. X 24.500.

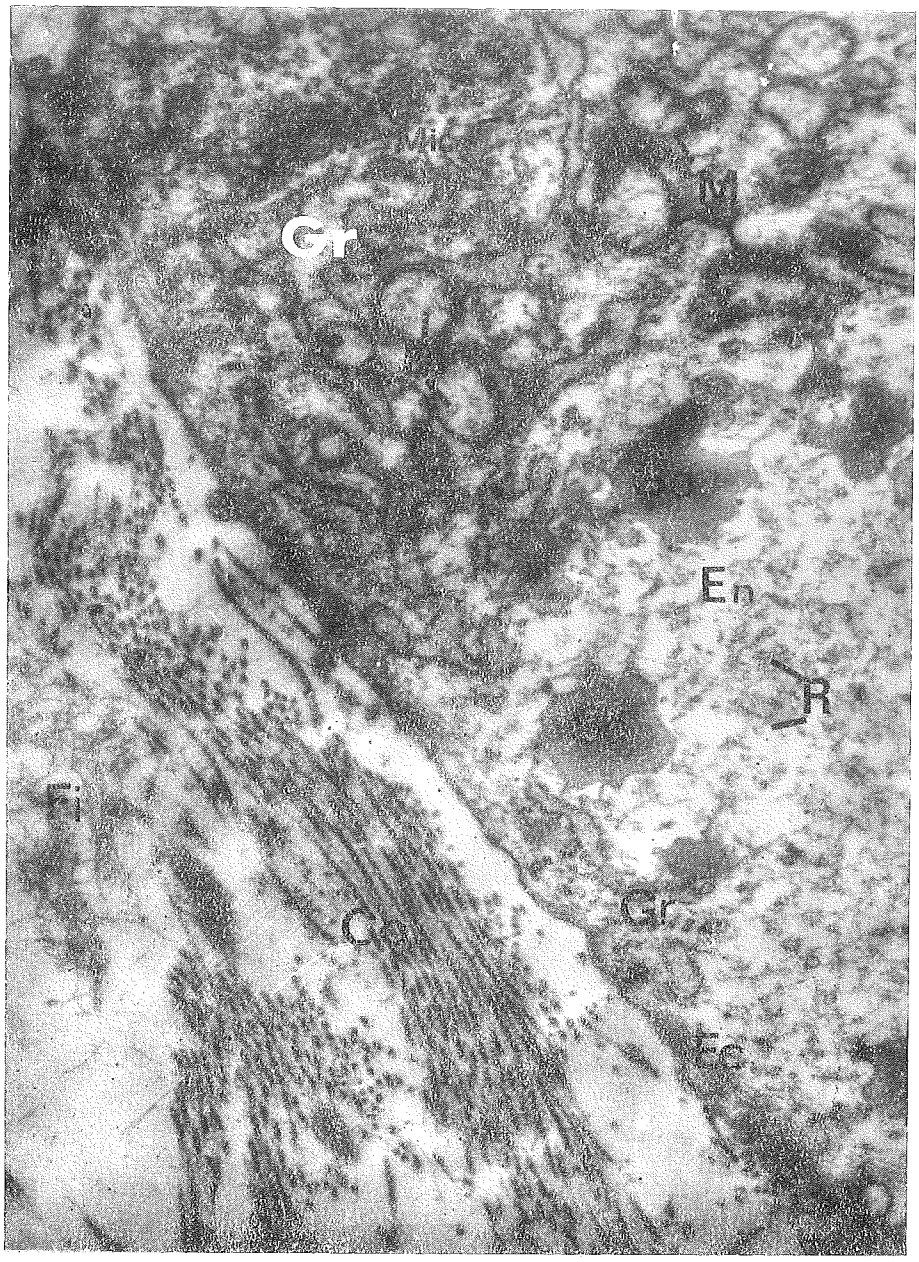

Figure 23

Detailed ultrastructure of one part of a fibroblast. The tubuli of the granular endoplasmic reticulum (Gr), mitochondria (M) and the microvesicules (Mic) in between them are observed in one part of the cell. Large secretion granules (Se) together with ribosomes (R) are observed in the endoplasm (En). A filamentous structure exists in the ectoplasm (Ec) at the periphery of the cell, particularly outside the tubuli of the granular endoplasmic reticulum. In addition, both fine filaments (Fi) and collagen fibrils (Co) displaying periodicity are observed together outside the cell. X 24.500.

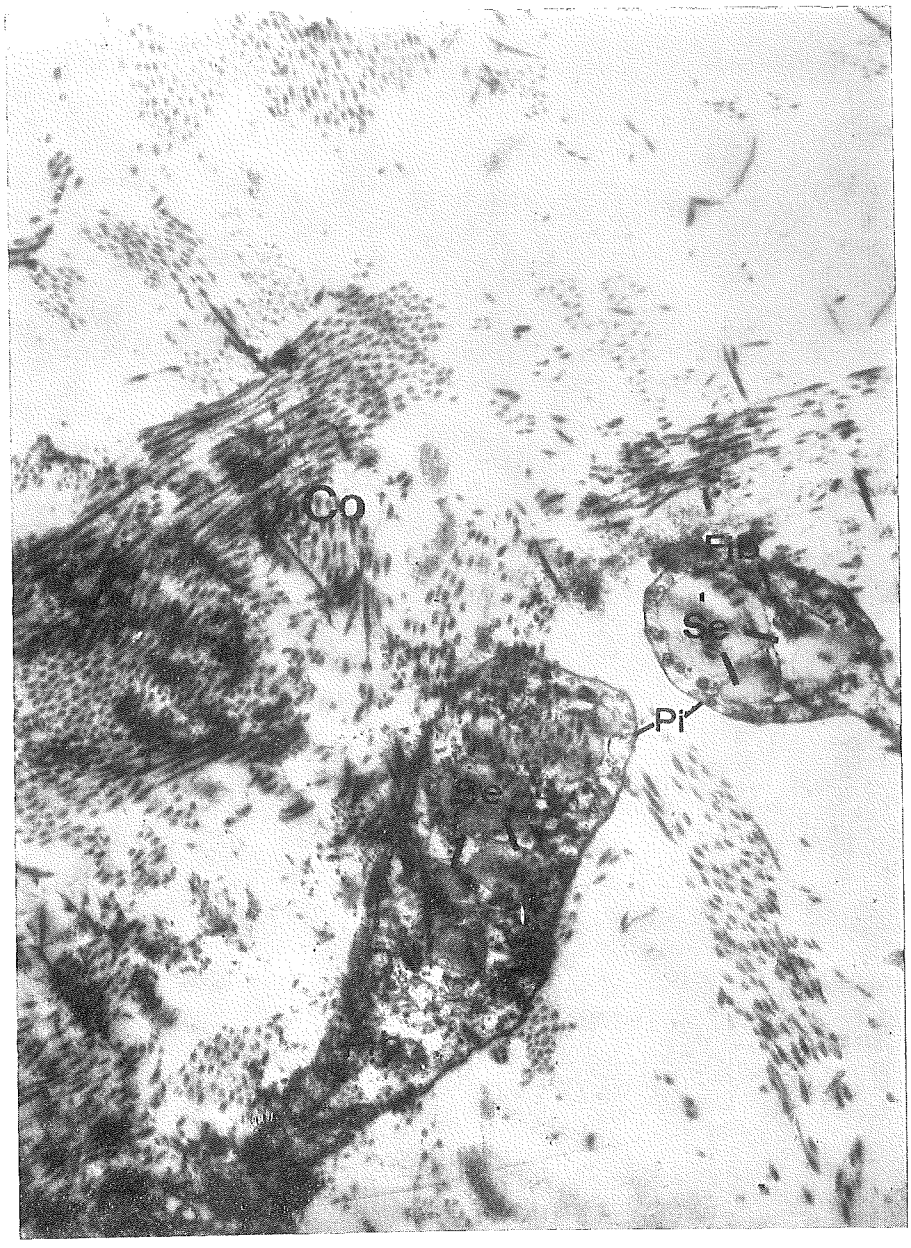

Figure 24

Two fibroblast (Fib) extensions and numerous bundles of collagen fibrils (Co) found in between them are observed. Some collagen fibrils are superimposed on the cell extensions. Large secretion granules (Se) fill up these extensions. Numerous micropinocytotic (Pi) vesicules occur in the periferic region, related to the cell membrane. X 24.500.

The second group of studies were carried out on cell cultures obtained during the embryogenesis of the amniotic epithelium [9 12 13 14 15 16]. The morphology of the amniotic epithelium at this stage is as follows:

There are flattened cells with elongated nuclei and microvilli all along the surface. Their cytoplasms are poor in organelles, i.e. the Golgi complex is underdeveloped and endoplasmic reticulum elements are scarce. A few mitochondria exist. Rather numerous ribosomes can be found and few or abundant bundles of tonofilaments are spread throughout the cell. Furthermore, it is pointed out that in the primary and secondary cultures of human amniotic cells, a quick proliferative phase exists in the beginning, followed by a rather prolonged stationary or degenerative phase, and then a recovery phase at the end[14]. In addition, cultures of human amniotic cells are found to be heterogenous in cell propagation[16]. Amniotic cells reconstruct desmosomes among themselves during cultivation so as to have interrelations[12]. The tonofilaments in the cells may increase as the culture ages[13].

The position of the amniotic epithelium covering the umbilical cord, is different and rather interesting. No ultrastructural research has been conducted on the epithelial covering of the umbilical cord.

According to several other authors and our own work carried out at light microscopy level, the morphology of the epithelium covering the umbilical cord undergoes large alterations along its entire length from the umbilical region of the fetus to the placental region. The epithelium is stratified squamous and has the characteristics of epidermis in the region very close to the umbilical cord at full-term. There are skin adnexa in the derma. After a little distance the stratified squamous epithelium is suddenly replaced by a single-layered epithelium. Nevertheless in the region close to the placenta, a stratified squamous epithelium may beobserved[17]. Our studies at light microscopy level (unpublished), proved the existence of several layered or pseudostratified types of epithelial coverings close to the placenta regions. In the material obtained from a region close to the placenta we established that the amniotic epithelial covering is three-layered.

The anatomohistological and functional features of the umbilical cord are different from each other. It is stressed that the umbilical cord seems to be a continuation of the chorion and the amnion from the anatomohistological point of view, and it appears as an active organ of fluid exchange from the functional standpoint[18]. The morphological characteristics of the three-layered epithelial covering, the ultractructure of which we observed in our studies, have led us to accept that the epithelial

cells are adapted in their structures to the functions outlined above. It may be thought that the cells containing metachromatic granules in the mucoid connective tissue under the epithelium could have a rôle in the exchange of material[19]. The ultrastructural study of cells in the amniotic fluid has proved that two types of cells exist. The morphology of the first type was identical with that of the superficial cells in our studies. The second type are identical to the macrophages and can produce phagocytosis. It is difficult to understand the mechanism by which these cells pass into the amniotic lumen. It seems impossible to us that they are driven from the amniotic epithelium as suggested by one author[20]. It is definitely known today that the origin of the amniotic epithelium and periderm is the same, i. e. the ectoderm[15].

The ultrastructural characteristics of the parietal amniotic cells both under normal conditions and in such cultures as in the above literature are identical to those of the superficial cells of the three-layered amniotic epithelial covering of the umbilical cord close to the placenta which were taken up in our studies. As in the single-layered parietal amniotic epithelial cells, the superficial cells of the three-layered epithelium in our studies were rather flattened, their nuclei were oval and centric, and their chromatin was rather dense and homogenous. Bundles of tonofilaments 50-70 A thick occurred in the cell cytoplasms. There were junctional complexes among the cells. Bundles of tonofilaments originating from desmosomes spread into the cells, which were very poor in organelles.

The ultrastructures of the middle and basal layer cells are different from those of the superficial cells and very interesting. The tonofilaments in these decreased greatly, and a highly developed, enlarged granular-type endoplasmic reticulum with a Golgi complex and large membrane-invested secretion granules were found. These were real secretive cells from the morphological point-of-view. It was shown that fine unmyelinated nerve fibers terminated in the amniotic epithelium[21]. Another non-secretive type and an undifferentiated cell which we termed an intermediary cell was also observed in the middle layer. It is difficult to decide only by morphological criteria whether these are the initial forms of secretive cells. Abundant free ribosomes existed in the discontinued basal layer cells, along with the granular endoplasmic reticulum. It cannot be argued that the non-secretive types change into secretive cells. While the non-secretive type of cells rested on the basal lamina with a smooth basal surface, the secretive cells were placed on the basal lamina in a deeply indented manner looking like a labyrinth by which their morphological distinction was made possible. No research on the study of the ultrastructure of the multi-layered amniotic

epithelial covering of the umbilical cord exists. Future studies in this field will contribute towards understanding of this subject. The identification of the histochemical characteristics of the epithelial secretion and a closer approach to their physiological aspects may well be of the utmost help in more constructive studies of this subject.

Fibroblast and Collagenogenesis

For a century authors have accepted that collagenous fibers are made by fibroblasts, but it is still debated how the collagen gains a fibrillar structure and is excreted by the fibroblast. Developments in electron microscopy technique over the last ten years supported by methods of physico-chemical molecular analysis and histo-autoradiography, have shown that collagen formation takes place in the phases shown below:

1. The clarification of the synthesis of the unit molecule, i. e. tropocollagen in the cell particularly through the use of physico-chemical and histo-autoradiographic methods. The synthesis occurs in accordance with the usual biosynthetic phases in forming the normal proteins within the cell [22 23 24 25 26 27]. The amino acids such as glycine and proline, each forming one-third of tropocollagen macromolecule, were labelled and the way they followed in the fibroblast, osteoblast and chondroblast in the connective tissues was observed [27 28 29 30 31]. As a result of these observations, the labelled amino acid was first collected in the endoplasmic reticulum tubuli within fifteen minutes, then passed into the Golgi complex and after about one hour reached the maximum amount. After two hours the labelling material was observed in the collagenous fibers outside the fibroblast. Although it was possible to trace the labelled amino acid in the cell during collagen formation by the use of histoautoradiography, it was not possible to identify the figurative details of the morphology of this procedure.

2. Chain formation of collagen macromolecules so as to constitute the single collagen fibril. It is still debated whether the triple polypeptide chain of the tropocollagen macromolecule is first formed in single chains coming together eventually, or triple in its origin and attached together [26 32 33 34]. It is still doubtful whether the periods of 640 A long observed in the collagen fibrils are initially formed, and come together even tually, or whether 3000 A long tropocollagen macromolecules are lined up one after the other to form the chain [26 32].

3. Excretion of collagen fibrils from the fibroblast into the intercellular space and the first site of aggregation of the definitive fibrils. The discussion is whether the collagen fibrils are excreted from the fibroblast

in the form of very fine filaments, or as non-fibrillar, soluble or granular secretion material [33 34 35 36 37]. Recently authors have been inclined to study the fibrillar precipitations of purified collagen solutions at electron microscopy level so that they could identify closely how the collagen fibrils are constructed [26 38 39 40 41]. Two types of collagen precursors were obtained from the solubilized extracts of the collagenous connective tissues, as follows:

A. Tropocollagen is a fibrillar protein, 3000 A long and 15 A wide. It can be extracted from the collagenous tissues with light akaline or neutral saline solutions. There is a tendency to accept this as the smallest unit of the native collagen, but as it is extracted from the solutions of collagen fibers, it cannot definitely be considered a prepolimerization precursor.

B. Procollagen which has a length of 7000 A, was extracted with citrate solutions. It displayed the periodicity of 640 A long. The new collagen forms obtained from the extracts were termed SLS (segment long spacing) and FLS (fibrous long spacing) and their morphological details were defined [38 39].

In spite of the above-mentioned data, there is as yet no unanimously accepted definition of a precursor molecule. The initial aggregation site of the precursor molecules from the morphological standpoint remains obscure. Generally the collagenous connective tissue in the skins and tendons of various animals have been used as material in the studies of formation and the internal structure of collagen fibrils at electron microcopy level. Detailed studies of the ultrastructure of collagen formation in the fibroblast cell befo the collagen has been excreted are really scarce[36 42 43 44 45 46 47 48] Various authors have pointed out that during collagen formation the endoplasmic reticulum tubuli in the fibroblast increases and enlarges as cisternae, with filaments occurring in the cells [32 37 42 43 45]. The existence of dense bodies[44] and lipoid and glycogen granules was reported[49].

The ultrastructure of fibroblasts in the mucoid connective tissue that we studied displayed very interesting morphological changes in various steps of the collagen formation, not thus far observed in the literature. In these fibroblasts the ultrastructures of the endo- and ectoplasms in the initial phase of collagen formation were very different from each other. Numerous ribosomes and polysomes filled the endoplasm. The ectoplasm was filled with coarse filaments about 60 A thick arranged in bundles parallel to the surface. A highly developed barrier of tubuli of the granular endoplasmic reticulum occurred continuously between the two regions of the cell and sometimes this was observed to spread towards

the nucleus. The cell was poor in other organelles and those which could be observed were located in the ectoplasm. In this phase the cell membrane investing the body and extensions of the cell was discontinued from place to place. In addition to the morphological picture above, large and pale secretion granules invested with membrane were observed in part of some cells. Furthermore the granular endoplasmic reticulum tubuli and ribosomes were abundant and the Golgi complex was highly developed.

Some fibroblasts contained no filaments and the cell membrane was not discontinued. The large secretion granules and the organelles occurring in between them filled up the cell.

In the later phases during which collagen formation advanced and abundant filaments were excreted from the cell, the ectoplasm was enlarged, and the filaments in it increased and aggregated densely. The barrier of tubuli from the granular endoplasmic reticulum disappeared as did the cell membrane in some places, and the filaments aggregating in a parallel manner seemed to sprout from this region. In very great magnifications the existence of a typical periodicity could be noted in the filaments aggregating within the cell and the small scale collagen subunits located either just on or outside the cell membrane.

In the only research made on the ultrastructure of the mucoid connective tissue filling up the umbilical cords of a four-month embryo and a full-term fetus, the granular endoplasmic reticulum was observed to be in the form of much enlarged cisternae spreading through the cell in a very irregular manner[4 9]. It was stated that the filaments formed in the fibroblasts during the last month and spread through the cell at random, and this was considered a symptom of aging and degeneration. The filaments occurring in the fibroblasts and their rôle in collagen formation is undoubtedly a subject calling for further research. It has been reported that definitive collagenous fibers occur in the fibroblast of invertebrates.[4 6]

According to the purely morphological findings obtained from our studies, the formation of a precursor molecule in the first phase of collagenogenesis is brought about in the endoplasm filled with abundant ribosomes. The formation of chains of these to form a filamentous structure in the ectoplasm can only be achieved on the outer wall of the barrier of tubuli of the granular endoplasmic reticulum. We thus concluded that the formation of chains as filamentous structures is brought about mainly the barriers of tubuli of the granular endoplasmic reticulum. The existence of typical periodic structures in the subunits of the collagen fibrils

occurring in the outer region of the ectoplasm and immediately within the cell membrane region has led us to believe that the most effective system of making a periodic intermolecular order in the native collagen fibrils is possibly located in the fibroblasts. Accordingly the definitive collagen fibrils reach their final forms with the help of the fibroblasts in the periphery of the ectoplasm or in the discontinued cell membrane region. From there they are directed into the intercellular space. It is certain that the fibroblasts play a significant rôle in securing the order of periodic structure. We also noted that bundles of collagenous fibers in between the fibroblasts were arranged irregularly, and therefore differed from the collagenous tissue of derma, the collagenous fibers of which are arranged regularly and may vary or be active[50 51]. It has been reported that capillaries exist in the mucoid connective tissue of the human umbilical cord[52], but no such capillaries could be noted in our studies. It should be accepted that diffusion plays the main rôle in the maintenance of the mucoid connective tissue. As mentioned in the discussion of the amniotic epithelium, the mucoid connective tissue is a region of active fluid exchange. The amniotic fluid absorbed by the amniotic epithelium is diffused throughout the mucoid connective tissue to the lumen of the umbilical vessels in the center.

Morphological studies on the interrelation of fibroblast collagen formation at electron microscopy level such as the present one will aim at clarifying the second and third phases of collagenogenesis, i. e., the initial site of the formation of subunits and definitive collagen fibrils. Our findings have led us to believe that the arrangement of filaments in the space in the definitive periodic structure forming the collagen fibril on the memb ane surface of the fibroblast is directed by the fibroblast itself.

Summary

In this study, the amniotic epithelial covering and the mucoid connective tissue filling up the umbilical cord of the full-term human fetus are considered.

It was observed that the amniotic epithelium was three-layered, and that the morphological characteristics of the cells found in each layer were interesting and varied immensely one from another The dense tonofilament bundles found in the cytoplasms of the cells of the surface layer, as well as numerous short and irregular microvilli on the surfaces facing the amnion, were noted in this study. It was also observed that although the epithelial cells in the middle and basal layers generally preserved their

surface covering epithelial characteristics from the morphological point of view, they also displayed the ability to secrete from place to place.

The amniotic epithelial covering of the umbilical cord was observed to be of an interesting ultrastructural morphological nature. The surface covering and absorptive and secretive epithelial characteristics could be wholly identified.

Fibroblasts in various stages of collagenogenesis and collagenous fiber bundles found among these in the mucoid connective tissue fill the interior umbilical cord. It was observed that the ultrastructures of the ecto- and endoplasms of fibroblasts in the early stages of collagenogenesis varied greatly. The endoplasm was filled with numerous ribosomes and polysomes, while the ectoplasm had parallel bundles of thin filaments. A barrier of the tubuli or the granular-type endoplasmic reticulum was observed between the ecto- and endoplasms. The filaments were secreted at the regions where the cell membrane was discontinued. Most of these turned into periodic collagen fibrils just outside the cell surface. It was observed that in the most active stage of collagenogenesis all the filaments in the ectoplasm seemed to sprout out towards the exterior of fibroblasts. This attracted attention to the fact that the barrier of the tubuli of the granular-type endoplasmic reticulum between the ecto- and endoplasms disappeared at this stage of collagen formation.

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The Importance of X-Ray, Gastric Cytology and Endoscopy in the Early Detection of Gastric Carcinoma

Hasan Telatar, M. D.*

Gastric carcinoma is a relatively common disease all over the world, but according to statistics it is most prevalent in Japan, Norway and Finland. Although the mortality rate due to this disease has decreased recently for reasons not well understood, gastric carcinoma still ranks first among the gastrointestinal malignancies.[1] It can occur at any age, but the highest incidence is after the fifth decade, and men are affected twice as frequently as woman.

The value of early diagnosis is very great in the treatment of gastric cancer. In spite of the recent progress made by the use of the fibroscope, early detection of this illness is not always easy. In this study the usefulness of the most outstanding diagnostic methods such as x-ray, gastric cytology and gastrocopy is evaluated.

Materials and Methods

Fifty-five male and 35 female patients, aged 37 to 82 years, were included in this study. For all the patients with symptoms and signs referrable to the gastrointestinal tract, upper GI series were obtained, and selection of the patients was done on the basis of the radiologic examination. Gastric cytology and gastroscopy were performed in every case demonstrating

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a benign, malignant or possibly malignant lesion on x-ray films. The final diagnosis was made by autopsy in five cases, by surgery in 62 cases, and by complete healing of the ulcer by medical treatment in the remaining 23 cases. Patients whose final diagnoses remained obscure were excluded from the study.

In every patient, gastroscopic examination was done with a fiberoptic gastroscope. [2] [3] The lesions were classified as benign, malignant and possibly malignant (Figure 1).

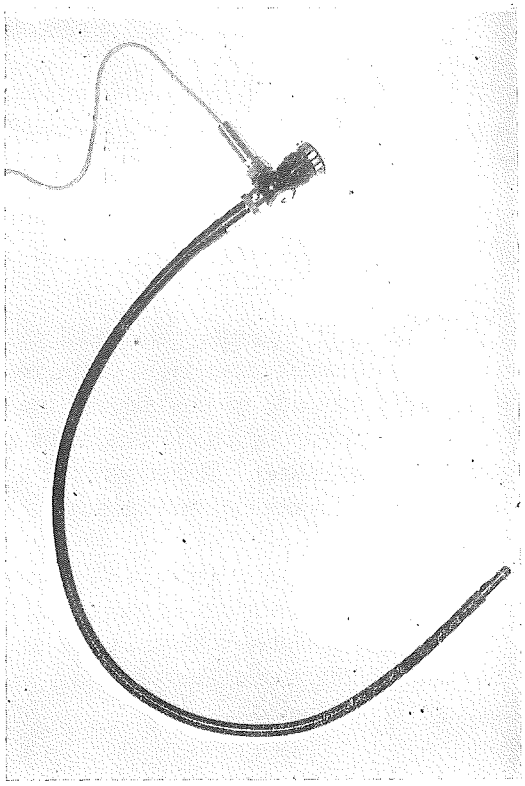

Figure 1. Fiberscope

An Ayre rotating gastric brush was used for gastric cytology (Figure 2). After a 12-hour fast, the stomach was brushed, and the material obtained was smeared on six slides, and immediately fixed in a mixture of equal quantities of 95 per cent alcohol and ether. In 75 patients, the slides were stained by the Papanicolaou method, and in 15 patients by the Türk method, and they were all studied under a light microscope. [4] [5] [6] The cells were classified as benign, malignant and suspected as malignant.

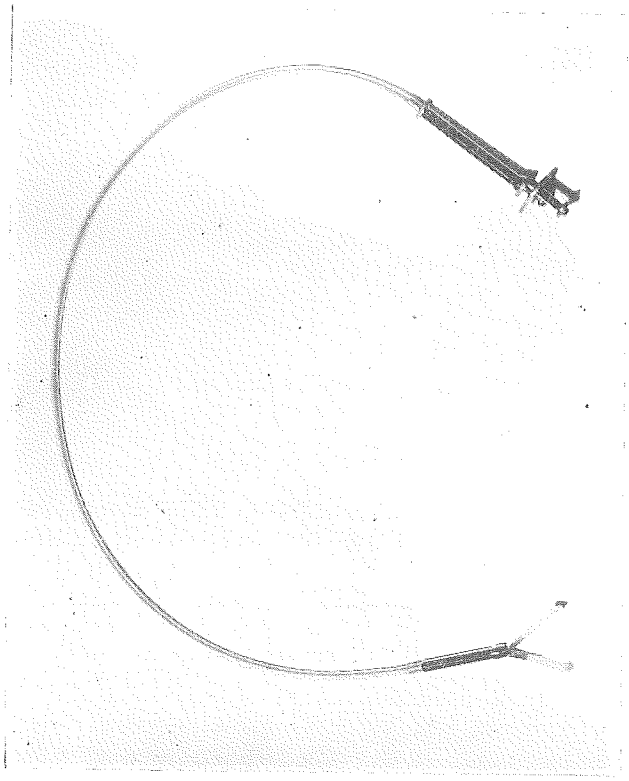

Figure 2. Ayre rotating gastric brush

Results

Of 40 cases of gastric carcinoma, only 34 were diagnosed correctly by x-ray examination. Four patients were diagnosed radiologically as having possible malignancy, and two were thought to have benign gastric ulcers. On the other hand, in 45 of a group of 50 patients, a correct diagnosis of benign gastric ulcers was made by x-ray examination; three patients were reported to have lesions in which malignancy was suspected, and two were thought to have malignant lesions (Tables I and II). Thus the diagnostic value of x-ray examination alone was 87.8 per cent.

Cytologic examination revealed malignant cells in 31 patients out of 40 with gastric carcinoma (Figure 3). Of the remaining nine patients, five had suspicious cells and four had gastric cells with no evidence of malignancy (Figure 4). Of 50 patients with benign gastric ulcers, 44 had normal results on cytologic examination (Figure 6). Suspected malignant cells were seen in five patients, and malignant cells were reported in one case. The diagnostic accuracy of gastric cytology was 84.5 per cent.

TABLE I

RESULTS OF THE ROENTGENOLOGIC, CYTOLOGIC AND GASTROSCOPIC EXAMINATION OF 40 PATIENTS WITH GASTRIC CARCINOMA

Diagnosis	X-ray	Gastric Cytology	Gastroscopy	
			Visualized	Non-visualized
Carcinoma	34	31	29	0
Suspected Carcinoma	4	5	4	0
False Negative	2	4	2	5

TABLE II

RESULTS OF THE ROENTGENOLOGIC, CYTOLOGIC AND GASTROSCOPIC EXAMINATION OF 50 PATIENTS WITH BENIGN GASTRIC ULCER

Diagnosis	X-ray	Gastric Cytology	Gastroscopy	
			Visualized	Non-visualized
Benign Ulcer	45	44	40	0
Suspected Carcinoma	3	5	4	0
False Positive	2	1	2	4

Gastric carcinoma was detected by gastroscopy in 29 patients out of 40 with the disease. In four cases the lesions were suspected as malignant, in five cases no lesion could be demonstrated, and in two cases it was thought to be benign. Of 50 cases of apparently benign gastric ulcers, gastroscopic diagnoses showed them to be benign in 40, suspected as malignant in four, and malignant in two cases. In four patients the lesion was not visualized. According to the above findings, the diagnostic accuracy of gastroscopy in 90 patients was 76.7 per cent.

Of six patients in whom roentgenologic studies revealed either benign ulcers or suspected malignancy, four were diagnosed as having malignant lesions by gastroscopic and cytologic findings. Gastroscopy and gastric cytology conformed with radiologic diagnosis in five patients in whom upper GI series were interpreted as showing malignant or possibly malignant lesions. So when the three methods were used together in the diagnosis of gastric carcinoma, accurate results were obtained for 97.8 per cent of the patients.

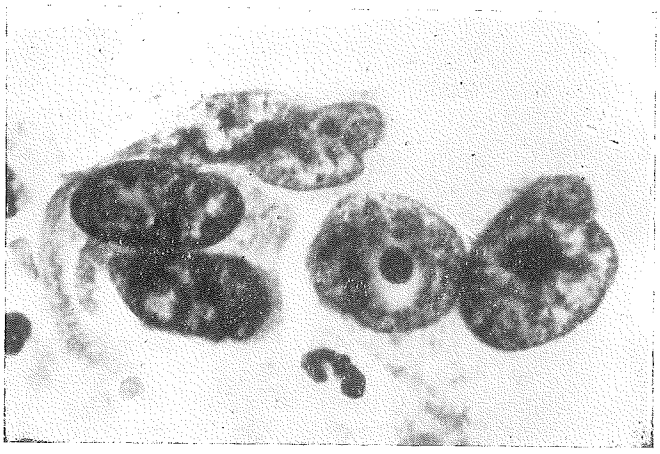

Figure 3. Malignant gastric cells

Discussion

The importance of early diagnosis of gastric malignancy is beyond argument. Despite recent advances in diagnostic methods, diagnosis is not always easy. Although x-ray examinations, gastroscopy and gastric cytology are the most outstanding methods of our time, when any one of them is used alone the results can frequently be misleading. X-ray examination is still one of the most dependable tools, however upper GI series do not always lead to a definite diagnosis because many surperficial

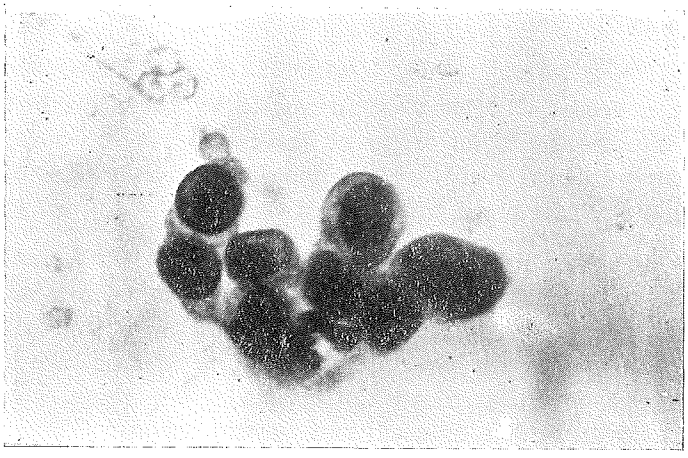

Figure 4. Suspected malignant gastric cells

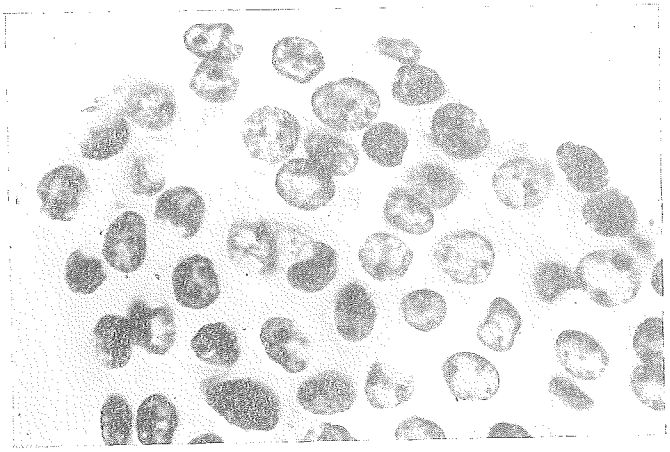

Figure 5 Normal gastric cells

lesions may not be shown, and the radiologic appearance of some malignant lesions can mimic benign ulcers.[7]

Gastric cytology, introduced by Papanicolaou in 1947, has become more and more important in the early diagnosis of gastric cancer.[8 9 10 11] However, the accuracy of the results depends on the experience of the cytologist, the localization of the lesion and the care taken in the technique. A correct diagnosis cannot be reached by cytology if the lesion is submucosal, intramural or covered with necrotic material; therefore failure to show malignant cells never rules out cancer.[1 2] On the other hand, positive results obtained by gastric cytology in superficial malignant lesions, which cannot be shown by x-ray examination, increase the diagnostic value of cytology.

With the recent addition of the fiberoptic gastroscope to the diagnostic methods, direct visualization of gastric lesions has become possible.[13 14] Unfortunately though, even with this type of gastroscope blind areas still remain, and this fact limits the value of the procedure to some extent.

This study and many others have revealed that although x-ray examinations, gastric cytology and gastrocopy are useful methods, when used alone none of them has reached a standard high enough to lead to correct diagnosis in every case. When the three methods are used together, however, correct diagnoses can be made in a significant number of patients with benign or malignant gastric lesions. Gastric cytology and gastroscopy together with roentgenologic examination are considered to be the safest procedure in the detection of gastric carcinoma.

Summary

Forty patients with gastric carcinoma and 50 with benign gastric ulcers were investigated with the aid of x-ray examination, gastric cytology and gastroscopy, and the usefulness of these methods was evaluated.

According to this study, the diagnostic value of x-ray examinations in gastric lesions was 87.8 per cent, of gastric cytology 84.5 per cent, and of gastroscopy 76.7 per cent. However, when these methods were used together, accurate diagnoses were reached in 97.8 per cent of cases. It was therefore concluded that in gastric carcinoma all three methods should be used together.

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Traditionally Generated Conflicts and Their Manifestations in Certain Belief Systems *

Orhan M. Öztürk, M.D.**

I

It is probably impossible to speak of a national character, a basic personality structure, or a modal personality in a society which is undergoing significant cultural and economic changes. In this presentation our purpose will not be in the direction of exploring the personality characteristics of a society at large. We shall rather try to show the development of some more or less commonly shared characteristics of the personality of the traditional section of the Turkish population, and relate them to certain belief patterns and folk practices encountered in it. The sources of information were special studies, as well as clinical and naturalistic observation. The characteristics described do not imply psychopathology, although such an impression may be inadvertently given due to the terminology used.

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* This paper was presented at the International Conference on Subjective Culture, Athenian Institute of Anthropos, Athens, June 1968.

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Present Turkish society is not homogenous, but neither does it have any serious class or caste barrier systems between the various sections or levels. In Turkey it is possible to distinguish traditional, transitional and modern societies,[1] which have distinct as well as common characteristics. With a high migration rate from the rural areas to urban and metropolitan centers, and with an increase in the literate and urban population, the body of transitional and modern society is rapidly growing. Unlike relatively homogenous, so-called primitive cultures, Turkey's traditional society does not lend itself to clear and easy cross-sectional study because of its heterogeneity and the fact that the stability of its traditions has been shaken. However, certain important characteristics can be specified. The traditional Turkish family is typically extended, patriarchal, patrilocal and patrilineal[2 3], and belongs mainly, to an agrarian community. In Kluckhohn's terminology[4], the man-nature orientation is in the form of subjugation to nature; time focus is more on the present and past than on the future; activitiy orientation is more in the form of «being« than «doing», and relational orientation is more lineal and collateral than individualistic. The primary interests of the typical traditional family, usually living in a rural area, are land, crops and religious activity. The thought content is predominantly determined by agriculture and by the precepts of the Moslem religion. The concepts of time and space, and social and work activities are shaped and regulated by agricultural and religious traditions. Stereotyped and monotonous relations with the soil and God, and their extensions, constitute the major part of the phenomenal field of the villager.

II

Although there have been some critical writings on the relationship between child-rearing and personality development[5], [6] there is ample clinical and experimental evidence to justify the use of knowledge of child-rearing practices to achieve any kind of formulation of the individual or the modal personality development. However, we believe that taking one specific circumscribed area of child-rearing and relating it causally to a specific personality characteristic may lead to false conclusions. Therefore, we shall try to take child-rearing patterns as a whole and use the knowledge pertaining to them within the framework of the psycho-social developmental concepts, particularly along the lines formulated by Erikson. [7 8]

Child-rearing practices are not uniform throughout Turkey. Certain fundamental and common aspects of these practices in the traditional society will be briefly described. The social role of the women which, we believe, greatly determines the type and quality of child-rearing,

is characteristically designed to fit a patriarchal society in which women are actively responsible for working in the fields, animal care, housework and child care. In spite of legal equality with men and educational endeavors for the past 40 years, the traditional women is still exploited by the male. It is our impression that the woman's social position deprives her of time, energy, self-esteem and security, and that such a situation must have some effect on the amount and quality of basic trust and security that will be assimilated by the child during the early years of life. This impression can only be valuable if one can find evidence of such a lack of internal security in adult personalities. More will be said about this later.

In general, babies are swaddled for six to nine months, and are breastfed for at least a year, and not uncomonly for two or three. Prolonged breastfeeding is not necessarily an indication of traditional maternal generosity in love. In some areas it is practiced for economic reasons, or because it is believed that continued breastfeeding is a means of birth control. Evidence of deprivation on a biological level can be observed in the high frequency of infectious diseases, malnutrition and the high infant death rate.

When the child reaches the developmental stage of walking, talking and asserting his own autonomy, the characteristic style of training can be described as coercive and constrictive. Those who are obedient, compliant and silent are rewarded. To be active, mobile, curious and talkative is discouraged and even punished. During this psycho-social stage of autonomy[7] [8], although no unduly rigid or early training exists[9], autonomous will and activity are discouraged. The child is preferred and rewarded if he behaves as an extension of his parents. During the next stage of initiative, or in psychoanalytic terms the phallic stage, curiosity and aggressive intrusion in play and in other life situations are inhibited for exactly the same reasons. Beating, shaming, threats of castration and frightening tales of supernatural beings are among the most common methods of punishment. Religious and mystical tales filled with jinns, fairies and superstitions enter the fantasy world of the child as soon as he begins to comprehend, and his fantasies become easily identified with those of the adults.

Circumcision of boys is done without anesthesia during this or the following period, usually between the ages of four and eight. Through the influence of many compensatory and counterphobic devices such as verbal preparations, ceremonies, gifts, masculinity status, etc., this potentially traumatic operation becomes something so strongly needed by the ego that the lack of it could be severely traumatic.[10] Girls are not circumcised, but they witness the experience not without overt envy. In additon to circumcision, which is a social landmark in the development of a sexual

identity, it should be emphasized that society's treatment of boys and of girls is highly differentiated from early childhood onwards. This is seen clearly not only in later training patterns, but also in early feeding and caring attitudes. For example, in certain areas boys are breastfed beyond the age of two or three because it is believed that they will grow with mother's milk. This sort of attitude toward sex differences, with the child's early witnessing and later experience of the circumcision phenomenon, are largely responsible for a precocious sex-role differentiation and consciousness of sex-related matters in early childhood. This sex-role differentiation is reinforced by introducing the boy and the girl into the world of work and responsibility at around the age of six or seven. The little girl is now, particularly, her mother's important helper in housework and in child care.

III

Naturalistic observation of the life-style of the traditional family and several meaningful studies [1 2] indicate certain common personality characteristics prevalent in traditional Turkish society. These characteristics have been condensed in the term «constricted self"[1 8], which refers to a lack of curiosity, initiative, empathy and changeability. Several studies [2 11 12] support this concept of the constricted self and also add to it the aspect of passive-dependent-expectation within the core of the villager's ego. This latter attribute can be seen in his attitudes towards the father-figure, the elders, the state, God and in his beliefs pertaining to many natural phenomena like birth, death, illness, and the catastrophes of life. This passive-dependent-expectation* and the characteristics of the «constricted-self" seem to have an almost identical meaning as a relative lack of basic trust, autonomy and initiative, which are basic psycho-social gains to be acquired during the course of healthy ego development.[7]

Children can apparently learn to adapt themselves to many modes of traditional care, and be satisfied up to a point with what they are given by their earliest providers. It can be assumed that the village child learns to modulate his needs and frustration thresholds, and to live with what can be given. It has been pointed out that «the amount of trust derived from early infantile experience does not seem to depend on absolute

* The term passive-dependent-expectation does not necessarily imply laziness or reluctance to work as some may tend to believe. Heavy working and a passive dependent attitude may coexist, and in fact this is usually what happens with the Turkish peasant.

quantities of food or demonstrations of love, but rather on the quality of the maternal relationship" and that " a traditional system of child care can be said to be a factor making for trust, even where certain items of that tradition, taken singly, may seem unnecessarily cruel."[7] Tradition and religion have thus been viewed as living psychological forces which, through the kind of faith and conviction created in the parents, reinforce the child's basic trust in the world's reliability.[7] However, this view should not prevent us from formulating certain important sources of frustration and conflict, although they may be culturally determined and traditionally established. These may be the areas related to what Fromm called "socially patterned defects".[13] We suggest that in the Turkish villager - and probably in other traditional agrarian communities with similar characteristics - religion and tradition play a compensatory role rather than a reinforcing one in the establishment of internal security or "basic trust", which is essentially a sense of trust or security derived from and toward the self and the environment. A high degree of passive-dependent-expectation in adult life is not compatible with the presence of adequate "basic trust". Therefore, we assume the existence of a nuclear deficiency in basic trust as an outcome of the obviously frustrating aspects of child-rearing during the early phases of life. In the adult the manifestation of this deficiency is seen in the pervasive passivity, that is, in the feeling of helplessness and dependence. In other words, what is not provided adequately by the human and natural environment is provided, or it is hoped, provided by the authority figures and by faith and conviction in what lies beyond the human and the natural. Neither the human nor the natural environment can be considered trustworthy. As will be described below, this can be clearly observed in folk understanding of illness. Besides, such a situation in child rearing would necessarily lead to the accumulation of aggressive impulses in accordance with the frustration-aggression rule.[14] To be more specific, acceptance of helplessness and passive-dependent expectation on one hand, and accumulation of aggressive drives on the other, become inevitably rooted during the oral-incorporative stage. These become further reinforced by the training attitudes and adult expectations during the following stages of childhood, in which we see predominatly a severe and diffuse suppression of behavior modalities of "autonomy and initiative". These suppressive measures would not only mean an inhibition of autonomy and initiative, and hence a "constricted-self", but also a further increase of aggressive drives due to the continuous frustration of such needs as autonomous will and activity, independent vigorous motility, intrusion and curiosity. Granted that all of this suppression is carried on in a "traditional system of child care", the increase in agressive drives in accordance with the frustration-aggression sequence still persists. Besides, early differ-

entiation of sex-roles and sex-appropriate behavior is another important pattern that leads to additional drive-load and responsibility burdens which the child finds difficult to cope with. I mean that in such a traditional society there is a medium which nourishes early and intense identifications with parents of the same sex, together with strong inhibitions of childhood curiosity and initiative, resulting in premature adoption of traditional adult roles and responsibilities and an early abandonment of childhood proper. The abundance of psychopathology related to oedipal conflicts in such a society may find an explanation in this aspect of child-rearing.

In brief, it can be said that the passivity, the inhibition of autonomy and initiative, the accumulation of drives, and an ever-increasing social need and demand for powerful mastery over one's own drives become essential areas of conflict within the traditional villager's ego.

IV

An attempt will now be made to show the relationship between these characteristics and certain folk beliefs concerning illness and treatment. It has previously been shown that the content of existing folk interpretations of illness in a society are closely related to, and probably determined by, certain culturally pervasive and predominant anxieties experienced during early childhood. [15] [16] The characteristics of passive-dependent-expectation, constriction and accumulated aggression can be traced in folk beliefs which have persisted in spite of serious legal and educational endeavors to change or eradicate them since the foundation of the Turkish Republic in 1923.

The information which has been presented in greater detail elsewhere[17] is drawn from the literature, and from a study of 100 patients in a university medical center in Ankara. Sixty per cent of these patients had tried various folk cures, all of them came from various parts of Anatolia and could be considered members of Turkey's traditional society, although naturally not a representative sample of it. Obviously there is no one single institutionalized method in Turkey. As would be expected, due to the variety of influences on the present culture, the number of beliefs and practices is great. Nevertheless, certain generalizations can be made. It should be emphasized that in 1925 all religious and quasi-religious educational and therapeutic institutions were abolished, and the practice of magic and religion for therapeutic purposes was outlawed and prosecuted. However, such practices do continue in the traditional segments of society, suggesting the existence of certain needs which have not been adequately met by endeavors toward modernization. Undoubtedly under-

standing of, and attitudes towards, physical or mental illness are changing as modern educational, medical and communication facilities become more readily available to the rural people. It is therefore difficult to achieve a clear sample of folk interpretation of illness in this changing society.

A traditional villager's understanding of illness is in accordance with his faith. Although the Moslem religion disapproves of belief in magic, sorcery and supernatural human powers, it does include concepts that can nourish tendencies towards magical thinking and practices. "God gave an illness", is a common expression which the villager uses concretely. How and why God gives an illness is not explained and is considered beyond human curiosity. As long as the patient is not depressed he does not feel or consider that his illness is a punishment sent by God. God knows best, and there is no need to ask questions of Him, but one can wish and hope from Him. The Moslem religion also accepts the existence of spiritual beings like jinns, which are recognized as aggressive, mobile, deceptive, seductive and punitive agents. Villagers commonly believe that various illnesses, especially mental ones, are the result of being possessed or "mixed-up" by the jines. Possession is believed usually to occur by accident, but sometimes as a punishment for performing daily activities without taking ritual precautions against jinns. For example, failure to take a ritual bath after sexual activity (whether heterosexual, autoerotic or in dreams) may be a cause for a jinn to strike. The result may be aphonia, aphasia, strokes, epileptiform, reactions, sexual impotence or delusional depressions. We have observed that many traditional patients with free-floating anxiety or repetitive anxiety dreams claim that "they are frightening me", referring to jinns.

Belief in the evil-eye, and to a much lesser extent in sorcery, is common not only among the illiterate traditional population, but also among many individuals of the literate transitional and even modern societies. Many illnesses, especially unexpected diseases of children or accidents, failure in life, and physical or personality defects, are explained by reference to the evil eye. Successful, progressing, intelligent, healthy and attractive people are believed to be particularly vulnerable. Paradoxically it is sometimes believed that the eyes of the closest and dearest person may have an evil influence. For example, some people believe that mothers are not supposed to praise their children or look at them with admiration. Villagers usually hide their newborn babies from the eyes of other people in order to protect them from evil influences.

Since such supernatural powers are believed to be responsible for illness, therapeutic and preventive measure take the from of abiding by the believed requirement of these powers. Praying, following religious orders and carrying amulets are among the common precautions. The

muska, a very widely used agent, is an amulet inscribed by the folk healer for the treatment or prevention of such evils as illness, the evil eye and sorcery. Among other methods of combatting the evil eye, eye-shaped blue beads, hanging unattractive objects on the clothes of children or on valued belongings, imperfection, inconspicuousness, or hiding from the looks of other people can be mentioned.

The therapeutic agents commonly seen in the villages are the hodjas, the odjaks and the yatırs. Hodjas are Moslem priests, and in the past they were both priests and teachers. They conduct religious ceremonies in the mosques and at funerals. Not all of these hodjas act as healers, and the great majority of those who do, have no particular skill or specialized ritual. Many of them have no religious training or function, but are able to find a medium through which they introduce themselves as men of religion and wisdom, using a good deal of quackery. Their practices are rather stereotyped, in that they write muska, pray, breathe or lay their hands on patients, massage, and pray to the water and offer it as a conjured drink. These practices are usually carried on without much exploration of the patient's problem, and only a few hodjas seem to be closely and genuinely interested in understanding the individual problem.

The other group of common treatment agents besides hodjas are the odjaks and the yatırs. The odjak (literally the hearth) may be a specialized hodja, or it may be a tomb or any other place where magical influence is believed to be precent to ward off evil or illness. The yatir (the laying one) is not radically different from the odjak, except that it is the tomb of a highly honored, revered and spiritually gifted individual around whom many elaborate fables are constructed. The sick villager visits the yatır, brings gifts to the keeper of the tomb, prays there and sacrifices a rooster, a goat or a sheep.

Besides these there are other types of folk beliefs and ideas about illness, some which are based on common-sense explanations. Life's miseries, frustrations, conflicts, fatigue and loss of love or unrequited love are some of the beliefs which are not as institutionalized as the ones described above.

The homeostatic significance of magical and animistic thinking associated with such concepts as evil spirits, the evil eye, sorcery, amulets and prayers has been descibed by many writers. [16 18 19 20] More specifically, sorcery and magic have been shown to be institutionalized instruments of covert aggression. [16 18 21 22] The homeostatic significance on psychological and sociological levels of these beliefs concerning the etiology of illness or evil, lies not only in the provision of socially sanctioned means of projecting, displacing and expressing aggression, but also in reduc-

ing the degree of helplessness and insecurity through attempts at mastering the unknown, which is intensified in the presence of a deficiency in basic trust. Vague and intangible threats are rendered recognizable and concrete through belief in evil spirits, the evil eye and sorcery, in a similar way that the concretization process takes place in individual symptom formation.[23] In a world where helplessness, dependency and insecurity are felt more than security, autonomy and initiative, these beliefs "make the unfamiliar familiar, and permit the individual to say to his fears and conflicts, "I see you! I recognize you!,"[18]

In the material described above the presence and development of the lack of trust and curiosity about the human and natural environment can be observed. The autonomy of the individual is reduced. There seems to be a shift of esteem and responsibility from the self toward what is omnipotent and omniresponsible. Where there is a deficiency in one's basic trust of the self and the environment, with a diffuse attitude of passive-dependent-expectancy, such belief patterns acquire important homeostatic significance. What is not explained or provided by the human and natural environment is explained and, it is hoped, provided by faith and conviction in what lies beyond the human and the natural.

The attribution of active, aggressive, changeable and seductive characteristics to the jinns seems to parallel the need for denial and suppression of comparable needs in the villager. This observation is supported by the fact that behavior motivated by such needs, i. e. by aggressive and intrusive needs, is discouraged or punished in childhood except in certain culturally sanctioned areas. The extremely common belief in the evil eye shows almost conscious recognition of the destructive potential of looks and wishes, that is of unconscious hostility and envy. All glances, and therefore all wishes, can be destructive, but as long as one can take precautions one does not have to feel guilty about one's own eyes. This is the point where we believe that internally accumulated aggression is both denied and also suitably expressed. The belief that a demonstration of love and admiration should be preceded by a precautionary word or phrase indicates the presence of hostility behind these pleasant demonstrations. It is interesting to note that a strong denial of guilt is probably connected with this diffuse denial of aggression. When "God gives" an illness or when the jinns "strike" or take hold of a person, there is usually little or no implication of consciously accepted guilty action or fantasy. Human will and responsibility have nothing to do with human anxieties and fears.

The healing and protective principles based on the etiological agents that I have mentioned briefly contain elements converging with what we have described of the traditional villager's ego, and the conflicts therein.

On one hand, there is the passive-dependent-expectation, and on the other the need for adequate mastery of one's own ever-increasing agression. Therapeutic measures seem to take advantage of these two facts, and they may be persisting in the society for that very reason, even though their results are not always satisfactory. It has been pointed out that "the medium through which a mental cure is achieved is the psychological apparatus of the recipient"[24]. Passive-dependent-expectation is seen in the villager's attitudes towards God, the state, parental figures, folk healers and medical doctors. The faith healer's social role is actually structured by this aspect of the individual's psychology. In the case of the hodjas odjaks and yatırs, this element is automatically supplied by the expectation of the villager. It is interesting to note that, as a very intuitive Turkish writer has shown,[25] the time a folk healer emerges coincides with social and economic crises which intensify already present feelings of insecurity and helplessness, and which lead to more passive-dependent needs.

The other facet, that of accumulated aggression, is handled by therapeutic measures through reinforcement of projections, displacements, and concretization. For example, the villager who suffers from an attack of anxiety believes that he is being frightened by external forces (the jinns). He expects a cure from the hodja, who reinforces the belief that the jinns have taken hold of him. The defence-strengthening aspects of this approach is obvious. The next step is dependence on the hodja's attributed powers to fight the jinns or the evil forces.

In this presentation we have tried to show some of the socially patterned conflicts which are acquired through certain child-rearing practices, and which are reflected in beliefs and practices concerning the etiology and treatment of illness. Undoubtedly other cultural, historical, political and economic factors may be related to what we have described, but these were kept beyond the scope of our study, with full recognition that their contributions also need to be considered and explored.

Summary

In this article an attempt has been made to formulate the psychodynamic interrelations between traditionally generated and maintained inner conflicts and certain belief systems pertaining to illness. The psycho-social developmental model of Erik Erikson has been used to integrate the knowledge obtained from the studies of child-rearing and the characteristics of a traditional society. Such studies are indispensable for carrying on preventive or therapeutic work and epidemiological research in the field of mental health.

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Allergic Reactions Following Insect Bites and Their Management

A Report of Eighteen Cases of Insect Allergy

Kemal Özkaragöz, M.D. *

Insect allergy dates back to the time of King Menes of Egypt who died following a wasp sting in 2641 B. C.,[1] but the subject did not appear in medical literature until 1833 when Brandt and Ratzenburg did a study of bee venom. In 1914 Waterhouse was the first to notice the similarity of the allergic reactions to anaphylactic shock in experimental animals.

An authoritative paper written by Benson and Seminon in 1930 in which they discuss the clinical manifestations of insect stings and treat them with adrenalin, also shows the possibility of desensitization with insect extract. Numerous studies[2] [3] [4] have appeared since knowledge of immunology has been obtained in this field.

Insect allergy may range from mild to severe urticaria or mild to severe anaphylactic reactions that can progress to loss of consciousness or death. Though not frequently seen in our allergy clinic, reactions to insect stings which may lead to death make this subject universally important. In a study[5] conducted among primary schools in different communities of Ankara during the year 1966-1967, of 1,163 children studied, 235 (20.2 per cent) were found to be atopic, and six (2.5 percent) of them had insect allergy.

The aim of this paper is to emphasize the importance of insect allergy and present 18 cases seen at our allergy clinic.

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Materials and Methods

The insect allergic patients seen at Hacettepe Faculty of Medicine Allergy Clinic during the years 1964-1968 are reviewed. Eighteen cases of insect allergy were seen during this period. The age and sex of these patients and the kind of insect causing the allergic reations are summarized in Table 1.

TABLE I

AGE, SEX AND KIND OF INSECTS CAUSING ALLERGIC REACTIONS

Insect	0–5 Years old		5–15 Years old		15+ Years old	
	♂	♀	♂	♀	♂	♀
Mosquito	1	1		1	1	
Common fly	1		1			1
Bee				1	2	7
Flea			1			

Three of the patients had a history of loss of consciousness and generalized angioneuro-edema, of whom brief case reports follow:

A boy aged 12 (case 15) complained of itching and swelling for two or three years after a bee sting. The patient started to develop symptoms of generalized itching, burning and flushing with erythema - he showed significant positive reactions to scratch tests of bee extract 1/100 and 3+ to intradermal 1/100.000 concentration; he received hyposensitization treatment for 18 months. After the patient had been on this treatment for 16 months, he received another bee sting, and had only local swelling without any generalized reactions.

A male aged 32 (case 17) was stung by a honey bee on the arm and face. One hour later, he swelled all over, particularly on the head, felt dizzy and lost consciousness. After 24 hours he came to himself and was referred to the allergy clinic for treatment. The patient was tested with an extract made from honey bee, and a positive 1+ reaction was indicated by scratch tests. An intracutaneous test with 1/100,000 concentration revealed a 4+ reaction

An 83-year-old male (case 8) with a long history of previous bee stings on the arm with only local reactions, said that in the last five or six years he had completely lost consciousness following bee stings. After every sting the patient collapsed within an hour or so, and was taken to a local hospital where he remained unconscious for two days, and was treated with intravenous dextrose and adrenalin. According to his statement this had recurred four or five times in the last six years.

Results and Discussion

During the last five years we have seen 18 insect allergic patients. The clinical data, kind of insect, type of reaction and drugs used are summarized in Table 2.

The reactions of children under 10 are mostly papular urticaria (Figure 1, cases 4, 7 and 16). and are easily treated with elimination and antihistaminies. Among the 10-15 age group, reactions develop into generalized edema and urticaria (cases 1 and 3). After the age of 20, insect allergic reactions become more severe and generalized, causing difficulty in breathing and shock. The type of reaction depends also on the kind of insect. Mosquitoes, common flies and fleas, for example, mostly cause papular urticaria and angioedema (Figure 2).

After a period of local symptoms, bee stings usually result in coma or shock. Out of the 18 patients, 10 (55 per cent) had cases of bee allergy of which four resulted in shock. Two of these patients had histories of recurrent shock and coma lasting for one to 48 hours.

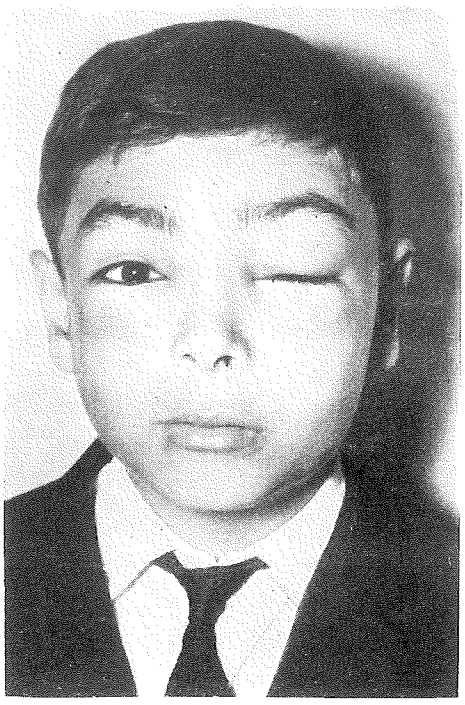

Figure 1

TABLE 2

CLINICAL DATA, TYPE OF REACTION AND DRUGS USED ON TWENTY-TWO INSECT SENSITIVE PATIENTS

Patient	Atopy	Insect	Reaction	Treatment
1 – E. S. 12.0	No	Mosquitoes	Angioedema, papular urticaria and ithing	Periaction, prednisone
2 – I. T. 22.0	No	Mosquitoes	Papular urticaria + itching, generalized erythema	Antihistamine
3 – B. Y. 13.0	Yes	Common fly	Papular urticaria + itching, angioedema	Anthistamine
4 – B. Ö. 2.0	No	Common fly and flea	Papular urticaria + itching	Antihistamine
5 – V. S. 15.0	No	Honey bee	Generalized edema, urticaria + itching, laryngeal edema, difficulty in breathing	Antihistamine, prednisone, adrenalin
6 – N. A. 38.0	Yes	Honey bee	Angioedema, generalized urticaria + itching	Antihistamine
7 – E. C. S. 4.0	Yes	Mosquitoes	Papular urticaria + itching	Antihistamine
8 – H. T. 83.0	No	Honey bee	Dyspnea, cyanosis, coma (recurrent) laryengal edema, difficulty breathing	Adrenalin, prednisone, antihistamine
9 – Ö. A. 17.0	Yes	Common fly Contact with eyes	Conjunctivitis + itching PAR, Laryngeal edema, coma, difficulty breathing	Prednisone, adrenalin
10 – G. I. 32.0	No	Honey bee	Angioedema, tachycardia + itching, vomiting, urticaria, fainting	Prednisone, antihistamine, adrenalin
11 – M. G. 53.0	No	Honey bee	Coma	Prednisone, adrenalin
12 – N. H. 25.0	No	Honey bee	Generalized edema, urticaria + itching, tachycardia, difficulty breathing	Prednisone, Antihistamine
13 – G. D. 26.0	No	Fleas, mosquitoes	Papular urticaria + itching	Antihistamine
14 – G. A. S. 35.0	No	Honey bee	Coma, urticaria, gneralized edema, dyspnea + itching	Prednisone, antihistamine
15 – C. B. 12.0	No	Honey bee	Shock, angioedema + itching	Adrenalin, antihistamine
16 – A. A. 4.0	Yes	Mosquitoes	Papular urticaria + itching	Antihistamine
17 – K. A. 32.0	No	Honey bee	Coma, difficulty breathing, generalized articaria + itching	Adrenalin, antihistamine
18 – T. O. 7.0	No	Fleas	Papular urticaria	Antihistamine

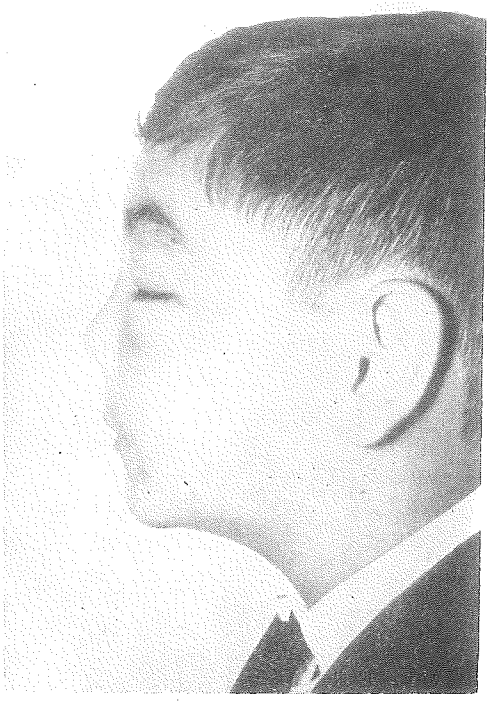

Figure 2

Local allergic reaction, urticaria and angioedema responded well to antihistamines and local cortisone ointment, but cortisone and adrenalin were necessary for systemic reactions or shock. Only two patients (cases 15 and 17) had skin testing with bee extract* diluted at 1/100,000, and both were positive. Hyposensitization treatment started with a concentration of 1/1,000,000. In two cases (Nos. 11 and 9) the patients had generalized reactions without any previous local or other reactions to stings. Once a person has been sensitive to insect stings he is likely to experience reactions of increasing severity, and occasionally, in cases of several insect stings, the results following each sting may be of equal severity. One of our patients had several stings followed by coma, but without fatal results.

Types of reactions are summarized in Table 3. Fourteen cases out of 18 had urticaria and itching. Eight had angioedema, six had difficulty in breathing, three shock and one vomiting.

* Obtained from the Hollister-Stier Laboratories

TABLE 3

PATIENTS WITH SYMPTOMS OF GENERALIZED REACTION

Type of Reaction	Number of Patients	Percentage
Urticaria	14	77.5
Itching	15	83.5
Angioedema	8	44
Difficulty in breathing	6	33
Shock	3	16.6
Vomiting	1	5.5

Allergic reactions to insect stings are similar to anaphylactic reactions. Usually when venom or any insect allergen is injected, there is no reaction from the first injection, but as the stings are repeated, delayed-type hypersensitivity develops. This is followed by immediate local reaction, and finally an immediate systemic reaction occurs. Occasionally after the second injection systemic reaction develops. Several antibodies can be produced by insect allergens.

The evidence of this hypersensitive reaction is based on the following clinical features:

1. The history of previous stings without reactions suggesting a process of sensitization;
2. The interval between the sting and the onset of symptoms;
3. The close resemblance of the symptoms to classical anaphylaxis;
4. The response to epinephrine and antihistamines.

More convincing proof of hypersensitivity mechanisms are:

1. Identification of the offending antigens in the insect;
2. Demonstration of the presence of antibodies in susceptible persons;
3. Evidence that the antigen-antibody reaction is actually operative in the production of clinical symptoms.

Practically all normal individuals experience some local reactions to toxin after a sting or bite, but it is necessary to distinguish normal reactions from hypersensitive reactions. When multiple stings have occurred, there may be generalized urticaria, dizziness, headache, fever, muscle spasms, sometimes seizures and syncope from shock.

Hypersensitivity to insects may be produced by first insect stings or by repeated sensitization; inhalation of insect dust has also been suggested.[6] The number of types of insects that cause allergy is very large, and they are found everywhere from the tropics to the frozen north. Moths, butterflies, bees, wasps, hornets, yellow jackets, flies and mosquitoes are the chief ones. Three sources of sensitizing antigen from hymenoptera have been reported: pollen[7], insect body protein,[8] and venom.[9]

It has been reported and shown that sensitizing antigen is located throughout the whole insect and not simply in the venom[10]. Reisman[11] demonstrated hemagglutination, skin sensitizing and precipitating antibodies in human serum with insect allergy. These antibodies can be demonstrated by skin testing, passive transfer skin testing, conjunctival testing, tanned-cell hemagglutination, immuno-electrophoresis, and gel diffusion. Allergic manifestation to stings may be different in adults and in children. Some children have delayed reaction consisting of swelling at the site of the sting for 24 hours, generalized urticaria, angioedema, and at times arthralgia. They may also show immediate reactions such as generalized erythema, urticaria, swelling of the eyelids, lips, cheeks, ears and hands, with occasional difficulty in breathing.

Severe general anaphylactic reactions may occasionally be seen in children. A fatal Schönlein-Henock syndrome[12] and connective tissue disease[13] have been reported.

Allergic reactions to insect stings or bites vary considerably. In 1959 Mueller[14] classified the clinical severity of sting reactions as follows:

1. Slight general reactions: Generalized urticaria, itching, malaise, anxiety;
2. General reaction: In addition to the above, generalized edema, a constricted feeling in the chest, wheezing, abdominal pain, nausea or vomiting and dizziness;
3. Severe general reaction: In addition to 1 or 2, dyspnea, dysphagia, hoarseness or thickened speech, marked weakness and confusion;

Patients who have sustained reaction to the stings of hymenoptera insects frequently show immediate wheal and flare skin reactions to hymenoptera extracts[15].

In 1967, Barnard[16] reported 50 insect sting fatalities; he stated that 70 per cent had engioedema with respiratory obstruction and that 60 per cent of those patients died within six hours, while the others died several days after the stings from delayed reactions.

If the history is not clearly known, skin testing may be performed in order to determine which insect was responsible. A regular warming for allergic skin testing is also a necessity during this process. Scratch tests should be carried out initially and followed by intracutaneous tests.

Skin testing is performed for two reasons:

1. To attempt to correlate the individual stinging insect with the clinical history;
2. To determine the reactivity of the patient.

There are different opinions and methods regarding skin testing and hyposensitization. Skin sensitivity at higher dilution occurred with much greater proportional frequency in the group with a history of other allergies than in the one without such a history. It is also a fact that negative skin tests do not rule out hypersensitivity. Some investigators prefer scratch testing first, others deny its value. Mueller[17] suggested the use of intracutanous testing with insect extracts diluted from 1:100 to 1: 100,000,000, starting with the 1:100 million in doses of 0.02 to 0.03 ml each. If a positive reaction occurs with a minimum erythema size 2.0 cm diameter, no further testing is done; if there are no reactions within 20 minutes the next lower dilution is applied.

Treatment and Management

The general treatment for acute allergic reaction is also applied to insect sting allergies; namely, elimination, hyposensitization and symptomatic treatment, though elimination is most impractical.

Symptomatic treatment

Since it takes a bee approximately two to three minutes to inject the total amount of its venom through its fine stinger, the quicker the venom sac is removed the less venom will be injected. Care should be taken not to press on the sac to prevent more venom being given. Early use of ice packs and elevation of the limbs are helpful.

Patients who have had previous reactions to insect stings are well aware of the possibility of fatal reactions and the need for rapid treatment. Death generally occurs within minutes, so that it is of vital importance that the following emergency treatment be given immediately:

1. Apply a tourniquet close to the sting area;
2. Take antihistamines orally as soon as possible;

3. Carry a syringe to inject 1 cc adrenalin diluted at 1/1000 into any area that the patient can reach easily.

The tourniquet should be loosened for a brief period every 10 minutes, or at 3-15 minute intervals in the event of severe reactions. Some, allergists advise that the patient also be instructed to swallow four 5 mgm tablets of prednisone immediately after the sting,but if possible this should be given intramuscularly or intravenously. In the event of shock, the patient should be stretched out with his head elevated and be kept warm, and general shock treatment should be given.

If patients with insect allergy are old enough, they should carry an insect-sting first aid kit containing an epinephrine hydrochloride ampule and syringe, ephedrine tablets, antihistamines and a tourniquet.

For unconsciousness and shock, 500 cc five per cent dextrose in water containing 25 mgm soludacorten, aminophylline (12 mgm per kg per day) and and an antihistamine should be promptly administered intravenously.

If a vasopressor is also indicated, levarterenol bitartrate (levophed) with epinephrine 0.5 cc in the liquid may have a similar effect.

Laryngeal obstruction may call for immediate tracheostomy, as fatalities have been known to occur from laryngospasm. If the patient survives the first half hour, the prognosis is good.

Hyposensitization

The starting concentration of hyposensitization correlates to the dilution of the skin testing materials. Usually treatment is started with 1:10,000,000 solution of insect antigen, increasing gradually until tolerance, or a maximum dose of 1.0 cc of the 1: 100 dilution, is reached. The technique and procedures of hyposensitization are essentially the same as in asthma bronchial treatment.[18]

It is generally believed that hyposensitization is quite successful. Injections may be given once or twice weekly, and treatment should continue for at least four years; one of our patients has continued for two years. The specificity of sensitization has been studied widely in order to achieve proper treatment. Many different procedures have been tried to determine which allergens are common to a number of insects and which are specific to a single one. Polyvalent extracts of the whole bodies of mixed bees, wasps, yellow jackets and hornets are used.

Loveless[19] in 1956 demonstrated that all venom contains some common antigen. For the treatment of unknown insects where others are not feasible, Loveless preferred yellow jacket extract alone, since it contains the common antigens.

Ninety per cent of sting-sensitive patients improved after hyposensitization treatment.[20 21] If a child has shown a generalized reaction to a hymenoptera sting, whether mild or severe, hyposensitization is mandatory. A history of repeated stings with progressively larger local reactions emphatically indicates a program of hyposensitization.

Preventive measures

In 1943, Shannon[22] reported that thiamine hydrochloride was effective against most mosquitoes.

Perlman[23] reported that doses of 150 mgm thiamine hydrochloride daily was also effective against fleas. Mueller[24] found that of 100 insect sensitive patients, 70 per cent seldom or never had ill effects from a daily dose of 75-150 mgm of thiamine hydrochloride.

Summary

Eighteen insect allergic patients seen in the Allergy Clinic of Hacettepe Faculty of Medicine during the last five years are reported.

A generalized reaction is most likely to occur in the over five age group. A short case history of three patients with anaphylactic shock following insect stings is presented. The treatment of insect allergy is tabulated, the importance of prophylactic and emergency measures are emphasized, and the literature regarding types of insects, antigens and reactions following insect stings or bites is briefly reviewed.

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An Electron Histochemical Study: Distribution of Adenosine Triphosphatase Activities in the Rat Heart at Neutral and Alkaline pH Levels

Meral Uysal, M.D.*

Introduction

With the introduction of electron microscopy techniques in the field of histoenzymology in 1960, various enzymes in tissues could be identified in a clear and descriptive manner, thus marking a new phase of research in this direction. As a result, it has been possible to observe not only the coarse distribution of enzymes *in situ*, but also their delicate interleations with the subcellular elements.

Electron microscopy techniques have been applied particularly in the histoenzymological investigation of some esterases, namely phosphatases. Research on the adenosine triphosphatases in the tissues at electron microscopy level has gained significance during recent years. The activities of this enzyme display a very interesting morphological distribution in all the tissues, especially in the muscular tissue where the existence of at least two adenosine triphosphatases with different functional characteristics at neutral and alkaline pH levels has been quite clearly seen. The adenoise triphosphate activities in the skeletal and

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heart muscles at different pH levels have been studied by several authors using various methods. However, the results obtained are not in full agreement. Studies on the complex details of the subcellular structure of striated muscle cells are still in progrress.

In this study an attempt is made to investigate adenosine triphosphatase activities in the rat heart at neutral and alkaline pH levels and to clarify the interrelations between the different morphological localizations of the enzyme and its functions.

Materials and Methods

In our experiments the Wistar species of adult male albino rats, weighing 350 grams each, were used. While the rat was under nembutal narcosis the thorax was cut open, and the heart was extirpated and put in a physiological solution. After the blood had been cleaned off, it was placed in fixatives of 4 per cent and 6 per cent glutaraldehyde solutions which were freshly prepared one day prior to the experiment. These were buffered with phosphate solutions to pH 7.15-7.25 (Beckman R pH meter) and preserved at O°–4°. C The phosphate buffer was prepared in the following manner:

Stock I: 0.2 M aqueous solution of mono sodium phosphate ($Na H_2 PO_4$), 27.585 gr/100 cc distilled water.

Stock II: 0.2 M aqueous solution of di sodium phosphate Na_2HPO_4), 28.38 gr/100 cc distilled water.

(All weights were measured by an analytical Sartorius balance).

39.0 cc from Stock I and 61.0 cc from Stock II were obtained in order to get an approximately neutral pH, and water was added to increase the volume to 200 cc. When necessary acid or alkaline was added so as to achieve a value of 7.15-7.25 on the pH meter. When the whole heart was in 4 per cent fixative, pieces from the left atrium, interatrial septum, interventricular septum and those obtained from the points adjacent to the right and left valves, were divided into two groups. One group was placed in 6 per cent fixative while the other remained in the 4 percent fixative. All the pieces were carefully sliced into 1 mm^3 blocks with very sharp blades, and prepared for electron microscope studies. The pieces in the 4 per cent fixative were termed Group I, and those in the 6 per cent fixative Group II, and different but parallel procedures were applied to both groups at the same time.

First they were all fixed at O°–4° C for half an hour in their respective fixatives. They were then washed three times for half an hour in a 7.5 per cent sucrose solution, buffered with phosphate to pH 7.25, and kept at O°–4° C. The pieces in Group I were incubated in a neutral medium of 6.9-7.1 pH. Substrate and some metallic ions were used for the precipitation of enzymatic activity products in order to establish the enzymatic activity in tissues in neutral pH. The method used in this study has been termed the Magnesium-Lead Method because magnesium is used as the activator and trapper, and lead as the precipitator. The incubation medium was mainly prepared in accordance with Wachstein-Meisel's principles as follows:

Stock I: 0.2 M tris buffer, 2.4228 gr /100 cc. aqueous solution.

Stock II: 0.1 M Magnesium sulphate, 2,4650 gr /100 cc aqueous solution.

Stock III: Adenosine triphosphate, 0.125 gr /100 cc, aqueous solution. (Adenosine-5-triphosphate, pure, Fulka, Buchs, SG)

Stock IV: Lead nitrate (concentration dropped by half), 1 gr/ 100 cc aqueous solution.

Two hours before the experiment, 20 cc from Stock I,5 cc from Stock II, 20 cc from Stock III and 3 cc from Stock IV at O°C were mixed together with 2 cc of distilled water for the incubation medium. Another medium was prepared without adding adenosine triphosphate and used as a control. The pH of each was adjusted to 6.99-7.15 using 0.1 M HCI solution. The mixture was regulated to 37°C in an oven, and Group I was subjected to incubation at 37°C for one hour. The tissue pieces were placed in small tightly-closed tubes containing the main and the control media, and attached to the rotary mixer at 25 revolutions per minute. Incubation in the rotary tubes lasted for one hour, during which the solutions were renewed once.

The pieces in the main and control groups were washed four times in 15 minutes with distilled water following incubation. A mixture of two volumes of glutaraldehyde and three volumes of osmium tetroxyde (normally used in our institution) was applied for postfixation. Although this mixture is generally buffered with S-collidin, the phosphate buffer at pH 6.99-7.15 was preferred so as to maintain uniformity in the use of buffers at different stages. The main and control groups were subjected to a second fixation at O°–4°C for one hour in the above mentioned fixaitve, and were then washed three times in 15 minutes with distilled water.

The tissues in Group II were incubated in an alkaline medium at a pH of 9.25 – 9.45. Substrate and some metallic ions were used for the precipitation of enzymatic activity products to determine the enzymatic activity, as in the case of neutral pH. This time the Calcium-Cobalt Method was used in accordance with Gomori's principle, in which calcium is the activator and trapper, and cobalt the precipitator. The medium was prepared as follows:

Stock I: 0.1 M Sodium barbiturate, 2.062 gr /100 cc, aqueous solution.

Stock II: 0.18 M Calcium chloride, 1.998 gr / 100 cc, aqueous solution.

Stock III: Adenosine triphosphate (powder).

The stock solutions were prepared one day prior to experimentation at O°–4°C, and placed in the oven at 37°C two hours before the experiment. Adenosine triphosphate was regulated at room temperature. Before the incubation of the pieces, the medium was prepared with 20 cc fom Stock I, 10 cc from Stock II, 152 mgr from Stock III with an addition of 30 cc distilled water, and the same medium free from subsrate was prepared for control. The pH in both cases adjusted to 9.24 – 9.45 using 0.1 M NaOH. The tissue pieces were then placed in the substrate and control media, and the same procedures were applied to these as in the case of the Group I pieces. The tubes were rotated in the oven for one hour at 37°C, they were then washed three times in 15 minutes in one per cent calcium chloride, and following this they were placed in two per cent cobalt chloride for 15 minutes. They were washed two or three times in 15 minutes in distilled water, buffered with phosphate, and then fixed for the second time in a modified osmium tetroxyde glutaraldehyde mixture at O°–4°C, just like the Group I pieces. Later on they were washed three times for a total of one hour in distilled water buffered with phosphate.

The Group I and Group II pieces at this stage were thus prepared for the electron microscopic studies usually carried out at this institute. They were first dehydrated in graded alcohols and then embedded in araldite in the usual manner. After this, they were placed in OO gelatine capsules (Eli Lilly and Co., Indianapolis, U.S.A.), processed for sectioning, so that silver-tone sections could be obtained using a diamond knife (du Pond 44°–45°) at the Porter Blum MTI ultramicrotome. They were carefully examined under a Carl Zeiss EM 9 Type electron microscope, and electron micrographs were obtained using Agfa Agepe and Gevaert films.

Observations

Observations on tissue sections of blocks incubated in neutral and alkaline pH media, first containing substrate and then free from substrate, for both main and control purposes, were made through a comparison of all the findings.

Tissue sections of blocks incubated in neutral pH media:

Enzymatic activity was only observed in the heart muscle cells, and opaque and dense mitochondria filled up with activity products were noted at first sight in these cells. The outer membranes and inner cristae of the mitochondria were masked with precipitations (Figure 1). Dense

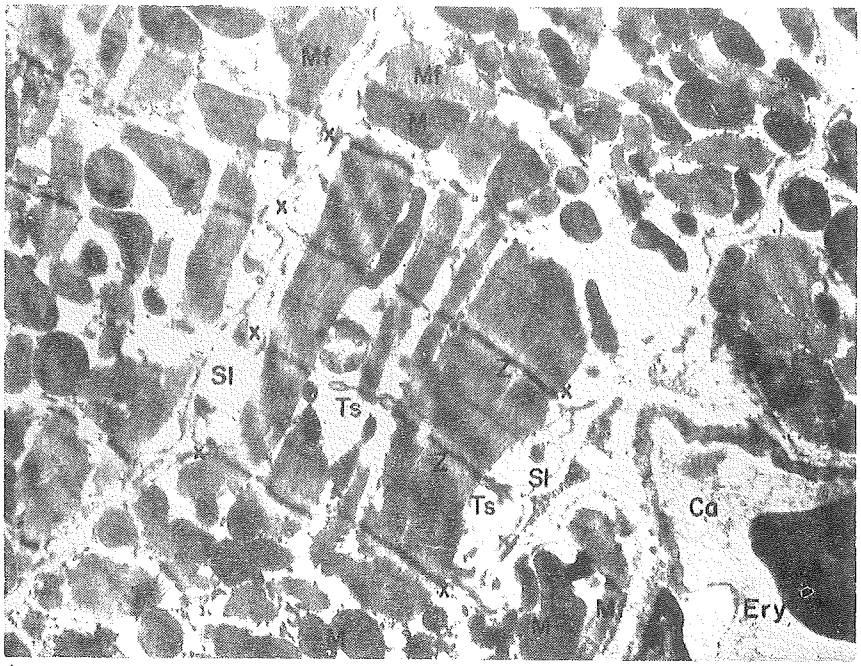

Figure 1: ATP'ase activity in atrial region of rat heart at pH 7.2. Prefixed in 4 % glutaraldehyde buffered with phosphate to pH. 7.2, enzyme determined by Magnesium-Lead Method and postfixed in modified glutaraldehyde-osmium tetroxyde mixture. Greater part of heart muscle cell is in center of micrograph, slightly oblique. Adjacent to it, is a capillary wall, R. and another heart muscle cell L. Cell in center contains main organelles. Enzymatic activity is seen as dark thickenings on sarcolemma (Sl), especially at Z band levels (Z) where T systems (Ts) continued with sarcolemma (X). Enzymatic activity is seen in the sarcomere, condensed at Z band region. Numerous mitochondria (M), particulary at upper and lower sites of cell, are dense and homogenously filled with precipitations of enzymatic activity products. Bundles of myofilaments (Mf) can be distinguished by filamentous structures in longitudinal and transverse sections. Capillary wall (Ca), (lower right of micrograph) containing erythrocyte (Ery) in its lumen exhibits no positive enzymatic activity. X 8500

enzymatic activity cumulations were only seen on the Z band along the sarcomere. The Z bands were observed to maintain the same homogenous and dense activity cumulations both in the case of contracted sarcomeres, the I bands of which had disappeared, (Figures 1,2, and 3) and those of relaxed sarcomeres with clearly-visible I bands (Figures 4 and 5). No

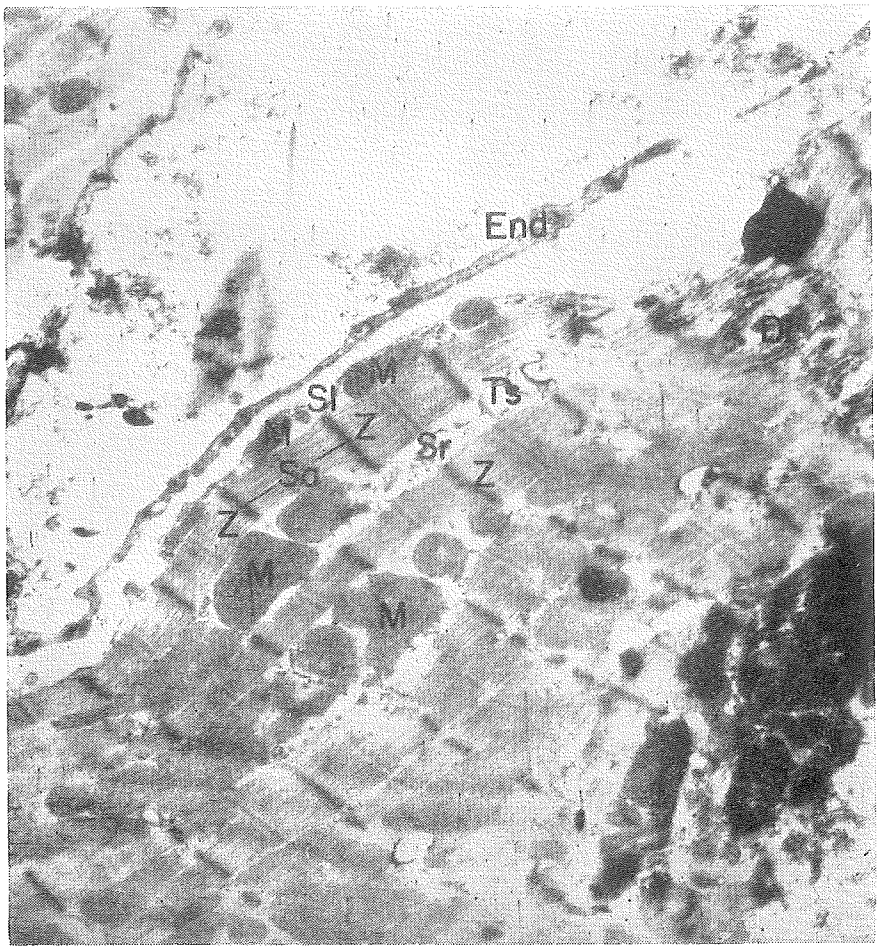

Figure 2: ATP'ase activity in atrial region of rat heart at pH 7.2. (Technique as in Fig. 1.). A part of a heart muscle cell which has contracted sarcomeres (Sa). Enzymatic activity is condensed over Z band (Z). Little subssarcolemmal (Sl) and large internal mitochondria (M) have intensely positive enzymatic activity products in form of dense precipitations. In sarcoplasmic reticulum (Sr) enzymatic activity can only be seen in T system (Ts). In course of discuss intercalaris precipitations are seen displaying enzymatic activity. Capillary endothelium (End) is free from precipitations, suggesting positive enzymatic activity. X 8500.

enzymatic activity could be seen in the longitudinal tubuli of the sarcoplasmic reticulum, but it was positively determined in the vesicles appearing as sections of the T system at Z band level, and in the regions (marked "X" in Figure 1) of the T systems extending towards the sarcolemma. In areas which were far from the transverse system, it was impossible to observe any precipitation.

Figure 3: ATP'ase activity in atrial region of rat heart at pH 7.2 (Technique as in Fig. 1). Micrograph shows interior of heart muscle cell with contracted sarcomeres (Sa). Enzymatic activity is condensed along Z band (Z). Dense cumulation of precipitations caused by enzymatic activity in mitochondria (M) and on the walls of T system (Ts) is seen in more detail at higher magnification X 90. 000.

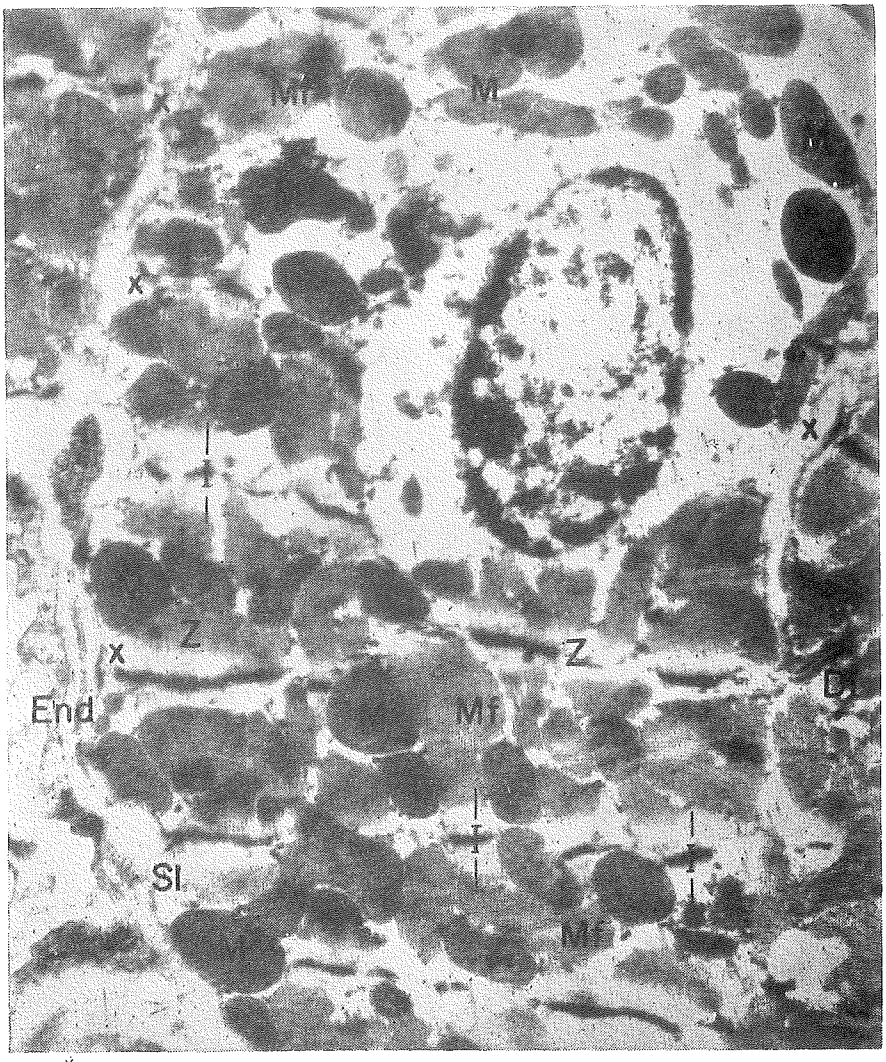

Figure 4: ATP'ase activity in atrioventricular septum of rat heart at pH. 7.2. (Technique as in Fig. 1). Part of a heart muscle cell with nucleus, sarcomeres are in relaxed position. I region (I) is seen. Enzymatic activity precipitations are condensed along Z bands (Z). Sarcolemma (Sl) exhibits positive enzymatic activity, especially at Z band levels corresponding to T systems (X). Positive activity is seen in course of discuss intercalaris (DI). Vascular endothelium (End) is free from enzymatic activity. Mf; myofilaments, N; nucleus, X 24 000.

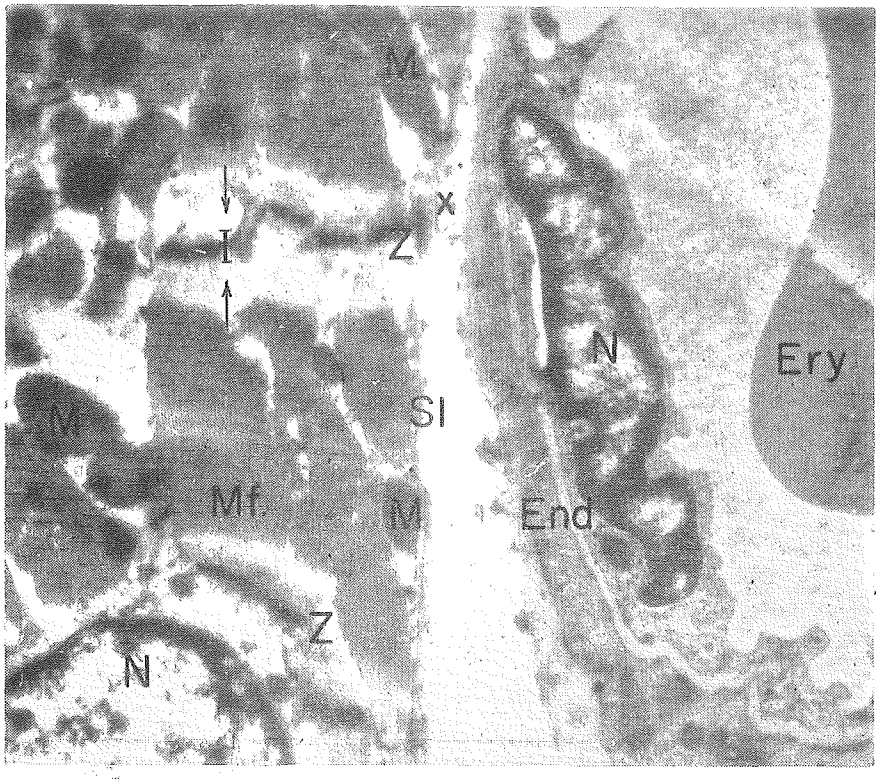

Figure 5: ATP'ase activity in the internal region of rat heart at pH 7.2 (Technique as in Fig. 1). In relaxed sarcomere enzymatic activity precipitations are cumulated on Z band (Z) region as seen in contracted sarcomere. Cell membrane regions corresponding to Z band levels (X) are more clearly determined by positive enzymatic activity at this magnification. Mitochondria (M) in different sites of cell have enzymatic activity. Capillary endothelium (End) and Erythrocyte (Ery) are free from enzymatic activity products. I; I band, N; nucleus, Mf; myofilaments. X 30 000

There was no enzymatic activity in any cells with the exception of the heart muscle cell. No precipitation could be noticed in the cytoplasms of the endothelial cells lining the lumens of the capillaries, the larger vessels, or the endocardium. Furthermore, the erythrocyte observed in the lumen of the capillary was found to be free of enzymatic activity (Figures 1 and 5).

In the tissue sections from blocks incubated in neutral medium free from substrate for control purposes, all the above-mentioned enzymatic activity precipitations were found not to have formed at all. The outer membranes and inner cristae of the mitochondria could be clearly dis-

tinguished, and were completely free from precipitations (Figures 6,7,8, and 10). As observed in the case of tissue sections of blocks incubated in a medium containing substrate, no enzymatic activity could be determined in the control, in the endothelial lining of the walls of the vessels and the endocardium (Figures 7 and 9).

Sections of tissue blocks incubated in alkaline pH media:

It was observed that enzymatic activity generally occured outside the heart muscle cell. In the cytoplasms of the endothelial cells lining the inner walls of the endocardium, the capillaries and the larger vessels, an intensive enzymatic activity in the form of rod-like and small granular

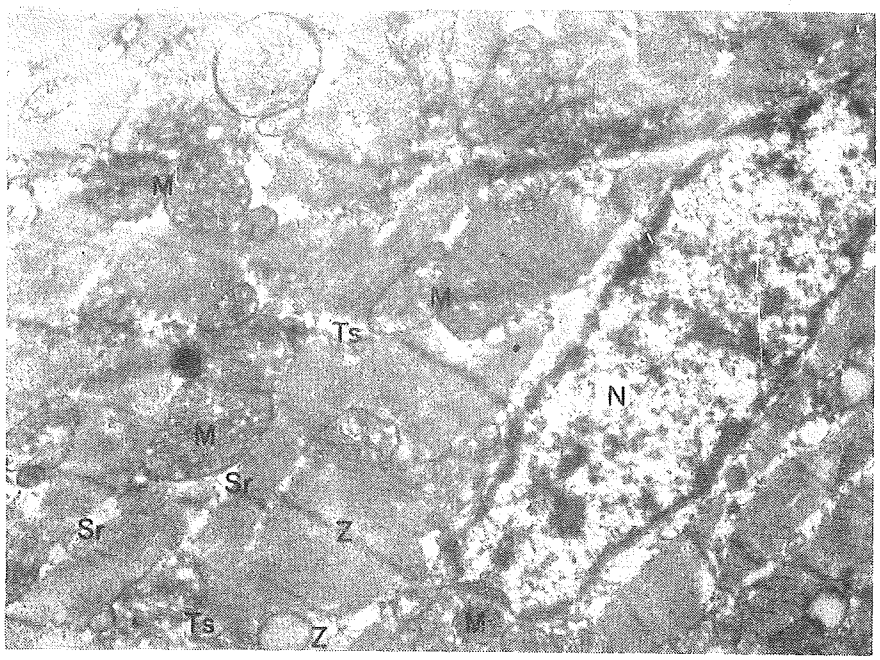

Figure 6: ATP'ase activity in atrial region of rat heart at pH 7.2 CONTROL. Prefixed in 4 % glutaraldehyde buffered with phosphate to 7.2, enzyme determined with-Magnesium-Lead Method using incubation medium without ATP, postfixed in modified osmium tetroxyde-gluteraldehyde mixture. Nucleus and perinuclear region of heart muscle cell is considered. Adjacent to nucleus, myofibrils and mitochondria (M) in between them are seen. Neither Z bands (Z) of contracted myofibrils nor mitochondria have precipitations to suggest enzymatic activity. Outer membranes and inner cristae of mitochondria are seen with no enzymatic activity. As compared with previous micrographs Z bands are less dens in appearance. Enzymatic activity products cannot be seen in longitudinal (Sr) and transverse (Ts) components of sarcoplasmic reticulum. Li, Lipid granules.X 24 000.

Figure 7: ATP'ase activity in atrial region of rat heart at pH 7.2. CONTROL. (Technique as in Fig. 6). A part of heart muscle cell adjacent to a capillary. No enzymatic activity is noticed throughou sarcomere (Sa) or in mitochondria (M). The endothelium (End) containing erythrocyte (Ery) have no precipitation suggesting enzymatic activity. X 24 000.

cobalt salt precipitations were abundant (Figures 11 and 12). Contrary to the homogenous and opaque appearance of lead precipitations in neutral pH, the cobalt salt precipitations were observed as particles with distinctive outlines. Enzymatic activity could clearly be noted in all the endothelial cells, particularly throughout the cell membranes and in the periphery of the micropinocytotic vesicles (Figure 13). The erythrocyte in the capillaries were all surrounded by enzymatic activity bands (Figures 11 and 12).

It was observed that abundant enzymatic activity precipitations occurred in both the inner and outer sides of the membrane faces of the heart muscle cell from place to place in close connection with the functional situation (Figure 14). While the sarcolemma of some heart muscle cells adjacent to the subendocardial connective tissue displayed a positive enzymatic activity, others showed no such precipitations, and this was identified as evidence of the close interrelation betwen the morphology and the functional situation (Figures 15 and 16). The activity precipita-

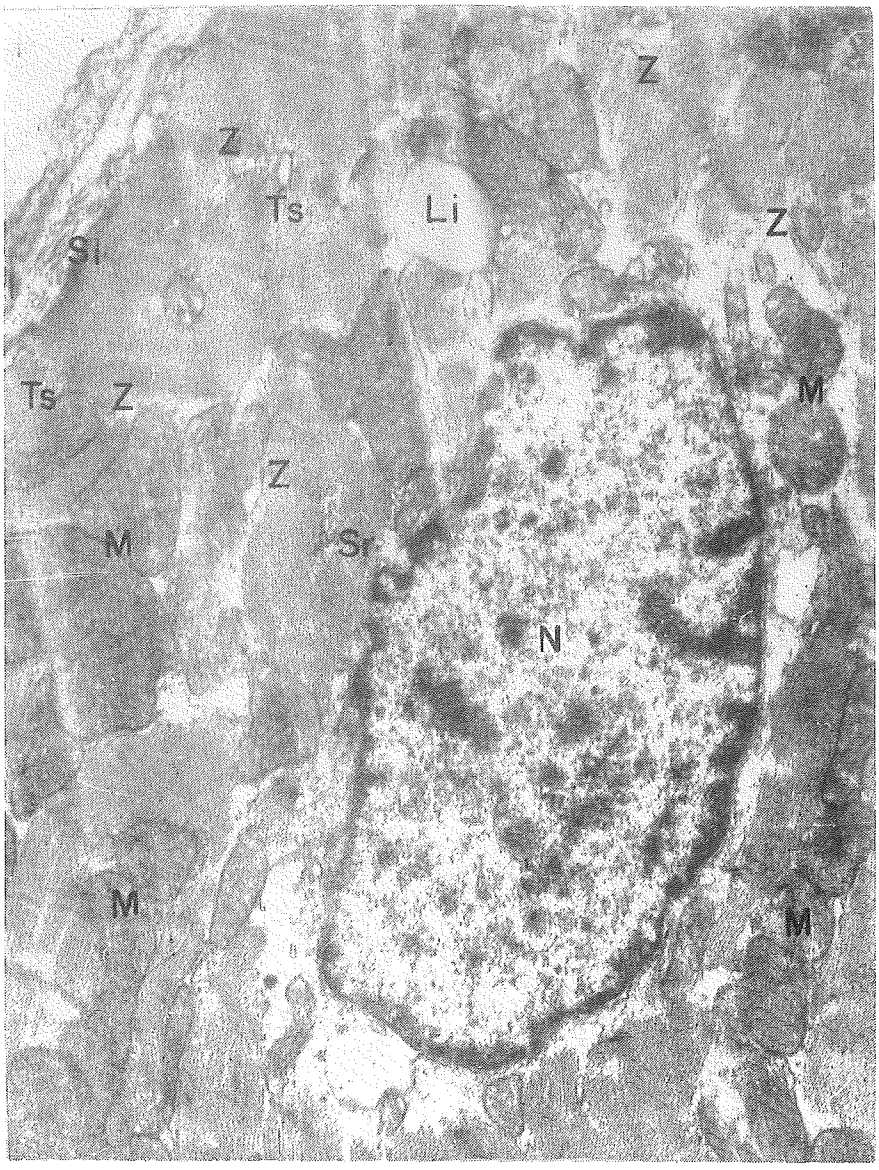

Figure 8: ATP'ase activity in atriventricular septum of rat heart at pH 7.2. CONTROL. (Technique as in Fig. 6). Large portion of heart muscle cell with nucleus, perinuclear and peripheral zones of cytoplasm with all main organelles can be seen in this micrograph. Adjacent to periphery of cell a capillary is observed. No enzymatic activity is seen at Z Band (Z) levels, transverse (Ts) or longitudinal (Sr) elements of sarcoplasmic reticulum and along the sarcolemma (Sl). Membranes of mitochondria are free from precipitations displaying enzymatic activity. Li, Lipid granule. X 24 000.

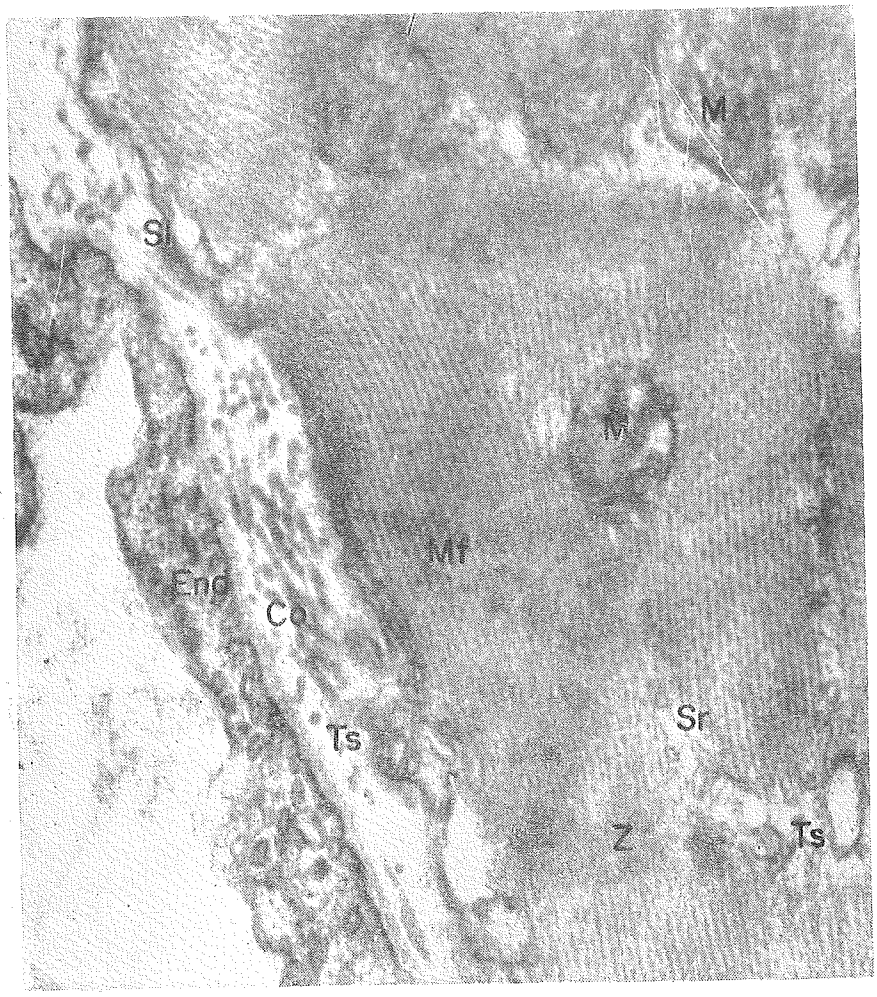

Figure 9: Further magnification of Fig. 8. In contracted sarcomere no activity product is seen at Z band (Z) level. Activity is not observed both in transverse (Ts) and longitudinal (Sr) elements of sarcoplasmic reticulum. Sarcolemma (SI) and mitochondrial membranes (M) are free from precipitations. No activity is seen in vascular endothelium (End). Mf; myofilaments; Co; collagen. X 150.000.

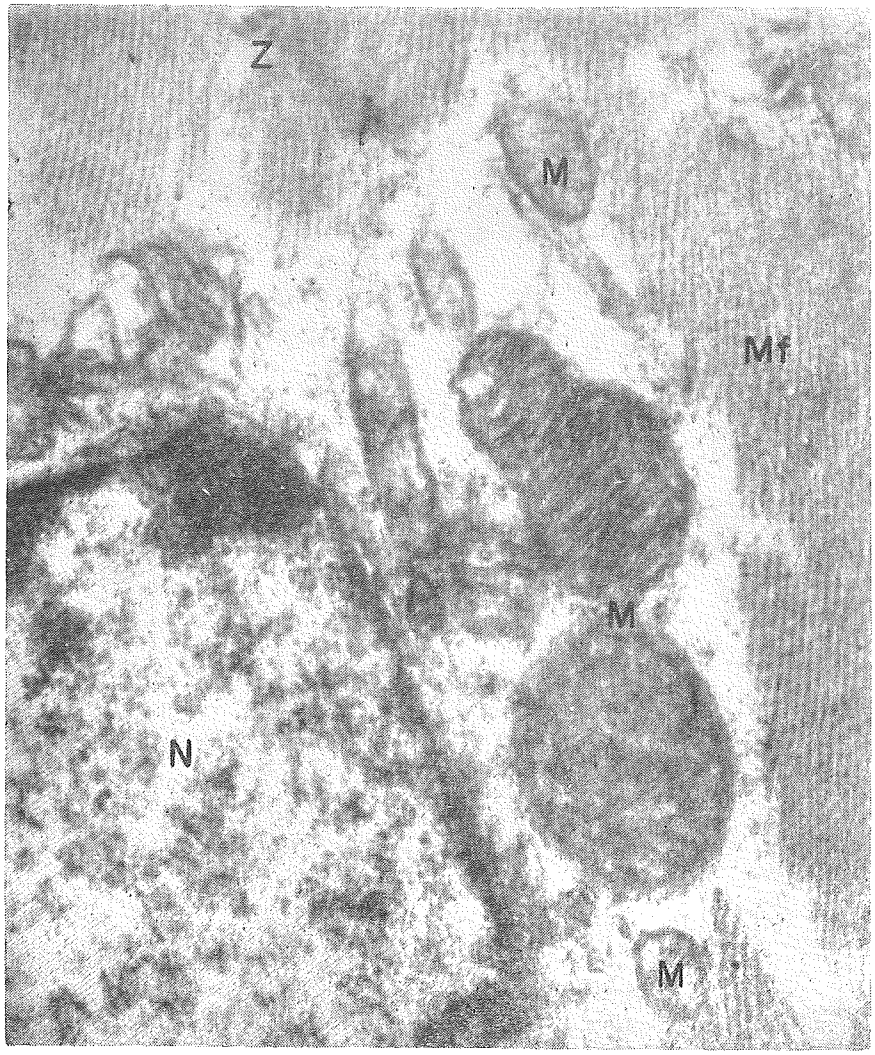

Figure 10: Magnification of another part of Figure 8. Mitochondria (M) in close periphery of heart muscle cell nucleus(N) exhibit no enzymatic activity product. The clearly visible longitudinal and transverse sections of internal cristae of mitochondria reveal no precipitation between them. Even in contracted sarcomere, Z bands (Z) are pale and free from products. Mf; myofilaments. X 150 000

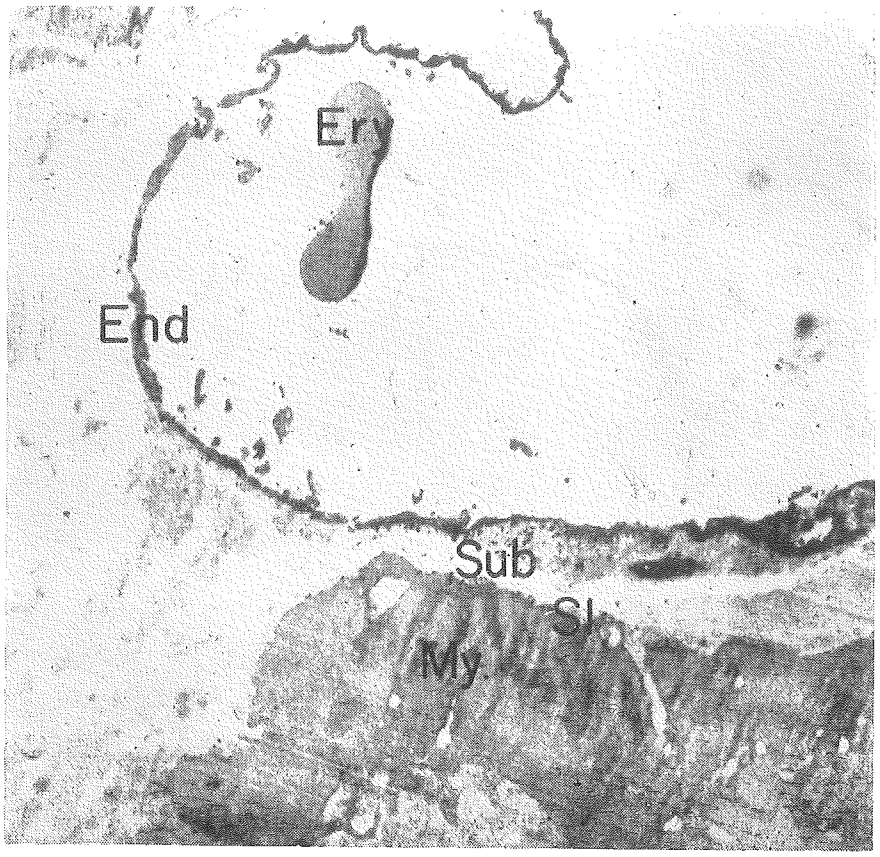

Figure 11: ATP'ase activity in auricular region of rat heart at pH. 9.4. Prefixed in 6 % glutaraldehyde buffered with phosphate to pH 7.2, enzyme determined with Calsium-Cobalt Method and postfixed in modified osmium tetroxyde-glutaral dehyde mixture. In endothelial cells (End) lining endocardium, marked positive enzymatic activity is seen. Products of enzymatic activity have diffused slightly into subendocardial region (Sub). Erythocyte (Ery) in lumen, punctuated with activity precipitations throught course of membrane at the periphery of cell can be noticed. Enzymatic activity is positive throughout the course of sarcolemma (SI) of heart muscle cell (My) beneath endocardium, giving morphological appearence of ionic transportation. No enzymatic activity is seen in the inner side of heart muscle cell. X 6.500

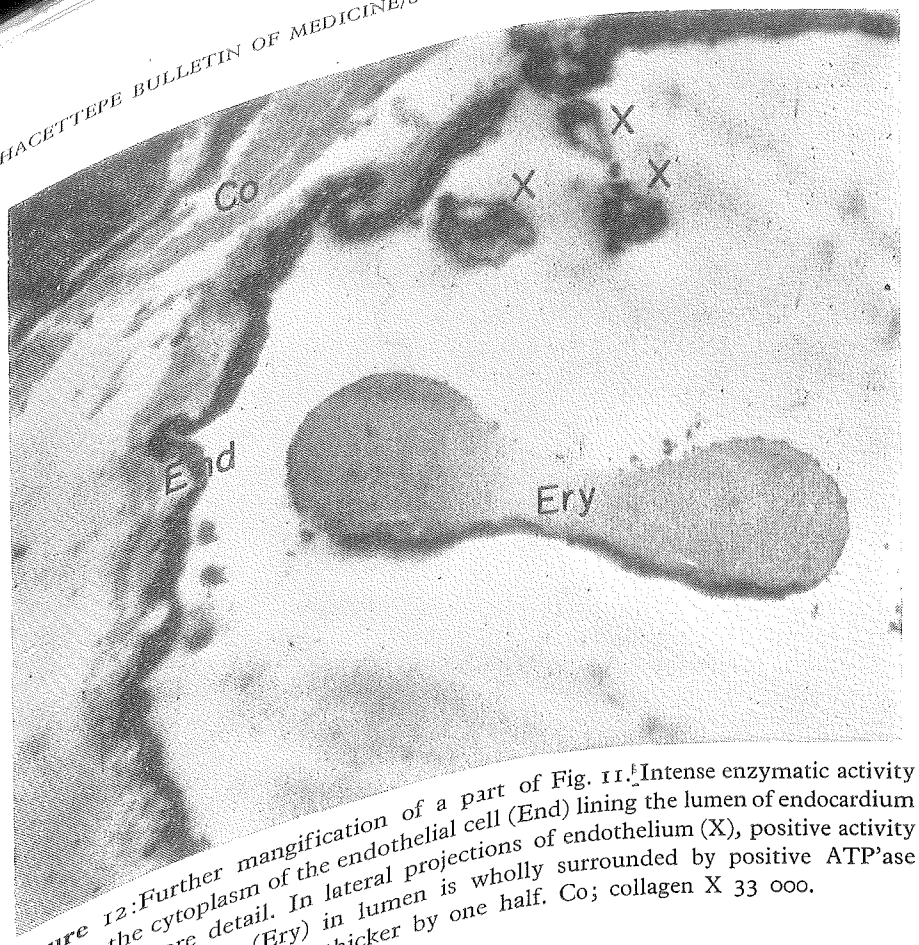

Figure 12: Further mangification of a part of Fig. 11. Intense enzymatic activity found in the cytoplasm of the endothelial cell (End) lining the lumen of endocardium observed in more detail. In lateral projections of endothelium (X), positive activity is seen. Erythrocyte (Ery) in lumen is wholly surrounded by positive ATP'ase activity band at periphery, thicker by one half. Co; collagen X 33 000.

tions in the membrane systems were observed to diffuse outwards to some extent, (Figures 14 and 15).

With the exception of the vesicles of the transverse system, enzymatic activity could neither be observed in the mitochondria nor along the sarcomeres of the muscle cell. In other words, the Z bands displayed no precipitation (Figures 14 and 16). However, occasional enzymatic activity was clearly noted in the T system vesicles (Figure 17).

The control sections from the blocks incubated at alkaline pH level in a medium free from subtrate did not exhibit any enzymatic precipitation in the cytoplasms of the endothelial cells lining the inner faces of the endocardium and the vessels, or at the periphery of the erythrocyte found in the lumens of the endocardium. Furthermore, no enzymatic activity could be observed in any of the organelles of the heart muscle cells (Figures 18 and 19).

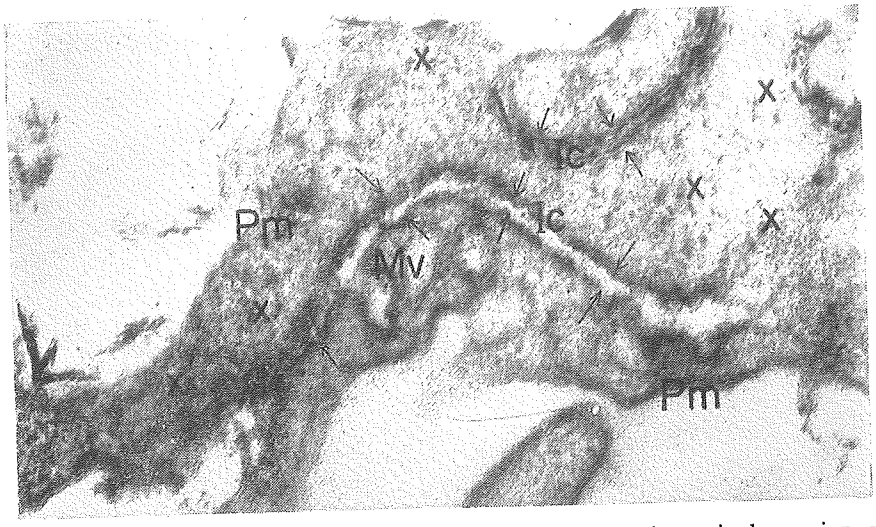

Figure 13: ATP'ase activity in endothelium of capillary in auricular region of rat heart at pH 9.4. (Technique as in Fig. 11). Tiny rod shaped precipitations resulting from enzymatic activity are seen between cells (Ic) and attached to cell membranes (Fm) throughout their courses and in micropinocytotic vesicles (Mv) beneath cell membranes. Enzymatic activity products can be noticed as granular precipitations (X) scattered all through interior of cytoplasms of endothelial cells. X 150. 000.

Discussion

Of the nucleoside phosphates, adenosine triphosphate is biochemically split up in a unique manner, catalyzed by adenosine triphosphatase, a highly substrate specific enzyme which is also specific to the tertiary phosphate bound. Therefore, the tertiary phosphate is broken off and, as a result, high energy (8000 calories) is freed by each molecule. The enzymatic splitting of adenosine triphosphate occurs continually in all the tissues of the organism. The energy is used in the synthesis of proteins, lipids and carbohydrates, and such mechanical processes as cell division and muscle contraction,[1] active transportation [1-3] conducting of nerve impluses [2-4] or as direct heat enegry. The enzymatic catalysis constitutes the most important point in the metabolic chain.

Many studies have been made on the biochemical properties of this enzyme, using developed chemical analyzing methods applied to tissue components. In this way, the functions, types and optimal pHs and the place in the metabolic steps of the enzyme have been clearly identified[5 6]. But it is impossible to argue this from the histochemical viewpoint. Re-

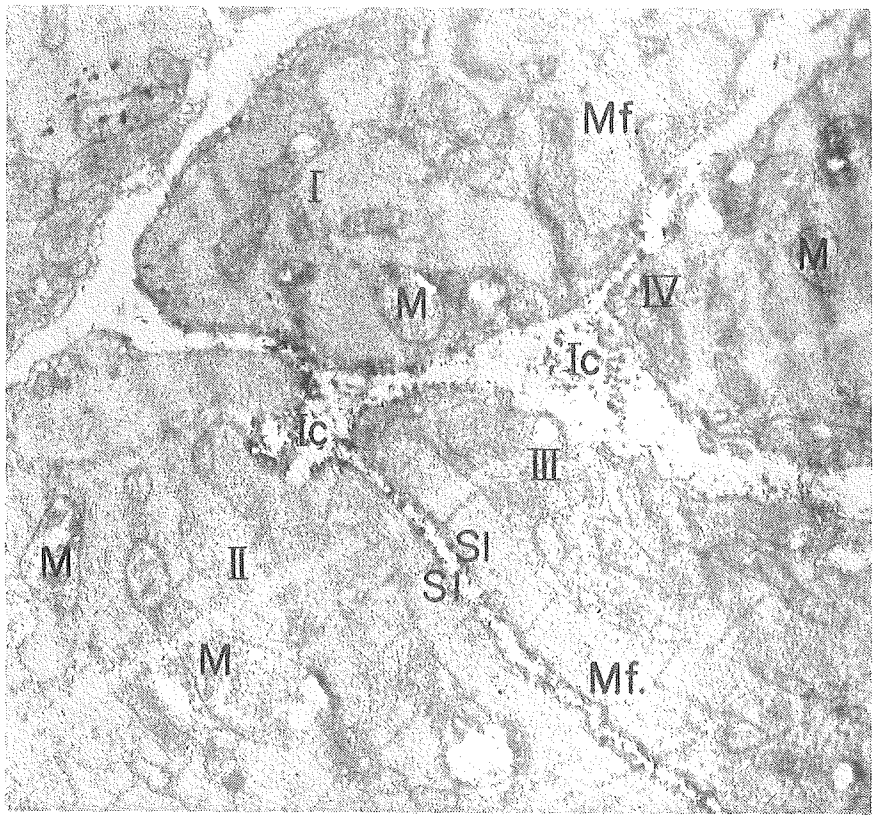

Figure 14: ATP'ase activity in deep regions of atrioventricular septum of rat heart at pH 9.4. (Technique as in Fig. 11). Numerous precipitations of positive ATP'ase activity are seen between (Ic) the four heart muscle cells (I, II, III, IV). Activity products are seen attached to inner and outher faces of sarcolemma (SI). There is considerable diffusion of enzymatic activity products in loose connective tissue among cells I, II, III. No activity is seen in mitochondria (M) or in the course of myofilaments(Mf) of all cells. X 24 000.

search on the morphological localization of adenosine triphosphatase in tissues and cells dates back only 15 years, and on tissues at light microscopy level followed by histochemical studies under the electron microscope about five years. An endeavour has been made to establish the localization of the enzyme and its interrelations within the cell.

The first important step in determining the histochemical localization of adenosine triphosphatase in the striated muscle cell at light microscopy level was taken in 1955 by Padykula and Herman,[7] [8] who made two studies using adult albino rats as material. They studied the enzymatic

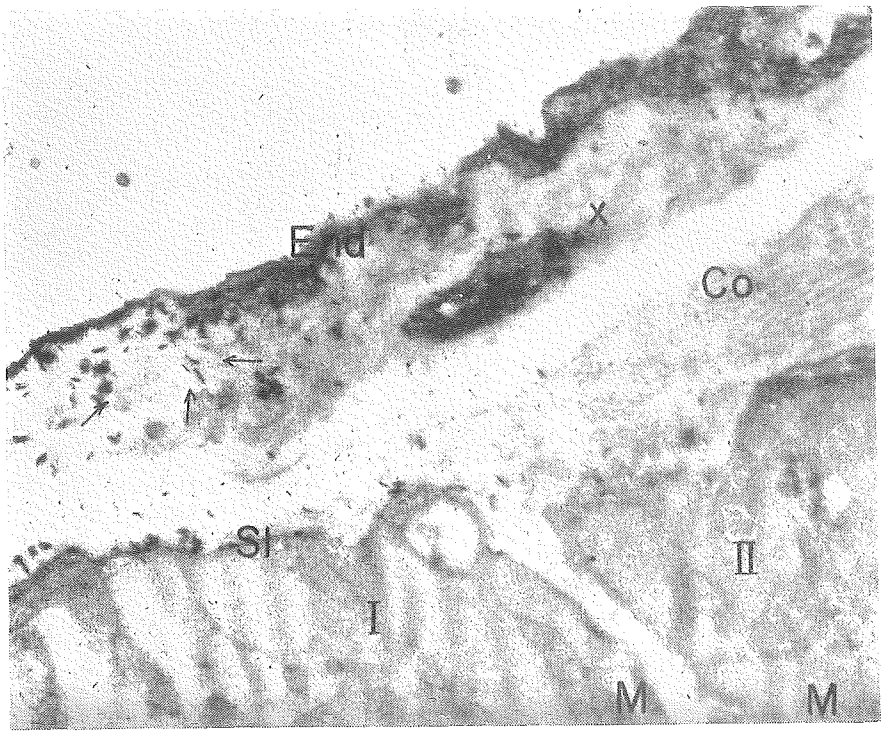

Figure. 15 ATP'ase activity in atrioventricular septum near valvular region at pH 9.4. (Technique as in Fig. 11). Enzymatic activity is positive in endothelial cells (End) lining inner face of endocardium and in subendocardial projections (X) of same cells. Moderate diffusion of enzymatic activity products is seen between collagen fibrils(Co) in subendothelial region (short arrows). Two heart muscle cells are placed beneath subendothelial layer (I, II). While the sarcolemma (SI) of the lst display positive activity products along its course, the 2nd does not owing to stages of metabolic transportation. This clarifies the close connection between morphology and functions. X 24 000.

activity in the rat heart and limb muscles, as well as various other tissues, and considered different methods for the precipitation of adenosine triphosphatase activity products. They used different metallic salts, and chemical elements effecting the enzyme as inhibitor and activator and tried to find optimum pH and temperature, testing every possible alternative. The work of these authors continued at light microscopy level to find the distribution of adenosine triphosphatase in the striated muscles, using normal electron microscopic sections for indirect comparison only. [9-10]

The greatest difficulty in determining the enzymes in tissues morphologically arises from the fact that well-preserved tissue sections must

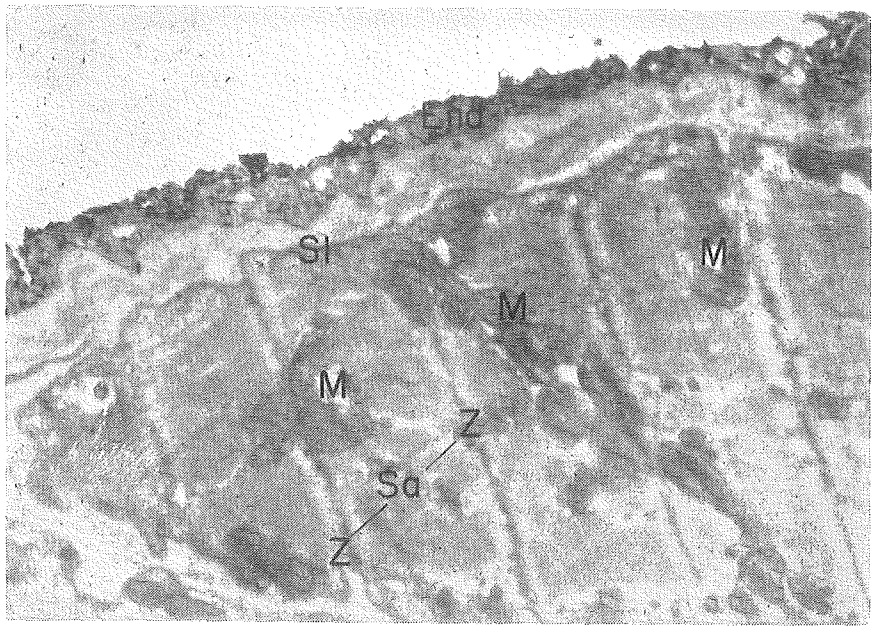

Figure 16. ATP'ase activity in atrial region of rat heart at pH 9.4. (Technique as in Fig. 11). Numerous rod-like precipitations of intesnsely positive ATP'ase activity are seen in endothelial cells (End) lining inner surface of endocardium. Enzymatic activity is negative in mitochondria (M) and sarcomeres(Sa) of heart muscle cell, partly seen beneath endocardium. X 24 000.

be obtained without harming their enzymatic activity. Although fixation is necessary for a satisfactory observation of tissue morphology, the fixative may greatly inhibit the enzymes in the tissues, sometimes so much so that sufficient enzymatic activity precipitation cannot be obtained. On the other hand, if no fixation is carried out the tissue is spoiled and a detailed study of the interrelation between the enzyme and the tissue components cannot be made. Besides, well-preserved tissue sections should be obtained for studies at molecular level through fixation which is necessary for electron microscopic research.[3 11-13]

Various aldehydes have been tested in morphological and enzymatic morphological studies at electron microscopy level, and it has been argued that glutaraldehyde is the best fixative [14-19]. Glutaraldehyde was therefore, used in this study, and it had a greater effect [16-17] as inhibitor on the active enzyme at neutral pH; consequently a 4 per cent concentration was applied in the case of tissues to be incubated at neutral media and a 6 per cent solution before incubation in alkaline media.

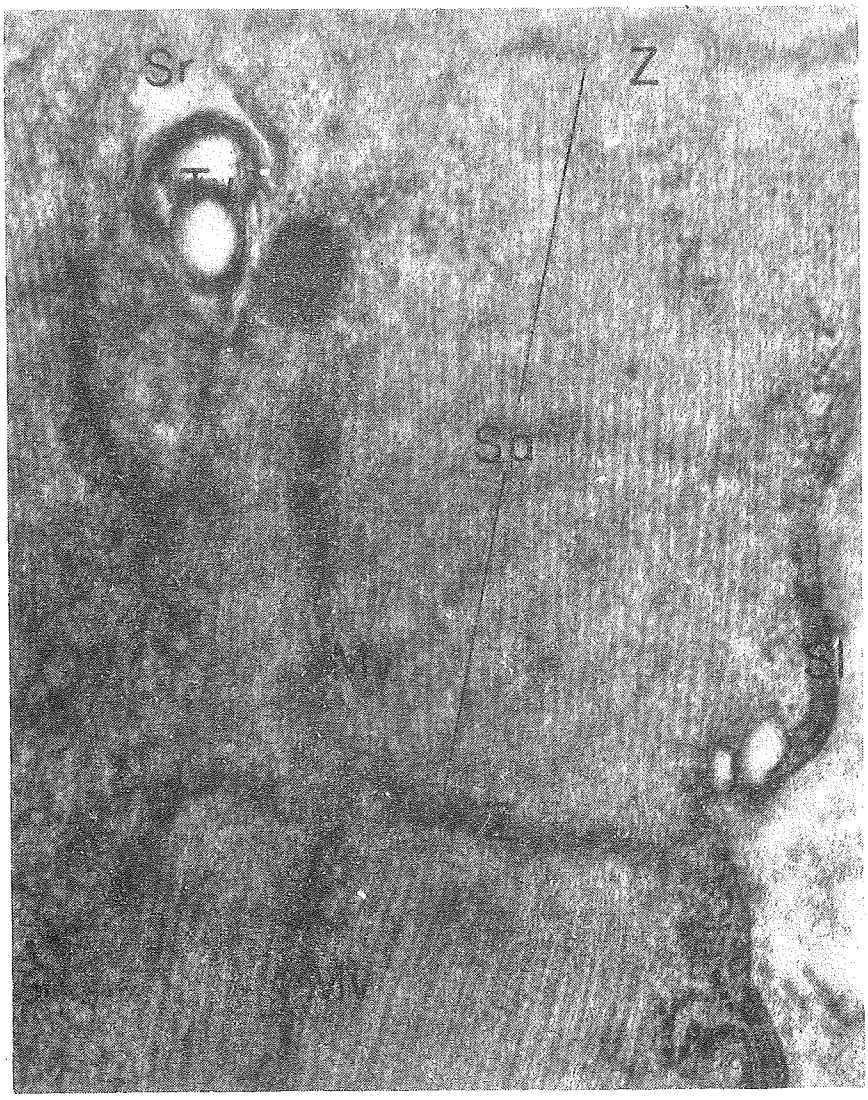

Figure 17. ATP'ase activity in atrial region of rat heart at pH 9.4. (Technique as in Fig. 11). Positive enzymatic activity can be seen only in vesicles of transverse system (Ts), and in micropinocytotic vesicles (Mv) attached to inner surface of sarcolemma (SI) throughout its length. Sarcomeres (Sa) are free from activity. Z; Z band. X 125 000

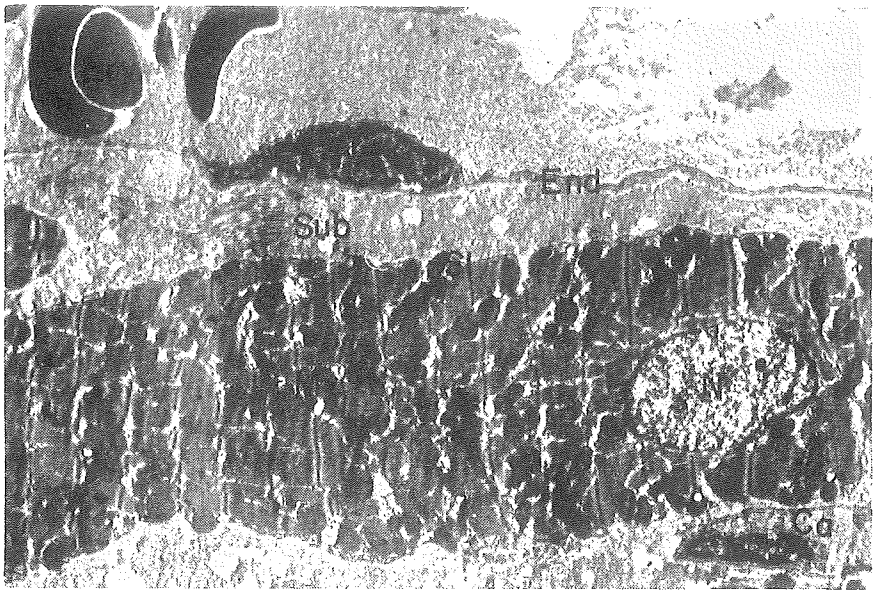

Figure 18. ATP'sae activity in atrial region of rat heart at pH 9.4. CONTROL. Prefixed in 6 % glutaraldehyde buffered with phosphate to Ph 7.2, enzyme determined with Calcium-Cobalt Method using medium free from adenosine triphosphate, postfixed in modified osmium tetroxyde-glutaraldehyde mixture. No enzymatic activity is seen in squamous endothelial cells (End) lining inner surface of endocardium, in subendothelial loose connective tissue (Sub) or in orgenalles of heart muscle cell (My) beneath endocardium. Lower R. of micrograph a capillary (Ca) is seen with no endothelial enzymatic activity. Erythrocytes (Ery) in its lumen are free from peripheral activity band. SI. sarcolemma. X 6 500.

Following the first fixation, the material was washed in cold and buffered sucrose [13 20 21]. As the substrate adequately penetrated the tissue in this way, cold sucrose washing was applied three times for a considerable duration.

In selecting the neutral and alkaline incubation media, the findings of all the research made were taken into consideration [3 13-15 2130]. Accordingly, the use of metallic cations for the precipitation of enzymatic activity products was found appropriate for this study. The main chemical procedure is that tertiary phosphate anions released from the adenosine triphosphate of the medium as a result of enzymatic activity, are trapped by the metallic cations in the tissue. The metallic phosphate salts are deposited in the sites where enzymatic activity exists in the form of dense precipitations.

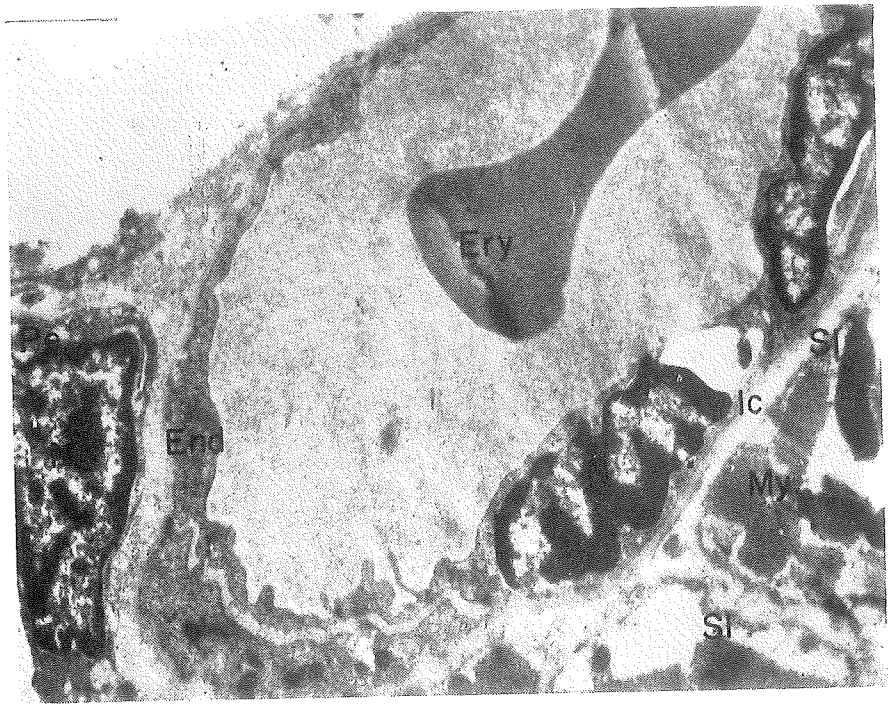

Figure 19: ATP'ase activity in atrial region of rat heart at pH 9.4. CONTROL (Technique as in Fig. 18). Showing transverse section of capillary and pericyte (Pe). No activity product can be seen in endothelium (End), lining capillary lumen or in organelles of heart muscle cell (My) at lower R, or in narrow space between endothelium and heart muscle cell. SI; sarcolemma, Ery; erythrocyte, N; nucleus. X 30 000.

The neutral media were prepared according to Wachstein-Meisel's method, modified by dropping the lead salt concentration by half [31-33]. Gomori's techniques, in which the enzymatic activity at neutral pH is considerably inhibited by the calcium cations, were applied in full in the preparation of the alkaline media [6-8]. Thus, two different methods were used in studying the enzymatic activity at two different pHs in the same tissue. Fixation and incubation were applied on 50 microns thick cryostate sections and tiny 1 mm3 tissue blocks [14 24 36-38] Tissue blocks were preferred in this study and yielded as good a result as the cryostate sections.

One per cent solutions of osmium tetroxyde adjusted to neutral pH using different buffers were generally applied for the second fixation following incubation.[16 17]. The mixture of osmium tetroxyde and glutaraldehyde described above was preferred in the postfixation, as extremely

well-preserved tissues and satisfactory contrasts were maintained. In all steps in the preparation of tissues, phosphate was used as the only buffer for keeping the homogenity of all the chemical solutions in which the tissues were processed.

The distribution of adenosine triphosphatase activity in the rat heart has not been studied as a whole, though various authors have made studies on it using different methods, but each considering the distribution at one pH step only. A careful study of the results found in the literature shows that two main kinds of adenosine triphosphatase exist in all tissues [9 14 24 34 35 39-54].

The optimal pH of adenosine triphosphatase, Type I, is between 6.9 and 7.8, and is activated by magnesium cations. Sodium and potassium cations increase its activity, and the enzyme maintains its activation at 17--37° C, while it is fully inhibited by calcium cations. The tertiary phosphate bounds in the adenosine triphosphate and inosine triphosphate are hydrolyzed by the enzyme, this is called "real adenosine triphosphatase" in the literature[20 21 35 52 55-63]. With the chemical elements giving the sulphydryl groups such as PCMB (parachloromercurybenzioate) and L-cystein and the excess of lead cations, Type I is inhibited [33 56 59].

The optimal pH of adenosine triphosphatase, Type II, varies between 9.2 and 9.8, and is activated by calcium cations. It hydrolyzes the phosphate bound both in adenosine triphosphate and inosine triphosphate, and in cytosine triphosphate, beta glycerophosphate, adenosine monophosphate and inosine dishosphate, and is active only at 37°C. It is not inactivated by the chemical components which give the sulphydryl groups, and is therefore a more widespread type of aenosine triphosphatase[8 12 20 27 43 64 65].

In the sections incubated in appropriate media at neutral pH, it was found in this study that Type I, or real adenosine triphosphatase activity, was limited to the organelles of the heart muscle cell, i.e., at the Z band evels of the sarcomeres along the myofibrils, in the mitochondria, the transverse system of the sarcoplasmic reticulum adjacent to the Z band and sites of the sarcolemma extending towards the tubuli of the T system.

There exists no unanimous agreement on the location of adenosine triphosphatase activity in the sarcomere along the myofibrils, and the precise interrelations between adenosine triphosphatase, myosine and actin filaments have not thus far been brought to light morphologically. Research has shown that there is positive enzymatic activity on the A

Band [8 34 39], in the A-I junction [15 41 43 66] on the Z band and on both sides of the latter. [47 66-68]

The present writer believes that these different findings must have resulted from the various methods used. Tice and Barnett[34] who used the homogenates of heart muscle myofibrils as material, located the enzymatic activity on the A band, but stressed that its interrelations with the myofilaments could not definitely be established. In this author's opinion fractionation methods are not suitable for the subjection of tissues to histochemical treatment. It is possible that the precipitations on the A band might have resulted from vigorous procedures when the enzyme spread from its real site towards the A band. In well-preserved tissue sections the enzymatic activity is located on the Z band, or adjacent sites,66-68 and this is supported by our findings.

Various authors have visualized the enzymatic activity precipitations in different shapes, sometimes as coarse and homogenous cumulations and at others as fine granules or rod-like particles. The formation of precipitations may be effected at all steps of the techniques applied. [20 21 56 69].

In our opinion the *in situ* forms of precipitations are characterized by the molecular structures of the metallic salts, and the molecular structure of the tissue itself should also be noted. In this study lead salt precipitations at neutral pH were observed as homogenous and opaque cumulations and alkaline precipitations in the form of fine rod-like particles and granules.

Little electron histochemical research has been made on the determination of adenosine triphosphatase in the mitochondria of the muscle, but the enzymatic activity has been visualized as a few cristalloid precipitation in the mitochondria.[33 35 51 54 63] In our study abundant opaque precipitations of enzymatic activity were established in the mitochondria.

The findings on the localization of adenosine triphosphatase in the sarcoplasmic reticulum of the striated muscle cells are contradictory. While some authors have observed that enzymatic activity was present all along the tubuli of the sarcoplasmic reticulum [10 35 68 70] others have stated that positive activity existed only in the transverse tubuli,[14 24 53 60] and occasional positive activity has also been observed just beneath the sarcolemma[53 60]. No description has been written of any histochemical study of adenosine triphosphatase in the sarcoplasmic reticulum of the heart muscle cell at electron microscopy level. Ultrastructural studies on the detailed morphology of the sarcoplasmic reticula of the

striated skeletal and heart muscle cells of various animal species are still being made. [71-95] According to the data in all the literature, the tubular system of the endoplasmic reticulum consists of two different systems in the embryological period: The longitudinal tubuli, originate from the smooth-surfaced vesicles in the sarcoplasm, and the T system develops with the invagination on the sarcolemma into the sarcoplasm. In the developed heart muscle cell the longitudinal tubuli overlie the A bands having a prevailing longitudinal orientation, but anastomose freely with each other, extending between the Z bands. The transverse sarcoplasmic reticulum is made of flattened tubuli running transversely across the myofibrils in close proximity to the terminal vesicles of the longitudinal tubuli at the Z band level (Diagram 1). The transverse and longitudinal tubuli never have direct joint continuation, but have a triple structural complex, called the triads of the muscle cell[71 72 74 59-93]. These data

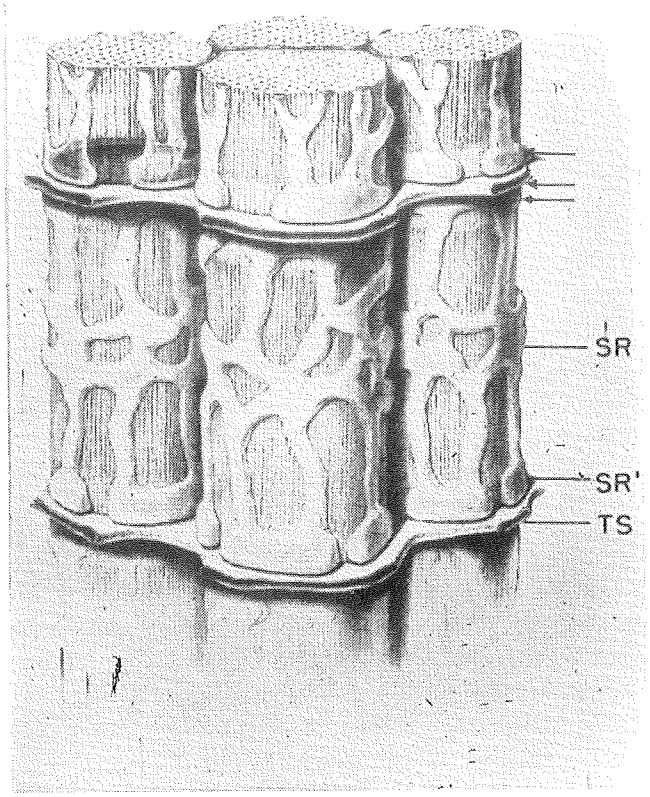

Diagram 1: Arrangement of longitudinal and transverse tubuli of endoplasmic reticulum in muscle cell. Sr.; longitudinal tubuli, Sr; enlarged endings of longitudinal tubuli adjacent to transverse system, Ts; transverse tubuli, Triad region is shown by arrows (from Porter and Bonneville).

explain why in our study the positive enzymatic activity precipitations were only visualized in the T system tubuli as separate components.

The three main organelles of the heart muscle cell in which we observed adenosine triphosphatase activity were the myofilaments, mitochondria and the sarcoplasmic reticulum elements which bring about and regulate the contraction and relaxation phenomena through their close functional collaboration. This functional coupling is achieved by the inner structure of the sarcomeres (Diagram 2) based on the "sliding filaments theory" described by Huxley[1].

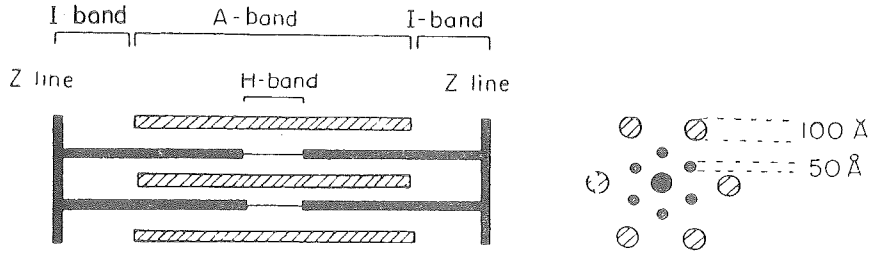

Diagram 2: Three dimentional order of action and myosin filaments in the sarcomere (from de Robertis, Nowinsky and Saez).

The energy required for contraction is supported by the enzymatic hydrolysis of adenosine triphosphatase in the mitochondria. Therefore visualization of abundant enzymatic activity in the mitochondria in this study agrees with Huxley's theory. However, the role of the sarcoplasmic reticulum in the contraction-relaxation coupling is also quite important. The simultaneous activation of the sarcolemma and the deep myofibrils by the stimulation of the sarcolemma can be accomplished by the tubuli of the transverse system, which supports our visualization of positive enzymatic activity in the T system.

Consequently, while the contraction and relaxation phenomena follow each other in the sarcomere, adenosine triphosphatase continuously hydrolyzes the tertiary phosphate bound of adenosine triphosphate and then links up again. In the light of this, the enzymatic precipitations, positively found at neutral pH on the Z band in the sarcomere and the related organelles such as the mitochondria, the T system and its continuation up to the sarcolemma, constitute the real adenosine triphosphatase. The precipitations at alkaline pH are located in the endothelia of the endocardium and the vessels related to active transportation, forming a chain in general metabolism. The enzyme does not insist on any specific sub-

trate, namely, the general adenosine triphosphatase, but acts on various substrates [7 8 20 21 35 52 58 59 63 96].

Specific and general adenosine triphosphatases have been located in various tissues in the organism, generally at light microscopy levels. Enzymatic activity has been studied in the intestines, [97 98] the liver, [13 29 36 49 97 99] the pancreas, [99 100] the kidney, [31 97 101] the skin, [1 37 102] the spermium, [103] the nervous system [4 25 104] the ciliated epithelia,[105] the thymus,[22] the parathyroid,[26] the thyroid[106], the erythocytes,[107] the bone marrow,[61] and in embryonal and tumoral tissues,[108 110].

Summary

By the electron histochemical methods and techniques used in this study it was possible to establish that adenosine triphosphatase activities at neutral and alkaline pH levels in the rat heart are located in sites with different morphological set-ups.

Positive enzymatic activity at neutral pH level is limited to the heart muscle cell, and is found on the Z bands along the sarcomeres, in the mitochondria and the transverse tubuli of the sarcoplasmic reticulum adjacent to the Z band at the same level. As these organelles bring about the contraction and relaxation coupling through their functional collaboration, the positive activity in all has been termed "the real adenosine triphosphatase activity" relating to the specific function of the heart muscle cell.

Activity at alkaline pH level changes its morphological localization, becoming positive in the endothelia of the endocardium, the vessels and in the periphery of the erythrocytes Owing to the in *situ* localization of the activity at the alkaline pH level, this enzyme has been considered to have a role in the active transportation of ions, which forms a chain in the general metabolism.

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Multiple Cardiac Pacemaker Complications in a Patient With Heart Block

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The use of pacemaker generated electrical stimuli has become a generally accepted method for the treatment of complete and intermittent, heart block. Zoll,[1] Chardack[2] and Kantrowitz[3] pioneered this field, and countless others followed. This lifesaving treatment can fail at times, and dangerous complications may occasionally occur with both endocardial and myocardial electrodes, and with any type of generator. The correction of dysfunctions of endocardial electrodes is rather easy, but myocardial electrodes are difficult to handle, because they require open thoracotomy. Today complications of pacemakers are readily detected and corrected early enough to prevent death in most instances,[4] because of this, patients with pacemakers usually die from other causes not directly related to electrical pacing.

In this paper we will summarize pacemaker and present a case of complications.

These can be classified as follows:

Generator Dysfunction

Pulse generator failure

Runaway with or without ventricular response

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Electrode Malfunction

Fracture of the electrode

Cardiac perforation

Threshold elevation

Electrode malposition or dislodgement.

Other Complications
Pulse Qenerotor Failure

The longevity of pacemaker pulse generator has been difficult to establish. Fixed rate generators have generally been more satisfactory than more complicated adjustable rate and amplitude or atrial synchronous models. Earlier claims by manufactures of five years' life have not been realized so far. In our experience and that of others most generators last about two years,[4-6] and atrial synchronous models have even shorter lives. Cessation of pacing has occurred very frequently despite efforts to detect impending, as well as actual, failure. With improved models the incidence of early failure of the generators has been on the decline.[7]

Runaway Pacemakers

This complication can occur in two ways: firstly by decreasing in amplitude as the rate increases, so that the myocardium fails to respond, and complete heart block returns; secondly, the ventricles respond to the stimulation, and a ventricular tachycardia ensues. This second type is more dangerous than the first, and may lead to death;[5] a return of heart block can be better tolerated by the patient.

Fracture of the Electrodes

Any kind of electrode may fracture; in this case the stimulation of the myocardium is no longer possible, and heart block returns. The fracture may be in the wires or at the tip of the electrode, as documented in our case report below (Figure 6). Another complication of this type is the oxidation of the myocardial end of the electrode, preventing electrical stimulus from being transmitted into the myocardium. This also occurred in our patient three months after the operation on first implantation, and we have not been able to find a similar incident in the literature.

Perforation

Another electrode complication is the perforation of the myocardium, as has been seen rather frequently by other authors [5 8 9] and in one of our patients. This occurs with endocardial catheter electrodes. Fortu-

nately it rarely causes hemopericardium or threatens the patient's life, as the catheter is quite easily repositioned in the ventricle by simply pulling it back. Some such perforations are probably not even recognized if the pacing continues.

Threshold Elevation

Threshold elevation may be caused by either histological changes at the electrode contact area, or by dislodgement of the catheter. In the latter case, the catheter tip floats in the ventricle or the atrium, or it may pass beyond the pulmonic valve into the pulmonary artery. This increases the distance between the electrode tip and the myocardium. In the former instance dysfunction can be corrected by increasing the pacemaker out put amplitude, but a dislodged catheter should be repositioned. Thresh old elevation, which occurred in our patient's case, may be due to the character of the catheter tip.[5 10]

Electrode Ejection or Malposition

In earlier series, catheter electrodes were positioned at the right ventricular output near the pulmonic valve. The tip can then pass the pulmonic valve into the pulmonary artery or float back into the right atrium causing the cessation of pacing.[5 11] The latter was observed once in our patient, and corrected by careful repositioning of the catheter into the apical trabecular area.

Other Complications

These include infections[4 11] which are infrequently seen, competitive rhythms and pacemaker induced arrhythmias, diaghragmatic irration, interference with other electrical equipment, and radio frequency pacemaker malfunctions.[5]

Competitive rhythms and pacemaker induced arrhythmias may cause ventricular tachycardia and fibrillation[7 11-13] which can lead to death. However, in a large series this could not be seriously incriminated.[5]

Diaphragmatic irritation is mostly caused by perforating catheter electrodes, but myocardial implanted electrodes may also cause this by irritating the phrenic nerve which lies on the pericardium.[11 14] We saw this complication resulting in troublesome singultus in one of our patients.

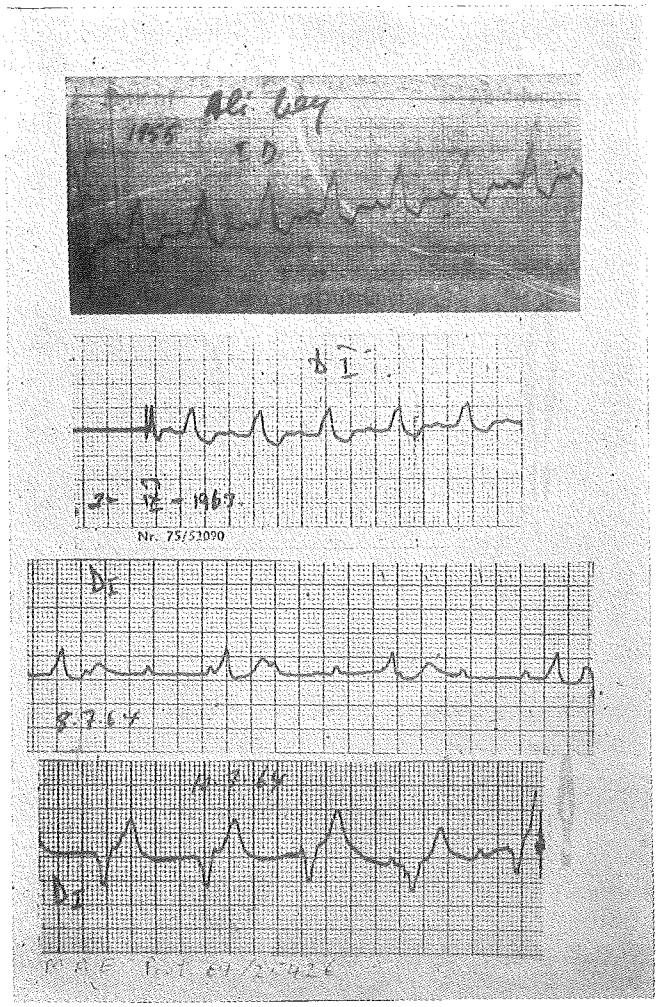
It has been reported that cessation of the pacemaker stimuli occurred during a transurethral prostatectomy, while the cutting edge of the cautery was being used.[15] Another problem arises when an electrical DC shock needs to be given to a patient with an implanted pacemaker.[16] Application of diathermy directly over the pacemaker genarator may also be hazardous,[7] and pacemakers should therefore be carefully protected against other electrical impulses.

Case Report

M. A. E., Case No. 64/25426, a 68-year-old male, gave a history of fainting spells starting in 1955, repeated once or twice a year until 1961. He had none between 1961 and 1963, then one in 1963 and two in 1964. Another occurred in May 1964, at which time he noticed for the first time that his pulse rate was down to 30; thereafter it remained between 30 and 40. We first saw the patient on June 30, 1964. Electrocardiogram revealed a complete A-V heart block with a ventricular rate of 30 per minute; the atrial rate was 90. Previous electrocardiograms taken elsewhere showed that he had NSR with left bundle branch block (LBBB) (Figure: 1-A) in 1955, first degree A-V block with LBBB (Figure 1-B) in 1963, and NSR with LBBB on March 16, 1964 (Figure 1-C).

A-V block and the heart rate were not affected by medical treatment, and so an electrodyne pacemaker with myocardial electrodes was implanted. As soon as the electrodes were connected to the generator the myocardium started to contract with each pacemaker stimulus at 70 per minute (Figure 1-D).

The patient returned on October 26, 1964 with a pulse rate of 40 per minute stating that he had had several fainting fits during the previous four days. There was no pacemaker activity on the electrocardiogram, and complete A-V block was found to be present with a ventricular rate of only 40 per minute. There was no breakage in the system; the generator was explored and found to be potent. A thoracotomy was then performed which revealed that the needle tip of one of the electrodes was dark in appearance, and that a dark brown material came off upon cleaning with a sponge. After this, the electrode transmitted the generator originated stimuli to the myocardium. As one cannot be sure that this complication will not occur again in the future, we decided to change the whole system, and a Medtronic Chardack-Greatbatch pacemaker with myocardial electrodes was implanted. The heart immediately started to be driven by this new pacemaker system, beating at 72 per minute (Figure 2). Next day a bronchoscopy was required for an atelectasis, resulting in satisfactory expansion of the lungs.



Figures 1:

On follow-up studies, the patient seemed to be progressing satisfactorily until June 12, 1967, when he returned saying that he had had influenza 10 days previously, and noticed that his pulse rate dropped to 30. Examination confirmed this, and an electrocardiogram showed a pacemaker activity of 28 per minute with corresponding ventricular response; there were also ventricular escape contractions following 1.7 seconds after the pacemaker stimuli. In some instances occasional non-conducted pacer stimuli were seen, because they fell into the refractory period of pre-

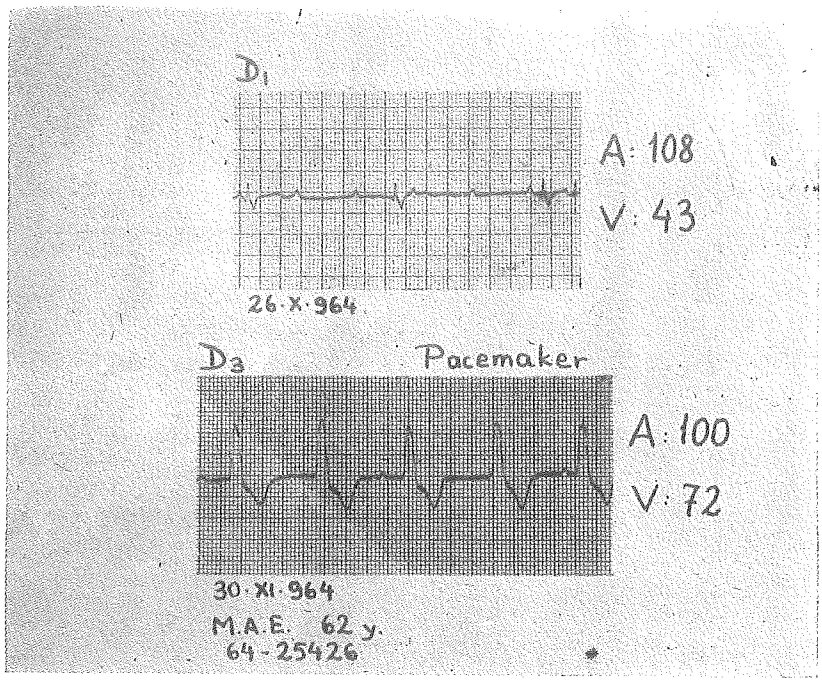


Figure 2:

vious escape beats as seen in Figure 3-B. These missed QRS complexes returned after giving a B- adrenergic agonist, A pent (Figure 3-C).

The electrode wires were again found to be normal, and increase in the pacemaker amplitude and rate caused no change in the heart rate; it was thus concluded that the generator had completed its normal life after 31 months, and that it should be changed. After incision, when the generator was exposed, the silicone cover of one of the electrode wires was found to have almost melted and become very fragile, so that it came off easily at the slightest touch, and a portion of the wire had become uncovered during this procedure (Figure 4). The generator was replaced by another rate and amplitude adjustable Chardack-Greatbatch, and the heart began to respond to pacemaker stimulation. We then opened the accessory tail and left the proper wire open under the skin to give the system a chance to work in a unipolar way in case the electrode wire should break in the fature.

After one uneventful follow-up the patient returned on December 20, 1967 complaining of bradycardia of two months' duration. Pacemaker

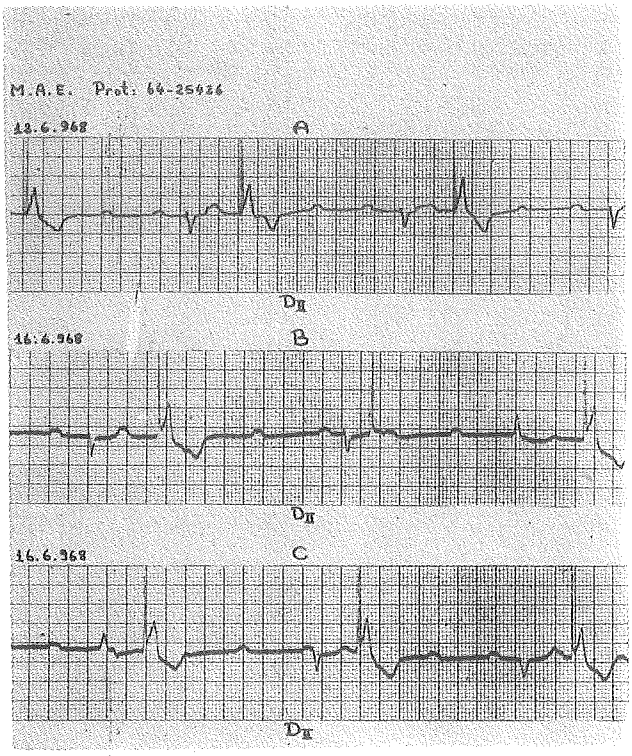


Figure 3:

a: Complete Heart Block with Slow Pacemaker Activity
b: Missed Pacemaker Conduction
c: Restored Pacemaker Conduction after Alupent

activity was 72 per minute on the electrocardiogram, with no ventricular response. There was an idioventricular rhythm of 40 per minute (Figure 5). AP-A and lateral x-ray showed that the tip of one electrode was broken and separated at its angle (Figure 6).

Increasing the amplitude of the generator did not have any effect. We suspected that there was still contact between the remaining part of the electrode tip and the myocardium, but this did not provide an effective transmission. On January 25, 1968, an endocardial electrode was passed into the right ventricle and placed at the apical trabecular area; the generator which had been implanted previously was removed from the left hypochondrial area and replaced in an area under the right clavicle after the exposed tail wire had been covered with special cement. The pacing continued well for 24 hours and then ceased; this was found to

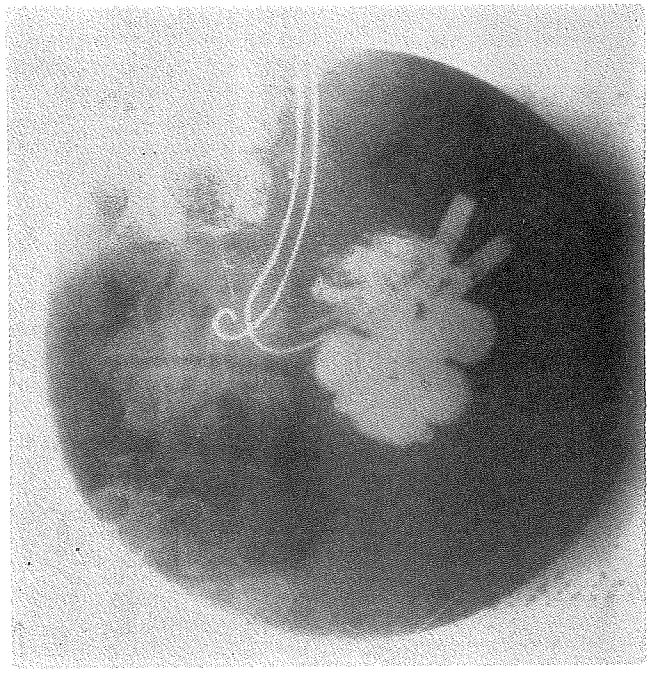


Figure 4:
Destroyed Silicone Sheet of One of the Electrodes

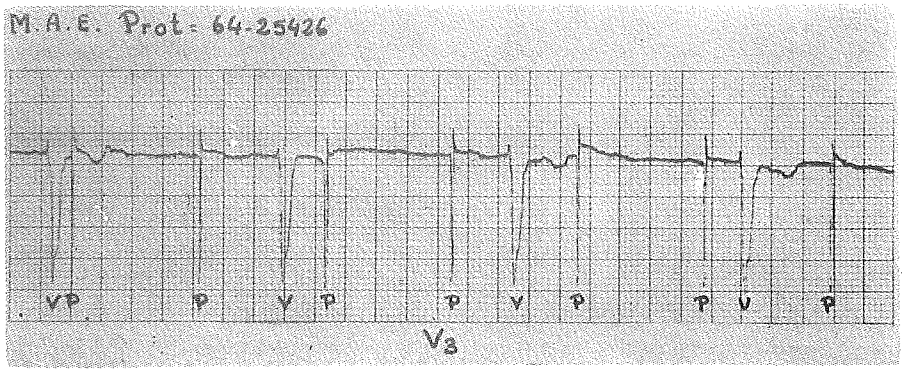


Figure 5:
P: Pacemaker Stimuli not Conducted to the Ventricles
V: Idioventricular Rhythm QRS Complexes

be because of rejection of the catheter tip at the apical trabecular area. The patient was discharged from the hospital with his heart beating with the pacemaker.

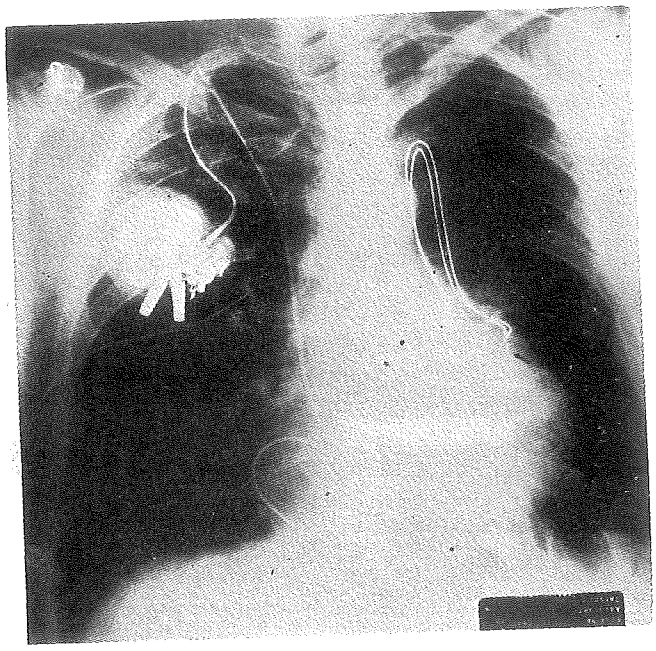


Figure 6:
PA and Lateral Views of Broken Myocardial Electrode Tips

The last time he was admitted, on May 23, 1969, the patient claimed that he had been feeling lightheaded and having slight fainting spells for the whole month, and had found his pulse rate as low as 30 per minute on several occasions, while at other times it was a regular 74. On this admission electrocardiogram revealed a regular and satisfactory pacing with ventricular response of 74 per minute, and occasional premature ventricular beats. He was monitored, but no pacemaker dysfunction was detected for the first 48 hours. After this period, although the patient had no symptoms there were no ventricular responses to pacemaker stimuli. An idioventricular rhythm was present with a heart rate of 35 per minute. Comparison of previous and present x-rays showed the catheter and tip positions to be the same, and there were no breaks in the wires. On May 25, 1969 we increased the amplitude of the pacemaker percutaneously, but this did not lead to ventricular response. We concluded that the generator's life had ended after 21 months, and there was probably an increase in the myocardial threshold. It was not possible to manipulate the catheter to ensure its position, because it was strongly adhered to the jugular vein. By using an adjustable portable external pacemaker we confirmed that the threshold was elevated, since no ventricular response was obtainable

with less than 7.8 m. a., regular ventricular QRS complexes followed each pacemaker stimulus when output was increased above this level. We then replaced the generator, and the new one was adjusted to deliver an amplitude of 11.2 m. a. Satisfactory pacing was obtained after this, and the patient was discharged from the hospital ten days later in good condition, his heart beating regularly at 72 with each electrical stimulus.

Discussion

In the beginning pacemaker manufacturers stated that the life of a battery was about five years, but today they give a lifetime of three years with some reservations. Even this seems to be an optimistic claim, since general clinical experiences have shown that generator life is around two years with most popular models. [4-6] This has also been our experience, and we have yet to see a generator which lasts more than 31 months.

When patients are treated with implanted pacemakers there are three ways to manage follow up.[17]

1. To wait until the unit fails and then to replace it;
2. To replace the unit after a fixed length of time;
3. To follow up the patients in a special pacing unit.

Impending failure is best detected in a pacing unit, but this requires that electronic engineers and a properly equipped laboratory be on hand. For the time being Hacettepe has no such facilties, and we have to choose between the first two methods. In fact, for practical reasons we usually have to be satisfied with the first.

Premature battery exhaustion is suggested by a change in heart rates, decay ratio, duration of impulse, or a fall in stimulus amplitude at the limbs which can be recorded by an electrocardiograph. [6 17] A simpler x-ray method has also been recommended as a reliable means of detecting impending battery failure. [18]

The first complication in our patient was when the generator was unable to pass into the myocardium. This was due to an oxydasic process, since the myocardial needle electrode (Electrodyne) was covered with dark material which was easily removed with a sponge. After its removal the electrode tip looked very much like the other normal one, and readily transmitted the stimulus with satisfactory myocardial response when touching the epicardium. Since it is impossible to be sure that this complication will not reoccur in the future, we decided to change the whole system, including the generator. We used myocardial electrodes again because the thorax was already open, and endocardial electrodes were not as widely accepted in 1964 as they are now. We have not noticed any other such complication reported in the literature.

On follow-up examinations we saw that this patient had no further complaints, and the pacing system was found to be functioning satisfactorily. Thirty-one months after the second implantation, however, he returned with a pacemaker rate of 28. He claimed that he had stopped taking his pulse several months previously because he "felt very well". Consequently, we did not know if he had had a period of increased heart rate before the generator slowed, but slowing had probably occurred at least 12 days prior to his arival. We immediately decided to change the generator, and at that point were faced with another complication, as described in the case presentation. Even though a portion of the electrode had lost its silicone covering, transmission of stimuli was statisfactory after the replacement of the generator. We left the electrodes in place, and prepared the system to work as a unipolar one in case this wire broke.

We saw the patient again after six months, and questioning revealed that his heart had occasionally been irregular and that his pulse rate had varied between 40 and 50 per minute during the previous two months. Although he was an intelligent man, he had ignored this and a little lightheadedness had not bothered him much. There were no ventricular responses to the pacemaker stimulation, and this was found to be due to the breakage of one the myocardial electrode tips. An endocardial electrode catheter was inserted and pacing was resumed. There was also an electrode ejection complication which was easily corrected.

Fractures of the electrode system are usually heralded by an intermittent ventricular response associated with variable absence of the pacer spike on the electrocardiogram.[5] In our case the fracture probably caused improper contact with the myocardium. Although there were spikes on the electrocardiogram, these stimuli were not strong enough to evoke ventricular responses. Local threshold elevation might have been an additional factor. Detection of the fractures by cinerontgenography was claimed to be superior to simple x-ray films.[5]

Twenty-one months later it was necessary to replace the pacemaker generator because of failure; we also found that threshold elevation had occurred and the generator had been calibrated to deliver three m. a. more than the threshold value. There is now a special Chardack-Greatbatch pacemaker (Model 5852) on the market which can give an amplitude of up to 23 m. a. if needed, but these have a shorter lifespan.

Besides the direct measuring of the threshold, there are several other ways of measuring. For example, increasing the pacemaker rate produces a linear decrease in pulse energy, so that at 120 per minute it is approxi-

mately half of its basic rate.[11] This simple test gives a gross estimate of threshold, and other tests have also been recommended.[5 17]

Our patient had his first pacemaker implantation five years ago, and had several subsequent complications. He had two sets of myocardial electrodes implanted, and finally an endocardial catheter electrode which has been functioning well for the last one and a half years. During this five-year period three pulse generators were replaced, and he is now carrying the fourth.

In spite of all these complications, the patient is alive and doing well. During the periods of complications he survived with his own slow ventricular rhythm with little discomfort. This shows that his myocardium had enough mechanical power, and only required electrical activity; a pacemaker system is the best means of suplying such activity at a desired rate. However, they are not free of complications, though they are becoming more and more efficient and reliable.

Our patient had several complications, but these were successfully treated with step-by-step conclusions without the aid of a special pacemaker follow-up laboratory.

Summary

Complications of cardiac pacemaker systems are briefly reviewed. A case is presented of a patient who had several complications related to pacing systems over a five year period.

Successful handling of different types of complications in this patient ere discussed in detail,.

Acknowledgement

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The Effects of Advanced Maternal Age and Parity

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"Age will not be defied" -Francis Bacon (1561-1626)

In recent years it has become quite evident that difficulties during pregnancy and parturition, and the frequency of developmental defects, are often correlated with maternal age. These are known as maternal age effects. Let us look briefly at the periods of female life, which are traditionally recognized as infancy, childhood, adolescence, nubility, adulthood and senility. It is now reasonably well established that the average female does not enter her optimum developmental readiness for reproduction until she is about 23 years old ±2. Maturity commences when the growth of the organism comes to an end, and the period between 23 and 28 years of age inclusive remains in every respect the best time for conception.

Hedberg, Holmdahl and Pehrson[1] found that the incidence of malformed children was significantly higher in mothers over 35 years of age. Schlesinger and Nesbitt concluded in their article on the causes of stillbirths that there was a relatively high ratio, 31 per 1,000, in the under 20 age group which progressively ascends in successive 5-year age groups to 47.0 among mothers of 40 years of age or older.[2] A study of infant loss in the Netherlands correlated to these findings.[3]

Mukherjee and Biswas,[4] in a study of 1,054 consecutive births to Indian women, observed a consistent increase in birth weight with the mother's age in primiparas up to 25 years of age, followed by a gradual but consistent decrease with advancing age.

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In 1934, when considering the possible caused of mongolism, Dr. Adrian Bleyer of Washington University[5] suggested that the condition might be due to an impairment of the ovarian function in the aging female, involving a disturbance in the chemistry of the ovum with resultant physiological variations and anatomical changes in the form or number of gene-containing chromosomes within the nucleus of the germinal cell. Maternal age is a factor in the production of anomalous movements of the chromosomes at meiosis.

Ford et al[6] in 1959 reported a case of Turner's syndrome, and Jacobs and Keay[7] also reported that Bonnevie-Ullrich syndrome has 44 autosomes and an XO sex chromosomal constitution. In 1959 Jacobs et al[8] reported a triple-X (miscalled "super female") where the mother's age at conception was 41 and the father's probably 40; the pregnancy was full-term and normal.

TABLE I

MATERNAL AGE EFFECTS IN DIFFERENT ABNORMALITIES

Condition	Children born after the mother had reached 40 years of age
Mongolism	39.5 %
Chorion-epithelioma	33.3
Vesicular mole	33.0
Achondroplasia	13.6
Central placenta praevia	17.9
C.N.S. malformations	11.8
Twins of unlike sex	6.3
Anencephaly	5.6
Control populations	3.5 to 5.0

(Penrose, L.S.: Mongolian idiocy (mongolism) and maternal age, Ann. N.Y. Acad. Sci. 57: 494, 1954.).

Haggstrom[9] found that there was a total of 432,000 ova in both the ovaries of a 22-year-old woman. Block[10-12] found that in the six to 15 age group the total number in both ovaries was 439,000 ova, but in women of 36-45 years of age the number was reduced to 34,000. There is, of course, a great deal of variability, but the trend is clear.

There is some evidence that the mothers of mongoloid offspring exhibit a higher incidence of endocrine insufficiency than those of normal children. The suggestion is that an inadequate hormonal intrauterine en-

vironment, in addition to nutritional deprivations, and the failure of endocrine stimulation on an already dysplastic ovum, result in anomalous development. Delayed fertilization of a deteriorating egg in a female of optimum reproductive age may have similar consequences, but mongolism occurs more frequently in the offspring of older mothers. Presumably the accident of nondisjunction is more likely to occur in older ova, too. Paternal age per se has no such correlation with the frequency of mongolism (McKusick[13]).

Two-egg twins occur least frequently in young mothers, becoming progressively more frequent with the increasing age of the mother, until 37-38 years and thereafter when the number drops sharply. One egg, or identical, twins occur with equal frequency in all maternal age groups. Waterhouse[14] has shown that the cause of the increasing number of unlike-sex twins in relation to all twin births with the advancing age of the mother is probably due to the fact that the frequency of abortion for these twins is comparatively less than for twins of the same sex, many of which are one-egg twins. This is less in urban than rural areas; presumably, he points out, this is a reflection of the generally higher fertility of rural-dwellers.

The tendency to monozygotic or disygotic twinning is often inherited, but maternal age is sometimes responsible for two-egg twinning. Multiple births, quintuplets and sextuplets are extremely rare in first pregnancies, and occur more frequently with older mothers than younger ones. Out of 39 records of quintuplets, 13 of the mothers were between 35 and 40 years old.

Book[15] wrote a report on the findings drawn from a total of 70,962 births in Stockholm and Upsala, Sweden. The total incidence of malformations was 1.3 %, but the incidence among the children of a sample of 1,030 mothers of over 42 years old was 4.5%. In our clinic the incidence of malformations was 2.2% and 9 % respectively. Mitchell's study[16] on 21,518 births, of which 2,149 or slightly less than 10%, were to mothers aged 36 years or over, showed that the incidence of abnormal deliveries in both primipara and multipara was higher in mothers over 36 years old.

Another interesting effect of maternal age is the sex ratio (the proportion of males born to females). In the United States it is 106 boys born in the white population to every 100 girls in the negro population. Investigations throughout the world show that the ratio of boys and girls tends to decline with the advancing age of the mother. There is a similar, though not quite so marked, decline in the sex ratio with the advancing age of the father.

TABLE II

EFFECTS OF MOTHER'S AGE ON THE SEX OF HER OFFSPRING

Mother's age	Female	Male	Total
Less than 15	–	–	–
15 – 19	31	33	64
20 – 24	141	128	269
25 – 29	121	128	249
30 – 34	60	68	128
35 – 39	22	36	58
40 – 44	3	4	7
45 – 49	–	1	1
Over 49	–	–	–
Unknown	9	1	10
Total	378	398	786

Lowe and McKeown[17-19] found in England and Scotland that the sex ratios of total births decreased with maternal age, whereas the sex rations of stillbirths increased. In young mothers the risk of stillbirths is much higher for twins than for single births, and there is little difference in the stillbirth sex ratios; but as maternal age increases the risk to the male fetus becomes progressively greater. Unlike-sex twins' stillbirth rates are lowest for mothers under 25 years old, and, as in the case of single births, they increase with maternal age.

TABLE III

LIVE BIRTHS BY AGE OF MOTHER, SEX OF CHILD AND COLOR

	White			Non-White		
Age	M	F	R	M	F	R
Less than 15	1,354	1,336	101	2,254	2,266	99.4
15 – 19	236,546	223,286	105.9	64,934	62,814	103.3
20 – 24	614,696	583,168	105.5	104,138	101,286	102.8
25 – 29	441,950	421,134	104.9	73,420	72,038	101.9
30 – 34	266,488	253,372	105.0	47,160	45,934	102.6
35 – 39	139,026	132,450	104.9	25,318	25,364	99.8
40 – 44	38,240	36,512	104.7	6,874	6,876	99.9
45 – 49	2,020	1,974	102.3	418	412	101.4
50 +	38	38	100.0	16	18	88.8

(Vital Statistics of the U.S.A. 1962, Vol. 1, Natality 1-112, Dept. of HEW, Public Health Service).

Cardiac Malformations: Except for those associated with mongolism these are apparently not associated with maternal age.

Harelip and Cleft Palate: Maternal age has no influence.

Anencephaly, Spina Bifida, Hydrocephalus: These show a steeply rising trend with maternal age, the relative liability at 40 to 44 years of age compared with that at 20 to 24 being doubled in anencephaly and hydrocephalus, and trebled in spina bifida.

Mental Deficiency: Dayton et al[20] reported that the age of the mother was a significant factor in all types of mental retardation. He concluded with a plea for early marriage and the completion of child-bearing before the mother has reached 35 years of age.

In 1945 Barry of Harvard[21] studied the relationship between maternal age and the number of children who later became neurotic or psychotic; from this one can make the interesting observation that the more severe the mental illness, the more often the mother was of advanced age, compared with mothers of children whose illnesses were less severe.

Prematurity: According to the figures published by the National Office of Vital Statistics, about 200,000 premature babies are born each year in the United States, about half of whom die during the first three days of life. Prematurity is closely associated with maternal age, the youngest and oldest mothers tending to give birth to the greatest number of prematures.

Abortions and Stillbirths: These are closely correlated with maternal age, and follow virtually the same pattern as the prematurity maternal age rates.

TABLE IV

INCIDENCE OF STILLBIRTHS ACCORDING TO AGE OF MOTHER

Age	Total births	Stillbirths
Less than 20	56	1
20 – 24	185	3
25 – 29	200	4
30 – 34	91	3
35 – 39	37	2
40 – 44	11	1

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Mitchell[16] gave the following figures on prematurity:

TABLE V

PREMATURITY RELATED TO AGE OF MOTHER

Age	Total births	Total Premat.	% incidence	Perinatal Dx.	Rate/100
Less than 35	19,369	123	6.2	290	14.9
36 - 37	948	61	6.4	26	27.4
38 - 39	603	60	9.9	18	29.8
40 +	540	5	9.4	22	40.7

Neonatal Deaths: Judd at the University of Southern California[22] stated that "the highest number of neonatal deaths occurs in infants born to women who are very young or very old. Infants of mothers 15 years old and under, and 45 years old and older have three and four times repectively the mortality rate of infants whose mothers are between the ages of 20 and 30".

Post Maturity: Over five per cent of pregnancies end at or about 301 days after conception. Such babies are more frequently born to mothers who have passed the optimal period for childbearing than to younger mothers.

Prolonged Labor: Labor that last for more than 24 hours is regarded as prolonged. This is much more likely to occur in older than in younger mothers. Baird[23] found that in his series of 2,694 pregnant women, the proportion of labors lasting for more than 24 hours rose from 10 per cent for mothers less than 20 years old to 25.5 per cent for those aged 35 or over.

Cesarean Section: Hellman[24] reported that there was a four per cent incidence of cesarean sections in primiparas. The ratio for those between 35 and 39 years inclusive was 16 percent, and 44 per cent for those between 40 and 45 years of age. According to Baird's series,[23] the frequency of termination of labor by cesarean section is strikingly higher in older patients. Of course, in the case of the older woman the baby is regarded as more valuable than that of younger women, for the simple reason that time is running out, and therefore the obstetrician tends to take fewer risks, and is more prone to do a cesarean section.

Growth: The relationship between maternal age and fetal growth is still obscure.

TABLE VI

INFANT WEIGHT AT BIRTH ACCORDING TO AGE OF MOTHER

Age of Mother	Weight in grams					
	Less than 2500	2500–2999	3000–3499	3500–3999	4000–4499	4500 and over
Less than 15	–	1	–	–	–	–
15 – 19	6	13	21	11	2	–
20 – 24	11	37	81	43	8	1
25 – 29	14	40	93	45	7	1
30 – 34	7	14	41	20	8	2
35 – 39	–	8	10	9	6	–
40 – 44	–	3	4	3	–	–

Toxemias: Toxemias of pregnancy are notably more frequent in older than in younger mothers. McClure-Browne of Hammersmith Hospital, London[25] indicates that there is strong evidence of slowing of the blood in mothers who are beyond the age of 30. Thomson in Aberdeen referred to an unpublished study of clinical records which shows that the incidence of ectopic pregnancy and placenta praevia in primigravidas rises steadily with age.

The incidence of toxemias in our patients clearly shows that they are seen frequently in the younger age bracket, and in gravidas aged over 30 years (Table VII).

TABLE VII

INCIDENCE OF TOXEMIA ACCORDING TO AGE OF MOTHER

Mother's age	Number of cases	Number of Toxemias	%
Less than 15	1	–	–
15 – 19	55	6	10.9
20 – 24	185	14	7.5
25 – 29	200	18	9.0
30 – 34	91	14	15.4
35 – 39	37	4	10.8
40 – 44	11	2	18.1

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Hydatidiform Mole: According to Eastman,[26] age has an important bearing on the frequency of hydatidiform moles, since this condition is seen more often at the very beginning, and more particularly toward the

end, of the childbearing period. Age shows its most pronounced effect in gravidas older than 45, when the frequency of the neoplasm is more than 10 times greater than at ages 20 to 40.

We concluded that maternal age effects are considerably more in primipari than in multipari. Does the number of a mother's previous pregnancies (parity) effect the development of her child? Eastman[26] emphasizes than any woman whose parity has reached IX or more can be considered a grand multipara; the most recent investigators, however, have regarded the para VII female as grand multipara.

TABLE VIII

PARITY AND MATERNAL AGE

	Para VI or less (128,568)		Para VII or more (5,551)	
	White	Non-White	White	Non-White
34 and under	85,219	31,907	1,406	2,155
35 and over	9,321	2,121	991	999

Neonatal Deaths: Children born at the end of a long series of pregnancies tend to be less viable than those born first, irrespective of maternal age. Firstborn children are characterized by the lowest death rates, while with increasing births the risk of deaths mounts steadily. As McKeown and others[28-32] have shown, the reasons for this increase are almost wholly attributable to deaths from infectious diseases. Later-born children are exposed to an increased risk of infection at vulnerable ages from older brothers and sisters, and the greater susceptibility to infection associated with the poor economic circumstances of large families. However, Ziel[33] states that increasing maternal parity does not cause a significant variation in the incidence of neonatal deaths.

Stillbirth rates are highest with the shortest and the longest birth intervals. It should be mentioned that the interval between births probably varies with maternal age and social class; the relation between stillbirth rates and birth intervals may, therefore, be no more than indirect.

Labor and Delivery: There is also an undoubted increase in the incidence of both primary and secondary inertia.

TABLE IX

INCIDENCE OF INERTIA

Age of mother	Number of Cases	Inertia		Total	%
		Primary	Secondary		
Less than 15	1				
15–19	55	–	4	4	4.8
20–24	185	9	13	22	11.8
25–29	200	14	7	21	10.5
30–34	91	6	5	11	12.0
35–39	37	4	4	8	21.6
40–44	11	2	1	1	27.2

(Department of Obstetrics and Gynecology, Hacettepe University Faculty of Medicine).

Maternal Death: The definition of maternal death (adapted from the Committee on Maternal and Child Care of the Council of Medical Services of the American Medical Association) is "the death of any woman from any cause whatsoever while pregnant or within 90 days of the termination of pregnancy, irrespective of the duration of the pregnancy, the time of the termination or the method by which it was terminated."

Lorens et al,[34] studying 80, 403 live births counted maternal deaths. He found that there were 22 white and 75 non-white, the total mortality rate being 12.1 to 10,000 live births; 13 per cent of these were registered, and 68 per cent were not.

The causes of death were classified as follows:

I. Deaths attributed to *direct* obstetrical causes: 66 (68%)

Hemorrhage	16
Rupture of the uterus	7
Abruptio placentae	4
Ectopic pregnancy	5
Uterine inversion	2
Toxemia	16
Infection	23
Other (non-septic pulmonary emboli, anesthesia, amniotic embolis, etc.)	1

II. Indirect causes: These were obviously aggravated by the physiological effects of the pregnancy: 17

III. Unrelated causes 12

IV. Undetermined causes 2

The maternal mortality rate increases as parity increases. For patients who were para IV or less the maternal mortality rate was lower than the clinic average for those who had five or more pregnancies, which was greater than the clinic average. When parity was greater than seven, the maternal mortality rate was doubled.

TABLE X

INCIDENCE OF MATERNAL MORTALITY

Age	Clinic Average
Less than 30	Less than average
More than 30	More than average
35 – 39	Rate was 3 ½ times higher
More than 40	Rate was 4 ½ times higher

Ziel[33] stated that in 1940 Eastman called attention to the increased maternal mortality among women who had had at least six previously terminated pregnancies of 20 or more weeks' gestation.

TABLE XI

CAUSES OF MATERNAL MORTALITY

Parity	Major causes of maternal mortality
Low parity High parity	Puerperal infections, toxemia, hemorrhage ruptured uterus, chorion epithelioma, hypertation, placenta praevia.

Infant Weight: Ziel[33] also gave information regarding birth weight as follows:

TABLE XII

BIRTH WEIGHT AND PARITY

Weight	Para I-IV	VI +	Grand multipara Age of 41 +
2,500 gm. or less		Same	
Average	3,290	3,380	
10 lbs. (4,536 gm.)	2.02 %	4.76 %	
Stillborn	1.31 %	2.86 %	7.14 %

Parity influences the rate of fetal growth, which is greater in later-born than in firstborn children. McKeown and Record[30] point out that the rate of fetal growth at about 36 to 37 weeks' gestation is greater in later born than in firstborn children.

Maternal Complications

Toxemia: Preeclampsia showed a significant increase in relation to the parity of the mother, reaching 16.12 per cent in grand multipara, and increasing even more in mothers of 41 years old or more to 21.6 per cent.

For abruptio placetae and placenta praevia there is no significant variation. There is an increase in primary uterine inertia, but no significant difference in secondary uterine inertia.

TABLE XIII

PRENATAL ANEMIA

Parity	% of anemia
I – IV	9.15
VI +	13.79

(Here there was no significant variation in relation to age).

Scharfman and Silverstein[35] state that the frequency of obstetric complications of all sorts in grand multipara was found to be small in their series, with the exception of primapara, which was 1.5 per cent, or approximately three times the general incidence; the incidence of post-partum hemorrhage was 2.17 per cent, which is five times greater than is found in the non-grand multipara.

Sex Ratio: This is found to be higher for firstborn than for later born children, and decreases as parity advances.

Oxorn[36] says, "There must be no sense of false security or neglect. Every patient must be thoroughly examined and carefully followed throughout her pregnancy, labor and puerperium."

Conclusion

When maternal age and parity are considered together it is found that the elderly (over 35 years of age) multigravida presents more problems to herself and to the fetus than does the elderly primigravidas.

Evidence is clear that the grand multipara is nowadays being cared for with no greater risk to life than that of any other pregnant women.

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Results Obtained with Lomotil (Diphenoxylate Hydrochloride) in the Treatment of Acute and Chronic Non-Specific Diarrheas, and a Comparative Study of Lomotil and Halquinol

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Introduction

Diarrhea is one of the most frequent complaints which forces patients to visit their doctors, and may sometimes be a hazardous condition, as acute diarrhea can cause loss of water and electrolytes which sometimes leads to acute tubular necrosis and renal insufficiency. The latter conditions are difficult to manage clinically. [1 2]

Furthermore, it is often impossible to find a specific cause for the complaint. In all cases of acute and chronic diarrhea, whatever their etiology it is important that they be brought under control so that dehydration, loss of electrolytes, renal insufficiency and malnutrition may be prevented. [3-4].

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Preparations containing charcoal, kaolin and bismuth, when used as anti-diarrheal preparations, are only effective in mild cases. Drugs such as atropine and belladonna when used in adequate doses invariably cause side-effects such as dryness of the mouth and blurring of the vision, and although they inhibit gastro-intestinal motility, they are not entirely satisfactory in preventing diarrhea. Opium preparations have a long history in the treatment of diarrhea, but these have the disadvantage of being habit-forming, especially in chronic use.[5]

Earlier research has established the efficacy of Lomotil as a synthetic anti-diarrheal agent in acute and chronic diarrhea.[6] It is not an antibacterial agent, but acts on diarrhea by lowering the gastrointestinal motility, and its action on the smooth muscle of the intestinal tract is similar to that of morphine. The amount of atropine present in Lomotil is sub-therapeutic, and the anti-diarrheal action is not attributable to its atropine content.[7] A trial of 60 patients was carried out in order to extend these studies, and to compare the effects of Lomotil with those of another anti-diarrheal agent, Halquinol, in acute non-specific diarrheas.

Materials and Methods

Sixty patients with non-specific acute and chronic diarrhea were studied. In 40 of them diarrhea was acute, and in 20 it was of a more chronic type. The patients were divided into three groups of 20 each.

The first group had acute non-specific diarrhea; three were women and 17 were men, and their ages ranged from 22 to 60 years. All these patients received Lomotil in daily doses varying between six and eight tablets (15–20 mg) according to the severity of the diarrhea.

The second group consisted of 20 patients with acute non-specific diarrhea, four of whom were women and 16 men, whose ages ranged from 18 to 65 years. They were given eight to 10 tablets (400-500 mg) Halquinol daily, according to the severity of their diarrhea.

The duration of diarrhea in both groups before treatment varied from one to three days.

Of the 20 patients with non-specific chronic diarrhea, nine were women and 11 were men, whose ages ranged from 18 to 60 years. Fifteen of them suffered from irritable colon syndrome, two had ulcerative colitis, two had malabsorption syndrome, and one had carcinoid syndrome. All these patients were treated with Lomotil in doses varying between 10 and 20 mg daily, and were given a low residue diet.

Liver function tests, including bilirubin, thymol, CCF, SGOT, SGPT and alkaline phosphatase, were made on the 40 patients with acute diarrhea before and after treatment with Lomotil. In addition stool cultures were obtained from all 60 patients before treatment, and radiological and sigmoidoscopic examinations of the gastro-intestinal system were made where indicated.

Results

Group 1– Acute non-specific diarrhea treated with Lomotil. (See Table 1) In eight patients bowel action returned to normal on the first day of treatment, and in a further nine patients normal function returned on the second day. In the remaining three patients the diarrhea had been controlled by the third day, and tenesmus and abdominal pain disappeared. Seventeen patients required treatment for three days, and the other three for only two days. Medication was then discontinued and diarrhea did not reoccur in any cases.

TABLE I

RESULTS OBTAINED WITH LOMOTIL IN THE TREATMENT OF ACUTE NON-SPECIFIC DIARRHEA

Number of Patients	Age	Sex	Dosage Daily	Duration of treatment	Number of Bowel Actions Before Treatment	Number of Bowel Actions After Treatment 1st day	2nd day	3rd day
1	57	M	15 mg.	3 days	8–10	2	1	1
2	24	M	15 mg.	4 "	10–12	3	2	1
3	36	M	15 mg.	3 "	8–10	2	1	0
4	27	F	15 mg.	3 "	7– 8	1	1	1
5	25	M	15 mg.	3 "	4– 6	2	1	1
6	39	M	15 mg.	3 "	8–10	3	1	1
7	25	M	15 mg.	4 "	5– 6	2	1	1
8	26	M	15 mg.	3 "	6	1	1	1
9	24	M	15–20	3 "	10–12	3	1	1
10	35	F	15 mg.	3 "	8–10	1	1	1
11	25	M	15–20	3 "	10–12	2	1	0
12	30	F	15 mg.	3 "	9–10	1	1	1
13	29	M	15 mg.	3 "	7– 8	3	1	1
14	22	M	15 mg.	3 "	7– 8	1	1	1
15	26	M	15 mg.	3 "	4– 6	2	1	1
16	32	M	15 mg.	2 "	6– 7	1	1	1
17	23	M	15 mg.	2 "	8–10	1	1	1
18	22	M	15 mg.	2 "	6– 8	1	1	1
19	41	M	15 mg.	4 "	7– 8	3	2	1
20	36	M	15 mg.	4 "	8–10	4	2	1

Group 2– Acute non-specific diarrhea treated with Halquinol.

As seen in Table II, normal bowel action had not been restored in any of the 20 patients at the end of the first day of treatment, although the frequency of bowel actions was reduced in 17 of them. On the second day of treatment four patients had a normal frequency, by the third day a total of 10 had returned to normal, and on the fourth day this figure had increased to 17. In the remaining three patients diarrhea could not be controlled even though the dosage of Halquinol was increased. After five days this treatment was discontinued in three cases, and 10 mg of Lomotil daily was given instead. These patients then made a normal recovery in three days.

TABLE II

RESULTS OBTAINED WITH HALQUINOL IN THE TREATMENT OF ACUTE NON-SPECIFIC DIARRHEA

Number of Patients	Age	Sex	Daily Dosage Tablets	Duration of Treatment	Number of Bowel Actions Before Treatment	Number of Bowel Actions After Treatment 1st day	2nd day	3rd day	4th day
1	32	M	8	4 days	7– 8	7	4	1	1
2	25	M	8	5 "	6– 7	4	3	2	1
3	26	M	8	3 "	5– 6	3	1	1	1
4	31	M	8–10	5 "	5– 6	5	5	4	5
5	29	M	8	4 "	10–12	8	4	1	1
6	41	M	8	5 "	7– 8	5	3	2	1
7	25	M	8	5 "	12–13	8	5	2	1
8	29	F	8	5 "	10–12	7	4	2	1
9	28	F	8	4 "	5– 6	4	1	1	1
10	63	M	8	5 "	7– 8	6	4	2	1
11	37	M	8–10	5 "	8– 9	8	7	5	6
12	28	M	8	5 "	6– 7	6	4	2	1
13	65	M	8	5 "	8– 9	7	4	2	1
14	31	M	8	4 "	6– 7	4	3	1	1
15	22	M	8–10	5 "	10–12	10	8	6	6
16	20	M	8	4 "	7– 8	6	3	1	1
17	34	M	8	4 "	6– 7	3	2	1	1
18	18	F	8	3 "	7– 8	3	1	1	1
19	23	M	8	3 "	8– 9	3	1	1	1
20	42	F	8	4 "	6– 7	3	2	1	1

The difference between the average number of bowel movements in two groups of patients treated with Lomotil and Halquinol is statistically significant ($P<0.001$).[8]

Group 3– Chronic non-specific diarrhea treated with Lomotil.

In Table III the results obtained have been evaluated as excellent, good or poor. In 11 out of the 15 patients with irritable colon syndrome, the number of daily bowel actions returned to normal with formed feces. In addition, abdominal pain, the feeling of fullness and mucus in the stools disappeared. When the symptoms had been controlled the daily dosage of Lomotil was reduced to 7.5 mg per day and the symptoms did not recur. This response to the treatment was evaluated as excellent. In three patients the results were classed as good, for although the number of bowel actions returned to normal the stool was not completely formed, even though the feeling of fullness, the abdominal pain and the fecal mucus had disappeared. In the one remaining patient improvement did nor occur even though the dosage was increased.

TABLE III

RESULTS OBTAINED WITH LOMOTIL IN THE TREATMENT OF CHRONIC NON-SPECIFIC DIARRHEA

Disease	Number Of Patients	Duration Of Treatment	Results Of The Treatment		
			Excellent	Good	Poor
Malabsorption Syndrome	2	20–25 days		2	
Ulcerative Colitis	2	7–10 days		1	1
Carcinoid Syndrome	1	20 days		1	
Irritable Colon Syndrome	15	30–62 days	11	3	1
Total	20		11	7	2

In the two patients with malabsorption syndrome (due to intestinal resection and non-tropical sprue), the patient with carcinoid syndrome, and in one of the two patients with ulcerative colitis, there was a significant decrease in the number of bowel actions per day and in the patients' complaints. One patient with ulcerative colitis did not respond to therapy even though the dosage was increased.

Thirty-eight of the 40 patients receiving Lomotil treatment did not have any side effects. The remaining two both experienced mild nausea which lasted for the first two or three days of treatment. There were no adverse effects of Lomotil upon liver function tests.

Discussion

Although this study is not based on a large group of patients we believe that it confirms that Lomotil is an effective preventive agent in acute and chronic diarrheas of non-specific origin.

A comparison of the results obtained in treating patients with acute non-specific diarrhea (Tables 1 and 2) shows that Lomotil controls the symptoms more quickly than Halquinol does. Good results obtained with Lomotil in three patients where Halquinol had been ineffective reinforced this belief.

The difference between the average number of bowel movements in two groups of patients treated with Lomotil and Halquinol was statistically significant. In other words, patients treated with Lomotil had fewer bowel movements than the patients treated with Halquinol.

Although satisfactory results were obtained in cases of malabsorption syndome, ulcerative colitis and carcinoid syndrome, it is difficult to come to any conclusion from this study, since the number of patients was limited. But the beneficial effects of Lomotil in irritable colon syndrome are self-evident, particularly when it is noted that no therapy other than Lomotil and a low residue diet was given. The fact that Lomotil was not habit-forming and does not cause any significant side-effects increases its practical value.

Summary

A study was made of the effects of Lomotil and Halqunol in 60 patients with acute and chronic non-specific diarrhea. It was established that Lomotil is an effective anti-diarrheal agent in both acute and chronic non-specific diarrheas, and that it acts more rapidly than Halquinol in acute cases.

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Intrauterine Contraceptive Devices and Their Biological Effect*

Nuri Sağıroğlu, M.D. / Emel Sağıroğlu, M.D.*****

The discovery presented in this paper has opened the door to several new avenues of research in preventive medicine and the basic sciences.

Because they can easily be placed in the uterine cavity, are inexpensive and are an almost perfect protection against pregnancy with very few undesirable side effects, intra-uterine devices (IUD 's) are being used increasingly for contraceptive purposes, and are the best method of temporary sterility.[1] They have been used successfully in almost every country for the last two decades, but the mechanism of their action is still unknown. Although many scientists employing different techniques have studied the subject, the results have only been speculative, and remain insufficient to explain the physical and chemical processes involved.

Most researchers have used histological, biological and chemical methods to study the implantation bed of the fertilized ovum, the endometrial tissue, which comes in close contact with the device. The physiology of the uterine muscle and the fallopian tubes while the IUD is

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in place, the ovarian hormones, and their influence on the uterus and ovulation, the ovum itself and the speed at which it travels between the ovary and the uterine cavity, and the speed of the spermatozoa, their motion through the genital canal and the change in their quantity have all been studied. These investigations have been carried out repeatedly on both humans and on experimental animals, the results obtained have indicated extreme differences, and the question of how the intra-uterine device prevents pregnancy still remains unanswered.

The solution to this problem would fill an extremely important gap both in medicine and in general science,[2] since the anticonception device has been found effective against trophoblastic cells which have invasive and destructive characteristics similar to those of malignant cells. Though the studies made to detect the IUD's mechanism of action have not achieved their target, they have produced the following valuable information on which to base new review studies of the subject:

1. The presence of the IUD in the uterine cavity does not stop ovulation.[3-5]

2. It neither blocks the oviducts nor slows down peristalsis.[6]

3. The IUD does not intercept all the spermatozoa, and some pass through the endometrial cavity even when it is correctly placed allowing fertilization to take place in the fallopian tube.[7-10]

4. The fertilized ovum, continuing primary cell division (cleavage) and producing the blastocyst in the zona pellucida, may reach the uterine cavity.[6]

5. The endometrial tissue show normal cyclic changes[5 11 12].

6. Ovarian hormones, endometrial enzymes and acidity (pH) do not show any significant changes which might cause degeneration of the cells of the blastocyst[13-15].

7. The IUD performs a perfect contraceptive action starting a few days after insertion and ending a few days following its removal.[16 17]

8. If the device is much smaller than the endometrial cavity, or if there is a partial displacement in the endocervical canal so that the fundal section of the endometrium becomes free of it, implantation and pregnancy become possible.[17 18]

9. When a suitable and correctly placed IUD covers less than one fifth of the implantation area, and an extremely large endometrial surface

area remains free between the curves of the Lippes loop, the ovum cannot implant itself and the cells of the blastocyst degenerate and disappear.[6 19-21]

10. Because it is made of chemically inert material, the IUD itself is not, and cannot be, a mechanical ovum killer.[18 22]

The above facts indicate that a woman using an IUD is still in a condition to conceive; why, then, in a vast implantation area of which only one fifth is covered by the device, is the blastocyst not embedded?

The cause of the blastocysts' degeneration lies between the endometrial surface and the IUD. The true cause of antifertility cannot lie in the endometrial tissue as this was found to be within normal limits from every point of view. The unknown factor which causes the degeneration of the blastocyst or the trophoblastic cells would be present in the endometrial cavity in the immediate neighborhood of the device.

Once we reached this conclusion, we decided to study the device itself to learn the basic mechanism of intra-uterine contraception.

Materials and Methods

Fifty-seven young women (virgins and non-virgins) were used for this study. The IUD's of 22 of them had been inserted by their physicians for contraceptive purposes, and were studied when the women came to our cancer prevention clinic with complaints, desiring to have the device removed or changed. The devices were inserted solely for experimental purposes, in the remaining 35 women in 30 of whom they were placed in the uterine cavity and removed at 24 hour intervals (the first after one day of use, the second after two days and so on until the last was removed after 30 days.) The remaining five women were young virgins, in whom the devices were placed in the vaginal cavity near the fornices through the annular or semilunar hymenal opening, and removed after varying periods of time, the first after 24 hours; the second after 5 days; the third after seven days; the fourth after 15 days; and the fifth after 21 days of insertion.

The duration of IUD use for intra-uterine contraception varied from 5 hours to 39 months, and those used in this study were Lippes loops made from radio-opaque polyethylene[23 24] (Figure 1) Clinical, cytologic, histologic and cytohisto-chemical methods of investigation were used. The clinical observations and findings will be presented in a subsequent paper.

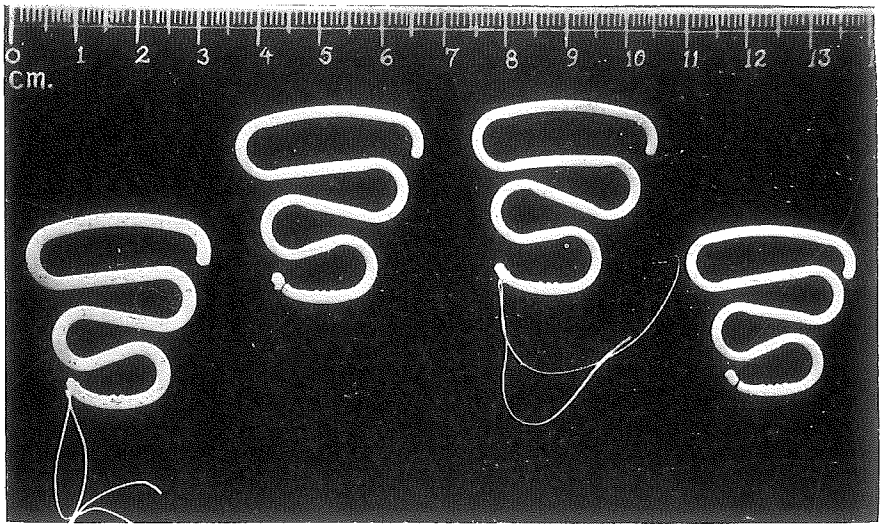


Figure 1: Lippes loops used as IUD. From right to left, (A, B, C, and D) sizes and thicknesses are increased for easy identification nylon threads are colored: A-Black; B-Yellow; C:Blue; and D-White.

Cytologic Methods:

Just before inserting the device into the uterine cavity a series of cytological specimens were prepared from the urethra, vagina (right, posterior and left fornices separately, but spread on the same slide), ectocervix, endocervical canal, endometrium and peripheral blood, and again just before its removal with the exception of the endometrial smear material, which was obtained following the removal of the loop. Urethral, ectocervical and endocervical smears were prepared with material obtained by a specially designed spatula called a "cytologic panscraper" or, for short, "cytopan"[25-28], vaginal smears taken with a 5 to 8 mm wide, 1.5 mm thick, jet-shaped wooden blade, and endometrial smears with a Cary's aspirator metal cannula, (Figures 2 and 3).

Three endocervical smears were prepared from each patient and stained by Papanicolaou, Feulgen and PAS stains, and the vaginal fornices, especially the endocervical canal, were then carefully cleaned several times with sterile gauze to prevent, or at least minimize, cellular contamination of the device during its removal. The loop was then removed very slowly and carefully.

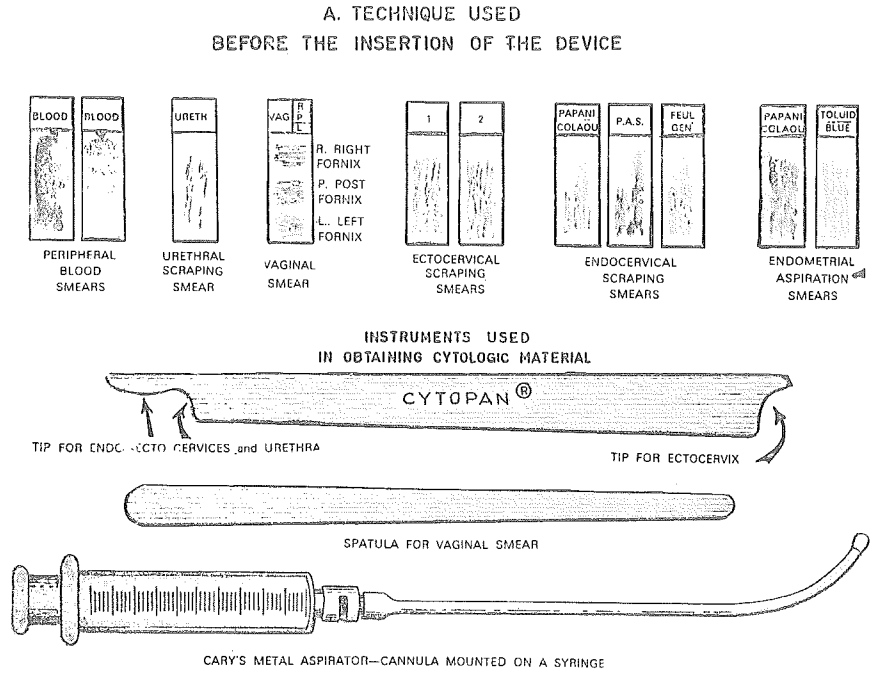


Figure 2: Technique used in the investigation.

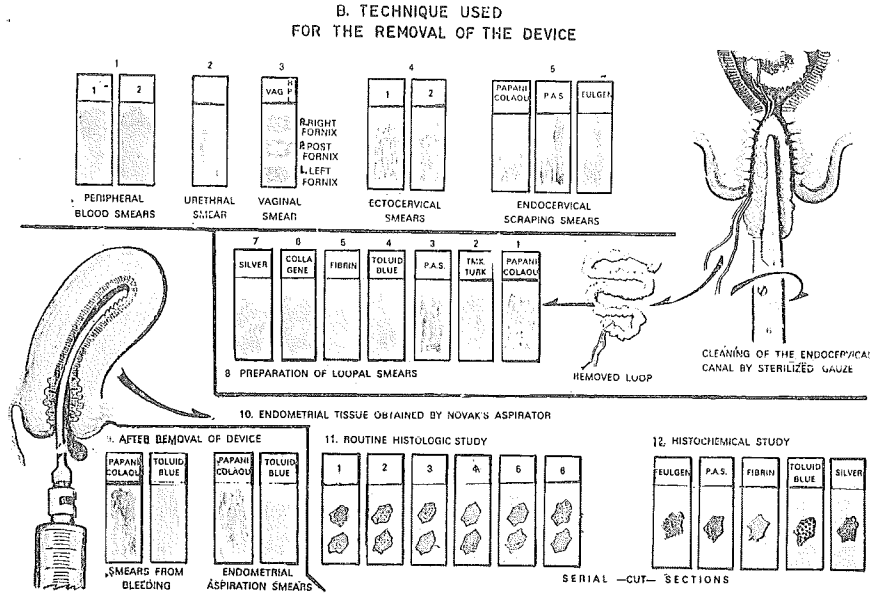


Figure 3: Technique used in the investigation.

Loopal Smear: The IUD was immediately placed on a clean dry microscope slide, and a smear was prepared by rapidly pulling and pushing it across the surface. This slide preparation, is called a "Loopal smear" (Figure 4), six to nine of these were prepared from each loop and stained by the standard Papanicolaou, "TMK-101, TÜRK" 26-29, Feulgen, PAS, toluidin blue, silver impregnation and Van Giezon's methods. The loop was then labeled and placed in pure alcohol to be kept for photographic purposes and further study.

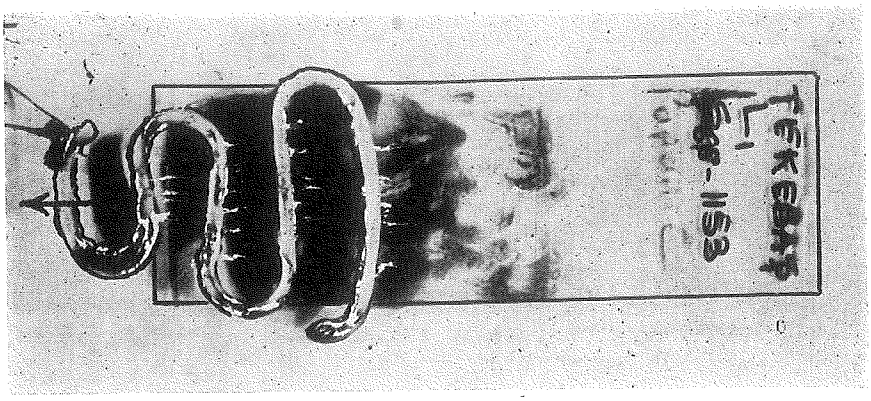


Figure 4: Preparation of the "Loopal smear". The loop was removed very carefully and immediately placed on a clean dry microscopic slide. A smear was prepared by rapid pulling and pushing movements of the loop across the slide surface.

Endometrial Blood Smears: Even the gentle removal of the loop scrapes the surface of the endometrium causing bleeding and contractions and when the latter occurs, blood and other uterine contents are forced downward, and appear at the endocervical os. Two smears of this bloody fluid, which is generally less than 1 cc in volume, and very seldom as much as 3 cc, were prepared from each patient, one stained by the Papanicolaou method and the other by toluidin blue.

Endometrial Aspiration Smears: A sterilized Cary metal cannula mounted on a 10 cc syringe was inserted into the uterine cavity, fragments were aspirated without disturbing the endometrial bloody liquid and small tissues and two smears were prepared and stained similarly to the endometrial blood smears.

Endometrial Tissue Studies: A sterilized Novak's scraper cannula mounted on the same 10 cc syringe was inserted into the endometrial cavity, and the lower anterior surface in anteversion, or the lower posterior surface in retroversion, which are the surface of the endometrium that comes in

closest contact with the device, were carefully scraped and aspirated. These tissue fragments were then placed in formalin for histopathological study. Two or three paraffin blocks were prepared from each case and 12 serial sections from each block, six of which were stained by hematoxylin and eosin; and the remaining six by Van Giezon, silver impregntation (Laidlaw) and fibrin (phosphotungstic acid and hematoxylin) for fibroblastic production, by periodic acid-Schiff (PAS) for glycogen, mucin and mucopoly-saccharides, by the Feulgen technique to demonstrate nuclear material (DNA), and by toluidin blue to demonstrate or separate the "mast cells" (those showing metachromasia).

Peripheral Blood Smears: When the intra-uterine or intra-vaginal devices were inserted and removed, two peripheral blood samples were obtained from the tip of the finger, smeared on a glass slide and stained with Wright's staining, and leukocytes were studied.

Double-Staining Technique: Following study and photomichrography, the loopal smear, previously stained by the supravital TMK-101, TÜRK method, was restained by the Papanicolaou method, by immersion in a solution of 4 per cent TMK-101, after which the cover slip was removed, the slide was transferred to the fixative (either ether and alcohol or 80 per cent alcohol) and stained by the standard Papanicolaou procedure[29]. This method helped to bring out the intracellular inclusions and fine fibrous threads of connective tissue which may not appear during direct fixation and staining using a dye solution prepared in lytic fluid.

Results

All the women in this study who were using IUDs had various complaints during the first 6-8 weeks of use, but only a few continued to complain of disturbances after that time. The clinical effects of and reactions to IUD use, the mechanism, and the interpretation of these will be presented in a subsequent paper.

Devices placed intra-vaginally in young virgins did not cause any clinical or cytological changes.

Cytological Findings In IUD Use

The urethral, vaginal, ectocervical and endocervical smears showed findings normally associated with the cyclic hormonal status of women. In addition, all smears were bacteriologically free apart from Doderlein's acidophylic bacilli with less inflammatory changes than are usually seen.

Trichomonas or monilia infestations, which are frequently observed in routine vaginal smears, were not seen in our cases. Endocervical smears made before removal of the device showed abundant spermatozoa, even when the last coitus had occured two or three days previoulsy. No cancerous or precancerous cells were observed in any of the slides.

The most interesting and significant cytologic pictures were observed in the loopal smears, in each of which three types of cells were found: 1) macrophages, 2) fibroblasts and their fibrinal threads, and 3) blood cells (erythrocytes and leukocytes).

Macrophages were the most prominent feature, being abundant in number and striking in shape related to their phagocytic function. When the loop was removed within five days of insertion, macrophages were generally the size of a blood monocyte or slightly larger, but no giant forms or mitotic figures were observed; however, when the IUD had been in place a week or longer, 30,000 to 50,000 macrophages were seen on each slide (Figure 5), the majority of which showed some degree of phagocytosis. Neutrophilic or eosinophilic polymorphonuclear leukocytes, smaller mononuclear cells, spermatozoa, and, in most of the macrophages, several lightly stained inclusions which could not be identified were recognizable in their cytoplasm. In addition to this type of individual phagocytosis, another form was observed: an amorphous, or polyhedral, giant body which seemed to be in its original cellular form, surrounded by numerous macrophages in "collective phagocytosis" (Figure 6), but this was seen only once in all the loopal smears we examined, and could, therefore, be the phagocytosis of an ovum or blastocyst.

The loopal smear showed one of the most dramatic pictures of the phagocytic phenomenon, which can only be seen in a living tissue culture specimen. In one of these, a multinucleated giant macrophage was found in a position of attack with its petal-like pseudopods at the end of the cytoplasmic elongation, resembling the hose of a vacuum cleaner (Figure 7). The pseudopods had separately engulfed three small cells, (the beginning of phagocytosis). Mitotic figures representing all the stages of macrophageal cellular division were observed in the loopal smears, and in some cases, up to 100 were counted on one slide (Figure 8).

Foreign body giant cells were numerous in the loopal smears from patients who had used a device longer than three months, and these macrophages also showed phagocytic inclusions in the cytoplasm and mitosis of several nuclei in a single cell (polyploid) (Figure 9). Macrophages were more numerous in the first slides than in the last ones made from the same loop.

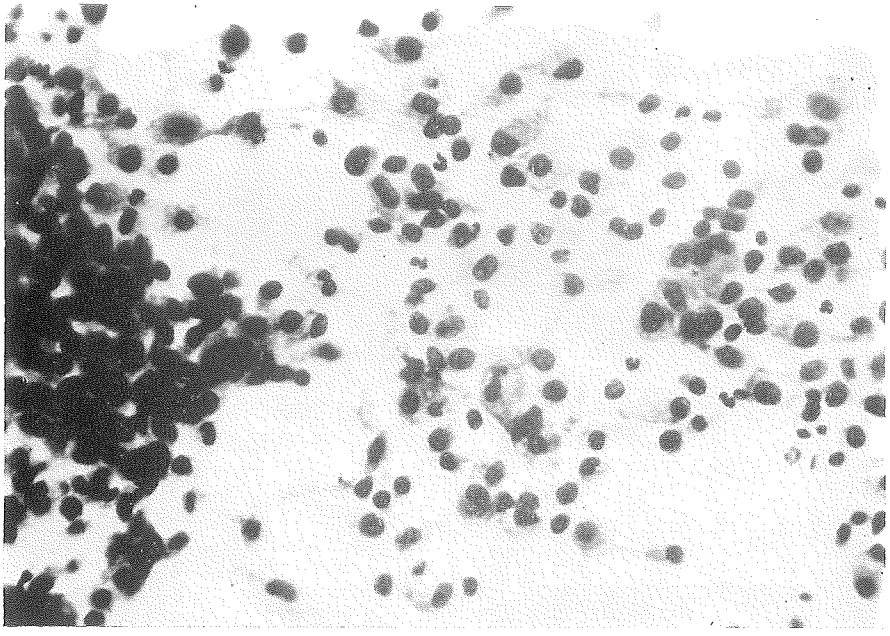
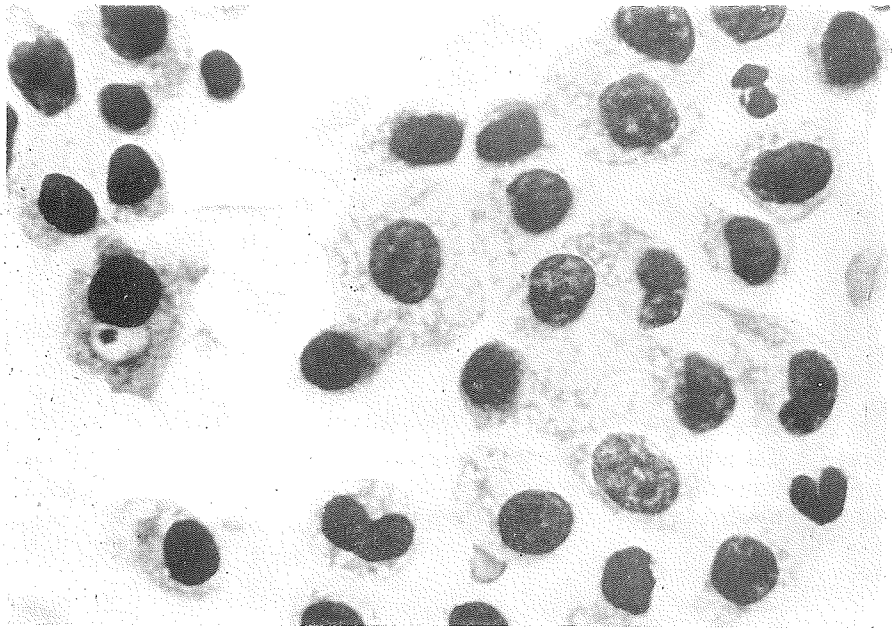


Figure 5: The edge of giant cluster of macrophages in a loopal smear (Top, 500 X); Some of the macrophages presents strong phagocytic action. Some of the material phagocytized resemble to the head of a sperm. (Bottom 1.500 X).



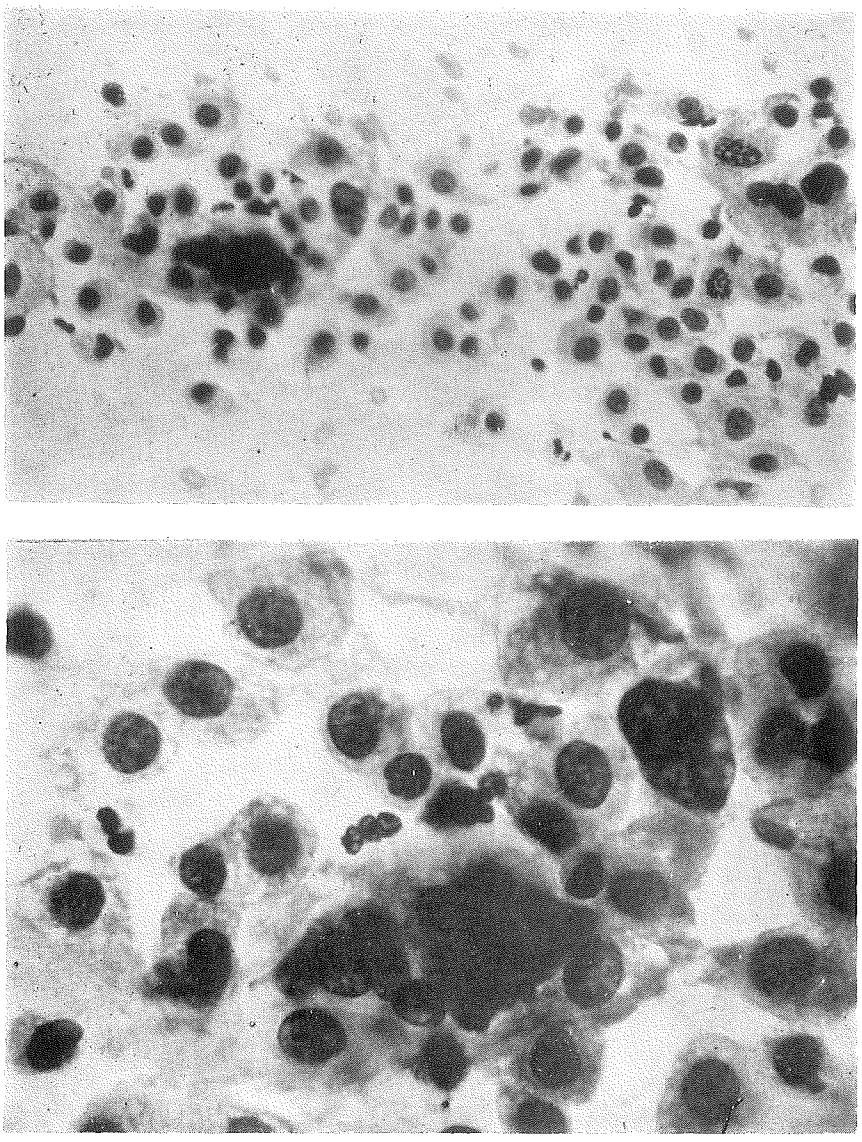


Figure 6: Collective phagocytosis of a giant-dark material (Top, left). At the right side of the same figure two mitotic divisions (metaphases) of macrophages, 500X. Bottom. Enlarged (1 500X) collective phagocytosis seen on the top. Macrophages spherically surround the unknown dark material. Ova or blastocyst probably to be phagocytized in this manner.

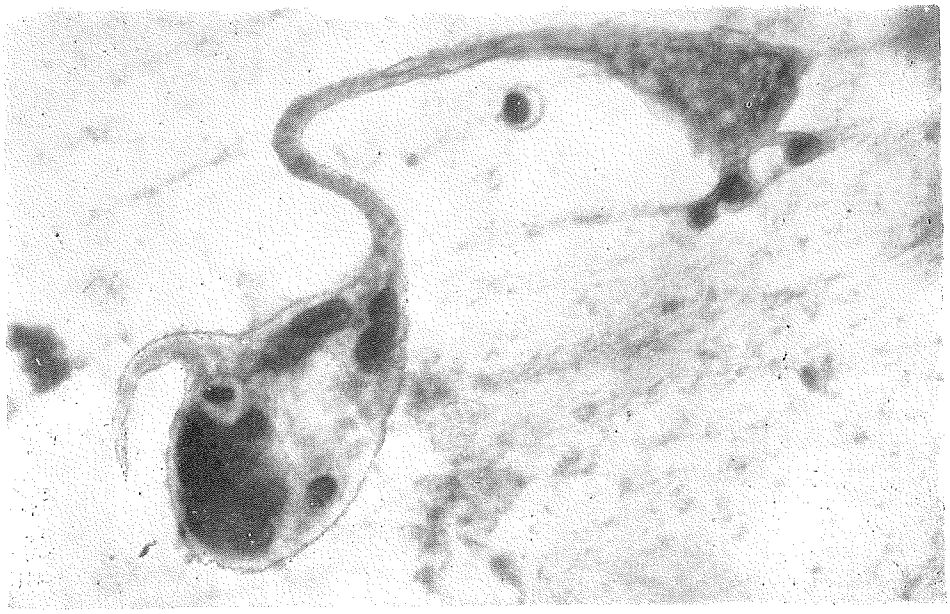


Figure 7: A multinucleated giant macrophage in action of phagocytosis, observed in loopal smear. Three fine petal-like protrusions at the end of a big cytoplasmic elongation resembles the hose of a vacuum-cleaner, grasp three pieces of globules to phagocytize. This picture proves that the cells were alive and functioning normally on the cytologic slide, prior to fixation, 100X.
From Sağıroğlu, N., and Sağıroğlu, E., Biologic Mode of Action of Lippes loop in Intrauterine Contraception, American Journal of Obstetrics and Gynecology.

Cytochemical Studies: The nuclei of the macrophages and of the phagocytized cells were stained light purple (DNA) by the Feulgen technique. Except for the very young and small macrophages which were stained light reddish-violet, they were PAS negative. The cells carrying metachromatic bodies were seldom observed in loopal smears when toluidin-blue was used, and the macrophages remained negative with Laidlaw's silver staining method (Figure 10). Macrophages took no color with phosphotungstic acid-hematoxylin to demonstrate fibrin or Van Geison's connective-tissue stain.

TMK–101, *TÜRK Stain*: Using this method, the macrophages stained a deep orange-red. Their cytoplasms were finely granular and frequently showed light yellow, lipid-like particles, and occasionally contained inclusion bodies surrounded by a clear halo. The cytoplasmic granulation and globules disappeared following fixation in a mixture of ether and alcohol, so it was concluded that they were lipids (Figure 11).

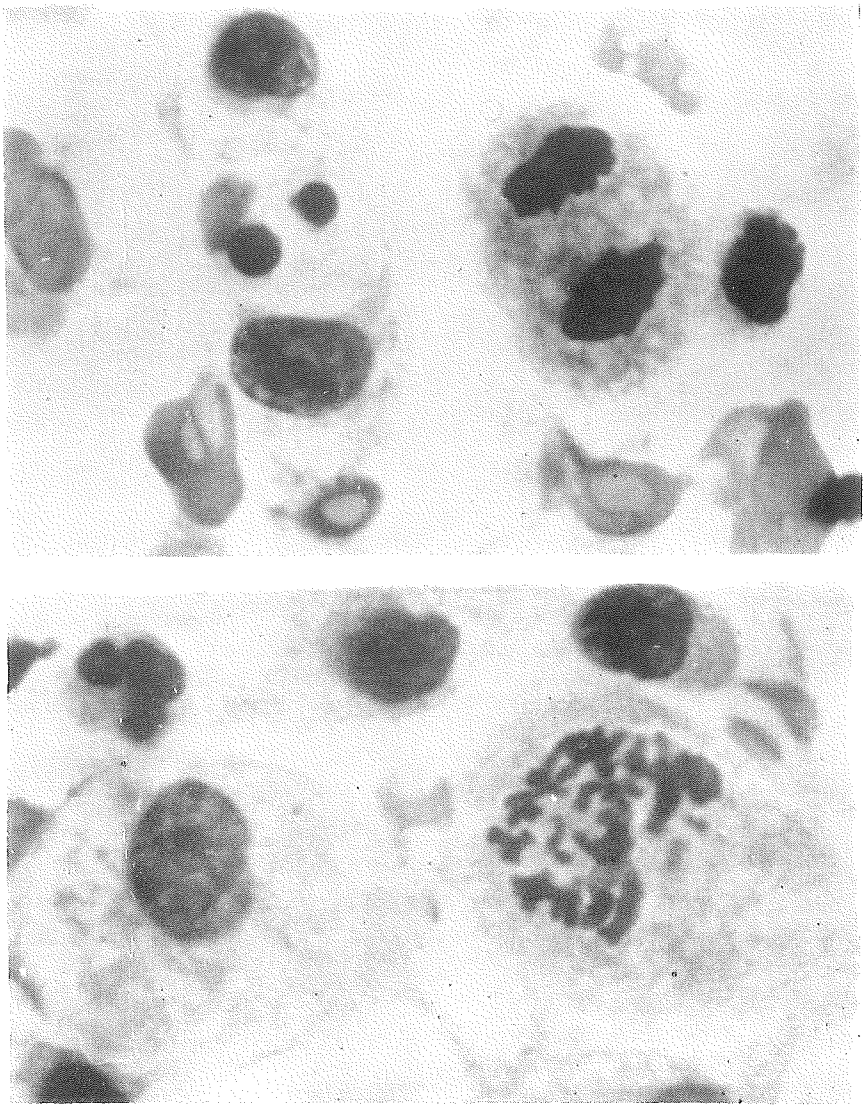


Figure 8: Mitosis of macrophages in a loopal smear. Top, a metaphase, bottom an anaphase, 200X. Macrophages placed around the loop live and multiply continually creating a loose tissue.

Double-Staining Method: Smear specimens were stained by the TMK-101, TÜRK method and, following microscopical study and photomicrography of the cells, fixed in an ether and alcohol mixture. They were then restained by the standard Papanicolaou technique, in which, more

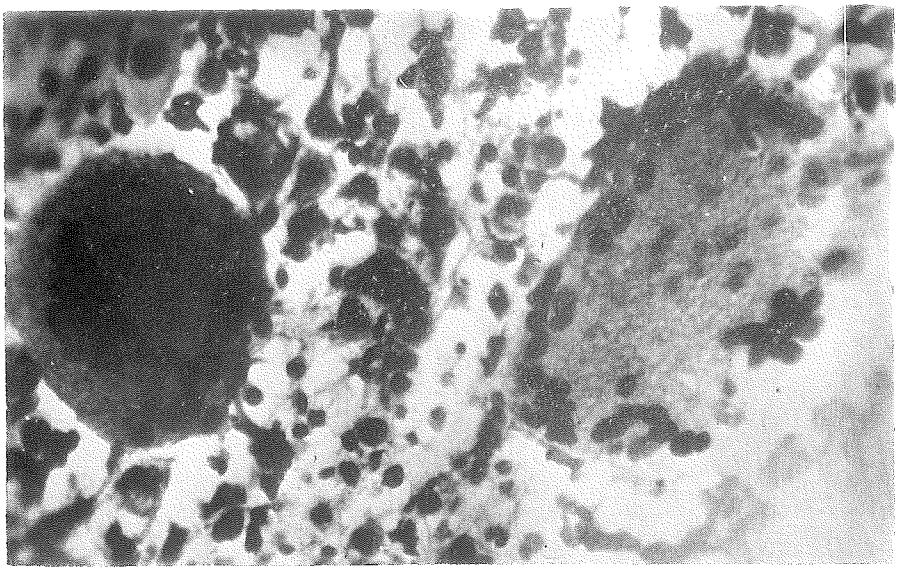


Figure 9: Different sized foreign body giant-cells observed in a loopal smear. In some slides hundreds of such cells are counted.

phagocytic inclusion-bodies were observed in the macrophages than could usually be seen with other techniques (Figure 11).

Fibroblasts in the Loopal Smears: Fibroblasts (young fibrocytic cells) were profuse, and second in number to the macrophages in smears from loops used longer than one month. They were elongated and polyhedral in shape, with a single large nucleus. Their cytoplasms were thinner and smoother than those of macrophages, inclusions were rare, and fine fibrous threads were frequently observed originating in them (Figure 12). These threads were occasionally seen in a nest-like network formation which included macrophages (Figure 13). The nuclei of the fibroblasts were larger than those of the macrophages, their membranes were thicker, their chromatin coarser, they had one or more large nucleoli and mitotic figures were occasionally observed among the fibroblasts. These were usually connected at the cellular borders or by fibrous elongations, forming small or large groups like those seen in tissue culture specimens of connective tissue. Fibroblasts were more numerous in the final loopal smears, it was thus concluded that they were placed very close to the loop, between its surface and the macrophages.

Cytochemical Studies: The cytoplasm and fibrous elongations of fibroblasts became visible with PAS positive stain; only the nuclei stained

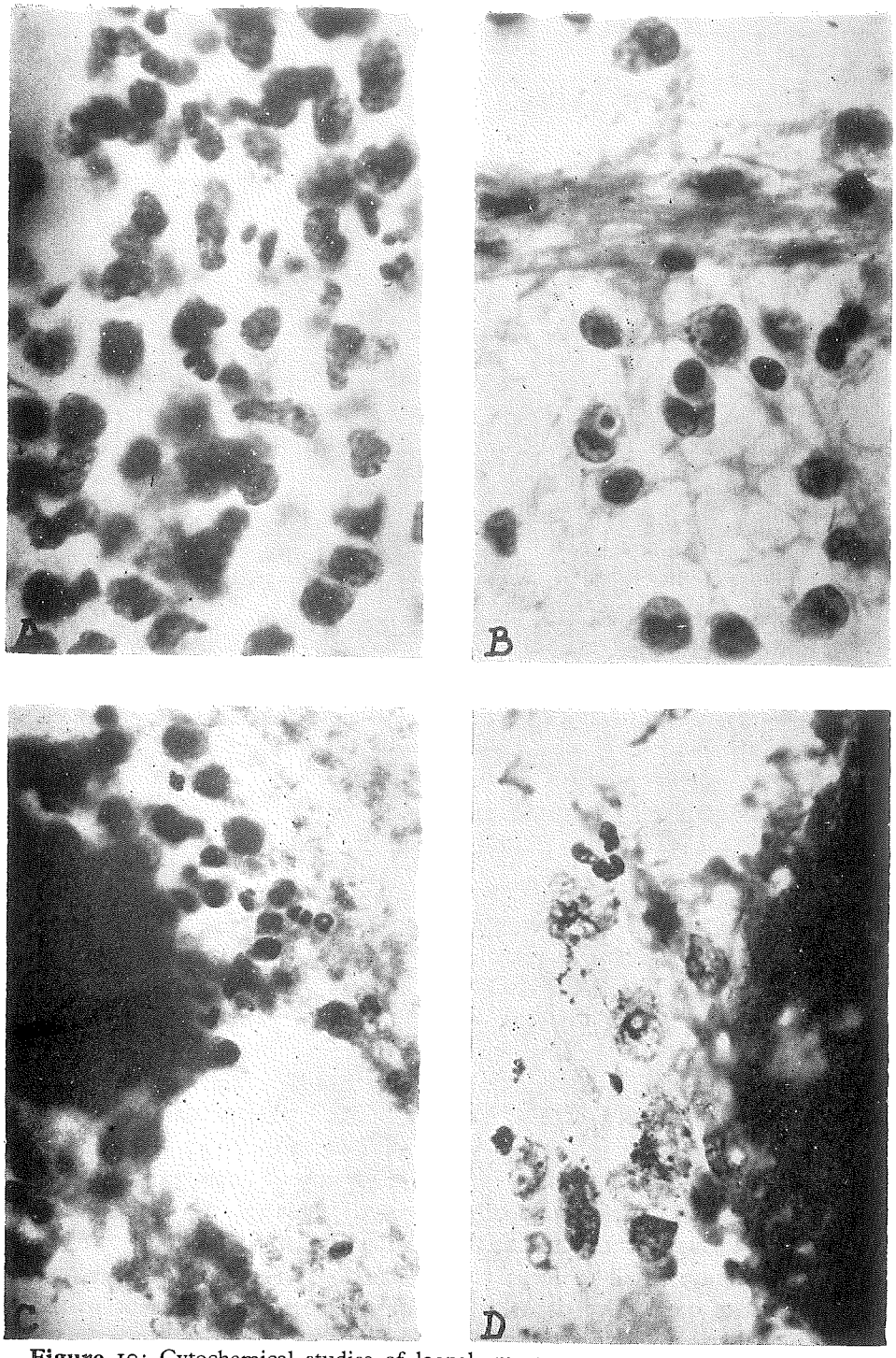


Figure 10: Cytochemical studies of loopal smear.

A– by Feulgen method only nuclei are stained

B– P. A. S. staining. Many threads of fine fibers are observed.

C– Toluidine blue staining: Cells carrying metachromatic bodies are not observed except in the earliest few day of loop use.).

D– Silver staining using Laidlaw's method. Many fine reticular fibers are observed in loopal smear.

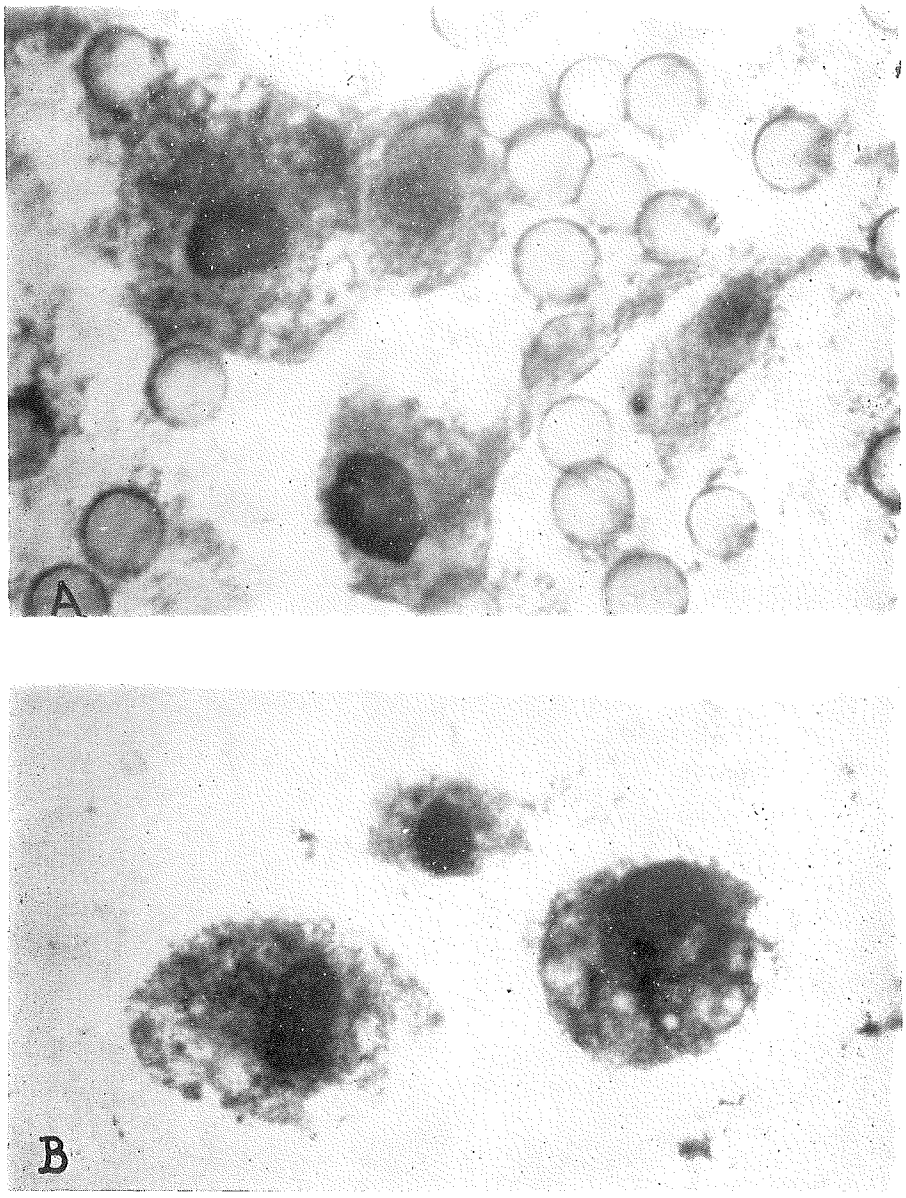


Figure 11: TMK–101, TÜRK method of staining (Top). Four macrophages are observed among erythrocytes. Many lipid-like materials in the shape of small droplets are observed in the cytoplasms of macrophages Bottom: Double-staining method demonstrates a better visualization of several inclusional bodies and their vacuoles (phagocytosis) within the cytoplasm of macrophages. 1000X.

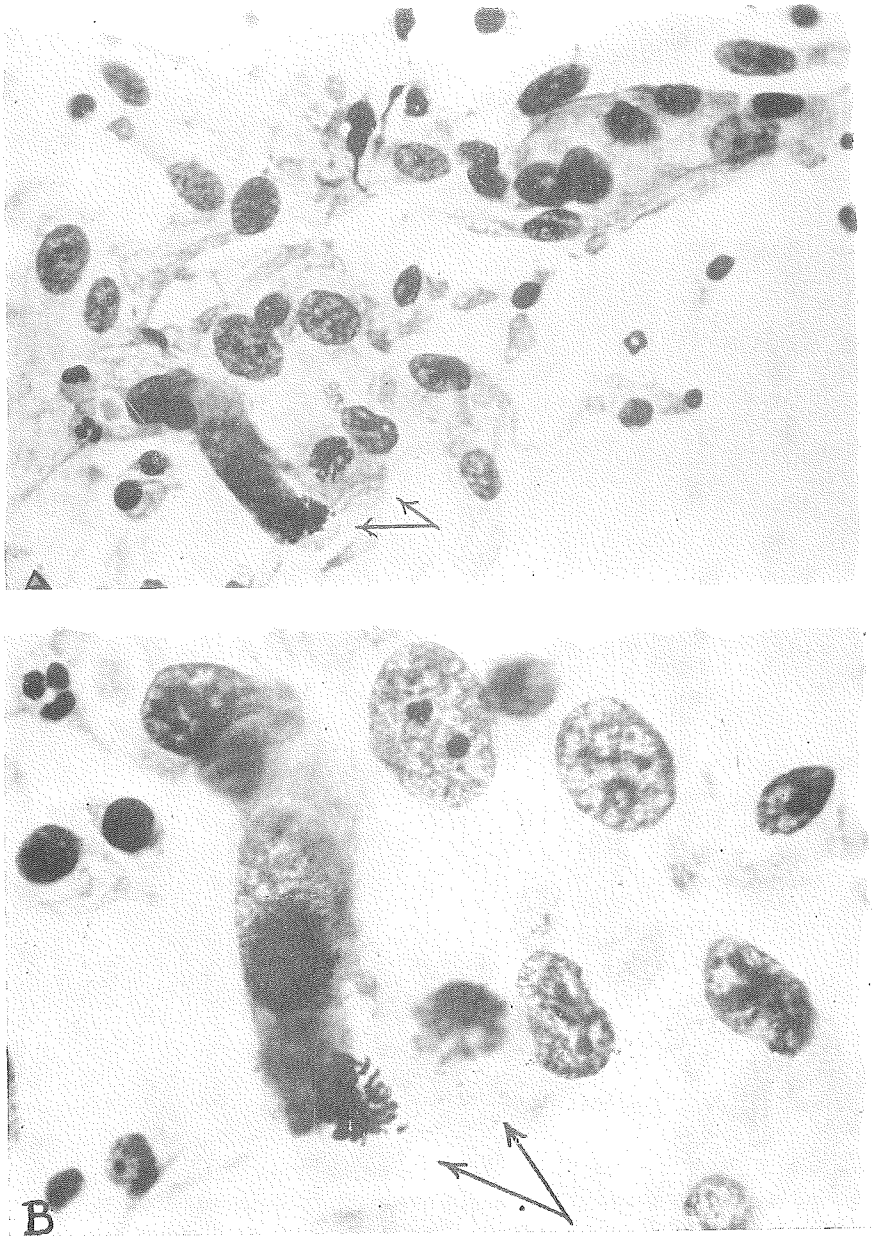


Figure 12: Fibroblasts in loopal smear, top: 600X. bottom: the left-lower section of top figure, 1500X. Arrows indicate an anaphase stage of the mitosis of a fibroblast.

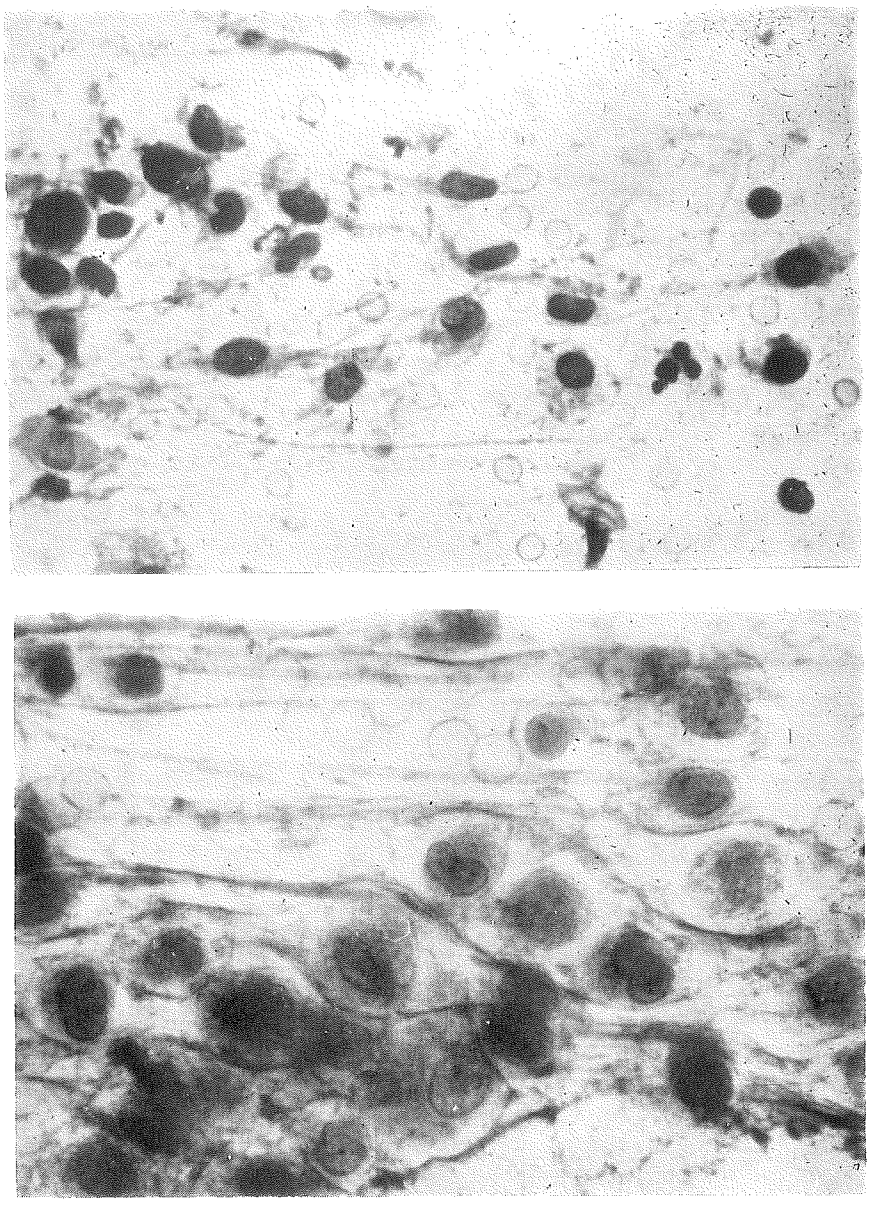


Figure 13: Loopal smear stained by the TMK–101, TÜRK Method. Top; fibroblasts and fibrinal threads, 500X. Bottom: Macrophages are seen in the nest-like spaces of a network made by fibrilar structures, 1000X.

with the Feulgen technique; toluidin blue stained the nuclei and fibrous threads from the cytoplasm deeply, and using phosphotungstic acid and hematoxylin, Van Giezon and Laidlaw's silver stains, the fibrous elongations stained clearly, showing that collagen, reticulum and fibrin threads surrounded the loop.

The TMK-101, TÜRK method stained the fibroblasts bluish-gray or light purple, while the fine fibrous elongations stained a darker blue-gray; thus, a differentiation between fibroblasts and orange-red stained macrophages was obtained, by leaving the slides in the TMK-101, TÜRK solution at room temperature for several days. Fibrinal threads become even more distinct with the double-staining procedure.

Blood Vessels: In some smears from loops which had been used for more than a year, newly forming blood vessels were found, the walls of which were made of a single layer of very young fibroblasts. No erythrocytes or leukocytes were seen in the lumen which was filled with pinkish amorphous material (Figure 14).

Blood Cells: Red blood cells were profuse in loopal smears. Polymorphonuclear leukocytes, lymphocytes and monocytes were also seen in proportions equal to those of a normal blood spread.

Neutrophilic polymorphonuclear leukocytes were abundant only when the loop had been used for a few days, and gradually disappeared subsequently. However, neutrophils were profuse in the loopal smears of one patient who showed clinical evidence of a subacute infection caused by the insertion of the loop by an inexperienced physician under septic conditions.

Eosinophilic Polymorphonuclear Leukocytes: These were observed in all loopal smears in larger quantities than are usually seen in the blood, and were even more numerous in those from loops used for less than three months (Figure 15).

Spermatozoa were occasionally seen when they were in the loopal smears, but were absent in the majority of cases, even when they were abundant in the endocervical smear; those seen were generally degenerated, and many were phagocytized by the macrophages (Figures 16).

Microorganisms: Few bacteria were observed in loopal smears from uncomplicated cases, except in those from loops taken within five days of use where coliform bacteria were seen. In all other cases except one a few similar bacteria were present which were probably the results of contamination during removal of the loop. In the one case excepted, the loop had

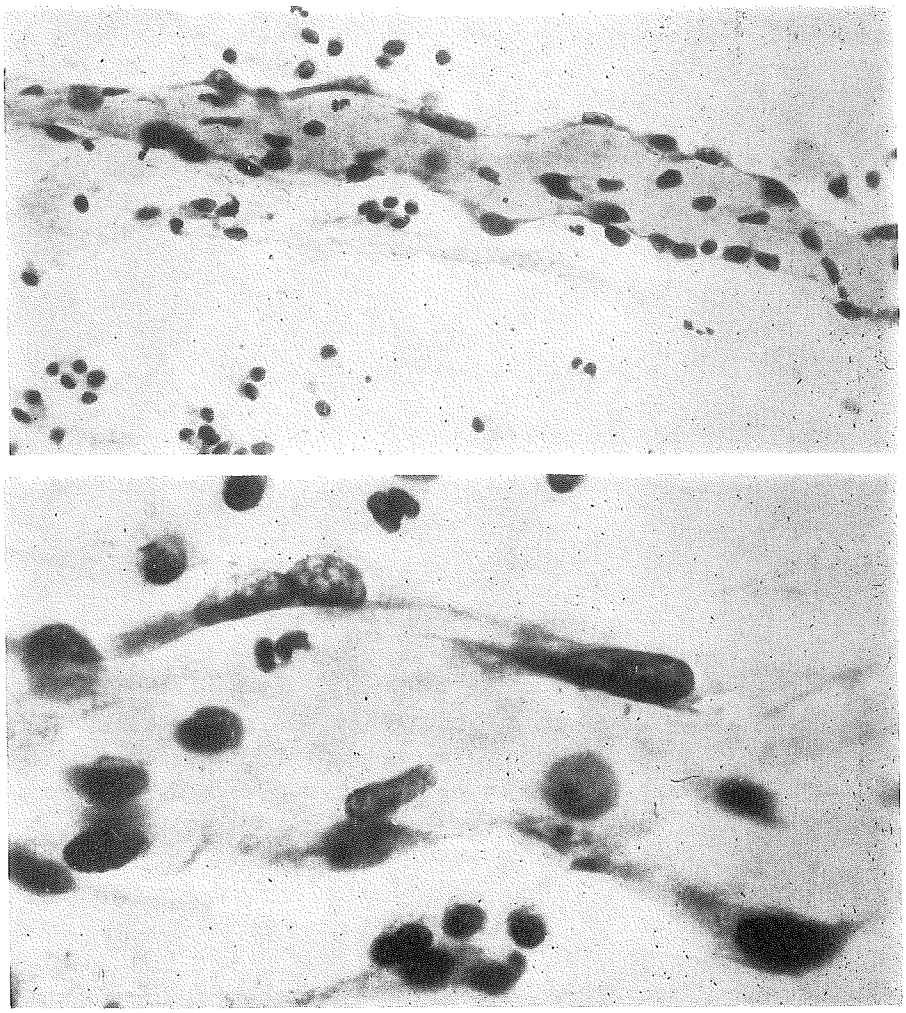


Figure 14: newly developing blood vessel observed in loopal smear, top 400X; bottom, 1000X. Young fibroblasts or reticular cells are seen at the wall of the vessel. Inner area of the vessel is filled by an amorphous material.

been inserted by an inexperienced physician two months prior to our examination and there had been abnormal bleeding continuously since its insertion, and the distal part of the device extended 1.5 cm into the endocervical canal. The loop was removed and smears made from it contained many scattered coliform, thick, short bacilli, and predominately polymorphonuclear leukocytes and macrophages.

Cytologic Studies of the Blood Collected from the External Os of the Endocervical Canal and Endometrial Aspirate Following the Removal

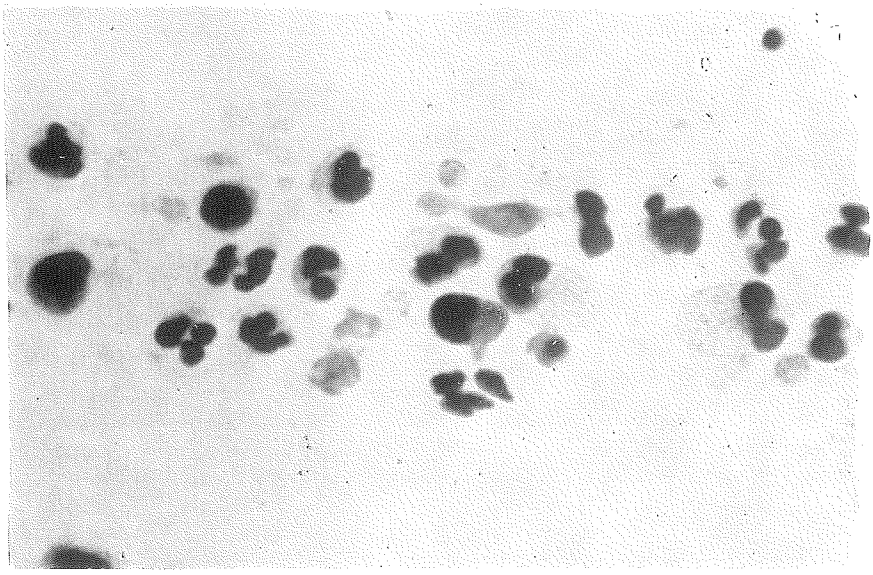


Figure 15: Large eosinophilic polymorphonuclear leukocytes frequently observed in loopal smears, 1500X.

of the Loop. When the loop was removed it scraped the endometrial surface slightly stimulating myometrial contraction, and causing blood to accumulate at the external os of the endocervical canal. In the smears prepared from this blood, enormous numbers of macrophages, nearly 50,000 on each slide, and small quantities of endometrial glandular cells and fibroblasts were seen. In those from the endometrial aspirates, huge numbers of macrophages, fibroblasts and a few endometrial glandular cells were found. Smears from the same material stained with toluidin-blue showed no metachromatic coloration (mast cells) in uncomplicated cases.

Endometrial Aspirate Smears in Control Cases

These were obtained using Cary's metal cannula from 50 symptom-free women who came to our cancer prevention clinic for uterine cancer control. Except for two patients on whom the aspirations were performed at the end of menstrual bleeding, where small groups of macrophages were observed scattered among the erythrocytes, the number of macrophages observed in these smears was not remarkable.

The IUD During Removal

During careful extraction of the loop, in every uncomplicated case where the device had been in place more than six months it appeared to

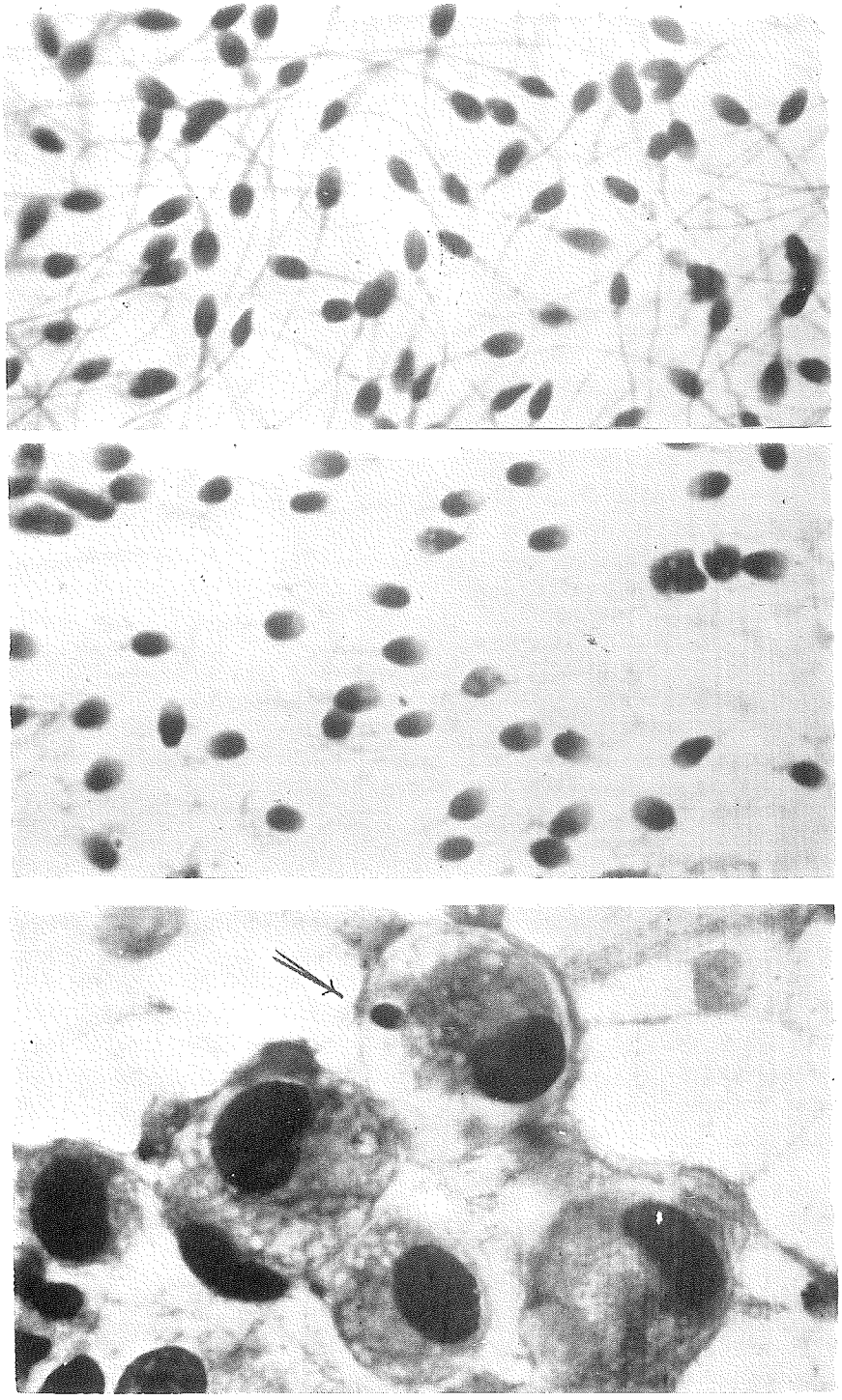


Figure 16: Top and center, spermatozoa are seen in endocervical smear. Top last coitus was 2 days ago; Center, last coitus was four days ago, 200X. Bottom, macrophages in a loopal smear. A head of sperm indicated by an arrow, is phagocytized by one of the macrophages, 1500X.

be held in the uterine cavity by numerous fine threads, resembling a three dimensional spider's web, and could only be removed by breaking these threads.

After removal all the loops were covered with irregular, thick, mucus-like opaque material. When they were removed early, this was scant and consisted of loosely attached macrophages, fibroblasts and fibrin threads, but after six months to one year in the uterus, it was abundant and firmly attached to the surface of the loop. When the IUD had been used over two years there were small fragments of tissue-like material here and there under the mucus cover, firmly attached to the surface of the loop, in which fibroblasts, fibrocytes and fibrinal threads predominated, and a few macrophages were seen. There were also some microscopically visible areas of erosion on the surface of the loop, under and between the tissue-like fragments, so that the surface resembled that of a file, and the loop looked like a piece of rusty iron wire (Figure 17.)

Histologic Studies of the Endometrium

Serial sections of endometrial tissue were studied using standard hematoxylin-eosin and histochemical staining techniques. Regular endometrial changes were observed in all material relative to the day of the cycle on which the loop was removed and the tissue obtained. In addition, a slight degree of round cell infiltration, enlargement of blood vessels, superficial fibrosis of the stroma and, in one case, chronic endometritis were noted. Bleeding into the stroma was frequently observed, but this was probably the result of scraping the tissue.

With chemical stainings (PAS, Van Giezon, phosphotungstic acid-hematoxylin, toluidin blue, and silver-Laidlaw's) the threads of connective tissue, called collagen, fibrin or reticulum, were found to have increased in some areas of the section near the surface, Figure: 18. In the slides stained by toluidin-blue occasional cells were noted with metachromatic bodies (mast cells).

Comment

In this investigation a new cytologic technique, a "loopal smear"[30] (direct contact smear of the Lippes loop freshly removed from the uterine cavity), was employed as well as the classical cytologic and histologic methods. The advantages of this new method are as follows:

As indicated above, when the device is in the uterine cavity it is surrounded by foam-like loose cells (macrophages and fibroblasts) within a

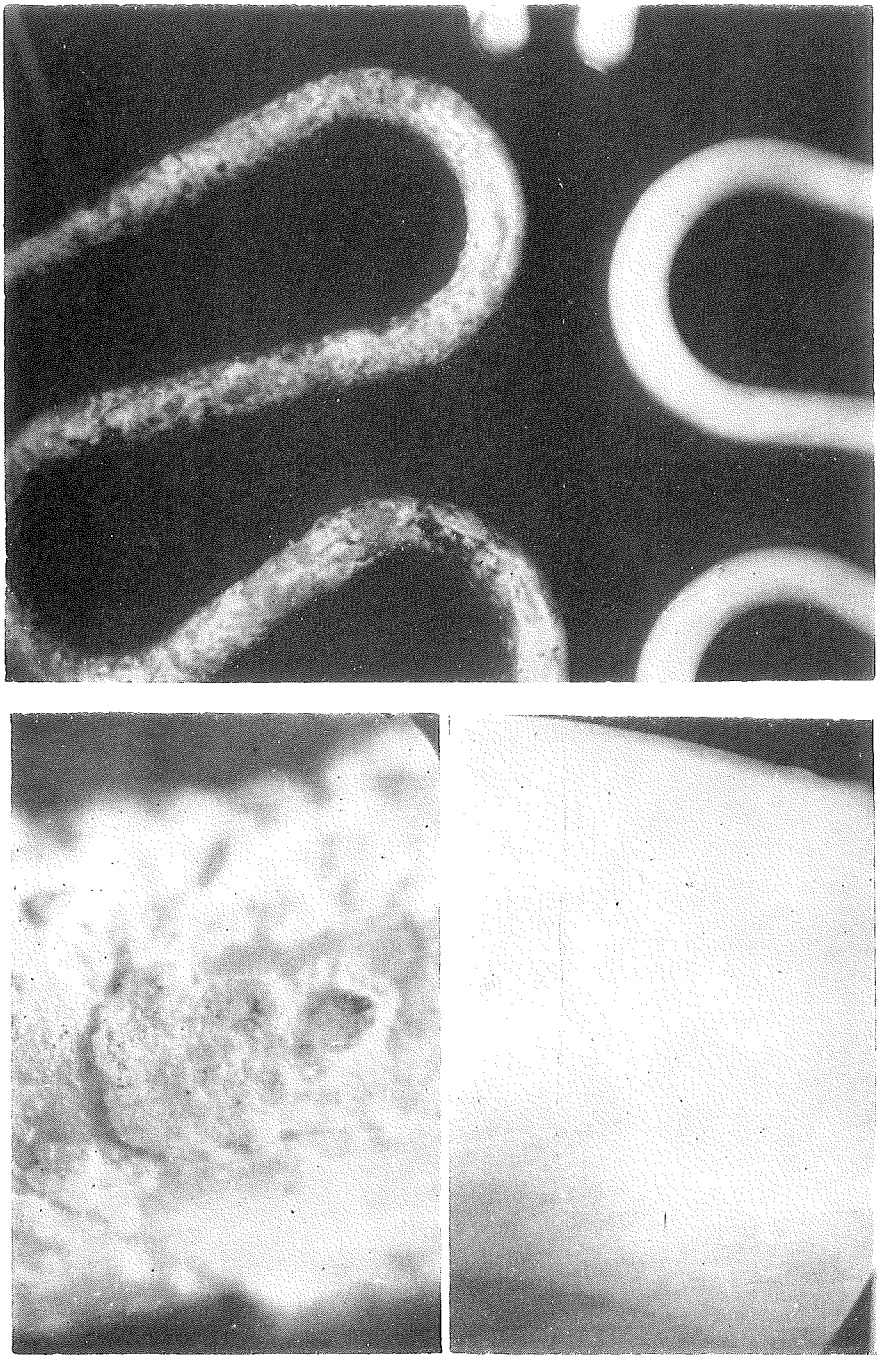


Figure 17: The Lippes loop used for 26 months continually as IUCD. Top, direct photography comparing with unused one, 2X. Bottom photomicrography, comparing with an unused one at right, 28X.

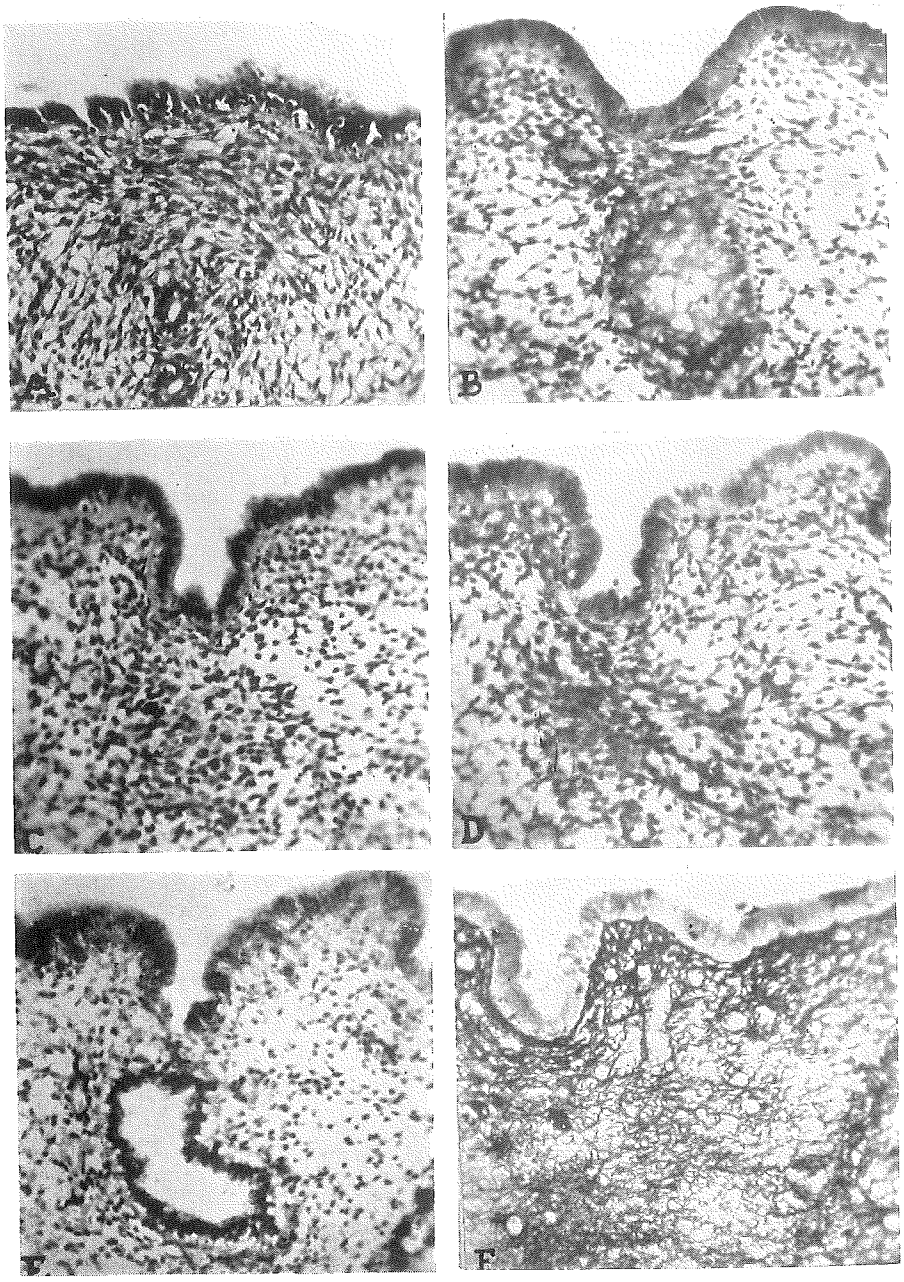


Figure 18: Studies of endometrial tissue after a long during intrauterine device use. Serial sections: A- H+E staining shows local fibrosis; B- PAS staining C- Van Giezon's staining, D- Toluidin blue, E- Phosphotungstic acid- Hematoxylen; F- Silver staining. Fibroblast, fibrocytes and reticular fibers are slightly increased at the surface area of endometrial connective tissue, 250X.

web of fibrous threads and fluid exudate. When the device is extracted a portion of this foam-like material remains attached to the loop, but in a collapsed form. Spreading the fresh, unfixed material surrounding the IUD onto a glass slide causes the cells and fibrin threads to reopen, simulating their natural loose morphology in the uterine cavity; thus, the material may be unequivocally seen and studied. Using this technique for the first time in the study of the IUD's effects, we were able to find a huge number of macrophages and their action, which is phagocytosis.

From the average 100,000 macrophages counted on the four slides made from one loop, and the same number counted in two slides made from the blood and endometrial aspirate after the removal of the devices, it was concluded that there are millions of macrophages in the endometrial cavity when the Lippes loop is in place (Figure 5). Previous studies have failed to demonstrate this because the material was not examined in its original and natural position, and the most suitable techniques, direct contact smears and cytology, for mobile and loose cells were not employed. Potts and Pearson[31], combining light and electron microscopy, studied the correct material, namely the cells in contact with the IUD, but their method was rather destructive to the fine, foam-like loose tissue. They put the device into a fixative and then cut sections for histological examination.

In another report, Sammour, Iskander and Rifai[32] combined histology and cytology in their study of intrauterine contraception, but they investigated the endometrial tissue and cervico-vaginal cells. Ours is therefore the first proper use of cytology to study the material in the endometrial cavity.

Macrophages are the most powerful phagocytic cells of the body;[31-33] they ingest live and dead cells, cellular debris and nonabsorbable materials including polyethylene[23 34 35] from which the Lippes loops used in this study were manufactured. The phagocytic and/or digestive effect of the macrophages on the loop can be seen and measured (Figure: 17). Live mycobacterium tuberculosae, an extremely resistant (*in vivo* and *in vitro*) acid-fast pathogenic bacteria can only be actively caught by macrophages and digested by phagocytosis.[36] Macrophages are mobile, rapid and with ameboid flexibility; they produce an enzyme, "protease", which is active in the digestion of organic material, and especially productive in an acid medium such as the endometrial surface.[31 36]

When the Lippes loop is inserted into the uterine cavity, macrophages emerge from every point of the endometrial surface surrounding the

device. Leukocytes are also present for the first four to five days, and endometrial fluid-exudate, which is increased, pours into the cavity.

These phenomena are a typical reaction against foreign matter, and the human body rejects the loop as it does a surgical organ transplantation. The first such reaction against the IUD is the contraction of the myometrium followed by endometrial bleeding and increased secretion of the tissues. These, however, are not enough to expel the loop from the uterus through the narrow internal os of the endocervical canal. Because of this failure, the body sends her "light brigades", the macrophages, to charge and isolate the foreign body. In a relatively short time millions of macrophages accumulate in the endometrial cavity and surround the device, and fibroblastic activity follows next in closer contact with it. Thus, the human body applies a rather long-lasting and gradual absorption policy against the foreign matter.

The Contraceptive Action of the Device

Under normal conditions, as supported by the findings in the control cases, there are no macrophages in the endometrial cavity, except in the post menstrual phase when the macrophages appear to eliminate the debris. These disappear completely before ovulation. Progesteron puts the endometrium into a condition where there is no cell or secretion present to inhibit implantation of the ovum.[30]

The fertilized ovum or blastocyst is in fact, a foreign matter, and an iso or a heterozygote because of the protein of the sperm,[37] not much different from a parasitic egg. It does not, however, cause a reaction against itself because it is covered by perfect isolators: the cells of the corona radiata and the shell of the human ovum (zona pellucida), both belong to the host. Thus, it travels safely through the fallopian tube and attaches to the surface of the endometrium. At the moment of implantation, the protective coverings disappear and free trophoblasts attack the unaware endometrium.

Implantation thus becomes possible through unavoidable damage to the unprotected endometrium, and from this moment until the fourth month of pregnancy the complaints and derangements, which are physiological, result from the body's awakening defensive reactions against the foreign matter. These, however, are adapted to and controlled after the fourth month.

In contrast, if the device is in the uterine cavity, the endometrium is no longer a welcoming bed for implantation, but a hostile environment,[38] filled with millions of macrophages. When the ovum or blastocyst enters

the uterine cavity, it is not the trophoblast which attacks the endometrium, but the macrophages, piranha-like bodyguards, which attack the ovum. (Figure 19)

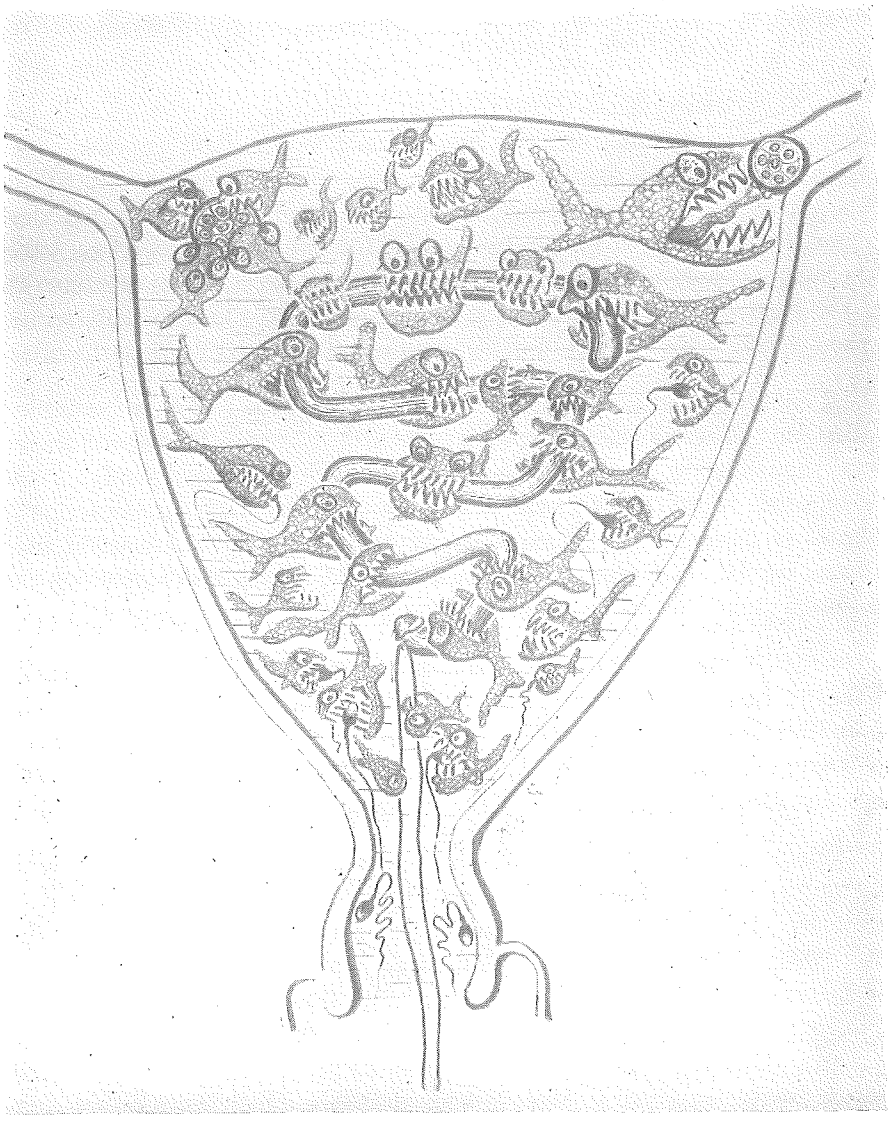


Figure 19: The macrophages filling the endometrial cavity during the use of IUCD, trys to destroy all foreign material by their phagocytic and anzymatic actions. Resembling the piranha they attack the IUCD continually and phagocytize the spermatozoa and the ova whenever they enter the endometrial cavity.

Implantation cannot occur because:

1. Foam-like, spreadable, cellular bodies composed of enormous numbers of macrophages completely cover the endometrial surface - the implantation area - and separate it from the ovum.

2. The macrophages isolate the blastocyst and eliminate it through collective phagocytosis. (Figure 6).

3. The macrophages secrete protease, which is active in an acid medium like the endometrium[31]. Protease is a strong lytic enzyme which acts on protein and organic exudate, and probably helps to digest the zona pellucida, an exudate and protein. The trophoblastic cells lose their protective covering when they are in an immature state, and can easily be phagocytized and destroyed by the macrophages. The degeneration of the blastocystic cells observed in the endometrial washing material obtained while the device was in place was probably the result of this action.[6]

4. Macrophages are believed to play an important role in immunity, and at least possess a reaction related to it[33 34 39 40-43] It has been shown that they carry "cytophilic antibodies" which attack the antigen, the IUD, stick to its surface and accumulate around it. A similar action is performed against the fertilized ovum, and the ovum or blastocyst is thus isolated by the macrophages, which prevents the trophoblast's invading the endometrium. This could be termed "rejection of the blastocyst".

5. Spermatozoa are also foreign bodies or foreign protein to the female tissue.[44] Because of their speed, they do not mobilize and attract the macrophages during their passage from the cervix to the abdominal cavity following ejaculation into the vaginal fornices. When they lose their speed, they are mostly phagocytized by the peritoneal macrophages.

When an IUD is present, the endometrial cavity is filled with macrophages, and the avenue leading to the ovum is blocked, at least partially, by the mechanical and bio-chemical-enzymatic presence of the macrophages. The majority of spermatozoa are unable to enter the endometrial cavity, and only a few are able to pass through the hostile environment and reach the fallopian tubes.[32] In some patients in this study, large numbers of spermatozoa were observed in the endocervical canal and only a few, when present, were found in the endometrium (Figure 16). Some investigators have observed spermatozoa in the fallopian tubes when the device was in place.[8 14 45] Others concluded that they disappear more rapidly when there is a device in the endometrial cavity[46]. When a device is present spermatozoa are arrested and destroyed in the

uterus by the presence of millions of macrophages (Figures 5 and 16) and the fertilization phenomenon becomes very rare or impossible, which prevents pregnancy.

The preventation of pregnancy due to a device and the macrophages in the uterine cavity, may be explained in the following manner: the millions of macrophages resemble vaginal foam contraceptives (i.e. Emko) which are injected into the vagina before coitus. The difference is that they are located in the uterine cavity, and destroy the spermatozoa and ovum biologically as well as chemically.

The eosinophilic polymorphonuclear leukocytes and lymphocytes observed in the loopal smears are part of the reaction against the loop, as well as an immunity reaction,[31 36 47] which supports our theory. Increase of these cells and monocytes in the circulating blood prove that both a local reaction in the endometrial cavity, and a systemic or generalized one against the IUD develop. Eosinophilic luekocytes are also phagocytic cells, and stimulate this function by the secretion of histamine.[47]

After long-term use, loose fibrinous threads (distally) and fibrin producing fibroblasts and fibrocytes (proximally) were observed around the device. The presence of these connective tissue elements and their product, the new blood vessels (Figure 14), may also be interpreted as the permanent isolation of foreign matter.

Thus, whenever a foreign body (e. g. a Lippes loop) is inserted into the uterine cavity, the human body's immediate reaction is to send the polymorphonuclear leukocytes lymphocytes, eosinophils and a seepage of serum fluid exudate against the invader. This is followed within a few hours by migration of the macrophages into the cavity.

From the fourth to the eighth day, macrophages loosely fill the endometrial cavity encountering the device, assuming of course, that there are no complications such as displacement of the device or considerable bleeding. Infiltration of fluid exudate and macrophages were obtained experimentally by placing a piece of glass - an inert foreign body - in a window opened into the skin.[35 48]

Loose fibro-cellular tissue surrounding the device and filling the uterine cavity may be damaged and partially lost by each menstruation, but it is probably rebuilt more strongly because of the stimulation of the debris. The presence of a huge number of macrophages (hundreds of mitotic figures of macrophages were present on each specimen slide from patients where the device had been used for one month to one year) supports this interpretation (Figure 8). Following the sixth month of use,

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the device is covered by well-organized sheets of tissue containing blood vessels constituting permanent isolation of the foreign matter (Figures 12 and 14). The uterus containing a device surrounded by many millions of loose cells and fluid exudate will naturally be heavier than normal, but they misinterpreted this weight increase as hyperplasia of the myometrium.[4]

ENDOMETRIAL TISSUE STUDIES have contributed very little to understanding the effects of IUD use.[3-5 10-14 19 49-51] During device use the endometrium showed regular cyclic hormonologic, enzymatic and morphologic changes.[11 15] Functionally, myometrial activity, which was reported to increase during device use by some authors,[52 53] was considered within normal limits by other.[54]

In our experience, local fibrosis was occasionally observed in patients who had used the device for more than one year. Histochemically there was no significant change found using PAS and Feulgen techniques, but Van Giesen, TMK-101, TÜRK, Fibrin and Silver staining techniques revealed an increase of young connective tissue elements, fibroblasts and fibrous threads in some superficial foci of the endometrium and in the surrounding area of superficial blood vessels (Figure 18). Toluidin blue staining showed mast cells in relatively normal numbers.

Intra-vaginal use of the device in young virgin produced no cellular differences in smears made from vaginal, urethral and peripheral blood materials. Vaginal and urethral smears showed complete accordance with normal, regular cyclic changes, from which it was concluded that the device cannot create a reaction through the stratified squamous epithelium of the vagina.

Discussion

As was clearly shown in this study, all reactive phenomena occurring in IUD use takes place in the space between the surfaces of the endometrium and the device in the uterine cavity, and not in the endometrial tissues. It is therefore natural that no measurable organic or functional reaction of the latter could be found when it was investigated to determine the device's mode of action.

Because the material in the uterine cavity is composed of loosely constructed foam-like cells and fibrous threads in a fluid exudate, cytologic rather than histologic techniques must be used to study it.

None of the many papers published on this subject mention the large number of macrophages found in the uterine cavity related to the IUD

One or two authors reported a few macrophages seen in the inflammatory exudate, but their presence was not discussed. In one such report where electron microscopy was used, failure to demonstrate the millions of macrophages surrounding the device probably resulted from unsuitable handling of the foam-like material. These researchers fixed the material in its collapsed form attached to the device and used the histological sectioning method[55].

There is much speculation on the mode of action of IUD's. One theory is that the device causes inflammation of the endometrium making it unsuitable for implantation,[6 50 51] but this can easily be refused because it has repeatedly been shown that an IUD aseptically and correctly inserted does not cause inflammation, and may even cure mild genital infections.[56 57]

Rapid and therefore immature transportation of the ovum, because of increased peristalsis caused by the device, was another of the theories widely believed to prevent pregnancy.[4] Evidence for this in human beings is insufficient, however animals wearing IUD's have shown that increased tubal motility has no contraceptive effect.[22] In cases where ovum transportation is slowed down in the tubes, the percentage of tubal pregnancies should increase but instead, a relative decrease has been observed among women using devices[58].

The motility of the IUD, under the influence of increased myometrial activity, may cause disturbance of the ovum when it arrives in the uterine cavity, so that the fertilized ovum or blastocyst cannot implant itself.[16 59] On the other hand, a device big enough to completely fill the uterine cavity is almost immobile, and a large one is the most successful in preventing pregnancy.[17] Other writers have shown experimentally that there is no chronic myometrial movement to cause continual displacement of the device.[54]

Change of endometrial pH from acid to alkaline by seepage of the alkaline endocervical mucus in an upward direction through the endocervical part of the device, has been thought to prevent implantation,[16 38] but an alkaline pH in the endometrium has not been proved by other workers. Endocervical mucus secretions easily flow in a downward direction; the endometrial secretion filling the uterine cavity (described in this paper) forces the fluid in a natural downward direction, and the fluid in the endometrial cavity keeps its acid character. In addition to this, the metabolic activity of millions of cells in the uterine cavity will produce acid and remain acid throughout device use.

Increased myometrial-prelabor-like contractions, which disturb the fertilized ovum under the local influence of the device, has also been considered as a cause of contraception.[52 53] If this theory was correct, some pregnancies with lower segment implantation and/or abortion should be observed; but none has yet been reported, neither has an effective chronic movement of the uterus.

Some minor structural alterations of the superficial stroma due to the direct influence of a foreign body in contact with the endometrium has also been reported to cause antifertility.[3 10 59] We did not find any remarkable change in the endometrium which would prevent implantation, neither is there evidence that a fertilized ovum can implant itself at almost any point between the endocervical canal and the ovary. Though many of the regions where ectopic pregnancies develop are extremely unsuitable compared to the minor alterations of the endometrium, the pregnancy may reach term.

Prevention of hormone use or creation of a hormone imbalance by altering the permeability vascularity or enzymatic pattern of the uterus with an IUD,[49 50] has also been thought to prevent conception, but neither can be supported. In our study the endometrium of women using devices quite frequently showed regular cyclic changes.

Histamine was found in increased amounts in the endometrium where there was an IUD; its influence on the endometrium may cause the mast cells to discharge heparine and histamine globules[60] during the early weeks of use. These two chemicals may cause dilatation of the superficial vessels[61] and serum and/or blood infiltration into the tissue.[49] Slight contraction of the myometrium may also be expected, but these changes are not sufficient evidence to prove antifertility. Almost all our patients reported that following the second month of device use, spotting, discharge, and pelvic pain or discomfort disappeared.

Probably the most significant theory before our presentation was that the spermatozoa were destroyed by polymorphonuclear leukocytes in the uterine cavity in the toxic-inflammatory secretion caused by the devices.[62] However, there was no evidence to support this speculation. In fact, spermatozoa are destroyed in the uterine cavity, as we shown, not by polymorphonuclear leukocytes and/or toxic-inflammatory exudate, but by the phagocytic and enzymatic actions of the macrophages.

No hyperplasia or carcinomatous changes were observed in the cervical, endocervical or uterine epithelial tissues in the cases studied. Similar results have been obtained by others,[58 63] and one can conclude that the IUD does not cause malignancy.

Summary

This systematic study has solved the question of how an intra-uterine device acts as a contraceptive in human beings. The mechanism of this action is biological and based on the principles of cellular immunity. The device, a giant antigen inserted into the uterine cavity, creates a strong foreign body reaction resulting in the rapid accumulation of millions of macrophages. These cover the endometrial surface and surround the foreign body, including the blastocyst, resulting in double sheets of isolation of the ovum from the implantation surface. Macrophages actively phagocytize the fertilized ovum and the spermatozoa in the uterine cavity, thereby preventing pregnancy in the most natural and biological way.

The IUD which creates this amazing phenomenon, is only a bait in the stimulation and accumulation of the macrophages in the uterine cavity.

The discovery presented in this paper has opened the door to several new avenues of research in preventive medicine and the basic sciences.

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An Electron Microscope Study of the Mast Cell of the Inguinal Lymph Node of the Rat

Aysel Şeftalioğlu, D.V.M., Ph. D.*

Introduction

Ever since the identification of the mast cell[1], innumerable studies carried out in this field have failed to yield satisfactory results concerning the morphological characteristics of the mast cell. Observation on the main organelles of the mast cell, i.e. mitochondria, the Golgi complex, and cell and nuclear membranes, have in particular remained obscure. As a result of electron microscope studies of ultra-thin sections of various mammalian tissues, it has been possible to obtain extensive data during the last 20 years about the morphology of a normal mast cell. Such studies have been made on mast cells of the peritoneal cavity of the rat, mouse and hamster,[2 3 4] the dermis of the mouse[5], subcutaneous [6 7] tissues and skin of the mouse, rat and hamster, the spleen, mesentery and liver capsules[2], and thymus of the rat[8], the lungs, omentum, submaxillary gland and pleura of the rat and man[9], the tonsils of man[10], and skin, the gastric mucosa and colonic mucosa of man.[11 12]

Although various histochemical and ultrastructural aspects of the mast cell have been identified, the development of staining and electron microscope techniques has maintained the already existing interest in these cells. [13-20]

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Burton[21], using the astra blue-safranin staining method on the adult and embryonic connective tissues of the rat, described the development of the mast cell according to the characteristics of its staining properties. He observed that there are three types of mast cells in adult tissues:(I) Astra blue positive mast cells or "blue" cells (2) Safranin positive mast cells or "red" cells and (3) Intermediate forms or "Mixed"cells.

At the suggestion of Burton's findings, a study was conducted on the mast cell of the inguinal lymph node of rats stimulated with histamine liberator 48/80 in order to investigate what histochemical and morphological changes would take place as a result of applying the same staining method.[22] It was established that the 48/80 histamine liberator caused a decrease in the number of mast cells at first but that a regeneration of mast cells occurred after a while.

The result obtained supported Burton's findings, so it was concluded that the blue (astra blue-positive) cells were immature, the red (safranin-positive) ones adult, while the mixed cells represented a transitional form in between the two types. It was therefore decided to study the three types of mast cells of the inguinal lymph node of the rat with different staining properties under the electron microscope.

Materials and Methods

In this work normal adult male rats, each having an average weight of 250-300 grams, were used. The left and right inguinal lymph nodes of the rats were obtained in good condition under chloroform narcosis and were studied in the following manner:

1. Tissues which had been placed in a few drops of fixative solution in a special wooden container were sliced into extremely thin pieces with a razor blade under the stereomicroscope.

2. The preparation of the fixative solution: 4 to 24 hours at 0°-5°C in six per cent unpurified gluteraldehyde in 0.067 M Sorensen phosphate buffer at pH 7.5 into which 100 miligrams of astra blue chroma 10110 (Fisher scientific CO.) dye was added just before use.

3. The tissue slices were kept four to 24 hours at 0° - 5°C in 0.1 M phosphate buffer - 7.5 per cent sucrose wash pH 7.4.

4. These were subjected to post-fixation for one hour at 0° - 5°C in two per cent osmium tetroxide in 0.2 M s-collidine buffer, pH 7.4. [23]

5. The tissue slices were kept at room temperature for dehydration according to the following process[17]:

50 % Alcohol	15 minutes
60 % Alcohol	15 minutes
70 % Alcohol	1 hour
80 % Alcohol	15 minutes
90 % Alcohol	15 minutes
96 % Alcohol	30 minutes
100 % Alcohol	2 x 30 minutes

6. The tissues rotated for one night (about 14 hours) in a rotator with 25 revolutions per minute in a mixture of Araldite 502 and Dodecenyl Succinic Anhydride (DDSA) 1: 1.

7. The tissue slices were transferred into a mixture of Araldite 502 and DDSA (1:1) and two per cent Benzyldmethylamine, and first rotated at room temperature for two hours then in a paraffin oven at 40°C for two hours.

8. The tissues embedded in OO gelatine capsules * filled with the second mixture were subjected to polymerization for 24 hours in a paraffin oven at 40°C and for 48 hours in paraffin oven at 60°C.

9. The paraffin oven at 60°C was turned off after 48 hours and the capsules containing the tissues were left to cool down gradually in the oven for 24 hours.

10. The silver sections were obtained by using Porter - Blum MTI ultramicrotome and glass knives. These sections were collected on unsupported grids (Netze Grill) three mm in diameter, with 200 holes.

11. These sections, stained with uranyl acetate and Reynolds[24] lead citrate staining method, were studied under electron microscopes, Carl Zeiss EM 9 and Carl Zeiss EM 9A.

12. The electron micrographs were taken using Kodalith LR film (Ester Base) and prints were made on Ilford 5 and Agfa Rapid.

Observations

It was possible to identify the mast cells in the tissue sections with the help of the existing characteristic granules. The shapes, granular struc-

* Elly Lilly and Co. Indianapolis, U.S.A.

tures and organelles were all taken into consideration, and as a result three types of mast cells were observed. These were as follows:

I. Immature mast cells.

II. Mixed (intermediate) mast cells,

III. Mature mast cells.

I. The immature mast cells: This type, resulting from the differentiation of the primitive reticular cells, probably corresponds to the astra blue positive mast cells under the light microscope.

They are generally oval in shape, their surfaces show no specialization and are surrounded by a unit cytoplasmic membrane. Numerous ribosomes, polysomes and granular endoplasmic reticulum with narrow cisternae, many oval mitochondria, and a Golgi complex including tubuli, vesicles and vacuoles can be observed in the cytoplasms of the cells (Figures 1, 2, 3, 4, 5 and 6).

In this type of cell, described as immature mast cells, characteristic morphological findings were noted in their cytoplasmic granules. These granules, which are few in their first phase of maturation, seem to migrate towards the cell periphery from the the Golgi complex region (Figure 1). They are generally oval in shape, have various diameters, and are surrounded by a perigranular membrane.

The maturation of the cytoplasmic granules existing in the immature mast cells can be seen in six successive phases, as mentioned below (Table I):

1. Formation of materials, undergoing a synthesis in the granular endoplasmic reticulum as granules,begins in the form of progranules in the vesicles of the Golgi complex (Figures 1, 2, 3, 4, and 5).

2. Various progranules aggregate inside a common membrane generally near the Golgi complex, but both single and aggregated progranules are observed in the periphery of the cell from time to time (Figures 5 and 6).

3. The granular endoplasmic reticulum passes the fine granular material found in its cisternae on to the vacuoles containing progranule aggregates. These progranules, and the fine granular material, then take a new form looking like dense cords embedded in fine granular material (Figures 3, 4, 5 and 6).

4. The dense cords and fine granular material are reorganized forming differently shaped granules containing massive strands (Figures 5 and 6).

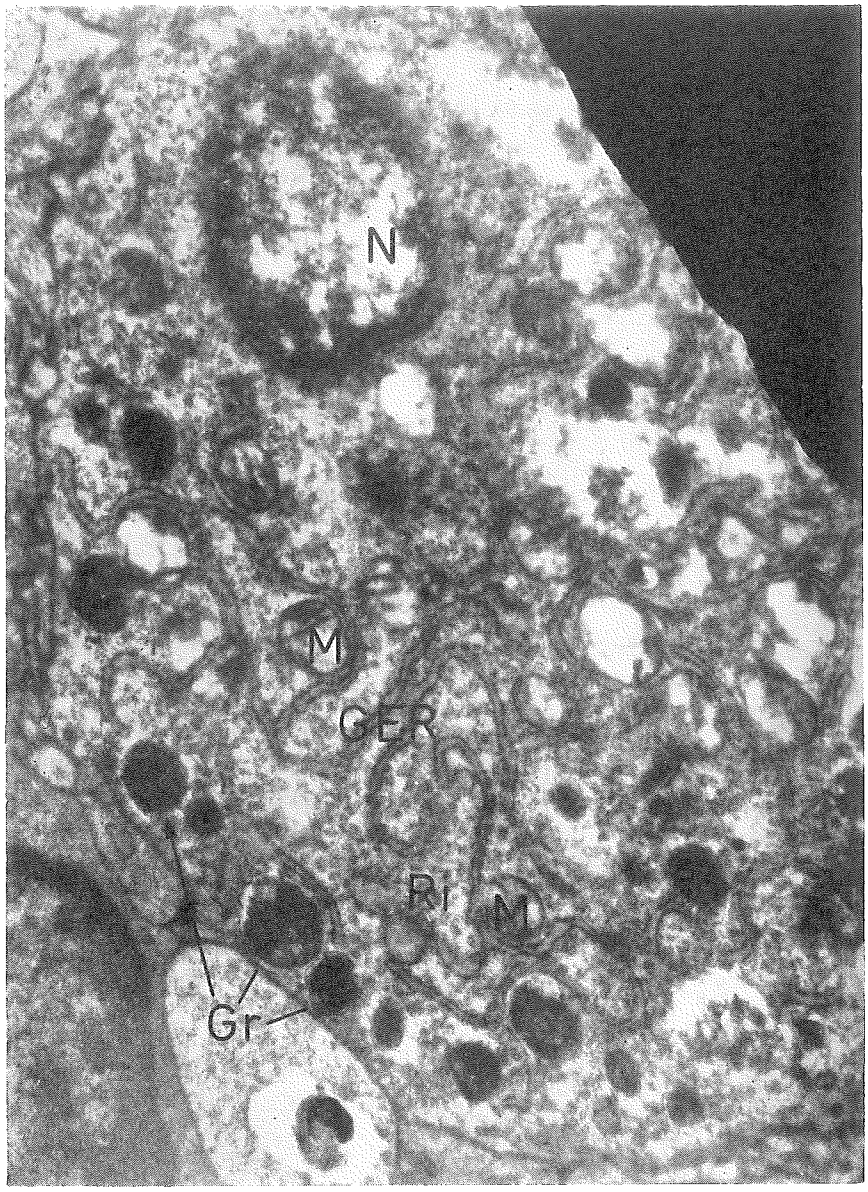


Figure 1. An immature mast cell. N, nucleus; M, mitochondria; GER, granular endoplasmic reticulum; Gr, granule; Ri, ribosomes. X 18,000

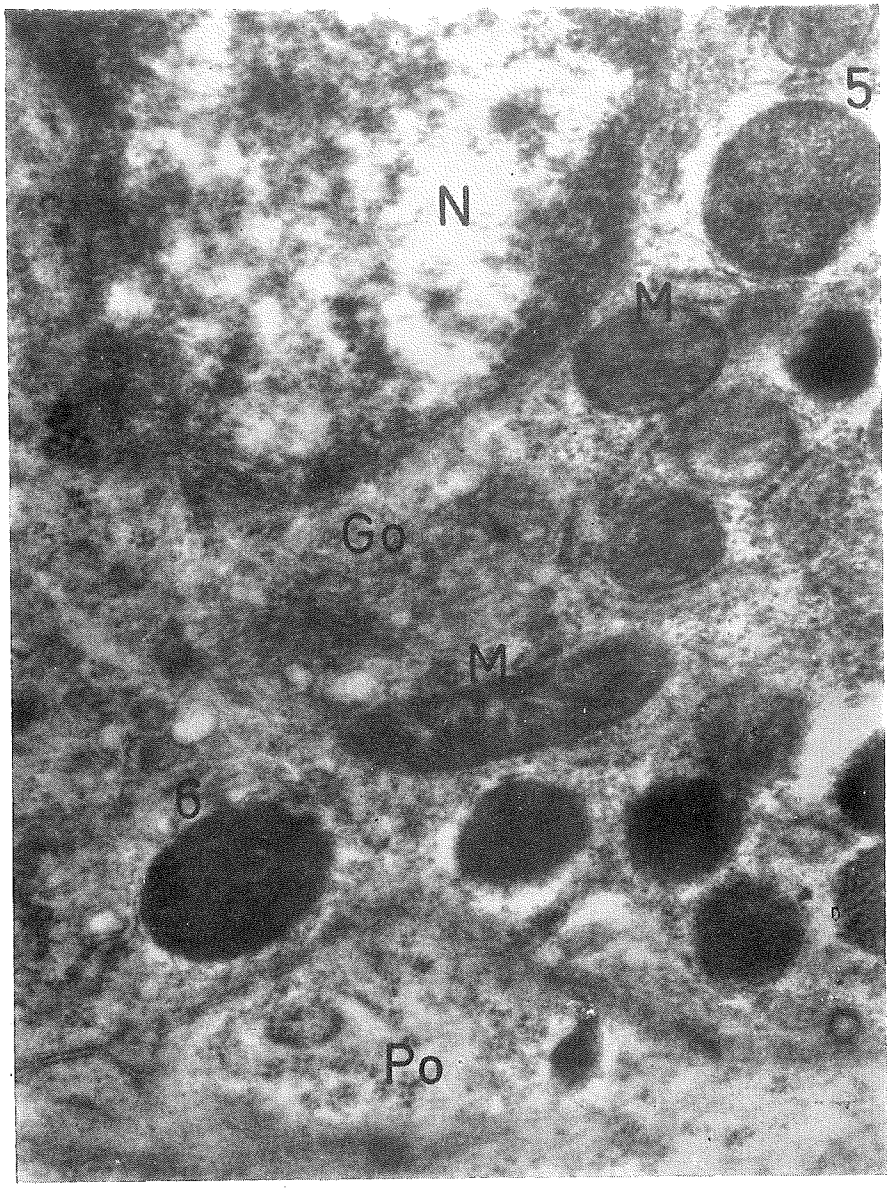


Figure 2. An immature mast cell. Go, Golgi complex; M, mitochondria; Po, polysomes; 5 and 6, granules in different stages of maturation. X 63,000

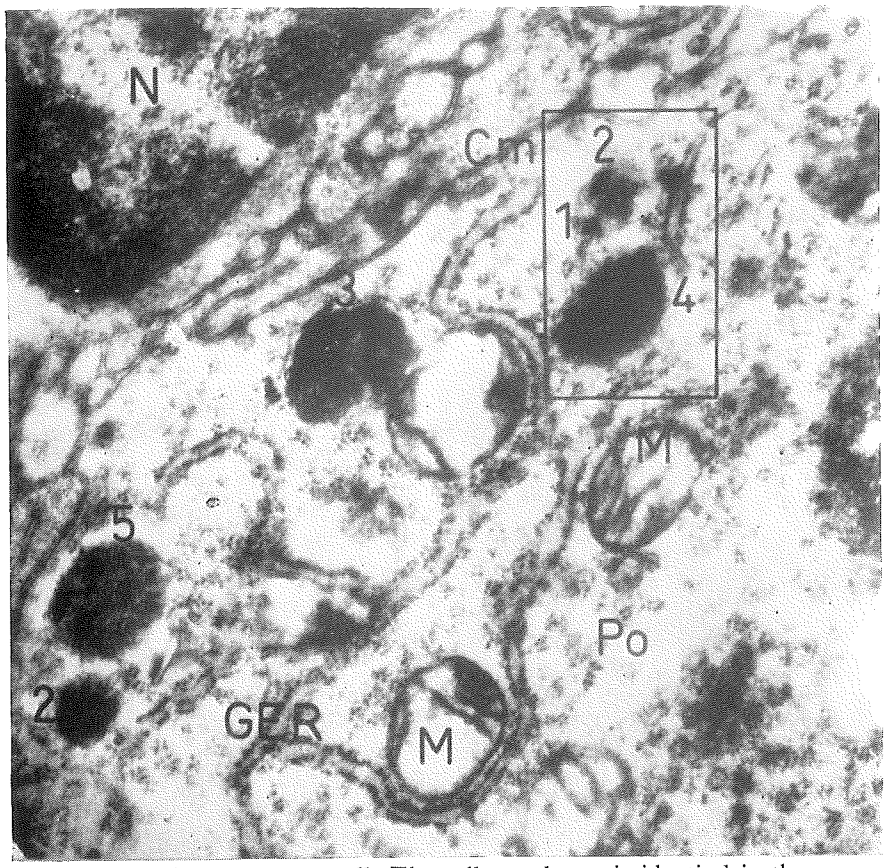


Figure 3. An immatur mast cell. The cell membrane is identical in the upper-left corner (Cm); 1, 2, 3, 4, 5, granules in different stages of maturation are seen M, mitochondria; Po, polysomes. X 63.000

5. The aggregates of massive strands turn into granules having dense and beaded granular structures (Figures 1, 2, 3, 5 and 6).

6. Finally, the granules undergoing compaction are seen to be dense, homogenous and chemically mature mast cell granules (Figures 5 and 6). However, granules at this stage are very rare in immature mast cells.

The immature cells have nuclei surrounded by unit membranes. Generally they are located on one side of the cell and are regularly oval in shape (Figures 1 and 2). The distribution of chromatin is irregular.

II. Mixed (intermediate) mast cells: These are thought to correspond to the astra blue - safranin - stained mast cells containing both blue and red granules and are round, oval or irregular in shape (Figures 7, 8, 9 and 10).

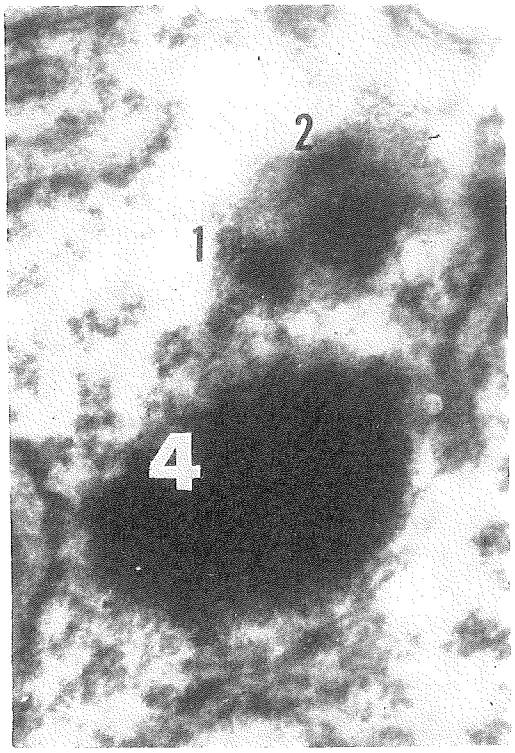


Figure 4. Magnifaction of granules Nos. 1, 2 and 3 seen in Figure 3 (in square). X 120,000

Numerous microvilli are developed by the plasma membrane. Some of these extend towards the mast cells, and others, point away from them in various directions. Both the cell membrane and the microvilli are surrounded by a unit membrane. Sometimes it is seen that the granules are extruded from the cell surface in a saccule covered with membrane (Figure 7). Compared with immature mast cells, there are fewer ribosomes, polysomes, less granular endoplasmic reticulum and even fewer mitochondria in the cytoplasms of mixed mast cells. However, a well-developed Golgi complex is noted in this type of cell (Figures 11 and 12). These cells have more granules than the immature cells and the granules which are scattered through the cytoplasm are either undergoing maturation or are mature. In other words, in addition to the small granules located in the vacuoles of the Golgi complex (Figure 12), granules passing through the second, third, fourth, fifth and sixth phases are seen,(Figures 7, 8, 9, 10, 11, 12, and 13). In these cells, the endoplasmic reticulum cisternae and the granules in the process of maturation are interrelated (Figures 9 and 13).

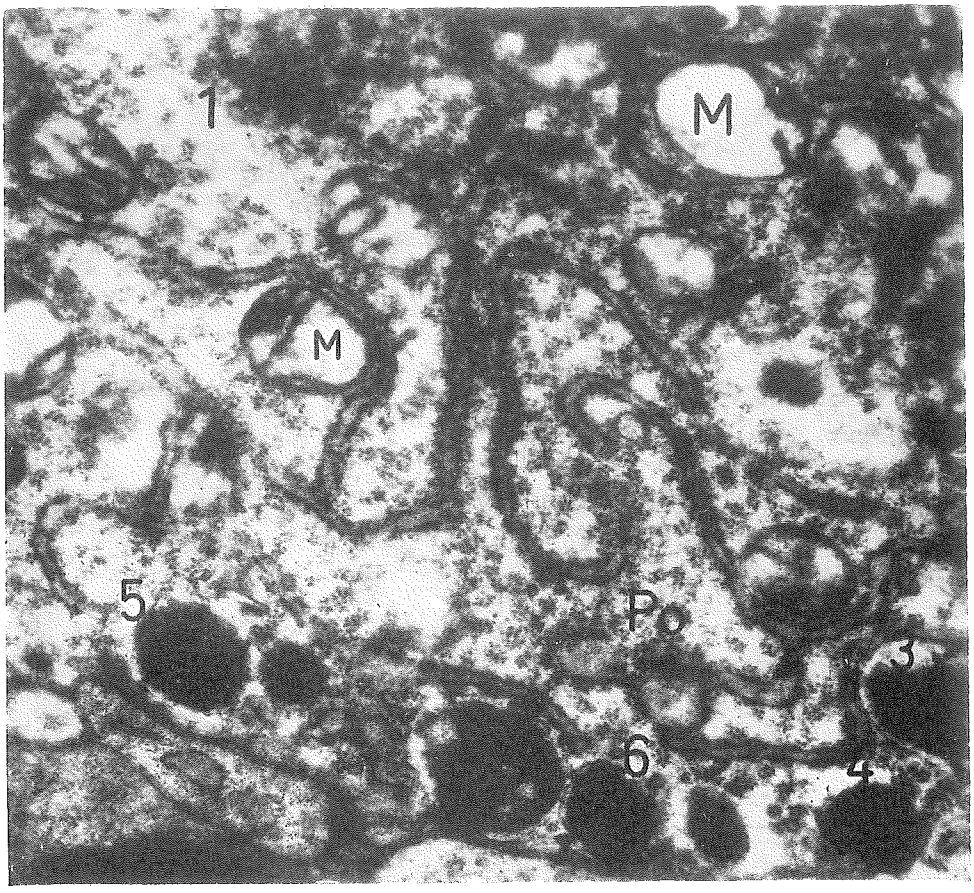


Figure 5. An immature mast cell. 1, progranules in vesicules are observed. 3, 4, 5, 6, granules in different stages of maturation, M, mitochondria; Po, polysomes. X 44,000

The nuclei of mixed cells with a unit membrane are rather large, regularly oval (Figures 8 and 9) slightly indented (Figures 7 and 10). They are generally located in the center, and contain one or two nucleoli (Figures 10 and 11). The distribution of chromatin is denser and more regular in the periphery of the nucleus than in that of immature cells.

III. Mature mast cells: These cells, thought to be the same as the safranin mast cells, are regular and oval in shape. Numerous microvilli exist on the cell surface (Figures 14,15 and 16). The cell membrane and microvilli are covered by a unit membrane, and though a few ribosomes are spread in the cytoplasm, there are no ribosomal aggregates (Figures 14, 15, and 16). The granular endoplasmic reticulum is very rarely seen

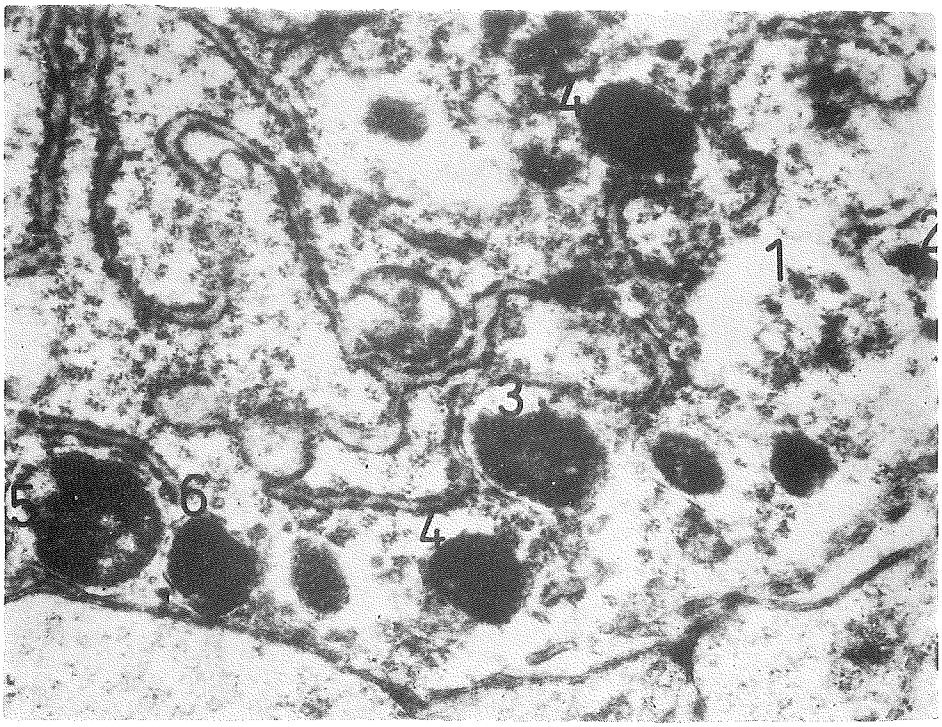


Figure 6. immature mast cell. The existence of granular endoplasmic reticulum (GER), polysomes (Po) and granules in different stages (1, 2, 3, 45, 6) are the most outstanding characteristics of these cells. The interrelation of granule No. 4 with the endoplasmic reticulum is clearly seen. X 44,000

(Figure 15), and oval mitochondria situated among the granules in this type of cells are also very rare (Figure 15). The Golgi complex is noted in some mature mast cells (Figures 14 and 15).

The most characteristic feature of the mature mast cells, distinguishing them from the other two types, is that they are packed with dense and homogenous cytoplasmic granules (Figures 14, 15 and 16). However, granules noted in the other two types of mast cells can also be seen among these granules (Figure 15).

The nuclei of mature mast cells are generally situated in the centre and are smaller than those of the mixed mast cells. They are oval, regular and slightly indented, with unit membranes, and the distribution of chromatin is dense and regular. Generally, one or two nuclei are noted in the cells (Figures 14 and 15).

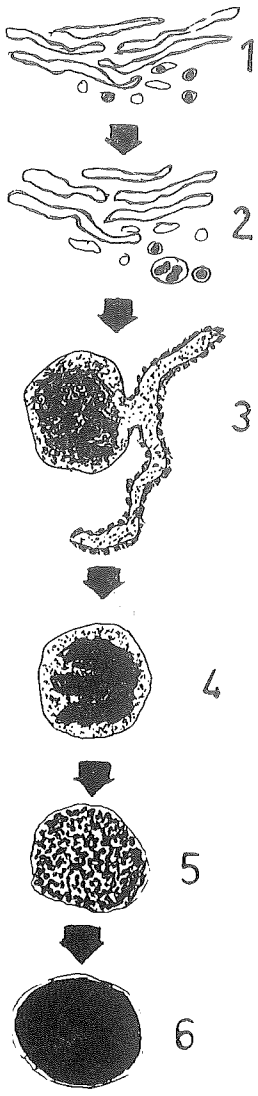


Table 1. The maturation stages of the mast cell cytoplasmic granules, as described by Combs.

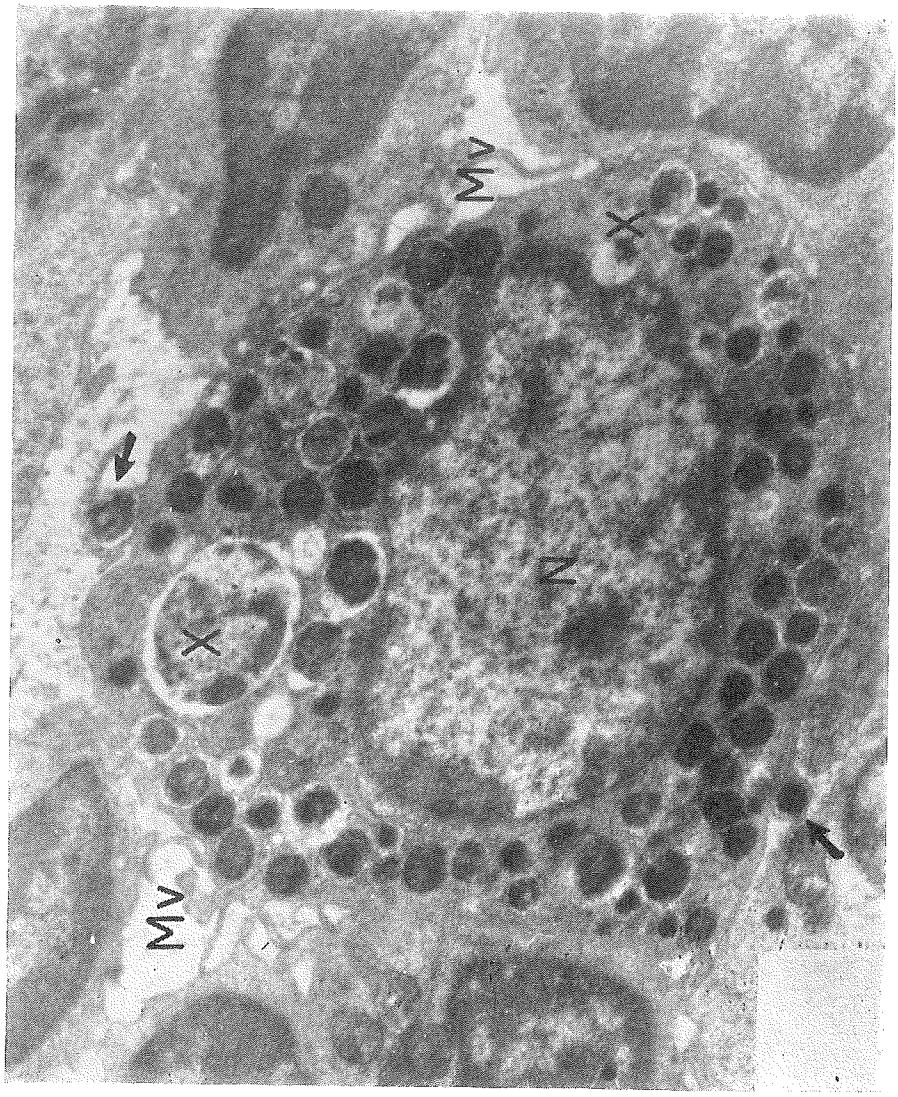


Figure 7. A mixed (intermediate) mast cell. It is clearly seen that there is litle endoplasmic reticulum but abundant granules in different stages, with microvilli (Mv) on the cell surface in these cells. Two granules being extruded from the cell are noted in the region marked by an arrow. Granules (X) impossible to classify. in any type, probably deformed because of insufficient fixation, are observed. X 21, 000

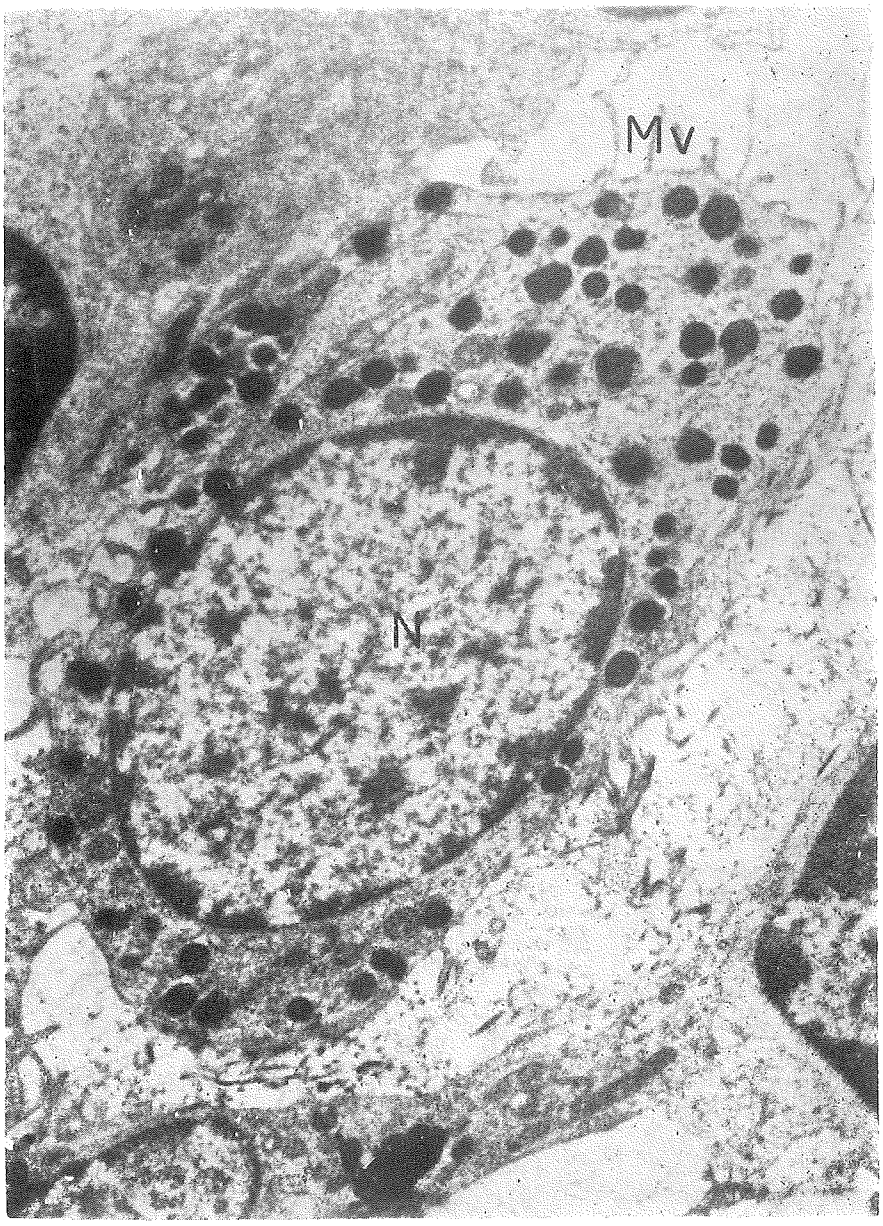


Figure 8. A mixed type of mast cell. The oval nucleus (N) of the cell, rather numerous granules and microvilli on the cell surface are clearly seen. X 16,500

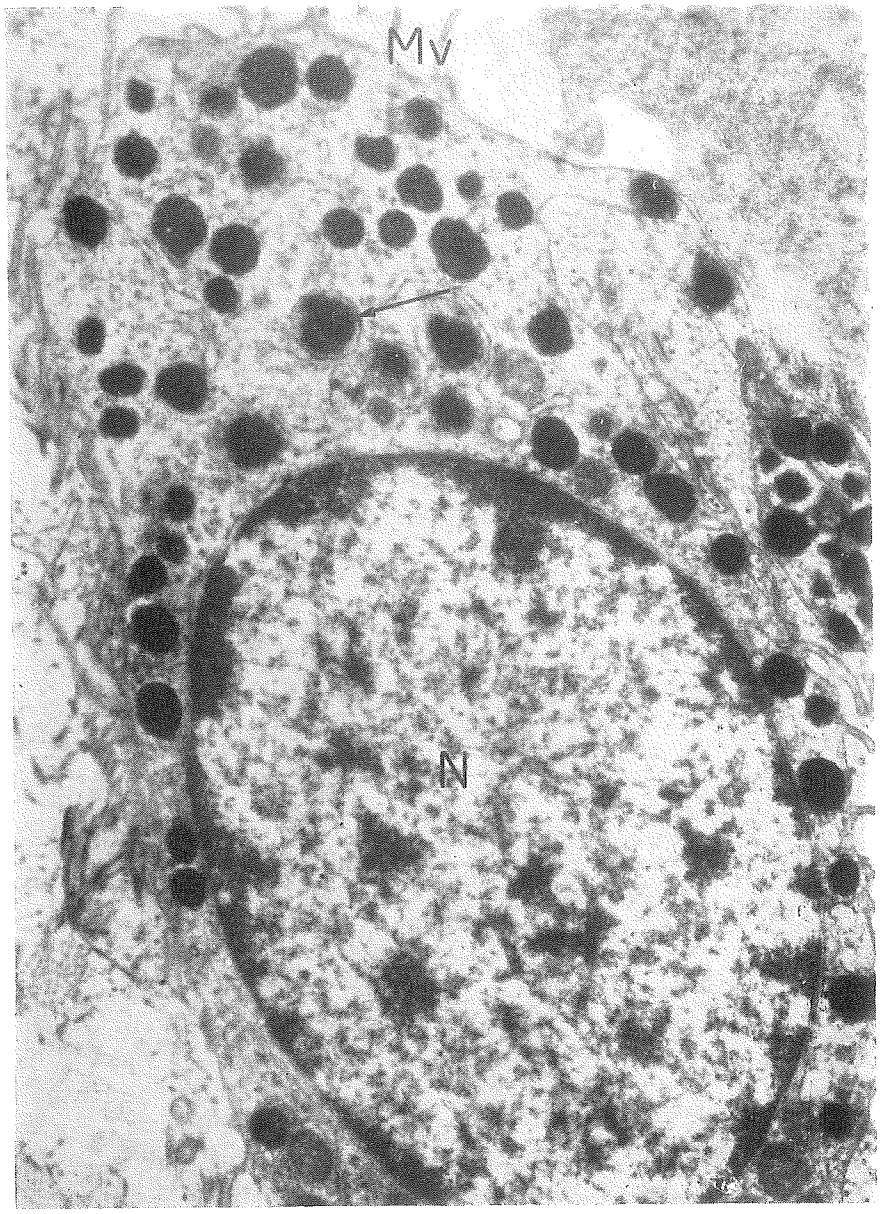


Figure 9. Magnification of cell observed in Figure 8. The microvilli on the cell surface, decrease of endoplasmic raticulum in the cytoplasm, ribosomes and numerous granules in the stage of maturation can be identified. The arrow indicates a perrigranular membrane. X 24,000

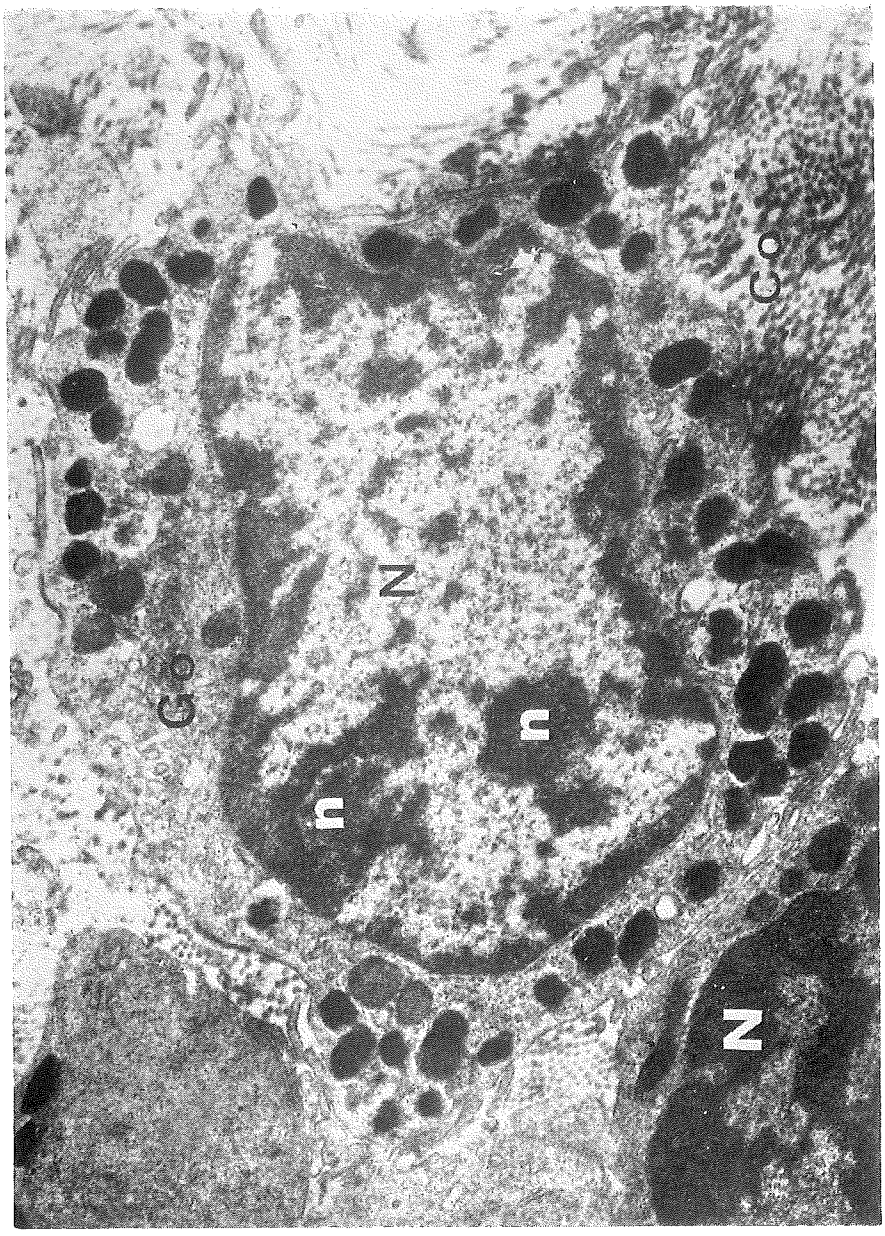


Figure 10. An intermediate mast cell, having a large nucleus (N), microvilli (Mv) and rather numerous granules. The nucleus has two nucleoli (n). In the right corner below, cross sections of collagen fibrils (Co) of the lymph node stroma are seen. X 16,500

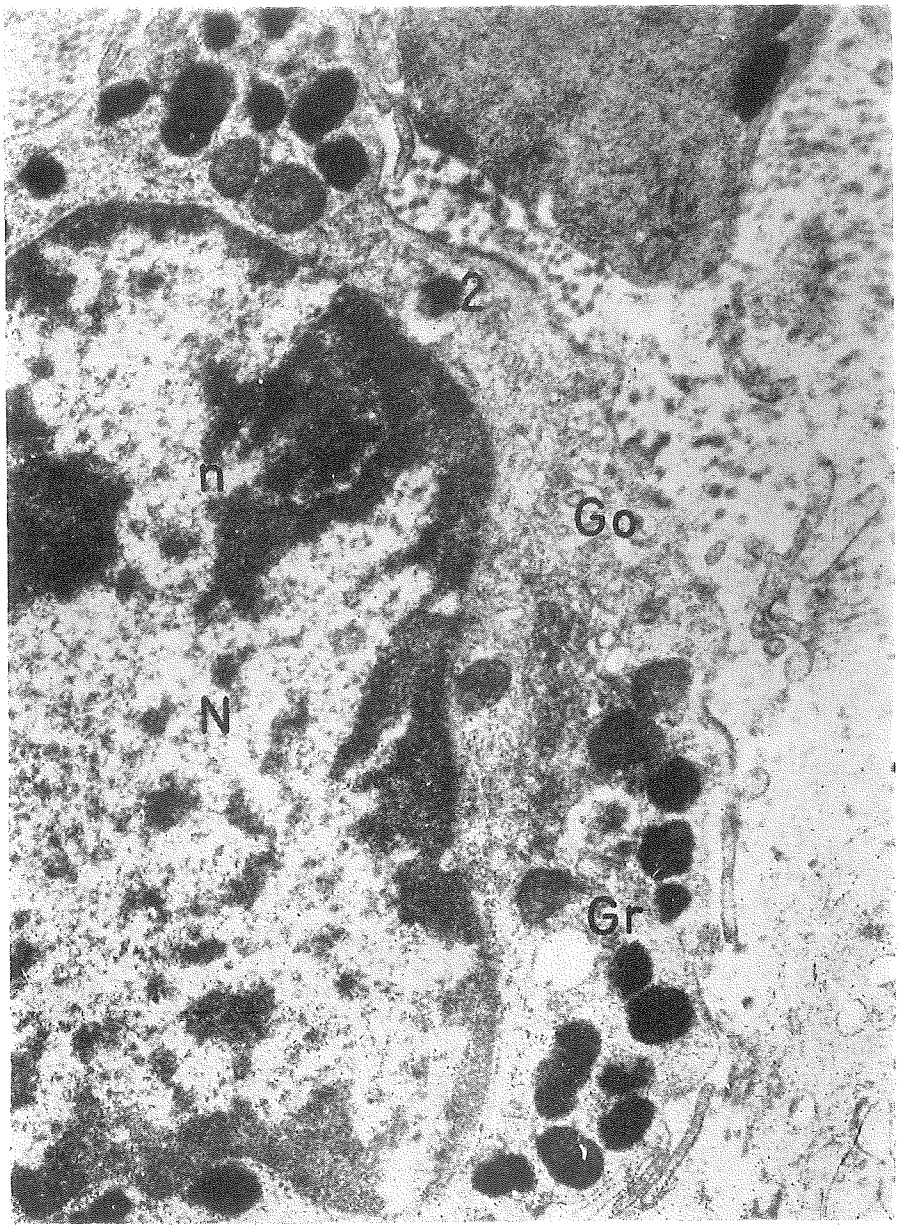


Figure 11. Mangification of electron micrograph of the upper half Figure 10. The Golgi zone (Go) and granules (Gr) in different stages of maturation are observed. Various progranules in granule No. 2 fuse together in an identical manner. X 36,000

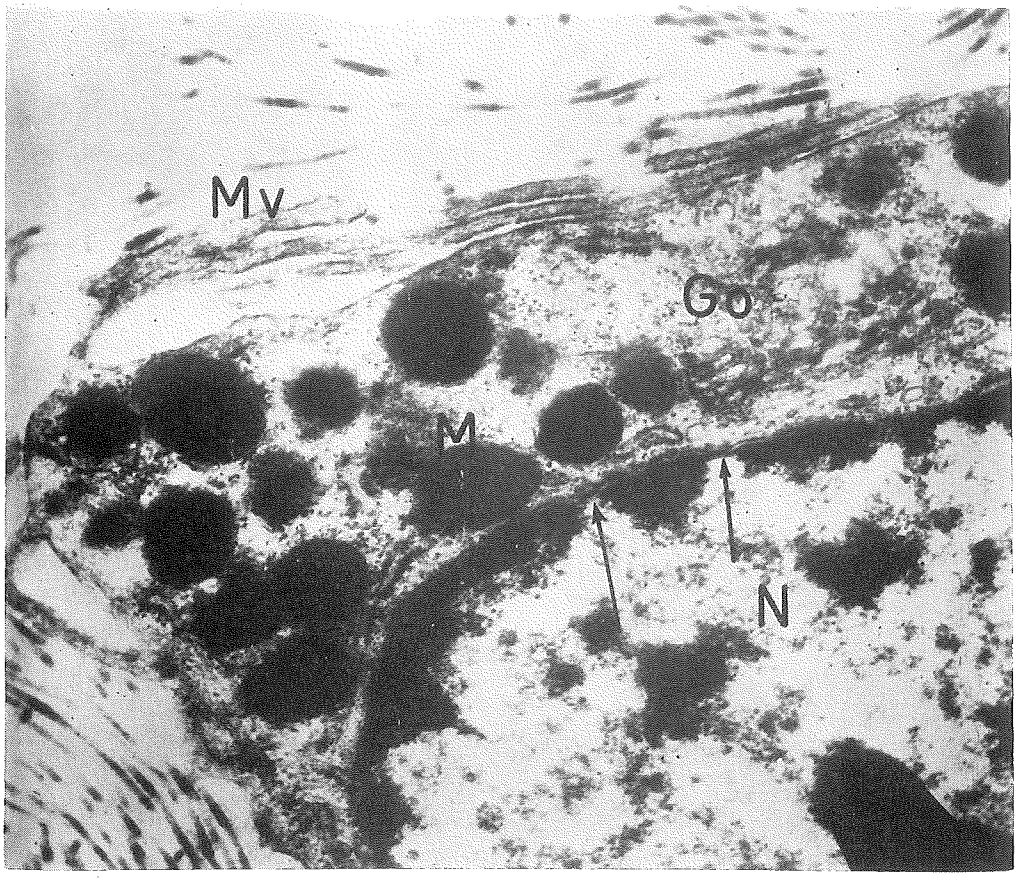
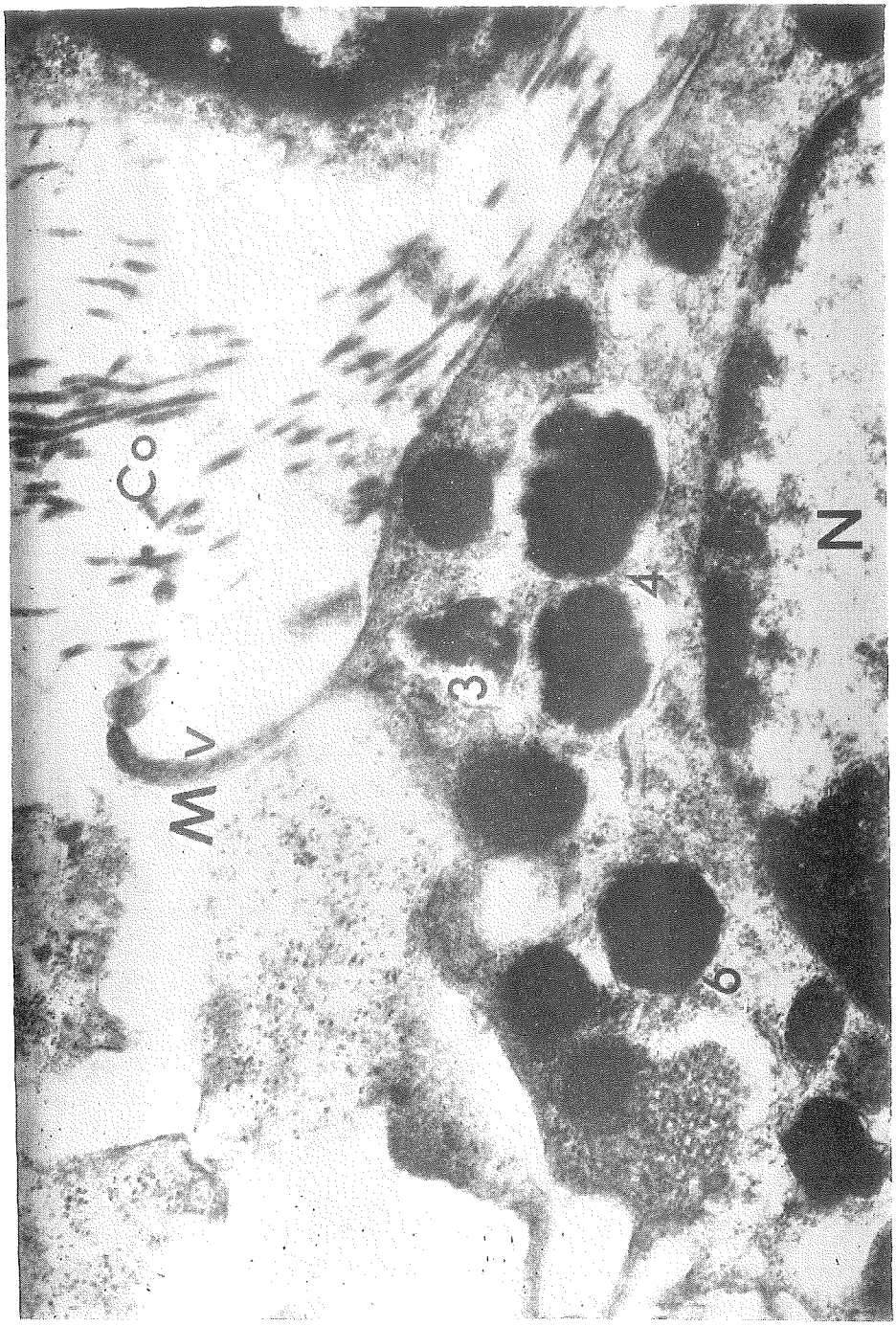


Figure 12. The unit membrane of the nucleus and pores (arrow), Golgi complex (Go), mitochondrium and granules of an intermediate mast cell are observed. X

Sometimes round granules of small or large diameter are noted in all three types of mast cells, which are reticular or vacuolar in structure and display empty spaces between their granular material and the perigranular membranes. In some cases these granules have no perigranular membranes (Figures 1, 7 and 14).

Discussion

Our findings in the cell membrane, granular endoplasmic reticulum ribosomes, polysomes, Golgi complex, nucleus and nucleolus of the three types of mast cells are identical to those of Smith and Lewis [2], Rogers [5],



63,000 **Figure 13.** A small part of the intermediate mast cell. Mv. microvilli; granules in stages 3,4 and 6 and Co, collagen fibrils, are clearly seen. X 63,100

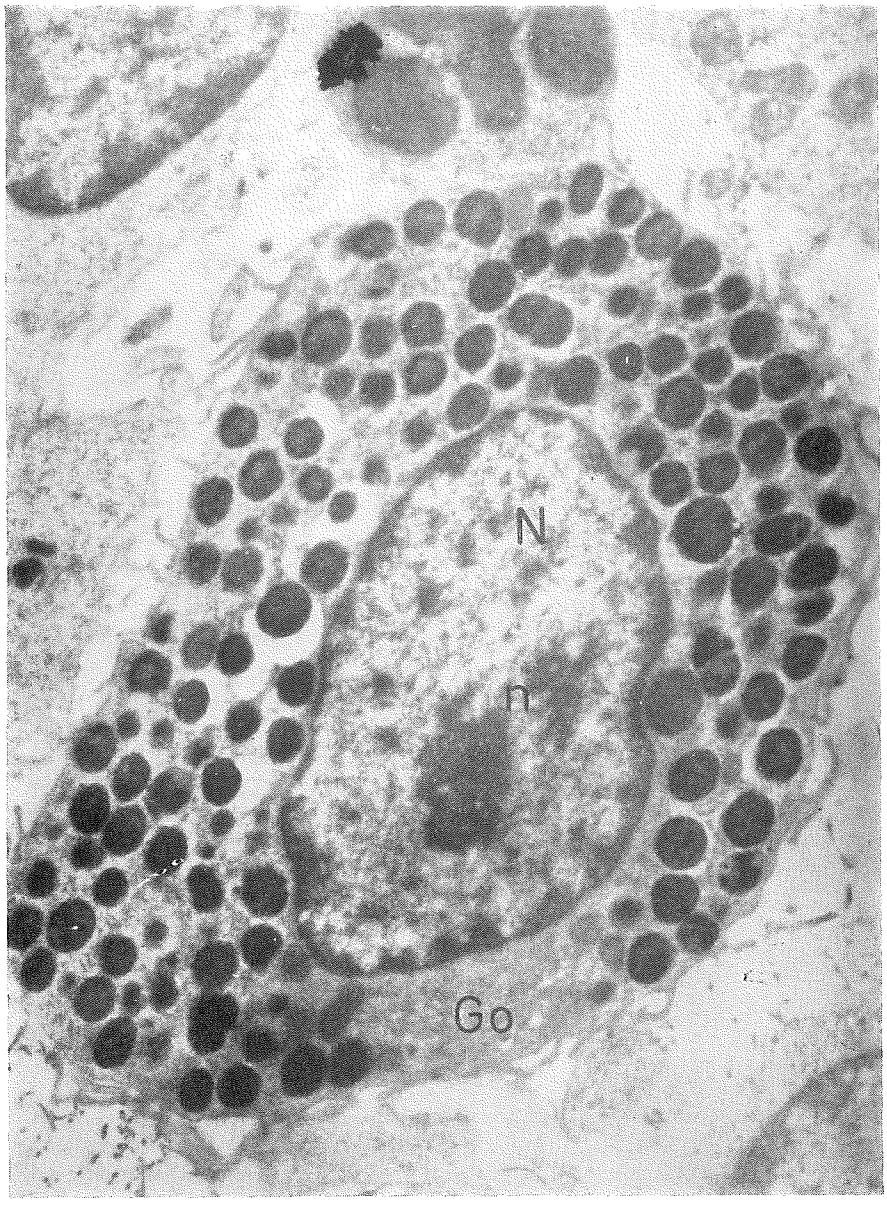


Figure 14. A mature mast cell. The nucleus containing one nucleolus (n) is rather large. The cytoplasm seems densely packed with numerous granules, highly advanced in maturation. A definite Golgi complex (Go) in the right side of the nucleus and numerous microvilli on the cell surface are noted. X 24,000

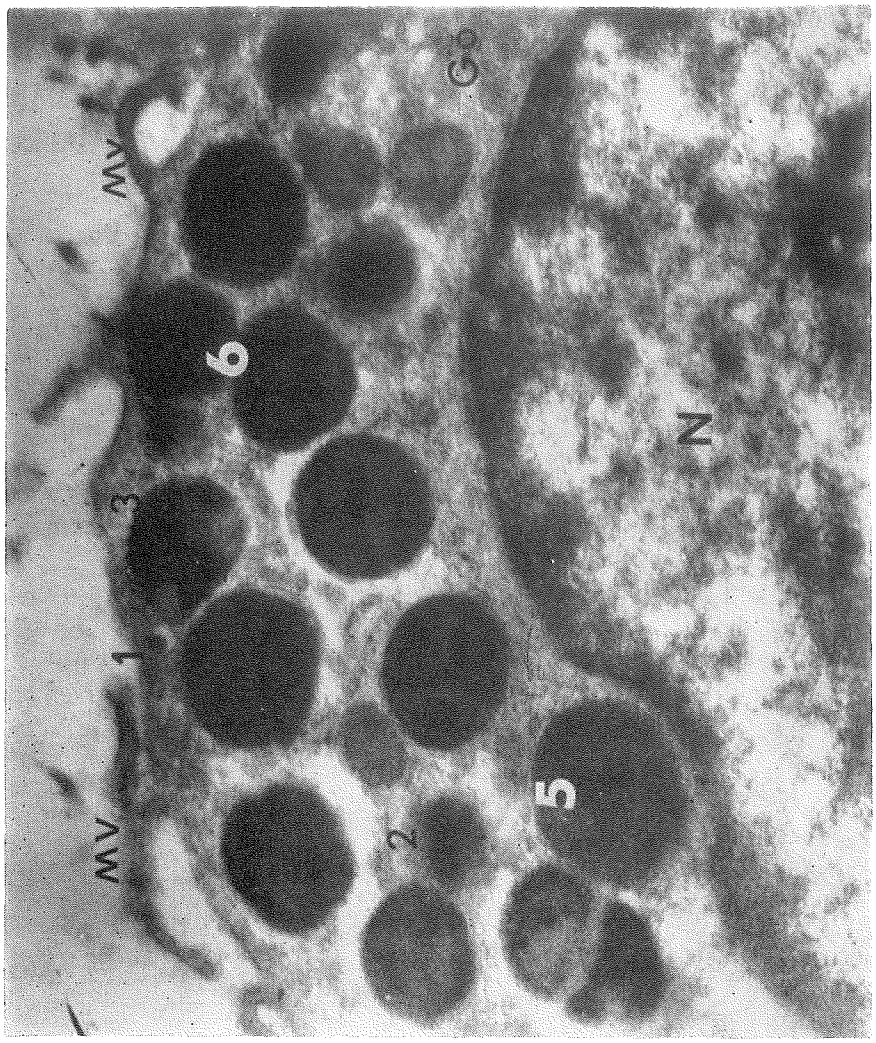


Figure 15. Magnification of one part of mature mast cell in Figure 14. Although the majorty of granules are in the 5th and 6 th stages of maturation, granules at earlier stages are also seen. A single progranule (1) and granules in the 2nd and 4th stages of maturation can be distinguished in this region of the mature mast cell. Granules Nos. 5 and 6 are mature On the right, the Golgi zone (Go) and the microvilli on the cell surface are clearly indentified. X 63,000

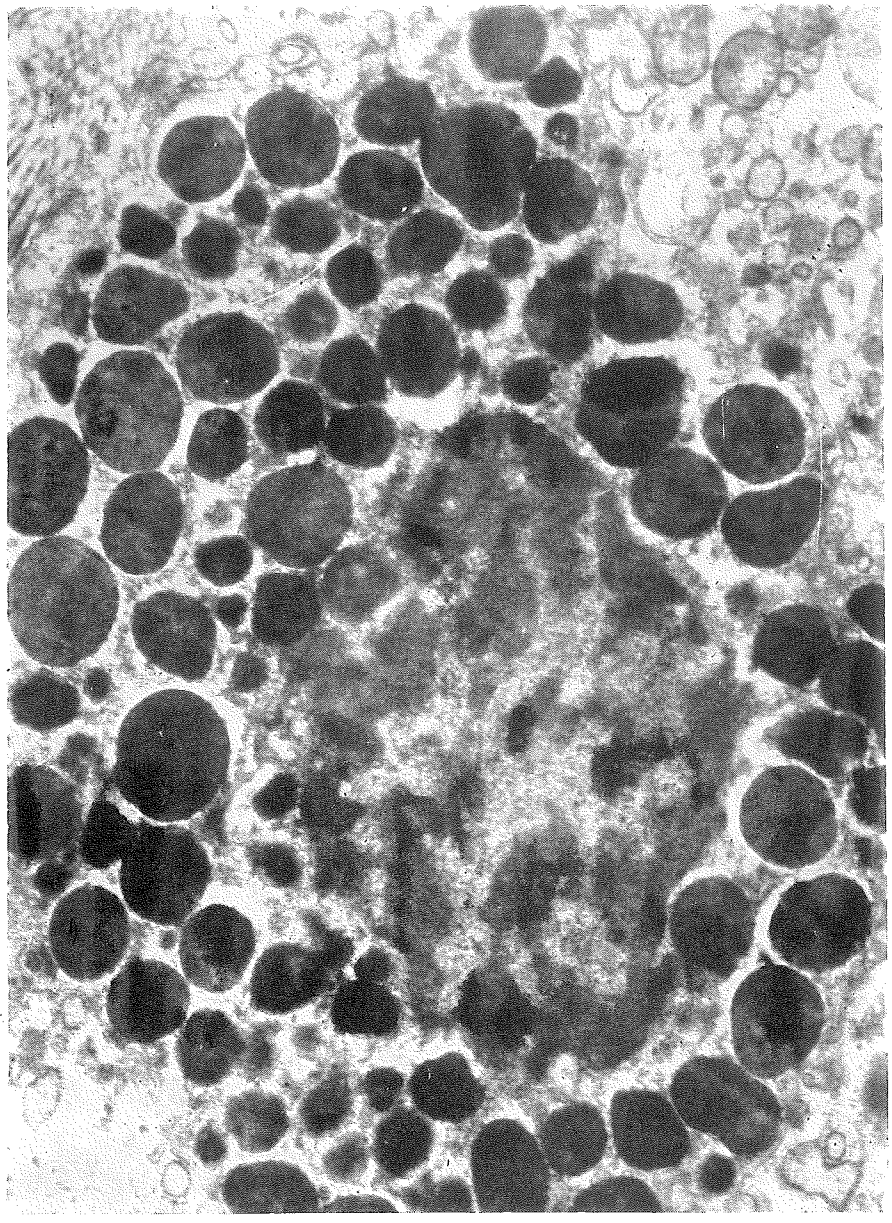


Figure 16. A mature mast cell. Although there is an absence of granular endoplasmic reticulum and polysomes in the cytoplasm, an abundance of mature granules is clearly observed. X 27,000

Policard et al[9]., Gusek[25], Klug[8], Smith[26], Fernando et al[13], Thiéry[14 15] Fujita[27], Weinstock et al[18], Kerse [17], Gustafan et al, [19] Geoffreyl [20] and Takasi et al[28].

In a study on the tissue mast cells of newborn and adult rats[13], few cytoplasmic granules, well-developed Golgi complexes, and granular endoplasmic reticula and numerous mitochondria and ribosomes have been observed in immature cells. On the other hand, a lesser granular endoplasmic reticulum, small Golgi complex and fewer mitochondria and microvilli on the cell surface have been identified, as well as the closely packed dense granules in the mature mast cells.

In the study made on the subcutaneous mast cells of normal rats at various ages [27], it was seen that the specific mast cell granules form in the vacuoles of the Golgi complex, and regularly become mature in a membrane. It was also noted that immature rat mast cell granules vary in shape and diameter, speedily achieve a formation and are extruded from the cell surface. It has been identified that the mast cells of adult rats have aggregates of packed granules and numerous microvilli on the surface, and indicated that there is little activity in this type of mast cell.

The differentiation and proliferation of the embryonic rat mats cells have been studied from the standpoint of histochemistry and autoradiography and the differentiation of the mast cells has been considered in the following four stages [29]:

Mast cells in stage I: These have a few cytoplasmic granules and are always stained blue, using the alcian blue safranin staining method. They incorporate radiosulfate in a very low quantity, thus displaying that they contain very little sulfated polysaccaride.

Mast cells in stage II: These have more cytoplasmic granules than Stage I mast cells. A few safranin positive granules, besides the numerous alcian blue-positive granules, appear as a result of the alcian blue safranin staining method.

Mast cells in stage III: Although a few alcian blue-positive granules are still present, Stage III mast cells contain a majority of safranin-positive granules.

Mast cells in stage IV: These contain a large number of granules, and are always stained metachromatically with safranin.

In the same study it was also observed that the speed of radiosulfate incorporation with the mast cell and granule staining increases after shifting from alcian blue to safranin. In the process of the formation

of mast cell granules, it has been identified that synthesis and accumulation of a heparin precursor in the alcian blue positive granules take place at first, followed by the synthesis and accumulation of highly N-sulfated heparin with mast cell chymase, and finally histamine in safranin-positive granules.

Combs[30] carried out a study on the maturation of mast cells in embryonal and adult rat tissues at the ultrastructural level based on the histochemical and autoradiographic findings on the differentiation and proliferation of the mast cell study mentioned above. According to the author, the mast cells that are identified earliest look very much like the surrouding mesenchymal cells that are not differentiated, and can be recognized by their granules. No specialization is seen on the surface of the cell,which contains very few cytoplasmic granules. Highly-developed granular endoplasmic reticulum, ribosomes, polysomes, a small Golgi complex and round mitochondria are seen in their cytoplasms. As differentiation proceeds, numerous short microvilli develop on the mast cell surfaces, a Golgi complex associated with dense granules of 70-100 millimicrons in diameter, mitochondria and an increasing number of cytoplasmic granules associated with the cisternae of the granular endoplasmic reticulum are observed. With the continuation of granule formation, the granular endoplasmic reticulum, mitochondria and ribosomes in the intergranular cytoplasm decrease, and the granules are closely packed in the cell, thus completing maturation. Combs described the process of granular maturation of the cells at the ultrastructural level with their hypothetical chemical correlates in the following manner:

1. The formation of single progranules in the membrane-limited vacuoles in the central part of the Golgi complex marks the granule synthesis. (Heparin precursor formation; o-sulfation).

2. The progranules, which seem to migrate towards the periphery of the Golgi zone, become denser, and aggregate inside a common membrane, usually at or by the Golgi zone periphery. (Heparin precursor accumulation in order to form the future mast cell granules).

3. The fine granular material, which is possibly derived from the granular endoplasmic reticulum, is added to the vacuoles containing granular endoplasmic reticulum cisternae and progranule aggregates. (Addition of basic protein including mast cell chymase).

4. The progranules then seem to fuse and dense ropy cords of 70-100 millimicrons in diametar embedded in fine granular material start to form. (N-sulfation).

5. The dense and fine granular elements then seem to reorganize and form a mass of electron-opaque strands of 20-30 millimicrons in diameter. This mass of strands appears to have a beaded structure and to be tightly packed inside the perigranular membrane. (N-sulfation).

6. Finally these granules form the dense, homogenous and chemically complete mast cell granules. (Maximum ionic bindings between basic proteins and heparin).

We, too, observed in our study the maturation stages of the mast cell granules of the inguinal lymph node of adult rats as Combs did in the embryonic and adult rats under electron microscopy. We described the immature mast cells as cells showing no specialization in their membranes, with well developed endoplasmic reticula, abundant ribosomal aggregates, progranules containing heparin precursors and granules which were just beginning to mature. Combs' findings, showing that cells having such characteristics at the ultrastructural level may be identical to the alcian blue positive granular mast cells, led us to believe that the immature mast cells observed in our study have the same ultrastructural characteristics, corresponding to astra blue positive granular mast cells.

We defined the cells which form the transition between immature and mature mast cells as mixed mast cells. These have numerous microvilli on the cell surfaces, reduced ribosomes, polysomes, mitochondria and granular endoplasmic reticula in their cytoplasms, as well as both mature and maturing cytoplasmic granules. In other words, sulfated polysaccharides and polysaccharides in the process of sulfation at the stage of active granule synthesis. Consequently, we thought that if the mast cells had these characteristics at the ultrastructural level, they may be histochemically identical to both those stained with alcian blue-safranin and with astra blue-safranin, containing both blue and red granules.

As a result of our findings we arrived at the conclusion that the mature mast cell has no granular endoplasmic reticulum, a small Golgi complex, and an abundance of dense and homogenous granules, which all indicate a cellular maturity and a synthetic inactivity. In addition, we thought that these chemically complete cells, or cells completely filled with basic proteins and sulfated polysaccharidic granules, may correspond to those stained metachromatically with alcian blue safranin and astra blue safranin.

It has always been a problem to find an appropriate fixative solution for studying the mast cells under an electron microscope, therefore the usefulness of the electron microscope in carrying out these studies has been limited owing to the difficulty of obtaining sufficeint fixed mast cells.

The reticular, or vacuolar, appearance of the granule structure, and the space left between the granular material and its surrounding perigranular membrane, have been described by many authors as sensitivity occurring during the electron microscopic procedures, and because of the osmic acid. [5 9 13 26 27 31]

Combs[30] added alcian blue, particular to the mast cell polysaccharides, to gluteraldahyde in a suitable buffer, and fixed the mast cells which were later subjected to post-fixation in osmium tetroxide. Besides the well-preserved mast cell granules of different structures and densities at the ultrastructural level, reticular and coarse granules, surrounded by empty halos and with indistinct margins were also seen, particularly in the immature mast cells. Such an appearence has been described as degranulation and histamine release in mast cells resulting from granule dissolution because of inappropriate procedures and insufficient fixations. Our findings supported Combs' observations, as we too applied Combs' techniques, but with small modifications in our preliminary procedures for study under the electron microscope. We occasionally observed in our micrographs, small and large cytoplasmic granules with reticular and vacuolar structures and indistinct margins, separated from their perigranular membranes, and well-preserved mast cell granules, with perigranular membranes, of different structure and density.

It has been suggested that the microvilli on the surface of mast cells have a role in the extrusion of the cytoplasmic granules[9]. Some other authors have pointed out that the extrusion of granules may occur either by way of the cell membrane [14 26] or by separation of the perigranular vacuoles from the mast cell or by extrusion of the granules together with their perigranular membranes following the dissolution of the mast cell membranes[27].

The observation of granules surrounded by membranes especially on the surfaces of mixed mast cells in our micrographs, has led us to believe that mast cell membranes open, after which the granules are extruded in their perigranular membranes.

Summary

The inguinal lymph nodes of adult male rats were fixed in a solution of astra-blue and gluteraldehyde mixture, subjected to a postfixation in osmium tetroxide and studied under the electron microscope. When the shape, organelles and granules of just the mast cells of the tissue were considered, three types could be indentified.

1. *Immature Mast cells*: These are oval in shape with no specialization in their surfaces. They have abundant granular endoplasmic reticula, ribosomes, polysomes, mitochondria and a few granules at their cytoplasms at the beninning of maturation. Possibly they correspond histochemically to astra-blue positive granular mast cells.

2. *Mixed* (*intermediate*) *Mast Cells*: These have numerous microvilli on their surfaces. Compared with the immature mast cells, they have abundant mature and maturing cytoplasmic granules in their cytoplasms, but ribosomes, polysomes, endoplasmic reticula and mitochondria are fewer. These cells correspond to those with both blue and red granules when using the astra-blue safranin staining method.

3. *Mature mast cells*: These are closely packed with dense and homogenous cytoplasmic granules, but are poor in cytoplasmic organelles and correspond to safranin-positive mast cells.

Acknowledgements

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Key to Symbols

Cm	: Cell membrane
GER	: Granular endoplasmic reticulum
Go	: Golgi complex
Gr	: Granule
M	: Mitochondrium
Mv	: Microvillus
N	: Nucleus
n	: Nucleolus
Po	: Polysome
Ri	: Ribosome
1	: Progranule
2, 3, 4, 5, 6	: Granules in different stages of maturation.

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Individual Variations in the Rate of Isoniazid Inactivation

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It has been recognized now that in a normal population some people inactivate isoniazid rapidly and other slowly.[1-3] The genetics of this variation have been investigated in various ethnic groups, and the ratio has been found to vary greatly from one to another; Eskimos and the Japanese have only a small proportion of slow inactivators, while whites (the United States and Canada) and negroes have a near equal distribution.[4-7] A study of the distribution of slow and rapid inactivators in the Turkish population, which would be interesting not only from a genetic point of view but also from the clinical standpoint, has never been made.

It has been known for many years that large doses of isoniazid result in convulsion both in man and in experimental animals.[8] This is due to the interference of the drug at the metabolism of pyridoxine in the nervous system,[9] and it can effectively be prevented by simulataneous administration of pyridoxine.[10] However, this is considerably hindered by restrained use of this expensive vitamin. Hughes et al reported that slow inactivators of the drug were more apt to suffer from the neurotoxic side effect of this compound,[2] and it has been suggested that there is most likely a correlation between blood level and toxicity. Hence the rates of isoniazid inactivation and excretion are determinants for its effectiveness, and it is agreed that isoniazid inactivation status and clinical response to the drug correlate to a certain extent.[5]

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Although even the smallest therapeutic dose is usually effective, as isoniazid is a potent tuberculostatic drug,[11] and isoniazid resistent **m. tuberculosis** strains are usually less virulent and pathogenic,[12] it is important to know the distribution of slow and rapid inactivators in order to avoid the neurotoxic side effect from excessive dosage, and to help in the choice of the optimum dose of drug that would be safe for a large population in the treatment of tuberculosis.

Materials and Methods

Two hundred and four normal male and female volunteers of all ages were selected at random, and care was taken to choose subjects who were not receiving any drugs. They were given 10 mg/kg body weight isoniazid, and a blood sample was taken six hours later.

J. F. Peter's method of analysis was used.[13] There are some 11 colorimetric procedures, two microbiologic assays and one radiocarbon method for measuring isoniazid;[14] however that developed by Peters is more sensitive and reliable than the others. It is also less time consuming, has a high power of resolution and allows the measurement of total and free isoniazid simultaneously. This method involves extraction of isoniazid and its acetylated product from serum with an alkaline isoamyl alcohol-ethylene dichloride medium, which is further extracted by acid after cleaning. A sample of this acid extract is used to determine total hydrazides by the use of Erlich's reagent, while another portion is reacted with cyanogen bromide. The free form is estimated on this latter sample by its characteristic fluorescence in an Aminco-Bowman spectrophotofluorimeter. The acetylated compound is not fluorescent.

Standard biostatical methods were used to evaluate the results.

Results

The results of total isoniazid after six hours of ingestion of the drug are given in Figure 1. Three groups are clearly seen, and they were tentatively classified as rapid, intermediate and slow inactivators. Biostatistical analysis revealed that the three groups did indeed represent distinct variations, and values for their identity were highly significant (Figure 1). (The overall correlation coefficient $r_{x,y}$ was found to be 0.60). Free isoniazid value distribution was investigated in each of the groups according to this classification, and in individual groups this varied from 0.47 to 0.52, with a small s_r and highly significant t values (all of the latter were $>$ 10).

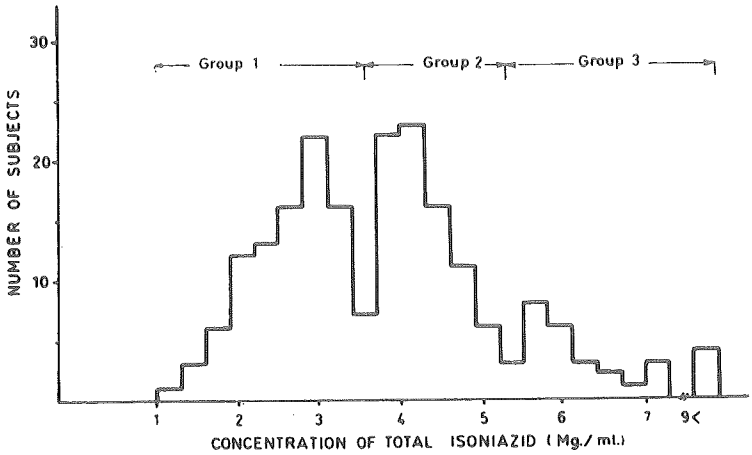

Figure 1. Concentration of total isoniazid after six hours of 10 mg/ kg administration. Three groups are discerned: Group 1: rapid inactivators, mean value 2.58 mg./ml. (S. E.: ± 0.05 S. D. ± 0.52); Group 2 (intermediate) mean value 4.18 mg/ml. (S. E.: ± 0.05, S. D.: ± 0.45); Group 3 slow inactivators; mean value 6.46 mg/ml. (S. E.: 0.22, S. D.: 1.13). t values related to the significant difference between groups are: t= 12. 3 (for groups 1 and 2), t=29.7 (for groups 1 and 3); t= 16.6 (for groups 2 and 3).

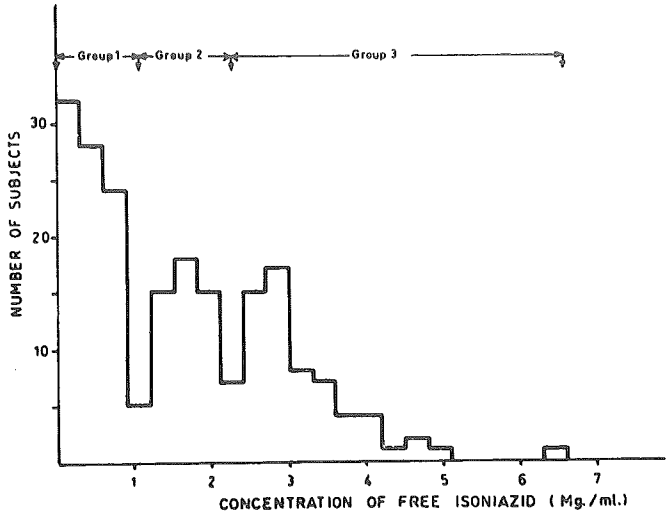

Figure 2. Concentation of free isoniazid after six hours of 10 mg/kg administration. Three groups are discerned: Group 1 (rapid inactivators): mean values 0.40 mg/ml (S. E.: 0.02: S. D. 0.03); Group 2 (intermediate): mean value 1.66 mg./ml. S. E. ± 0.01, S. D.: ± 0.04); Group 3 (slow inactivators): mean value 3.25 Mg./ml. (S. E.: ± 0.11, S. D. ± 0.80). t values related to the significant difference between groups are; t= 8.2 (groups 1 and 2) t= 16.4 (groups 1 and 3); t= 9.2 (groups 2 and 3).

The results of free isoniazid after six hours' ingestion of the drug are given in Figure 2, in which three groups may also be discerned, and these were also assigned as rapid, intermediate and slow inactivators. Again, t values for their identity were found to be highly significant (Figure 2).

Statistical analysis of various phenotypes are presented in Table 1. It can be seen that although significantly different groups may be obtained if the data are analyzed according to their distribution in the total isoniazid content, the distinction is not very clear if they are analyzed according to the free isoniazid content where the total content of the rapid and intermediate groups overlap, and no statistical significance could be obtained. For this reason the values in Figure 1, are used to estimate the gene frequency, and this frequency is compared with that of other ethnic groups in Table II.

TABLE I

STATISTICAL ANALYSIS OF VARIOUS PHENOTYPE GROUPS. ANALYSIS OF THE DATA GIVEN IN FIGURES 1 AND 2 IS PRESENTED. THE PHENOTYPES ARE EXPRESSED AS R = RAPID AND S = SLOW INACTIVATORS.

Fig: 1	RR	RS	SS
Free isoniazid	0.94 ±0.08 *	1.83 ±0.11	2.65 ±0.32
P value	$P < 0.001$	$0.001 < P < 0.01$	
Total isoniazid	2.58 ±0.05	4.18 ±0.05	6.46 ±0.22
P value	$P < 0.001$	$P < 0.001$	
Number	84	93	27
Fig: 2			
Free isoniazid	0.40 ±0.02	1.66 ±0.01	3.25 ±0.11
P value	$P < 0.001$	$P < 0.001$	
Total isoniazid	3.35 ±0.13	3.53 ±0.09	4.92 ±0.19
P value	P: n. s.	$P < 0.001$	
Number	86	61	57

* Standard error: ± S. E.; n. s.: not significant

Discussion

Analysis of the results reveals three distinct groups, regardless of whether it is made of the free or the total isoniazid content. Although in the past some authors, such as Knight et al[15], analyzed their data for

TABLE II

COMPARISON OF SLOW AND RAPID INACTIVATORS OF ISONIAZID IN VARIOUS ETHNIC GROUPS:

Ethnich group	Rapid inactivators	Slow inactivators
Japanese *	86.7%	13.3%
Eskimo *	95.4%	4.6%
American Indian *	78.5%	21.5%
Latin American *	67.2%	32.8%
White (USA and Canada) *	44.9%	55.1%
Negro (USA) *	47.5%	52.5%
Turkish	63.9%	31.1%

* Data taken from Kalow[14]

the presence of two groups, Evans et al.[7] proved the existence of heterozygotes (intermediates) in an extensive survey of families, and gave statistical evidence that they can be discerned by their intermediate blood level of the drug. The method of Peters[13] yields lower values than that of Evans el al, because whereas the latter could detect about 1.5 μg hydrazid/ml blood in subjects who did not receive the drug, none could be found in the more specific method of Peters. A wide variety of values depending on the method of chemical analysis, is found in various investigator's.[14 15]

In view of the authenticated presence of intermediates, all investigators are faced with assigning their groups according to the statistical analysis of distribution. We adopted the distribution of the values for total hydrazid for the estimation of gene frequency, as it correlates well with that of free isoniazid values (Figure 1). If gene frequency is estimated by the distribution of free isoniazid values alone, a similarly close values can be obtained (R=0.57, S=0.43).

According to our findings, the distribution of rapid and slow inactivators in the Turkish population is very close to that found in Latin Americans, the frequency of rapid inactivators being relatively high.

Summary

A groups of 204 normal subjects was investigated for the presence of rapid, intermediate and slow inactivation of isoniazid. The genetic frequency was calculated according to the distribution of the population.

Acknowledgment

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The Relation of Portal Hypertension to Histological Changes in the Intestinal Mucosa

Hasan Telatar, M.D. *

The present advances in intestinal biopsy technique have enabled the clinician to investigate the histologic changes in the intestinal mucosa in various disorders. Intestinal malabsorption rather frequently occurs in liver cirrhosis, and this observation has evoked special interest in the histologic study of the intestinal mucosa of these patients.[1 2 3]

Since Astraldi and Strosselli published their findings of histological changes in the jejunal mucosa of 10 patients with advanced cirrhosis in 1960, the studies in this field have progressively accumulated.[4 5 6]

However, these authors did not discuss the factors leading to the histologic changes they observed.

The present study was made to investigate the importance of portal hypertension in the development of histologic lesions observed in the jejunal mucosa.

Materials and Methods

Twenty-four patients with portal hypertension and four control cases were investigated. The control group consisted of two males and two females ranging in age from 22 to 55 years.[7 8] Sixteen of the cirrhotic patients were male and eight were female, and their ages ranged from 35 to 65 years.

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Jejunal biopsy was performed in each case with a Ross-Moore biopsy capsule under fluoroscopy.[9 10] The biopsy specimen was placed on a piece of paper, with the cut surface in contact with the paper, and then put in Bouin solution. After four to 24 hours it was transferred to 70 per cent alcohol. Paraffin sections were stained with hemotocylin-eosin and periodic acid-Schiff, and studied under a light microscope.

In every case portal pressure was recorded percutaneously through the splenic pulp, and values below 150 mm of water were considered normal.[11]

Reults

The histologic appearance of jejunal mucosa in all the control cases was normal (Figure 1). The 24 patients with portal hypertension were classified as follows according to the histologic findings:

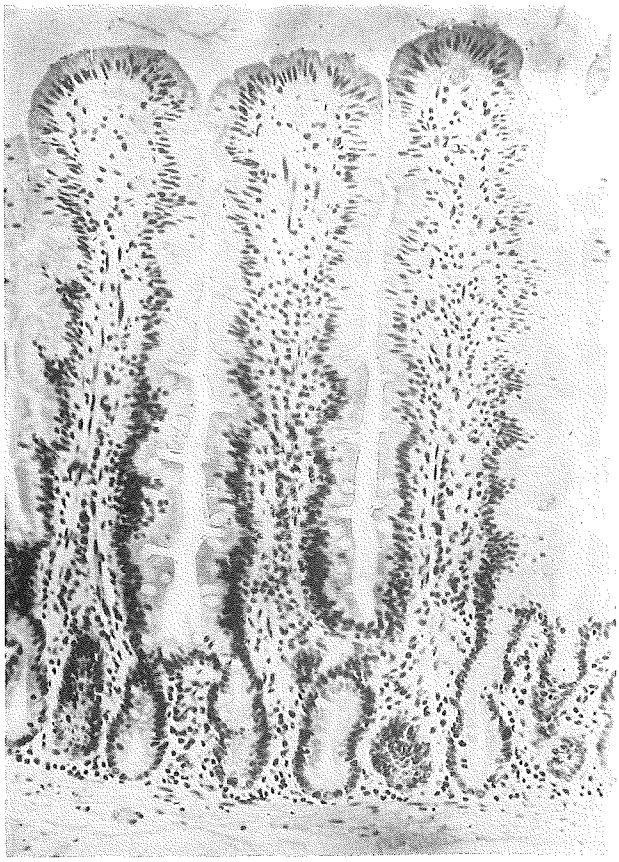
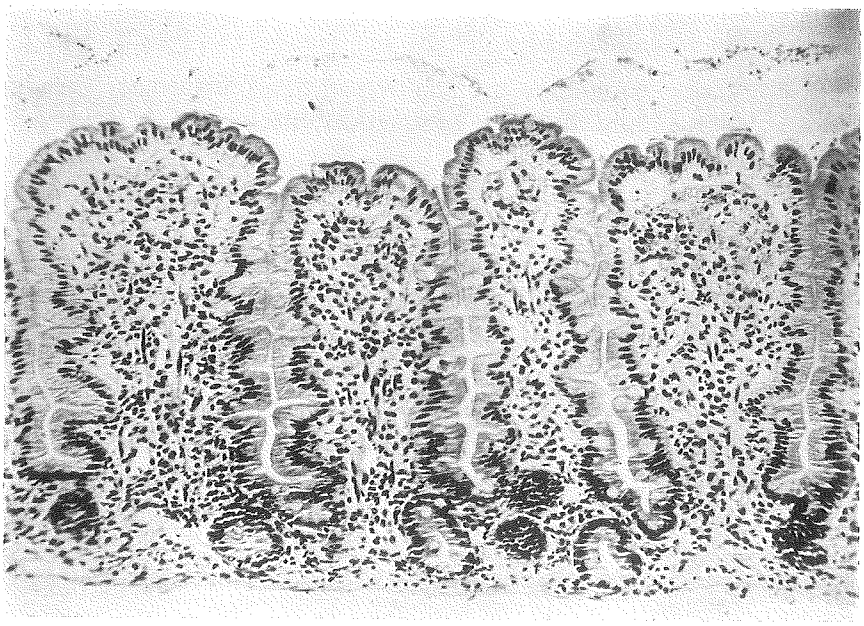
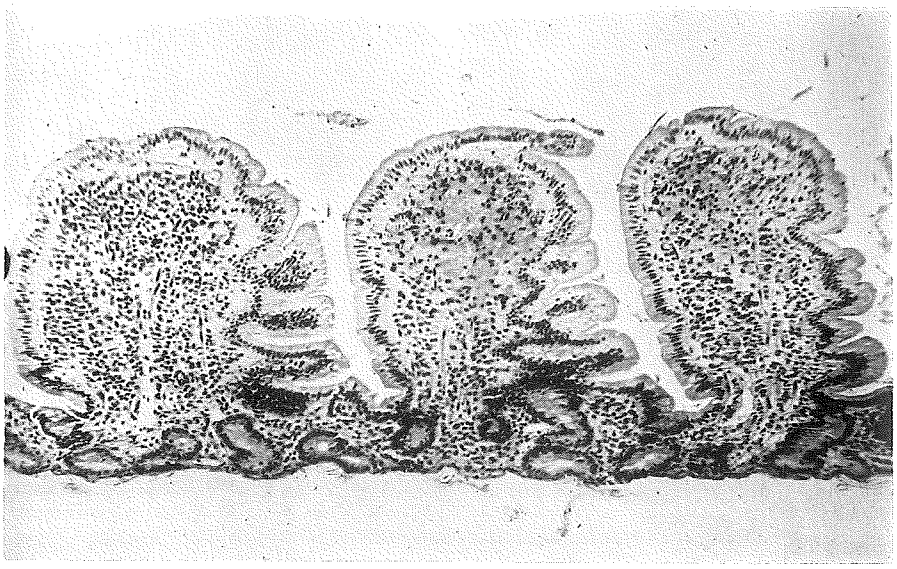


Figure 1: Jejunal mucosa of a control case

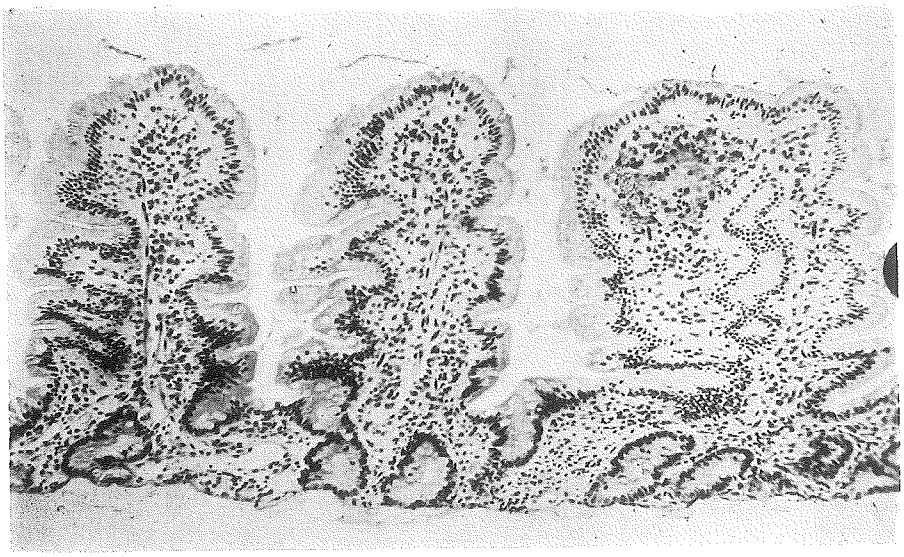
Group A: In six patients the jejunal mucosa manifested marked histologic changes. Portal pressure in this group read from 335 to 425 mm of water (Figures 2 and 3).

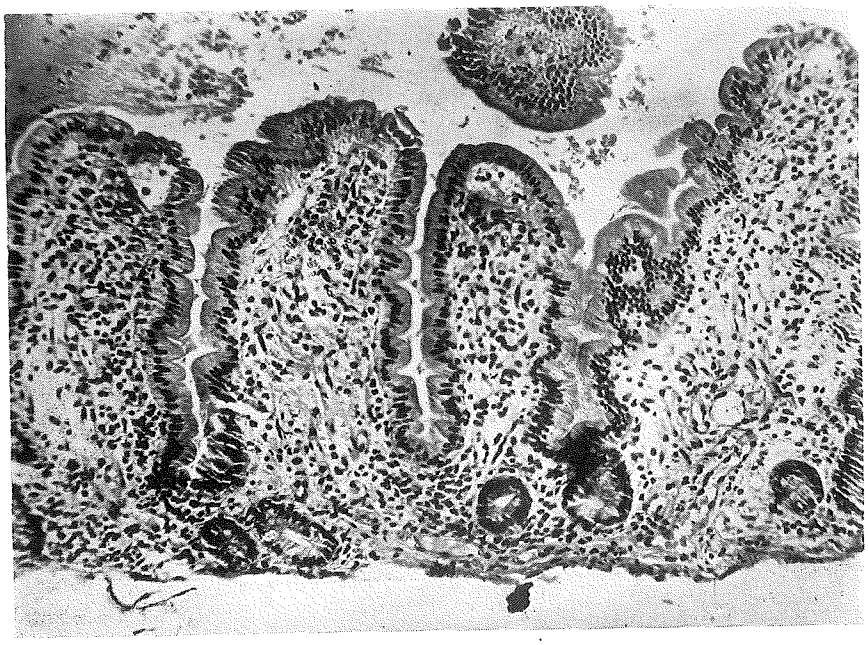


Figures 2 and 3: Jejunal mucosa of patients from Group A showing marked histologic changes



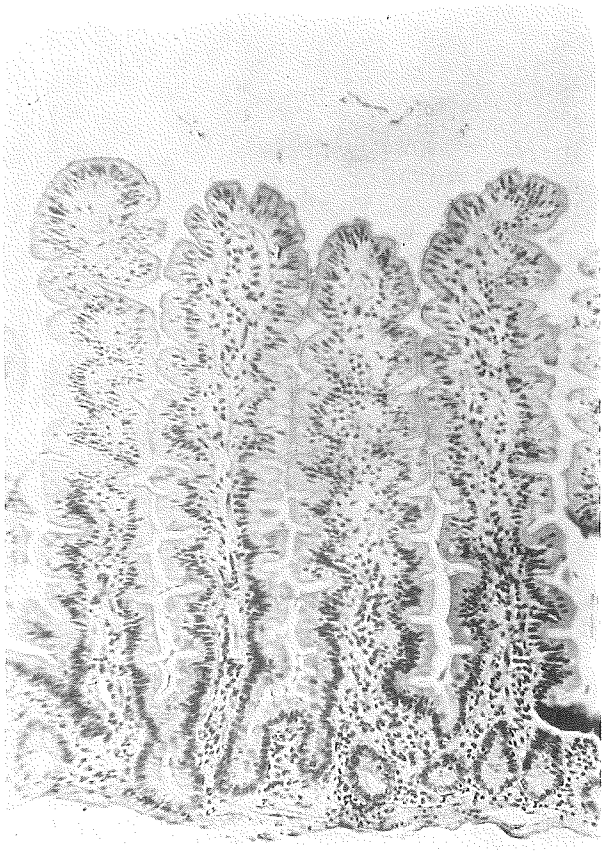
Group B: Seven patients had moderate changes in the jejunal mucosa and their portal pressures varied between 240 and 295 mm of water (Figures 4 and 5).





Figures 4 and 5: Jejunal mucosa of patients from Group B showing moderate histologic changes

Group C: Nine patients with portal pressure from 184 to 200 mm of water had normal histology (Figure 6).



Figures 6: Jejunal mucosa of a patient from Group C without histologic changes

Group D: In two patients the jejunal mucosa appeared histologically normal, however, the portal pressures were as high as in Group A, i.e. 300 mm and 410 mm of water respectively (Figure 7).

The histologic findings observed in the intestinal mucosa were summarized as follows:

1. Shortening and widening of the villi;
2. Hyperplasia and round-cell infiltration in the tunica propria;
3. Edema and hyperemia in the villi;
4. Detachment of the villus epithelium from the stroma;

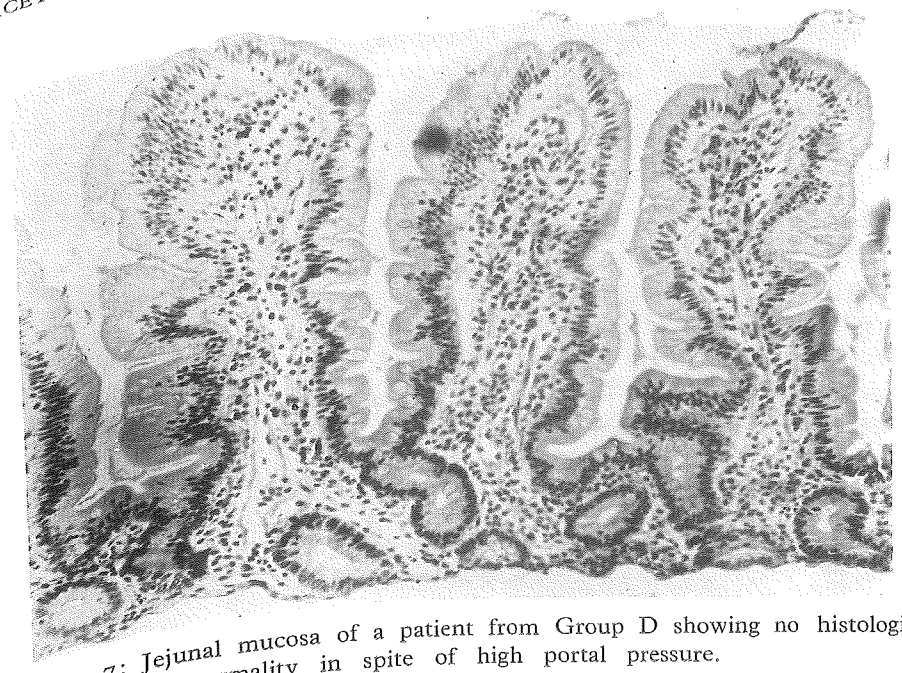


Figure 7: Jejunal mucosa of a patient from Group D showing no histologic abnormality in spite of high portal pressure.

5. Destruction of the vessels;
6. Increase in goblet cells;
7. Dilatation of the lymphatics.

Discussion

The factors leading to the histologic changes observed in the intestinal mucosa of cirrhotic patients have not been clarified, and the presence of lesions in some of the patients, and their absence in others, further complicates the subject.

Nevertheless, the observation of histologic changes in 13 patients with marked portal hypertension (Groups A and B), and the absence of such lesions in patients with minimal elevation of portal pressure, suggests the possibility that portal pressure might be one of the etiologic factors. Besides, the histologic findings such as edema in the villi, detachment of the villus epithelium from the stroma, hyperplasia of the tunica propria and vascular destruction in the villi are considered secondary to increased portal pressure. The normal histology of jejunal mucosa, in spite of the high portal pressure in Group D, does not contradict this opinion,

since the occurrence of mucosal lesions may be related not only to the degree of portal hypertension, but to its duration as well. It is probable that in the two Group D patients the duration of portal hypertension had not been long enough to cause histologic changes.

Summary

The relation of histologic changes in jejunal mucosa in liver cirrhosis to portal pressure was investigated. Of 24 patients, 15 had portal pressures ranging from 335 to 425 mm of water, and in 13 of them marked histologic changes were present. In the remaining nine patients portal pressures were recorded between 184 and 200 mm of water, and jejunal biopsies were histologically normal. As a result of these findings, portal hypertension was thought to be one of the etiologic factors producing histologic changes.

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The Effect of Irradiation on Plasma Pseudocholinesterase Levels in Men and Guinea Pigs

Muhlise Alvur M.D.* / Pınar T. Özand M.D.**

Introduction

In man and other species transient variations in serum activity of various enzymes in postirradiation period have been reported[1 2 3 4 5 6 7 8 9 10 11]. The purpose of this paper is to investigate the plasma pseudocholinesterase levels in man and experimental animals.

We have found that plasma levels of this enzyme increases after cranial irradiation in man, and decreases significantly if the irradiation is given to the abdominal region. We have been able to confirm the decrease in guinea pigs. The results suggest pseudocholinesterase as a suitable plasma enzyme to investigate the effects of irradiation.

Materials and Methods

Forty-five adults of both sex who were irradiated for treatment of various malignancies were selected. They received various doses of R's for 10-40 days. Venous blood samples were obtained before (beginning) and after irradiation. The sample collection was discontinued at the end of irradiation. The blood PCE, LDH and SGPT levels varied over a wide range both among patients and experimental animals; for this reason

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the findings were expressed as the percent of the sample obtained before irradiation. Blood samples obtained from control patients (n=10) for the same period of time did not show significant variation in PCE, LDH, and SGPT levels.

Experimental Animals:

An experimental model for irradiation was looked for and about 20 type of experimental animals were screened for their blood level of plasma pseudocholinesterase activity. Among them guinea pigs were found to be suitable. Blood samples (about 0.5 ml) were obtained through intracardiac puncture. However, guinea pigs could not resist more than 5-6 intracardiac punctures. For this reason during each group of experiments a control group was maintained without irradiation and blood samples were obtained from them together with the experimental group (control group). The values for animals who died during the experiment were rejected. When effect of long term irradiation was investigated, blood samples were obtained only starting after the 12th day of irradiation.

The serum was separated after centrifugation and enzyme analysis was performed immediately in most instances. When assayed later they were kept frozen at-20°C. Hemolytic sera were discarded. Radiation was administered from an X-ray unit which produces a radial beam at 250 kvp, with a target distance of 25 cm. The rates determined in air in the center of cages with a calibrated ratemeter. The control animals underwent sham treatment.

Plasma pseudocholinesterase was determined as described previously [12 13]. Lactic dehydrogenase determination method was as described by Kornberg[14]. SGPT determination was according to Karmen *et al*[15]. All PCE and LDH assays were run in triplicate and results averaged.

Results

The effect of irradiation on patients:

The PCE, LDH, and SGPT levels obtained in patients irradiated for various types of malignancies are shown in Table I. In patients where cranium was irradiated there was a very prominent and statistically significant increase in PCE; whereas no significant change was observed in LDH and SGPT. In patients where liver and kidney irradiation took place (abdominal irradiation) PCE level decreased significantly, and LDH level increased to a slight extent. The changes observed were statistically significant (in all cases mentioned p was < 0.02).

The effect of irradiation on animals:

Similar to the findings of patients with abdominal irradiation there was a statistically significant decline in plasma PCE levels of guinea pigs irradiated for 25 days (Figure 1). The decline observed for PCE was paralleled with an increase in LDH (Figure 2). In another group of animals the body was shielded and irradiation was given only to abdominal region. The findings were similar to whole body irradiated animals i.e., PCE level decreased whereas LDH level increased (Figure 3).

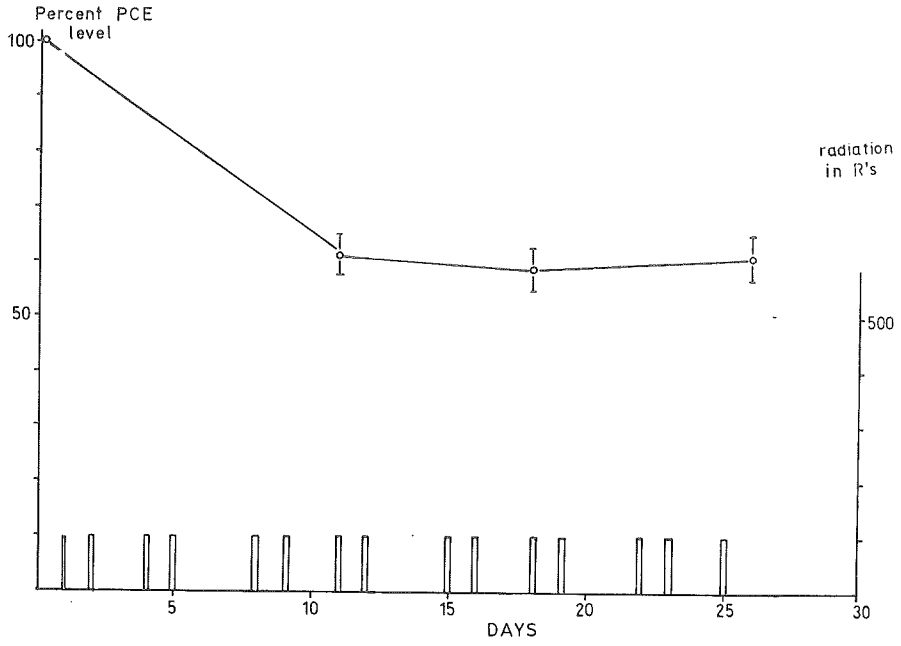

Figure 1
The Effect Of Long Term Irradiation

18 guinea pigs were irradiated with 100 r at one time, 15 times during a period of 25 days. The results are expressed as PCE percent that was found, together with the standard error.

The X-ray irradiation dosage response curve is shown in Figure 4. At low levels of irradiation the change observed in PCE level was transient and returned to normal level in a short period of time; whereas when doses higher than a total of 400 r in eight days were administered there was a steady decline in PCE level.

Previous administration of cysteine did not prevent the decrease in PCE level (Figure 5).

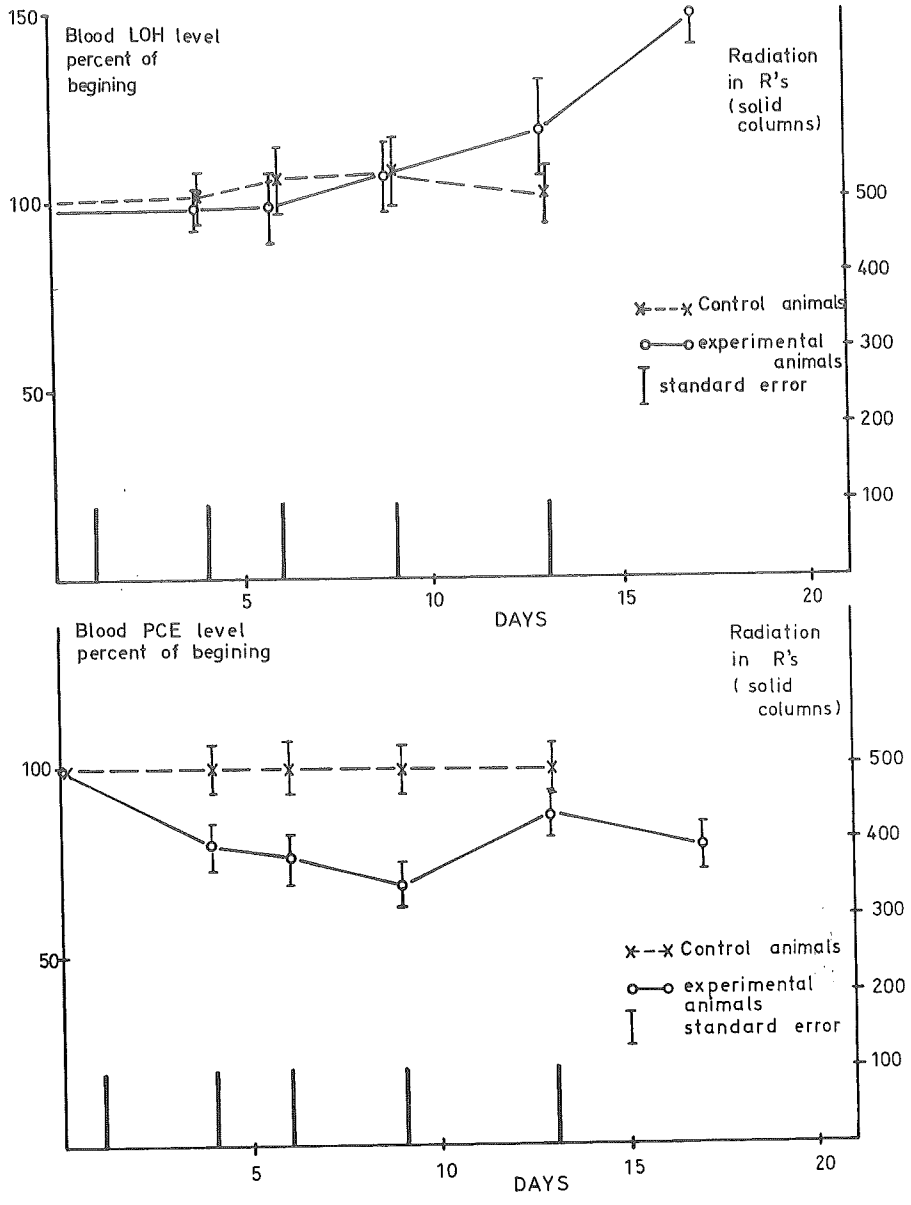

Figure 2

The Effect Of Irradiation On Plasma PCE And LDH In Guinea Pigs

18 guinea pigs were given 500 R over a period of 13 days. The results are expressed as PCE and LDH percent found, together with the standard error.

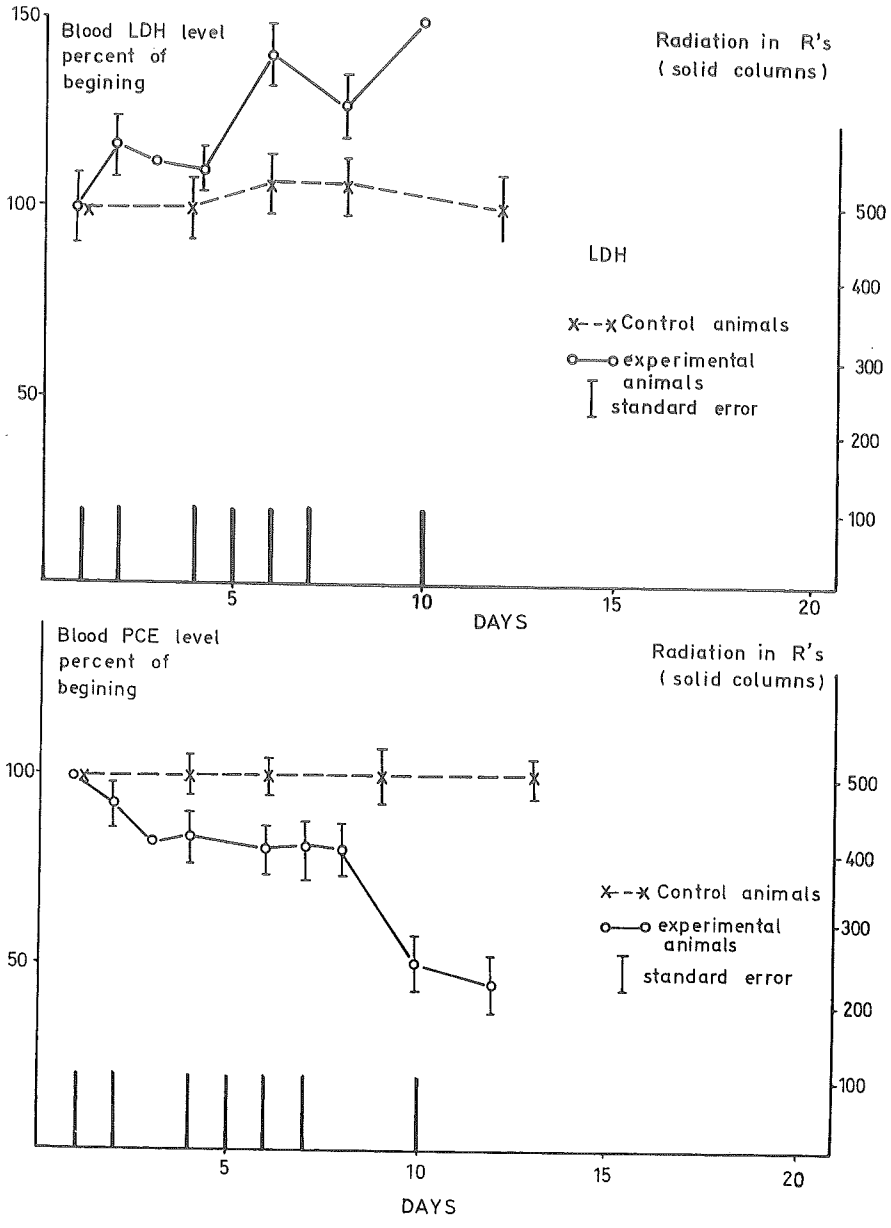

Figure 3

The Effect Of Abdominal Irradiation On LDH and PCE In Guinea Pigs
18 animals whose bodies were shielded were irradiated at the abdominal region. 700 R's total were given over a period of 12 days. The results are expressed as PCE and LDH percent found, together with the standard error.

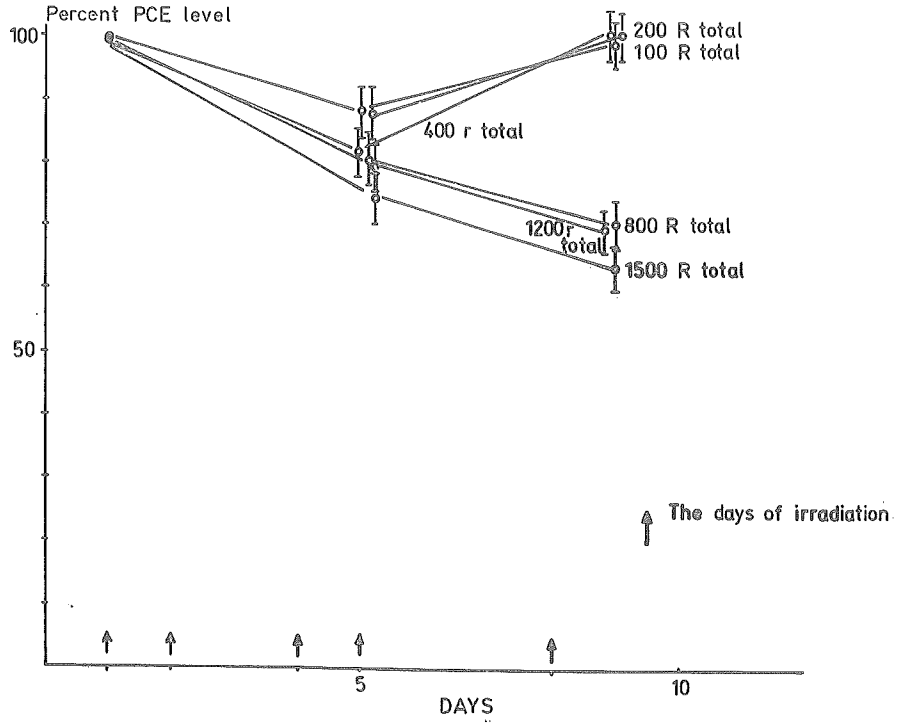

Figure 4 The X-Ray Irradiation Dosage Response Curve
Six guinea pigs were used for each experiment. Results are expressed as percent of the PCE present initially together with standard error. The indicated dosage is total, and is given in five times (each irradiation dosage being 1/5 th of the total dose indicated).

Discussion

The postirradiational PCE level in man showed a decrease upon abdominal irradiation. Lundin *et al* reported a decrease in blood cholinesterase in guinea pigs. This decrease seemed to be related to abdominal irradiation both in guinea pigs and in man. A concomitant rise in LDH or other serum enzymes[1 9] in guinea pigs would suggest liver injury and hence a decrease in liver synthesis of this enzyme. Other studies indicate liver as possible source for the enzyme circulating in plasma[1 6]. If the results were due to increased permeability of peripheral cells to enzymes or due to increased peripheral destruction, simultaneous decrease in LDH and SGPT should also be detected. Hence in guinea pigs the PCE decrease could be to due to decreased rate of synthesis in liver due to liver injury or due to a specifically increased destruction of PCE. In man abdominal irradiation specifically led to a decrease in PCE, LDH and SGPT levels

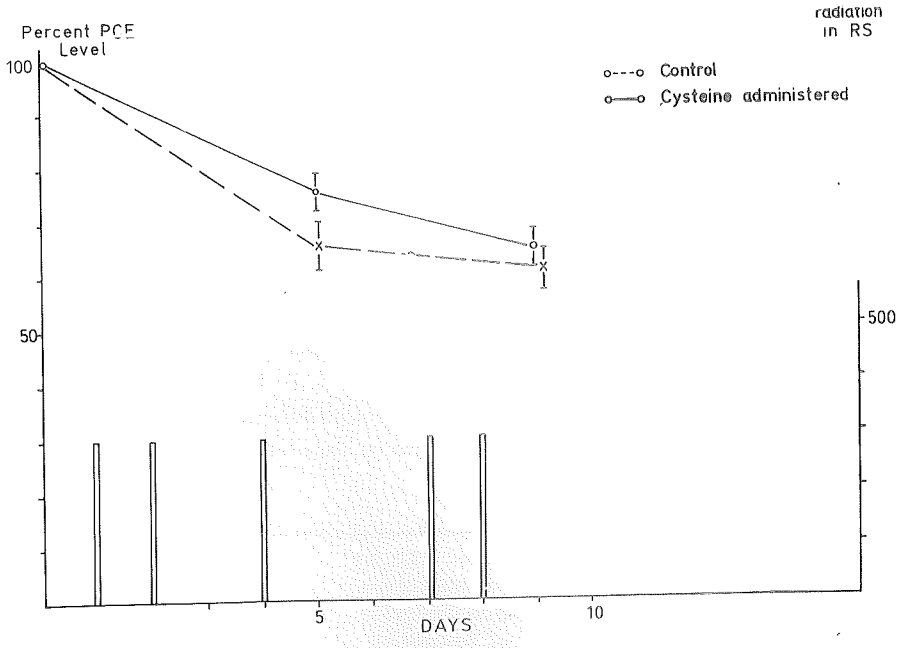

Figure 5 The Fffect Of Previously Administered Cysteine

18 guinea pigs were used for each group of experiments. Results are expressed as the percent of the initial value together with standard error. The experimental animals were given intraperitonecally neutralized cysteine solution at a dose of 0.5 gram/kg. animal weight. One hour after the cysteine administration the irradiation was given.

did not change. This finding supports a postirradiational decrease in liver synthesis without any gross liver cell injury evidenced by a lack of serum LDH and SGPT increase; or again we could postulate a specific peripheral destruction of PCE by irradiation. However, irradiation of other parts of the body did not lead to a decrease in serum PCE in man.

All these findings suggest either a decrease in enzyme synthesis or a conformational change of enzyme in the liver upon irradiation.

The postirradiational increase in PCE levels in man upon cranial irradiation suggest the liberation of enzyme from central nervous system. Enzymes have been shown to be lost from cells without morphologic alterations[17]. It has been demonstrated that central nervous system tissues and capillaries contain a cholinesterase similar to plasma pseudocholinesterase in substrate specificity[18,19]. The findings of cranial irradiation may be explained by a loss of the enzyme from its binding sites in the central nervous system.

TABLE I

THE EFFECT OF IRRADIATION UPON ENZYME LEVELS IN PATIENTS

The plasma pseudocholinesterase (PCE); lactic dehydrogenase (LDH); and glutamic-pyruvic transaminase (SGPT) levels were measured in patients with various types of malignancies irradiated. The irradiation duration was between 10-40 days. Patients were divided into groups according to the site of irradiation. The total R range is indicated. The average of the enzyme level was calculated and reported as percent of the beginning value with ± S. E. for every determination day; n denotes the number of patients in the group. Determinations were discontinued at the end of irradiation. Blood specimens were obtained from adult patients of both sex.

Group of Patients	The range of R	Days of Enzyme Determination after the onset of irradiation	PCE %	LDH %	SGPT %
Upper and Lower Extremities and inguinal and axillary fossa irradiation n = 8	3000–8000	Beginning	100	100	100
		1	105 ±5	103 ±8	102 ±1
		3	109 ±12	115 ±8	105 ±5
		6	105 ±5	120 ±10	100 ±5
		12	115 ±12	110 ±5	100 ±5
		16	116 ±12	100 ±3	105 ±6
		25	113 ±15	105 ±5	98 ±3
		34	120 ±20	108 ±5	103 ±5
Pelvic irradiation n = 8	3000–7000	Beginning	100	100	100
		1	100 ±2	105 ±5	100 ±2
		4	96 ±3	110 ±5	100 ±5
		8	106 ±5	115 ±5	100 ±3
		15	98 ±3	100 ±3	105 ±4
		21	107 ±5	103 ±3	98 ±5
Irradiation of thorax n = 8	3000–9000	Beginning	100	100	100
		2	96 ±2	100 ±4	100 ±2
		4	92 ±8	116 ±7	109 ±15
		6	83 ±7	98 ±12	110 ±8
		9	107 ±3	116 ±8	100 ±10
		14	94 ±3	105 ±13	104 ± 3
		24	103 ±2	108 ±2	100 ±3
		38	114 ±6	95 ±8	100 ±5

Group of Patients	The range of R	Days of Enzyme Determinations after the onset of irradiation	PCE %	LDH %	SGPT %
Cranial irradiation		Beginning	100	100	100
n = 8		1	120 ±5	100 ±5	
		5	133 ±13	110 ±5	
		9	193 ±10	115 ±5	
		15	180 ±10	120 ±5	
		22	210 ±10	120 ±15	
		35	240 ±10	110 ±8	
Abdominal irradiation					
n = 13	3000–5000	Beginning	100	100	100
		3	98 ±3	95 ±5	105 ±5
		6	83 ±5	124 ±10	110 ±5
		9	71 ±7	120 ±10	100 ±8
		16	60 ±6	125 ±5	109 ±5

The dose response curve as shown in Figure 4 suggests a complicated multiphasic process involved in the effect of irradiation upon PCE. The lack of cysteine protection affect on the enzyme may also be related to more than one type of effect present leading to the PCE level change in guinea pig. These processes can be best understood by kinetic behavioral change and by the establishment of the mode of biosynthesis of the enzyme. Such studies are not conducted during these experiments. A clear answer should wait the clarification of these latter points.

Summary

1. Abdominal irradiation of man and guinea pigs led to a significant decrease in plasma PCE.

2. Cranial irradiation in men led to an increase in plasma PCE.

3. The results suggest a decreased rate synthesis in the former and an increased liberation from tissues in the latter case.

4. The dose response curve of the enzyme level in guinea pigs suggests a complicated process underlying the change of level observed.

Acknowledgements

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