hacettepe medical journal

A QUARTERLY PUBLICATION

VOLUME 16 / NO. 1 / JANUARY 1983

PUBLISHED BY HACETTEPE UNIVERSITY PRESS

SUBSCRIPTION RATES

TURKEY :	Annual subscription (four issues forming one volume including postage)	1500.00 TL.
	Special annual rate for students, interns and residents	500.00 TL.
	Single issue (including postage)	500.00 TL.
FOREIGN :	Annual subscription (including postage)	$ 20.00 or 50 D.M.
	Special annual rate for students, interns and residents	$ 10.00 or 25 D.M.
	Single issue (including postage)	$ 6.00 or 15 D.M.

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HACETTEPE TIP DERGİSİ/HACETTEPE MEDICAL JOURNAL
HACETTEPE ÜNİVERSİTESİ TIP FAKÜLTESİ DEKANLIĞI HACETTEPE-ANKARA

Printed by
Hacettepe University Press
Printing Division

VOLUME 16 / NO. 1 / JANUARY 1983

hacettepe medical journal

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FOREWORD

Beginning with this issue, Hacettepe Medical Journal, (formerly Hacettepe Bulletin of Medicine/Surgery) appears with a new face. The changes will be not only in style but also in content. A new editorial board will try to assure the delivery of best in basic and clinical medical sciences to our readers with differing disciplines and interests. The journal, in its English edition, hopes to establish a bridge between the world and Turkish medicines, of which Hacettepe has traditionally been a prominent representative. It is our sincere wish that the journal will accomplish this important feat with the dedicated work of our authors, referees, publishers, but most of all with the continuous support of our readers.

Editor

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An Echocardiographic Study of Diabetic Patients without Clinical Heart Disease*

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Introduction

Recently, it has been suggested that a specific type of cardiomyopathy might develop in diabetics in the absence of large coronary artery involvement, hypertension or valvular disorders. In addition, it has been thought that small vessel disease might be responsible for this myocardial dysfunction and recent studies have directed us to detect this disorder in the preclinical phase by non-invasive techniques.[1-8]

In this study, we performed a detailed echocardiographic examination in diabetics without clinical heart disease to determine the value of this non-invasive technique in early detection of clinical cardiac abnormalities in diabetic patients.

Materials and Methods

The echocardiographic examination was performed on 24 diabetic patients aged under 40 and 18 age and sex matched healthy control subjects. In the diabetic group the mean age was 26.9 (with a range of

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16-38). None of these patients had any cardiorespiratory symptoms or signs and all of them had no evidence of any systemic disease that could have affected the heart. Standart ECG's taken in all patients revealed no abnormality. In order to exclude a latent coronary artery involvement exercise tests with thread-mill were performed and evaluated by the criteria described elsewhere in the literature and all were within normal limits.[9, 10] The chest x-rays and additional evaluation of cardiac silhouette by measuring transvers diameter, cardiothoracic ratio and oblique diameter according to the previously defined criteria revealed no abnormality.[11, 12] In the control group the mean age was 25.9 (with a range of 17-37) and none had a family history of diabetes mellitus or any evidence of cardiopulmonary disease. Fasting and postprandial blood sugar levels were within normal limits. Standart ECG's, plain chest X-rays and maximal exercise tests revealed no abnormality.

In the study group, 16 of the diabetic subjects were insulin dependent and appeared to have juvenile onset diabetes (were known diabetic by the age of 25), 8 were on oral hypoglycemic agents and appeared to have adult onset diabetes (were known diabetic after the age of 25). According to the degree of blood sugar control they were divided into three groups: well-controlled group (12 patients), lightly-controlled group (3 patients) and uncontrolled group (9 patients).[13] The fluorescein angiography in 11 patients revealed diabetic retinopathy and 13 had normal retinal findings. The known duration of diabetes was less than 1 year in 7 patients,1-5 years in 7 patients and more than 5 years in 10 patients.

Electrocardiograms, phonocardiograms and echocardiograms were simultaneously recorded with a "Smith-Kline Ekoline 20 Diagnostic Ultrasonoscope" utilizing a 0.5 inch diameter 2.25 mHz transducer focused at 10 cm and an "Electronics for Medicine Dr-12 Research Monitor". Left ventricular, mitral and aortic valve echograms were recorded with a technique and criteria reported previously.[14] After obtaining technically excellent echograms we calculated the following parameters according to the definition elsewhere in the literature:[10, 14, 15] Left ventricular end-diastolic diameter (EdD), left ventricular end-systolic diameter (EsD), stroke volume and stroke volume index, cardiac output and cardiac index, ejection fraction, fractional shortening, velocity of circumferential fiber shortening (VCF), mitral valve E-F slope, the ratio of left atrial diameter/aortic diameter.

Results

The echocardiographic parameters measured in 24 diabetic patients and 18 normal subjects are shown in Table I. As indicated in the table

we found a significant difference in cardiac index ($p < 0.01$), VCF ($p < 0.01$) and mitral E-F slope ($p < 0.01$) of the diabetic patients group as compared to normal subjects. The cardiac index was 33.54 %, VCF was 26 % and mitral E-F slope was 16.16 % higher in the control subjects. The difference in the other echocardiographic parameters were not found to be statistically significant.

TABLE I

THE ECHOCARDIOGRAPHIC PARAMETERS MEASURED IN 24 DIABETIC PATIENTS AND 18 NORMAL SUBJECTS

Echocardiographic Parameter	The Study Group*	The Control Group*	p Value
End Diastolic Diameter	4.89 ∓ 0.59	5.01 ∓ 1.12	$p > 0.5$
End Systolic Diameter	3.34 ∓ 0.53	3.36 ∓ 0.91	$p > 0.8$
Stroke Volume Index	42.34 ∓ 17.49	50.97 ∓ 16.43	$p > 0.1$
Cardiac Index	3.19 ∓ 1.21	4.26 ∓ 1.43	$p < 0.02$
Ejection Fraction	66.08 ∓ 11.51	69.88 ∓ 10.06	$p > 0.2$
Fractional Shortening	31.04 ∓ 8.58	35.00 ∓ 6.61	$p > 0.1$
VCF	1.00 ∓ 0.29	1.26 ∓ 0.23	$p < 0.01$
Mitral E-F Slope	93.29 ∓ 20.25	108.65 ∓ 24.33	$p < 0.05$
PR-AC	0.796 ∓ 0.286	0.939 ∓ 0.128	$p > 0.05$
Left atrial diameter/Aortic diameter	0.974 ∓ 0.152	1.043 ∓ 0.103	$p > 0.1$

* Mean ∓ S.D.

In evaluating according to the type of diabetes, we found significant difference in EsD ($p < 0.01$), EdD ($p < 0.05$) and mitral E-F slope ($p < 0.05$) between the juvenile and adult onset diabetics (Table II).

There was no significant difference between those with and without retinopathy (Table III). There was also no association between the known duration of diabetes (Table IV) or quality of previous diabetic control (Table V) and the left ventricular function measured echocardiographically.

TABLE II

EVALUATION OF THE ECHOCARDIOGRAPHIC FINDINGS ACCORDING TO THE TYPE OF DIABETES MELLITUS

Echocardiographic Parameter	Juvenil Onset Diabetes*	Adult Onset Diabetes*	p Value
End diastolic diameter	4.72 ∓ 0.54	5.24 ∓ 0.56	$p < 0.05$
End systolic diameter	3.12 ∓ 0.44	3.78 ∓ 0.43	$p < 0.01$
Stroke volume index ml/m²	43 71 ∓ 20.67	39.61 ∓ 8.81	$p > 0.50$
Cardiac index liter/min/m²	3.29 ∓ 1.37	2.99 ∓ 0.85	$p > 0.50$
Ejection Fraction %	68.25 ∓ 13.20	61.75 ∓ 5.47	$p > 0.10$
Fractional shortening %	32.87 ∓ 9.79	27.37 ∓ 3.66	$p > 0.10$
VCF cycle/sec	1.05 ∓ 0.34	0.91 ∓ 0.15	$p > 0.20$
Mitral E-F slope mm/sec	87.50 ∓ 17.09	104.87 ∓ 23.35	$p < 0.001$
PR-AC sec	0.768 ∓ 0.297	0.852 ∓ 0.275	$p > 0.50$
Left atrial dia/ Aortic dia.	1.00 ∓ 0.148	0.920 ∓ 0.155	$p > 0.50$

* Mean ∓ S.D.

TABLE III

THE ECHOCARDIOGRAPHIC FINDINGS IN DIABETICS WITH AND WITHOUT RETINOPATHY

Echocardiographic Parameter	Diabetic Retinopathy*	Normal Retinal Findings*	p Value
End diastolic diameter cm	4.88 ∓ 0.58	4.91 ∓ 0.62	$p > 0.8$
End systolic diameter cm	3.31 ∓ 0.52	3.38 ∓ 0.57	$p > 0.50$
Stroke volume index ml/m²	41.99 ∓ 14.68	42.70 ∓ 20.59	$p > 0.8$
Cardiac index liter/min/m²	3.06 ∓ 0.87	3.31 ∓ 1.50	$p > 0.50$
Ejection fraction %	67.16 ∓ 10.43	65.00 ∓ 12.88	$p > 0.50$
Fractional shortening %	51.50 ∓ 7.30	30.58 ∓ 10.00	$p > 0.50$
VCF cycle/sec	1.04 ∓ 0.34	0.97 ∓ 0.25	$p > 0.50$
Mitral E-F slope mm/sec	95.50 ∓ 20.38	91.08 ∓ 21.59	$p > 0.50$
PR-AC sec	0.795 ∓ 0.314	0.798 ∓ 0.270	$p > 0.80$
Left atrial dia/ Aortic dia.	0.975 ∓ 0.134	0.973 ∓ 0.174	$p > 0.80$

* Mean ∓ S.D.

TABLE IV

THE EVALUATION OF THE ECHOCARDIOGRAPHIC FINDINGS ACCORDING TO THE KNOWN DURATION OF DIABETES MELLITUS

Echocardiographic Parameter	Less Than 1 Year*	1–5 Years*	More Than 5 Years*	p Value
End diastolic diameter cm	5.10 ∓ 0.59	4.90 ∓ 0.51	4.66 ∓ 0.63	**
End systolic diameter cm	3.51 ∓ 0.47	3.40 ∓ 0.57	3.10 ∓ 0.53	**
Stroke volume index ml/m²	45.89 ∓22.20	40.73 ∓16.96	39.75 ∓ 13.02	**
Cardiac index liter/min/m²	3.21 ∓ 1.54	3.08 ∓ 1.08	3.25 ∓ 1.03	**
Ejection fraction %	64.77 ∓12.73	64.14 ∓13.34	69.25 ∓ 8.97	**
Fractional shortening %	30.33 ∓ 9.65	30.00 ∓ 9.79	32.75 ∓ 6.94	**
VCF cycle/sec	0.95 ∓ 0.30	0.93 ∓ 0.16	1.12 ∓ 0.37	**
Mitral E-F slope mm/sec	101.11 ∓ 22.8	90.28 ∓ 23.8	87.12 ∓ 13.9	**
PR-AC sn	0.952 ∓ 0.3	0.710 ∓ 0.3	0.697 ∓ 0.2	**
Left atrial diameter/Aortic diameter	0.957 ∓ 0.17	0.935 ∓ 0.17	1.026 ∓ 0.10	**

* Mean ∓ S.D.
** Not significant for all groups.

TABLE V

THE EVALUATION OF THE ECHOCARDIOGRAPHIC FINDINGS ACCORDING TO THE QUALITY OF THE PREVIOUS DIABETIC CONTROL

Echocardiographic Parameter*	Well Controlled	Lightly Controlled	Uncontrolled	p Value
End diastolic diameter cm	4.79 ∓ 0.54	4.37 ∓ 0.33	5.20 ∓ 0.59	**
End systolic diameter cm	3.31 ∓ 0.62	3.12 ∓ 0.17	3.46 ∓ 0.48	**
Stroke volume index ml/m²	37.80 ∓ 9.76	27.39 ∓ 5.13	53.38 ∓ 22.2	**
Cardiac index liter/min/m²	3.07 ∓ 0.89	2.10 ∓ 0.21	3.71 ∓ 1.33	**
Ejection fraction %	65.58 ∓11.95	63.00 ∓ 3.00	67.77 ∓ 13.2	**
Fractional shortening %	30.75 ∓ 8.89	28.00 ∓ 2.00	32.44 ∓ 9.79	**
VCF cycle/sec	1.02 ∓ 0.35	0.95 ∓ 0.40	1.00 ∓ 0.28	**
Mitral E-F slope mm/sec	98.25 ∓18.62	83.00 ∓ 4.16	89.88 ∓ 25.7	**
PR-AC sec	0.698 ∓0.249	0.820 ∓ 0.33	0.92 ∓ 0.30	**
Left atrial dia/ Aortic diameter	0.96 ∓ 0.15	0.81 ∓ 0.13	1.05 ∓ 0.12	**

* Mean ∓ S.D.
** Not significant for all groups.

Discussion

Most of the recent investigation on the diabetic heart disease have been limited to the assessment of left ventricular function either by systolic time intervals or by evaluation of a few echocardiographic parameters.[3, 4, 6, 7, 16] For that reason, in the present study we performed a detailed echocardiographic examination hoping to reach more precise conclusions.

Echocardiography has become one of the most important non-invasive methods for cardiac diagnosis. A close relationship of ultrasound with angiography in measurements of left ventricular volumes and contractility was demonstrated in early echocardiographic studies.[9, 14, 17, 19] Thus, echocardiographic assessment of left ventricular performance is reliable as well as non-invasive.

Among the assessed echocardiographic parameters we found a statistically significant difference in cardiac index, VCF and mitral E-F slope between the diabetic and control groups ($p < 0.01$).

Our findings which reflect both systolic and diastolic events of the cardiac cycle is consistent with the previous idea of the left ventricular dysfunction in diabetics without clinical heart disease. It seems far from the fact that all of the abnormalities that we found were due to a premature coronary artery disease because of the strict selection of the study group (free of angina, normal ECG's, maximal exercise tests and chest x rays). In addition, our findings failed to show any influence of diabetic control on impaired myocardial performance. Accordingly one can rule out metabolic factors as a cause of myocardiopathy.

Thus, it seems to be reasonable to postulate that the diabetic small vessel disease plays an important role in the pathogenesis of the impaired myocardial performance described above. However, in our study there was no statistically significant difference between the patients with and without diabetic retinopathy. In addition, the recent studies of diabetics suggest that small vessel lesions in diabetes may have little or no relation to cardiac abnormalites.[5, 20]

On the other hand, according to the recent morphological studies the change in cardiac function may be related to altered muscle composition in the form of interstitial glycoprotein and collagen accumulation.[5, 21] Although the mechanism is not clear, the amount and distribution of glycoprotein and collagen in the interstitium may determine the progression from the functional cardiac abnormality to clinical cardiac decompensation.[1]

In conclusion, our study confirms the occurrence of a relative reduction in myocardial performance in the absence of major coronary artery involvement, hypertension and valvular heart disease. However, we have not reached a precise pathophysiologic explanation. The echocardiographic assessment of left ventricular performance seems to be a useful method to detect this abnormality in the preclinical phase. Since all of our patients were asymptomatic invasive diagnostic procedures such as cardiac catheterization and angiography were not performed. Further studies of diabetic patients will describe the factors that may contribute to this abnormality and its natural course.

Summary

In this study we performed a detailed echocardiographic examination in 24 diabetic patients without clinical heart disease and in 18 healthy person. We found a statistically significant difference in cardiac index, VCF and mitral E-F slope between the diabetic and control groups ($p < 0.01$).

The possible pathogenetic explanations of these abnormalities are discussed and it is concluded that the echocardiographic assessment of left ventricular performance is a useful method to detect this myocardial dysfunction in the preclinical phase.

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Hacettepe Medical Journal
Volume 16 / No 1 / January 1983 / pp. 9 – 14

Effect of Proximal Gastric Vagotomy on Gastric Mucus Secretion*

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Proximal gastric vagotomy (PGV) have been extensively used in the treatment of duodenal ulcer in recent years. The effect of proximal gastric vagotomy on gastric acid secretion have been studied in numerous reports. Gastric acid secretion is reduced significantly after PGV,[1-4] but its effect on gastric mucus secretion have not been studied extensively.

Wise and Ballinger found a 75 % fall in the basal mucus output after truncal vagotomy in dogs.[5] On the otherhand, again Wise and Ballinger found that vagotomy caused a change in the quality of gastric mucus rather than the quantity.[6]

The aim of this study was to evaluate the effect of proximal gastric vagotomy on gastric mucus secretion in the early postoperative period.

Material and Method

Basal gastric contents were collected for one hour in 14 patients who had proximal gastric vagotomy preoperatively and postoperatively on the first and seventh day. The total dry weight of gastric mucus, total proteins, protein bound hexose, hexosamine, sialic acid and fucose concentrations were determined in the hourly gastric aspirates as previously described.[6-10]

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Basal acid output (BAO) was also determined preoperatively and postoperatively on the seventh day. Gastric contents were collected at 15 minutes interval for two hours to determine BAO.

The statistical analysis was done with Student t test.

Result

Dry Weight of Total Gastric Mucus (mg %): The mean dry weight of total gastric mucus was 649.42 $\pm$ 25.92, 1062.20 $\pm$ 69.56 and 1007.15 $\pm$ 108.29 mg % preoperatively and on the first and seventh postoperative day respectively. PGV caused a significant rise in the dry weight of gastric mucus on the first and seventh postoperative day ($p < 0.01$). The difference between the first and seventh day was not significant ($p > 0.05$).

Total Proteins (mg %): The mean total protein concentrations in the gastric juice was 254.24 $\pm$ 40.02 mg % preoperatively. On the first postoperative day the total proteins were elevated significantly to a level of 479.46 $\pm$ 61.48 mg % ($p < 0.01$) and decreased significantly to 262.38 $\pm$ 41.76 mg % ($p < 0.01$) on the seventh postoperative day. The difference between the preoperative and postoperative seventh day values was not significant ($p > 0.05$).

Protein Bound Hexose (mg %): The mean gastric juice hexosamine concentration was 34.51 $\pm$ 4.09 mg % preoperatively. On the first post PGV day a non-significant rise ($p > 0.05$) was determined. On the otherhand the rise on the seventh postoperative day, 56.48 $\pm$ 12.18 mg %, was significant ($p < 0.01$). The difference between the first and seventh postoperative day was not significant ($p > 0.05$).

Hexosamine (mg %): The mean hexosamine concentrations in the gastric aspirates were 11.44 $\pm$ 1.31, 16.62 $\pm$ 2.24 and 24.64 $\pm$ 3.65 mg % preoperatively and on the first and seventh postoperative day respectively. This rise was not significant on the first ($p > 0.05$), but was found to be significant on the seventh postoperative day ($p < 0.01$). The difference between the first and seventh postoperative days was also significant ($p < 0.05$).

Sialic Acid (mg %): The mean sialic acid concentrations were 12.99 $\pm$ 0.76, 18.40 $\pm$ 2.39 and 18.05 $\pm$ 1.44 mg % preoperatively and the first and seventh postoperative day respectively. This rise was significant on the first postoperative day ($p < 0.05$) and remained significant on the seventh day ($p < 0.01$).

Fucose (mg %): The mean fucose concentrations were 19.31 ± 2.20, 22.14 ± 2.18 and 21.40 ± 3.72 mg % preoperatively and the first and seventh postoperative day respectively. The differences between pre-and postoperative values were not significant ($p > 0.05$).

Basal Acid Output (BAO) (mEq/hr): The mean BAO was 3.90 ± 0.69 mEq/hr preoperatively and 0.78 ± 0.22 mEq/hr on the seventh postoperative day. This reduction in the BAO was significant ($p < 0.01$).

The results are summarized in Table I.

TABLE I

EFFECT OF PROXIMAL GASTRIC VAGOTOMY ON GASTRIC MUCUS AND BAO

	Preoperative (mean ± S.D.)	Postoperative 24 Hours (mean ± S.D.)	Postoperative Seventh day (mean ± S.D.)
Dry weight of total gastric mucus (mg %)	649.42 ± 25.92 (n : 14)	1062.20 ± 69.56 (n : 10)	1007.15 ±108.29 (n : 13)
Total protein (mg %)	254.23 ± 40.02 (n : 14)	479.46 ± 61.48 (n : 13)	262.38 ± 41.76 (n : 13)
Protein Bound Hexose (mg %)	34.15 ± 4.09 (n : 12)	51.96 ± 7.17 (n : 10)	56.48 ± 12.18 (n : 10)
Hexosamine (mg %)	11.44 ± 1.31 (n : 12)	16.62 ± 2.24 (n : 10)	24.64 ± 3.65 (n : 11)
Sialic Acid (mg %)	12.99 ± 0.76 (n : 12)	18.40 ± 2.39 (n : 13)	18.05 ± 1.44 (n : 13)
Fucose (mg %)	19.31 ± 2.20 (n : 10)	22.14 ± 2.18 (n : 10)	21.40 ± 3.72 (n : 10)
BAO (mEq/hour)	3.90 ± 0.69 (n : 11)	–	0.78 ± 0.22 (n : 11)

Discussion

The layer of mucus which covers the gastric mucosa is formed by glycoprotein molecules and carbohydrate complexes.[11] The glycoprotein molecules are synthesized and secreted by gastric mucosal cells, particularly surface epithelial cells, foveolar cells, mucous neck cells and cells in pyloric gland.[12] Gastric acidity, serotonin, secretin and pentagastrin infusions, parathyroid hormone injections and direct gastric stimulation appear to increase mucus secretion.[13]

The effect of the vagi nerves on the gastric mucus secretion is controversial. In some studies the vagal stimulation caused a rise in mucus output,[6, 13] and in some it did not.[14] Although some investigators have found a decrease in total gastric mucus output after vagotomy, others found a change in the quality rather than the amount of the gastric mucus.[5, 6]

Proximal gastric vagotomy (PGV) have been used extensively in the treatment of duodenal ulcer since early 1970's. This operation has been effective in reducing the gastric acidity.[1-4] But its effect on mucus secretion is not known. The effect of PGV on mucus secretion might be expected to be different from truncal vagotomy because of the innervation of the antrum and pyloric region which contain cells secreting and synthesizing glycoproteins.

We have found a significant increase in the total dry weight of gastric mucus after PGV which starts early in the postoperative day too. This elevation have been in the non-protein, carbohydrate, portion of the gastric mucus components. The proteins increased significantly after PGV on the first postoperative day. This increase returned to the preoperative values on the seventh postoperative day. The early rise of the proteins in the gastric juice might be secondary to the surgical trauma to the stomach. Devascularization of the proximal stomach during PGV might play a role in this findings. As it has been shown by some investigators the increase in the proteins of the gastric juice might be secondary to mucosal damage.[15, 16]

As seen in our study proximal gastric vagotomy have reduced the basal acid output significantly. This effect seems to be independent from the effect on mucus secretion.

Proximal gastric vagotomy caused an increase in the non-protein / protein ratio of gastric mucus. This effect on the quality of the gastric mucus was more prominent on the seventh postoperative day. Alteration of the physical and chemical properties of the proteins in the gastric juice may be protective to peptic digestion.[6] Thus, the effect of PGV on the quality of the gastric mucus might be beneficial in the early healing of the ulcer.

Stimulation of gastric mucus after PGV seems to be another advantage of this operation. This effect of PGV on mucus secretion might be secondary to the preservation of the antral and pyloric innervation. This innervation might play a role in secreting and synthesizing gastric mucus. Beside this role, keeping the pylorus functioning PGV does not cause

bile reflux. Bile and bile acids are known to be destructive to the mucous cells.[17]

Although, this beneficial effect of PGV is demonstrated only in the early postoperative period we believe it is significant. It will be necessary to asses the late effects of PGV before definite conclusions could be made.

Summary

The effect of proximal gastric vagotomy on gastric mucus secretion was evaluated on 14 patients in the early postoperative period. The gastric mucus and some of its components increased significantly in the early postoperative period. This increase was more prominent in the non-protein components. Although, these are the early effects they seem to be beneficial for the ulcer healing.

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Hacettepe Medical Journal
Volume 16 / No 1 / January 1983 / pp. 15 – 23

Antiplatelet Antibodies in Chronic Idiopathic Thrombocytopenic Purpura*

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It is generally accepted that the thrombocytopenia in chronic idiopathic thrombocytopenic purpura (Chronic ITP) occurs as a result of antiplatelet-antibodies of IgG type. Yet a practical and reliable assay to demonstrate the presence of these antibodies has not been developed. The major problem with such an assay is the inability of the antiplatelet antibodies in chronic ITP to agglutinate or bind the complement.[1-7] This obstacle could be overcome since it has recently been shown that these antibodies have opsonic activities. This property has been used to detect the antiplatelet antibodies[8] as opsonizing antibodies in the ITP sera leads to the phagocytosis of normal platelets and thus increase in the NBT (nitroblue tetrazolium test) reduction by the leucocytes. An alternative procedure may be to precipitate the leucocytes which ingest Cr^{51}-labelled platelets. But ingestion of Cr^{51}-labelled platelets and increase in NBT reduction may simply result from non-phagocytic events such as adhesion of platelets to leucocytes.[9] Besides, platelets, themselves, are shown to cause NBT reduction.[10] If the antiplatelet antibodies in chronic ITP do have opsonic activity they must activate the hexose-monophosphate shunt in the leucocytes. This property may be used for a specific and sensitive assay to test their presence. It has been reported that platelets sensitized by the sera of children with acute ITP activated the hexose-monophosphate shunt in the leucocytes.[11] There has been no report in the literature that such an investigation had been done in chronic ITP.

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In this study we tried to develop a method to demonstrate the presence of antiplatelet antibodies. We also investigated the alterations in the hexose-monophosphate shunt in the leukocytes during their phagocytosis of the autologous normal platelets which were sensitized with the sera of chronic ITP patients.

Materials and Methods

Subjects: Twenty-two patients with chronic ITP (5 male, 17 female; ages variying between 19 and 65) who fulfilled the following criteria were included in this study (Table I):

TABLE I

AGE, SEX, PLATELET COUNTS AND CLINICAL EVALUATION OF THE CASES

Case No.	Age-Sex	Platelet Counts ($x10^9/l$)	Clinical Evaluation
1	36,F	43	Relapse
2	25,F	50	Relapse
3	21,F	32	Relapse
4	32,F	38	Relapse
5	65,F	42	Relapse
6	27,F	40	Relapse
7	45,F	38	Relapse
8	23,M	35	Relapse
9	24,F	37	Relapse
10	19,F	48	Relapse
11	19,F	32	Relapse
12	28,F	29	Relapse
13	48,M	30	Relapse
14	22,F	50	Relapse
15	27,F	35	Relapse
16	59,F	63	Relapse
17	30,F	170	Remission
18	28,F	180	Remission
19	39,M	220	Remission
20	36,M	220	Remission
21	44,F	180	Remission
22	24,F	220	Remission

a) Peripheral platelet counts less than 100 x 10^9/l,
b) Thrombocytopenia lasting more than 6 months,
c) Bone marrow smears considered to be within normal limits except for increased megakaryocyte counts,
d) Absence of secondary factors to produce thrombocytopenia (such as drugs, leukemia, disseminated intravascular coagulation, infectious diseases, sarcoidosis, tumours, collagen diseases) in medical history, physical examination and laboratory findings.

At the time of collection of the sera 16 of the patients were in clinical relapse while the other six were in remission.

Controls: 5 healthy individuals without any previous blood transfusions and pregnancy were taken as controls in all experiments.

Donors for leukocytes, platelets and sera were also selected among healthy individuals.

Separation of the Sera: Complete blood was either left at room temperature for 3-4 hours or at + 4°C overnight. Isolated serum was heated up to 56°C for 30 minutes and further supplemented with 2.5 mM EDTA (0.3 mg/ml). In the case that the serum was not used immediately it was preserved at –20°C until used. Donor serum, normal control serum, positive control serum and patients' test serum were used separately. Donor serum is the serum taken from the leukocyte and platelet donors. Normal control serum is the pooled sera of the controls.

Positive control serum taken from a chronic ITP patient proved to bear anti-platelet antibodies previously. In order to standardize the experiments, the same normal control and positive control sera were used in all experiments as an intraexperimental control. In the assays for hexose-monophosphate shunt activation of leukocytes, donor sera were used without heat inactivation (donor fresh serum).

Preparation of Platelets: Blood was taken into plastic tubes containing 1 mg EDTA per 1 ml blood and was kept only in plastic material. Upper section of crythrocyte sediment was taken and centrifuged at 2200 rpm. Pellet was diluted with 1 mM EDTA-0.15 M Na Cl in minimal volume.

Separation of Leukocytes: 50 ml blood was collected into tubes containing 17.5 ml of the following solution (Glucose 15 gm, EDTA 100 mM 200 ml, Dextran-70 500 ml) and left for gravity sedimentation for 60-90 minutes. Buffy coat was separated and centrifuged at 800 rpm.

Pellet was resuspended in 0.87 % Ammonium Chloride in order to lyse the erythrocytes and then washed once with 0.15 M NaCl. Leukocytes were finally adjusted to 4-7x10^7 cells per ml in Krebs-Ringer Buffer (pH: 7.4, NaCl 0.154 M 100 ml, KCl 0.154 M 4ml, $MgSO_4$ 0.154 M 1 ml, phosphate buffer 0.1 M 20 ml) Wright-stain smears from this suspension revealed 93-97 % granulocytes.

Radioactive Material: Glucose-1-C^{14} (specific activity 50 mc/mmol) was purchased from The Radiochemical Center, Amersham, England.

Polystrene Latex Particules: Original solution consist of 0.71 ∓ 0.005 mu polystrene latex particules. This was diluted with physiologic saline so that solid phase was 2 % and 0.1 ml of this solution provided 400-500 particules per leukocyte.

Detection of Anti-Platelet Antibodies: Investigation of alterations in the activation of the hexose-monophosphate shunt in the leukocytosis of the normal donors during the phagocytosis of the normal platelets which were sensitized with the sera of chronic ITP patients was done as follows (Table II).

Experiments were carried in metabolic bottles (Kontes Glass Co, Vineland) which were first described by Saba and Di Luzio.[12] These 50 ml bottles were connected to a counting bottle via plastic adaptors and contained Whatman 40 filters absorbed with 10% KOH. After centrifuging the platelets which were previously diluted In 1 mM EDTA 0.15 M NaCl at 2200 rpm, the pellet was incubated for 20 minutes: with 0.5 ml of one of the following sera donor serum, normal control serum, positive control serum or one of the test sera. After the incubation the platelets were collected with centrifugation and 10^8 platelets in 0.1 ml were put into metabolic bottles together with 10^7 leukocytes in 0.5 ml so that the leukocyte: platelet ratio was 1:10. Each bottle was supplemented with 1 ml HBSS and 0.05 ml donor serum. After the addition of polystrene latex particules and 0.1 uc Glucose-1-C^{14} the bottles were incubated at 37°C shaking water-bath for one hour. Reaction was ended by adding 62.5 % (w/v) citric acid. Thereafter it was allowed to stay for an extra 30 minutes so that dissolved $C^{14}0_2$ could vaporize. At the end of incubation, counting bottle was removed and dried in a vacuum dessicator. These were counted in a Liquid Sintillation Counter (Packard Model 3380) in 20 ml PPO, POPOP, toluen mixture (5.5 gr. Permablent ™11-Packard solution in Toluol). Results were expressed and used in statistical evaluation as counts per minute (cpm). Scheme for assay is summarized in Table II. In each group one bottle which is

TABLE II

ASSAY SCHEME

Leukocytes (ml)	Platelets (ml)	Sera Used to Sensitize the Platelets				Donor serum (ml)	Radioactive Glucose (Glu-1-C^{14}) (μcurie)	Latex Particules	Incubation
		Donor serum (ml)	Normal control serum (ml)	Positive control serum (ml)	Test serum (ml)				
0.5**	–	–	–	–	–	0.05*	0.1	0.1	****
0.5	–	–	–	–	–	0.05	0.1	–	****
0.5	0.1***	0.5	–	–	–	0.05	0.1	–	****
0.5	0.1	–	0.5	–	–	0.05	0.1	–	****
0.5	0.1	–	–	0.5	–	0.05	0.1	–	****
0.5	0.1	–	–	–	0.5	0.05	0.1	–	****

* In some assays inactivated donor sera was used
** 10^7 leukocytes
*** 10^8 platelets
**** Incubation

supplemented with latex particules formed an intraexperimental control so that if the phagocytosis activation ratio was less than 4 the experiment was dropped off. Experiments were made in duplicates and reproducibility was tested using the positive control serum.

Results

Detection of Anti-Platelet Antibodies: Alterations in the activation of the hexose-monophosphate shunt in the leukocytes of normal donors during the phagocytosis of the normal (autoloqous)platelets which were sensitized with the sera of chronic ITP patients, are presented below: Table III shows the results of the $C^{14}0_2$ counts which formed as an oxidation product of glucose-1-C^{14} by the leukocytes that are phagocyting the donor platelets sensitized by the sera. Additionally phagocytosis stimulation ratio (i.e. patient's (chronic ITP) count: donor's count) is presented.

TABLE III

DETERMINATION OF THE ALTERATIONS IN LEUCOCYTE HEXOSE-MONOPHOSPHATE SHUNT ACTIVATION DURING THE PHAGOCYTOSIS OF NORMAL PLATELETS SENSITIZED WITH THE SERA OF CHRONIC ITP PATIENTS

Patient No.	Donor (cpm)	Patient (cpm)	Normal control (cpm)	Phagocytosis Stimulation Ratio Patient cpm / donor cpm
1 Relapse	1064	2681	1045	2.52
2 Relapse	1064	1961	1045	1.84
3 Relapse	1064	1667	1045	1.56
4 Relapse	1064	1176	1045	1.10
5 Relapse	1064	1113	1045	1.04
6 Relapse	1250	1675	1190	1.34
7 Relapse	1250	1280	1190	1.02
8 Relapse	1280	1296	1240	1.02
9 Relapse	1280	1442	1240	1.12
10 Relapse	1121	1227	1079	1.10
11 Relapse	1121	1409	1079	1.25
12 Relapse	1240	2728	1223	2.20
13 Relapse	1250	1330	1190	1.06
14 Relapse	1293	2116	1206	1.63
15 Relapse	1064	1682	1045	1.58
16 Relapse	1064	1383	1045	1.29
17 Remission	1121	2118	1009	1.89
18 Remission	1250	1900	1190	1.52
19 Remission	1064	1498	1045	1.45
20 Remission	1250	1400	1190	1.12
21 Remission	1280	1500	1250	1.17
22 Remission	1280	1856	1240	1.45

Difference between the results of donor serum and normal serum is not significant (t-test, Table IV) Conversely the difference between patient serum and donor serum is significant ($p < 0.001$, Table V). Phagocytosis stimulation ratio ranges between 1.02 and 2.52 (mean = 1.419). Even though the phagocytosis stimulation ratio is always greater than 1.00, values greater than or equal to 1.10 was regarded as positive for the reliability of evaluation.

TABLE IV

EFFECT OF SENSITIZED PLATELETS ON THE ACTIVATION OF LEUKOCYTE HEXOSE-MONOPHOSPHATE SHUNT-TEST OF SIGNIFICANCE BETWEEN THE MEANS OF DONOR AND NORMAL SERA

	Mean	Standard Error	t	p
Donor (n: 22)	1171.72	20.67	1.37	> 0.05
Normal (n: 22)	1133.00	19.23		

TABLE V

EFFECT OF SENSITIZED PLATELETS ON THE ACTIVATION OF LEUKOCYTE HEXOSE-MONOPHOSPHATE SHUNT-TEST OF SIGNIFICANCE BETWEEN THE MEANS OF DONOR AND PATIENTS' SERA

	Mean	Standard Error	t	p
Donor (n: 22)	1171.72	20.67	4.96	< 0.001
Patients' (n: 22)	1656.27	95.36		

Thus, a factor –an antibody– that leads to the activation of hexose-monophosphate shunt in leukocytes is detected in 18 out of 22 chronic ITP patients (% 81.8). This antibody is observed in all of the cases in remission whereas 12 of the 16 cases in relapse (75 %) were positive. Difference between the phagocytosis stimulation ratios of the remission and the relapse cases is not significant (Mann-Whitney U test, U: 71)-

Discussion

An factor which opsonizes the platelets and leads to their phagocytosis by the leukocytes is detected in 18 out of 22 patients (81.8 %). The nature of this factor must be elucidated. The factors which have opsonic properties can be classified as heat-labile opsonic activity (HLO) due

to complement and the heat-stable opsonic activity (HSO), which is low and is related to antibodies, especially Ig G'in nature.[13, 14, 15] Though some Ig M antibodies have heat-stable opsonic activity, they are few and so far are known to opsonize only certain bacteria, i. e. E. Coli.[16] There has been no evidence for the opsonic activity of Ig A. Since the sera was inactivated at 56°C for 10 min.in these experiments the heat-labile complement activity abolishes. The activation of hexose-monophosphate shunt of leukocytes due to opsonization the opsonic activity should be heat-stable and must essentially be due to Ig G.

It has been reported that anti-platelet antibodies exist in chronic ITP patients during their remission periods, besides megathrombocytes and increased numbers of megakaryocytes are found.[17] Özsoylu et al., have found the platelet life-span to be significantly shortened in children with chronic and acute ITP (3 and 5 cases respectively) during their remission periods.[18] Therefore it may be concluded that these antibodies found in this study may point out a thrombocytolytic state in chronic ITP patients in remission.

This work demonstrates that measurement of activation of hexose-monophosphate shunt of leukocytes while they are phagocyting the sensitized platelets could be used to detect the anti-thrombocyte antibodies present in the sera of chronic ITP patients.

Summary

Twenty-two chronic ITP patients were evaluated for the opsonic activity of their sera for normal platelets. Opsonization of normal platelets provoked phagocytosis by the autologous leukocytes (of the patients) and this was measured via $C^{14}0_2$ counts which formed as an oxidation product of glucose-1-C^{14} through the activation of the hexose-monophosphate shunt in the leukocytes. Patients' sera activate this shunt significantly greater than normal control or donor's own sera ($p < 0.001$). Anti-platelet antibodies which were shown by this method were present in 81.8 % of the tested patients. Anti-platelet antibodies were also present in remission and there was no significant difference whether the patients were in remission or in relapse.

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Hacettepe Medical Journal
Volume 16 / No 1 / January 1983 / pp. 24 – 30

Serum Migration-Inhibitory Activity in Patients with Nonsuppurative Sequelae of Streptococcal Infections

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The pathogenesis of the nonsuppurative sequelae of streptococcal infections has been the subject of investigation for many years. Such extensive studies re-emphasize the futility of current attempts to eradicate the high incidence of the streptococcal infections.[1]

While primary prevention fails, because of high number of asymptomatic carriers, secondary prevention is the medical approach to rheumatic fever prophylaxis.[1]

Recent studies strongly suggest that hypersensitivity to streptococcal antigens may play a role in the initiation of events leading to the rheumatic fever. Read et al. report that cellular reactivity to the streptococcal membrane antigens, measured by the inhibition of migration of peripheral leucocytes in capillaries, was significantly enhanced in those patients with rheumatic fever when compared to cells from normal subjects or patients with uncomplicated streptococcal infections.[2]

A number of recent studies have shown that it is possible to detect migration inhibition factor (MIF) or MIF -like activity in the sera of animals exhibiting delayed hypersensitivity reactions to tubercle bacilli and the sera of patients with lymphoproliferative diseases.[3, 4]

If under appropriate circumstances, MIF-like activity could be detected directly in the sera of patients, the procedure would have obvious practical advantages in the collection, preparation and handling of test materials.

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Because of these advantages, the sera of the patients with streptococcal infections, rheumatic fever and rheumatic heart diseases were studied in this study. These diseases are associated with immunologic status, spread all over communities and in some cases need intensive follow-up for secondary prevention of rheumatic diseases.[1]

The purpose of this study is to determine if the leucocyte migration inhibition activity test is the right assay for rheumatic fever prevention in community programmes.

Materials and Methods

Subject: The subject population included 33 patients from the outpatient clinic of Cardiology Department in the Hacettepe University Hospital at Ankara, 12 out of 33 patients (group II) having acute rheumatic fever and 21 out of 33 patients (group III) having rheumatic heart disease. 27 patients with streptoccocal infections (group I) and 72 healthy controls (group IV) from the nursing school were matched for age and sex.

Migration Inhibition Assay: Ten to 20 ml. of venous blood was collected from each subject. The blood was centrifuged at 3000 rpm for 30 minutes, the serum was collected aseptically, heated at 56°C for 30 minutes and frozen at minus 20°C for up to six months.

Peripheral white blood cells were isolated from 20 ml. of venous blood that was drawn from one of the normal subjects into a 30 ml. plastic syringe which contained 0.5 ml. heparin (liquemine) and 2 ml. of 6 % dextran (macrodex).

The syringe was gently shaken twice to mix the blood, heparin and dextran and the mixture incubated at 45°C for 30 minutes at room temperature.

The supernatant, containing mostly leucocytes was removed, placed in a sterile centrifuge tube and spun at 1000 rpm for 5 minutes.

The cell pellet was resuspended in 3 ml. of 0.83 % ammonium chloride, 0.1 M Tris buffer and incubated for 7 minutes in 37°C to lyse the contaminating red cells. The cells were spun at 1000 rpm for 5 minutes and the cell pellet washed in 10 ml. RPMI 1640 medium at 1000 rpm.

The cells were resuspended in RPMI 1640 medium adjusted to 35 or 40 x 10^6 leucocytes/ml. and packed into capillary tubes as previously described.

Leucocyte migration from the capillaries was assayed in RPMI 1640 medium containing 50 % serum from the patient to be tested.

Leucocyte migration in medium containing 50 % serum from normal subjects served as controls.

The migration-inhibition index was calculated as previously described.[5]

$$MI = 100 \times \frac{\text{migration area in medium containing serum sample}}{\text{migration area in control serum}}$$

Results are expressed as migration index $\pm$ standard error. For each serum sample, at least two independent assays were carried out, each assay consisting of a minimum of three capillary tube preparations.

Results

A total of 72 serum samples from healthy subjects were tested as control. These sera had an average migration index (MI) of 87.6 $\pm$ 0.86.

The patients with streptococcal infection had an average MI of 61.8 $\pm$ 1.04 %. After ten days of oral penicilin treatment the index increased to 74.5 $\pm$ 1.19 %. These values are significantly different ($p < 0.001$).

Patients with acute rheumatic fever have higher leucocyte migration inhibition activity than other patients. The average MI was 35.9 $\pm$ 3.18 % in the first week of the illness. One month after treatment, the average MI was 39.1 $\pm$ 3.91 %. These values are not significantly different.

Patients with rheumatic heart disease had an average MI of 41.5 $\pm$ 2.28 %.

These results are summarized in Table I. In accordance with usual criteria, a migration index MI $<$ 80 % is taken as significant migration inhibition.[3, 5]

The average MI of the normal controls was significantly different from all patients studied.

Group I had significantly different MI than the other groups studied, while the MI of group II were not significantly different from group III.

Of the 12 patients with acute rheumatic fever the case five had lowest MI (17 %). The third case had a migration index of 20 %; both cases had carditis.

TABLE I

MIGRATION INHIBITION INDEX (MI %) AND COMPARISON OF THE STUDIED GROUPS

Groups : The date of MI assay	No. of subject.	MI % Min.	MI % Max.	Average MI Mean ± SE	Statisitical Analysis.
Group I : Before treatment,	27	51	73	61.85 ± 1.04	
					(P < 0.001)
After 10 days treatment.	26	61	88	74.50 ± 1.19	
					(P < 0.001)
Group II : In the first week.	12	17	52	35.91 ± 3.18	
					(P > 0. 05)
After one month.	9	17	50	39.11 ± 3.91	
					(P > 0. 05)
Group III :	21	22	61	41.57 ± 2.28	
					(P < 0.001)
Group IV :	72	68	99	87.62 ± 0.86	

Group I : first MI – Group II: first MI (P < 0.001)
Group I : first MI – Group III : second MI (P < 0.001)
Group IV : – Group I, II, III, MI (P < 0.001)

The patients with acute rheumatic fever who have migration indices higher than 20 % did not develop carditis.

The sedimentation rate, ASO and CRP of the patients with rheumatic heart disease showed no statistically significant association with the average MI ($r > 0.60$).

Discussion

Many studies have demonstrated that the inhibition of migration of human blood leucocytes in the presence of their specific sensitizing antigens is an accurate reflection of the state of delayed type hypersensitivity.[2]

A number of authors have reported studies on cellular reactivity to streptococcal antigens in both normal healthy subjects and rheumatic individuals.[6–9]

Most authors noted a generalized response to streptococcal antigens in the majority of individuals tested, indicating a broad cellular response to those antigens in man. The main disagreement among these studies centres around the question of specificity of the cellular response.[7, 8, 10]

These conflicting results may well be related to both the streptococcal strain used in a given study and the type or the quantity of the antigens employed.[11]

In has been shown that only specific antigens give consistent and accurate results in this direct migration inhibition system.[12]

The sera of patients with streptococcal infections instead of peripheral blood leucocytes of these patients were tested in our laboratory to lessen these conflicting results.

The present study documents significantly different migration inhibition activity between the sera of the patients with streptococcal infection and the normal controls.

Using another test system, it was shown that the patients with streptococcal infection and normal control subjects exhibit the same degree cellular reactivity to streptococcal antigens.[11]

The sera of the patients directly reflect activity of migration inhibition factors released in vivo by sensitized leucocytes.

In addition, the sera of patients can be stored for comparing the migration inhibition activity in the clinical course of the illnesses.

The highest migration inhibition activity was found in the sera of two patient with acute rheumatic fever. These two patients had carditis. This finding gives an obvious indication to observe closely the patients with acute rheumatic fever for secondary prevention.

In summary, the detection of MIF activity in serum is an accurate reflection of the disease activity. It is a simple and convenient assay since it does not require collection and separation of living cells from the patient with streptococcal infections disease. Storage availability gives possibility of comparison in the clinical course of the disease. Finally, many sera can be tested in one assay.

It is important to re-emphasize that these findings do not provide direct information about the cellular sources of the MIF-like activity observed or the nature of the stimulus for the production of the factors involved.

Serum MIF's may be involved in the underlying immunopathology of various sequelae of the streptococcal infections especially in relation to the altered immunoresponsiveness frequently observed.

It remains a distinct possibility that serum MIF determinations will prove to have diagnostic or therapeutic implications in acute rheumatic fever.

Acknowledgement

I am indebted to Dr. A. İzzet Berkel for a review of the manuscript and for many helpful suggestions. (Division of Immunology, Department of Pediatrics, Hacettepe University), and Dr. Korkut Özerkan for his valuable contribution (Division of Haematology, Department of Internal Medicine, Hacettepe University).

Summary

Leucocyte migration-inhibitition activity can be detected in the sera of the patients with nonsuppurative sequelae of streptococcal infections.

Average migration inhibition index (MI) was found 61.85 $\pm$ 1,04 % in the patients with streptococcal infection, 35.91 $\pm$ 3.18 % in the patients with acute rheumatic fever, 41.57 $\pm$ 2.28 % in the patients with rheumatic heart disease and 87.62 $\pm$ 0.86 % in the normal controls.

This activity was found significantly different in the patients with streptococcal infection, acute rheumatic fever and rheumatic heart disease than controls ($p < 0.001$).

The sedimentation rate, ASO and CRP of the patients with acute rheumatic fever showed no statistically significant association to the average migration index ($r > 0.60$).

The presence of such activity in the sera of these patients did not seem to be related to any laboratory measurement available.

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Hacettepe Medical Journal
Volume 16 / No 1 / January 1983 / pp. 31 – 35

Treatment of Cardiac Tamponade Due to Isolated Chylopericardium Following Open Heart Surgery

A Case Report

Cihat Bakay, M.D.* / Theodorus S. Wijers, M.D.*

Haselbrock first described isolated chylopericardium in 1888.[1] Isolated chylopericardium following cardiac surgery was first reported by Thomas and Mc Goon[2] in a female patient after repair of pulmonary atresia, patent ductus arteriosus and ventricular septal defect.

Since then, 5 cases have been reported after cardiac surgery[2–6] but only 3 have been reported in connection with open heart surgery.[2, 5, 6]

Case Report

A 41 year old man presented with a history of typical angina during exercise for two months. After the initial attack, he complained of pain on light exertion (NYHA class III). Physical examination revealed no abnormality. Radiological examination of the chest showed a normal cardiothoracic ratio of 14:33 and normal lung fields. ECG showed a sinus rhythm of 70 bpm. and normal QRS-T complex. Ischemic repolarisation was detected on ergometric examination. The patient was treated with coumarin derivatives, metoprolol isosorbide and nitroglycerine, but the chest pain on exertion persisted. It was decided to perform cardiac catheterisation and coronary angiography. Angiography revealed a left main coronary artery stenosis of 90 % with a good left ventricular function.

Two days after this investigation, the patient was operated upon, three distal and two proximal anastomoses were made using reversed saphenus vein.

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Just before the operation, a central venous line was inserted via the left internal jugular vein. An intravenous canula was also placed in the left arm.

Postoperative treatment included 3 x 75 mg dipyridamole, 500 mg salicylate per day.

After a good recovery, the patient was discharged on the 10 th post-operative day.

Five weeks after the operation he complained of restlessness and dyspnea. Because of this and because of an enlargement of the cardiac silhouette on his chest X-ray, (cardiothoracic ratio of 24:34) he was readmitted. Bloodpressure was found to be normal (130/90 mmHg). There was a pulsus paradoxus of 15-20 mm Hg and distended jugular veins. It was difficult to hear the heart sounds on auscultation.

Electrocardiography and vectorcardiography studies showed a sinus rhythm with low voltages.

Echocardiography revealed a large quantity of pericardial effusion, particulary behind and under the heart. Late cardiac tamponade was diagnosed. An emergency operation was performed. Two and a half liters of milky fluid was aspirated through a sub-xyphoid incision. In order to prevent subsequent tamponade or excessive loss of chylus, a fistula was created by suturing the pericardium to the peritoneum.

Laboratory studies of the aspirated fluid showed it to consist of a total protein level of 39 g/l; LDH level of 15 U/L; a large component of triglyceride and cholesterol level of 3.10 mm ol/1. Specific gravity was 1.037. No growth was obtained in cultures. The diagnosis of chyloperi-cardium was confirmed. The patient was discharged on the 7 th post operative day. The recovery period was uneventful and his chest X-ray was normal. Ten months after his operation, he is free from chyle fluid collection in the pericardium.

Discussion

23 patients with isolated primary chylopericardium have been reviewed by Dunn,[7] Charnilas,[8] Ross and their associates.[9] Ofthese, 12 had uncertain aetiology and 6 had either pericardial or mediastinal lymphangiomas.

Charnilas et al[8] suggested that a generalised anomaly of lymphatic vessels is the underlying cause of idiopathic chylopericardium. Cardiac

lymphatics form a common junction under the arch of the left pulmonary artery and main pulmonary artery and then pass to the left of the aorta, posterior to the mediastinal lymphatics and finally join the thoracic duct. Also, the pericardial lymphatics, which are posterior to the paraesophageal lymphatics and inferior to the diaphragmatic lymphatics, can drain into channels in the superior mediastinum before joining the thoracic duct.[10]

Cardiac and pericardial lymphatic vessels can be injured during cardiac surgery. For this reason, the surgeon must be prepared for isolated chylopericardium, however there seems to be only one possible explanation for this rare complication following cardiac surgery. As suggested by Thomas et al,[2] even if major cardiac lymphatic vessels are transected, chyle would not be expected to leak back through the nodes and lymphatic valves unless the intra-thoracic duct pressure was elevated.

The thoracic duct empties at the junction of the subclavian and jugular veins. A venous thrombosis at this point could increase pressure in the thoracic duct. Indeed, Thomas and Mc Goon have demonstrated in a venogram that recanalization of thrombosis did occur at the confluence of the internal and subclavian veins in their patient.[2] Normally there are one-way valves in lymphatic tributaries.

The pressure within the thoracic duct must exceed 15 cm H_2O to result in reflux.[11] The occurrence of chylopericardium in the 3 cases following open heart surgery seems to possess the same aetiologic factors: injury to the pericardial lymphatics and an elevation of intrathoracic duct pressure secondary to venous thrombosis.[2, 5, 6]

In the single case of Thomas and Mc Goon,[2] venous thrombosis secondary to jugular vein catheterisation was demonstrated using venography. Our patient had jugular venous catheterisation and although no venogram was performed, we may assume that the same aetiologic factors were present.

An early diagnostic clue to the nature of the fluid in cases of suspected chylopericardium can be gained by visualising the cardiac border on a plain chest X-ray. The relative transradiency of chyle, compared with that of the heart and the blood is due to the fatty composition of the chyle.[9]

Diagnosis is made by pericardiocentesis after aspiration of the milky fluid.[2, 3, 6, 7, 8, 10, 12, 13] Analysis of the fat is done either by direct microscopic examination or with a Sudan III stain and high concentrations of triglicerides and protein.[6]

In differential diagnosis one should consider cholesterol pericarditis. However, this has a golden hue and is characterised by cholesterol crystals on microscopic examination.[14]

It seems essential, in the treatment of chylus pericardial effusion due to lymphangiectasia and lymphangioma, to ligate the thoracic duct and to perform a partial pericardectomy, because in 3 cases where the duct was not ligated, tamponade reoccurred and the patients died.[9] We made a pericardio-abdominal tunnel in our patient but did not ligate the thoracic duct. He has now been free of pericardial effusion for 10 months. Of the cases reported in the literature only 1 in 5 had ligation of the thoracic duct and none of these patients has had a reoccurrence of tamponade.[2, 3, 5, 6, 13]

In summary chylopericardium following cardiac surgery is a rare complication. Etiology seems to be related to an injury to the pericardial and cardiac lymphatics together with an elevation of the thoracic duct pressures secondary to a venous thrombosis at the junction of the internal jugular and subclavian veins. Diagnosis is made by pericardiocentesis. Decompression of the pericardial effusion is enough to relieve this problem. Ligation of the thoracic duct is necessary in patients who develop chylopericardium due to lymphangioma and lymphangiectasia.

Summary

A cardiac tamponade due to chylus fluid following open heart surgery in a 41 year-old man, is presented. To our knowledge, this is the fourth reported case in English literature. Treatment included aspiration of the chylus fluid through a subxiphoid incision and creation of a fistula between pericardium and peritoneum to prevent loss of chylus fluid.

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Hacettepe Medical Journal
Volume 16 / No 1 / January 1983 / pp. 36 - 40

Intrathoracic Rib and Fibrous Band*

A Case Report

Mustafa Artvinli, M.D. / Meltem Altınörs, M.D.*** / A. Altay Şahin, M.D.*** / Y. İzzettin Barış, M.D.******

Intrathoracic rib is a very rare congenital anomaly. Only 15 cases having been reported in the literature up to 1972.[1] We report an another case, emphasizing the need for clinical recognition of this uncommon entity, avoiding an unnecessary thoracotomy.

Case Report

A 31 year-old white male, who presented with a complaint of left chest pain of one year duration, extending from his left scapula downwards, was examined in the Department of Chest Diseases. He was a non-smoker and had no other complaint than the above-mentioned pain which he described as mild.

Physical Examination: Findings were within normal limits except for an unimportant degree of excavation in the midthoracic region; and an equally mild degree of humping of the right hemithorax from the back.

A postero-anterior chest x-ray showed the presence of an opacity in the right parahilar region extending through the middle of the right hemitorax laterally and another shadow in the right paracardial area was also seen which resembled that of a right middle lobe atelectasis (Figures 1,2). Right lateral chest roentgenogram showed that this opacity,

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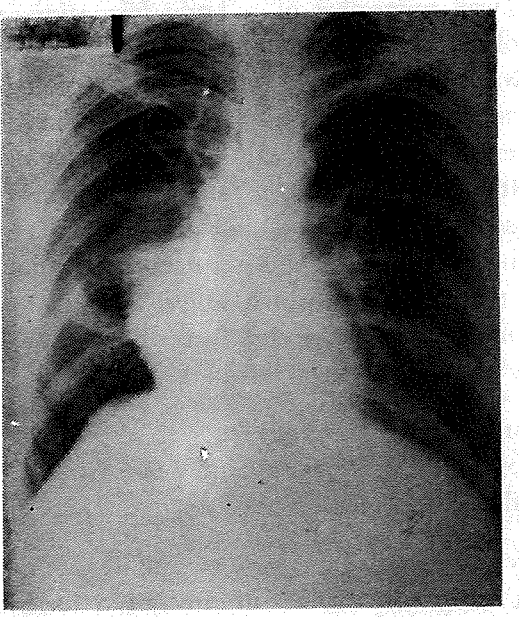

Figure 1

Postero-anterior chest roentgenogram showing the extra rib extending laterally and upwards from the right parahilar region; suggesting a right middle lobe atelectasis.

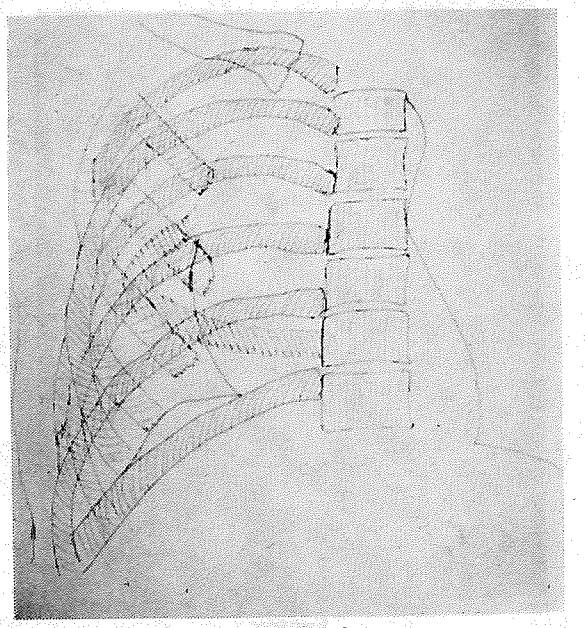

Figure 2

Diagramatic explanation of the condition of the abnormal rib in relation to the structures of the right hemithorax as seen on the P-A chest roentgenogram.

whose density was suggestive of a bony formation, was originating from the body of the 9th thoracic vertebra. It was extending laterally and upwards, and was ending at the postero-sternal region as a fibrous band (Figure 3). As can be seen from the diagrams these findings suggested the presence of an extra intrathoracic rib in the right hemithorax, (Figures 2,4). The results of the pulmonary function tests were within normal limits. Bronchoscopy was performed but failed to show any endobronchial pathology. Bronchography also did not demonstrate any obstruction in the bronchi of the right lung which would account for a middle lobe atelectasis. A tomography of the chest was obtained and this confirmed the presence of an intrathoracic extra rib.

The patient's clinical appearance was also compatible with this diagnosis. The other ribs and the remaining bony structures of the thorax were all normal.

No therapy was given to the patient, and he was informed that this condition was a benign one, which did not need further investigations and treatment.

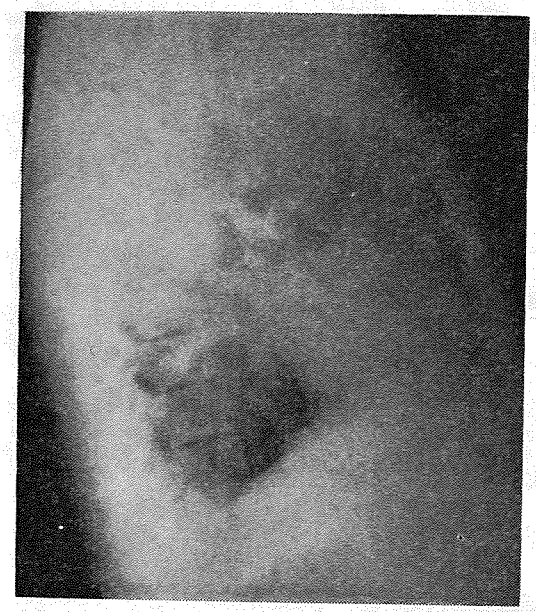

Figure 3

Right lateral chest roentgenogram showing the extra rib extending as a fibrous band to the back of the sternum.

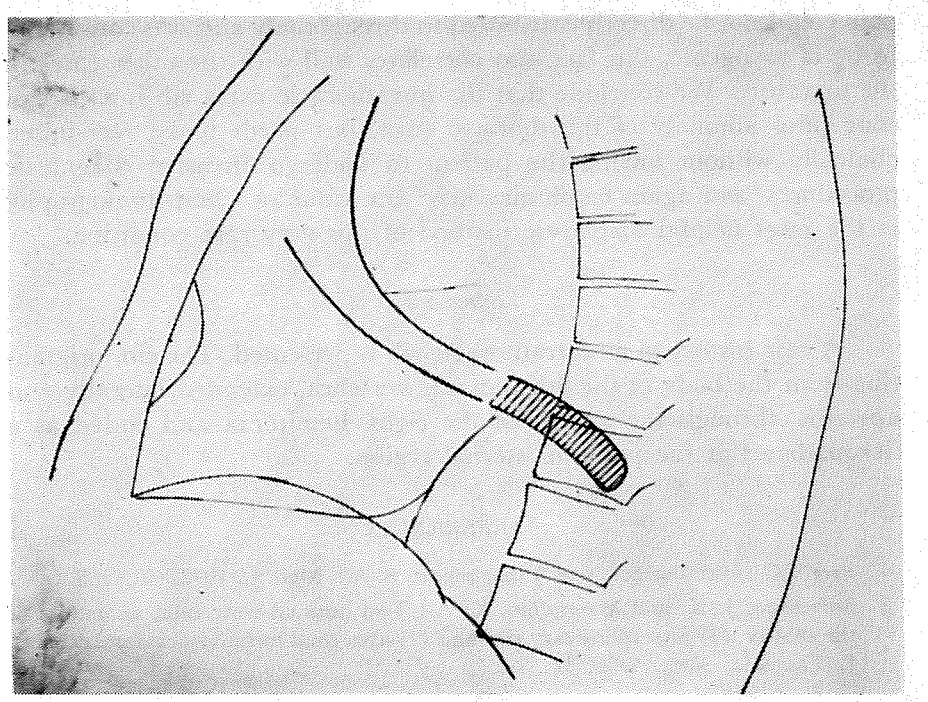

Figure 4
Diagramatic explanation of the condition of the anomaly in relation to the right lateral chest roentgenogram.

Discussion

Freed, has reviewed the literature, explaining the prominent features of this extremely uncommon condition along with his own case[1] Stevenson and Merendino, have considered an embryologic basis for the occurrence of this condition.[2] They have reported two cases, one having a extra ribs and the other having an abnormal intrathoracic fascial band, both being diagnosed during thoracotomy. According to our opinion our patient's condition represents a combination of these two entities, namely, an intrathoracic extra rib and an abnormal intrathoracic fibrous band. As expressed by Freed, clinical recognition of this condition seems important, as otherwise the patients can undergo an unnecessary thoracotomy with false diagnoses of atelectasis of unknown etiology, as mediastinal mass, anomalies of the vessels or cystic conditions, etc.[1] The most valuable method of clinical investigation of this condition is chest tomography. Some of the cases reported in the literature have been diagnosed after tomography, thus avoiding an explorative thoracotomy. As most of the reported cases the extra rib in our patient was unilateral and right sided. His left chest

pain could not be directly attributed to this anomaly and was considered to be of myogenic, this fact also correlates well with the other cases in the literature. We conclude that the intrathoracic extra rib is a benign congenital anomaly of the thoracic cage, but needs to be recognised clinically without having the patient to undergo invasive diagnostic procedures and again emphasize here the value of chest tomography as the most helpful diagnostic method of this very rare condition.

Summary

A case history of an intrathoracic rib is presented. The rib, originating from the body of the 9th thoracic vertebra, extended laterally and upwards through the middle of the right hemitorax and ended as a fibrous band at the posterior sternal region.

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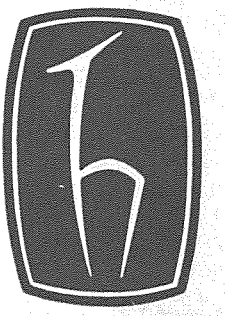

PUBLISHED BY HACETTEPE UNIVERSITY PRESS

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Printed by
Hacettepe University Press
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VOLUME 16 / NO. 2 / APRIL 1983

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VOLUME 16 / NO. 2 / APRIL 1983

Human Trabecular Endothelial Cell Culture

Murat İrkeç, M.D.* / Ceylâ İrkeç, M.D. / Altan Günalp, M.D.*** / Meral Sakızlı, Ph.D.******

Summary

Details of a tissue culture method for establishing human trabecular endothelial cell lines were given in this study. Careful dissection of the trabecular endothelial meshwork was found to be the most important prerequisite for establishing human trabecular endothelial cell cultures. Light and electron microscopic studies of cultured trabecular cells demonstrated that these differed from keratocytes, corneal endothelial cells and scleral fibrocytes particularly in surface morphology, cytoplasmic and nuclear features. Conclusively, trabecular cell culture appears to be a critically valuable means which may add much to our knowledge regarding pathogenesis and therapy of various types of glaucoma.

Key Words: Aqueous outflow, trabecular cell culture, trabecular meshwork.

Introduction

In human eyes the most important outflow route of aqueous humor is the conventional pathway through the trabecular meshwork and Schlemm's canal. Trabeculae forming the uveal, corneo-scleral and endothelial meshwork are lined with phagocytotic endothelial cells which in some aspects resemble fibroblasts.[1,2] Trabecular endothelial cells are capable of removing and destroying foreign material and debris entering the meshwork from the anterior chamber. In this respect, the trabecular meshwork may be assumed to be a self-cleaning filter which, undoubtedly, has an utterly significant role in the regulation of the outflow of aqueous humor.

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Most of the resistance to aqueous outflow has been thought to reside in the endothelial part of the trabecular meshwork which consequently attracted much attention particularly in primary open-angle glaucoma.[3] Regarding this fact, it would not be a deception to attribute a critical function to the endothelial cells of the trabecular meshwork. Recently, much work has been done relevant to functions of the trabecular endothelial cells,[4-6] and yet more remains to be done. It has been proposed that alteration in trabecular synthesis of connective tissue elements or phagocytosis may contribute more or less to the pathogenesis of primary open-angle glaucoma and of some obstructive glaucomas.[2, 4, 7, 8] Therefore, establishment of human trabecular cells in culture would provide a valuable means to study the structural and functional features of these cells in vitro.

In the present study, a method for establishing human trabecular cells in culture is described and morphological, growth and migratory characteristics of trabecular cells in vitro are given in detail.

Materials and Methods

Five human trabecular endothelial cell lines were established from trabecular specimens obtained after death of patients with no personal or family history of glaucoma and ages 21, 32, 48 and 55 respectively. The first three subjects were killed in traffic accidents, whereas the fourth patient died due to acute myocardial infarction. Trabecular specimens were obtained from both eyes of the patient, age 21 years. All of the material was taken within 8 hours following death and with a surgical procedure similar to trabeculectomy.

Explants of trabecular tissue were first placed in sterile glass dishes containing 10 ml of a mixture of Eagle's "Minimum Essential Medium" (MEM), 25 % fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin. Following delivery to sterile 4 cm Petri dishes with the same tissue culture medium, explants were examined under an Olympus inverted microscope to make sure that they did not consist of corneal or scleral tissue. A further fine dissection of the trabecular specimen with surgical microscissors and forceps was found necessary under microscopic observation to obtain uniform and small (about 1 cu. mm) explants consisting of almost pure trabecular meshwork tissue.

Explants were then washed several times with PBS to remove blood elements and debris in the meshwork. Afterwards, explants were delivered into Falcon (3012) tissue culture flasks containing a culture medium composed of MEM, fetal calf serum, 100 U/ml penicillin and 100 µg/ml

streptomycin. pH of the culture media was adjusted to 7.4 and was monitored by phenol red indicator (concentration 0.01 g/litre). To promote cell division, initial concentration of fetal calf serum was kept 25-30 %, but later it was reduced to 10 % during maintenance of cell cultures. Explants were incubated at 37°C under a 5 % CO_2 atmosphere and were left undisturbed for 1 or 2 weeks. By the third week in culture, a spontaneous migration of cells from all areas of the explant was generally noticed.

After 1 week of plating, the cells were passaged following the removal of the explant. In subculturing, trabecular cells were detached from the culture flask and from each other with 0.025 % trypsin. Six serial subcultures were performed and confluent trabecular cell cultures were obtained. Cultures were examined under an inverted light microscope and media were changed twice a week. Besides serial subculturing, a slide culture technic[9] was also employed to provide material for morphological studies with histological stains. Cultured cells were photographed with an inverted Nikon phase-contrast microscope and a Zeiss photomicroscope was used to photograph Papanicolau stained slide cultures.

In order to determine ultrastructural characteristics and to confirm purity of cultured cells, an electron microscopic study was also made on subcultures according to a method described previously.[10] Ultrastructural morphology of the endothelial cells was photographed with a JEOL-100 C electron microscope.

Results

The most important prerequisite to establish human trabecular cells in culture is an extremely careful dissection of the trabecular meshwork from surrounding tissues, namely the sclera and the cornea. This is best accomplished in our study by the dissection procedure performed under an inverted light microscope.

A typical explant consisting mainly of the meshwork is shown in Figure 1. After a period of 1 or 2 weeks, propagation of the trabecular endothelial cells may be observed from all areas of the explant (Figure 1). Initially, the endothelial cells migrating from the explant exhibited close attachments to each other and rounded or ruffled edges.

Cells subcultured from initial trabecular tissue cultures were prepared for cytologic studies via a slide culture technic and Papanicolau stained cells were examined under light microscopy. Figure 2 shows various morphological types of cultured human trabecular endothelial

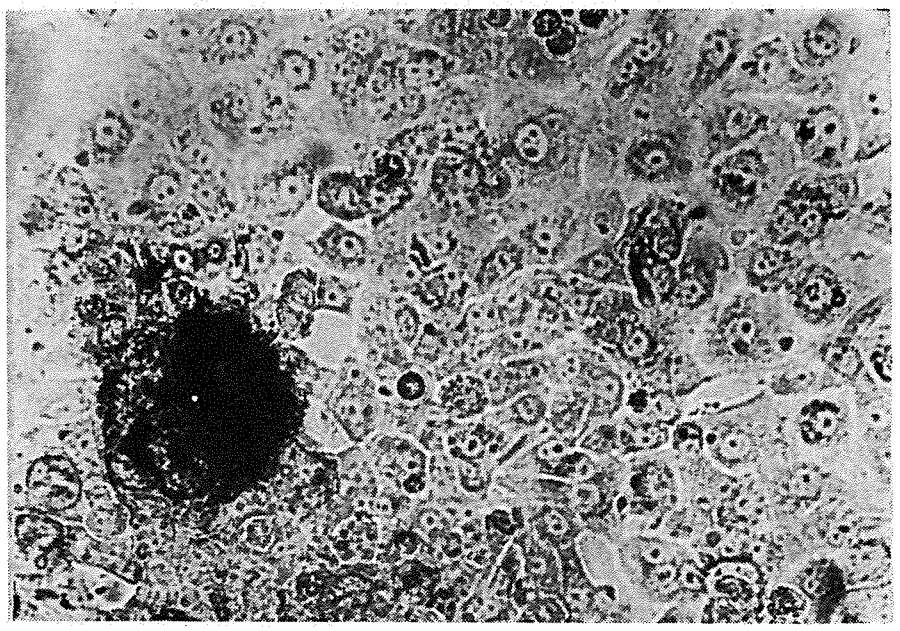

Figure 1
Human trabecular endothelial cells propagating from all areas of the meshwork explant.

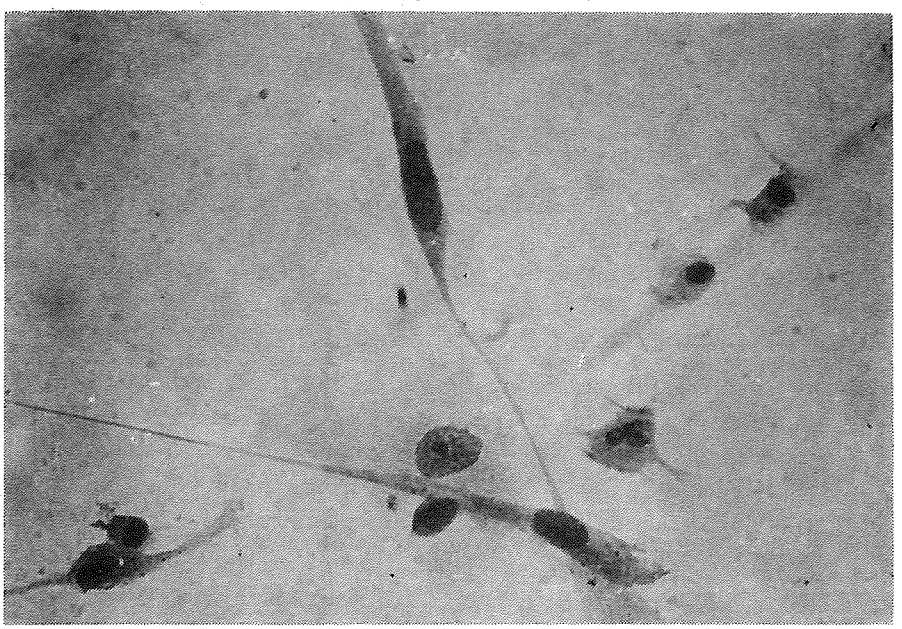

Figure 2
Cellular morphology of human trabecular cells in culture (Papanicolau's stain, X 400).

cells which, indeed, are quite different in cellular morphology from corneal endothelial cells and keratocytes that are possible contaminants in trabecular tissue cultures. One of the most outstanding features of the trabecular endothelial cells is cell processes which extend to neighbouring endothelial cells as if to form a network. Another characteristic of human trabecular endothelial cell is the rounded appearance when it is away from the mass of cells. Trabecular cells often showed branching of their cell bodies, whereas no cellular branching was observed in keratocytes and fibrocyte cultures.[4, 10]

An electron microscopic counterpart was added to the present study to enlighten ultrastructural aspects of human trabecular cells in culture. Cell surface, cytoplasmic and nuclear features of a typical trabecular endothelial cell are depicted in Figure 3. Endothelial cells typically have numerous villous projections, which in fact may be used as a criterion to distinguish these from cultured fibrocytes. The trabecular cell cytoplasm consists of a considerable type and number of cell organelles. Golgi

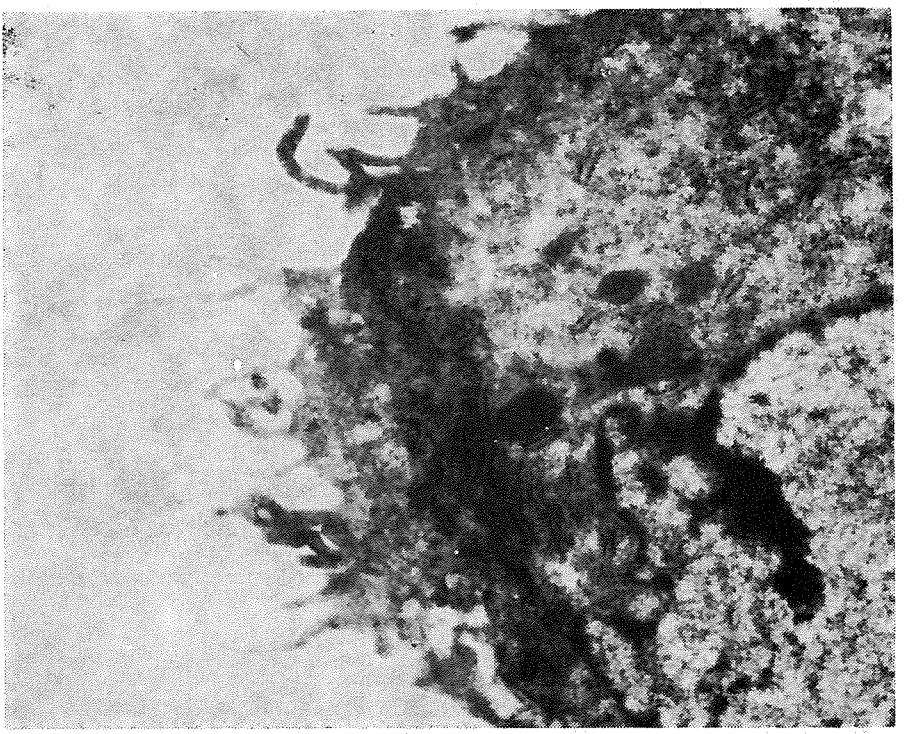

Figure 3

Electron microscopic ultrastructure of a typical human trabecular endothelial cell (X 16 000).

apparatus, lysosomes, microfilaments and microtubules are especially conspicious in the dark staining, osmiophilic cytoplasm. The nucleus of the trabecular cell had a dark, spiked band of heterochromatin on electron microscopy, whereas very prominent nucleoli were noted Papanicolau stained sections (Figure 3 and Figure 2).

After 1 week of plating, trabecular cells were passaged according to the methods. The passaged cells which appeared to be homogeneous were further subcultured after they reached confluency. Although the morphology of the early-passage cells seemed comparable to that of later passages, an increase in the number of abnormal cells was notable in later subculturing. Figure 4 shows that a considerable number of cells may exhibit serious signs of degeneration after the sixth passage. There was an increased number of lysosomes and intracellular vacuoles and sometimes disappearance of the nucleus, which as a whole revealed a process of extensive intracellular digestion.

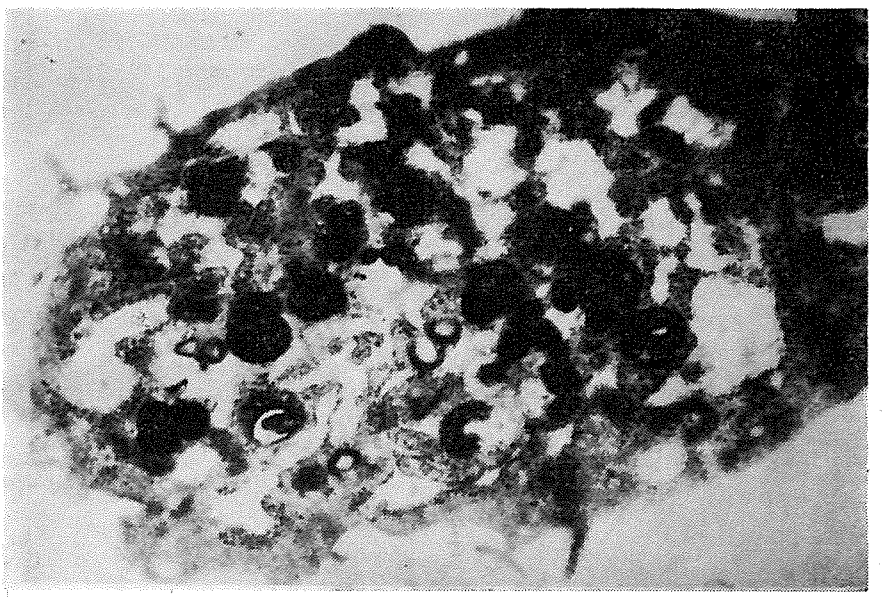

Figure 4
A sixth passage trabecular endothelial cell exhibiting ultrastructural changes of degeneration (X 13 000).

Discussion

Postmortem trabecular meshwork tissue is a useful source of explants to establish trabecular cells in culture. The present and previous studies have clearly shown that an extremely careful dissection of the trabecular

meshwork is obligatory for success in the establishment of human trabecular endothelial cell cultures.[4, 10] In addition to this, a special importance should be given to aseptic technic, use of antibiotics in the maintenance solutions and to the pH control.

Human trabecular cells exhibited a propagation from all areas of the explant when left undisturbed for 1 or 2 weeks at 37°C and under a 5 % CO_2 atmosphere. In our study, no other factors such as FGF (fibroblast growth factor) were used to promote mitosis, but a satisfactory growth pattern was observed in our cultured cells. We are also of the same opininon that fetal calf serum in considerably high concentrations in primary culturing is a potent stimulator of mitosis,[4] however in media changes for culture maintenance concentrations of fetal calf serum may be reduced or even human serum may be substituted for it. It has been stated that fetal calf serum may interfere with some hormonal studies on trabecular endothelial cells.[4]

As reported by other investigators,[4, 10] homogeneity of cells in cultures were also confirmed in our initial plating, which in fact might be a source of error in the following investigations on trabecular cells. As shown under light and electron microscopy, trabecular endothelial cells had quite a different cellular morphology in comparison with keratocytes, scleral fibrocytes and corneal endothelial cells. Trabecular endothelial cells have villous projections and cell processes which are morphologically striking. In addition to these features, cytoplasmic and nuclear characteristics of trabecular endothelial cells vary considerably in comparison with those of keratocytes and scleral fibrocytes.[10]

Subcultures of human trabecular cultures are efficient sources for various studies that may be performed on these cells. Our study clearly demonstrated that upto six subcultures might be safely used for such purposes, but an increase in the number of cells showing degeneration and abnormal morphology conspiciously appeared if further passages were performed.

As a conclusion, we may state that human trabecular cell cultures may allow a variety of studies to be performed on these cells that might not be possible in vivo. In this respect, biochemical, morphological and hormonal studies may be readily performed with trabecular endothelial cells grown in tissue culture and much may be added to the pathogenesis and treatment of glaucoma.

Received : February 16, 1983
Accepted for Publication : April 28, 1983

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Hacettepe Medical Journal
Volume 16 / No. 2 / April 1983 / pp. 49 - 55

Growth Characteristics of Trabecular Cells from Glaucomatous Patients

(An in Vitro Tissue Culture Study)

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Summary

Trabecular endothelial cells from five glaucomatous patients were established in tissue culture and were studied for growth characteristics. It was notable that the glaucomatous meshwork was hypocellular in comparison with the normal trabeculum and primary cell cultures could be established probably with the propagation and mitosis of relatively normal endothelial cells in the explants. Morphologic studies of these cells under conventional and inverted light microscopy depicted a close resemblance to human trabecular endothelial cells in culture from normals.

Key Words: Glaucoma, trabecular cell culture, trabecular meshwork

Introduction

Recently it has been reported that human trabecular endothelial cells might be established in culture and subcultures with a high density of trabecular cells might be used efficiently to carry out research on the endothelial cells.[1-3] Trabecular endothelial cells line trabeculae of the meshwork which intercommunicates the anterior chamber and Schlemm's canal. The most important feature of trabecular endothelial cells is phagocytosis by which foreign material and debris entering the meshwork from the anterior chamber are engulfed and destroyed.[4] In this respect, trabecular meshwork has been resembled to a self-cleaning

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filter which in case of obstruction may play an important role in the pathogenesis of some clinical forms of glaucoma.

The critical anatomical place and function of the trabecular meshwork make it subject to extensive investigations aimed to enlighten some of the obscure pathogenetic aspects of particularly obstructive glaucomas. Taking this into consideration, in the present study trabecular endothelial cells from glaucomatous patients were established in culture and their growth and morphological features were compared with those from normals.

Material and Method

Trabeculectomy specimens from 9 patients undergoing operations for medically uncontrolled glaucoma were used to establish trabecular cell cultures. Five cases were operated on at the Department of Ophthalmology of Hacettepe School of Medicine, whereas four trabeculectomies were performed in the Eyc Clinic of Ankara Mevki Military Hospital. Trabecular tissues were dissected under an operation microscope and explants were first taken into dishes with 10 ml of a mixture of Eagle's Minimum Essential Medium (MEM), 25 % fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin. Following delivery to sterile 4 cm Petri dishes wtih the same tissue culture medium, explants were examined under an Olympus inverted microscope to make sure that they did not consist of corneal or scleral tissue. A further fine dissection of the trabeculectomy specimens with microsurgical scissors and forceps was performed under microscopic observation to obtain uniform and small (about 1 eu mm) explants consisting of almost pure trabecular meshwork tissue.

Explants were then washed several times with PBS to remove blood elements and debris in the meshwork. Afterwards, explants were delivered into Falcon (3012) tissue culture flasks containing a culture medium composed of MEM, fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin. pH of the culture media was adjusted to 7.4 and was monitored by phenol red indicator (concentration 0.01 g/litre). To promote cell division, initial concentration of fetal calf serum was kept 25-30 %, but later was reduced to 10 %. Explants were incubated at 37°C under a 5 % CO_2 atmosphere and were left undisturbed for 1 or 2 weeks. A spontaneous migration of cells from all areas of the explant was generally noticed by the third week in culture.

After 1 week of plating, the cells were passaged following the removal of the explant and after treatment with 0.025 % trypsin. Six serial subcultures were performed and culture flasks were examined under an

inverted light microscope and media were changed twice a week. Trabecular cells were also grown on glass coverslips to provide monolayers[5] to be used to study cellular morphology under light microscopy after staining with Papanicolau's stain. Cultured cells were photographed with an inverted Nikon phase-contrast microscope and a Zeiss photomicroscope was employed to photograph stained preparations.

Results

Adequate growth of trabecular cells was notable in five out of nine trabeculectomy specimens used in the study. By the third week of initial culturing, a spontaneous propagation of endothelial cells from all areas of the explant was observed (Figure 1). During the third week, endothelial cells increased in number and reached confluency (Figure 2).

Morphological characteristics of trabecular endothelial cells from patients with glaucoma resembled closely those from normal cases. Figure 3 depicts morphological features of cultured trabecular endothelial cells from glaucomatous patients, particularly in the periphery away from the main cell mass surrounding the explant. Trabecular cells in culture showed many cell processes which seemed to contribute to adhesion of the cell body to the surface of the culture flask. Fusiform and triangular cells were the salient morphological figures in the cultures (Figure 3).

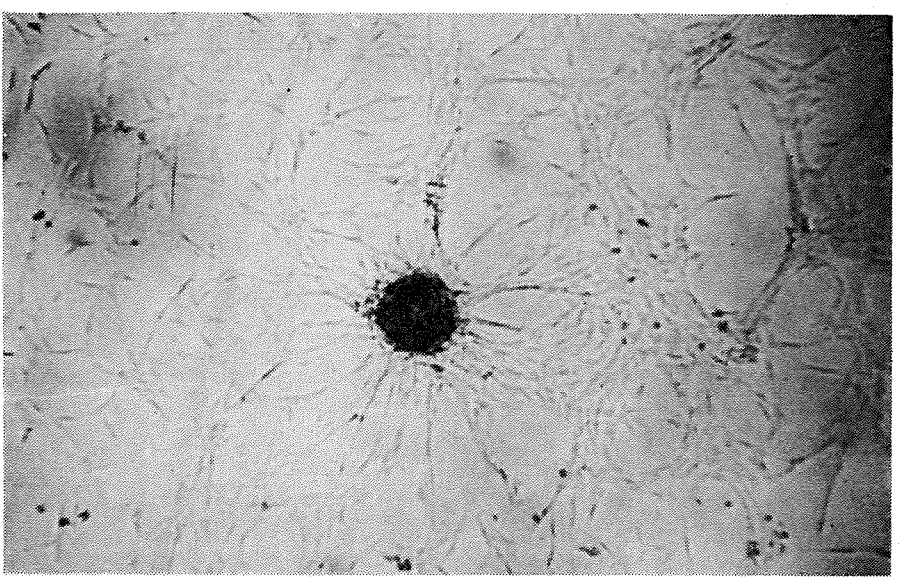

Figure 1
Initial propagation of trabecular endothelial cells from the explant.

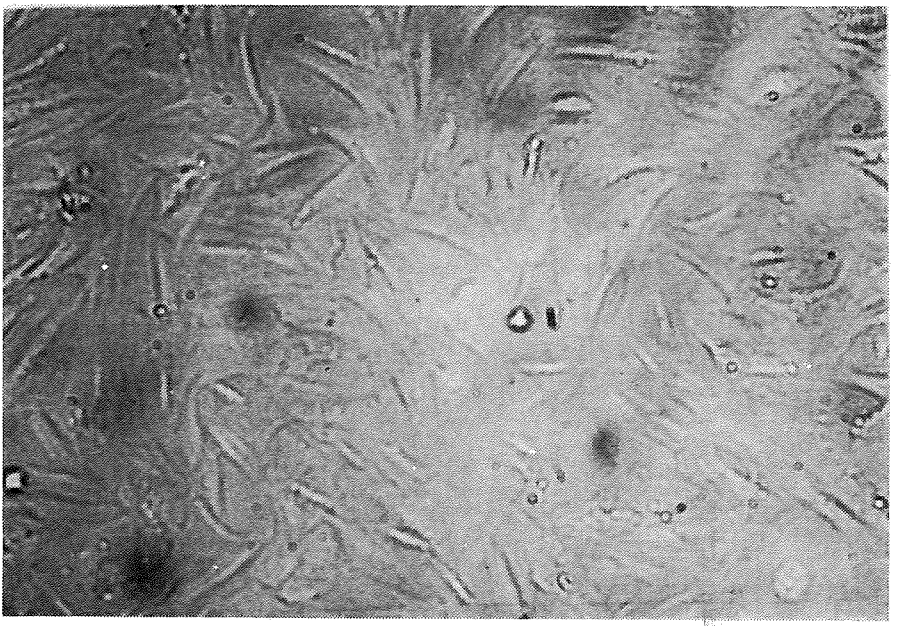

Figure 2
Human trabecular endothelial cells established in culture and reaching confluency.

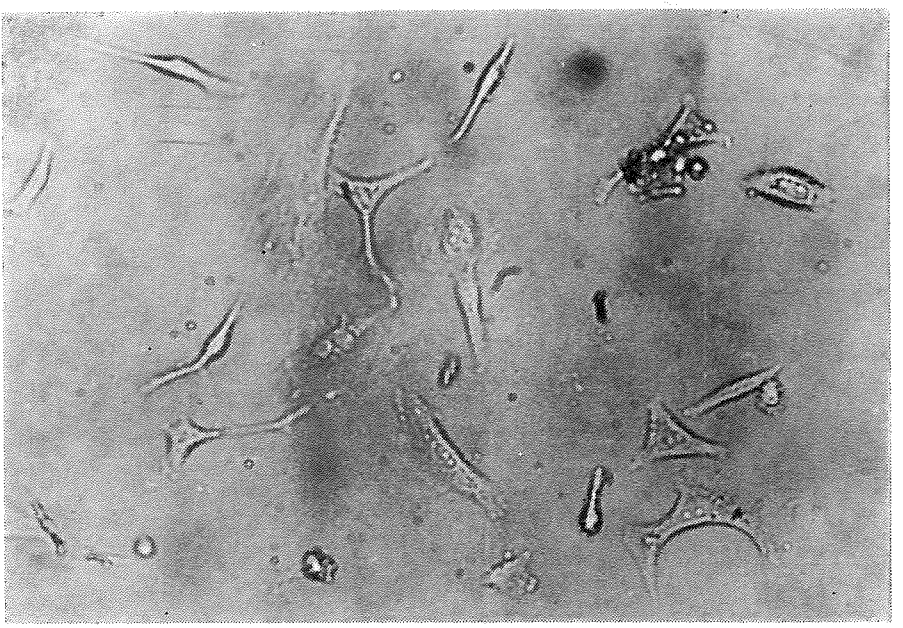

Figure 3
Various morphological patterns of cultured trabecular endothelial cells from a glaucomatous patient observed in the periphery of the culture dish.

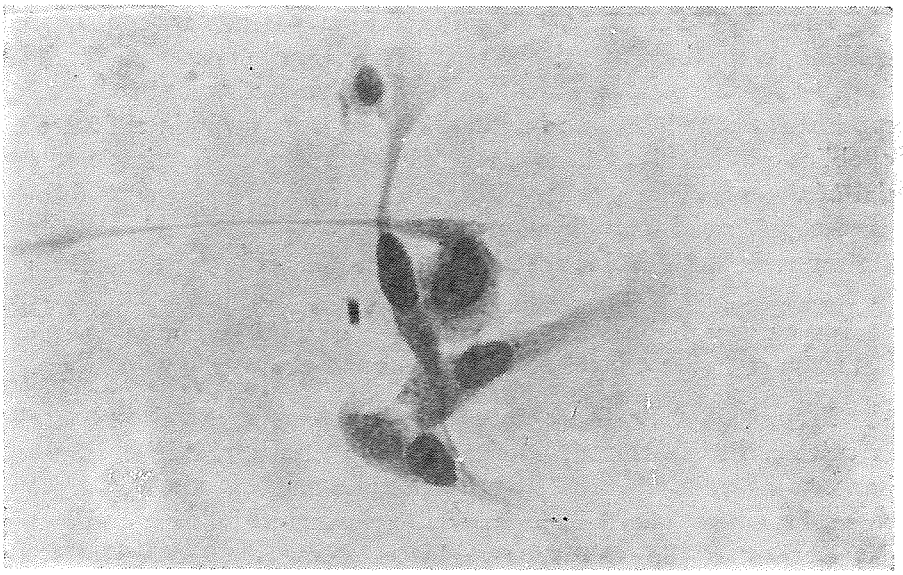

Figure 4
Cellular extensions and nucleoli are prominent features of cultured trabecular endothelial cells from patients with glaucoma (Papanicolau stain, X 425).

Papanicolau stained preparations of the trabecular cells demonstrated clearly that cell morphology might vary due to intercellular junctions. Cellular projections were conspicious in these preparations and prominent nucleoli were quite outstanding (Figure 4).

Morphology of trabecular endothelial cells from glaucomatous patients seemed quite comparable to those from normals. Cells were closely packed with narrow spaces between them and grew in a monolayer despite high density of endothelial cells (Figure 2).

Discussion

Aqueous humor leaves the eye mainly by the conventional route of outflow, namely the trabecular meshwork and Schlemm's canal. Trabecular meshwork, lined with endothelial cells, stands out to be at least an effective filter with its openings of various diameters for foreign and particulate material entering from the anterior chamber.[4, 6] It has been proposed that a defect in trabecular endothelial cell function may result in some types of glaucoma. In this respect, phagocytic and metabolic characteristics of these cells need elucidation both in disease and health. As a consequence, much attention has been paid to establishing trabecular cells in culture so to perform further biochemical, morphological and pharmacological studies on these with the purpose of adding more to elucidation of pathogenetic mechanisms in glaucoma.[1, 3]

In the present study, cell cultures from patients with glaucoma exhibited a striking similarity to cell lines grown from explants obtained from non-glaucomatous patients.[3] It was obvious that trabeculectomy specimens might be sparse in endothelial cells to result in a failure to establish cells in culture. Another possibility is decrease in mitotic potential of these cells in patients with glaucoma, which may result from a disease process of long duration or medications employed in therapy. However, it is reasonable that some trabecular cells might retain their normal cellular and metabolic features to grow in culture and to allow various passages to be done. This may form a rationale to morphological similarity of trabecular cells from normal and glaucomatous patients.

Prominent nucleoli are an outstanding feature of trabecular cells in culture. In this regard, no significant difference could be observed between the trabecular cells from the normals and from those with glaucoma. Prominent nucleoli which are also encountered during embryonic development of the trabecular meshwork[7,8] may be indicative of an increased synthetic activity. This, in other words means that cellular metabolism is quite active even in trabecular cell cultures from glaucomatous patients. This can also be taken as an evidence for the mitosis of particularly healthy endothelial cells of the glaucomatous meshwork to result in confluent cell cultures.

Another common characteristic of trabecular cell cultures of the glaucomatous and normal meshwork is the villous projections and cellular processes. It has been stated that the length of apical villous processes and cell extensions may reflect a difference of trabecular cells in vivo and in vitro.[2] Trabecular endothelial cells have to span aqueous channels to contact neighbouring cells in vivo, so villous processes and cell extensions are considerably longer.

Though impetus for further investigations on the pathogenesis of glaucomas is provided by employing trabecular cell cultures, certain limitations should be borne in mind:

1- Trabecular cell cultures from glaucomatous subjects may not depict the characteristics of abnormal endothelial cells, for propagation may occur only from the remaining normal cells in the meshwork.

2- Some of the features of cultured trabecular endothelial cells differ considerably from those in vivo. These differences may result in confusion particularly in biochemical and hormonal studies.

3- Fetal calf serum used in high concentration seems to be a significant stimulator of mitosis for the meshwork explants from normals, but

also for cultures established from glaucomatous subjects. This factor by itself may also give rise to some serious errors in hormonal studies carried on these cultures.

All of these limitations should strongly provoke an appropriate investigative effort to check their hypothetical significance. Only in this way would it be possible to use human trabecular cell cultures as in vitro models to deduce pathogenetic conclusions for mechanisms and clinical aspects of various types of glaucoma.

Received : February 16, 1983
Accepted for Publication : April 28, 1983

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Hacettepe Medical Journal
Volume 16 / No. 2 / April 1983 / pp. 56 – 65

Familial Mediterranean Fever*

Analysis of 71 Cases

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Summary

In this report we reviewed the records of 71 patients with the diagnosis of FMF followed at the Hacettepe University Hospital during the years 1971-1981. We analysed the clinical and laboratory features, complications and treatment with colchicine and also reviewed the literature.

Key Words: Familial Mediterranean Fever.

Introduction

Familial Mediterranean fever (FMF) is an inherited disease of unknown etiology characterized by idiopathic, recurring, self-limited attacks of febrile serosal inflammation involving especially the peritoneum, synovium or pleura. It is most frequently encountered in ethnic groups of Mediterranean ancestry, particularly Jews, Arabs, Armenians and Turks.[1-6] This clinical syndrome was first described as a distinctive entity by Siegal[7] as "Benign Paroxysmal Peritonitis" in 1945. The clinical picture of the disease has been expanded since then, and it has been shown that some individuals in certain ethnic groups (especially Turks and Sephardic Jews) develop amyloidosis which is nearly always fatal.[1, 3, 4, 8-11] Effective treatment of FMF became possible only after the emergence of colchicine as a therapeutic agent in 1972.[12]

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In this report we present a series of 71 cases followed at the Hacettepe University Hospital between the years 1971-1981.

Material and Methods

The records of 71 patients of Turkish origin with classical FMF according to the criteria reported previously were analysed retrospectively.[1, 13] The criteria of which the first three were obligatory for the diagnosis of FMF were as follows:[1] (1) Short attacks of fever recurring at varying intervals; (2) painful manifestations in the abdomen, chest, joints or skin; (3) absence of any causative factor or pathologic finding, in vivo or postmortem, capable in itself of explaining the picture; (4) amyloidosis; (5) features of autosomal recessive inheritence; (6) preference for people of Mediterranean stock. All of these patients had been diagnosed and followed at the Hacettepe University Hospital between the years 1971-1981. Most of them had been hospitalized at least once and many on numerous occasions and investigated in detail. The others had been seen regularly as outpatients.

Results

I. Clinical Features

Age and Sex: The patients comprised of 52 males and 19 females (ratio 2.7/1), ranging in age from 7 to 50 years with a mean age of 27.6 years. The age of onset of symptoms ranged from 2 to 39 years with a mean of 15.5 years (Figure 1). The clinical manifestations appeared at or before the age of 10 years in 24 (33.3 %) and at or before the age of 20 years in 47 (71.2 %) of these patients. The mean duration of illness has been 14.4 years, with a range of 1 to 48 years.

Family History: Twenty four patients (33.3 %) had family history of FMF. Data were not sufficient to show a specific mode of inheritence.

Geographic Distribution: The patients were from all parts of Turkey and no significant regional differences were noted.

Periodicity (Frequency of Attacks): The frequency of attacks ranged from once a week to 2 years. Although 55 patients (77.5 %) showed a certain periodicity, the attacks occurred irregularly in the rest.

Duration: The duration of the attacks varied from 2 hours to 7 days. It was less than a day in 7 patients (9.8 %), one to three days in 42 patients (59.2 %), more than three days in 10 patients (14.4 %). Duration of the attacks could not be determined clearly in 12 patients (16.9).

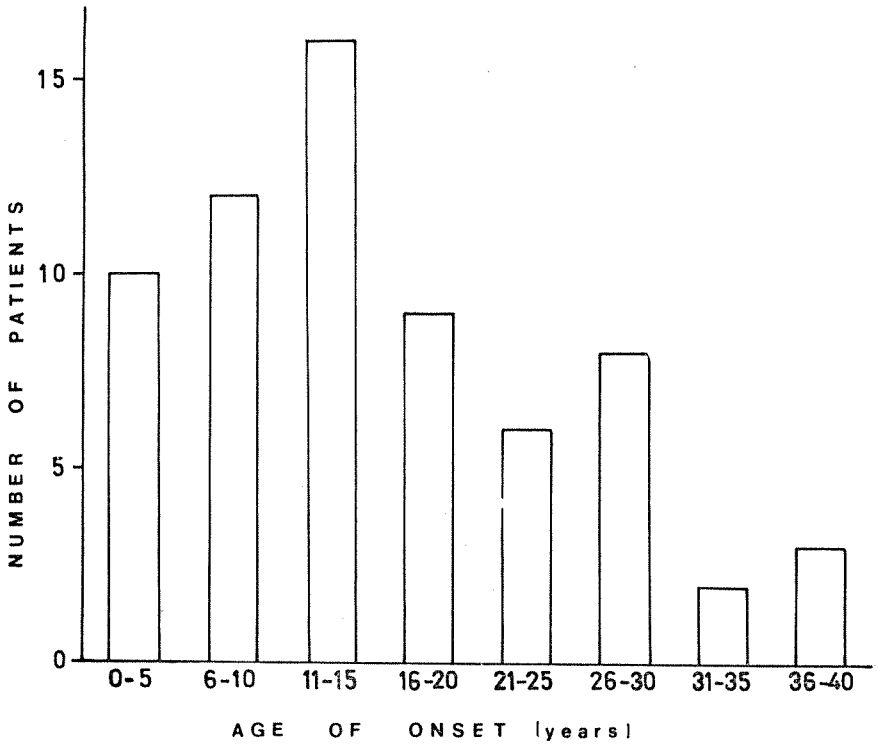

Figure 1
Familial Mediterranean fever; age of onset.

History of Abdominal Operations: Thirty one patients (43.7 %) had abdominal operations and six of them (8.5 %) had multiple laparotomies.

Fever: All of the patients had fever ranging between 38-41°C during the attacks, and in most of them chills accompanied the fever. Fever mostly subsided in a few hours, but in some it persisted up to the termination of the paroxysms. In one patient with amyloidosis, fever has been the sole manifestation of the disease (Figure 2).

Abdominal Pain: Severe abdominal pain with fever was the commonest form of the attacks. Sixty eight patients (95.8 %) experienced episodes of abdominal pain during their lifetime. The severity of the pain varied from a mild abdominal discomfort to a typical acute surgical abdomen (Figure 2).

Chest Pain: Eleven patients (15.5 %) suffered recurrent episodes of pleuritic chest pain, but in none of them this was the sole manifestation of their illness. Chest pain with varying duration and severity always accompanied abdominal pain and fever (Figure 2).

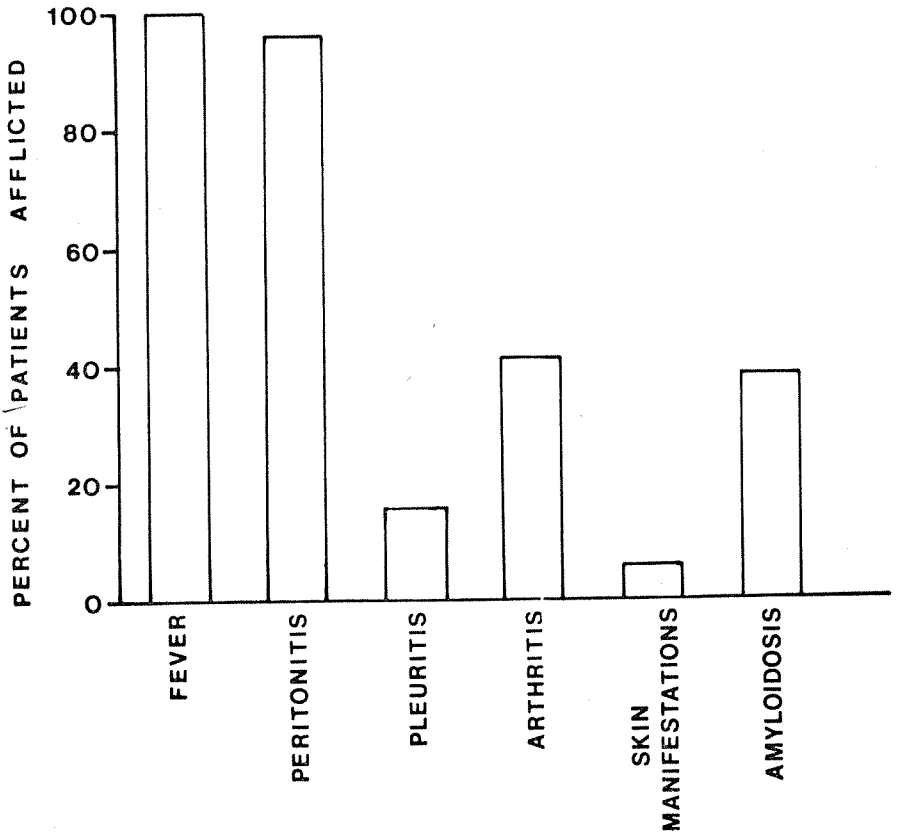

Figure 2
Familial Mediterranean fever; clinical manifestations in 71 patients.

Joint Involvement: The articular disease of FMF occurred as the second most common manifestation in our series. Twenty nine patients (42 %) showed synovial attacks sometime during the course of their illness. In three quarters of them the episodes were limited to arthralgias whereas they were true arthritis with no sequela in the rest (Figure 2).

Skin Manifestations: Skin manifestations were recorded in 4 patients (5.6 %). These were typically maculopapular or nodular, red, hot lesions occurring on the anterior aspects of the lower extremities (Figure 2).

Prominent Symptoms and Clinical Features: Abdominal pain was the prominent symptom in 42 patients (59.2 %), whereas it was fever in 4 patients (5.6 %), fever and abdominal pain in 15 patients (21.1 %), chest pain in 2 patients (2.8 %), fever and arthralgia in one patient (1.4 %), abdominal pain and arthralgia in one patient (1.4 %) and

undetermined in six patients (8.5 %). Nephrotic syndrome was the prominent clinical feature in 8 (11.3 %), and chronic renal disease in 2 (2.8 %).

Physical Examination: The vast majority of patients had no significant abnormalities on physical examination between the attacks except the ones with nephrotic syndrome or chronic renal disease. The classical physical findings of peritonitis, pleuritis and arthritis were usually evident during the attacks. Thirteen patients (18.3 %) had hepatomegaly, 4 patients (5.6 %) had splenomegaly and 12 patients (16.9 %) had hepatosplenomegaly. Generalized lymph node enlargement was recorded in two (2.8 %) and localized lymph node enlargement in three (4.2 %) patients.

II. Laboratory Manifestations

The erythrocyte sedimentation rates were found to be elevated in 49 out of 58 patients (84.5 %), whereas prominent leukocytosis was found in only 21 out of 65 patients (32.3 %) during the attacks. Thirty one out of 32 patients (96.8 %) showed raised plasma fibrinogen levels during the attacks. Ten patients (14.1 %) had anemia (hemoglobin level range 5.8-9.6 g/dl) whom 9 had amyloidosis with renal involvement. Serum proteins and protein electrophoresis values showed abnormalities (mainly hypoalbuminemia) in accordance with the presence of nephrotic syndrome or chronic renal disease. None of the patients had hyperbilirubinemia during or between the attacks. Only some of the patients with nephrotic syndrome showed hyperlipidemia and hypercholesterolemia. In six patients Schwartz-Watson test was performed during the attacks and found to be negative.

Routine urinalysis were performed in all patients. Most patients with chronic renal disease and nephrotic syndrome had white blood cells, red blood cells and various casts in their sediments. Twenty six patients (36.6 %) showed proteinuria with a range of 1.0-14.0 g/day. Mean protein excretion was 4.5 $\pm$ 0.6 g/day. Blood urea nitrogen was elevated in 12 patients (19.7 %); the levels ranged from 20.0 to 150.0 mg/dl (mean 60.0 $\pm$ 12.3 mg/dl). Thirteen out of 39 patients who were tested (33.3 %) had high serum creatinine levels with a range of 1.1-13.7 mg/dl (mean 4.0 $\pm$ 1.0 mg/dl). Creatinine clearences were estimated in 14 patients; ten of them (71.4%) were under the lower normal limits. These ten patients had either proteinuria or renal amyloidosis.

III. Biopsies

Renal Biopsies: A needle renal biopsy was carried out in 21 patients.

Amyloidosis was detected in 18 of them (85.7 %). Rectal amyloidosis was found in a patient who had negative renal biopsy.

Rectal Biopsies: A rectal biopsy was performed in 35 patients and revealed amyloidosis in 14 (40 %).

Liver Biopsies: A needle liver biopsy was performed in 4 patients and all of them were found to be negative for amyloidosis. In these patients, renal or rectal biopsies also did not reveal amyloidosis.

IV. Amyloidosis

Amyloidosis was found in 27 patients (38 %). This diagnosis was established by renal biopsy in 13 (48.1 %), by rectal biopsy in 9 (33.3 %) and by rectal and renal biopsies in 5 (18.6 %).

V. Miscellaneous Features

Seven patients (9.9 %) of whom 2 were amyloid positive experienced diarrhea episodes during the attacks. Two patients (2.8 %) with amyloidosis had chronic diarrhea. One of them had a well-defined malabsorption syndrome related to gastrointestinal amyloidosis.[14] Gross hematuria was present in 6 patients (8.5 %) during the attacks. Two of these also had renal amyloidosis. Two patients (2.8 %) experienced constipation, nausea and vomiting during some of their attacks. Meningitis, non-uremic pericarditis or hepatitis were not seen in these patients.

VI. Precipitating or Ameliorating Factors

Stress in four (5.6 %), fatty foods in three (4.2 %) and exposure to cold in two (2.8 %) patients seemed to be responsible for precipitation of their attacks. Menstruation, oral contraceptive drugs and physical activity were not found to be precipitating, and the favorable effects of pregnancy have not been noticed.

VII. Treatment

In our series, 35 patients (49.3 %) were given colchicine prophylactically in a regimen of 0.5-2 mg daily. Seventeen of them (48.5 %) could have been followed up between 6 months to 8 years and the results of therapy were satisfactory. This regimen was found to be effective both in ameliorating the attacks and in reducing their frequency. Even it abolished the attacks in some of the patients. It was noticed that proteinuria decreased considerably during colchicine therapy in some cases with renal amyloidosis. Among the patients who could have been followed up, one died of chronic renal disease.

Discussion

Although FMF has been described earlier as a genetic disorder restricted to Jews, Arabs and Armenians;[13, 16] recently it has been reported frequently from Turkey.[3-6, 16] This fairly common but not widely recognized disease must be sought for in every patient presenting with fever of unknown origin or systemic amyloidosis in our country.

Unfortunately, the diagnosis of FMF still depends entirely on a history of recurrent, self-limited attacks of fever and pain in the abdomen, chest and joints. Presence of positive family history and systemic amyloidosis support the diagnosis. Laboratory findings are mostly nonspecific and generally are used for ruling out other probable diagnoses. Therefore it is essentially a clinical diagnosis for which the criteria have been brought forward by the Israeli workers previously.[1]

The clinical and laboratory manifestations of our patients are generally similar to those reported by the others except in few points.[1, 2, 11, 13] Pleural attacks seem to rather rare (15.5 %) in this series while it is much more common in the others ranging from 38.0 % to 87.0 %.[11] Synovial attacks (40.8 %) and typical skin lesions (5.6 %) are also not so frequent as in the other reports.[11] Abdominal pain (59.2 %) or fever with abdominal pain (21.1 %) are the main presenting symptoms whereas only one patient had fever as the sole presenting manifestation. Owing to higher incidence of renal involvement, some patients present with the clinical features of nephrotic syndrome or chronic renal disease. In accordance with the medical literature, splenomegaly, hepatomegaly or both are not rare in our patients.[1, 2, 13] Rectal biopsies in 3 patients with splenomegaly, in 3 patients with hepatomegaly and in 4 patients with hepatosplenomegaly could not demonstrate amyloidosis. In 2 other patients with hepatosplenomegaly liver biopsies were negative in addition to rectal biopsies. Lack of amyloidosis in these patients may indicate that hepatomegaly, splenomegaly or hepatosplenomegaly may develop independent of systemic amyloidosis. Lymphadenopathy may be a feature of FMF as seen in 5 of our patients.[2, 13]

Laboratory manifestations of FMF are entirely nonspecific. The ESR, white blood cell count and plasma fibrinogen levels may be elevated during the attacks.Particularly plasma fibrinogen levels are found to be raised in 96.8 % of our patients. In cases with amyloidosis and renal involvement anemia, azotemia, hypoproteinemia and hyperlipidemia may develop as encountered in some of our patients.

Diarrhea may be a feature of FMF either during the attacks or in a chronic form.[2, 17, 18] We had 7 patients experiencing diarrhea episodes

during the attacks and 2 patients with chronic diarrhea. Interestingly malabsorption syndrome is a very rare manifestation of FMF amyloidosis and there are only a few case reports published in the medical literature. Chronic diarrhea and malabsorption is mainly due to gastrointestinal amyloidosis as shown in our case.[14, 17, 18]

Gross hematuria during the attacks may be encountered in FMF and we had 6 such cases whom 2 had amyloidosis. Gross hematuria has also been reported in the absence of amyloidosis.[2]

Several precipitating factors such as stress, fatty diet and exposure to cold described previously, seem to have some unfavorable effect in few of our patients.[2]

Amyloidosis is a well known and potentially fatal complication of FMF. According to the recent classification it is categorized as heredofamilial amyloidosis with clinical manifestations similar to secondary amyloidosis.[19] It also resembles secondary amyloidosis histologically and biochemically with AA-protein fibrils deposition.[19, 20] Amyloidosis associated with FMF has been known to be frequent in non-Askhenazic Jews; the prevalance being reported 12-42 % by different authors from Israel.[1, 10, 21] However, amyloidosis develops very rarely or not at all in Askhenazic Jews and Armenians.[2, 13] Interestingly, the prevalance of amyloidosis has been found in a rather high frequency in all of the previous reports from Turkey except a recent one.[3, 4] In these series the occurence has been found as 60 % whereas it is 38 % in our study. Surprisingly, Paykoç and Sümer[5] have reported a very low prevalance of amyloidosis (2.9 %) in their series. This inconsistency in frequency of amyloidosis among the Turks may be attributed to the limited case numbers and inevitable case selection in the previous reports. Nevertheless, the difference in the prevalance of amyloidosis in different ethnic groups has been observed by many authors and this fact most likely is related to the difference in genetic loading; but some artificial factors may be responsible.[10] Furthermore, the frequency of amyloidosis among the Sephardic Jews in Israel who must possess the same genetic loading has been found to be different by the two major Israeli groups.[9, 10]

There was no effective treatment of FMF until Goldfinger[12] showed colchicine markedly prevented the recurrence of the attacks in 1972. Since then, colchicine has been widely used successfully in preventing and ameliorating the attacks.[22-25] Recently it has been suggested that colchicine is also effective in decreasing the proteinuria and improving the prognosis of amyloidosis in FMF.[24-29] But Önen and Erek did not find any favorable effect of colchicine treatment on proteinuria.[16]

Our experiences, on 35 patients treated with colchicine also support the previous favorable observations and we suggest colchicine as a useful drug in the treatment of FMF and prevention of amyloidosis.

Received : February 10, 1983
Accepted for Publication : March 30, 1983

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Hacettepe Medical Journal
Volume 16 / No. 2 / April 1983 / pp. 66 – 72

Type III Radical Hysterectomy in Early Cervical Cancer*

The Evaluation of 147 Patients

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Summary

147 Patients who underwent type III radical hysterectomy were presented. Of the 147 patients, 103 had stage I cervical lesion, 39 had stage II cervical lesion, and 5 had stage II endometrial cancer. Lymph node metastases in stage Ia, stage Ib, stage IIa, and stage IIb were 5.3 %, 23.4 %, 30.4, and 50 %, respectively.

Five-year survival rate was 100 % in, in situ and microinvasive, 89.5 % in stage Ia, 84.4 % in stage Ib, 73.9 % in stage IIa, and 56.3 % in stage IIb disease. The operative mortality rate was 0.7 %.

Key Words: Type III Hysterectomy, Wertheim Hysterectomy.

Introduction

The radical surgical treatment of cervical cancer was started by Clark,[1] Reis,[2] and Wertheim[3] in the late 1800's. By the 1920's, radiation therapy had essentially replaced surgery in the treatment of cervical cancer. Meigs[4] reviewed the surgical approach to invasive cervical carcinoma in the mid 1940's. He combined radical hysterectomy with complete removal of pelvic lymph nodes and also recommended that surgery is limited to patients with early invasive disease. He reported an 85 % 5-year survival rate for stage I disease, with acceptable operative morbidity and mortality rates.[5]

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The purpose of this study is to determine the operative and postoperative complications, the incidence of lymph node metastases, and the 5-year survival rate in patients undergoing type III radical hysterectomy.

Materials and Methods

147 patients underwent radical hysterectomy and bilateral pelvic lymph node dissection (RHBPLND) for cervical and endometrial cancer (Table I).

The mean age at the time of surgical intervention was 53.54 ∓ 0.79 (range 16 to 75). The age of the first sexual intercourse in 55 % patients was 19 and less. The mean gravidity and parity were 6.06 ∓ 0.35 and 4.51 ∓ 0.49, respectively.

The indications for RHBPLND are given in Table I.

TABLE I
INDICATIONS FOR RHBPLND

Indications	No	%
In situ and Microinvasive	7	4.8
Stage IA	19	12.9
Stage IB	77	52.4
Stage IIA	23	15.6
Stage IIB	16	10.9
Endometrial Carcinoma	5	3.4
Total	147	100.0

Of the 147 patients, 94 had only surgery 19 had preoperative irradiation, and 34 had postoperative radiotherapy. Preoperative irradiation was given in 16 patients with stage IIB disease and in 3 patients with stage IB disease and heavy vaginal bleeding. 34 patients who underwent postoperative radiotherapy had pelvic lymph node metastases. In 2 young patients who had radical hysterectomy, bilateral oopherectomy was not performed.

The type III radical hysterectomy and bilateral pelvic lymph node dissection and vaginectomy as described by Meigs[4] and Piver et al.[6] has been performed for the treatment of stage I and IIA cervical cancer in our institution since 1978.

All patients undergoing radical hysterectomy since 1978 have received heparin in subclinical dosage preoperatively and 7 days after the operation.

Prophylactic antibiotic therapy (2 gr alphacillin 2 hours preceding and every 6 hours following the operation for 5 days) has been used since 1978. Extraperitoneal suction drainage of both obturator fossae by means of catheters (Hemo-Vac) is routinely performed. The mean number of bladder catheter drainage in this study was 14.

Results

Of the 147 patients, 129 had squamous cell cancer, 14 had adenocarcinoma, 2 had adeno-squamous carcinoma, and 2 had clear cell carcinoma.

Clinical staging was accurate in 79.4 % of stage I patients who underwent pretreatment surgical staging.

5.3 % (1/19) of the patients with stage IA disease, 23.4 % (18/77) with stage IB, 30.4 % (7/23) with stage IIA, and 50 % (8/16) with stage IIB had lymph node metastases.

Microscopic vaginal lesion was present at the time of surgery in 1 patient with stage IA (5.3 %) disease, 6 with stage IB (7.8 %), and 4 with stage IIB (25 %).

Large pelvic vessel injuries, bladder and rectal perforation, cutting of the urether and obturator nerve were observed in 8, 5, 1, 2, and 1 patients, respectively, during surgical procedure.

Of the 147 patients, 67 had 500 cm^3 and less blood loss, 47 had 501 to 1000 cm^3, 17 had 1001 to 1500 cm^3, and 16 had 1501 cm^3 and more blood loss. Blood transfusion was performed in a total of 105 patients 2 and less units in 63 patients, 3 and 4 in 27, and 5 and more in 15.

The operative mortality was seen in 1 (0.7 %) patient who had pulmonary embolism within the first 24 hours of the operation.

The types of postoperative complications are shown in Table II. The most common complications were urinary, tract infection, wound infection and temporary paralytic ileus.

Among the 142 patients with cancer of the cervix, the 5-year survival rates by stages were 100 % for insitu and microinvasive, 89.5 % for IA, 84.4 % for IB, 73.9 % for IIA, and 56.3 % for IIB (Table III).

The 5-year survival with positive pelvic lymph nodes in this study was 68 % compared to 75 % for patients with negative lymph nodes.

TABLE II
POSTOPERATIVE COMPLICATIONS

Type	No	%	Comment
Urinary Tract Infection	22	15.0	
Temporary Paralytic Ileus	18	12.2	
Wound Infection	15	10.2	
Vaginal Vault Stenosis	15	10.2	
Wound Seperation	10	6.8	
Lymphocyst	10	6.8	
Temporary Incontinence	8	5.4	
Pelvic Abscess and Hematoma	6	4.1	
Postoperative Hemorrhage	6	4.1	Two Intraperitoneal and Four Vaginal Bleeding
Urethero Vaginal Fistula	5	3.4	
Vesico Vaginal Fistula	4	2.7	One Had Previous Irradiation
Bladder Atonia	4	2.7	
Phlebitis	3	2.0	
Pulmonary Embolism	2	1.4	One Died Suddenly
Intestinal Fistula	1	0.7	

TABLE III
THE 5-YEAR SURVIVAL

Stage	Living/Total	%
In Situ -Microinvasive	7 / 7	100.0
IA	17 / 19	89.5
IB	65 / 77	84.4
IIA	17 / 23	73.9
IIB	9 / 16	56.3

Discussion

Radical hysterectomy and pelvic lymphadenectomy can be accomplished with low martality and morbidity .The mortality after radical hysterectomy is between 0.6 % and 2.7 % and most operative deaths are due to pulmonary embolism and hemorrhage.[7,8] The operative mortality rate in this study was 0.7 %.

Lymph node metastases in this study were present in 24 % of the patients. Various reports in the literature show the incidence of lymph node metastases in microinvasive carcinoma to be between 0 % and 7 %, in stage IA disease to be approximately 2-3 %, in stage IB to be as low as 4.3 % and as high as 34.2 %, in stage IIA to be between 17 % and 34 %, and in stage IIB to be 25-48 %.[7-13]

Preoperative external radiation therapy has been shown to decrease the incidence of pelvic node metastases. Patients with tumor in pelvic

nodes have lower survival rates than patients with negative nodes. The 5-year survival rate for stage IB disease was 45 % in patients with positive lymph nodes and 91 % in patients without positive lymph nodes. For stage IIA, these figures were 42 % and 77 %, respectively.[7, 8, 12] The 5-year survival rate with positive lymph nodes in this study was 68 % compared to 75 % for patients with negative nodes. In patients with positive lymph node metastases, postoperative radiotherapy should be given as 5000 Rad total pelvis plus one application with colpostats 4000 or 5000 Rad surface dose.[14]

The reported mean incidence of urethero-vaginal and vesico-vaginal fistulae in the collected series were 2.4 % (range 0-8.6 %) and 1.7 % (range 0-10.3 %), respectively.[7, 8, 15, 16] The same figures in this study are 3.4 % and 2.7 %, respectively. Approximately one-half of these vesico-vaginal fistulae close spontaneously when treated by continuous bladder drainage.[15, 16] Preoperative irradiation may increase the risk of fistula formation.[16] The incidence of recto-vaginal fistula has been reported to be 0.6 %.[7]

The incidence of severe vaginal vault stenosis after surgery in this series is 10.2 %. This figure has been reported to be 17 % in stage I and II disease.[7]

The incidence of lymphocyst formation after radical hysterectomy and pelvic lymph adenectomy has been reported to range from 12.6 % to 24 %.[17, 18] Lymphocyst occurred in 6.8 % instances in this series. Lymphocysts should be observed, since they may regress without treatment.[8]

The clinical staging of cervical cancer (FIGO) was reported to be inaccurate between 22 % and 39 % in patients with stage I disease who underwent pretreatment exploration.[7, 19, 20] Clinical staging in this study is accurate in 79 % of stage I cases. Although the role of staging laparotomy in patients with cervical cancer is controversial, it should be routinely performed before deciding on the definitive treatment modality.

The paraaortic lymph node metastases have been reported to be between 0 % and 8 % in stage I disease, to be 5 % to 44 % in stage II, to be 15 % to 38 % in stage III and to be between 44 % and 57 % in stage IV.[7]

The 5-year survival rates were 94 % for IA, 88 % for IB, 68 % for IIA and 44 % for IIB.[7, 8] These figures in this study were 89.5 %, 84.4 %, 73.9 %, and 56.3 %, respectively (Table III).

The 5-year survival rate in patients with cervical lesions less than 3 cm ranged from 72 % to 81.8 %, however in patients with lesions greater than 3 cm, the 5-year survival rate decreased to less than 42 %. In stage IB, the 5-year survival rates in women with lesions less than 3 cm ranged from 84 % to 90.1 %. However, in patients with lesions greater than 3 cm, the 5-year survival rate decreased to less than 66 %.[13] These figures in this series are 87 % and 65 %, respectively, in stage IB.

The prognostic factors stated in various reports are as follows; size of the cervical lesion, lymph node metastases, depth of invasion, histologic type of tumor, clinical staging (FIGO), vascular space involvement, lymphatic response, the treatment modalities, and coexisting pathology.[7, 8, 12]

Received : March 28, 1983
Accepted for Publication : May 25, 1983

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Hacettepe Medical Journal
Volume 16 / No. 2 / April 1983 / pp. 73 - 77

Pregnancy and Its Complications After Cardiac Valve Replacement

Report of 22 Deliveries

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Summary

The outcome of 22 pregnancies in 19 patients with prosthetic heart valves is analysed, and the literature about the subject is reviewed.

Key Words: Cardiac valve replacement, Cardiac valve prosthesis.

Introduction

Since Hufnagel and Harvey first implanted a prosthetic cardiac valve in the descending aorta of a human subject in 1952, refinements in prosthesis design and surgical technique have permitted successful insertion of artificial heart valves in several hundred thousand patients throughout the world.[1] The use of prosthetic heart valves has allowed women with cardiac disease to improve their functional capacity and undergo pregnancy with a very low rate of complications.[2]

In the last quarter of our century, many pregnancies in women with cardiac valve prosthesis have been reported in medical literature.[1, 3, 4] Morbidity and mortality for both the infant and the mother in such cases depend on many factors which include the functional cardiac capacity, the degree of hemodynamic disability and the management of anticoagulation. The management of anticoagulation probably has the most significant effect on the outcome of pregnancies.

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In an attempt to provide information that will assist in the evaluation and management of the problems associated with pregnancy after insertion of an artificial cardiac valve, at the Department of Obstetrics and Gynecology, Hacettepe University Hospital, the outcome of pregnancies in women with prosthetic valves was analysed.

Materials and Methods

Nineteen patients with artificial heart valves delivered 22 children at our institution from January 1970 to January 1983. The mean age was 26.8 ∓ 1.02 (range from 21 to 36) and the mean gravidity was 2.7 ∓ 0.4 at the time of delivery after surgical intervention. Of the 19 patients, 15 had mitral valve prosthesis, 3 had mitral and aortic valve prosthesis, and one had mitral valve prosthesis and tricuspid valve plasty. Starr-Edwards, L-Custar, and Bjork-Shiley were previously inserted in 12 patients, 6 and 2, respectively. All patients were receiving medical treatment, including anticoagulant drugs, restriction of physical activity and dietary sodium. Administration of oral anticoagulants was stopped 72 hours before delivery and restarted 72 hours after delivery. During this period, subclinical dosage of heparin was given in all patients. The prothrombin and coagulation times were controlled once a week in outpatients and daily in inpatients. Prophylactic antibiotic was used (1 gr. ampicilline every 6 hours during labor and for 7 postpartum days). Digitalis and diuretic agents were given to 15 patients. Breast feeding was discontinued when the patient was on coumarin.

Results

19 patients had 22 babies after surgical intervention. The mean gestational age was 38.9 ∓ 0.64 (range from 34 to 41.5 weeks). 18 babies were vaginally delivered and 4 patients were subjected to cesarean section for obstetric indications. Forceps was applied in 10 and vacuum was applied in one of the 18 vaginal deliveries. Postpartum pathological vaginal bleeding did not occur in any patient.

The mean weight of the babies was 3053.86 ∓ 391.76. Of the 22 neonates, 19 had 7 and more Apgar scoring and 3 had 6 and less.

Congenital anomaly was found to be 9.1 % (2/22) and perinatal mortality rate was 9.1 % (2/22).

Antenatal vaginal bleeding occurred in 2 (10.5 %) patients who had prolonged prothrombin time. The vaginal bleeding in these patients stopped after bed rest and a short interruption of anticoagulation.

Postpartum anemia was seen in three patients who required fresh blood transfusion.

Wound hematoma was observed in one (5.3 %) patient.

Maternal death was seen in one patient who had pulmonary edema.

Discussion

Advances in the surgical management of valvular heart disease have allowed women with cardiac problems to improve their functional capacity and undergo pregnancy with very few complications.[5] The degree of hemodynamic disability, the functional cardiac capacity and anticoagulation during pregnancy, labor, delivery and early postpartum carry the most important risks for the mother, fetus and newborn. Harrison and Roscke have found that 35 % of pregnancies in women with valve prostheses were associated with maternal cardiovascular problems, suggesting that strict cardiac management is essential in reducing maternal risks.[6] 15 patients in our study had digitalisation and one patient died because of pulmonary edema developed after delivery.

The incidence of arterial embolism in patients who do not recieve anticoagulant drugs is 5 to 10 % in those with an aortic valve prosthesis and 20 to 55 % in those with a mitral valve prosthesis.[7, 8] There was no arterial embolism in our series. The frequency of embolic episodes in patients treated with coumarin has decreased even more when cloth-covered prostheses are used, ranging from 3 to 20 %. The use of anticoagulant agents during pregnancy is still controversial. Out of the 135 patients reported in published series who had heparin therapy during pregnancy, significant maternal hemorrhage was reported in 14 (10 %), and death in 3 mothers (2 %), two were evidently secondary to treatment failure and one due to hemorrhage.[9, 10, 11] In contrast, only four reports (1 %) of significant maternal hemorrhage and two deaths associated with the use of coumarin derivatives was found in the literature.[10, 12, 13] In this series, maternal hemorrhage was found in two patients who were on coumarin, but there was no maternal death secondary to hemorrhage. Close monitoring will decrease the risk of maternal hemorrhage.

Anticoagulant therapy has additional risks for the pregnant women, also for the fetus and the newborn; since coumarin crosses the placenta and can induce hemorrhage and congenital malformations (Table I).[2]

In many series, a high rate of prematurity and stillbirth has been reported in women using heparin, although no hemorrhage was encountered.[14] Summary of fetal and neonatal complications associated with the use of coumarin derivatives and heparin is given in Table I.[14]

TABLE I

FETAL AND NEONATAL COMPLICATIONS ASSOCIATED WITH THE USE OF COUMARIN AND HEPARIN

Outcome of pregnancy	Coumarin and derivatives	Heparin
Live birth with no complications	293	86
Live births with problems	57	30
Embryopathy	11	–
C.N.S. effects	6	–
C.N.S. effects + embryopathy	5	–
Hemorrhage	7	–
Prematurity (normal)	8	19
Prematurity (died)	4	10
Prematurity + hemorrhage	4	–
Other problems	12	1
Spontaneous abortion	36	2
Stillbirth without hemorrhage	25	17
Stillbirth with hemorrhage	7	–
Total	418	135

Two neonates in this series had congenital anomalies; one had hydrocephalus and the other had nasal hypoplasia. Coumarin derivatives were given to their mothers. In this study, infant death occurred in two neonates. The cause of death was hydrocephalus in one and cerebral hemorrhage in the other.

This figure in the literature was 2.4 % for coumadin and 7.4 % for heparin and also persistent sequela was found to be 10.4 % and 0.7 %, respectively.[14]

Comments

1- Both heparin and coumarin carry substantial risks for the mother and the fetus during pregnancy.

2- If the patients have taken an oral anticoagulant in the first trimester, its teratogenic affects should be explained and the termination of pregnancy should be offered.

3- The administration of oral anticoagulants should be stopped 72 hours before delivery, and heparin should be used until oral anticoagulants are restarted 72 hours after delivery.

4- The use of low-dose heparin in the first trimester, followed by oral administration of coumarin until the 37th week of gestation and heparin again until 72 hours after delivery is also recommended.

5- Lately, low dose heparin therapy has been used in the first trimester and the last 3 weeks of the gestation to prevent systemic embolism.[15]

6- Nonthrombogenic valves, such as porcine heterograft or other types of tissue valves promise particular advantage for patients who plan to become pregnant, because this type of valves do not require continuous anticoagulation.

7- The type of delivery should be selected according to cardiovascular, obstetric and fetal conditions.

8- Prophylactic antibiotics should be routinely used.

9- Breast-feeding should be discontinued during oral anticoagulation.

10- The patient using anticoagulants, should be avoided from pelvic trauma.

Received : March 28, 1983
Accepted for Publication : May 25, 1983

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Hacettepe Medical Journal
Volume 16 / No. 2 / April 1983 / pp. 78 – 83

Polycystography in the Staging of Carcinoma of the Bladder

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Summary

An accurate knowledge about the morphology and the degree of infiltration of a bladder tumor is an essential prequisite to plan the appropriate therapy regimen. Among the radiological investigations, polycystography is one of the least invasive and simple procedures to detect the infiltration of the tumor. This procedure was performed on 48 patients with bladder carcinoma. The correlation between staging based upon clinical criteriae and those of roentgenologic criteria was investigated. In the patient group of non-infiltrating tumors, the accuracy rate of polycystographies in staging was 100 %, while in the group of infiltrating tumors it was estimated to be 88 %.

This procedure is offered as a supplementary investigation to predict the stage of the bladder tumor preoperatively.

Key Words: Polycystography, Staging of carcinoma, Carcinoma of the bladder.

Introduction

Progression in surgical and adjuvant treatment of bladder carcinoma, there is a demand for a greater accuracy in the staging at the initiation of treatment.

Temeliescu[1] first demonstrated that serial instillation of contrast material into the bladder results in a symmetrical expansion of the distensible part of the bladder. When a tumor infiltrates the muscular layer of the urinary bladder, the site of invasion loses its mobility and consequently its capacity to expand.

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The purpose of this study, which includes 48 patients with histopathologically confirmed transitional cell carcinoma of the bladder, is to determine the effectiveness of polycystography in staging as a supplementary procedure.

Materials and Methods

48 patients with carcinoma of urinary bladder were included in this study. 45 patients were male and three were female. The average age of all patients was 58.1 ∓ 3.4 years, with a range of 24-76 years.

Each patient had a through clinical examination and excretory urograms to assess the morphology of the bladder tumor and its effect on the upper urinary tract. The clinical examinations: cystoscopy, bimanuel palpation and biopsy were performed in all patients. These parameters were considered to classify the tumors according to TNM classification.[2] The patients with T_a and T_1 tumors according to TNM classification were grouped as non infiltrating tumors, while T_2 and T_3 tumors as infiltrating tumors.

Tumors were graded histopathologically using the specimens obtained from transurethral biopsies which were performed both from the tumor and its base.

Polycystographies were performed preoperativelly in order to assess the degree of infiltration of the tumor. The findings thus obtained from polycystographies were compared with those obtained from clinical examinations, cystoscopy, biopsy and bimanuel palpation.

Polycystography

Technique: A mild laxative is administered the day before the procedure, to clear the bowels and avoid gas shadows. The patient is catheterized on the X-ray table under aseptic conditions. The residual urine is drained off and the pelvis is strapped to the X-ray table to prevent its movement during the procedure. The bladder is slowly filled by gravity to the capacity with appropriate contrast material. The first exposure, an anteroposterior view of the bladder, is made when the bladder is full. Exactly one third of the contrast material is drained and a second exposure is made on the same film. Another one third of the dye is drained and the last exposure is made.[3]

The bladder wall collapses concentrically with each successive withdrawal of the contrast material from the bladder. When three exposures are made on the same film this concentric collapse of the bladder is seen on the X-ray plate as three concentric lines. This type of polycystographic finding is regarded as indicative of a non infiltrating bladder tumor (Figure 1).

When the bladder tumor infiltrates deep into the bladder wall, that side of the bladder wall becomes rigid, fixed to the surrounding tissues and can not collapse when the contrast material is withdraw from the bladder. This can be seen on the X-ray plate by the absence of the three lines at the tumor site which can be interpreted as an infiltrative bladder tumor (Figure 2).

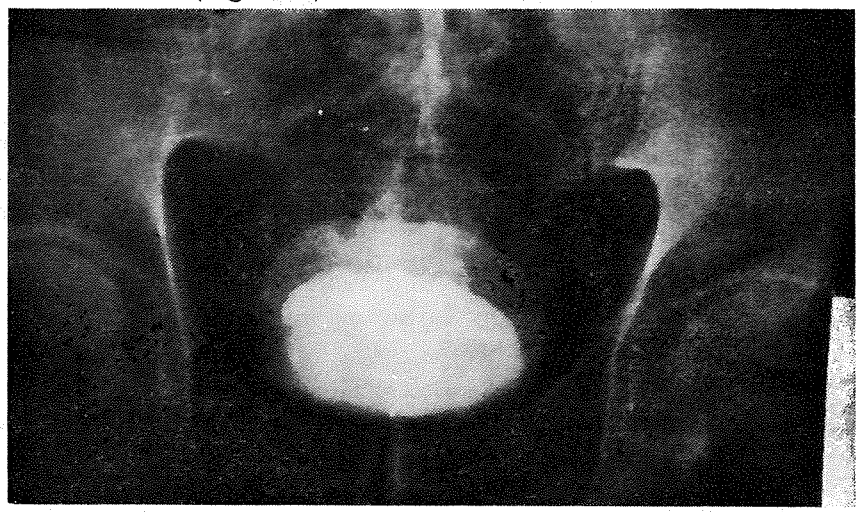

Figure 1
Clinically T_1 tumor with full expansion polycystography.

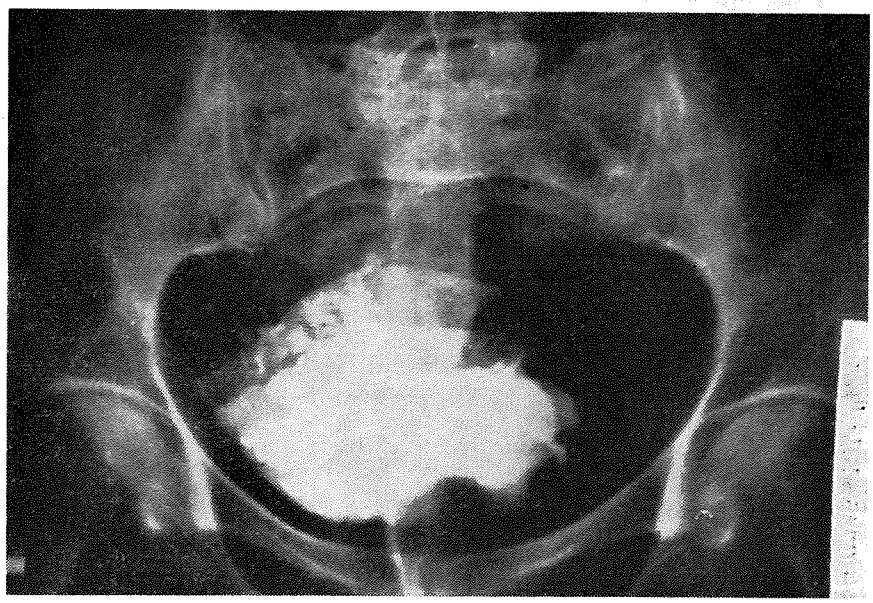

Figure 2
Clinically staged as T_3 tumor with poor expansion on the left side.

Results

48 patients with carcinoma of the urinary bladder were included in this protocol. Among the 48 patients investigated, 20 patients were clinically staged as infiltrative tumors, while the rest were non-infiltrative tumors.

We were not able to obtain sufficient polycystographies in four patients because of small bladder capacity and/or previous operations.

According to polycystographic findings, 25 patients had infiltrative tumors, while the remaining 19 patients fell into non-infiltrative tumor group.

19 of the 20 patients who were clinically staged as non-infiltrative had normal polycystographies, yielding a 100 % accuracy rate for this procedure in this group. In this group, we were not able to obtain a sufficient X-ray in one patient. (Table I).

TABLE I

THE CORRELATION BETWEEN CLINICAL STAGING AND POLYCYSTOGRAPHIC FINDINGS

	N	Normal Polycyst.	Abnormal Polycyst.	Insufficient Polycyst.
Clinically staged as non-infiltrating	20	19	–	1
Clinically staged as infiltrating	28	3	22	3

In the infiltrating tumor group three false negative results can easily be seen in Table I. In this group the positive correlation between roentgenologic and clinical staging was found to be only 88 %.

There were no major complications related to this specific procedure.

Discussion

An accurate pretreatment staging of bladder tumors has been recognized as a mandatory prerequisite for the appropriate selection of treatment modality. Emphasis is usually placed on that of invasion of the tumor. Although, histologic degree of differentiation effects the prognosis, the depth of penetration determines the mode of therapy.[4]

Urography, cystoscopy, biopsy and bimanual palpation under anesthesia are the conventional methods of staging. They yield an accuracy rate of approximately 80 % in staging.[5, 6]

The demand for a more accurate pretreatment staging has led to introduction of more sophisticated roentgenologic procedures, one of the least invasive method among which is polycystography. The others are double, triple contrast cystography, angiography, lymphography and C-T Scan.

Urography has an important place in the diagnosis and clinical staging of bladder tumors. Because of the multifocal nature of urethelial tumors, it is mandatory to visualise adequately renal pelvis and ureters. As a method of delineating bladder tumors it is less informative because the dye may get diluted in the bladder and may not outline the bladder and the tumor.

Conventional retrograde cystogram is not always informative in case of small papillary tumors because dense contrast medium may mask the filling defect.

The clinical stage of the bladder tumor can be estimated by bimanual palpation under anesthesia combined with transurethral biopsy. Although this is a reliable method in staging, the anterior wall immediately behind os pubis and a portion of posterior wall may be inaccessible.

There are reports in the literature, comparing the staging done by transurethral biopsy to that of entire tumor removed surgically, indicating that 20-30 % of the cases were underestimated.[7] But, still this method if properly done, gives an accurate picture of the stage of the tumor in most of the cases.

The other method of assessing the degree of infiltration is polycystography. In a study of 40 patients with bladder carcinoma Sakkas found a close relation between the polycystographic results and clinical staging.[8] Similarly, Raghavaiah[3] found in 96 % of the cases with bladder carcinoma polycystography accurately determined the degree of infiltration. In our study we estimated the accuracy rate of this specific procedure to be 93 %.

This method may not be very accurate in detecting the degree of infiltration of the tumors located in the bladder neck region, but we also are aware of the fact that it is equally difficult to assess the stage by bimanual palpation of the same area.

Great care must be taken in interpreting these radiographies because bladder fixation may be associated with various vesical and/or extravesical inflammatory conditions. A bladder which has been subjected to an open operation or radiotherapy may expand poorly. In addition, this tecnique is of little value in contracted bladders.

As a conclusion, the polycystography is not offered as a replecament for bimanual examination and transurethral biopsy in staging but rather as a supplementary procedure. In addition there is no need for sophisticated equipment and trained personel.

Received : March 2, 1983
Accepted for Publication : May 25, 1983

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Hacettepe Medical Journal
Volume 16 / No. 2 / April 1983 / pp. 84 - 92

Hydatid Disease of the Liver

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Hydatid disease has been known from the time of Hippocrates, who refers to the disease as "liver full of water".

The disease is endemic in South America, the Far East, Middle East, Eastern Europe. Turkey is one of these countries that the disease is endemic.[1-6]

General Features

Hydatid disease in man is most often caused by Echinoccoccus granulosus but may also result from infection by Echinococcus multilocularis. The mature adult organism, which is a parasite, is surprisingly small, measuring only 4-6 mm in length and is generally composed of four segments, a head and three proglottids. The third segment contains the male and female sexual organs of this true hermaphrodite. The last segment is the gravid proglottid, distended with up to 800 eggs. This segment or its eggs are released into the intestinal tract of the dog to be discharged to the outside every time the animal defecates. The host dog is uneffected by the tapeworm in its intestine.[6, 7]

The eggs of the parasite can not mature into adult worms without first passing thru the larval stage. Since this cannot take place in the definitive host, the eggs must find and enter an intermediate host to undergo metamorphosis. The indermediate hosts are sheep, cattle and deer. Man is an accidental or incidental host (Figure 1). Exposure takes place thru ingestion and very doubtfully, thru inhalation of the ova.[7]

The outershell of the ovum becomes lysed in the upper gastrointestinal tract of the intermediate host. This allows the embryo to penetrate the mucosa of the small bowel and reach the liver by the portal venous system. The liver acts as a filter. Most of these embryos are trapped in the liver and the rest pass thru the liver and are scatterred to the lung, brain and other organs. The liver is effected in approximately 60-80 % and the lungs in 10-30 % of the cases.[5, 7, 8]

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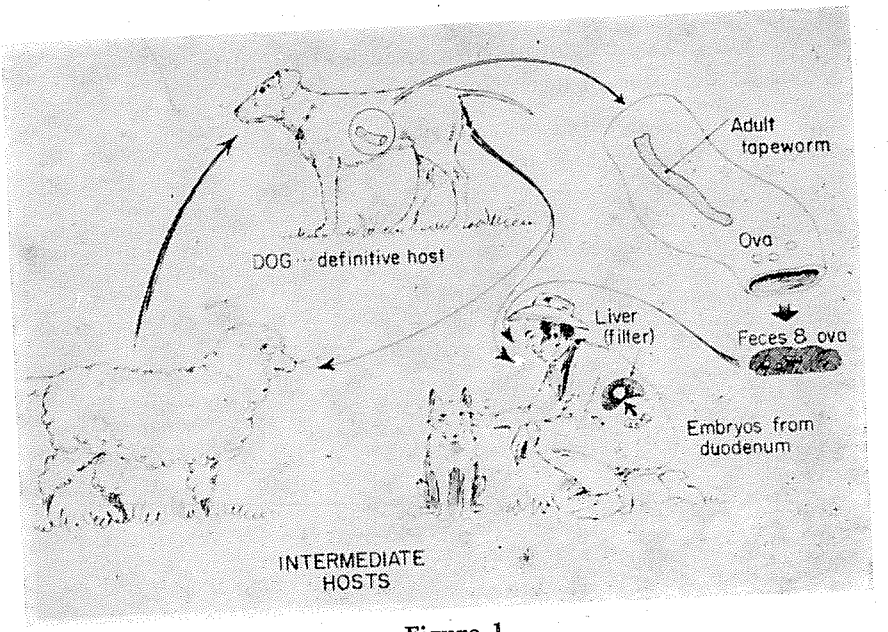

Figure 1
Life cycle of the parasite.

Lodged in the liver, the embryo either dies or develops into a cyst which has a characteristic architecture. The outer laminated whitish chitinous capsulle is lined by a germinal epithelium which is the active lange of the cyst, producing capsules in which the scolices are formed. A highly antigenic fluid "eau de roche" "Spring water" collects within the cyst. Daughter cysts are often found floating in this fluid under high pressure. Outside this acellular layer, there is a fibrous but vascular adventitial zone, called pericyst, that is surrounded by compressed liver tissue (Figure 2).

A scolex can remain attached to the germinal surface or it can float in the hydatid fluid. Free floating scolices and the capsules settle down at the bottom of the cyst as a whitish sediment. This is called hydatid sand. Each cubic cm. of the hydatid sand contains 400.000 scolices.[7] The hydatid fluid is one of the most characteristic features of unilocular hydatid cysts. This is a clear, colorless and odorless water-like fluid.

There is no question that the hydatid fluid protein has antigenic properties for mammalian hosts. The antigen quite likely enters the circulation of the intermediate host in the early vesicular stage; when the laminated membrane is just beginning to form. The mononuclear cells and eosinophils around the vesicle could be taken as a local immunolo-

gical response of the host. The antigen comes into contact with the host tissues when the cyst (laminated membrane) ruptures, either spontaneously or at the time of operation.[7]

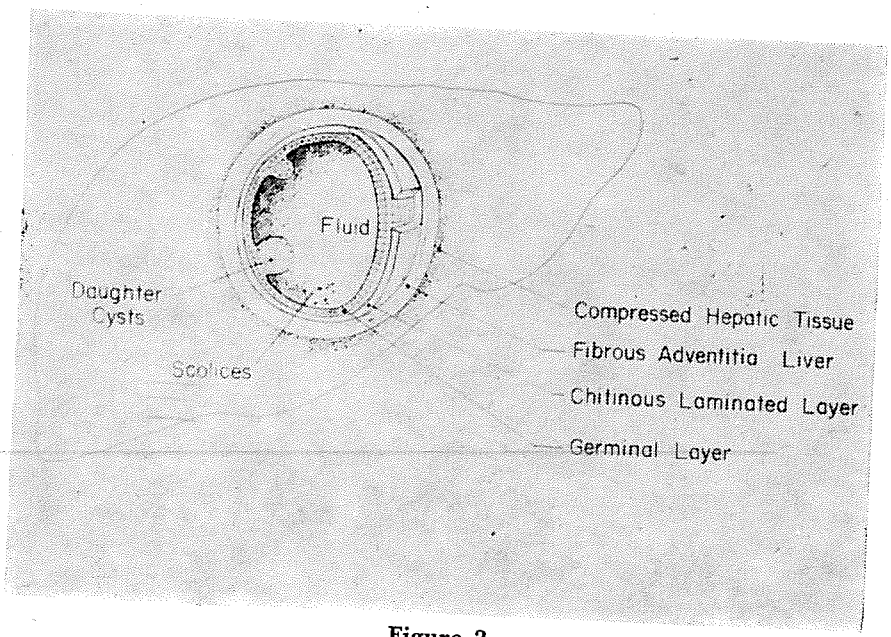

Figure 2
The architecture of the cyst in the liver.

In man the liver is the most commonly involved organ in hydatid disease. The liver is the site of involvement in about 70 % of the patients. The majority of the cysts are located in the right lobe (75-85 % of the cases) and are single.[5, 8, 9] The cysts might be univesicular or multivesicular.

Symptomatology and Diagnosis

The uncomplicated hydatid cysts are asymptomatic. The symptoms of hydatid disease depends on the location and the size of the cyst producing mechanical problems.[5] Abdominal pain, nausea and vomiting could be seen but they are not spesific. In endemic areas in cases of obstructive jaundice the disease must be kept in mind.

There is no test that will definitely establish the diagnosis of the hydatid disease of the liver. Casoni and Weinberg tests are positive in 87 and 69 % of the cases. Casoni test is an intradermal test in which 0.2-0.3 ml of antigen is given intradermally. A wheal appears in 15

minutes or an area of induration and erythema occurs in 24-72 hours when the test is positive. The Casoni test may remain positive after the parasite has died. Weinberg test is a complement fixation test. False positive results could be obtained in patients with cancer. Indirect hemagglutination and bentonite flocculation tests have been developed. Currently available tests make an accurate diagnosis possible in over 90 % of the cases.[7, 8]

Plain chest and abdominal X rays may be helpful. The diaphragm can be found elevated. Abdominal X-rays may reveal a concentric calcification in the right upper quadrant.

Contrast studies such as UGI-series, cholecystography, barium enema and IVP could be helpful. But these are not suggested as routine measures. Simply, compression effects of the cysts are found in these X-ray studies.

Liver scans are diagnostic in about 85-90% of the cases.[7] The cysts occurs as a filling defect within the liver. A compensatory hypertrophy of the liver could also be found on the scan. The liver scans are helpful to show whether the cysts are single or multiple.

Arteriography is very helpful and shows displacement of the vessels in the liver. But we believe that it is a very invasive procedure to be used for hydatid cyst of the liver.

Ultrasonography should be used more widely in this situation. Internal echos are typical of multivesicular cysts which are found in 80 % of the cysts. Ultrasonography will also give an idea about the multiplicity of the cysts and the bile ducts. Ultrasonography is diagnostic in about 90 % of the cases.[10, 11]

Complications

The main complications of hydatid cyst of the liver are:[6-9, 12]

1- Rupture into the peritoneal cavity: This complication gives rise to spillage of multiple daughter cyst, ascites, multiple small granulomas on the intestines and subsequent intestinal obstruction. About 20 % of the patients with free rupture into the peritoneal cavity will develop hydatid implantation in 5 years. But occasionally sudden rupture into the peritoneal cavity may cause a severe anaphylactic reaction and death.

2- Obstructive jaundice occurs in 5-10 % of the cases. In most of the cases this obstruction is secondary to compression of the bile ducts

and less often to rupture into the major biliary radicles with passage of daughter cysts. When there is a communication with the biliary tract it is with the minor ducts more often than the major ducts. In this situation jaundice will not be found.

3- Rupture into the pleural cavity may give rise either to pleural effusion or to a bronchobiliary fistula.

4- Secondary infection is an important complication. When suppuration develops the clinical picture is of liver abscess. Secondary infection develops after the laminated membrane is fragmented and a communication has developed either with the biliary tract and from there to the gastrointestinal tract or a bronchobiliary fistula has developed and finally if the cyst has been treated by external drainage. Very rarely a blood-borne infection can also develop.

The organisms usually cultured out from such infected hepatic hydatid cysts are gram-negative enteric bacteria, staphylococcus aureus and streptococci. Anaerobic bacteria could also be isolated from the supurating cysts.

5- Intravascular rupture is a very rare complication. Rupture into the major venous and arterial systems have been reported.

Surgical Treatment

The treatment of hydatid cyst of the liver is surgical. In selecting the surgical technique, the surgeon should be guided by the size and location of the cyst and the complication it has already sustained or caused.

Danger of rupture, infection and pressure on the surrounding organs and clinical symptoms are indications for surgery.[13]

The principal objectives to be met by the surgical maneveurs are the same in all modes of surgical technics. These objectives are:

1- Total removal of all parasitic elements,

2- Avoidance of spillage of cyst contents,

3- Management of the residual pericyst cavity,

4- Closure of biliary communications.

Either anatomical or partial hepatic lobectomies have been used in the treatment of hydatid disease of the liver.[2, 14] There is no question that with removal of the hepatic lobe bearing a large cyst, a number of surgical problems are solved in one stroke: The parasite is removed in toto, there is no spillage of cyst contents and hence no danger of anap-

hylaxis or recurrence of the cyst and finally there is no residual pericyst cavity to worry about. Hepatic resections should be considered only in single cysts. We believe it is a radical procedure for a benign disease and it should not be used.

Pericystectomy or radical cystectomy is removal of the cyst just outside the pericyst layer. It should be remembered that no such plane exists anatomically. This makes this procedure complicated with postoperative bleeding and bile leakage. We believe pericystectomy should be used only in peripheral and pedinculated cysts.

Evacuation of the cyst and management of the pericyst cavity could be performed in different ways. But during the evacuation of the cyst, the cyst should be handled carefully to prevent spillage of the viable scolices. Different scolicidal agents have been used to kill and inactivate all the scolices before they can be spilled into the surgical areas.

Years ago 40 % formaldehyde was used for inactivation, but during the past few years hypertonic saline solutions have been widely used for this purpose. The rationale of using hypertonic saline is simply to effect a sufficiently strong osmotic gradient across the outer cuticular membrane of the scolex to bring about its lysis. We had been using 3 % saline for this purpose. Some advocate that 15-20 % of saline should be used. Saidi have used 0.5 % solution of silver nitrate for this purpose.[7]

After the proper walling off the surgical field with lap pads moistened with the scolicidal agent, the hydatid fluid is aspirated with a large bore needle and the scolicidal agent is given into the cyst. After 5-10 minutes the cysts are aspirated with a trocar and the pericyst layer is opened. The germinative membrane and the daughter cysts are removed. The pericyst cavity then could be managed either by external drainage, internal drainage or obliteration of the cavity. External drainage could be accomplished either by marsupialisation or by simple tube drainage.

Obliteration of the cavity could be done either by reapproximation of opposing walls with rows of sutures (cappitonage), or by omentoplasty, where the cavity is filled with an omental pedicle. Omentoplasty could be used safely in almost all patients except those who have suppuration or a communication with a major bile duct.[4, 9]

In the presence of suppuration, external drainage is unavoidable. If there is a communication with a minor bile duct, this can be sutured; but if the communication is with a major bile duct T-tube drainage of the common bile duct, a choledochoduodenostomy or a sphincteroplasty should be performed. This will prevent formation of a biliary fistula and will definitely shorten the hospitalization period.

Results of Surgical Treatment at Our Institution

We have treated 158 cases of hydatid cysts of the liver surgically during the period of 15 years at Hacettepe University Hospitals. Of these 158 cases 103 (65.2 %) were women and 55 (% 34.8 %) were men. A peak is present between 20 and 50 years of age. The most common symptom was right upper quadrant pain. Hepatomegaly and a palpable mass were the most common findings (89 %). 102 (65 %) of the cases had single cyst and 75 % the disease was located in the right lobe of the liver. 23 patients had hydatid cysts out side of the liver concomitantly. The lungs, spleen and omentum were the most common affected organs beside the liver.

25 (15.8 %) patients had a communication between the cyst and the biliary tree. Nine of them were recognized during surgery and T-tube drainage was performed in 6 of them.

Suppuration of the cyst was found in 26 (% 16.5) of the patients. One patient had a free perforation into the peritoneal cavity.

Omentoplasty, marsupialization, tube drainage and cystectomy were the most commonly performed operations. In 14 patients tube drainage was added to omentoplasty.

The most common complication was infection in the residual cavity and biliary fistula.

All of the patients on whom omentoplasty of cystectomy was performed were discharged from the hospital without any residual problems. On the other hand 24 of the patients who had marsupialization and 10 of the patients who had tube drainage were discharged with drainage.

Omentoplasty and cystectomy led to a shorter hospitalization period than marsupialization and tube drainage. The results are summarized in Table I.

TABLE I

RESULTS OF THE SURGICAL TREATMENT

	No of Cases	Complication Rate %	Patients Discharged with drainage %	Mortality %
Omentoplasty	56	16.1	0	0
Marsupialization	37	78.4	64.8	5.4
Cystectomy	16	12.5	0	0
Tube Drainage	30	80.0	33.3	3.3
Omentoplasty + Tube Drainage	15	73.3	68.8	0
Other	4	75.0	0	0

The overall mortality was 1.8 %. All of these patients died of septicemia secondary to the infection in the cyst cavity.

With these results I believe that every effort should be made to perform omentoplasty and avoid external drainage in the treatment of hydatid disease of the liver.

Medical Treatment

Until recent years surgery was the only known therapy for hydatid disease. Mebendezole (Vermox(R)) have been used in the treatment of hydatid disease after it was found to be effective in mice.[15] Mebendazole is a potent antihelminthic. It is belived that Mebendazole damage the cyst wall which then ceases producing the antigenic material. There is then a fall in osmotic pressure and the cyst collapses and the cyst wall then break up.[12] It has been shown to degenerate the cytoplasmic microtubules and inhibit the glucose uptake of the parasite.[15]

Beard and Co-workers confirmed the success of Mebendazole.[16] Although the optimal dose and duration of Mebendazole therapy has not yet been established 40 mg/kg/day for period of 1-6 months have been used in most trials. The side effects are not known yet, but a risk of rupture of the cyst has been reported.[15] My personal experience in a small group of patients with hydatid disease of the liver was not very satisfying. I believe Mebendazole should not be used as an alterative to surgery, but reserved for only inoperable cases.

Received : March 17, 1983
Accepted for Publication : April 13, 1983

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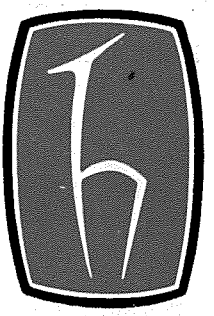

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Printed by
Hacettepe University Press
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VOLUME 16 / NO. 3 / JULY 1983

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Morphological Study of the Lysosomes by Acridine Orange in Human Trabecular Cell Cultures

Murat İrkeç, M.D.* / Ceylâ İrkeç, M.D.**

Summary

A study of lysosomes in trabecular cell cultures from patients with glaucoma and from normals via vital staining with acridine orange was undertaken in the present study. Trabecular cells in culture were shown to have numerous punctiform lysosomes which appeared morphologically normal both in cells from normals and glaucomatous eyes. A discussion about the role of the lysosomes in the regulation of outflow facility of the aqueous humour was made and possible contributions of lysosomal dysfunction to the pathogenesis of glaucoma were evaluated in the article.

Key Words: Acridine orange, glaucoma, lysosomes, trabecular cell culture.

Introduction

A major part of resistance to aqueous humour outflow has been stated to reside in the trabecular meshwork and in the inner wall endothelium of Schlemm's canal.[1,2] The extracellular spaces of these tissues consist of fibrillar and non-fibrillar amorphous materials which undoubtedly should have an important role in the regulation of the outflow of aqueous humour.[3,4] Demonstration of the complex extracellular polysaccharides on the surface of the cells and extracellular spaces of the trabecular meshwork[5] has provided strong evidence for their selective

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filtration effect on the passage of solute molecules and for their role in various diverse functions of the endothelial cells. On the other hand, lysosomes, cytoplasmic vacuoles containing acid hydrolases and bound by a trilaminar unit membrane are closely related to endocytosis and might have a function in turnover of intracellular and extracellular constituents, particularly the proteoaminoglycans.[6]

Establishment of human trabecular cells in culture has provided an efficient means of studying various aspects of these cells in an in vitro system.[7] Taking this into consideration, morphology of the lysosomes of cultured trabecular endothelial cells from normal and glaucomatous patients was evaluated by acridine orange vital staining in the present study.

Materials and Methods

In this study, trabecular endothelial cells established in culture according to a previously described method[7] were used to evaluate morphological characteristics of the lysosomes. Five cell cultures from normal subjects and four cell cultures obtained by trabeculectomy from patients with primary open angle glaucoma were employed as primary cultures and via a slide culture technique cells were passaged to and grown on glass coverslips in 35 mm Petri dishes or Leighton tubes.

An adequate number of cells was available generally on the third day of subculturing to be used for acridine orange staining. Acridine orange was freshly prepared and was normally used in the cell culture of 1 μg/ml. The details of the staining method with acridine orange has been given elsewhere[8] and used without any significant modification in our study.

For photographic documentation, an Ilford HP4 black and white and a Kodak High Speed Ektachrome (160 ASA) color film were used taking previously made remarks into consideration.[9] Acridine orange stained cells were examined and photographed under a Zeiss fluorescence microscope.

Results

Acridine orange selectively stained the lysosomes of trabecular cells yielding an orange-red fluorescence, whereas the nuclei exhibited a green and the nucleoli an orange color. Figure 1 shows lysosomes in the cytoplasm of trabecular cells from normals. It may be readily notable that these lysosomes are punctiform and have a diameter of less than 0.5 μm. Although lysosomes were numerous, there was quite a homogeneity in relation to their size and no particular pattern of polarisation could be detected around the nucleus. Lysosomes were also observed in cellular

extensions of the trabecular endothelial cells (Figure 2). Occasional intermediate lysosomes, sizes from 0.5 μm to 1.5 μm could be seen in the cytoplasm, but no difference was found regarding their fluorescence (Figure 2). A gradual increase in the fluorescence of the punctiform lysosomes observed as they were situated near the nucleus (Figure 1). Occasionally, cells with two nuclei in the same cytoplasm might be seen and numerous punctiform lysosomes generally gathered around the nucleus might be noted (Figure 3). A similar condition might be encountered during cell division, when the internuclear zone of the cytoplasm was occupied by punctiform and intermediate lysosomes following the division of the nucleus into two nuclei. Finally the lysosomes migrate, one half for each cell occupying the juxtanuclear zone of the daughter cells.

The morphological appearance of lysosomes in cell cultures from glaucomatous patients seemed to resemble to those from non-glaucomatous subjects (Figure 4). In these cells, lysosomes were generally of punctiform type and occupied the juxtanuclear region in the cytoplasm. The number of punctiform lysosomes were relatively fewer than those trabecular cells from normals in the cellular projections. Under higher magnifications, lysosomes of trabecular cells from glaucomatous patients were of amazingly homogenous size and intermediate lysosomes could rarely be observed in the cytoplasm without any conspicuous situational pattern (Figure 5).

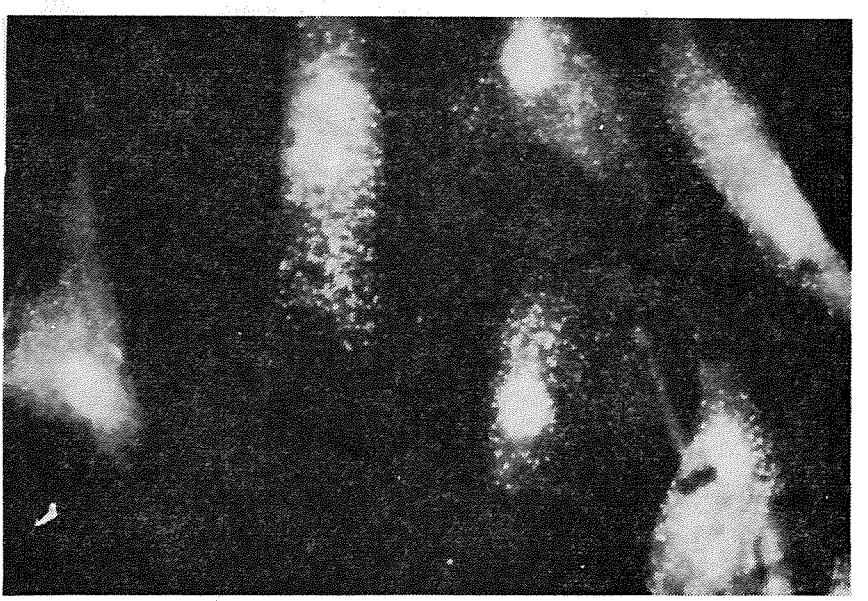

Figure 1
Lysosomes in the cytoplasm of normal human trabecular cells (Acridine Orange, x 500)

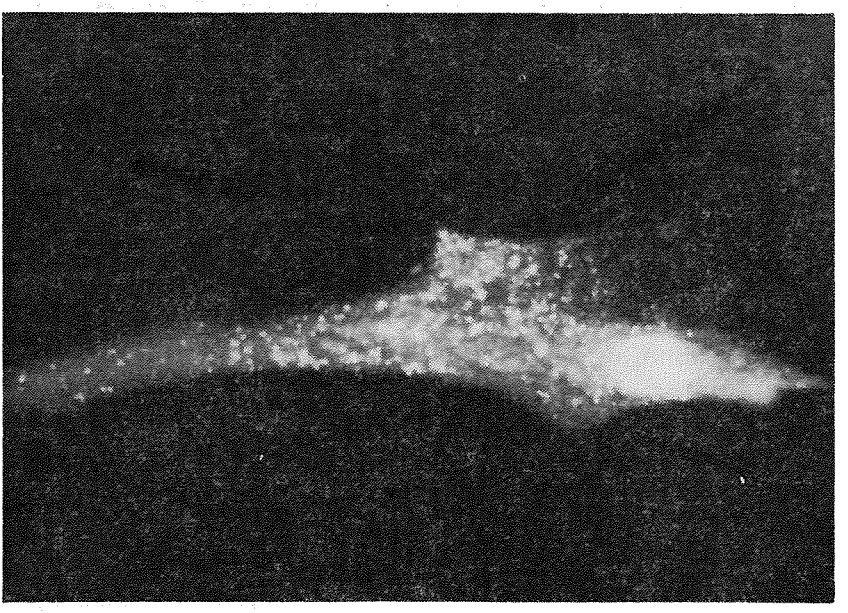

Figure 2
Lysosomes in the cellular projection of a normal human trabecular endothelial cell (Acridine Orange, x 525).

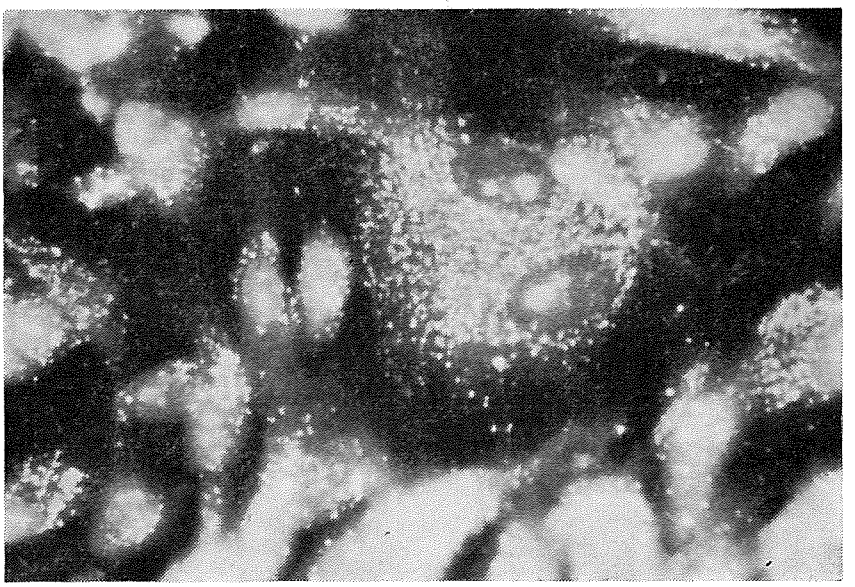

Figure 3
An unusual trabecular cell with two nuclei exhibiting an abundance of lysosomes in the juxtanuclear region of the cell (Acridine Orange, x 425).

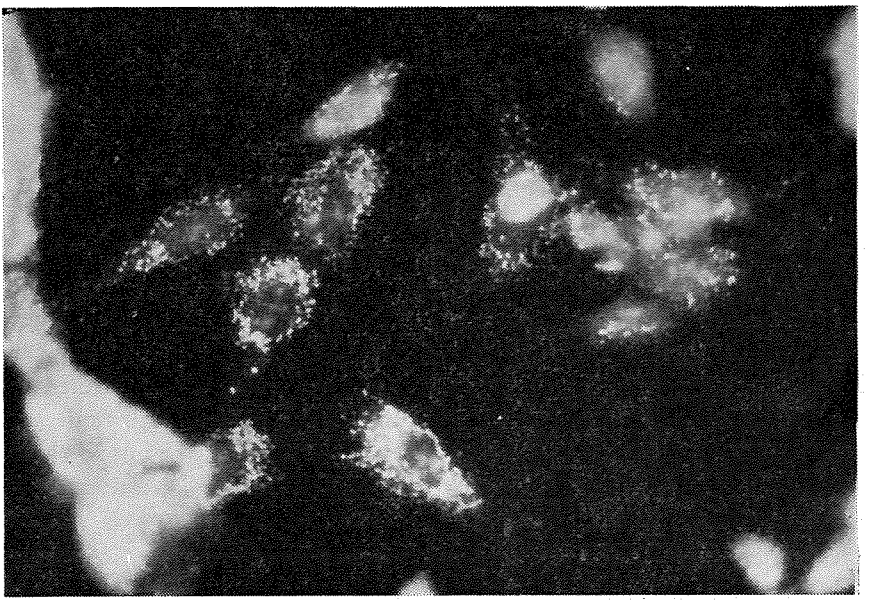

Figure 4
Morphologically normal lysosomes in trabecular endothelial cells grown in culture from a glaucomatous subject (Acridine Orange, x 375).

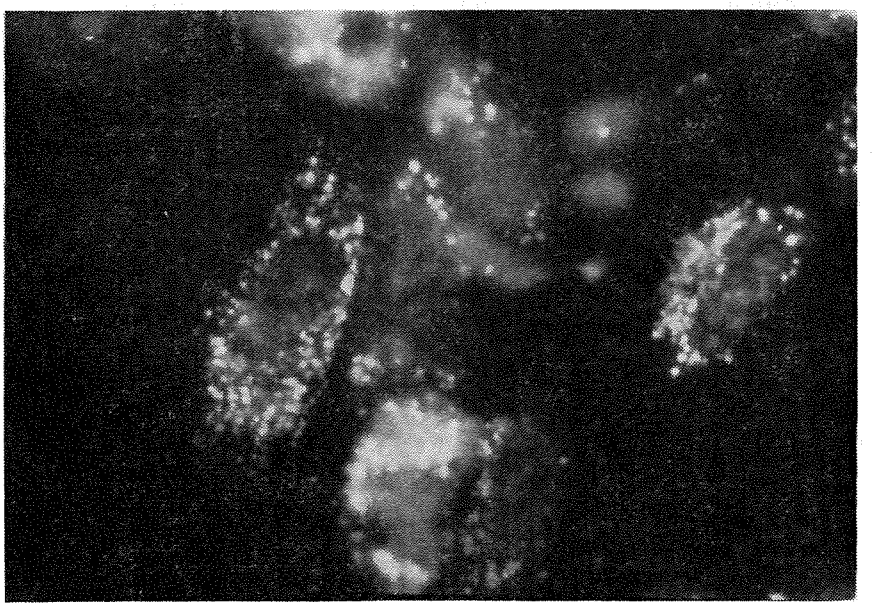

Figure 5
Amazingly homogenous lysosomes in endothelial cells from a patient with primary open angle glaucoma (Acridine Orange, x 525).

Discussion

In human trabecular cell cultures an endogeneous hyaluronidase and extracellular degradation of hyaluronic acid by these cells have been shown recently.[10] On the other hand, the presence of matrix vesicles and lysosomes in the trabecular meshwork of normal and glaucomatous human eyes[11] makes it necessary to explain the role of lysosomes in the regulation of aqueous outflow. Nevertheless, in previous studies on the cellular morphology of human trabecular cell cultures, it has been clearly shown that the cytoplasm of these cells had many lysosomes.[7, 12]

The results of our study using a vital staining method demonstrated clearly that particles representing the lysosomes were punctiform and normal in morphology. These features were also shared by trabecular endothelial cells cultured from patients with primary open angle glaucoma. It is well known that some important morphological alterations in lysosomes may occur in some storage diseases chiefly due to a dysfunction of these organelles.[13] Although lysosomal morphology was normal both in cultured normal and glaucomatous trabecular cells, it is not irrational to claim that lysosomal morphology might not reflect much about the lysosomal function of the trabecular cells and it might be prudent to perform enzymatic studies to reveal the functional status of the lysosomes. A preliminary study has shown that the acid phosphatase content of cultured trabecular cells from normals and subjects with glaucoma reached considerably high values.[14]

A recent consideration of the trabecular meshwork as a self-cleaning filter which in dysfunction might result or contribute to the development of glaucoma appears to be challenging.[15] If this hypothesis were valid, phagocytotic function of the trabecular endothelial cells would be extremely critical in the pathogenesis of various glaucomas. Since lysosomes actively contribute to the complex process of endocytosis, our results relevant to trabecular cells from glaucomatous subjects may at least in part acquit these cellular organelles of any notorious role in the pathogenesis of primary open angle glaucoma. It is, however, obvious that before fully endorsing this concept of pathogenesis of glaucoma much has to be done concerning the functions of the trabecular endothelial cells.

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Hacettepe Medical Journal
Volume 16 / No. 3 / July 1983 / pp. 100 – 104

A Method of Myocardial Protection During Coronary Artery Bypass Operations Using Cold Potassium Cardioplegia

Cihat Bakay, M.D.* / A. H. M. van Straten, M.D.* / Th. S. Wijers, M.D.* / Aydın Aytaç, M.D.**

Summary

To decrease the risk of myocardial injury during coronary bypass surgery in patients with stenosed coronary arteries, we have developed a technique of infusing a cold cardioplegic solution via the aortic root and through the vein graft to the distal coronary circulation.

Keywords: Coronary bypass, Myocardial protection, Aortic root venting.

Introduction

The first time cardioplegia was used in a clinical setting was by Dennis Melrose in 1955.[1] He used a cardioplegic solution with a high dose of potassium-citrate on children. This method was abandoned because of the high incidence of focal necrosis of the myocardium.[2]

During the 1960's cardiac operations were performed while perfusing the cannulated coronary arteries.[3] The operation took place on a fibrillating heart. The effects of fibrillating the heart were described by Buckberg et al. in 1975.[4] With this method the incidence of subendocardial ischemia and necrosis was high and the operating field was far from ideal.

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Topical hypothermia was used in the 1950's[5] and promoted by some surgeons during the 1960's.[6]

During the 1970's the use of cold cardioplegic solutions became the method of choice in protecting the myocardium during the ischemic period of operation. The cardioplegic solution described by Brettschneider[7] in 1964 containing a high dose of procaine is still used in some clinics. Others use cardioplegic solutions or blood with the same concentration of potassium as in the extra-cellular fluid. Still others use blood or solutions with a higher concentration of potassium. The type of cardioplegia and the method of delivering that gives the best myocardial protection is still under much debate. However acute electrical standstill and continued cooling seems to be the most consistent factor of importance.

The cardioplegia we use is described in Table I. We deliver this cardioplegia at a temperature of 4°C.

TABLO I

COMPOUNDS PER LITER GLUCOSE 5 % AND NaCl 0.45 %

KCL	30 mmol
MgCl	15 mmol
$NaHCO_3$	5 mmol
$CaCl_2$	2 mmol

Method of İnfusing the Cold Cardioplegia and Venting the Aortic Root: A 14 G. plastic cannula is placed into the aortic root. This cannula is connected with a line which is Y-shaped. One end of the Y-shaped line is connected to another tubing placed in a roller-pump. After cross-clamping the aorta we can vent the aortic root by sucking on this line via the roller-pump. By venting the aortic root, the aortic valve opens and thus the ventricular wall tension decreases. The operating field becomes bloodless. The other end of the Y-shaped line is connected to a bag of cold cardioplegia which is placed in a pressure bag. After crossclamping the aorta and venting the aortic root we infuse the cardioplegia into the aortic root and through that into the coronary arteries. In this way it is possible to deliver an exact amount of cardioplegia by controlling the pressure that is used to infuse the cardioplegia. We infuse with a pressure of 100-150 mmHg, resulting in an aortic pressure of about 80 mmHg. The moment the cardioplegia starts running, topical cooling is applied by pouring 2 liters of a cold (4°C) physiological solution into the pericardium.

After infusing 600 cc of cardioplegia less (500) with fewer distal anastomoses and more (700-800) with a greater amount of distal anas-

tomoses) the cardioplegic line is clamped off and the roller-pump is started. Another line is connected to the cardioplegia line before the point of this line has been clamped off. This line is connected to the vein graft. Through this line and the vein graft it is possible to deliver cardioplegia, after making a distal anastomosis to the myocardium distal from the stenosis in the coronary artery. After making each distal anastomosis, another amount of cardioplegia is again infused through the vein graft. By checking the flow of cardioplegia through the vein graft it is possible to control the potency and the leakage of each anastomosis immediately after making it. Distal anastomoses are performed by a running suture technique, to avoid a pursestring effect, cardioplegia is delivered at the moment of tying the anastomosis.

Results

In the period of April 1981 until August 1982 in the medical centre "de Klokkenberg" in Breda (the Netherlands) 745 patients with coronary artery disease were operated upon using this method of myocardial protection.

The mean age of the patients was 55.9 year.

The male-female ratio was 3:1.

In this group of coronary patients 4 patients (0,5 %) died within 30 days after surgery. A peri-operative myocardial infarction was diagnosed in 38 patients (5 %). The criterium for peri-operative infarction was a new Q-wave or loss of R-wave in the post-operative ECG that was not present in the pre-operative ECG plus an increase in serum transaminases.

Discussion

Cold cardioplegic solutions supply protection to the heart by reducing ischemic injury, by reducing the myocardial energy demands, by rapid cardiac arrest with cessation of energy demands and by washout of the myocardial metabolites of anaerobic metabolism.[8] In patients with critically occluded or stenosed coronary arteries, the distribution of cardioplegic solution is difficult.[8–10]

Oldham and associates[10] by using radionuclide techniques, have demonstrated interaoperatively, the inability of collateral vessels to provide blood or cardioplegia flow to regions of the heart served by occluded or stenosed coronary arteries. They have concluded that bypass graft flow is distributed to localised regions of myocardium, collateral

flow rarely occurs between adjacent areas of myocardium and sequential or multiple grafts are beneficial in completely revascularizing adjacent areas of underperfused myocardium. Dagget et al.[9] have also shown by mapping the regional temperature that cold solutions injected into the aortic root do not cool all areas of the myocardium equally. Thus the regions of the myocardium that are at highest risk for ischemic injury are also the regions receiving the least amount of cardioplegic solution.[11]

We perform distal anastomoses before and after finishing each anastomosis cold potassium cardioplegia in an amount of 100 cc is infused via the proximal end of the vein graft. In this way we can perfusate the ischemic areas of the myocardium that need most protection and we can see the flow through each distal anastomosis. If there is a leakage, it can be repaired immediately.

A similar method but without delivering cardioplegia through the vein graft has been described by Cantinella et al.[12] Another method in which the proximal anastomosis is made before the distal anastomoses was described by VanderSalm et al.[8]

Using this method of myocardial protection, per and postoperative monitoring of the patients using Swan-Ganz catheters, and performing complete revascularisation have made it possible that only 4 (0,5 %) died within 30 days after surgery.

The peri-operative myocardial infarction rate was 5 %.

The simplicity and good results of this method, besides the fact that it is a safe method, leads us to continue perfusating all distal anastomoses with potassium cardioplegic solution via the proximal end of the vein graft.

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Hacettepe Medical Journal
Volume 16 / No. 3 / July 1983 / pp. 105 - 112

The Effect of Propranolol on Secondary Hyperparathyroidism in Uremic Patients*

Sunay Sandıkcı, M.D. / Ünal Yasavul, M.D.*** / Şali Çağlar, M.D.******

Summary

The effect of propranolol on plasma parathyroid hormone (PTH) levels and calcium-phosphate metabolism in 16 uremic patients with an average glomerular filtration rate (GFR) of 22,5 ml/min are reported. An average dose of 80 mg (40-160 mg) propranolol in four separate doses was started. The patients were followed during five months of propranolol therapy. The plasma PTH level (–COOH fragment of PTH) decreased to the 73 percent of pretreatment level in the first and third month of the therapy. Plasma alkaline phosphatase and urinary phosphate levels decreased significantly in all measurements. Three patients had subperiostal bone resorption in radiographic examination and one of them improved in the fifth month of the therapy.

Key Words: Parathormone, Propranolol, Alkaline phosphatase, Phosphate.

Introduction

Secondary hyperparathyroidism is the most important complication of chronic renal failure.[1-3] At least two factors are responsible for its pathogenesis. The first is the hypersecretion of PTH. It is due to phosphate retention. Secondary to GFR reduction is the impaired production of

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1,25 dihydroxycholecalciferol and skeletal resistance to the action of parathyroid hormone resulting reduction in 1,25 and 24,25 dihydroxycholecalciferol.[4, 5] The second is the impaired renal degradation and clearance of PTH. Freitag et al have demostrated that in uremic patients as much as 80 percent of excess PTH (carboxyl terminal) was due to impaired renal clearance and the remaining 20 percent was associated with hypersecretion.

It has been suggested that PTH is a major uremic toxin and many manifestations of uremic syndrome such as renal osteodystrophy, numerous neurological abnormalities, itching, soft tissue calcification, soft tissue necrosis, anemia, hyperlipemia, impotence etc, may be related to the excess PTH.[7-10]

Because of its great importance, secondary hyperparathyroidism should receive medical treatment. Up to now many treatment methods such as diet, binding of intestinal phosphate, oral calcium, calcitonin, active vitamin D metabolites and subtotal parathyroidectomy have been applied. None, however, have been satisfactory.

Several in vivo and in vitro studies have shown that beta-adrenergic agonists increased serum PTH level.[11, 12] This effect is mediated through beta-2 adrenoreceptors and is independent of the effect of calcium.[13] Patients undergoing chronic hemodialysis were investigated for manifestations of secondary hyperparathyroidism retrospectively.[14, 15] In patients receiving propranolol for some reason, serum PTH and alkaline phosphatase levels were lower when compared to others not receving it. Furthermore, there was less radiological evidence of renal osteodystrophy in the group receving propranolol.

This study was undertaken to investigate the effect of propranolol on the serum PTH level and calcium-phosphate metabolism in chronic renal failure.

Metarials and Methods

Sixteen patients (mean age 33 yrs; range 22-52 yrs; 13 males and 3 females) with chronic renal failure were studied. The diagnosis of chronic renal failure was based on the clinical and laboratory findings. GFR's ranged 5-35 ml/min (mean 22,5 ml/min) and mean daily urine volumes were 2300 ml (range 1000-5000 ml).

Six patients had histological diagnosis (3 membranoproliferative glomerulonephritis, 1 chronic pyelonephritis, 1 sclerosing glomerulonephritis, 1 amyloidosis). Patients could not usually date the outset of their disease exactly and none of them described acute beginning or

rapid progress. Eleven patients were hypertensive and drugs given were Adelphan (reserpine + hydralazine) to 3 patients, alphamethyl dopa to 1, indapamide to 1, aluminum hydroxyde to 3 and oral calcium to 2.

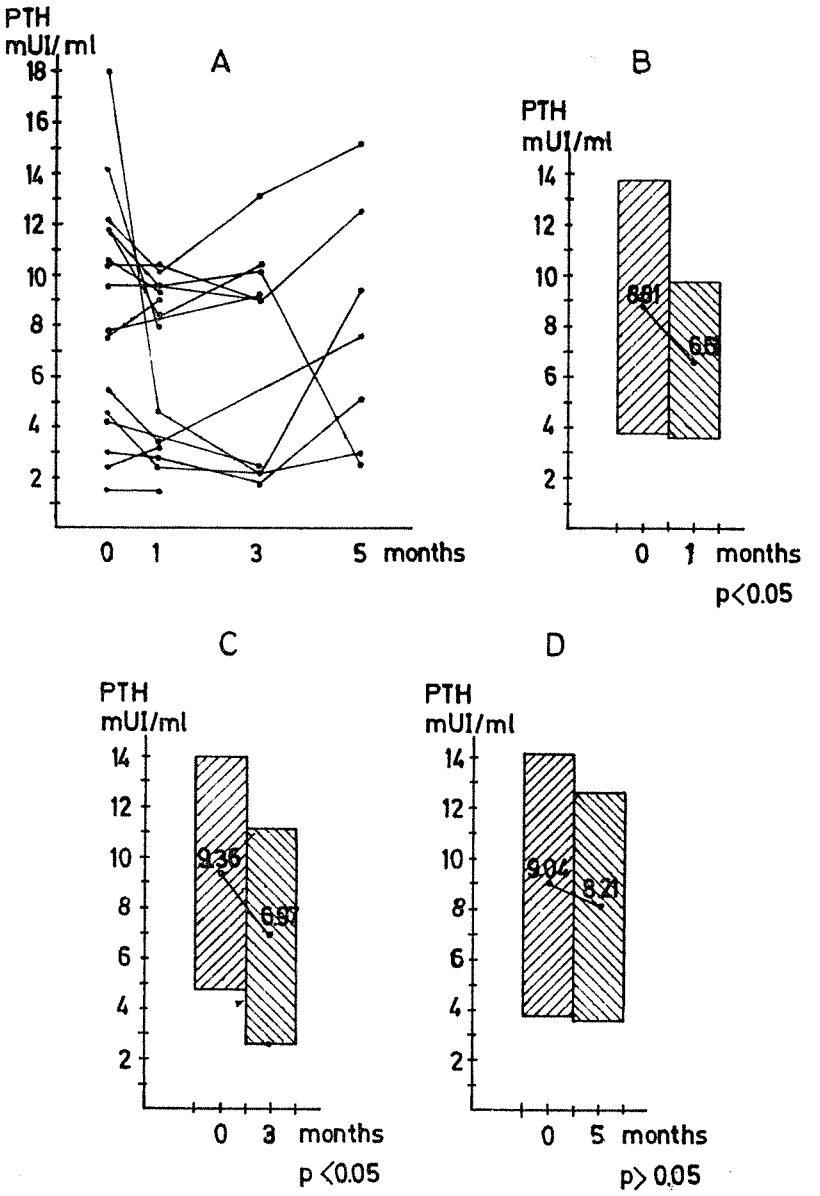

Figure 1

The effect of propranolol on serum PTH level. The PTH level decreased to 73 percent of pretreatment level in the first and third month of propranolol therapy ($p < 0.05$). But, the decrease in the fifth month was not significant. ($p < 0.05$).

Drugs given previously were continued on the same dosage. The patients had no cardiopulmonary contraindications to propranolol administration. An average dose of 80 mg (40-160 mg) propranolol in 4 divided doses was started. Before and during 1, 3, 5 months of propranolol therapy total serum calcium, phosphate, alkaline phosphatese, plasma PTH, urinary calcium and phosphate levels were determined. Plasma PTH levels were measured by radioimmunassay.[16-18] For this assay, the antiserum which had been prepared against the COOH fragment (Institute National des Radio Elements, Belgium) was used. High levels greater than 4.0 mUI/ml of PTH are found in more than 95 percent of the patients with primary and secondary hyperparathyroidism. Other tests were performed by an autoanalyser (SMA-12). Hand and clavicle X-rays taken initially and at the fifth month of the therapy were interupted by the same radiologist simultaneously. Wilcoxon's matched pairs signed ranks test was used to determine the significance of correlations.

Results

Results are shown in figures 1-3. Measurement could be performed on 14 patients in the first, 10 in the third and 7 in the fifth month.

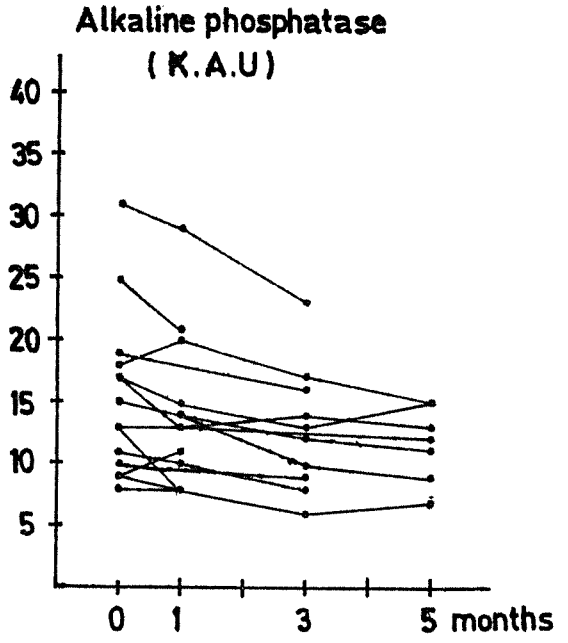

Figure 2

The effect of propranolol on serum alkaline phosphatase level. The average serum A. P level decreased significantly in the first, third and fifth month of therapy.

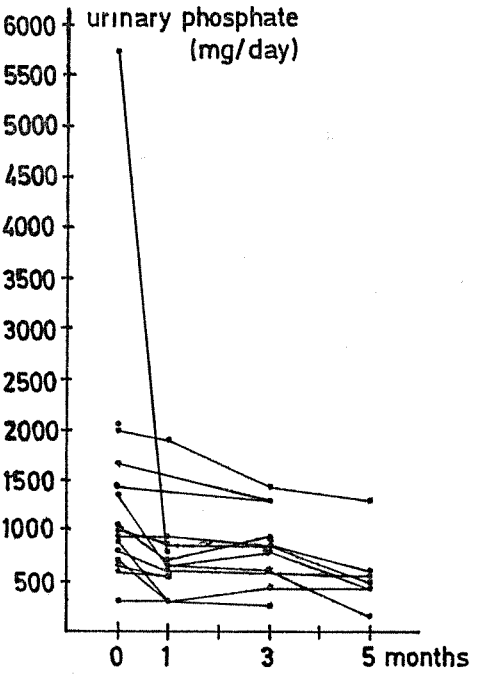

Figure 3

The effect of propranolol on urinary phosphate level. There was significant decrease in the first, third and fifth month of propranolol therapy.

Before treatment with propranolol, 2 subjects showed normal and 14 subjects showed elevated serum PTH levels (range 1,5-18 mUI/ml). Normal PTH level was 2,7 $\pm$ 0,8 mUI/ml. In the first and third month of therapy, the average serum PTH level decreased to 73 percent of pretreatment level (range 1,5-10,4 mUI/ml). This difference was singificant statistically ($P < 0,05$). The decrease in PTH levels was not related to the duration of treatment. No significant changes in serum calcium, phosphate or urinary calcium levels were detected. Alkaline phosphatese and urinary phosphate levels decreased significantly in the first, third and fifth months during the therapy ($P<0,05$). In x-ray studies 3 patients out of 16 showed minimal subperiostal resorption and one of them improved in the fifth month.

Discussion

Fournier[19] in uremics, and Hargis[20] in normals showed that propranolol infusion acutely decreased plasma PTH level. Caro[14] and later Brancacio[15] observed that in patients undergoing chronic hemodialysis, propranolol decreased plasma PTH and alkaline phosphatase levels.

Later, Caro[13] showed that plasma PTH and total calcium levels were significantly decreased following propranolol administration in patients with asymptomatic primary hyperparathyroidism. In this study, the plasma PTH levels decreased significantly after one and three months of propranolol therapy. Although our date are consistent with the previous findings, the degree of supression differs from one patient to another. This variable response may be related to the heterogeneity of GFR and propranolol doses among the patients. However, at the end of the fifth month, there was no significant decrease in PTH levels. This result may be related either to the small number of patients or to the progressive reduction of GFR in 4 patients out of 7. Because of the inverse relationship between GFR and PTH activity, the reduction of GFR may lead to an increse of PTH concentration relatively. Freitag[6] and several investigators have actually confirmed that the high levels of COOH fragments of PTH uremic patients is related mainly to impaired renal degradation and excretion of the hormone.

The marked reduction in plasma alkaline phosphatase and urinary phosphate levels are indirect evidence of PTH reduction and they are due to the decrease of bone resorption and phosphaturic effect. Leme and colleagues[21] have shown that propranolol does not change the response of the target organ to PTH and phosphaturic effect on PTH.

Although there was a decrease in serum PTH, no difference was found in serum total calcium. This result may be related to the following factor: (1) In uremic patients, the relationship between PTH and calcium is not closer than that of PTH and creatinine, therefore serum calcium level is not a good criterion for PTH. (2) Skeletal resistance to the calcemic effect of PTH. (3) Alteration in the response of hyperplasic parathyroid gland to calcium.

In x-ray studies, subperiostal bone resorption has been observed in 3 patients out of 16 (19 %). This ratio is consistent with findings of others.[22, 23] In uremic patients, Kumar et al[22] have found that symptomatic osteodystrophy is about 10-20 %. In Caro's study, the radiological evidence of renal osteodystrophy has been found to be 14/25 but their patients were undergoing chronic hemodialysis. Johnson, and then Tatler, have shown that frequency of renal osteodystrophy increases with prolonged hemodialysis.[24, 25] The following factors could be responsible for the normal roentgenologic findings in our 13 patients.

1. Development of roentgenographic findings take a long time.
2. X-ray is not a sensitive method for revealing bone changes.

According to Ritz[26] bone scanning is superior to x-ray. If we could have used bone scanning sensitive results might have been obtained.

At the end of 5 months, X-ray abnormalities improved in one of the three patients. Madsen,[27] Massry[5] and Ritz[28] have shown that renal osteodystrophy was reversible and could improve partially or completly with 1-alpha- hydroxycholecalciferol or transplantation.

The present study suggests that propranolol may be a new approach to treatment of secondary hyperparathyroidism in renal failure. However, we think further studies are needed for a practical therapeutical approach.

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Hacettepe Medical Journal
Volume 16 / No. 3 / July 1983 / pp. 113 - 124

Effect of Cyproheptadine on Basal and TRH-Stimulated Thyrotropin and Prolactin Release and Serum Thyroid Hormone Levels in Man*

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Summary

The effects of cyproheptadine, an inhibitor of serotonin receptors, on the TRH-induced TSH and PRL release and on the serum levels of thyroid hormones (total T_3 and total T_4) were investigated in eleven healthy volunteers. Cyproheptadine was given orally in a dose of 16 mg per day during 4 consecutive days.

Mean basal and TRH-stimulated serum TSH levels were not changed by pre-treatment with cyproheptadine.

Mean basal serum PRL concentration increased significantly following cyproheptadine; but mean PRL responses to TRH were not significantly different.

Also, the administration of cyproheptadine did not alter serum total T_3 levels, but decreased total T_4 levels.

The increment in basal serum PRL levels with cyproheptadine treatment was attributed to antidopaminergic or perhaps antihistaminic activity and the decrement in serum total T_4 levels was attributed to antiserotoninergic effect of the drug.

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Key Words : Prolactin, Thyrotropin, TRH, Thyroid Hormones, Cyproheptadine.

Introduction

There are a lot of studies dealing with the importance of the serotoninergic mechanisms in the regulation of pituitary hormones secretion. From these studies, which were based on the comparative effects of serotonin agonists and antagonists, rather controversial conclusions have been obtained with regard to thyrotropin (TSH) and prolactin (PRL) secretion.

Recent experiments in laboratory animals have demonstrated that the control of thyrotropin-releasing hormone (TRH) and/or TSH secretion are influenced by neurotransmitter monoamines and particularly by serotonin (5-HT).[1-3] Despite these evidences obtained from animals for a significant role of serotonin in TSH regulation, there are little and controversial evidences for such a role in humans. Woolf and Lee[4] could not demonstrate any effect of oral tryptophan (TP), the initial precursor of serotonin, on either basal or TRH-induced TSH release in healthy subjects. In addition, in patients with carcinoid syndrome and elevated peripheral serotonin concentrations, the thyroid function was normal.[5] MacIndoe and Turkington[6] reported small decreases in TSH levels after i.v. TP, while 5-HTP, the immediate precursor of serotinin, decreased TSH only in patients with high levels.[7] From some experiments in rats and mice, it was suggested that serotonin was an inhibitory transmitter regulating TRH secretion.[8, 9] Therefore, these data rise the question whether serotonin acts as an inhibitor or as a stimulator in the control of TSH secretion.

On the other hand, it was reported that under experimental conditions, serotonin is involved in PRL release in animals both in vitro and in vivo.[10, 11] Moreover, there are increasing evidences that serotoninergic mechanisms may play a role in the neurohormonal control of PRL secretion in man.[6, 12, 17]

In the present study , the effect of cyproheptadine, an inhibitor of serotonin receptors, on the TRH-induced TSH and PRL release was examined to clarify its possible action on the pituitary cells.

The another purpose of the present study was to see whether the treatment with cyproheptadine had any effect on the serum levels of thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4).

Materials and Methods

Four male and seven women, healthy volunteers, aged 22-45, participated in this study. None of them was taking any drug known to influence serum PRL levels and tests of thyroid function.

The TRH stimulation was performed on two separate days; the first TRH stimulation was performed under basal condition and the second test followed pre-treatment with oral cyproheptadine. As it is known TRH stimulates PRL release as well as TSH in man and may be considered as a physiological prolactin-releasing factor (PRF).[18-20]

Since several investigators[21, 22] have not found menstrual cycle differences in the PRL and TSH responses to TRH stimulation, the tests were done regardless the stage of the menstrual cycle in women.

All tests were performed in the morning after an overnight fast. For the first test, 200 μg of synthetic TRH (TRF "Roche") was administered intravenously as a bolus injection at time 0. Venous blood samples were obtained at 0,20 40, 60 and 120 minutes for TSH and PRL assays. Venous blood was also taken for determination of total T_3 and total T_4 at time 0.

Following the first test, cyproheptadine hydrochloride (Sipraktin) was given by mouth in a dose of 4x4 mg per day, during 4 consecutive days to each subject. After the last dose of cyproheptadine, the next morning, TRH stimulation was repeated and blood samples were obtained at corresponding times for TSH, PRL, T_3 and T_4 assays.

The blood samples were centrifuged and the sera were stored at-20° until analyzed. All of the samples from one subject were analyzed in the same assay and all assays were performed in duplicate.

Serum PRL, TSH, total T_3 and total T_4 were assayed with double antibody radioimmunoassay tecniques as previously reported, using "IRE Prolaction Radioimmunoassay Kit", "GammaDab ^{125}I Human TSH Radioimmunoassay Kit", "SPAC T_4" and "SPAC T_3".

The limit of sensitivity in the PRL assay was 34 $\pm$ 5 μg/ml and in the TSH assay was 1.0 μU/ml.

Statistical analyses of results were made by Wilcoxon's matched pairs test. P values more than 0.05 were considered nonsignificant in this study. All data in the text and figures were given as the mean $\pm$ SE.

Results

TSH Responses to TRH: No significant statistical difference in the mean basal TSH levels was found among the two tests: with and without pre-treatment with cyproheptadine.

Also, each subject responded to TRH stimulation with a significant increase in TSH levels in each test. The maximum increments in serum TSH concentrations following the injection of TRH usually occurred at 20 min. but occasionally at 30 min. and returned to baseline levels at 120 min. (Figure 1). The differences between the mean TSH responses during the two tests, were also not significant (Table I).

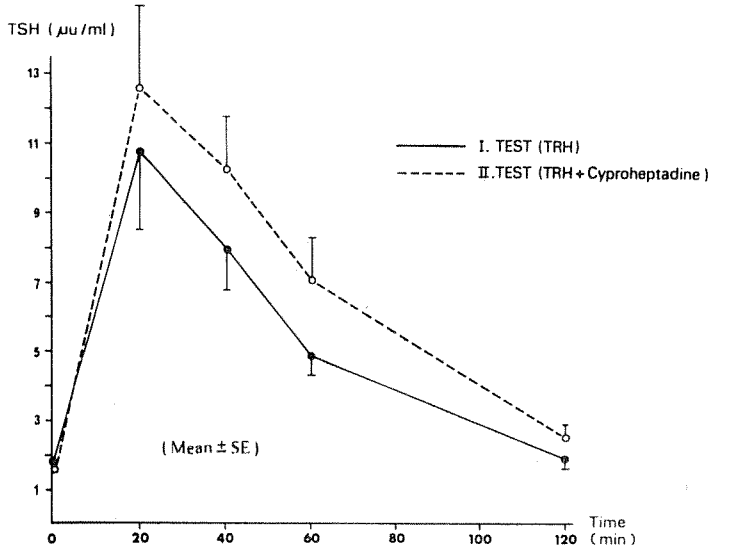

Figure 1

Mean responses of serum TSH to TRH injection. The intravenous administration of 200 μg TRH to 11 healthy volunteers, with and without cyproheptadine treatment, caused significant TSH responses. The differences between the mean basal TSH levels and mean TSH responses during the two tests were not significant.

TABLE I

THE DIFFERENCES BETWEEN THE MEAN TSH AND PRL RESPONSES TO TRH DURING TWO TESTS ($\bar{D} \pm S_{\bar{D}}$)

Time	Serum TSH	Serum PRL
(min.)	I. Test – II. Test	I. Test – II. Test
0	– 0.14 ± 0.26[a]	176.54 ± 73.77[b]
20	1.87 ± 2.27[a]	84.54 ± 115.23[a]
40	2.23 ± 1.42[a]	– 257.27 ± 282.98[a]
60	2.18 ± 1.78[a]	97.27 ± 83.54[a]
120	0.54 ± 0.37[a]	261.36 ± 224.19[a]

a) $p > 0.05$, b) $p < 0.05$

PRL Responses to TRH: The mean basal serum PRL levels in TRH and TRH + Cyproheptadine tests were 278 ± 29 μU/ml and 455 ± 82 μU/ml, respectively. The difference of means was significant ($p < 0.05$) (Table I). But the differences between the mean PRL responses to TRH stimulation in each test were not significantly different. The serum PRL concentrations almost always reached their maximum levels 20 min. after TRH injection (Figure 2) and returned to near the baseline at 120 min. in Test I and at 60 min. in Test II.

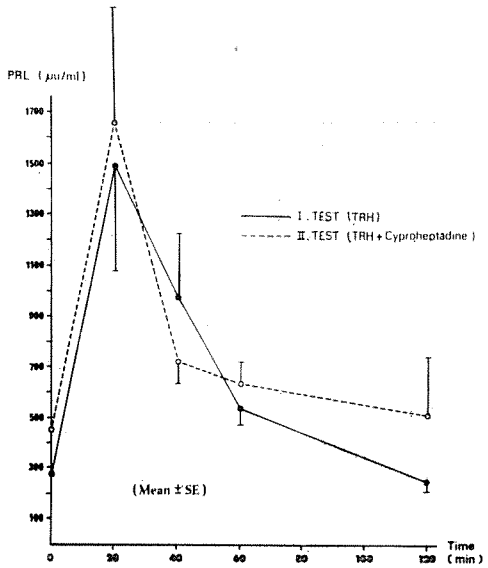

Figure 2

Mean responses of serum PRL to TRH injection. Mean basal PRL concentration was significantly higher after cyproheptadine treatment. The intravenous administration of 200 μ TRH to 11 healthy volunteers with and without cyproheptadine treatment, caused significant and similar PRL responses.

T_3 and T_4 Levels: The mean basal serum total T_3 concentration did not change with cyproheptadine treatment (1.26 ± 0.90 ng/ml and 1.24 ± 0.79 ng/ml, respectively) ($p > 0.05$).

The mean basal serum total T_4 concentration decreased significantly after cyproheptadine administration (9.79 ± 0.53 μg/dl and 9.14 ± 0.55 μg/dl, respectively; $p < 0.05$).

Discussion

From the results of animal experiments it has been proposed that the release of TRH is regulated by a double monoaminergic system, cate-

cholamines as promotors and serotonin as an inhibitor of TRH release.[8, 9] Later investigations refuted this proposal. In rats 5-HTP had no effect on serum TSH.[23] In another study on rats, 5-HTP produced a significant rise in serum TSH levels, whereas parachloroamphetamine (PCA), a depletor of serotonin, decreased it.[2]

In vivo and in vitro experiments in rats made by Jordan et al.[3] demonstrated that serotonin had no direct TSH-releasing effect, but strongly supported a direct action on TRH release. As serotonin is a hypothalamic and mesencephalic neurotransmitter, it seems reasonable that it may act on TRH at the hypothalamic level. However, the evidences obtained from healthy men and from patients with carcinoid syndrome are in contrast to these results.[4–6] Therefore, the effect of serotonin on the regulation of TRH and TSH secretion in man is not yet clear.

In contrast to the incongruities about the interrelationship between serotonin and TSH (or TRH), the stimulatory serotoninergic control of PRL secretion is almost certain. Although there are limited number of studies suggesting the lack of significant effects of serotonin precursors on basal and/or TRH-stimulated PRL release in humans [4, 24–26] the results of these studies are surprising. Because the existence of serotoninergic stimulatory effect on human PRL release was supported by several investigations including studies which showed that serum PRL levels were raised after intravenous or oral administration of serotonin precursors (L-tryptophan and 5-HTP)[7, 13, 17] and that serotonin antagonists strongly suppressed basal PRL concentration[13, 15, 18] sleep-related[14] or breast-emptying PRL release[16] and hyperprolactinemia due to various causes.[27–29] From these studies, serotonin appears to be involved in stimulation of PRL release. The mode of action by which serotonin exerts a stimulatory effect on PRL release is not clear at present. It may inhibit prolactin-inhibiting factor (PIF) or enhance prolactin-releasing factor (PRF). Because its site of action is in the hypothalamus, not in the pituitary. However, because 5-HTP significantly increased serum PRL levels in a patient with isolated TRH deficiency and there was no significant change in plasma TSH levels following 5-HTP administration in normal subjects, it was suggested that serotonin may stimulate PRL secretion by inhibiting dopaminergic neurons (PIF is believed by some investigators to be dopamine[30]) and also by enhancing PRF activity, aside from TRH.[12] Also, Lu and Meites[11] have reported that 5-HTP has no significant effect on the PIF content in the hypothalamus in rats. This possible mechanism was also evidenced by several observations.[31–33]

In this study, we investigated the effect of cyproheptadine, a serotonin receptor blocking agent, on basal and TRH-stimulated TSH and PRL release in man. Cyproheptadine, given orally during 4 consecutive days, did not change the basal and stimulated serum TSH levels. In other two experiments, cyproheptadine also did not modify the basal levels of TSH.[34, 35]

On the other hand, our observation that TRH-induced TSH release failed to show any significant changes following cyproheptadine administration is in accordance with that by Goldstein et al.,[35] but is at variance with the two previous observations[14, 34] in which a significant decrease in TRH-induced TSH concentration occurred. In one of these studies,[34] TRH was administered for 4 consecutive days which might explain the decrement in the TSH response. Haigler et al.,[36] showed that the inter-test interval is important for TSH response to TRH and daily TRH testing blunts the TSH response. But they could not explain the mechanism of the inhibiting action of cyproheptadine and suggested a direct effect of this drug at the pituitary level. Our results are also compatible with that by Woolf and Lee[4] who demonstrated the failure of oral L-TP to affect basal and TRH-stimulated TSH concentration in healthy man.

The present data thus suggest the absence of a direct or antiserotoninergic effect of cyproheptadine on thyrotropes and don't support a conclusive role for serotonin in either basal or TRH-stimulated conditions.

The data obtained from this study, as regards the effect of cyproheptadine on basal PRL release are contrary to the results of Goldstein et al.[35] They found that the basal levels of PRL were significantly lowered by cyproheptadine administration and they explained this with antiserotoninergic effect of the drug.

In the present study, basal PRL secretion significantly increased after cyproheptadine ($p < 0.05$). As it is known, cyproheptadine posseses antidopaminergic, antihistaminic and anticholinergic activity in addition to its definite antiserotonin action.[37-39] The obtained result can not be explained by the antiserotoninergic properties of the drug. It is reasonable to suggest that this effect results from the antidopaminergic effect of cyproheptadine; since the agents blocking dopamine receptors stimulate PRL secretion. Similarly, antihistaminic activity of the drug can explain this result. Since, it has been shown that cimetidine, a blocker of histamine H_2 receptors, stimulates PRL secretion.[40]

In addition, it has been observed that cholinergic drugs can also inhibit PRL release.[41] But, at the dosage employed in our subjects, the

anticholinergic effects of cyproheptadine would be expected to be very slight.

In our study, cyproheptadine treatment did not alter the PRL responses to TRH in healthy subjects. Comparable data have been obtained by others.[4, 14, 35] However, Egge et al.,[34] found a significant increase in TRH-induced PRL concentrations following cyproheptadine. The possible explanation suggested by them was antidopaminergic action of the drug. It has been shown that, TRH stimulates the production of PRL in functional pituitary cells in culture.[20] Failure of cyproheptadine to modify PRL responses to TRH as observed in this study, may be explained with this direct effect of TRH on pituitary lactotropes.

Serotonin has been the subject of numerous studies with respect to its action on iodine metabolism and the thyroid gland. The other aim of the present work was to study the effects of cyproheptadine on serum T_3 and T_4 levels. To our knowledge, antiserotoninergic effect on serum T_3 and T_4 levels has not been investigated until now.

The biogenic amines, serotonin and norepinephrine, have both been found in the thyroid gland of mammals. These are endogenous thyroid constituents and are endogenous physiological mediators of thyroid activity.[42] Actually, the presence of different amine-storing cell systems within the thyroid gland such as adrenergic nerve terminals, mast cells and parafollicular cells suggests that these biogenic amines play a physiological role.[43, 44]

In the action of TSH, the role of serotonin as an intermediate was first proposed by Clayton and Szego.[45] Clayton et al. and others have shown that exogenous and endogenous TSH rapidly mobilizes serotonin from thyroidal storage sites (perivascular and perifollicular mast cells).[45, 48] This effect is a rapid and target organ specific action of TSH and suggests that mast cells and serotonin are involved in thyroid function. In addition, TSH appears to promote the formation of mast cells within the thyroid. The long-term enhancement of TSH secretion is accompanied by an increased number of thyroid mast cells.[44]

Relatively low concentrations of serotonin stimulate the organification of iodine and the formation of iodothyronines in suspensions of isolated thyroid cells.[49-51] This effect of serotonin is not related to the increased vascularity of the thyroid gland that serotonin induces; on the contrary, it is a direct effect on the follicle cells and is mediated by alpha-adrenergic receptors in the follicle cells.[44, 50] In addition, the release of thyroid hormones is stimulated by serotonin. The effects of cate-

cholamines and serotonin on the in vivo endocytosis of thyroglobulin and the release of thyroid hormones were studied in mice and in rats[44, 52, 53] and was concluded that several aromatic monoamines can stimulate the endocytosis of thyroglobulin and the release of thyroid hormones. Similarly, this thyroid hormone-releasing effect does not result from intra-or extra-thyroidal vascular changes but is a consequence of a direct effect on the follicle cells and is mediated by follicular cell receptors of alpha adrenergic type.

In the present study, we found that the mean serum T_4 level decreased significantly after cyproheptadine treatment but the mean serum T_3 concentration did not alter. Depending on the present results, we propose that antiserotoninergic agents don't alter serum total T_3 level in man. As it is known, T_3 is the active thyroid hormone and most of the circulating T_3 is produced by monodeiodination of T_4 in peripheral tissues.[54] Thus, T_4 can be considered as a prohormone.

It is logical to speculate that, antiserotoninergic agent cyproheptadine decreases thyroidal T_4 synthesis and/or release but the more potent thyroid hormone, T_3, level remains unchanged probably as a result of increasing peripheral conversion from T_4.

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Volume 16 / No. 3 / July 1983 / pp. 125 - 129

Evaluation of Response to Stress with Plasma Cortisol Levels*

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Summary

It has been stated that various stresses lead to increased secretion of ACTH and plasma cortisol levels are indicators of this effect.

In this study patients who underwent elective surgery in different wards of the Faculty of Medicine of Cumhuriyet University were divided into two groups. These groups were divided according to the anesthesia received; either local or general anesthesia. The preoperative and postoperative blood cortisol levels were measured in 20 petients in each group. All of the patients received the same anesthetic agent and the blood samples were obtained at the same time of the day.

The postoperative blood cortisol levels were found elevated in both groups. The cortisol levels in the postoperative period were found to be higher in the group which received general anesthesia. Thus, it has been concluded that the stress due to general anesthesia is more intense than that of local anesthesia.

Key Words: Stress, Plasma cortisol.

Introduction

Stress is a psychological state which manifests itself through a series of symptoms. There are three main phases following one another in the organism suffering from stress. This condition is called "general adaptation syndrome" and it consists of three stages, (a) alarm reaction, (b) re-

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sistance phase and (c) the stage of collapse. If accomodation is secured, the second phase begins. This stage can be prolonged by administering drugs such as steroids.[1, 2]

The changes in the blood cortisol levels in response to stress have been studied extensively. The effects of emotional stress, anesthetic agents and various surgical procedures on blood cortisol levels have been investigated.[3–7]

In this clinical study standardized local and general anesthesia were applied and changes in the blood cortisol levels were measured.

Materials and Method

This research was carried out with the patients to be operated on at the clinics of the Faculty of Medicine of the Cumhuriyet University and who were not suffering any particular disease which would directly affect the ACTH and cortisol levels. The patients ages ranged from 18 to 50 years. They were divided into two groups based on the type of anesthesia they received, local or general.

Those who received general anesthesia constituted the first group:

Halothane pentothal, oxygen and nitrous oxide were used for general anesthesia in all patients with 100 mgr. of Dolantin, 10 mgr. of Diazepam and 0.5 mgr of Atropine as premedication.

The operative period ranged from one to two hours in all patients.

The blood samples for control studies were taken a day before the operation between 10 and II p.m and within thirty minutes after the operation.

Those who received local anesthesia constituted the second group. Citanest was used for local anesthesia with the same premedication as in the first group for all patients.

The duration of operations was from 30 to 90 minutes and the blood samples were obtained in the same way as in grup I. 10 cc. blood samples were centrifuged at 3500 rpm and preserved at –20 degrees C until all the samples were obtained. Later the blood cortisol levels were measured by the radioimmunoassay (RIA) method.* The cortisol level was measured twice in each sample.

Results

Twenty patients were selected for each group. There were 11 male and 9 female patients in the first group. The mean age was 34 and the

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main surgical operations performed were laminectomy, hernioraphy, thyroidectomy and cholecystectomy.

In this group the preoperative cortisol levels varied between 3.7 and 16.0 micro gm %. The mean level was found as 6.7 ∓0.6 micro gm %. The postoperative levels ranged between 8.2 and 30.0 micro gm % and the mean level was 18.7 ∓ 1.6 micro gm % ($p < 0.01$).

In the second group there were 20 patients. 14 of them were male and 6 of them were female. The mean age was 28 and the main surgical operations performed were nasal polyp excision, tonsillectomy, nasal septal deviation repair and cataract extraction.

In this group the preoperative blood cortisol levels ranged between 4.1 and 9.7 micro gm %. The mean level was 6.5 ∓ 0.4 micro gm %. The postoperative levels ranged between 5.6-27.0 micro gm % and the mean level was 14.4 ∓ 1.4 micro gm % ($p < 0.01$).

According to the results obtained above there was no significant difference between the preoperative blood cortisol levels of both groups ($p > 0.05$). However, in those who were subjected to general anesthesia, the postoperative cortisol values were quite higher than those of the second group ($p < 0.05$).

Discussion

In the body, the increasing ACTH and cortisol levels are the most important hormonal and metabolic changes caused by various emotional and physical stresses. Like ACTH, cortisol has a diurnal rhythm. In the morning it reaches its highest level, and at midnight it drops to its lowest level. Cortisol exists in three forms in the blood:

a) bound to transcortine,

b) bound to albumine and,

c) free cortisol.

Transcortine bound cortisol constitutes 75-80 % of total cortisol, albumine bound cortisol forms about 15 % and free cortisol forms 10 %. The half life of cortisol is 90 minutes. The secretion of cortisol begins two minutes after ACTH stimulation.

Plasma cortisol levels have been measured by many researchers to establish the effects of the various stresses and similar results have been obtained. The results have shown that plasma cortisol levels increased after trauma.

In a study, Davis and associates[4] showed that emotional stresses increased plasma cortisol levels. Likewise Bliss and associates[5] also show-

ed that emotional stresses resulted in continous but mild cortisol secretion which did not exceed the physiological limits.

After some time it was proved that the effect of emotional stress on plasma cortisol levels could be reduced by using sedatives. In 1970 it was stated that halothane, pentothal, nitrous oxide and oxygen have not supressed the adrenocorticol response and therefore these agents were used for anesthesia.

Hamanaka and associates[3] found that in 33 patients from different surgical clinics, the cortisol levels were 7.04 $\mp$ 1.77 micro gm % preoperatively and 14.01 $\mp$ 1.46 micro gm % postoperatively. Our results were similar to these findings. Moreover Hamanaka and associates[3] did not find any difference between the two groups consisting of 24 patients who underwent abdominal or thoracic surgical operations.

Lines and associates[7] compared the neurosurgical patients with the general surgical patients and found the blood cortisol levels elevated postoperatively in both groups.

There have been a few reports in the literature that standardized blood cortisol levels were measured after different operations concerning different systems and with various anesthetic agents.

We could not find any reports about comparing the effects of local and general anesthesia on blood cortisol levels in the last 20 years.

This research endeavoured to see that the mean ages of the patients were more or less the same and ensured that the anesthetic agents used were identical. Likewise the blood samples were obtained at the same time of the day and there was no significant difference in the duration of the surgical procedure.

By providing the conditions above, we carried out this research and found that the blood cortisol levels in the preoperative period were almost the same in both groups ($p > 0.05$).

Hence we came to the conclusion that our findings were the same as the result of others and stresses of the operations raised blood cortisol levels postoperatively.

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Clinical Analysis of Tuberous Sclerosis

A Case Report and Review of the Literature

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Summary

Tuberous sclerosis (Bourneville's Disease,Epiloia), is an uncommon neurocutaneous syndrome of autosomal inheritance. The classical features of this disorder are seizures, mental deficiency, and skin lesions including adenoma sebaceum, shagreen patches, and periungual fibromas. In this report, a case of tuberous sclerosis is presented and pertinent literature is reviewed.

Key Words: Tuberous sclerosis, Adenoma sebaceum, Shagreen patch.

Introduction

Tuberous sclerosis is a relatively uncommon disease of irregular but dominant autosomal inheritance occurring with an incidence of about 7 per 100,000. Tuberous sclerosis is classified as a neurocutaneous syndrome in which the brain, eyes, skin, heart, kidneys, lungs and bones may be affected. The full entity classically is defined by a triad of seizures, mental deficiency, and a variety of pathognomonic skin lesions. The latter include; adenoma sebaceum, shagreen patches, periungual and gingival fibromas and hypopigmented macules.[1]

Case Report

A 37 year-old male patient was referred to our Clinic with multiple cutaneous lesions located on his face. His medical history disclosed the

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first appearance of the facial skin lesions after his third birthday. Since then he had been hospitalized for several occasions and received medical treatments for the skin lesions. His family history was negative for similar lesions. On physical examination the patient was in good condition. His blood pressure was 110/80 mm.Hg. and his pulse rate was 78 beats per minute and regular. There were no abnormal neurological findings. The liver and spleen were not palpable. Ophtalmological examination revealed a retinal calcified hemartomatous glial tumor in one eye. Skin examination disclosed 1 to 4 mm dome-shaped fibromas with a smooth surface, pink to red in color, located in the nasolabial folds, cheeks, chin, around the finger nails, toes and on the gums (Figure 1, 2, 3). On the lumbosacral area, there were shagreen patches, seen as yellowish-orange plaques measuring 2 to 10 cm. (Figure 4).

The results of the following laboratory examinations were all normal or negative: Complete blood cell counts, serum electrolyte levels, urinalysis, erythrocyte sedimentation rate, blood urea nitrogen, total plasma proteins, fasting glucose levels, liver function tests, serologic tests for syphilis and chest-X-rays. The radiological surveys of the bones of the hands disclosed cystic-appearing lesions (Figure 5). Skull X-rays revealed multiple intracranial calcifications (Figure 6). Intravenous pyelography (IVP) disclosed an enlarged renal shadow and renal cortex distortions (Figure 7).

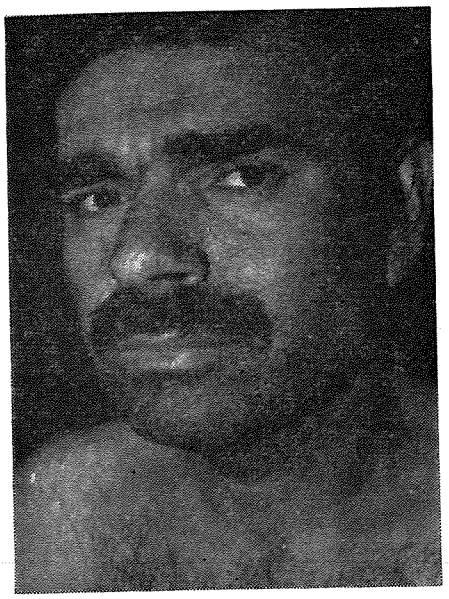

Figure 1
Adenoma sebaceum.

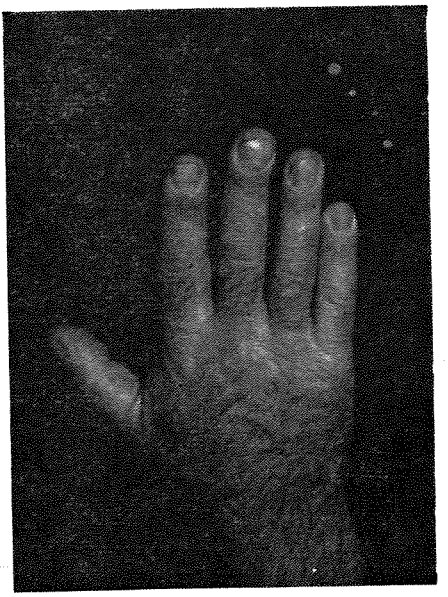

Figure 2
Koenen's tumor.

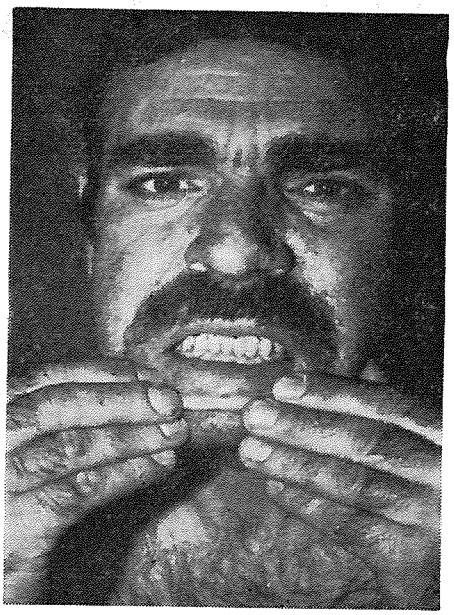

Figure 3
Fibromatous involvement of the gums.

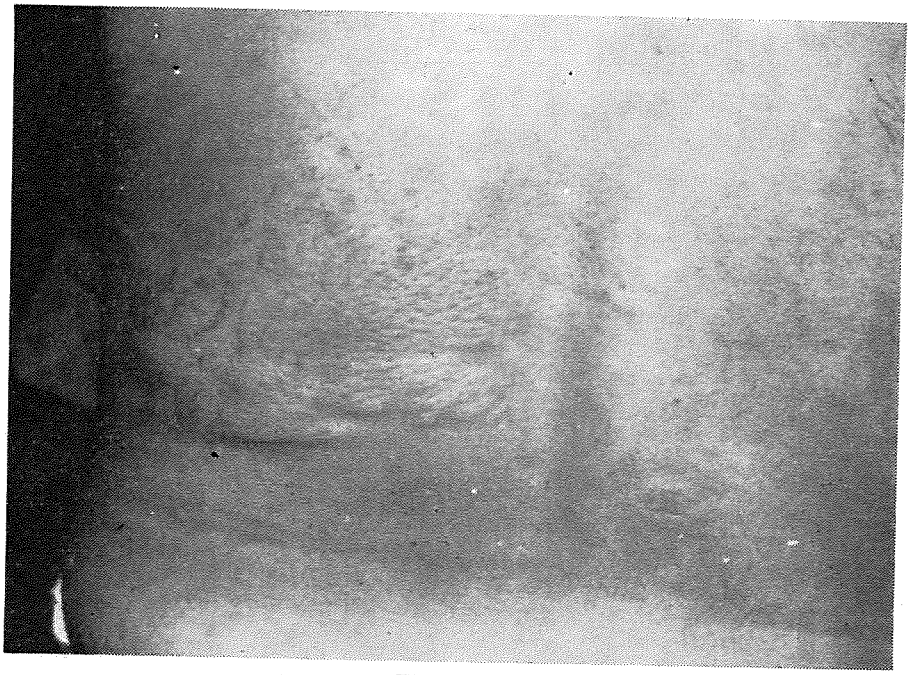

Figure 4
Shagreen patches.

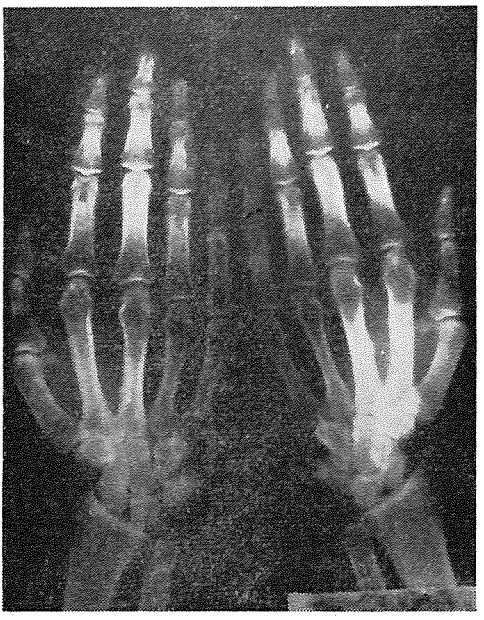

Figure 5
The cystic-appearing bone lesions seen in the X-rays of the hands.

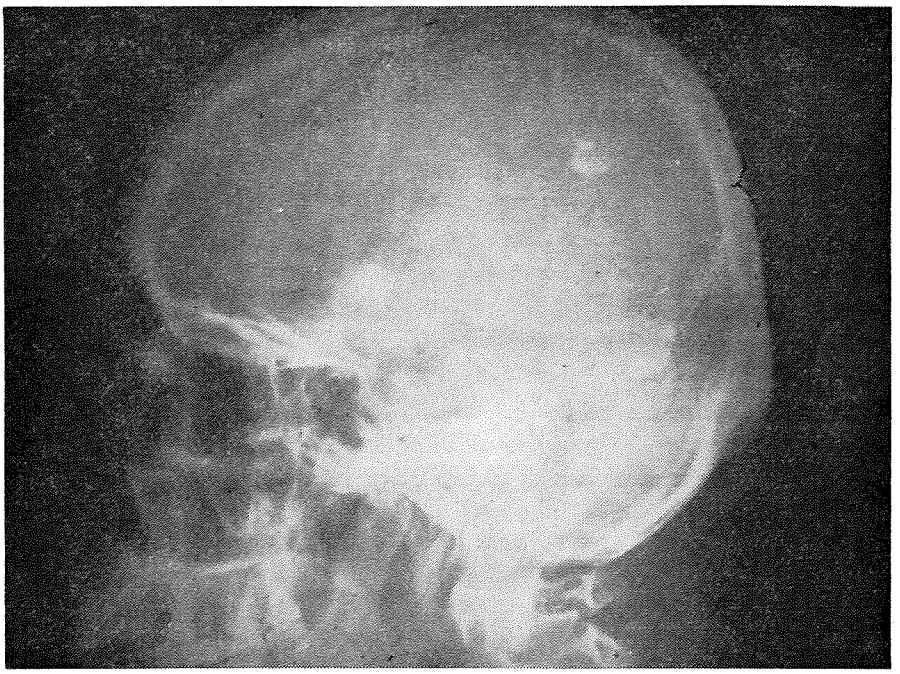

Figure 6
Skull X-ray shows sclerotic calcifications in the brain.

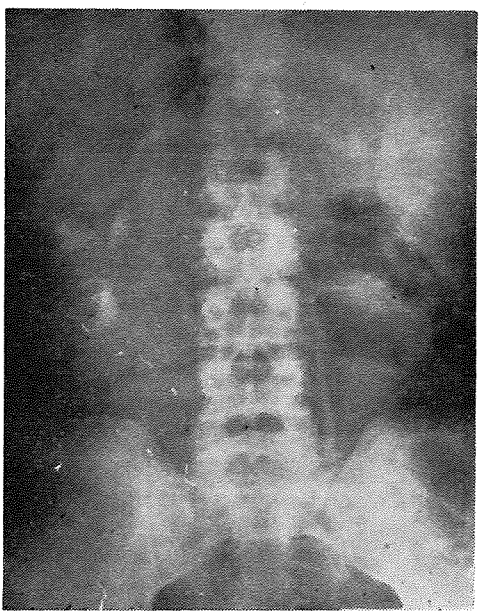

Figure 7
IVP shows an enlarged renal shadow and renal cortex distortions.

Since the patient had no history of seizures, no anticonvulsant therapy was given. The skin lesions required no treatment but for cosmetic reasons the fibromas were removed by electrodissiccation and curettage and a good result was achieved.

Discussion

Tuberous sclerosis is a genetic disease and its pathogenesis remains unknown. Although Virchow had noticed the scleromas of the brain in the 1860s, it was Bourneville who systematically presented the disease in his articles between 1880 and 1900. In 1885, Balzer and Menetrier described these lesions in detail. Later, Vogt put stressed the significance of neurocutaneous lesions and described the classic triad of adenoma sebaceum, epilepsy, and mental retardation. Morolton, conceives of the abnormality as a disturbance at an embrioyologic level of cellular differentiation, dependent on the relationship between cell competence for specialization and the amount of an organizer substance which normally provides the inductive stimulus for a differentiation.[2] How the trait underlying this disease is genetically transmitted remains a mystery. The disease may be present at birth, but more often within 2 to 3 years, focal or generalized seizures or retarded psychomotor development occurs and draw attention.[3] The characteristic tuberosc-

lerotic nodules of glial proliferation may occur anywhere in the cerebral cortex, basal ganglia and ventricular walls but are rare in the cerebellum, medulla or cord. Gliomas develop in the striothalamic region. The facial cutaneous lesions, the so-called adenoma sebaceum, appear usually between the fourth and tenth years and thereafter progress. In our case, these fibromatous skin changes appeared at the age of three. Although called adenoma sebaceum, these tumours are actually angiofibromas.[4] Typically, they are red to pink nodules located in the nasolabial folds, cheek, chin, and sometimes the forehead and the scalp. They are rarely found on the upper lip except for the central area, immediately below the nose. Since only 13 percent of children with tuberous sclerosis develop the facial lesions of adenoma sebaceum during the first year of life, it appears that this is not the best early marker of this condition.

A characteristic lesion that appears on the trunk is the shagreen patch found mostly in the lumbosacral region. These lesions are seen in 21 to 83 percent of patients with tuberous sclerosis. They develop during early childhood, usually between the second and fifth birthdays. The shagreen patch appears most often as a flat, slightly elevated area of skin, with an "orange peel" appearance. Such areas are in fact, plaques of subepidermal fibrosis.[5]

Another common site of fibromatous involvement is the subungual and periungual regions, and the gums. The periungual fibromas are also called "Koenen's tumors". These lesions are seen in about 50 percent of patients and appear at puberty as firm, flesh-coloured growths. These were prominent in our case. White macules are seen in 70 to 80 percent of patients with tuberous sclerosis. The cause of this pigmentary disturbance is unknown. These lesions are the earliest marker of this disease. They appear at birth, and persist throughout life. They are easily detectable by examination under Wood's light. The white spots range in size from 0,4 to 7 cm. or more. They are located over the abdomen, back, and anterior and lateral surfaces of the arms and legs. They are usually oval or semioval in appearance. Although ash-leaf-shaped hypopigmented macules have been described as the characteristic lesions of tuberous sclerosis, it has been noted that only 18 percent of the white spots are truly ash-leaf in shape.[6] There appears to be an increased incidence of tuberous sclerosis in patients with partial albinism. The presence of one or more tufts of white hair in an infant with seizures is suggestive of a diagnosis of tuberous sclerosis. Other skin changes include; soft fibromas, cafe-au-lait spots and portwine hemangiomas. Recent studies suggest that tooth-pits (seen as punctate, round or oval, 1 to 2 mm. randomly arranged enamel defects) are of diagnostic importance.[7] The

systemic lesions of tuberous sclerosis may produce severe symptoms and possibly death. Central nervous system involvement may lead to convulsions and mental retardation. Retardation may be mild or severe and appears in 62 percent of affected individuals and seizures are present in more than 80 percent of cases.[8] Our patient, did not have any convulsions and we found no sign of mental retardation. Sclerotic calcification in the brain is visible as "tubers" by X-ray in approximately 50 to 75 percent of individuals. In our patient, the skull X-rays were diagnostic. When diagnosis of tuberous sclerosis cannot be established by clinical findings in a young child, a computer-assisted cranial tomographic scan (CAT) can be utilized as an early helpful diagnostic procedure.[9] The kidneys may reveal tumors that are hamartomas.[10] These tumors are usually multiple subcapsular and benign. They are frequently asymptomatic. In our case, IVP showed enlarged renal shadow and a distorted renal cortex.

The eyes may have characteristics of retinal gliomas.[11] These lesions are seen as white streaks along the vessels or as rounded tumours near the disc. These are consistent with those of our case. Rhabdomyomas in the heart may be associated with congestive heart failure, murmurs, cyanosis or sudden death. Congenital rhabdomyoma is an abnormal and premature differentiation of embryogenic myocardium into atypical Purkinje cells. Cystic lesions in the lungs may rupture and produce spontaneous pneumothorax, often with a radiographic "honey-combed" appearance.[12] In our patient, X-ray films of the chest revealed no pulmonary deformities. The bones, particularly those of the hands and feet may demonstrate the presence of cysts and periostal thickenings.[13] In our case, there was evidence of cystic-appearing bone lesions in the radiological surveys of the bones of the hands. A variety of developmental abnormalities has been reported in a few cases of tuberous sclerosis. Patients with tuberous sclerosis show abnormal electro-encephalographic findings. Seizure disorders associated with tuberous sclerosis often respond to anticonvulsant therapy. Adenoma sebaceum requires no treatment but many patients benefit by cryosurgery, electrodissiccation and curettage or dermabrasion. Although surgery may be required for relief of symptoms from internal tumours of tuberous sclerosis, surgical removal is often unsatisfactory.

In general, the disease progresses so slowly that years must elapse before one is sure of the advancing course. Approximately 30 percent of the several cases die before the fifth year and 50 to 75 percent before attaining adult age. Death may result from status epilepticus, pulmonary or renal insufficiency or cardiac failure.

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Hacettepe Medical Journal
Volume 16 / No. 3 / July 1983 / pp. 138 - 145

Myelofibrosis with Granulocytic Sarcoma*

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Summary

A case of granulocytic sarcoma in a patient with agnogenic myeloid metaplasia is presented. This finding suggests that in myelofibrosis, soft tissue lesions resembling an infectious process should be investigated including a biopsy, imprints and cultures. In the case of granulocytic sarcoma, among different therapeutic modalities, radiotherapy may be more beneficial than chemotherapy.

Key Words: Myeloid metaplasia, Myelofibrosis, Granulocytic sarcoma.

Introduction

Granulocytic sarcoma is a tumor which consists of immature myeloid cells. Originally, these tumors were called "chloroma" because of their characteristic green color. However, there are also certain nonpigmented tumors infiltrated by immature myeloid cells and it has been suggested recently that these tumors be called myeloblastoma or "granulocytic sarcoma".

Granulocytic sarcomas may be seen in any site of the body.[1-5] Bones are frequently involved by tumors, especially the vertebrae, ster-

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num, orbit, and cranium.[1, 3, 6] In autopsy series tumor nodules have been frequently found in the dura, arms, lymph nodes and kidneys.[6] A high frequency of ocular granulocytic sarcoma (chloroma) with acute myelomonocytic leukemia has been reported in Turkish children.[7] Granulocytic sarcomas involving the eye and orbit also appear to be common in Ugandan children.[8] However, today these tumors are rarely seen in western countries.[9]

It was generally believed that granulocytic tumors developed in myelogenous type leukemia, especially in acute forms.[6, 7] However, in a series of 478 cases, Muss and Moloney reported that the incidence of granulocytic tumors in chronic myelogenous leukemia (CML) were twice as high than in acute myelogenous leukemia (AML).[10]

Granulocytic sarcoma may occur in patients without peripheral blood or bone marrow evidence of leukemia. Leukemia may evolve in these patients 2 to 24 months after the diagnosis of granulocytic sarcoma.[4, 5, 11, 12] The diagnosis may be particularly difficult when the granulocytic sarcoma is observed before peripheral blood or bone marrow abnormalities are recognizable and when it is not green. However, a recent report by Mc Carty et al. showed that light microscopic histochemical studies can facilitate the diagnosis of granulocytic sarcoma or chloroma in the absence of hematologic manifestations of leukemia.[13]

Granulacytic sarcoma is usually associated with leukemia, but it is rarely found together with myelofibrozis. Here, we have reported a case of myelofibrozis with granulocytic sarcoma.

Case Report

A fifty one year old man was admitted to Hacettepe University Hospital because of weakness and a mass on the left side of his neck in October 1980. He had been well until five months previously, when he began to complain of increasing weakness. Three weeks before admission, he noticed a rapidly enlarging mass on his neck.

The patient's temperature was 38°C, the pulse 114 per minute, the respiration 18 per minute, and the BP was 140/75 mm Hg. On physical examination the patient appeared pale and chronically ill. There was a medium sized, tender and firm mass on the left side of his neck and it was warm on palpation. The lungs were clear and the heart was normal. The abdomen was soft, the liver edge descended 9,5 cm below the right costal margin. The spleen was felt 11 cm below the costal margin. There was no peripheral edema. There were few ecchymoses on extremities. The patient was given penicillin intravenously to counteract the possibility of any deep neck infection.

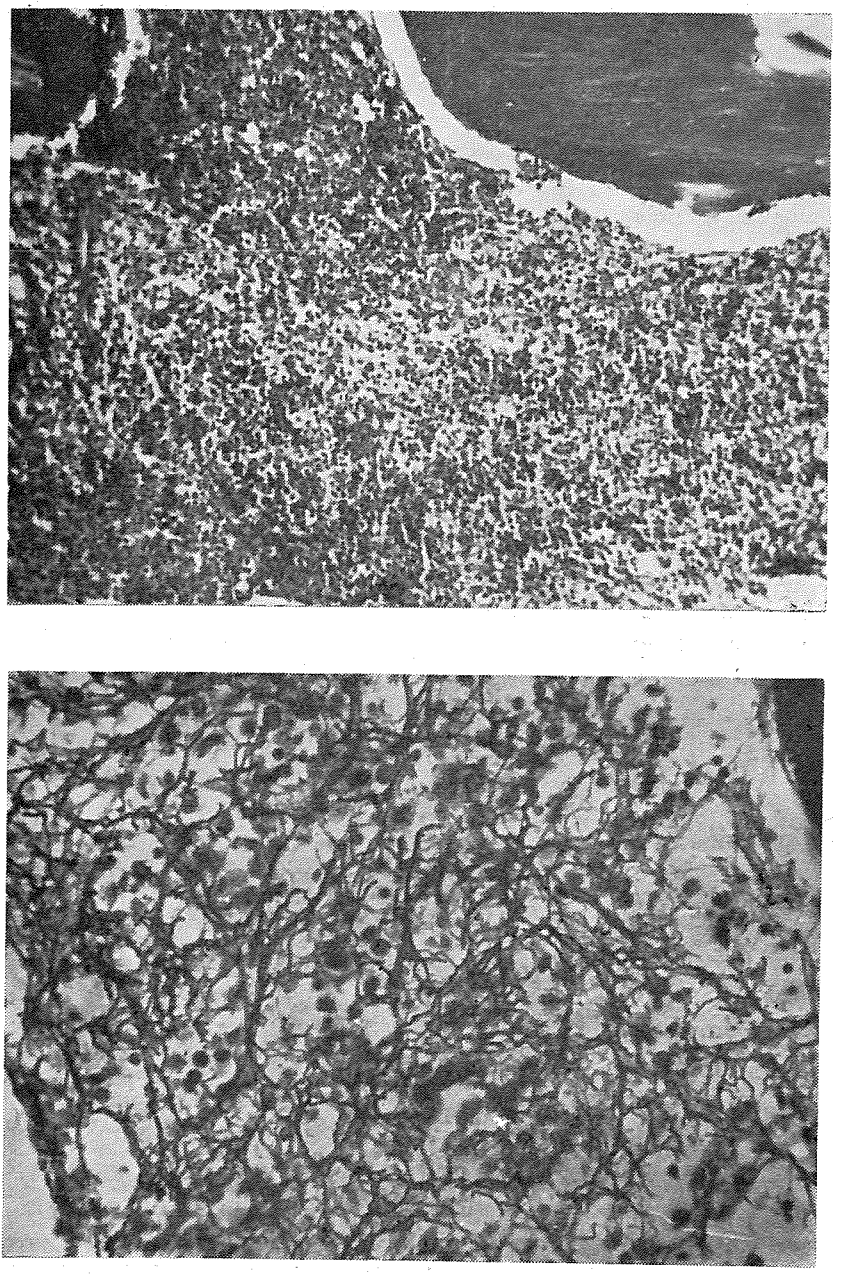

Figure 1

Hypercellular bone marrow (A) showing increased reticulin fibers (B) and pleomorphic cellular composition with numerous megakaryocytic figures.

A : H+E X 150

B : Gomori's silver impregnation X 600.

Laboratory findings revealed that the hemoglobin was 4,7 gm %; the white cell count was 49,800/mm3, with 12 % blasts forms, 14 % myelocytes, 2 % promyelocytes, 10 % band forms, 28 % neutrophils, 2 % basophils, 2 % monocytes and 1 % eosinophil. The platelet count was 50.000/mm^3, and the reticulocyte count 10,5 %, the peripheral blood smear showed marked anisocytosis, poikilocytosis, tear-drop cells and 4 % normoblasts, per 100 WBC.

During the course of his disease, hemoglobin values varied between 4,7 gm % and 7,7 gm %, white cell counts were in the range of 9200 to 35,200/mm^3, with similar differential counts as described. Two bone marrow aspirations were not satisfactory. Bone marrow biopsies showed the characteristic findings of myelofibrosis (Figure 1). Philadelphia chromosome was not found in the sample obtained from the marrow. Leukocyte alkaline phosphatase was 6.5 U/10^8 leukocytes/hour. (N: 50-150 u/10^8 leukocytes/hour). The prothrombin time was 16 seconds, with a control of 13 seconds. The urea nitrogen was 11 mg/100 ml., the glucose 94 mg %; uric acid 6,3 mg %, the bilirubin 1,4 mg %, the calcium 8,0 mg %, the phosphorus 3,3 gm % and the protein 6,2 gm % (the albumin 3,3 gm % and the globulin 2,9 gm %). The SGOT was 28 U/ml, the alkaline phosphatase 7,0 K. A. units. An electrocardiogram

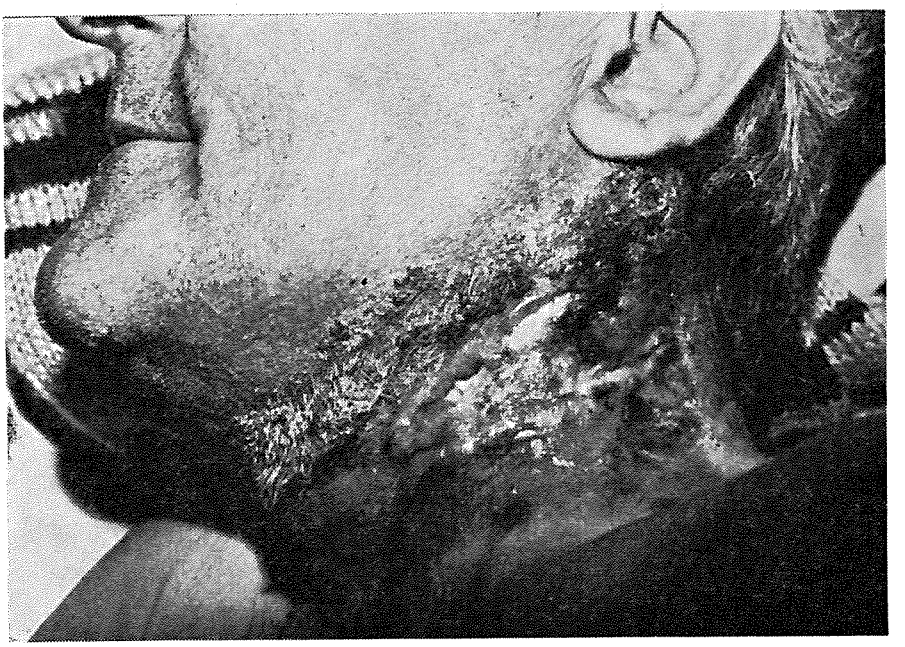

Figure 2
Lesion on the neck showing ulceration and local extension.

and an x-ray film of the chest were normal. The urine analysis was normal.

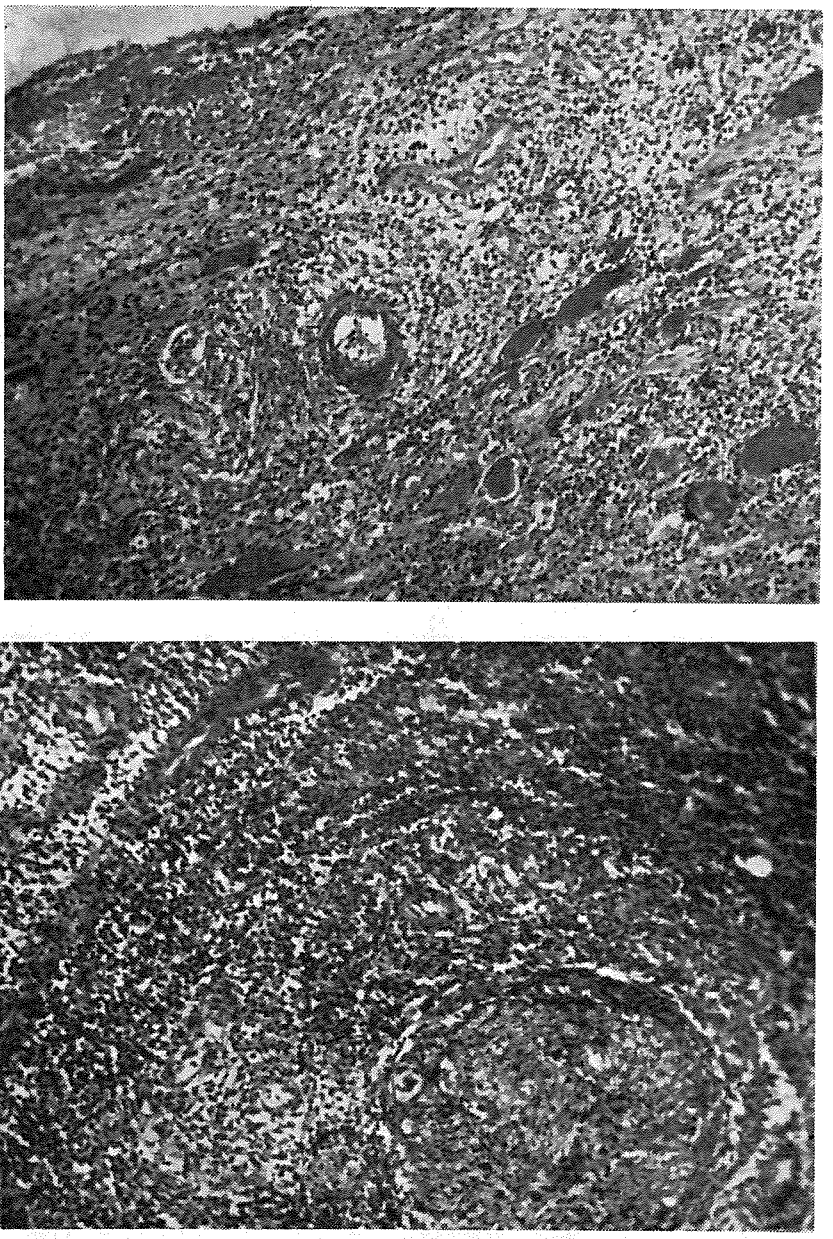

Figure 3

Sections of the tumoral mass in the neck showing infiltration of the skeletal muscle (A) and peripheral nerve (B) by uniform, round undifferentiated cells. H+E X 150.

The neck mass gradually became softer and finally drained. Cultures from the purulent material disclosed staphylococcus coagulase-positive organisms. Although local treatments and antibiotics were given, no significant improvement of the lesion was observed (Figure 2). A biopsy taken from this ulcerative lesion showed infiltration of the skeletal muscles, peripheral nerves and fibroadipose tissues by immature mononuclear cells (Figure 3). Numerous eosinophilic myelocytes were present within the infiltrate as demonstrated by the Giemsa stain. A Naphthol AS-D chloroacetate esterase stain confirmed the myelocytic origin of these cells. These findings were compatible with the diagnosis of granulocytic leukemia. The ulceration disappeared after 2000 rads were given to the involved region. He was sent home without further treatments. Later, on the right hand, a swelling of the first phalanx of the third finger was observed. The bone x-rays were normal. Shortly after, at the site of the previous bone marrow aspiration on the sternum, a tender, warm and erythematous mass was noticed. Needle aspiration from this fluctuating mass showed degenerating neutrophils and the cultures failed to grow any bacteria or fungi. The sternal mass gradually worsened with necrosis and deep ulceration in spite of intensive local treatments and systemic antibiotics. These two new lesions were also considered granulocytic sarcoma and he was given a course of oral busulphan (Myleran) 6 mg per day for 4 weeks. The lesions and his general status progressively became worse. His anemia did not respond to androgens and he continued to require very frequent packed red cell transfusions. The patient died at home in February 1981, an autopsy was not performed.

Discussion

The diagnosis of primary or idiopathic myelofibrosis in the patient described here was made on the basis of hepatosplenomegaly, anemia with teardrop forms, leukocytosis with shift to the left and fibrosis of the marrow. Two bone marrow biopsies which were done at different sites of the iliac crest revealed myelofibrosis. In our patient no evidence of other diseases such as tuberculosis or metastatic cancer which could cause myelofibrosis was detected. Myelofibrosis may also occur during the late phase of chronic myelocytic leukemia, and/or polycythemia vera and it is usually attributed to chemotherapeutic agents.[14–16] Diffuse fibrosis in the bone marrow can be rarely seen in patients with CML at the initial phase of their disease.[15] Our patient's myelofibrosis appeared to be primary and was not preceeded by any of the disorders mentioned previously.

Among the patients with agnogenic myeloid metaplasia, to our knowledge, there is only one case reported with "granulocytic sarcoma"[18] The patient described by Cehreli, et al. had agnogenic myeloid metaplasia with immature myeloid cell infiltration of the lymph nodes simulating malignant lymphoma. In contrast, our patient's granulocytic sarcoma was found in the soft tissue on the neck. Furthermore, he also had two different sites with similar involvements.

In myelofibrosis, myeloid metaplasia may develop in various tissues and organs.[19-21] However, development of "granulocytic sarcoma" is quite rare. The nature of the process by which granulocytic sarcoma develops in myelofibrosis remains obscure. Although the presence of immature myeloid elements in the lesion reminded us of the possibility of a metaplasia secondary to myelofibrosis, the absence of megakaryocytes and erythroid precursors, the presence of immature eosinophilic cells, and uniform chloroacetate esterase positivity of the infiltrating cells are all consistent with the diagnosis of granulocytic sarcoma. In this patient, the primary event was considered infiltration of soft tissues with immature myeloid elements, and it seems to occur in only a very small percentage of patients with myelofibrosis.

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A QUARTERLY PUBLICATION **VOLUME 16 / NO. 4 / OCTOBER 1983**

PUBLISHED BY HACETTEPE UNIVERSITY PRESS

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Printed by
Hacettepe University Press
Printing Division

VOLUME 16 / NO. 4 / OCTOBER 1983

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VOLUME 16 / NO. 4 / OCTOBER 1983

Editorial

Responsibilities of Medical Schools and Society

Doğan Taner, M.D.*

Universities have as their primary mission the generation and transmission of knowledge. Medical schools, as component parts of universities, have as their primary mission the educating of students to become physicians and the generation of biomedical knowledge.[1] Providing service for the society is the third function of medical schools. This third function can be described as the medical schools' most vital social responsibility.

These tasks of teaching, research and providing health service for the community are all equally important. But the extent of these tasks and the amount of time devoted to them are still matters of discussion. The most important question is to what extent these three activities should be interwoven in the health care system of a community without compromising a medical school's primary purpose.

The traditionally accepted aims of medical schools were teaching and generation of biomedical knowledge. But changing social problems related to health are forcing medical schools to recommend solutions and actively participate in the health care system of a society. In response to social changes and needs, medical schools are trying to adjust their educational programs. Undoubtedly it is very difficult for a medical school to balance the tasks of teaching, research and providing health service for the society. One could come up with very convincing arguments why priority should be given to any one of these three tasks with equal force.

Historically universities were regarded as ivory towers committed to teaching and research and insulated from the social needs of the community. In time, social changes, social needs and economic difficulties

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forced the universities to redefine their functions and aims. Probably the social pressures and changes in society influenced the medical schools more than the other disciplines.

Society at large with all its various institutions continuously influences medical schools and as a result of this many different written and unwritten aims and goals are defined. These aims and goals keep changing according to real or imagined needs of the society. There is seldom a consensus.

When new problems are identified many different solutions are offered by different people. Therefore, with time, medical schools trying to adopt to the new needs of the society started to redefine their roles. As is the case on many occasions the identification of the problems and the solutions offered are not necessarily the right ones. Certain problems may be exaggerated or stressed out of proportion. Before remedies or solutions are offered, problems must be identified correctly. The public has from time to time expected too much from medical schools. Expectations of the society must be realistic.

Medical schools should inform the public that, although they have a unique capability to identify the changing medical needs of society and to propose responses to them, they are only one of the elements of society that may be involved in responding.

Medical schools should continue to identify social problems related to health and should continue to recommend solutions, but they should participate in such solutions only when adequate resources are available, and when there is no hazard of compromising a school's primary purpose.[2]

Therefore I believe the functions of medical schools should be listed as follows:

1. The Mission of Educating Students to Become Physicians

This is the most important mission of a medical school. No other obligation should compromise this primary mission. In order to achieve this vital goal the following should be taken into consideration:

Striking increases in enrollment will inevitably threaten the quality of education. In terms of health care, unreasonable increases in enrollment probably will be more costly in the long run. Therefore medical schools should limit their enrollment and select their students carefully. Physical facilities of a medical school should be adequate. The student faculty ratio should be balanced.

2. Biomedical Research

"advancing the frontiers of knowledge through research, both basic and applied."

The importance of research is obvious. But research needs financia support. In developing and even in developed countries finding enough financial resources is becoming more and more difficult. Especially in developing countries most medical schools are faced with great financial difficulties. Therefore, the quality and quantity of research in many medical schools leave a lot to be desired.

3. Providing Health Service

Service to the sick and prevention of disease is the third main responsibility of medical schools. According to some, this is the most vital responsibility of medical schools. A lot may be said in favour of this function. It is true that medical schools should provide health care for the community. Medical schools are in a position to identify and address societal needs and changes. The importance of this function is unquestionable, but service to the community should not interfere with the other two main functions of a medical school. A fine balance should be kept between teaching, research and providing service to the community. Service to the community should not compromise a medical school's education or biomedical research.

Undoubtedly the main mission of a medical school should be "health for all". In order to achieve this, medical schools should provide research to solve the problems faced with today and will be faced within the future and educate the physicians of the future, according to the possible needs of the future. Today's medical schools should not only contribute to the social needs of today but also to the social needs of the future.

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Ultrastructural Observations on the Endocrine Cells

I - Types of Endocrine Cells in the Corpus of Stomach of Adult Mice

Aysel Şeftalioğlu, D.V.M., Ph.D.* / Deniz Erdoğan, Ph.D.**

Summary

Types of endocrine cells in the corpus of stomach of three-day-old and adult mice were investigated under the transmission electronmicroscope.

Five types of ultrastructurally different endocrine cells were identified: D_1, A, EC, ECL and D cells. A and ECL cells were predominantly observed.

D_1-cells had variable shapes and clear cytoplasm. Their secretion granules were small and scattered throughout the cytoplasm. Two types of granule were noticed. Type I granules were electron-dense. The others had light-filamentous texture.

A- cells were round and with dark cytoplasm. They were rich in secretion granules accumulated in the basal region of the cells and were large, electron-dense and uniformly round.

EC- cells were well characterized by their electron-dense and polymorphous secretion granules.

ECL- cells had abundant cytoplasmic organelles. At least three types of granule were detected. Bundles of filaments and cilias were often observed in these cells.

D- cells were oval and elongated in shape. Secretory granules of D-cells were variable in opacity and diameter. Their limiting membrane was discontinuous.

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Introduction

Endocrine cells of the adult gastrointestinal tract are believed to have the capacity of synthesizing and secreting low molecular weight polypeptide hormones and biogenic amines. Therefore they are classified as APUD (Amine Precurcor Uptake and Decorboxylation) cells.[1, 2, 3]

Investigators have attempted to characterize endocrine cell types morphologically and cytochemically in various adult animals in order to elucidate the cellular source of gastrointestinal hormones and biogenic amines.[4–6]

The fine structure of endocrine cells in mammalian gastric mucosa has been studided extensively.[7–18] Based on the presence of specific types of granules that may have endocrine functions, five types of endocrine cells have been described in the corpus of stomach of adult mammals: EC, ECL, A, D, and D_1-cells.[19]

In this paper the types of endocrine cells in the corpus of stomach of three-day-old and adult mice have been carefully investigated by electronmicroscopy with the aim of attaining a better identification and classification of developing endocrine cells in the second part of the work.

Materials and Methods

Female Swiss albino mice in estrus were mated overnight. The day following a successful mating, assessed by the presence of a vaginal plug, was considered the 1st day of gestation (normal gastation time is 21 days). From day 16 throughout day 20-21 of gestation, pregnant mice were anesthetized with ether and the embryos were removed from the uterine horns through an anterior abdominal incision.

The embryonic and adult stomach tissue slices were first fixed in a solution of 2,5 % glutaraldehyde and then in a solution of 1 % osmium tetroxide. After dehydrating at room temperature, the tissue slices were embedded in araldite (araldite CY 212, Taab). The thin sections were obtained from blocks of araldite, using LKB-Ultratom III Ultramicrotome and glass knives. Sections were collected on unsupported grids and stained with lead according to Sato[20] for 5-10 minutes and later with 70 % uranyl acetate saturated in ethanole for 10-15 minutes.

All the sections were studied under the electronmicroscope, Carl Zeiss EM 9 S-2, the photographs were taken using Dupont-Graphic Arts Films and prints were made on forte-BNO-Bromforts.

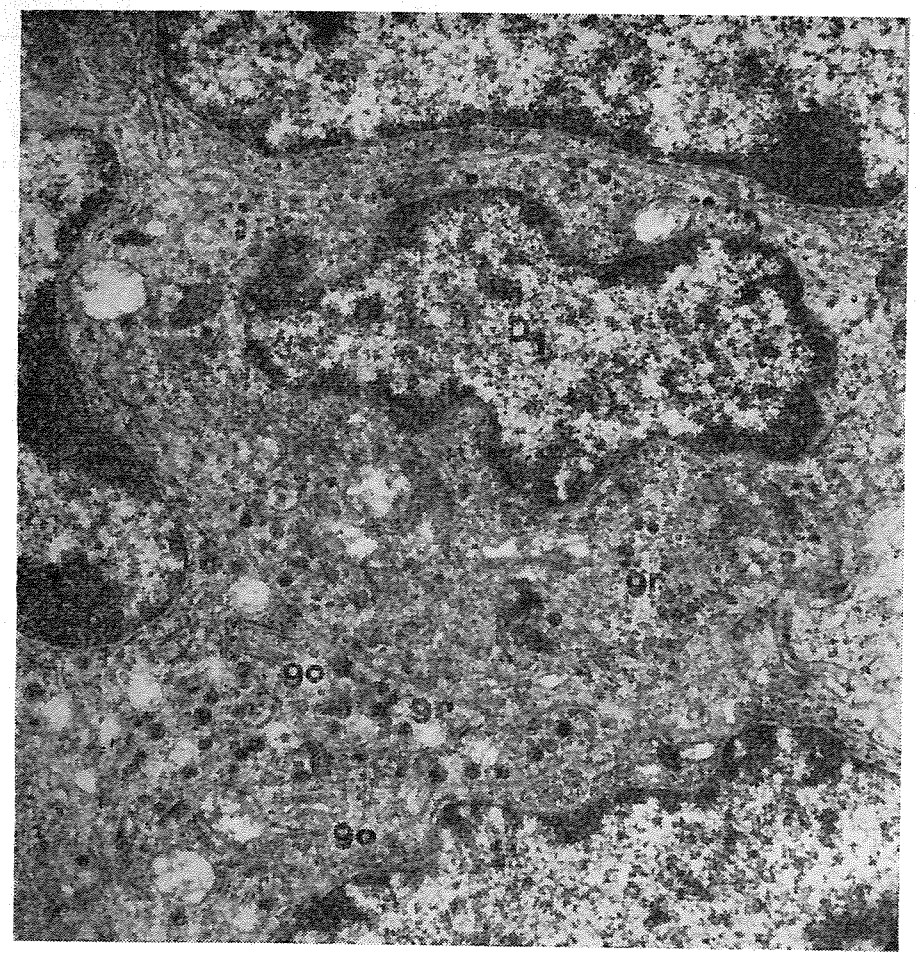

Figure 1
Two D_1-cells from the corpus of stomach of a three-day-old mouse. Secretion granules (gr) having small diameter and scattered throughout the cytoplasm and two Golgi complexes (go) are seen. X 25500

Results

Three-day-old and Adult Mice: Five types of endocrine cells were observed. These were D_1, A, EC, ECL and D cells.

Undifferentiated cells were small in number, scattered here and there in the corpus of stomach of three-day-old and adult mice. They were almost always observed in close relationship with other types of endocrine cells. These cells will be described and discussed in the II part of our work.

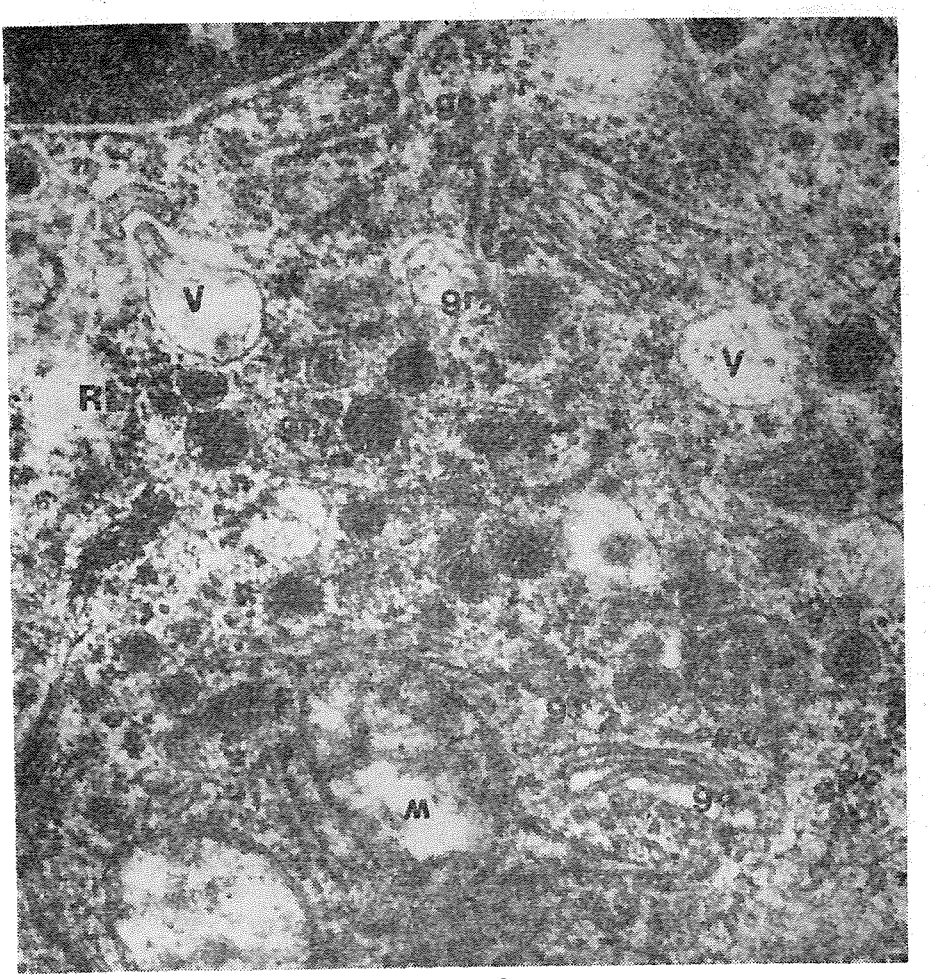

Figure 2

An electronmicrograph in higher magnification of one of the cytoplasmic areas of above D_1-cells. Two types of secretion granules (gr) are observed. Type I granules (gr_1) are filled with light-filamentous granular material. Type II granules (gr_2) are electron-dense. There is a narrow space between granular material and limiting membrane in both types of granules. A few granular endoplasmic reticulum (ger) and mitochondria (M), abundant ribosome and polysome, more than one Golgi complex (go), vacuoles (v), multivesicular bodies (Mv) and bundles of filaments (Fi) are also seen in the cytoplasm of D_1-cell. X 93000

D_1 - Cells: They were generally located in the basal region of the glands of the stomach. They varied in shape and had clear cytoplasm. Their secretion granules were predominantly small and round. They were scattered throughout the cytoplasm of the cell (Figure 1). Two types of granules were noticed. Type I granules were electron-dense.

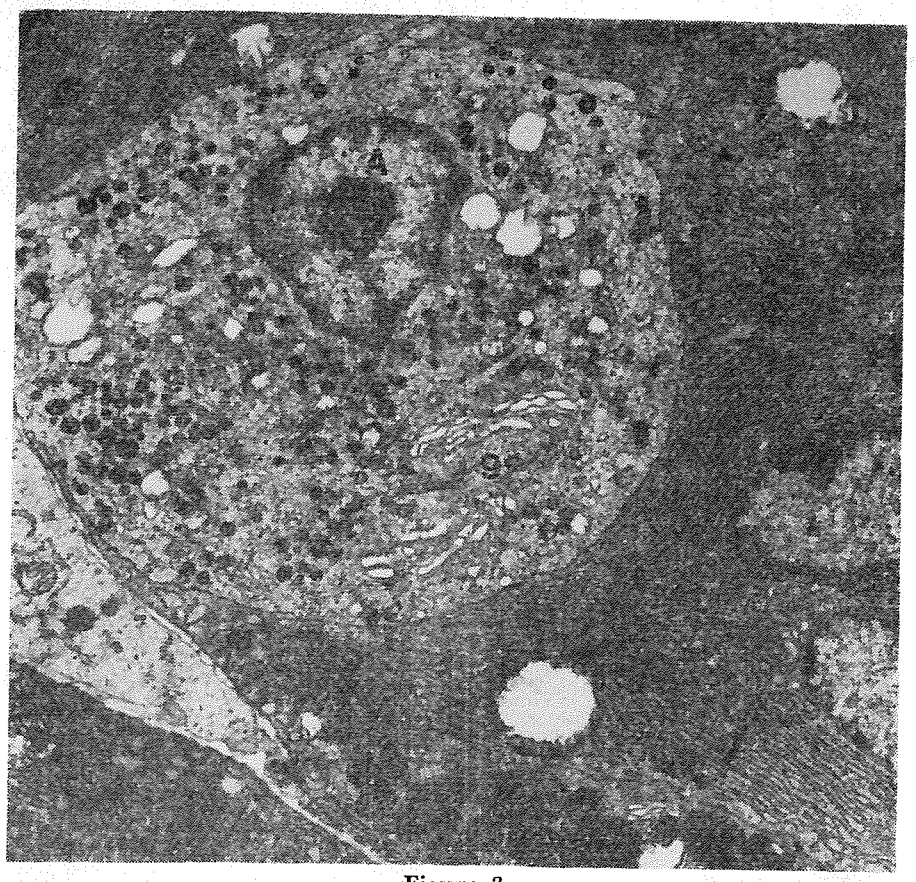

Figure 3
A-cell from adult mouse. It is located in the basal region of the gland of stomach. It has a round shape and indented nucleus (N). Highly electron-dense secretion granules (gr) and more than one Golgi complex (go) are situated in the basal region of cytoplasm of cell. X 14100

Type II ones were light-filamentous in texture. The limiting membrane of both types of granules was distinct and continuous. There was a narrow space between the limiting membrane and granular material of secretion granules. D_1-cells contain bundles of filaments, more than one well-developed Golgi complex, vacuoles, multivesicular bodies, polysomes, ribosomes and small amounts of granular endoplasmic reticulum (Figure 2).

A- Cells: They were round in shape and situated in the basal regions of the glands of the stomach. In general their indented nuclei were found in the center of cells. Their Golgi complexes and granular endoplasmic reticulum were well-developed. In some cells granular endoplasmic reti-

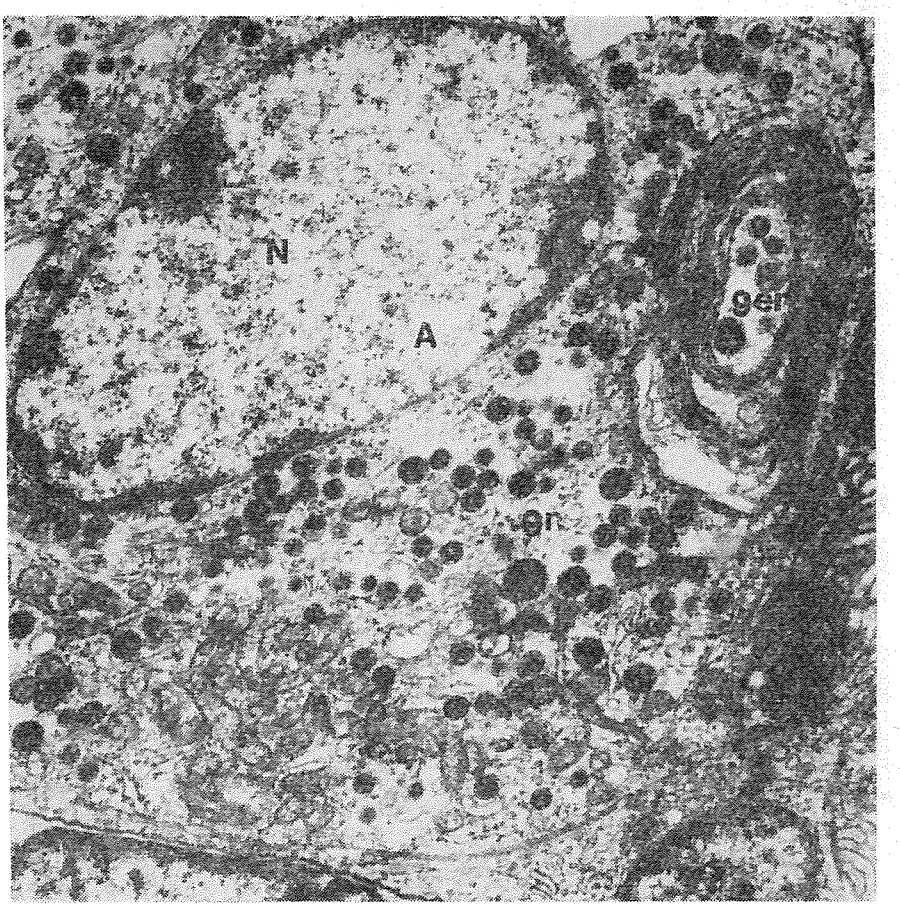

Figure 4

Adult mouse. A-cell contains abundant mitochondria (M), secretion granules (gr) and granular endoplasmic reticulum (ger) displaying concentric lameller structure. X 25500

culum displayed a concentric lamellar structure (Figures 3 and 4). A-cells had many mitochondria and abundant ribosomes, vacuoles, vesicles and coated vesicles. They were rich in secretion granules which generally accumulated in the basal regions of the cells. Secretion granules were mostly large and highly electron-dense, and of uniformly round shape. A fairly pale area was discernible between the membrane and granular content. Among the large granules there were small-electron-lucent granules having discontinuous limiting membranes. There were also type III granules that probably developed into large-electron-dense granules (Figures 5 and 6).

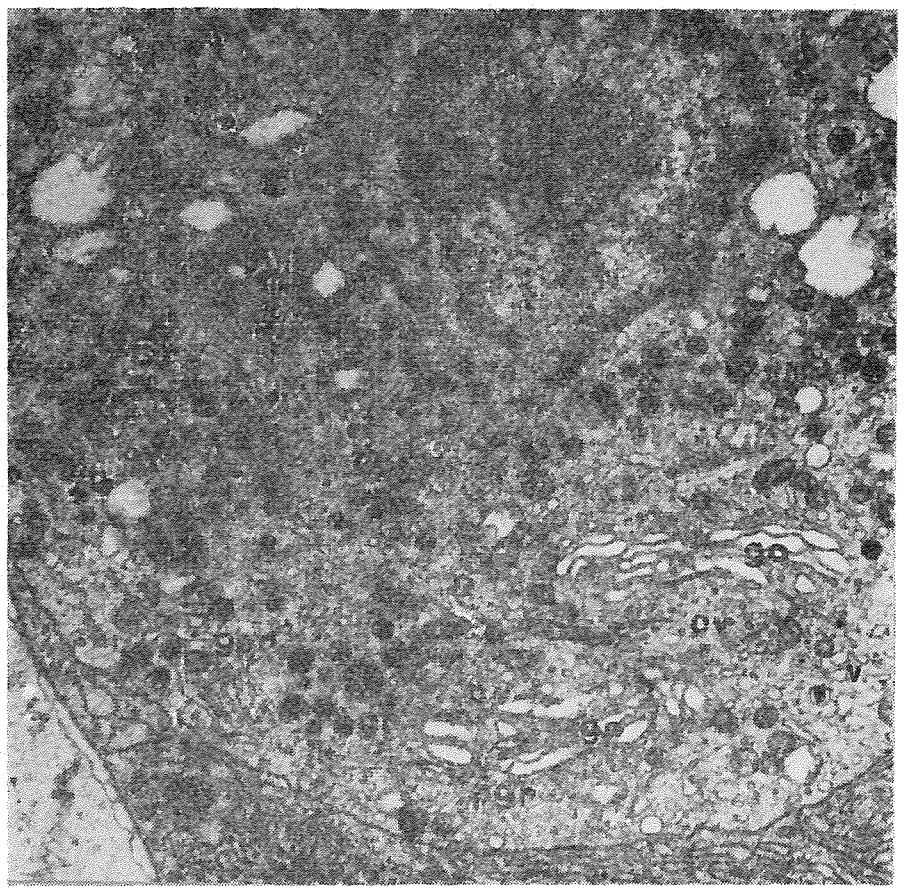

Figure 5

A-cell from the corpus of stomach of adult mouse. Indented nucleus (N) and well-developed nucleoli (n) are noticed in the center of the cell. A-cell is rich in secretion granules (gr), progranules (pgr), Golgi complexes (go), mitochondria (M), granular endoplasmic reticulum (ger), vesicles (v) and coated vesicles (cv). X 25500.

Enterochromaffin-Cells (EC): They were observed in the basal regions of glands. Their shapes were elongated and triangular. EC-cells contained well developed Golgi complexes, relative number of mitochondria, and a few cisternae of granular endoplasmic reticulum. They were rich in ribosomes. Their secretion granules were not abundant and were distribuated throughout the cytoplasm of the cells. These types of cell were well-characterized by their electron-dense and polymorphous secretion granules(Figure 7).

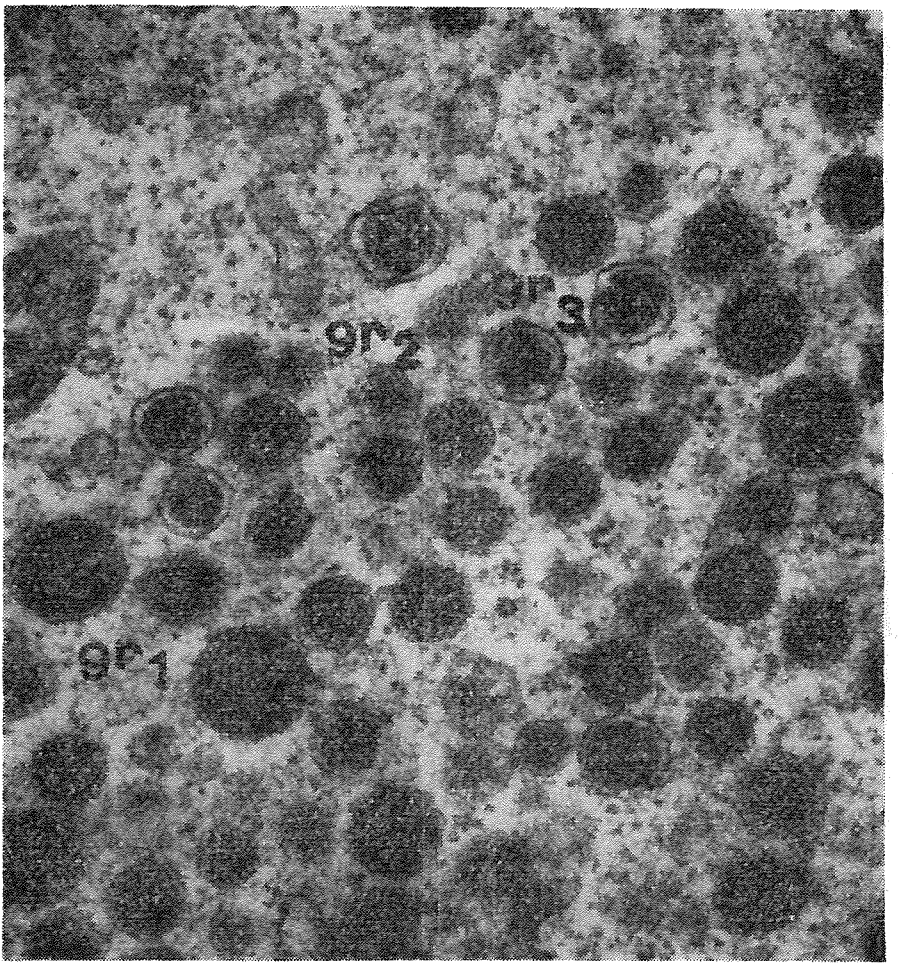

Figure 6
Three types of granules (gr) of A-cell are seen in a three-day-old mouse. Type I granules (gr_1) are large highly electron-dense. Type II granules (gr_2) are small-electron-lucent. Type III ones (gr_3) are granular in structure. X 93000

Enterochromaffin-Like Cells (ECL): Enterochromaffin-like cells were generally found among the epithelium of the gastric glands. Their shapes were oval and triangular. The cytoplasms of ECL-cells were richer in organelles than those of other endocrine cells. Granular endoplasmic reticulum, coated vesicles, Golgi complexes and vacuoles were well-developed. Mitochondria were abundant. Bundles of filaments and cilias were frequently seen in the cytoplasms of many ECL-cells. At least three types of granules were seen in these types of cells. The type I large granules filled with a light granular and filamentous material were limited by

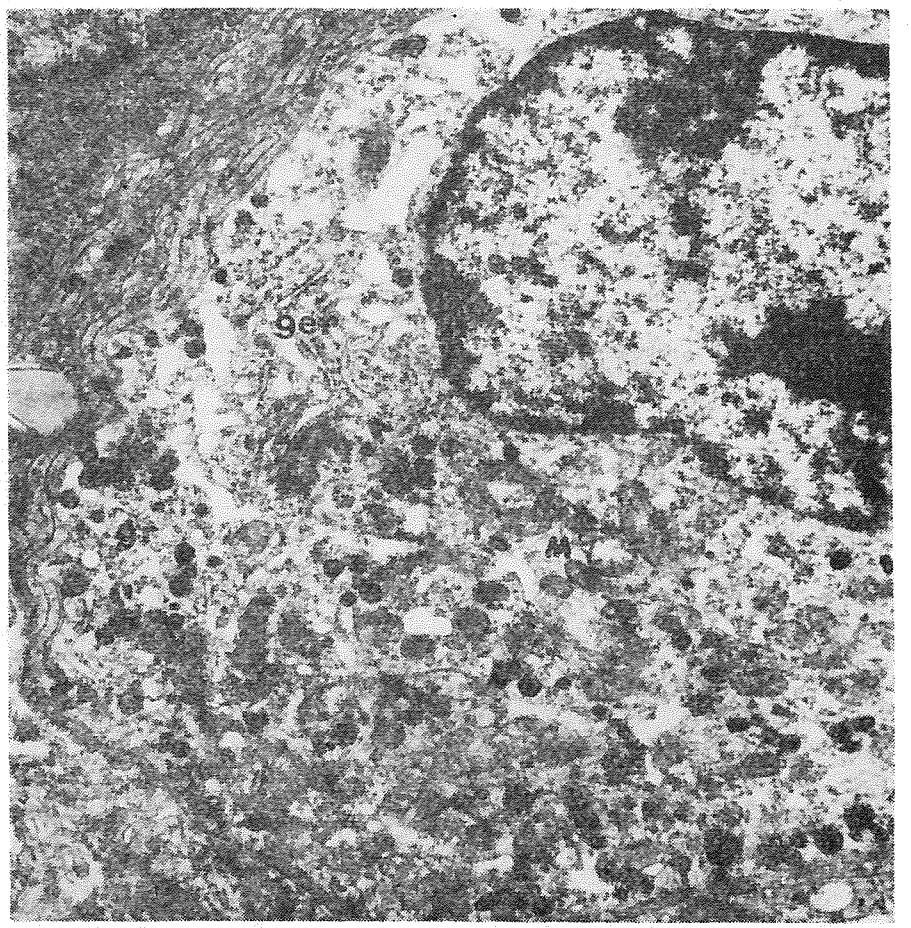

Figure 7
EC-cell. 3-day-old mouse. Few cisternae of granular endoplasmic reticulum (ger), many mitochondria (M) and electron-dense-polymorphous secretion granules (gr) are noticed. X 25500

a distinct membrane. There was no space between membrane and granular content. Type II granules were electron-dense, finely textured and variable in shape. These granules were separated from their limiting membrane by a narrow space. Type III granules had a granular core having variable density, inside an irregular-shaped vacuole. In some cells these bubble-shaped granules did not seem to contain any secretory product at all (Figures 8 and 9).

D- Cells: They were oval and elongated in shape and located in the basal regions of the gastric glands (Figures 10 and 11). Lysosomes seemed to be more common in the D-cells than in any of the other endoc-

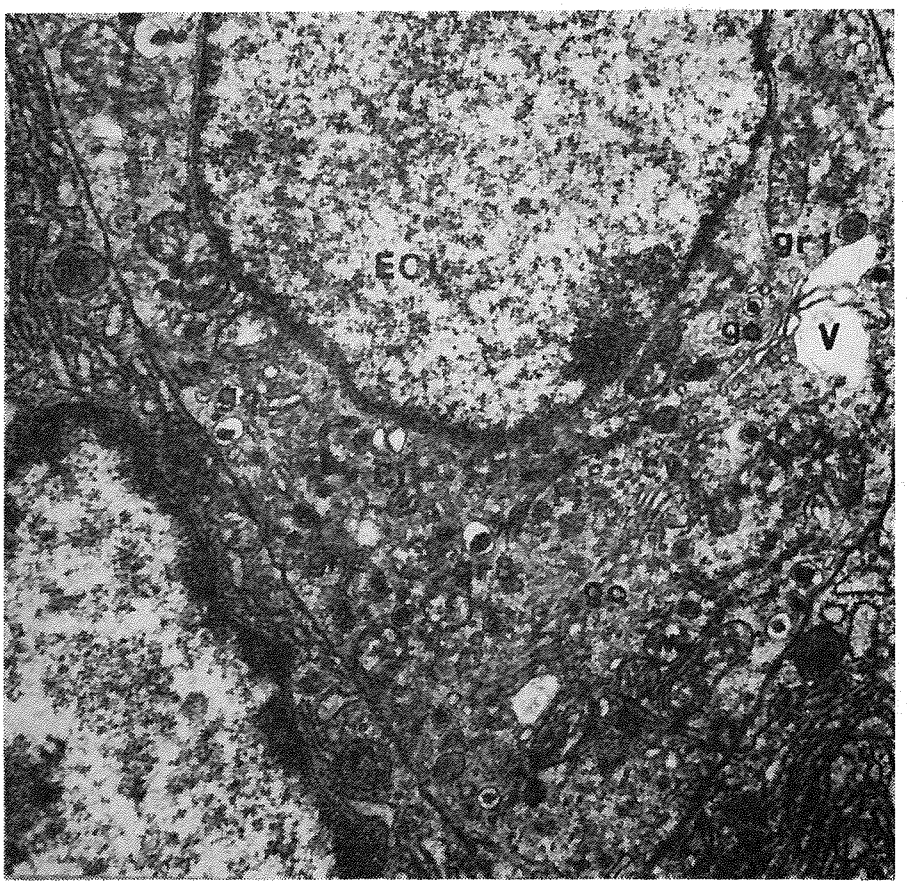

Figure 8

ECL-cell from adult mouse. It is rich in cytoplasmic organelles. Well-developed Golgi complexes (go) vesicles (v), coated vesicles (cv), vacuoles (V), mitochondria (M), bundles of filaments (Fi), cilias (Ci) and three types of secretion granules. X 25500.

rine cells. They had Golgi complexes. The granular endoplasmic reticulum and mitochondria were poorly developed. The secretory granules of D-cells were variable in electron opacity and diameter. The limiting membrane of granules often had a discontinuous and nibbled appearance. The formation of granules was frequently observed in the Golgi regions of the cells (Figure 11).

Discussion

Based on the above results and on those of De Lemos,[19] five types of endocrine cells were observed in the corpus of stomach of adult mice.

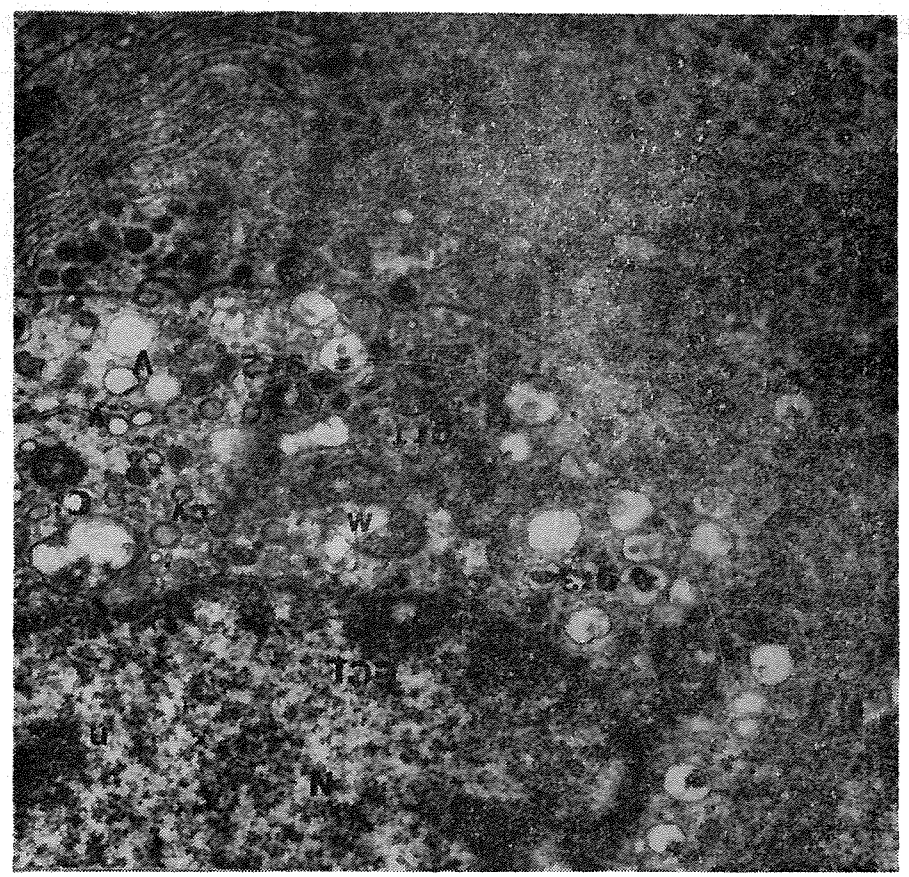

Figure 9

ECL and A-cells from adult mouse. Type I large granules (gr_1) filled with light-filamentous material, Type II granules (gr_2) being electron-dense, finely textured and variable in shape, and Type III granules (gr_3) having a granular core in variable density and situated in irregular shaped vacuole are seen. Cilia (Ci), vesicles (v), coated vesicles (cv), vacuoles (V) and mitochondria (M) are observed in the cytoplasm of ECL-cell. X 25500

D_1 - Cells: It has been noted that D_1-cells are found in the gastric mucosa of several species. As a whole the ultrastructural and/or staining patterns of such cells were clearly different from those of EC, ECL, G and D-cells.[10, 13, 19] On the other hand these cells have been labelled D_1-cells, because in the rat[11] and human[14] stomach cells have been described similar to the "D" cells owing to their ultrastructural similarity with rat pancreatic D-cells.

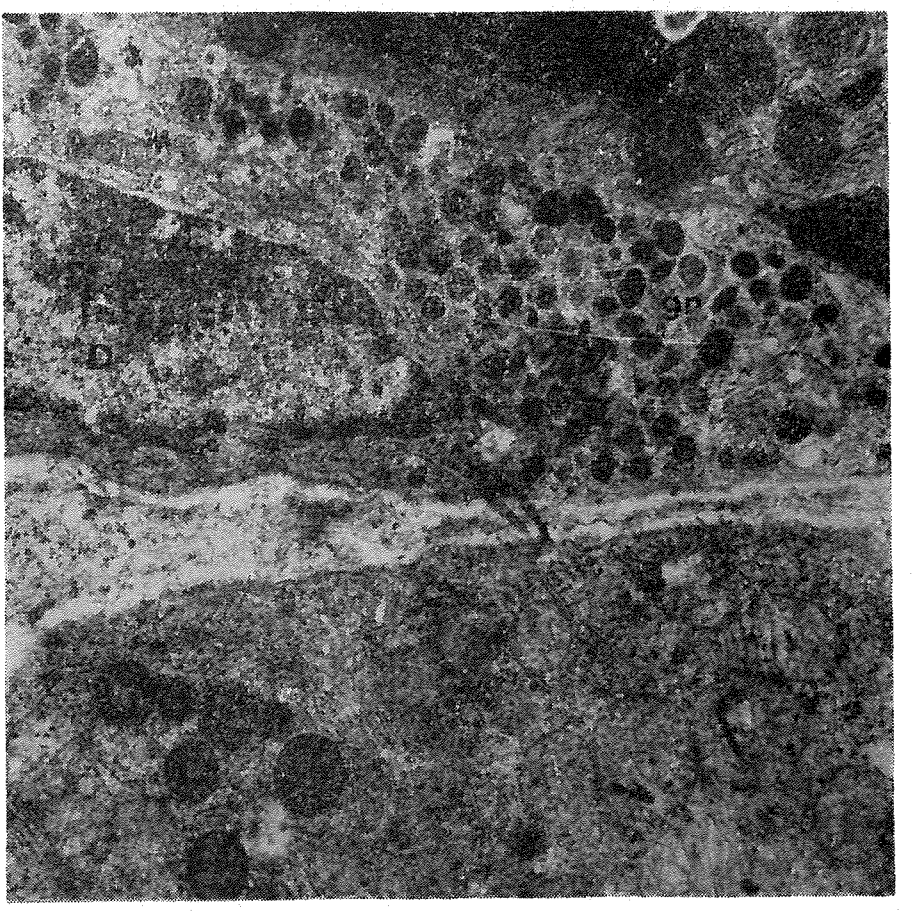

Figure 10

Three-day-old mouse. D-cell is elongated in shape and is found in basal region of the gastric gland. Many secretion granules (gr) having variable electron opacity, diameter and discontinous limiting membrane are noticed. X 25500

The most important ultrastructural features of D_1-cells are of secretion granules of small diameters and bundles of cytoplasmic filaments scattered throughout the cytoplasm.[13, 19]

The researchers investigating the function and the nature of secretion granules of D_1-cells in the gastrointestinal tract using immuno-electron-cytochemical techniques have reported that D_1-cells produced vasoactive intestinal peptide (VIP).[21-27]

In the present study findings confirming that the D_1-cell granules contained vasoactive-intestinal-peptide (VIP) could not be obtained.

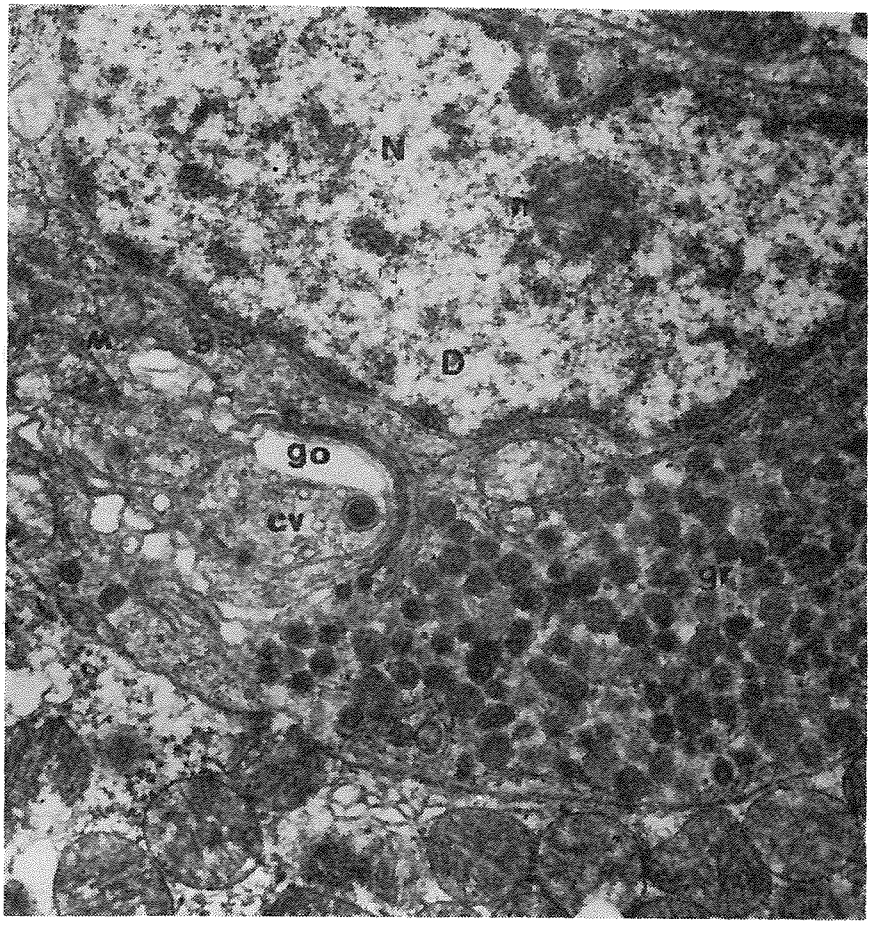

Figure 11

Adult mouse. D-cells contain secretion granules (gr), well-developed Golgi complexes (go), small amount of granular endoplasmic reticulum (ger), many mitochondria (M) and coated vesicles (cv) are observed. X 25500

But it was established that D_1-cells were morphologically identical to the ultrastructural findings of researchers.[10, 11, 13, 14, 19, 21, 25]

A-Cells: Although A-cells have been found in many other species,[10,12] Kobayashi and his workers[15] and De Lemos[19] have recognized them as few in number in adult human gastric epithelium. Forsmann et al[11] and Kobayashi et al[15] described these cells as "Endocrine cells of a second type" and it was noted that they were similar to the enteroglucagon-producing intestinal A and L cells.[11, 14, 19, 28–31] A-cells also appear morphologically comparable to the glucagon producing A-cells in pancreatic islet.[27, 31]

Polak et al[32] detected by immuno-fluorescence techniques that A-cells and L-cells of the dog stomach and intestine were responsible for glucagon-like immunoreactivity (GLI) and thus may contain enteroglucagon.

Beaten et al[33] reported that the cells situated in gastric mucosa, observed to be indistinguishable ultrastructurally, cytochemically and immunocytochemically from A-cells, were responsible for the secretion of the gastric-glucagon.

In this study A-cells attracted attention by their round shape, dark cytoplasm, highly electron-dense and uniform large secretion granules accumulated in the basal region of cytoplasm. They were often observed in our sections. Our ultrastructural findings in the A-cells were identical to those of findings obtained from light and electron microscopic[10–12, 14, 19, 27, 31] and immuno-electron-cytochemical studies.[32, 33]

Enterochromaffin Cells (EC): The ultrastructure of adult EC-cells is well characterized by their highly electron-opaque, polymorphous secretion granules.[10–12, 19]

In adult animals the gastrointestinal EC-cell granules have been regarded as the storage site of the biogenic amine serotonin (5HT)[34–38] and recently of the polypeptide motilin, stimulating gastric motility.[39, 40] Serotonin has been proposed as the hormone that may be involved in the regulation of intestinal peristalsis in the adult.[41]

The present study showed that the EC-cells were rarely seen in the corpus of stomachs of three day-old and adult mice. They were triangular and elongated in shape and had highly electron-dense and polymorphous secretion granules.

Enterochromaffin-Like Cells (ECL): In the fundus and corpus of adult stomachs of various mammals, ECL-cells have been observed as the most abundant endocrine cells.[10–13, 19]

Enterochromaffin-like cells having distinctive ultrastructural and staining properties have been shown to store histamine in rat and mouse gastric mucosa.[10, 42–45] A cell type with ultrastructural and staining patterns similar to those of rat and mouse ECL-cells also been identified in the gastric mucosa of the guinea pig, rabbit, cat, dog, pig and man. Unfortunately no histamine-storing cells other than mast cells have been found by Hakanson et al.[45]

We were able to establish that the ECL-cells were observed as the second most abundant endocrine cells in the adult corpus of the stomach

of mice. These cells were well-characterized by their richer organelles than those of other endocrine cells. Bundles of filaments, cilias and the characteristic three types of secretion granules were the most important ultrastructural features of ECL cells.

D- Cells: Adult gastric D-cells received their name because of their similarity to human pancreatic D-cells.[27, 31]

The identification of somatostatin-containing cells of the antral mucosa as D-cells was affirmed by Polak et al[46] and by Rufener et al[47] on the basis of immunofluorescent observation combined with histochemical and/or conventional electron-microscopy. In addition Rufener[47] reported the ultrastructural localization of somatostatin using peroxidase-labelled antibodies applied on monolayer cultures of rat pancreatic islets. In this study the polypeptide was demonstrated in the secretory granules and in the cytoplasmic matrix of the pancreatic D-cells, which are known to be structurally similar to the antral D-cells.

Using an alternate semithin-thin section technique, L'Eremite, et al[48] tried to identify the somatostatin-containing cells of the rabbit and mouse antral mucosa. Recently, Canese and Bussolati[49] showed that somatostatin, a growth hormone-release inhibiting factor, was localized in the endocrine granules of the D-cells using immuno-electron-cytochemical methods.

We also belive that D-cells observed in the corpus of the stomach of three-day-old and adult mice, and that all the ultrastructural features corresponding to those of findings of above researchers, were the site of storage of somatostatin, a growth hormone release inhibiting factor.

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Hacettepe Medical Journal
Volume 16 / No. 4 / October 1983 / pp. 168 – 185

Ultrastructural Observations on the Endocrine Cells

II-Development of Endocrine Cells in the Corpus of Stomach of Mouse

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Summary

Development of the endocrine cell types of the corpus of stomachs of 16,19 and 20-21-day-old mouse embryos and new-born mice was investigated at the electronmicroscopical level, taking into consideration their shapes, secretion granules and organelles.

Undifferentiated endocrine cells with clear cytoplasms were unique cell types in the 16-day-old embryos. They were very similar to basal cells of the stratified columnar epithelium of the developing stomach.

Starting from 19-day-old embryos and going up to new-born mice, three types of endocrine cells were identified; undiffentiated cells, D_1-cells and A-cells. In the new-born mice, enterochromaffin cells (EC) started to appear in addition to the cells mentioned above.

Four types of endocrine cells, undifferentiated, D_1, A and EC-cells, were identified in the corpus of stomach of 16, 19 and 20-21 day-old mouse embryos and new-born mice.

Introduction

Information concerning the presence of endocrine cells, their fine structural classification and their possible role in the embryonal or fetal gastrointestinal tract is scant. The few studies describing the fine structure of embryonal or fetal gastric endocrine cells are the observations on the human-gastrointestinal tract by Pick and De Lemos,[1] De Lemos[2, 3] and the works of Matsumoto[4] on the rat.

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The present study describes the types of endocrine cells in the corpus of the stomachs of 16, 19 and 20-21 day-old mouse embryos and newborn mice, taking into account their shapes, secretory granules and organelles.

Materials and Methods

These are the same as discribed in the first part of the work.

Results

16 - day-old embryos: The epitheliums of the stomachs of these embryos were stratified columnar (Figure 1). In some sections among these epitheliums the gland organizations of the stomach were observed. The

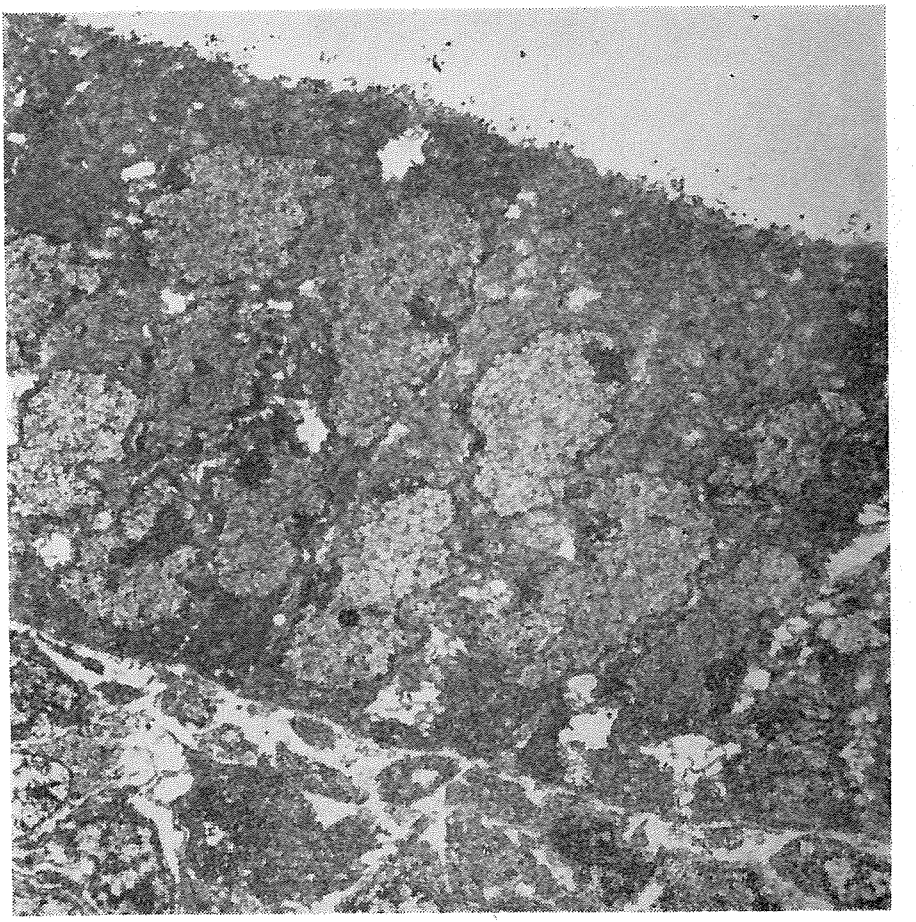

Figure 1
Stratified columnar epithelium of the corpus of stomach of 16-day-old mouse embryo. X 5700.

unique cell type of these embryos was the undifferentiated endocrine cell. They were small in number and were situated in the basal regions of the epithelium. They were identified by their characteristic clear cytoplasms and undeveloped secretion granulles. These cells were round, oval and elongated in shape, and very similar to basal cells of stratified columnar epithelium. Their cytoplasms had few secretory granules and organelles. Secretions granules of these cells were small and had irregular outlines. Limiting membrane of granules was discontinuous (Figure 2).

19 - day-old embryos: In these embryos, glands of stomachs were formed. Endocrine cells were often observed in the glands of isth-

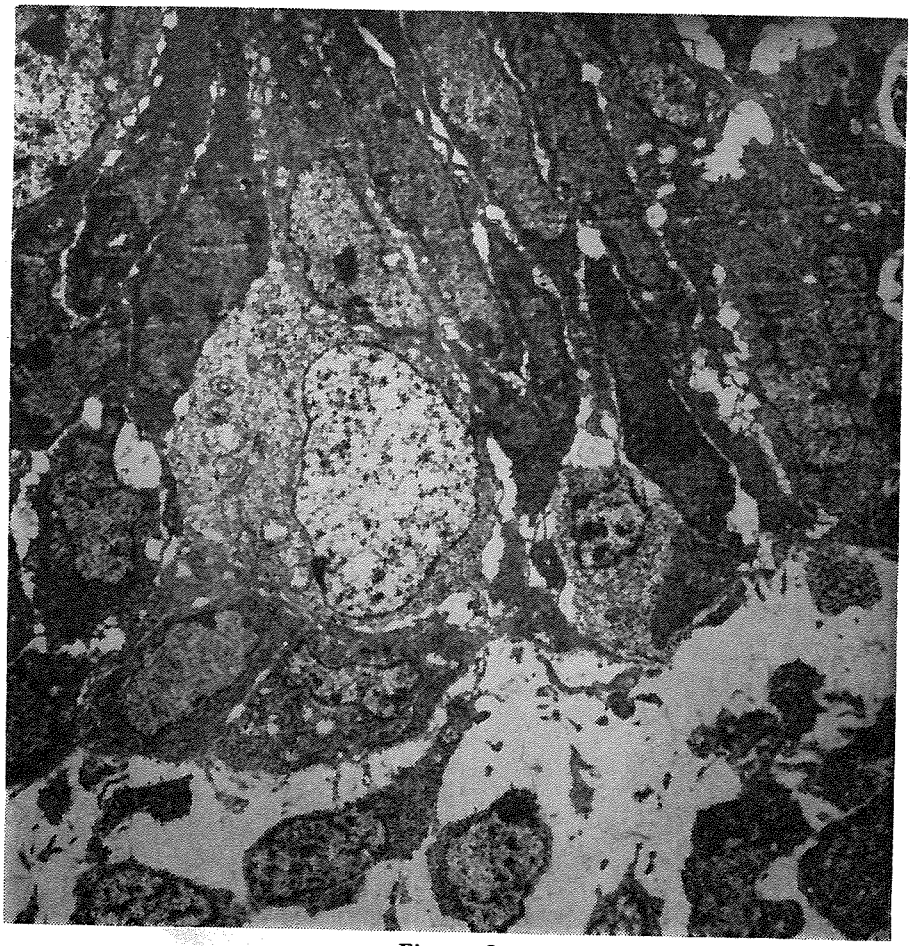

Figure 2
16-day-old embryo. Two undifferentiated endocrine cells in the basal region of the stratified columnar epithelium. X 5700

mus or necks. Three types of endocrine cells were found: Undifferentiated cells, D_1-cells and A-cells.

Undifferentiated cells having clear stoplasms, small number of granules and organelles were similar to those of 16-day-old embryos. In some sections these cells were found in the stages of mitotic division (Figure 3).

D_1 - cells were variable in shape and had clear cytoplasm. Their secretion granules were small and scattered throughout the cytoplasm. Two types of granule were noticed. Type I granules were round and electron-dense. Type II granules had light-filamentous texture. The

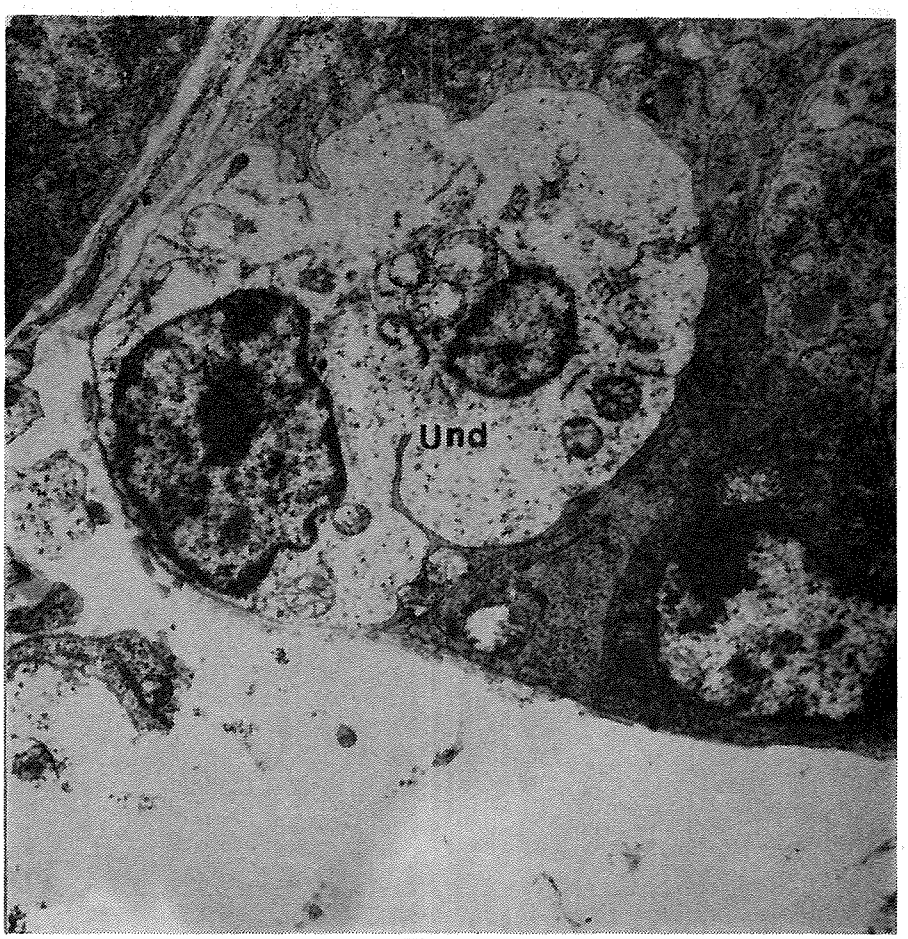

Figure 3
19-day-old embryo. Undifferentiated endocrine cells are observed in the mitotic division. X 14100

limiting membrane of both types of granules was distinct and continuous. Small amount of granular endoplasmic reticulum, a few mitochondria and variable amounts of ribosomes were observed in the cytoplasms of D_1-cells (Figure 4).

A - cells were oval shaped but irregular in contour. They had dark cytoplasms (Figure 5) and were rich in ribosomes, polysomes, coated vesicles and mitochondria. Granular endoplasmic reticulum and Golgi complexes were well-developed. Many mitochondria displayed myelin degeneration. Secretion granules in these cells were of two types: Electron-dense granules were larger than the small light ones. They were very similar to the granules of A-cells observed in the corpus of the stomachs

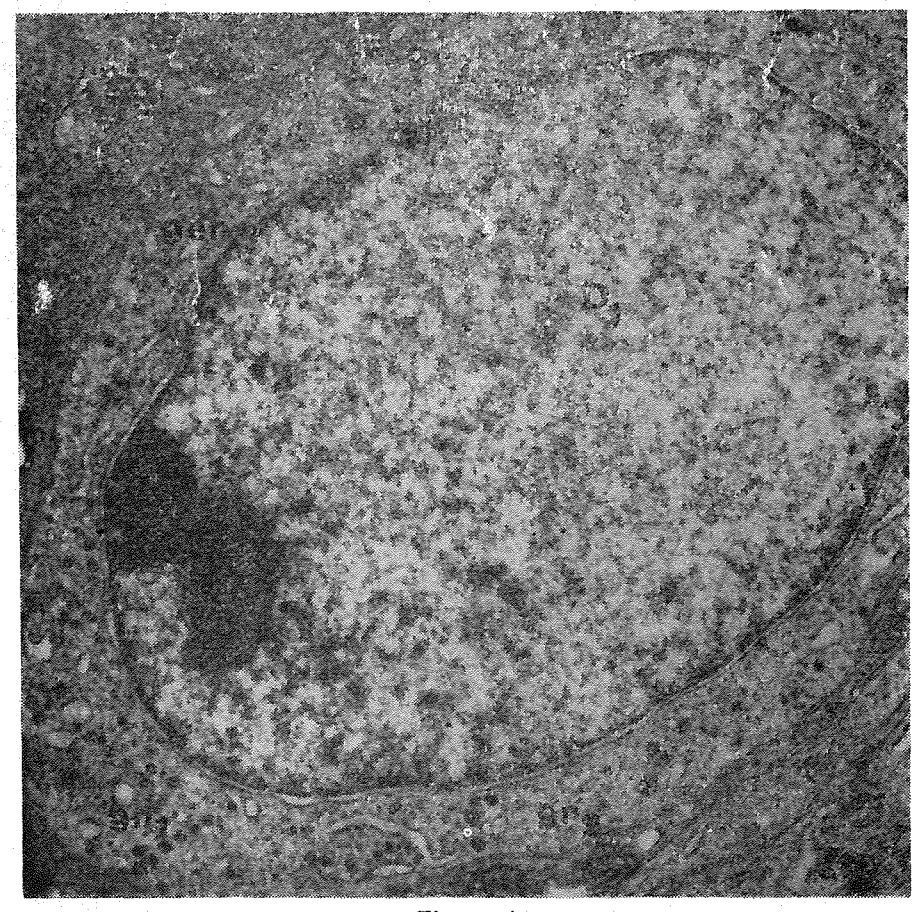

Figure 4

D_1-cell from 19-day-old embryo. Type I electron-dense secretion granules (gr_1), type II light-filamentous secretion granules (gr_2) and small amount of granular endoplasmic reticulum (ger) are seen in the cytoplasm of D_1-cell. X 25500

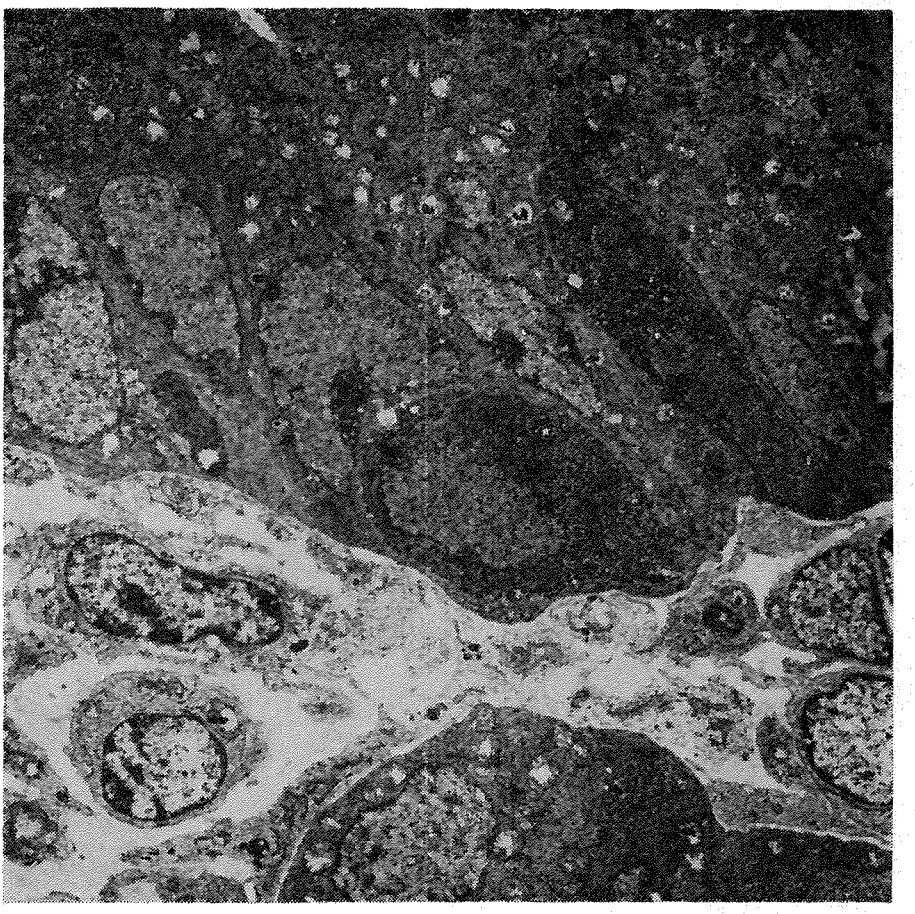

Figure 5
19-day-old embryo. A-cell having dark cytoplasm and irregular shape is observed. X 5700

of adult mice (Figure 6). In some cells, granules seemed to form by the pinching of cisternea of granular endoplasmic reticulum having electron-dense contents resembling secretory granules. These cells also contained bundles of filaments (Figure 7).

20-21 - day-old embryos: Three types of endocrine cells were observed in the corpus of stomachs of these embryos. They were undifferentiated endocrine cells, D_1-cells and A-cells.

D_1 and A-cells were more developed than those of 19-day-old embryos. Their secretion granules and organelles increased in number, and Golgi complexes and coated vesicles were well-developed (Figures 8, 9 and 10). In the cytoplasms of some D_1-cells large granules filled with

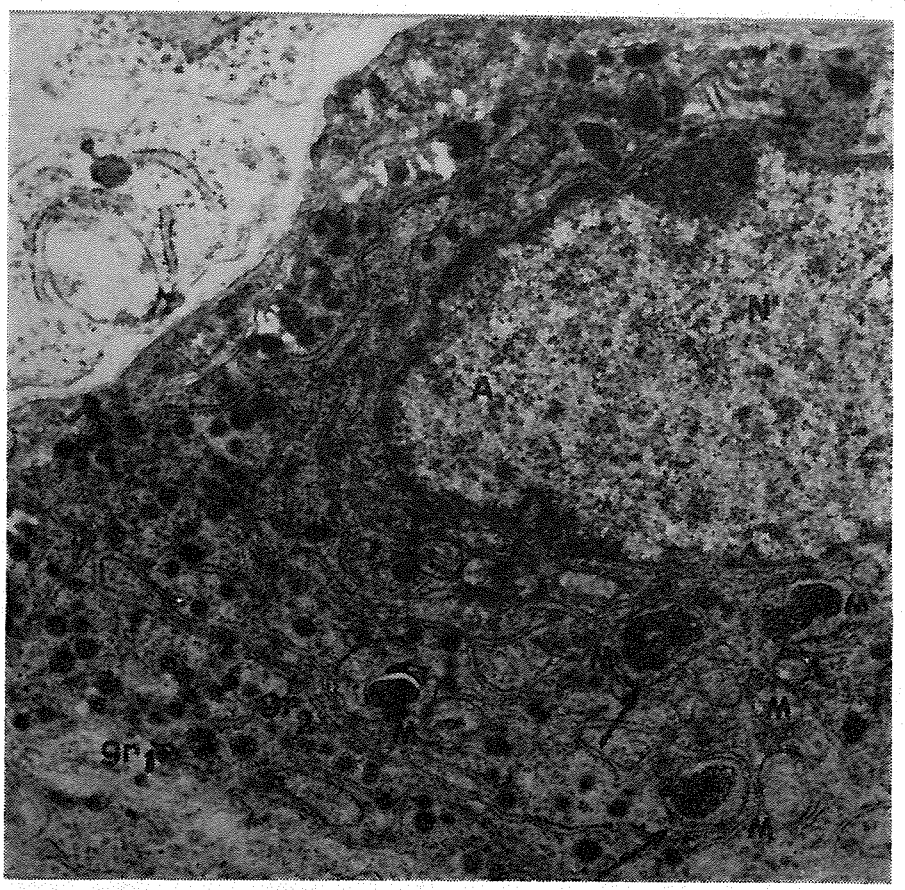

Figure 6

A-cell from the corpus of stomach of 19-day-old mouse embryo. Large-electron-dense secretion granules (gr_1), small-light secretion granules (gr_2), mitochondria (M) displaying myelin degeneration (arrows) and well-developed granular endoplasmic reticulum (ger) are detected in the cytoplasm of cell. X 25500

filamentous material were also noticed in these embryos. These granules were similar in appearence to the adult D_1-granules, having light filamentous texture. These D_1-cells also contained bundless of filaments, more than one well-developed Golgi complex, abundant coated vesicles, ribosomes, polysomes and a small amount of granular endoplasmic reticulum (Figure 9, 10).

New-born mice: Four types of endocrine cells were seen: Undifferentiated cells, D_1-cells, A-cells and enterochromaffin cells (EC).

Undifferentiated, D_1 and well-developed froms of D_1-cells were observed in close relationship in some sections (Figure 11).

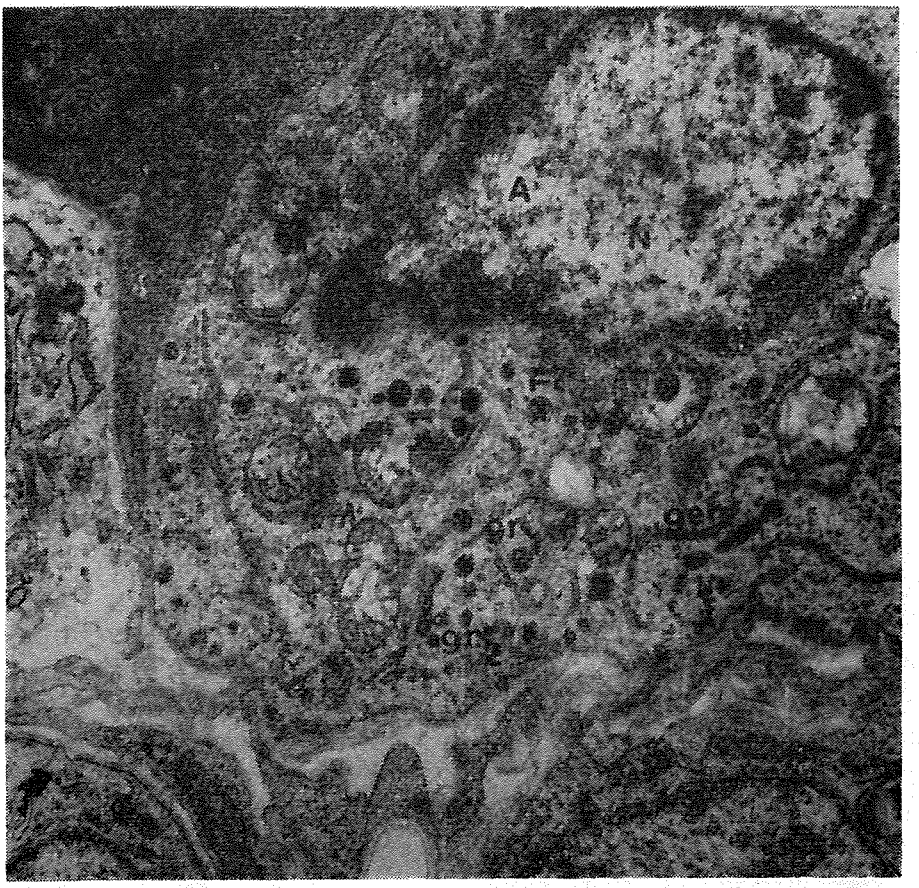

Figure 7

19-day-old embryo. A-cell. Secretion granules (gr_1 and gr_2), granular endoplasmic reticulum (ger), granule formation by pinching of cisternae of granular endoplasmic reticulum (arrows) and bundles of filaments (Fi) are seen. X 25500

A-cells had the appearence of A-cells observed in the adult stomachs of mice (Figure 12).

EC-cells having clear cytoplasms were poor in organelles and oval in shape. Their secretion granules were in the formation stage. Distended cisternae of granular endoplasmic reticulum contained materials of secretion granules. Secretion granules seemed to form by pinching of cisternae of granular endoplasmic reticulum (Figure 13 and 14).

Discussion

Endocrine cells are considered to be of mesodermal origin by Kull[5] and Dias Amado[6], of embryonic sympathetic origin by Danis[7] and

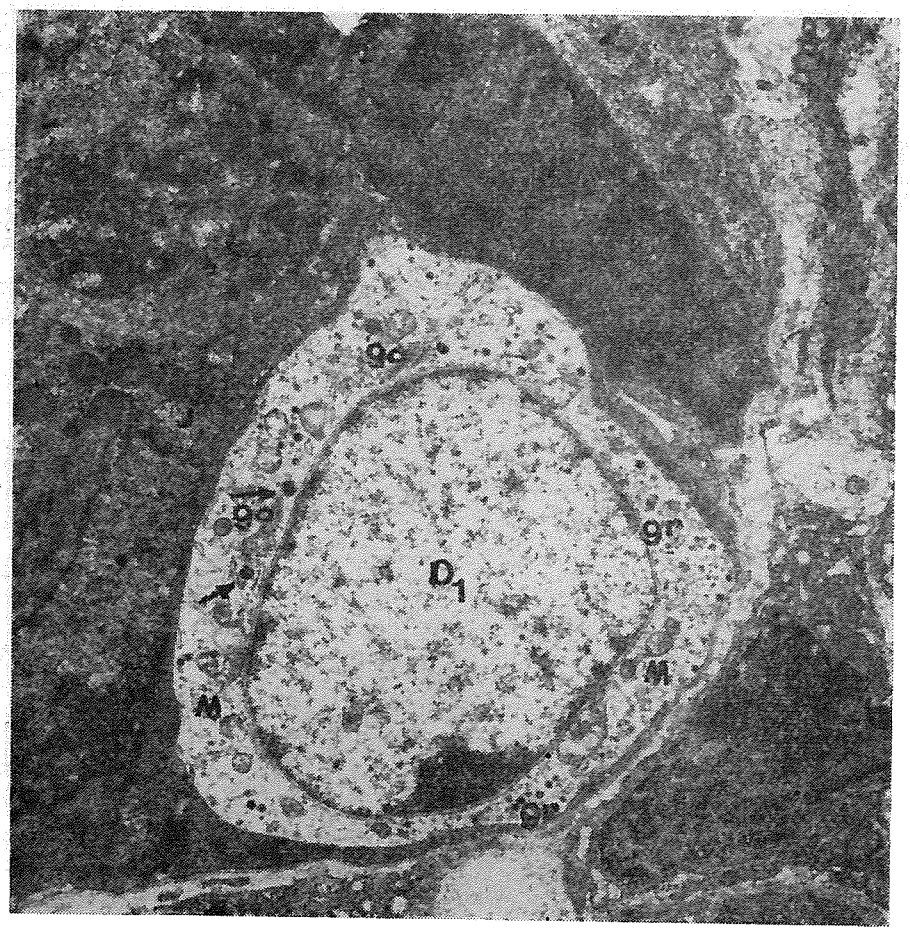

Figure 8

D_1-cell from 20-21-day-old embryo. Well-developed Golgi complexes (Go), many secretion granules (gr) and mitochondria (M) and large-filamentous secretion granules (arrows) attract attention. X 14100

Chung[8] and of neural crest origin by Pearse[9], but the majority of workers agree that they differentiate in situ in the epithelium of the gastrointestinal tract.[1, 2, 3, 10]

In this study, during the development of endocrine cells of the corpus of stomachs of mice, undifferentiated endocrine cells were very similar to basal cells of the stratified columnar epithelium in the 16-day-old embryos, and gland epithelial cells in 19 and 20-21-day-old embryos and new-born mice. In some sections, these undifferentiated endocrine cells were observed in different stages of mitosis.

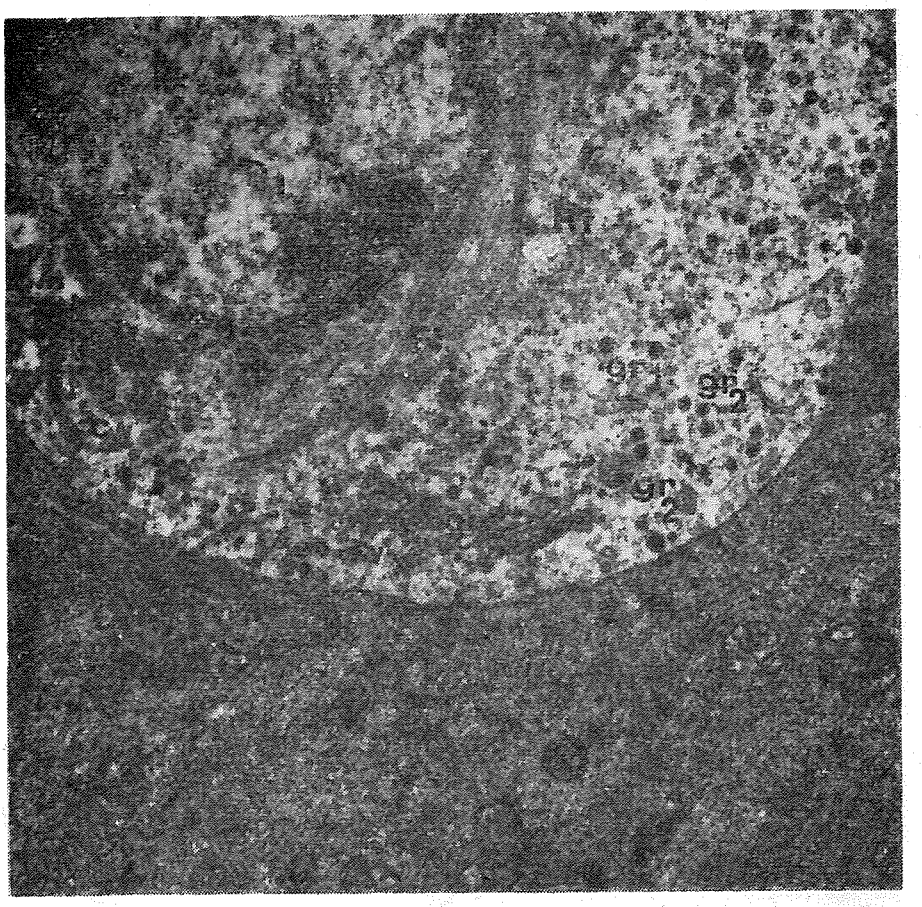

Figure 9

20-21-day-old embryo. D_1-cell containing type I electron-dense secretion granules (gr_1) and type II large and small-light filamentous granules (gr_2), well-developed Golgi complexes (go), bundles of filaments (fi), mitochondria (M) and coated vesicles (cv) are observed. X 25500

Matsumoto[4] reported the presence of clear cells with small mitochondria in the gastric epitheluim of 20-day-old rat embryos and granulated basal cells four days after birth. He equated the development stage of the gastric epithelium of 21-day-old rat embryos with that of the human fetus at 12 weeks of age.

According to the findings of De Lemos[3], the number and types of endocrine cells increase with development. The frequency of the distribution of the different types of endocrine cells appear to be D, EC, ECL cells. D_1 and AL-cells are the least frequent.

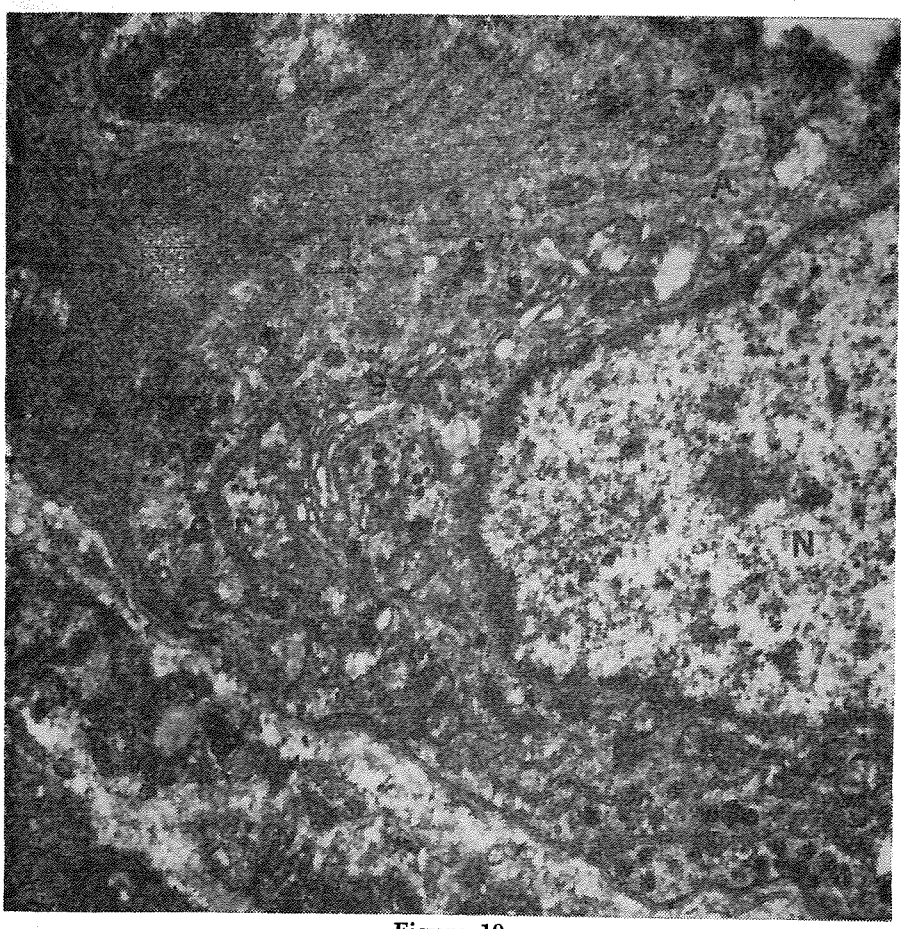

Figure 10

A-cell from the corpus of stomach of 20-21-day-old mouse embryo. Well developed Golgi complexes (go), granular endoplasmic reticulum (ger), many mitochondria (M), coated vesicles (vc) and large-electron-dense granules (gr_1) and small-light granules (gr_2) are seen. X 25500

The data reported in this paper indicated that the number and types of endocrine cells increased as development proceeded. However, undifferentiated endocrine cells, D_1 and A-cells were predominantly observed, starting from 16-day-old mouse embryos up to new-born mice. In addition to the cells mentioned above, EC-cells started to appear in the new-born mice.

The appearance of different endocrine cells in different developmental periods indicates that they are capable of producing specific biogenic amines and/or hormones. Therefore they must be compared to their adult counterpart individually.

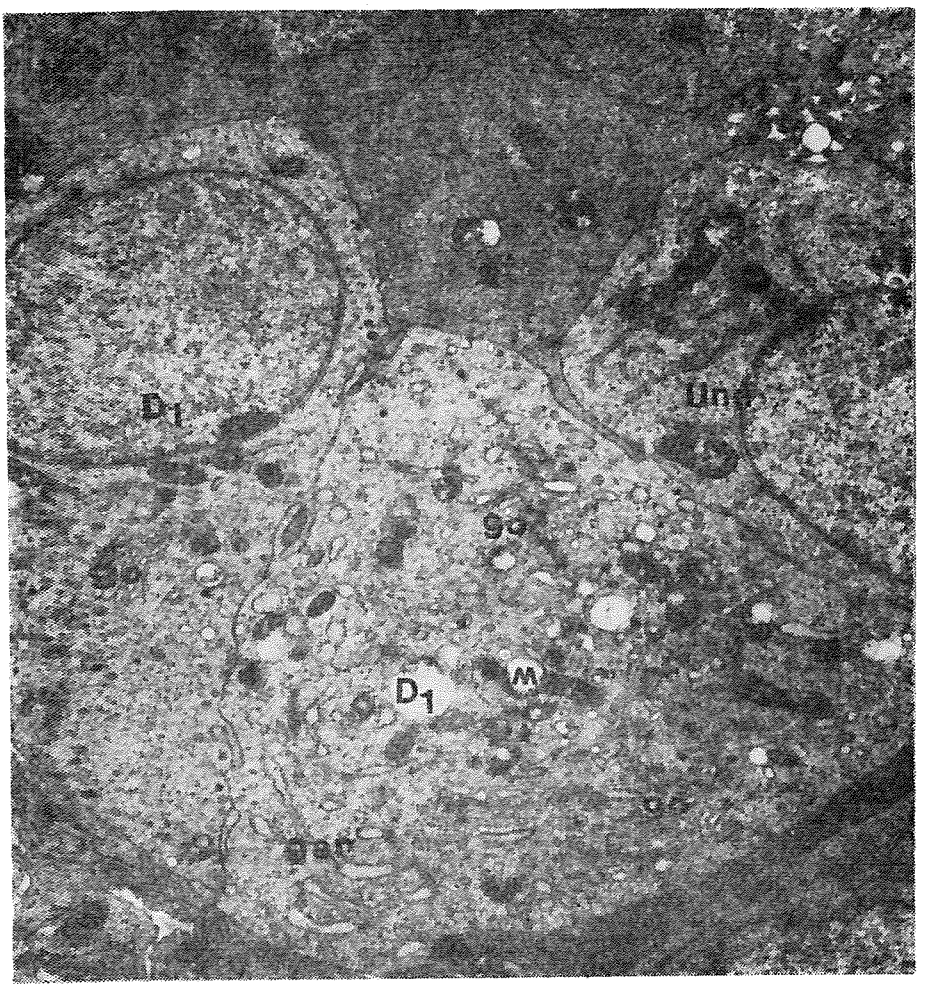

Figure 11

Newborn mouse. Two D_1-cells relating with undifferentiated endocrine cell (Und) and containing more than one Golgi complex (go), many mitochondria (M) and small amount of granular endoplasmic reticulum (ger) are noticed. X 14100

Undifferentiated Endocrine Cells: Studies on the developing endocrine cells in various mammalian gastrointestinal tracts have reported that the clear cells observed in the early gestation period and having no or only a few secretion granules in their cytoplasms were considered to be the immature or reserve cells of severel types of endocrine cells.[3, 4, 10, 11, 12]

Undifferentiated endocrine cells observed in this study were frequently seen throughout the period of development. Moreover there

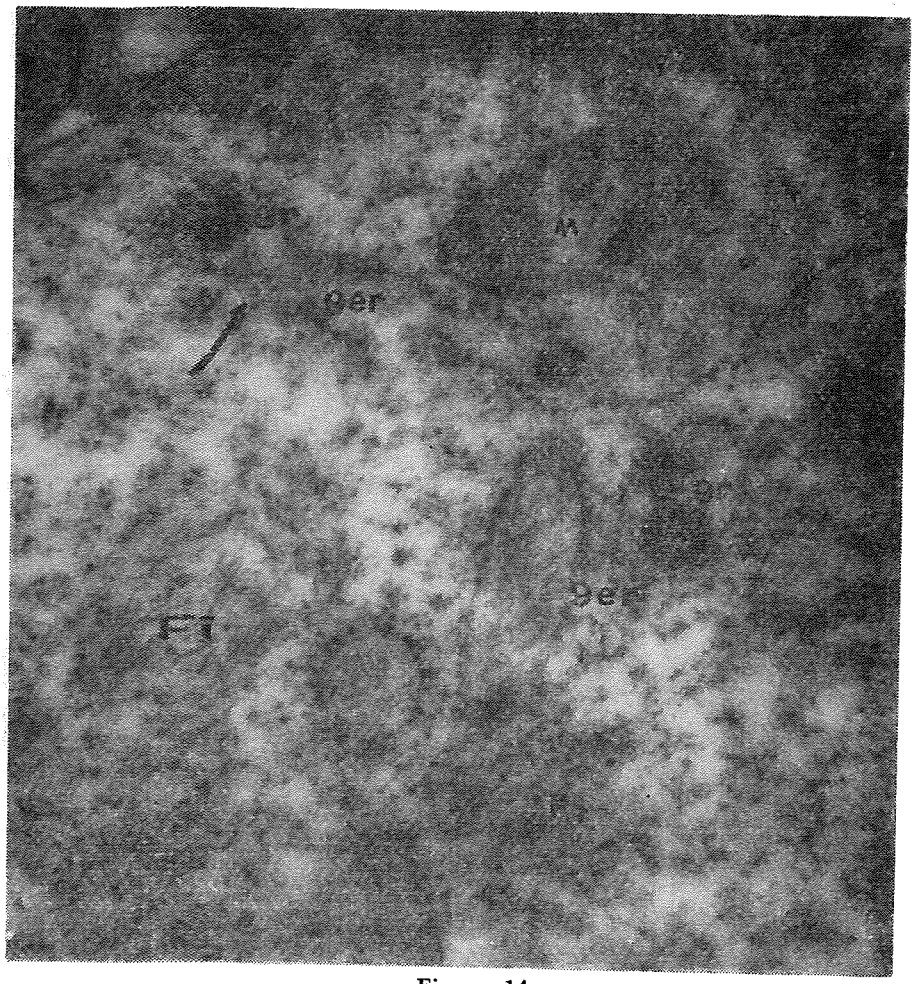

Figure 14

Newborn mouse. Detailed structure of formation of secretion granules in the cytoplasm of EC-cell. Secretion granules (gr) seem to form by pinching of distended cisternae of granular endoplasmic reticulum (ger and arrow), mitochondria (M), filaments (Fi) and ribosomes (Ri) are detected. X 93000

A-cells: The ultrastructure of the fetal A-cells is also like that of adult pancreatic A-cells except that the membrane of the fetal granules is apparently more tightly fitting.[13]

Fetal A-cells containing two types of granules, dense and light, have been described in the fetal human pancreas as a mixed type of A-cell[14] and in the corpus of fetal human stomach as AL-cells.[3] Two types of granules in fetal gastric AL-cells may represent different stages of matu-

ration of function of the cell and may be an intermediate type between A and D-cells in the pancreas.[3]

We were able to identify A-cells in the 19-day-old mouse embryos. In these cells secretion granules were of two types. Electron-dense-large granules were limited by a tightly fitting membrane. Light-small ones had discontinuous limiting membranes and there was a narrow space between membrane and granule content. As development advanced together with increase in other organelles and number of dense-large granules of A-cells, small-light granules decreased. These considerations have led us to conclude that the two types of granules in A-cells represent different stages of maturation.

In the study of human fetuses at from 6 to 26 weeks[15] a progressive increase in Glucagonlike-Immunoreactivity (GLI) was noted from about weeks 13 to 15 of gestation, with apparent peaks at approximately 17 to 12 weeks. Pollak et al[16] however found a few immunofluorescent cells in parts of the small intestine of 12-to 17-week-old fetuses, but not in the stomach.

In the present study developing A-cells were ultrastructurally identical to the adult ones. It is conceivable that the function ascribed to adult cells (Part I) may be applicable to fetal A-cells.

Enterochromaffin cells (EC): It has been reported that there might be two subtypes of EC-cells according to mammalian species, region of gastric intestinal tract and age of mammals.[3, 11, 16–24]

Swallowing of amniotic fluid and peristaltic movements of the gastrointestinal tract in human fetuses have been observed by 12 to 13 weeks of gestation.[25]

In 12-to 27-week-old fetal human stomachs, 5-HT has been detected in some cells by fluorescence microscopy.[26]

De Lemos[3] found EC-cells at the same time as gastrointestinal movements are known to commence. He reported that this situation represents the source of 5-HT stimulated muscular motility in the developing stomach. The relationship between EC-cells and 5-HT is also supported by the results of studies done with chicken embryo duodenums using fluorescence or electronmicroscopy.[27–29]

In the present study the EC-cells started to appear in the corpus of stomachs of new-born mice. They were poor in secretion granules and organelles. Their granules were in the formation stages. With those ultrastructural characteristics EC-cells in the corpus of stomach of newborn mice, may have the same function as the afore-mentioned adult form of EC-cells (Part I).

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Hacettepe Medical Journal
Volume 16 / No. 4 / October 1983 / pp. 186 – 190

Incidence, Prophylactic Chemotherapy and Malignant Transformation of Hydatidiform Mole*

Clinical Evaluation of 139 Patients

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Summary

139 patients with hydatidiform mole are presented in this report. The observed incidence of hydatidiform mole was 1/1171 pregnancies. Partial mole was found in 5 % of the patients. Malignant trophoblastic neoplasia developed in 2 % of the patients with prophylactic chemotherapy and in 6 % of all patients without chemotherapy. Mortality resulting from chemotherapy toxicity has been recorded for two cases. At the present time prophylactic chemotherapy is used in this institution in patients with high risk mole.

Key Words: Hydatidiform, Mole Prophylactic Chemotherapy, Malignant Transformation.

Introduction

In the past two decades advances have been made in the understanding and management of hydatidiform mole. This has been due to technical developments, better monitoring with hCG assays and the use of chemotherapy.[1] However, hydatidiform moles are well recognized to have a potential for local uterine invasion and distant metastasis.[2]

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About 80-90 % of moles have a totally benign outcome even without treatment, but severe morbidity and death have been reported after a single course of prophylactic chemotherapy.[3]

Today, although prophylactic chemotherapy has been advocated by some authors, it is routinely used only in patients with high risk mole.[4]

The purpose of this report is to evaluate our experience in patients with hydatidiform mole.

Materials and Methods

We reviewed the records of the 139 patients with hydatidiform mole treated at the Division of Gynecological Oncology, Department of Obstetrics and Gynecology, Hacettepe University Hospital, from January 1967 to April 1983. Of the 139 patients, 16 % (22) were 19 years or less and 8 % (11) were over 40 years old. Hydatidiform moles in 41 % (57) of the patients occurred in their first pregnancies.

Accurate diagnoses were made by pathologic examination of specimens obtained from each patient. Patients with hydatidiform moles are classified as low risk and high risk. Patients with high risk mole are characterized by having a serum hCG level above 100.000 mIU/ml a uterine size larger than usual for dates, and a number of associated medical conditions and epidemiologic factors, which include previous molar pregnancy, maternal age over 40 years, GHEP, coagulopathy, trophoblastic embolization and hyperthyroidism.

Before 1983, prophylactic chemotherapy was routinely used in all patients after molar evacuation. Today, it is used only in patients with high risk moles. 121 patients were treated with prophylactic chemotherapy administered daily in 5 day courses, utilizing methotrexate (25 mg. per day). Patients were followed weekly until hCG titers were normal for three consecutive weeks. Than, titers were obtained monthly for six months and bimonthly for the next six months. Oral contraceptives were used in all patients after molar evacuation.

Surgical procedures performed in patients with mole for molar evacuation are given in Table I.

Results

The incidence of hydatidiform mole was 85 per hundred thousand pregnancies (1/1171 pregnancies and 1/249 deliveries). Partial mole and tubal mole were seen in 5 % (7) and 1.4 % (2) of the patients, respectively.

TABLE I

SURGICAL PROCEDURES IN PATIENTS WITH MOLE

Surgical Procedures	Patients No.	%
Vacuum aspiration	111	80.0
Classical curettage	10	7.0
Hysterectomy	12	9.0
Hyseretomy	4	3.0
Salphingectomy	2	1.0
Total	139	100.0

Of the 139 patients with hydatidiform mole, 93 % (129) had vaginal bleeding with or without other symptoms, 14 % (20) had hyperemesis gravidarum, 14 % (20) had theca lutein cysts, 11 % (15) had GEHP prior to 20 weeks, 8 % (11) had hyperthyroidism (one clinical and ten biochemically) and 1.4 % (2) had acute abdomen due to the ruptured tubal mole. Although large uterine size for dates was 54 % (75), small and normal uterine size were 31 % (43) and 15 % (21), respectively. Initially the commercial pregnancy test was negative in 5 % (6)of the patients with moles who had high levels of hCG beta-subunite. The relationship between prophylactic chemotherapy and subsequently malignant trophoblastic neoplasia in patients with hydatidiform moles is shown in Table II.

TABLE II

THE RELATIONSHIP BETWEEN PROPHYLACTIC CHEMOTHERAPY AND MALIGNANT TROPHOBLASTIC NEOPLASIA

Prophylactic chemotherapy	No. of patients	Malignant trophoblastic neoplasia
Present	121	3
Absent	18	1
Total	139	4

Mortality due to prophylactic chemotherapy occurred in two patients. Recurrent mole in subsequent pregnancies was seen in three patients. One of these three patients had three consequtive moles.

31 patients had 44 later pregnancies which terminated in 30 full-term live births.

Discussion

The incidence of mole varies widely throughout the world. The highest incidence is reported in Taiwan at 1:120 pregnancies, while in the USA, it is reported to be between 1:1000 and 1:2000 pregnancies.[3,5] The incidence found in this report is 1:1171 pregnancies. It has been reported by Jacobs et al. that a total of 40 complete and 88 partial moles were identified by pathological and cytogenetic criteria in a population of 1602 spontaneous abortions.[6] Partial mole was found to be 5 % in this study. The majority of complete moles (excluding 3 to 13 % who had 46XY) have a 46XX chromosome constitution. However most partial moles are triploid.[6,7] The chromosome work-up in this series are not available for technical reasons. Although, after molar evacuation, the spontaneous remission rate is reported to be 80-85 %, the progression to malignant trophoblastic neoplasia requiring therapy is 15-20 %.[5] Goldstein et al., showed that malignant trophoblastic neoplasia after molar evacuation developed in 16 % of an untreated group and 2 % in the treated one.[4] The same figures for this study are 6 % and 2 % respectively. As indicated above, about 80-90 % of moles have a totally benign outcome even without treatment, but severe molar morbidity and death have been reported following a single course of prophylactic chemotherapy.[3] Two deaths due to chemotherapy toxicity occurred in this series. Therefore, today prophylactic chemotherapy is only used in patients with high risk mole, with high rate of aneuploid cells and with high levels of urinary log F_2/F_1 between 11 and 20 days after molar evacuation.[4,8,9] This protocol is supported by the Gynecological oncology group.[5]

Women with successfully treated moles have normal pregnancies with no increase in prematurity, congenital anomalies, perinatal or neonatal mortality and morbidity. However, there have been reports of increased partial placenta accreta and spontaneous abortions in patients previously subjected to chemotherapy.[3] In this series, of the 44 subsequent pregnancies in 31 patients with hydatidiform mole, 30 terminated with full-term live births. It has been reported that oral contraceptives do not appear to increase the risk of postmolar trophoblastic tumors and therefore may be safely prescribed after molar evacuation during the entire interval of gonadotropin monitoring.[2]

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Hacettepe Medical Journal
Volume 16 / No. 4 / October 1983 / pp. 191 – 197

Attitudes of Psychiatric Personnel and the Therapeutic Milieu*

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Summary

There is an increasing interest and effort in Turkish psychiatric services towards more therapeutic milieus in psychiatric wards. A prominent trend in the literature is to assess the attitudes of psychiatric personnel so that intervention for change in milieus can be programmed. The study is the first of its kind in the Turkish population, studying attitudes of psychiatric personnel and taking into account variables which may affect the outcome.

The Turkish adaptation of the "Custodial Mental Illness Ideology Scale" was used to assess attitudinal differences between three different institutions and three professional groups. Closed or open ward systems and staff-patient ratios per ward were investigated to see their effect on staff attitudes.

The results showed significant attitudinal differences among the three institutions but not within the institutions. The three professional groups showed significant differences favoring psychiatrists as the least custodial, nursing-aides as the most custodial and nurses in between the two groups. Open wards and high staff-patient ratios showed the least custodial attitudes compared to the high custodial attitudes of closed and low staff-patient ratio wards.

Key Words: Therapeutic Milieu, Attitudes, Psychiatric Personnel, Social Psychiatry, Psychiatric Wards.

Introduction

Mental health institutions and mental health personnel usually aim at a totally therapeutic environment and experience for their in-patients.

* Presented at VII. World Congress of Psychiatry, 1983.

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However, it is observed that quite a number of psychiatric institutions have social and physical environmental facilities which may negatively affect the therapeutic outcome.[1]

Under the influence of certain theoretical approaches which emphasize the individual and his/her environment in an interactive system, efforts to assess the social milieus of psychiatric hospitals and making them more therapeutic have increased considerably. Ego psychology, social psychology, social psychiatry and behavioral approaches have contributed most to this change of philosophy in psychiatric treatment.[2-7] These developments have led to the establishment of Milieu Therapy programs in both eastern and western psychiatric services.[8] This new approach, which started as a reaction to the custodial care systems of large psychiatric hospitals, stressed the etiological significance and the therapeutic effect of the patient's immediate social environment. Certain measures were taken to arrange the ward environment so that it would include therapeutic dimensions.

Besides initiating "Milieu Therapy" programs in psychiatric services, a large number of studies aimed to assess the social context of psychiatric wards. This social context was objectively and systematically investigated by studying psychiatric personnel and psychiatric patient groups in the wards. The general aim of the studies investigating the therapeutic milieu of psychiatric hospitals is to find out those milieu factors which decrease length of stay in the hospitals and the readmission rate of patients.

Currently there is increasing interest and effort in the Turkish psychiatric services towards more therapeutic milieus in psychiatric wards. Many principles of therapeutic milieu programs are applied, especially in university psychiatric hospitals. Yet there are no psychiatric institutions which have total commitment to all standards of a "Milieu Therapy" program.

A prominent trend in the literature is to assess the attitudes of psychiatric personnel so that intervention for change in milieus of wards can be programmed accordingly. This study is the first of its kind in the Turkish population studying attitudes of psychiatric personnel and taking into account variables which may effect the outcome.

Material and Method

The first aim of the study was to investigate the attitudes of personnel in six different psychiatric wards in Ankara. The second aim of the

study was to investigate the differences of attitudes between different occupational groups in psychiatric wards. As a third aim, closed or open ward systems and staff-patient ratios per ward were investigated to see their effect on staff attitudes.

The study was carried out in the psychiatric wards of the Ankara University Hospital, The Hacettepe University Hospital and the Gölbaşı Mental Health Institute. The subjects consisted of 65 personnel in these institutions. The Turkish adaptation of the "Custodial Mental Illness Ideology" scale was used to assess the attitudes of psychiatric personnel. Both custodial and humanitarian attitudes are included in this scale. Custodial attitude emphasizes the detention and security of patients who are regarded as potentially harmful to the society. On the other hand, humanitarian attitude emphasizes the individuality, freedom and responsibility of the patients. The reliability of the Turkish version of the scale computed by Kuder Richardson 20 technique is r = .68.

Results and Discussion

The attitudes of personnel in six different psychiatric wards were investigated. One-way analysis of variance and "t" tests were used to evaluate the data. The results showed that there were significant differences in attitudes of personnel among the six wards. However there were no significant differences in the attitudes of personnel within institutions (Table I).

TABLE I

DIFFERENCES OF ATTITUDES BETWEEN WARDS: ANOVA

Source of variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Within Groups	189.23	5	37.85	4.83**
Between Groups	461.91	59	77.83	
Total	651.14	64		

** $p < .01$

The differences of attitudes between three largely representative occupational groups were investigated. These groups were psychiatrists, nurses and nursing-aides. One-way analysis of variance and "t" tests were used to evaluate the data. There were significant differences between the three occupational groups. Further analysis showed that the nursing-aides were the most and the psychiatrists the least custodial in their attitudes, while the nurses were more custodial than the psychiatrists and less custodial than the nursing-aides. These results are parallel to vari-

ous studies showing that professional groups are the least custodial in their attitudes towards mental patients[9–17] (Table II, Table III).

TABLE II

DIFFERENCES OF ATTITUDES BETWEEN MEDICAL DOCTORS, NURSES AND NURSING-AIDES: ANOVA

Source of variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Within Groups	317.06	2	158.53	28.47**
Between Groups	328.49	59	5.57	
Total	645.55	61		

** $p < .01$

TABLE III

MEANS, STANDARD DEVIATIONS AND "t" SCORES OF DIFFERENT PSYCHIATRIC PROFESSIONAL GROUPS

Professional Groups	n	Mean	St. Deviation	Comparison of means	t
Medical Doctor	27	15.68	2.60	$\bar{X}_1 - \bar{X}_2$	3.95**
Nurse	14	12.57	1.95	$\bar{X}_1 - \bar{X}_3$	7.11**
Nursing-aide	20	10.55	2.26	$\bar{X}_2 - \bar{X}_3$	2.71**

** $p < .01$

Attitudes of personnel in open and closed wards were compared by "t" tests. The results showed that attitudes of personnel in open wards were less custodial compared to attitudes of personnel in closed wards.

Secondly, attitudes of personnel in wards with different staff-patient ratios were compared. One-way analysis of variance and "t" tests were used to evaluate the data. The results showed that attitudes of personnel in wards with high staff-patient ratios were less custodial compared to attitudes of personnel with low staff-patient ratios. Various studies done in other countries show that high rate of discharge and low rate of readmission is highly correlated with high staff-patient ratios in wards[18–21] (Table IV, Table V, Table VI).

TABLE IV

MEANS, STANDARD DEVIATIONS AND "t" SCORES OF PERSONNEL SCORES IN OPEN AND CLOSED WARDS

Wards	n	Mean	Standard Deviation	t
Open ward	47	14.32	2.81	4.40**
Closed ward	18	10.89	2.83	

** $p < .01$

TABLE V

DIFFERENCES OF PERSONNEL ATTITUDES ACCORDING TO STAFF-PATIENT RATIO: ANOVA

Source of variation	Sum of Squares	Degrees of Freedom	Mean Squares	F
Within Groups	102.12	2	51.06	5.77**
Between Groups	549.02	62	8.86	
Total	651.14	64		

** $p < .01$

TABLE VI

MEANS, STANDARD DEVIATIONS AND "t" SCORES OF PERSONNEL ATTITUDES ACCORDING TO STAFF-PATIENT RATIO

Wards	n	Mean	Standard Deviation	Comparison of Means	t
Ankara (% 44)	30	12.03	3.25	$\bar{x}_1 - \bar{x}_2$	2.02*
Hacettepe (% 54)	13	14.15	2.94	$\bar{x}_1 - \bar{x}_3$	3.23**
Gölbaşı (% 58)	22	14.73	2.57	$\bar{x}_2 - \bar{x}_3$	0.61

* $p < .05$
** $p < .01$

The results on the attitudes of psychiatric personnel in this study suggested that the measurement of attitudes was important in defining the therapeutic milieu of psychiatric settings. Significant differences of attitudes within institutions were not observed. Therefore it seems important to control the variable of treatment philosophy and orientation of wards in future investigations.

We can say that high staff-patient ratio and open ward systems contribute to therapeutic characteristics of the ward environment. Therefore future innovations in the Turkish psychiatric services should include plans for open ward systems and more psychiatric personnel.

The results on the attitudes of different occupational groups suggested that there should be more emphasis on the training of nurses and nursing-aides for attitude change. How to bring about attitudinal change in these two groups is another subject which should be closely investigated.

We can observe that it is important to assess the attitudes of psychiatric personnel towards mental illness and related ward characteristics which effect these attitudes. However, the perspective which equ-

ates attitudes of psychiatric personnel and the therapeutic milieu of wards is rather narrow and biased. A multifactorial approach to the assessment of therapeutic milieus seems essential to clarify the problem.

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Hacettepe Medical Journal
Volume 16 / No. 4 / October 1983 / pp. 198 – 201

Hypothyroidism Followed by Hyperthyroidism

Case Report

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Summary

The sequence of well documented hypothyroidism followed by hyperthyroidism has rarely been reported. We describe such a case and discuss the possible mechanisms.

Key Words: Graves' hyperthyroidism, hypothyroidism, TSH receptor antibodies.

Introduction

It is known that primary hypothyrodism requires lifelong therapy with thyroxine. Just as spontaneous remission from primary hypothyroidism is rare,[1] the sequence of well documented hypothyroidism followed by hyperthyroidism has rarely been reported.[2–11] In this report we describe a patient with hypothyroidism who became hyperthyroid 2 years later, and discuss the possible mechanisms.

Case Report

A 49-year-old man presented with non-pitting edema and puffiness around his eyes in September 1978. He had noticed decreased exercise tolerance and cold intolerance. He also complained of dry skin, a husky voice, and constipation. There was no past medical or family history of pertinent problems.

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On physical examination, the pulse was 64 beats per minute and regular. The patient was noted to have slow, slurred speech and physical lethargy. His skin was cold, pale and dry. There was periorbital puffiness. The thyroid gland was not palpable. The relaxation phase of his deep tendon reflexes was considerably delayed.

The diagnosis of hypothyroidism was confirmed with a T_3 of 0,35 ng/ml (normal range 1.2-2.3 ng/ml) and a T_4 of 1 µg/dl (4.5-12 µg/dl).

The ECG showed a sinus bradycardia with low voltages in all leads. A skull roentgenogram was normal.

Thyroid hormone replacement was started with levothyroxine, 25 µg/day; the dose was gradually increased to 0.15 mg/day. In July 1980, after 20 months of levothyroxine therapy, the patient presented with the complaints of tremor, heat intolerance, perspiration, tachycardia, and weight loss of 6 kg. The pulse was 120 per minute and regular. His skin was warm and moist, and the relaxation phase of his deep tendon reflexes was brisk. The thyroid was not palpable. Levothyroxine was discontinued and thyroid hormone levels were determined 21 days later; T_3 was 5 ng/ml and T_4 was 25 µg/dl. Treatment was begun with 300 mg propylthiouracil q.d. The patient discontinued his medication when symptoms of hypothyroidism reappeared one and a half months later. In November 1980 his T_3 was 3.9 ng/ml and T_4 was 3.7 µg/dl; propylthiouracil 200 mg q.d. was started again and the patient became hypothyroid one month later. His thyroid was diffusely enlarged to twice the normal size. Propylthiouracil was discontinued. Complete blood count, urine analysis, routine serum chemistries, and polytomograms of the sella were normal. Thyroid scanning showed diffuse hyperplasia with patchy uptake of the isotope. ^{131}I uptake was 51 % at 2 hours, 50 % at 6 hours, and 36 % at 24 hours. T_3 was 2.2 ng/ml and T_4 was 1.5 µg/dl. TSH was 2 µIU/ml (normal range 0-5 µIU/ml.)

The patient underwent thyroid surgery and a subtotal thyroidectomy was performed. Pathologic examination showed proliferation of the follicular epithelium that formed papillary projections. The epithelium had a high cuboidal character and the follicles were devoid of colloid. The walls of the interstitial vessels were thickened.

The postoperative course was marked with periods of hyperthyroidism, hypothyroidism, and euthyroidism. The patient was given propylthiouracil 300 mg/day when he was hyperthyroid and soon became hypothyroid. After discontinuation of propylthiouracil, he has remained euthyroid to the present.

Discussion

This case illustrates the unusual series of events in which Graves' hyperthyroidism develops after an unquestionable diagnosis of hypothyroidism has been made. There has been one report of spontaneous remission from primary hypothyroidism,[1] and others have described hyperthyroidism following either primary hypothyroidism or Hashimoto's thyroiditis.[2–11] One would not expect a gland with primary atrophy, or autoimmune ablation to ever recover and then hyperfunction; rather the patient is expected to need continued thyroid hormone replacement.

There are several possible mechanisms for the development of hyperthyroidism following hypothyrodism. Two previous reports proposed the stimulation of a thyroid nodule by an excess of TSH, leading to autonomous function of the nodule.[2, 7] Our case does not support this theory. In addition there was no evidence for transient exposure to a goitrogen or for hyperthyroidism secondary to the correction of iodine deficiency. Moreover, none of these mechanisms explain the preceeding hypothyroidism satisfactorily.

It appears likely that the initial hypothyroidism follows a chronic autoimmune thyroiditis in which part of the gland escapes the destructive process and remains responsive to thyroid stimulating antibody. Another possibility is the presence of TSH receptor antibodies that bind to and block the TSH receptor without stimulating the cell. In support of the latter hypothesis, TSH receptor antibodies have been detected in euthyroid patients[12, 13] and hypothyroid patients with Hashimoto's thyroiditis.[14, 15] Other antireceptor antibody diseases, such as myasthenia gravis, are known to involve antibodies that bind to a receptor and block its normal function. The subsequent development of hyperthyroidism would be due to the production of TSH receptor antibodies that stimulate the thyroid cell.

In predisposed patients thyroxine treatment may influence lymhocyte function sufficiently to precipitate a second autoimmune disorder. Thus, in patients with an underlying genetically determined abnormality of regulatory cells thyroxine treatment may lead to the development of Graves' hyperthyroidism if functioning thyroid tissue remains.

Our patient had a clinical course that suggested the presence of TSH receptor antibodies of two different types. When blocking antibodies were prevalent, the patient probably became hypothyroid, and later developed hyperthyroidism when stimulating antibodies reached high levels.

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Hacettepe Medical Journal
Volume 16 / No. 4 / October 1983 / pp. 202 – 206

Desmoplastic Fibroma

A Case Report

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Summary

A case of desmoplastic fibroma arising in the iliac bone and femoral head of an eighteen-year-old man is described. The lesion is discussed, together with a brief review of the literature.

Key Words: Fibroma, Bone Tumor, Arthrodesis.

Introduction

The term desmoplastic fibroma was first used by Jaffe[1] to describe an intra-osseous fibrous tumor. By the use of this term, Jaffe also differentiated it clearly from fibrosarcoma, non-ossifying fibroma and chondromyxoid fibroma. The disease occurs at any age. The lesion is uncommon, and about fifty patients with desmoplastic fibroma have so far been recorded in the world literature.[2–7]

The present paper reports one case of this tumor, which was located in both iliac bone and head of the femur.

Case Report

A boy of eighteen came to the hospital in May 1981 with a two-years' history of vague discomfort and pain around the left hip.

Physical examination showed that the patient walked with crutches. No mass could be palpated. The hip motion was restricted and painful. Laboratory findings were within normal limits.

A radiograph, showed an osteolytic tumor in the left ilium which expanded into the whole acetabuler area and superior pole of the fe-

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moral head. The lesion was well defined and had a sclerotic margin (Figure 1). No soft tissue mass was seen. The roentgenograms suggested either giant cell tumor or chondromyxoid fibrom. Biopsy at this time showed a desmoplastic fibroma (Figure 2). Macroscopically, it was whitish-grey in color, rubbery in consistency and showed no calcification. Microscopic examination showed there was an abundance of intercelluler tissue rich in collagen fibers, with small homogenous fibroblasts oval in shape with nuclei of benign appearence (Figure 2). Curretage, bone grafting and arthrodesis of the hip were advised to the patient and an operation was performed as described below.

Under general anesthesia anterior iliofemoral incision was made, left ilium and femoral head were widely explored. The periosteum of the affected bone showed no macroscopic abnormality. A window of about five to six cm. opened in the inner aspect of the ilium. The meduller cavity and also superior part of the femoral head were found to be filled with a dense, rubbery and whitish-grey mass.

After thorough curretage of the lesion, the defect was packed with chips of autogenous iliac bone and a graft from the ribs placed between the ilium and femoral head, and hip arthrodesis was performed. Recurrence was not evident after 18 months (Figure 3).

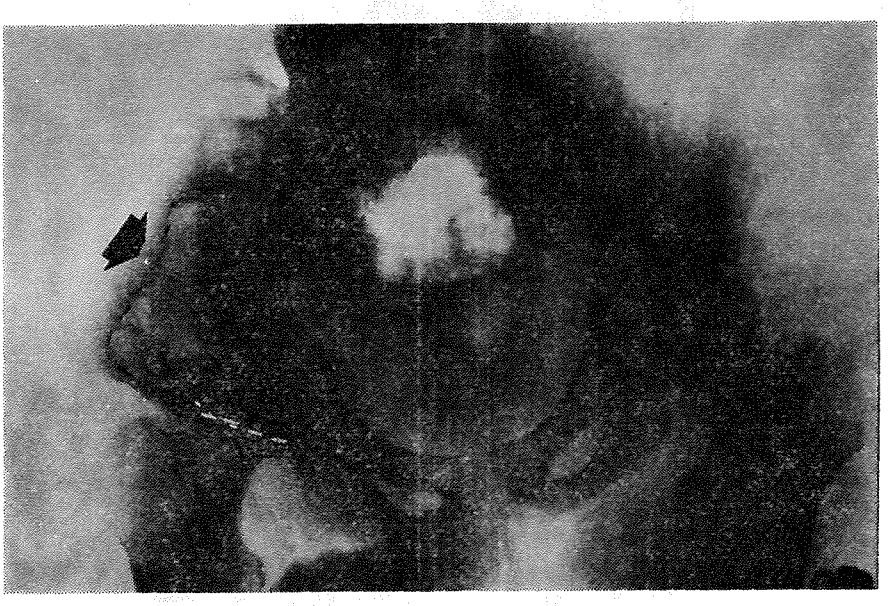

Figure 1

Radiograph showing iliac and femoral involvement by the osteolytic tumor.

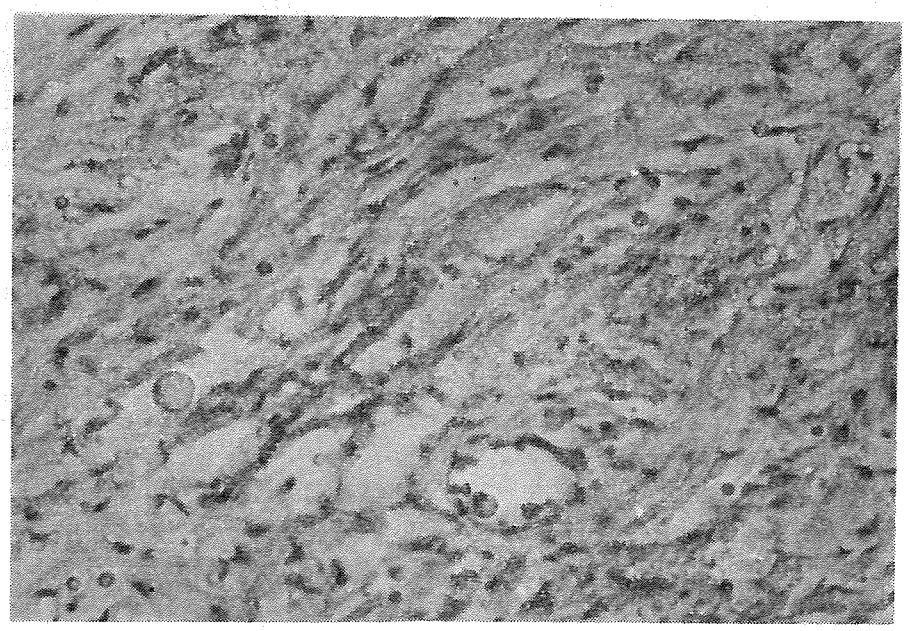

Figure 2
Photomicrograph showing an abundance of intercelluler tissue rich in collagen fibers, small fibroblast with oval innoculous nuclei (Hematoxylin - Eosin X 120).

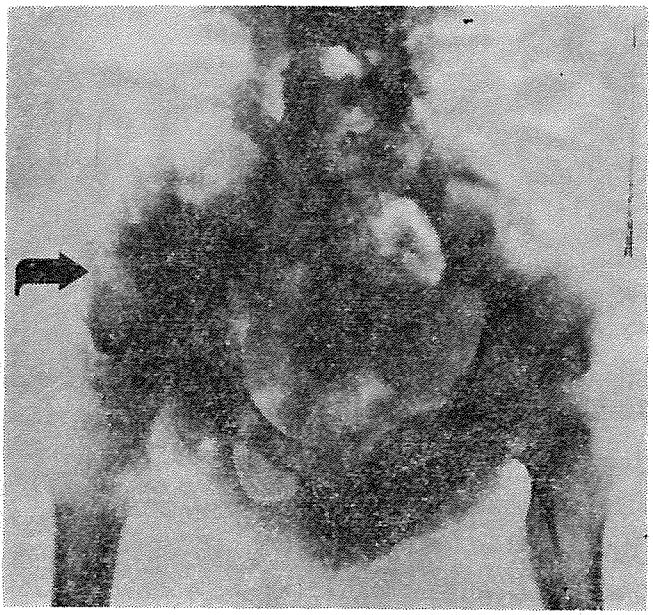

Figure 3
Eighteen months after operation.

Discussion

In 1958 Jaffe[1] used the term desmoplastic to distinguish this tumor from fibromas which are non-ossifying fibroma, chondromyxoid fibroma, periosteal fibroma and osteogenic fibroma.

This tumor was shown to have occurred in a wide variety of bones and more than one case was recorded in the reports of Jaffe,[1] Whitesides,[8] Cohen[3] and Nilsonne.[5]

In a typical case the tumor resembles the other benign fibrous tumors of bone. Although the tumor is seen in the long bones, it also has been noted in the flat bones such as the pelvis, scapula and skull. The physical findings elicited depend on the location and growth of the tumor.

The onset of the tumor is so insidious that the symptom is reproduced only when the tumor grows large. In the long bones, the tumor mostly affects the metaphysis. Sometimes it extends to the epiphyseal region.

The misdiagnosis of desmoplastic fibroma is common. Desmoplastic fibroma is roentgenographically similar to solitary bone cyst, aneurysmal bone cyst, giant-cell tumor, chondromyxoid fibroma and fibrous dysplasia. These tumors are easily differentiated from desmoplastic fibroma by histological appearence.

Histologically, desmoplastic fibroma must be distinguished from well differentiated fibrosarcoma. This distinction can be extremely difficult.[1,9] The presence of mitosis does not indicate malignancy.

The treatment of desmoplastic fibroma is wide resection followed by bone-grafting. In our patient, the treatment of choice was is curretage of the ilium and femoral head, followed by bone grafting and hip arthrodesis. Recurrence was not evident after 18 months in our case. If the lesion recurs, the wider resection may be indicated.

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Hacettepe Medical Journal
Volume 16 / No. 4 / October 1983 / pp. 207 – 215

Stem Cells and Hematopoietic Microenvironment*

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In human beings, clinical and several experimental observations suggest that virtually all the hematopoietic cells are normally produced in the bone marrow. The erythrocytes, granulocytes, platelets, monocytes and lymphocytes originate from a pool of self-perpetuating bone-marrow stem cells. This pool is believed to be made of multipotential stem cells which include the most primitive ones and later partially differentiated stem cells committed in the bone marrow which respond to humoral regulators such as erythropoietin and are primarily responsible for maintaining the level of mature blood elements. Within the last decade or so, we have observed some major developments in the field of stem cells which give some insights into the complex control of proliferation and differentiation processes during hemapoiesis.[1]

Recent developments in clonal cell culture techniques for hemopoietic progenitors have made it possible to study the mechanisms of stem cell differentiation and have provided quantitative methods for investigation of hemopoiesis.[2, 3] In 1961, Till and McCulloch demonstrated that the cells which form colonies in the spleens (CFU - S) of heavily irradiated syngeneic recipient mice are able to self-renew and produce cells that reveal terminal differentiation in red blood cell, white cell, platelet and monocyte lineages.[4] This colony-forming-unit-spleen (CFU-S) was a demonstration of a pluripotent stem cell. Later, growth of committed progenitors of both myeloid (CFU-C), megakaryocytic (CFU-M) and early (BFU-E) and late erythroid (CFU-E) cell lines were studied in "in vitro" assays utilizing semisolid media.[3, 5–7]

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In recent studies, Johnson et al., Hara and Ogawa and his co-workers described hemopoietic progenitors that are capable of differentiation in more than three cell lines in cultures of fetal-mouse-liver and adult mouse marrow cells.[8, 9] In 1979, Fauser and Messner described human multipotential hemopoietic progenitors in cell cultures that are capable of differentiation into granulocyte-erythrocyte-macrophage-megakaryocyte (CFU-GEMM) lineages.[10] It appears likely that CFU-S is located very close to CFU-GEMM and the exact terminology hasn't been clearly resolved. A lineage diagram of current thinking on hemopoiesis and colony-forming cells is outlined in Figure 1. In their report, Nakahata and Ogawa have described a class of hemopoietic stem cells that reveal extensive self-renewal capacities.[11] Their analysis of individual stem cell colonies had revealed concurrent and high incidence of spleen colony-forming units and macroscopic CFU-GEMM in culture. Their studies resulted in identification of a class of hemopoietic stem cells that appear to be more primitive than CFU-GEMM in the lineage of these stem cell colonies and provided an assay for the class of primitive hemopoietic progenitors.[11]

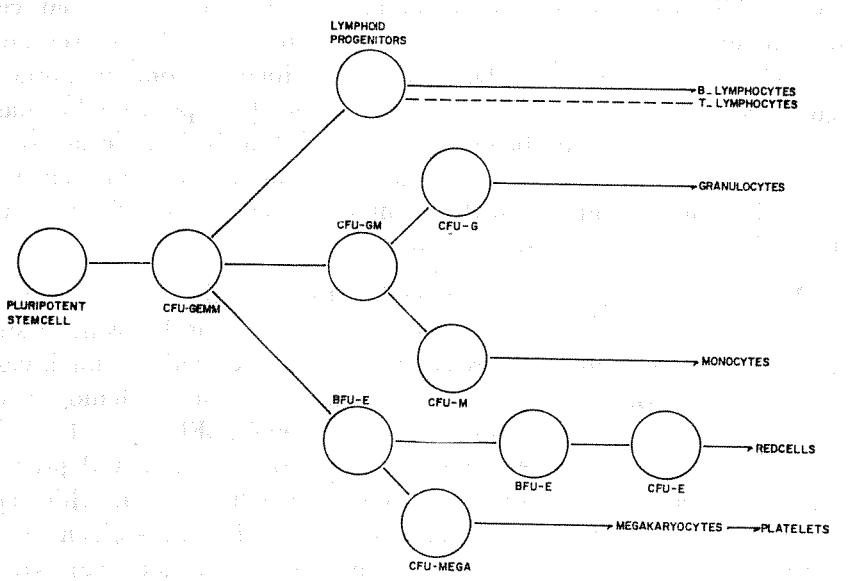

Figure 1
A diagrammatic model of hematopoietic activity.

The unipotential stem cells have been shown, by the use of "suicide" techniques, to be in active cell cycle and capable of self-renewal for a considerable period of time. The colonies designated as CFU-E (colony-forming unit-erythroid) are identical with unipotential and erythropoie-

tin responsive stem cells. In addition to these early colonies (CFU-E) occurring in cultures a second kind of colony which begins to appear late and grows to macroscopic size is called "burst" and the responsible cell is called "Burst-forming unit-erythroid-BFU-E." BFU-E represent erythroid progenitors earlier than CFU-E. Growth of committed progenitors of early (BFU-E) and late (CFU-E) erythroid cell lines have been studied in "in vitro" assays. The cells that appear in the final stages of erythroid progenitor development are capable of proliferative or differentiative responses to erythropoietin. The factors that influence the replication and maturation of immature committed or uncommitted progenitors have not been identified. The terminal differentiation of immature progenitors into morphologically recognized colonies requires certain inducer molecules "in vitro". This induction known as "Burst Promoting Activity" (BPA) could be a product of T-cells, monocyte lines or lectin-stimulated spleen cells.[12–14] Its in vitro presence is required to help erythropoietin to stimulated erythroblast formation from erythroid progenitors.

Granulocytes and monocytes (macrophages) appear to arise from a common progenitor, the CFU-GM. These large granulocytic colonies (CFU-G/M) consist of many thousands of mature cells. A smaller cluster of immature myeloid cell, a pre-CFU-G/M, has also been recently described in man.[15] The in vitro proliferation of myeloid progenitors is dependent on colony-stimulating factor or activity (CSF or CSA). CSA has been found in serum urine and many tissues, and is generally provided by a variety of conditioned media. This activity is capable of transforming granulocyte-committed unipotential stem cells to myeloblasts. Although it acts as a "leukopoietin" in vitro, its physiologic significance, if any, for in vivo granulopoiesis has not been resolved. In 1981, Chiao et al. demonstrated that the products of T-cell can induce the terminal differentiation of phagocytic cell lines.[16]

Megakaryocytic colonies designated as CFU-M originating from bone marrow have been described but their relationship to physiologic thrombopoiesis is also unknown.

All these above-mentioned stem cells, progenitors and their differentiated forms and mature blood cells constitute normal hematopoiesis and it occurs within a specialized physical and functional microenvironment in the bone marrow. In the embryo, hematopoiesis begins in the yolk sac, switches to the spleen and liver, and finally settles in the bone marrow. Marrow sinus endothelial cells are covered incompletely on their abluminal surface by adventitial reticular cells. Hematopoietic cells are supported in extravascular spaces by the reticular cells and reticular-cell derived fibrils (Figure 2).

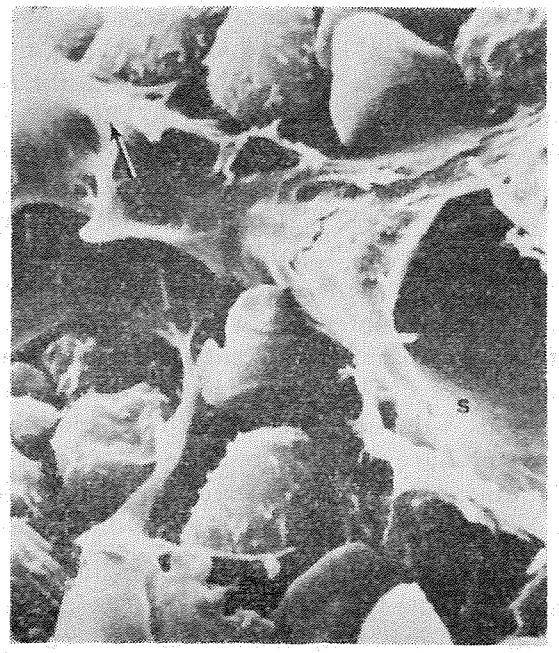

Figure 2

A venous sinus (s) crossing the field with luminal endothelium exposed but covered by adventitial reticular cells. The cytoplasmic projections (arrow) of these cells extend far into the hematopoietic cells on both sides. (From Weiss, L: reference No: 17).

Studies of the ultrastructure of bone marrow done in mouse or rats showed that the marrow is highly organized with a spoke-like pattern of venous sinuses and cords of hematopoietic tissue[17] (Figure 3). The cords are percolated by arterial blood draining into the central venous sinuses through a fenestrated basement membrane partly covered on the inside by endothelial cells and on outside by reticular cells. Projections from the reticular cells subdivide the cords and provide support for hematopoietic cells. (Figure 2). Human marrow does have a sinus system that is analogous to that of other mammals. The nonhemopoietic structures and cells of marrow can be outlined as follows:[18]

Neural Structures:

Myelinated and Unmyelinated Nerve Fibers, Schwann Cells

Vascular Structures:

Arteries
Veins
Sinuses
Endothelial Cells
Basement Lamina
Adventitial Reticular Cells

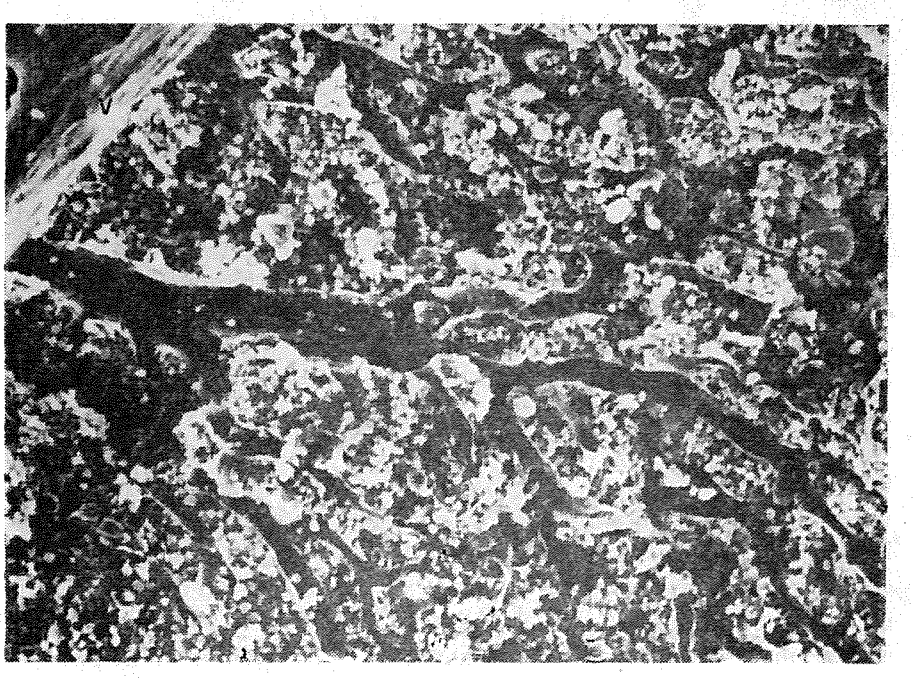

Figure 3

Cross section of bone marrow with spokelike sinusoids draining into a central longitudinal vein (upper left). Close view of a sinusoidal membrane and its components. (From Weiss, L: In Gordon, A.S (Ed): Regulation of Hematopoiesis. Vol. 1. New York, Appleton-Century Crofts, 1970).

Preadipocytes and adipocytes
Fibroblasts

Among these non-hemopoietic elements of marrow with the aid of ultrastructural techniques we can recognize the endothelial cells, basement membrane, adventitial reticular cells and adipocytes. Endothelial cells which are broad and flat form a complete covering for the inner surface of the sinus. In cross-sections, the cell junctions are distinct and often are overlapping or interdigitating. A very fine basement membrane underlies the endothelial cell layer. The adventitial surface of the vascular sinus is composed of reticular cells. They have extensive cytoplasmic processes which envelop the outer wall of the sinus and form an adventitial sheath. The cytoplasmic projections of reticular cells extend into the hemopoietic compartment and form a network on which the hemopoietic cells rest. These cells and their processes with their fibrils constitute the reticular skeleton of marrow.[18]

Adipocytes or fat cells in marrow develop by lipogenesis in cells that are probabaly closely related to fibroblasts. Adipocytes are of parasinal which would be also consistent with their origin in adventitial reticular cells, whose cell bodies are closely applied to the sinus wall. Fibroblasts, fat cells, macrophages, nerves, endothelial surfaces and other non-hemapoietic components of human marrow complete the marrow microenvironment.

Marrow stromal cells can form fibroblast colonies (CFU-F) in culture[19] and these cells are now thought to represent an important component of the hematopoietic microenvironment. Fibroblast colonies (CFU-F) are recognized and can be grown in liquid and short term human semi-solid culture systems. Fibroblasts grown from human marrow were used as underlayers for agar of methyl-cellulose cultures containing bone-marrow cells. It was found that fibroblasts enhance granulopoiesis when colony-stimulating factor was present in the cultures. The combination of fibroblasts and CSF stimulate more granulocyte/Macrophage colony forming cells (CFU-G/M) than are expected from their individual colony stimulating activities. In contrast, when we examine the BFU-E formation or colony numbers stimulated by erythropoietin in the presence of fibroblasts we find that the fibroblasts grown from human bone marrow suppress the formation of BFU-E and mixed colonies (granulocytic-monocytic-erythroid).[20] But all these studies have looked into the effect of fibroblasts on committed hemopoietic precursor cells and the results cannot be used to deduce what effects the various stromal cells might have on pluripotent stem cells in the marrow.

The other stromal cells commonly seen in the hemopoietic cords are macrophages. Macrophages are either surrounded by developing erythroid cells forming "erythroid islands" or they are seen in the vicinity of sinuses (parasinal macrophages). In some studies, it was found that the macrophages had no important effects on the growth of CFC-GM or BFU-E. Their presence in colony cultures may produce a slight reduction in CFC-GM numbers when CSF is present and did not stimulate colony formation in the absence of CSF. The presence of endothelial cells in continous cultures has been debated but the demonstration of Factor VIII antigen and other cell by products has provided good evidence for their presence.[21] Endothelial cell cultures proved to be a potent source of CSF and these cells are usually found to be efficient stimulators of CFC-GM colonies but their culture supernatants have no significant effect on either BFU-E or Mixed-CFC colonies.

Non-cellular components of the stroma have not been adequately studied in vivo. The presence of collagen fibers in developing marrow

is well substantiated, but the exact nature of reticulin fibers remains to be better determined.

The ground substance of the bone marrow probably consists of mucopolysaccharides which can be neutral or acidic. As may be seen from these studies, in normal hemapoietic marrow these stromal cell components namely fibroblasts, adipocytes, reticular and endothelial cells are found in close association with the potent stem cells and the differentiation of restricted progenitor cells.

In terms of the origin of the stromal cells forming the in vitro microenvironment, it was found that in vitro microenvironment in long-term cultures generated from marrow transplant recipients is donor-derived and in part consists of endothelial like cells.[22] Indeed, transplantation of human marrow has revealed the hematopoietic origin of the osteoclast, the Kupffer cell, the pulmonary macrophage, and the marrow microenvironmental cells.[22, 23]

The hematopoietic inductive microenvironment (HIM) model proposed by Trentin and his associates is based on their sequential cytochemical examination of spleen colonies.[24] They claimed that the commitment of pluripotent hemopoietic stem cells to unipotent ones is determined by a specific microenvironment. However, the recent observation by Magli, et al.[25] of the transient nature of early spleen colonies presented a need for re-interpretation of the data on which the microenvironment model was established. Recently, Nakahata and Ogawa[11] observed that a single hemapoietic stem cell colony reveals multilineage differentiation upon replating into a new culture dish. They showed that the replating of those colonies revealed their self-renewal capacity and the extensive ability to generate secondary colonies, many of which were multipotential hemopoietic colonies. These observations suggest that the microenvironment is not obligatory for stem cell commitment or at least cast some doubts on the instructive models of stem cell regulation. Clearly, much more work must be done before the complex interrelationships between hemopoietic cells, humoral factors and stromal cells can be fully understood.

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AUTHOR INDEX

Hacettepe Medical Journal, Vol: 16, 1983

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