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EDITORIAL

On A Fertile Soil...

A convenient link between East and West, Anatolia, with its beautiful nature, climate and inexhaustible resources, is the site where not less than a dozen civilizations were born or grew up. The history of the civilization of this land goes back 4,000 years, being one of the oldest in the whole world.

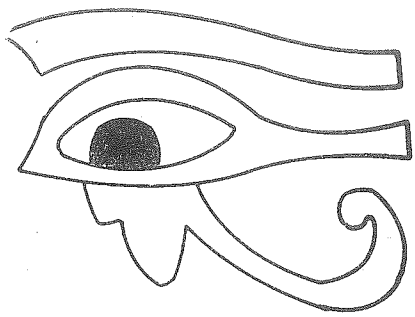
Almost everybody is familiar with the names of such places as Istanbul (Constantinople), Ankara (Angora), İzmir (Smyrna), Trova (Troy), Bergama (Pergamum) and Efes (Ephesus). In addition to these there are hundreds of others with equal importance from an historical and scientific point of view, such as Edirne, Boğazköy (Hattusas), Konya, Van, Sardes, Gordium and Istanköy (Cos). It is certain that at least six of these places were the locations of capitols of highly civilized peoples such as the Hittites, Byzantines, Lydians, Phrygians, Selçuk Turks and Ottoman Turks. Aegean and Mesopotamian civilizations were also formed on the very same soil. Ankara, the modern capitol city of the young Turkish Republic, is the last pearl on this wonderful necklace of immortal cities.

As a crucible of various cultures of all ages, Anatolia is a priceless treasure not only for the tourist's eye, but also for the real scientist, who, being removed from racial and religious considerations, is interested solely in studying the unknown aspects of the history of civilization in our world.

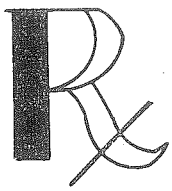
Some of the cities mentioned above are the birthplaces of outstanding figures in the history of medicine. Hippocrates, for example, usually called the Father of Physicians, was born in Istanköy in the western part of Anatolia. Galen, the Father of Experimental Physiology, as he is often called, is also a product of fertile Anatolian soil, born and having practiced in Pergamum. For about seven hundred years, the famous Aesculapium in this city was a

center of medicine for this area and was dedicated to Aesculapius, son of Apollo, the Chief God of Healing. The ruins of this sanitarium are still there, with case histories of cured patients and methods of treatment carved in stone. Today's symbol of men of medicine comes from the serpent-twisted staff which stood in the great square of Pergamum. To Galen we are indebted for the familiar sign R_x which evolves from the Eye of Horus, the mystic symbol which he used to impress his patients. Soranus of Ephesus, 2nd Century A. D., is the ancient authority on obstetrics, gynecology and pediatrics.

The Staff of Aesculapius



The Eye of Horus



The Symbol used on all Prescriptions

Starting with the Hittites, different communities built institutions, hospitals, bridges, aqueducts and schools of all types in Anatolia. The Turks, as they appeared on the scene of history, paid special attention to science and to medicine as a very valuable and superior profession. They practiced medicine and made many contributions to it. Yusuf Has Hacib (1069 A.D.), the Vezir of the Turkish State in Turkistan called Karahanlılar, when classifying different folk groups in the community, uses the following expressions in his world famous book *Kutadgu Bilig* : «One of them is the physician,» «He is the finder of remedies for all illnesses,» «Keep the physician close to you and treat him well,» «Keep his rights secured; he is the necessary man.»

As rulers of a stable, strong and highly civilized empire, it is not surprising that the Selçuk Turks built hundreds of bridges, schools, medical schools and hospitals, including mental hospitals and leprosaries. The Selçuks were experts at carving stone with beautiful designs, inscriptions and flowers; their buildings are still in good shape scattered all over this land today. One of the most

famous hospitals which they built in honor of Gevher Nesibe, a Selçuk princess, in 1205 A.D. is in Kayseri.

In the following centuries, during the Ottoman Turkish Empire, additional hospitals, at least three leprosaries, baths, aqueducts, arches and hundreds of mosques with connected medical schools and libraries were built. Turkish physicians wrote many books on different subjects and almost all of the famous books of medicine, starting from Hippocrates, were translated into Turkish. The libraries in Turkey are full of these books. In 1699, the first Turkish treatise on pediatrics, *The Book of Medical Measures for Children,* was written by Şâban Şifaî. It is worthwhile mentioning that one architect alone, Great Sinan, chief architect of Suleiman the Magnificent, built over 400 masterpieces, more than 300 of which are still standing in Anatolia as monuments to the Ottoman civilization.

Everyone will recall that preventive medicine and in particular smallpox vaccination, was practiced by Turkish physicians in Anatolia for several hundred years, a fact unknown until the famous letter of Lady Montagu, wife of the British Ambassador to Istanbul, in 1717 informed Europe that there was a preventive measure against smallpox in Turkey and that she had had her children vaccinated by the method used here. During those days, Anatolian civilization was equal to and even superior to the Western one in many fields.

Western authors are generally unaware of Turkish contributions to science and medicine, because they classify everybody according to their names and the languages used in their publications. Turkish scientists of those days used Arabic or Persian names and wrote their books mostly in these languages, because Ottoman culture was a mixture of Turkish, Persian and Arabic cultures. Although the Turkish Society was the ruling force, the scientific and poetic language was either Arabic or Persian. It is a great mistake to classify all outstanding persons in history according to their names or the language they used, without examining existing conditions. For instance, the editor of this *Journal* is not an Arab, just because his name is Arabic and means «successful.» Nor is he English nor American, because he has written this editorial in English. I am sure that a new look at the history of medicine in Anatolia from a scientific point of view would be of undeniable importance.

After 1923, under the leadership of Kemal Atatürk, the founder of the Turkish Republic, the Arabic alphabet was abolished and

the Latin alphabet was adapted to Turkish. Parallel to the establishment of new and modern institutions, medical faculties were founded in Ankara, İzmir, Erzurum (Atatürk University) and finally in Diyarbakır, in addition to the original Faculty of Medicine in Istanbul.

Hacettepe University, where this *Journal* originates, was founded as a Research Institute of Child Health, connected to Ankara University Faculty of Medicine, in 1957. In 1963, just like the budding of a yeast cell, it became another faculty of medicine, still attached directly to Ankara University. After a short period, it was organized as a third independent university in Ankara. This fertility of the Anatolian soil is still in existence. The first journal published by this institution, *The Turkish Journal of Pediatrics,* has already completed its tenth year. Hacettepe is now a university, with schools, clinics and institutes in fields related to pediatrics and medicine, and also the social sciences, humanities and basic sciences. For the scientists working outside the field of pediatrics, a necessity arose to publish independent journals to cover their fields of research. Professor İhsan Doğramacı, President and founder of Hacettepe University, and colleagues decided to publish three additional Journals, *Medicine and Surgery, Science* and *Social Sciences* in both Turkish and English.

This *Journal,* which you see for the first time, is another product of Hacettepe and we hope to publish your papers in the fields of medicine and surgery to create another link between Turkish and foreign scientists, and thus secure an exchange of knowledge and information for the better health of mankind.

I sincerely hope that our wish will come true.

Muvaffak Akman, M.D., M.P.H.
Editor

Studies on Red Cell Production in Adrenalectomized Rats

RESPONSE TO ERYTHROPOIETIC STIMULATION BY ERYTHROPOIETIN AND TO HORMONAL REPLACEMENT

Mithat Torunoğlu, M. D. *

The constancy of peripheral red cell and hemoglobin levels in healthy individuals is well established. This indicates a highly efficient homeostatic regulation. In the last three decades, studies demonstrated humoral control of erythropoiesis and led to the discovery of an erythropoiesis-stimulating factor in the plasma, later named erythropoietin. Subsequent work describing the site of production of this factor, its mode of action and its rôle in the control of erythropoiesis clearly established an important homeostatic function for erythropoietin.[1 - 8]

That erythropoietin is not the sole factor in control of erythropoiesis is evident from many clinical and experimental observations related to endocrine glands, including the adrenals.[9-13] Experimentally, removal of adrenals in the rat and mouse causes an anemia associated with a decrease of erythroid cells in the marrow.[14] Following administration of whole adrenocortical extract or cortisone to adrenalectomized rats, an erythropoietic stimulation has been observed in the marrow.[15 16] Yet, several investigations using ACTH[17] and cortisone[18] in intact rats have shown a depressive effect upon red cell production. Bone marrow metabolism studies demonstrated increased glycolysis following adrenalectomy in the rat.[19] After prolonged administration of ACTH, bone marrow cellularity reduces by 35 per cent.[17] Thus, a controversy exists as to the action of adrenal corticoids upon erythropoiesis and the rôle of the adrenals in red cell homeostasis still remains obscure. The present work deals with the rate of red blood cell production in adrenalectomized rats under the conditions of stimulation by

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erythropoietin and hormonal replacement, in an effort to elucidate the influence of the adrenals on red cell production and homeostasis.

Materials and Methods

Male and female albino rats, inbred in this laboratory, weighing 200 to 300 gm and 120 to 250 gm, respectively, and rabbits weighing 1850 to 3250 gm were used throughout the experiments. Animals were fed chow (product of Ankara Yem Fabrikası, Ankara), wheat, milk and greens, supplemented with I. M. injections of iron and B complex vitamins including vitamin B_{12} for a period of one week before starting the experiments.

Adrenalectomy was performed according to the dorsal approach method of Farris and Griffith [20] which was standardized in this laboratory. Adrenalectomized rats were given one per cent saline as drinking water, ad libitum, and were kept in individual metabolism cages in a room maintained at 24° to 28°C.

Anemic plasma filtrate containing high erythropoietin activity was obtained in rabbits by daily 1 ml subcutaneous injections of 2.5 per cent aqueous solution of phenylhydrazine hydrochloride (B.D.H.). The filtrate, prepared according to Borsook's method, [21] was diluted with distilled water. Intact and adrenalectomized rats were given daily subcutaneous injections of the filtrate in amounts corresponding to two per cent of body weight.

Radioactive iron incorporation studies in both normal and adrenalectomized rats were done by injecting one or two microcuries of Fe^{59} into the femoral veins of the rats with a precalibrated tuberculin syringe. Twenty - four hours later a one ml blood sample was obtained through a cardiac puncture and counted in a well - scintillation counter using a properly diluted aliquot of $Fe^{59}Cl_3$ solution as standard. Incorporation was calculated according to the following formula : [22]

$$Fe^{59} \text{ incorporation } (\%) = \frac{\text{net counts in 1 ml of blood} \times \text{body weight} \times 100 \times 0.05}{\text{net counts of standard.}}$$

Fe^{59} incorporation was similarly performed in both intact and adrenalectomized rats following stimulation by erythropoietin - rich anemic plasma filtrate for four days.

Peripheral blood values were determined by conventional methods on samples obtained from a tail wound. Hemoglobin was assayed by the oxyhemoglobin method,[23] hematocrits by the microhematocrit method using heparinized capillary tubes and cell counts by the Thoma counting chamber using Hayem's diluting fluid. Reticulocytes were counted in smears supravitally stained by one per cent aqueous solution of Nile Blue [24] and counterstained with Wright's stain. Erythrocytic indices were calculated by Wintrobe formulas. [25]

Adrenalectomized male and female rats were given I.M. injections of hydrocortisone (CIBA, 25 mg/ml suspension), in a dose of two mg per kg of body weight, while normal controls received just one mg. The former dose is physiologic for the rat, while the latter is pharmacologic. [20]

At the end of each observation period, femoral bone marrows were obtained from the rats and sections and smears were done. Sections were stained by routine histologic methods and smears with Wright's stain.

Results

Hematologic findings in male and female adrenalectomized rats are given in Tables 1 and 2. As Table 1 shows, in males the red cell counts increased progressively until the 19th day after adrenalectomy, while red cells decreased in both size and hemoglobin content. Within the same period there occurred insignificant changes in hemoglobin and hematocrit levels in contrast to a significant reduction of reticulocyte counts.

Between 19 and 77 days following adrenalectomy a different hematologic picture was observed. Both red cell counts and hemoglobin levels started to decrease reaching clearly anemic values on the 77th day. Conversely, the size and hemoglobin content of red cells increased to almost pre-adrenalectomy levels with return of reticulocyte counts to their original values. Thus, all red cell indices seemed to be restored, though a moderate degree of anemia was established in the peripheral blood (Figure 1).

The observed changes in the male suggest that the postadrenalectomy period could be divided into two stages. The first or earlier stage, which lasted nearly three weeks, was characterized by a marked elevation of red cell counts without corresponding changes in hemoglobin and hematocrit levels. This stage was also

TABLE 1

PERIPHERAL BLOOD VALUES AND RED CELL INDEXES IN MALE ADRENALECTOMIZED RATS

Days	Hemoglobin (gm/100 ml)	Hematocrit (%)	Red Cell Count (ml per cmm)	MCV	MCH	MCHC	Retic (%)
0	14.53 ± 0.522	50.5 ± 2.58	6.80 ± 0.464	74 ± 6.0	21.3 ± 1.78	28.8 ± 0.8	2.4 ± 0.64
7	14.10 ± 0.857	48.5 ± 3.20	7.48 ± 0.589	65 ± 6.3	18.9 ± 1.39	29.2 ± 1.7	1.2 ± 0.435
12	14.00 ± 1.3	49.0 ± 3.49	7.646 ± 0.65	65 ± 4.35	18.4 ± 1.36	28.4 ± 0.98	1.6 ± 0.75
19	14.25 ± 0.65	47.5 ± 0.25	10.50 ± 0.30	45 ± 1.0	13.6 ± 0.25	30.0 ± 0.1	1.5 ± 0.1
45	12.50 ± 0.1	48.0 ± 0.2	7.32 ± 0.49	66 ± 5.0	17.2 ± 1.15	26.2 ± 0.2	1.3 ± 0.3
77	11.90 ± 0.556	44.1 ± 0.985	5.84 ± 0.19	76 ± 2.5	20.5 ± 1.6	27.2 ± 1.0	2.3 ± 0.4

TABLE 2

PERIPHERAL BLOOD VALUES AND ERYTHROCYTIC INDEXES IN FEMALE ADRENALECTOMIZED RATS

Days	Hemoglobin (gm/100 ml)	Hematocrit (%)	Red Cell Count (ml per cmm)	MCV	MCH	MCHC	Retic (%)
0	12.7 ± 0.728	47 ± 1.72	7.512 ± 0.47	62.6 ± 5.0	16.9 ± 1.59	27.2 ± 1.7	1.4 ± 0.3
7	12.6 ± 0.829	48 ± 2.4	6.636 ± 0.645	73.4 ± 8.7	19.1 ± 1.9	26.1 ± 1.79	3.2 ± 0.56
21	11.0 ± 0.9	40 ± 2.4	6.80 ± 0.277	58.3 ± 6.2	16.2 ± 1.3	27.9 ± 0.9	3.0 ± 0.58
28	11.4 ± 0.75	44 ± 2.23	6.84 ± 0.345	64.0 ± 5.4	16.8 ± 1.6	25.9 ± 1.59	1.8 ± 0.36

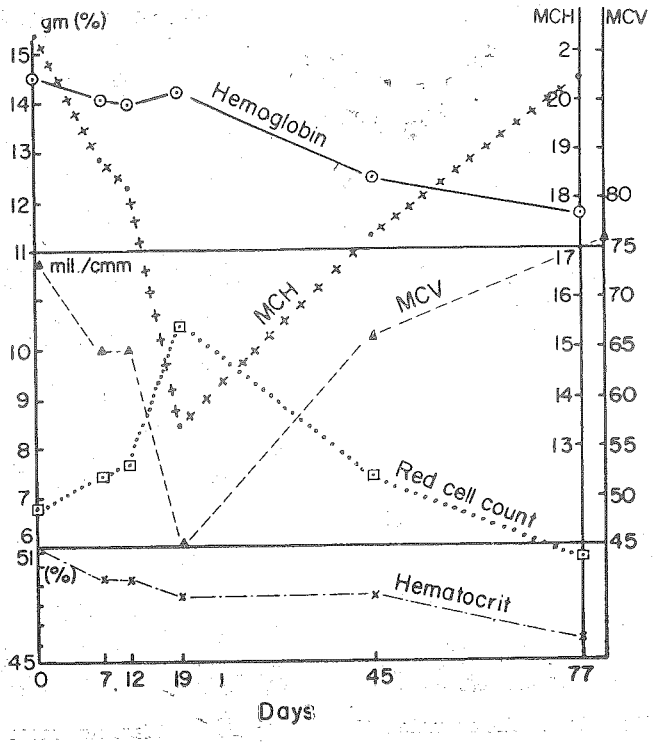

Figure 1. Peripheral blood changes in adrenalectomized male rats.

associated with a significant drop in reticulocyte counts and a substantial reduction in red cell indices leading to clearly microcytic and hypochromic characteristics in the cell. The second or late stage presented evidence of the development of a normochromic normocytic anemia, associated with a rise of reticulocyte counts to normal levels. Changes in the size and hemoglobin content of red cell in both stages were also detectable in peripheral blood smears (Figures 2 - 4). Examination of bone marrow sections and smears during the early postadrenalectomy period revealed relative hypercellularity and microcytic red cell precursors in the male bone marrow (Figure 5). Later bone marrow showed typical normoblastic hypocellularity.

In females, seven days after adrenalectomy, red cell counts showed a significant drop while little change occurred in hemoglobin and hematocrit values. Reduction of red cell counts was associated with considerable increases in MCV and MCH giving the cell hyperchromic macrocytic characteristics. Changes in MCV were roughly proportional to each other as well as to the reduction of red cell counts. Twenty - one days after adrenalectomy a marked

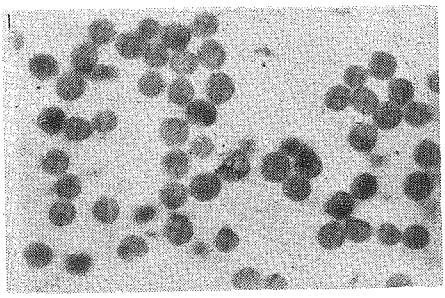

Figure 2. Peripheral blood smear from a normal male rat.

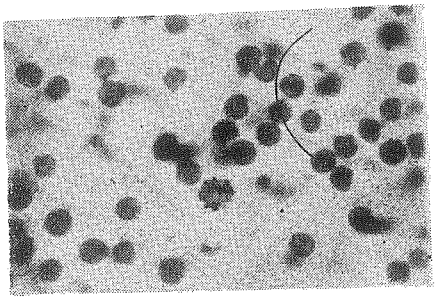

Figure 3. Red cells in the male on the 19th day following adrenalectomy. Red cells have decreased in size.

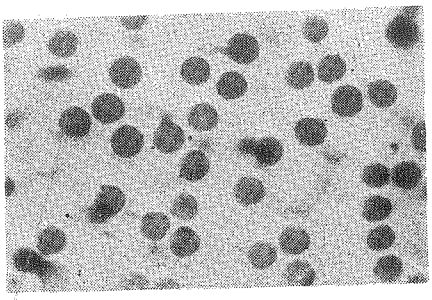

Figure 4. Red blood cells of male rat on 77th day following adrenalectomy. Note that the cells have acquired their pre-adrenalectomy characteristics.

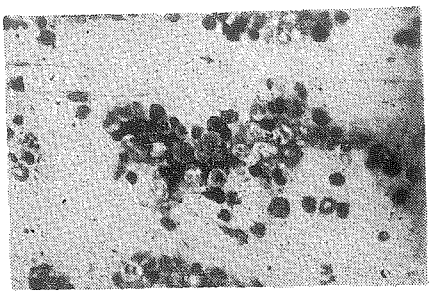

Figure 5. Marrow smear from adrenalectomized male. Smaller red cell precursors can be noted.

decrease in the hemoglobin level was observed. Red blood cell counts slightly increased but were significantly below those of preadrenalectomy values. These changes were associated with corresponding decreases of MCV and MCH to preadrenalectomy levels. Yet, erythrocytic indices were closer to their original values on the 28th day. Thus, four weeks after adrenalectomy a normocytic normochromic anemia of moderate degree was observed in the female rats. Animals were followed for 45 days, but no significant changes from those hematologic findings observed on the 28th day were detected (Table 2 and Figure 6). In female adrenalectomized rats two stages in the postadrenalectomy period were still discernible though they appeared earlier and were characterized by a different hematologic picture from that of the males. Early in the postadrenalectomy period, the bone marrow of the female rat showed hypocellularity with larger red cell precursors and blood smears revealed the existence of hyperchromic macrocytic erythrocytes (Figures 7 - 9).

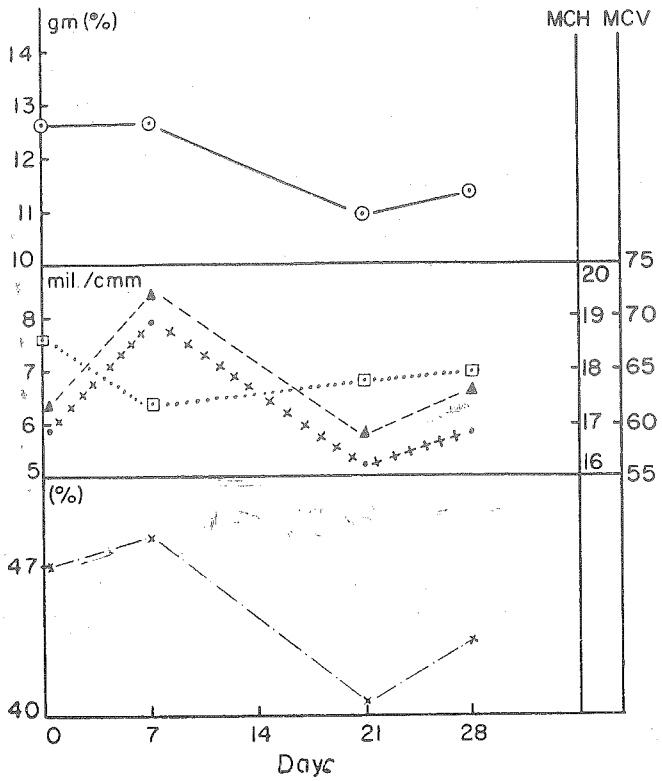

Figure 6. Changes in hematologic values and red cell indices in adrenalectomized female rats.

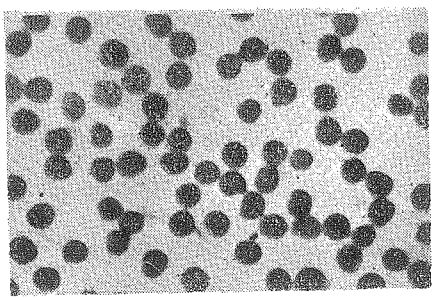

Figure 7. Red cells from the female rat before adrenalectomy.

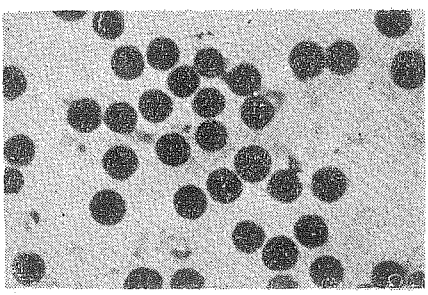

Figure 8. Red cells on the seventh day following adrenalectomy in the female. Red cells have increased in size and hemoglobin content (compare with Figure 7).

Erythropoietin Stimulation in Adrenalectomized Rats :

Male adrenalectomized rats were given anemic plasma filtrate injections with erytropoietin content for a week. Since adrenalectomy in males showed two quite different stages in regard to hematologic values and cell characteristics, erythropoietin stimulation was tested in two different periods. Thus, a group of rats received the injections on the 33rd postadrenalectomy day and another on the 77th. The response in the former group consisted of moderate elevation of hemoglobin and red cell counts and a marked rise in reticulocytes. Red cell indices varied very little and the cell maintained its previous hypochromic microcytic characteristics (Figure 10). In the latter group stimulation by erythropoietin produced comparable results with the exception of the red cells' being normal in size. In adrenalectomized males the magnitude of response to erythropoietin stimulation in both instances was considerably below that of normal controls. The increase in the red cell counts in the 77 - day group was higher than that of the 33 - day group, though the rise in the hemoglobin level was identical.

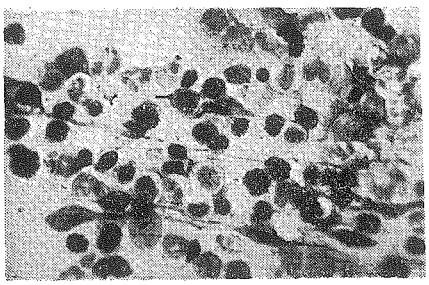

Figure 9. Bone marrow smear from female adrenalectomized rat. Larger red cell precursors are noted.

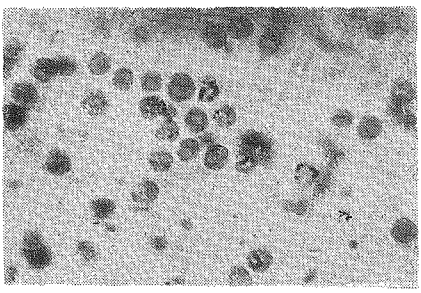

Figure 10. Red cells from a male rat after stimulation by erythropoietin on the 33rd day following adrenalectomy. Red cells are still microcytic.

Erythropoietic stimulation by erythropoietin in female adrenalectomized rats caused as good a hematologic response as in the control females. Data suggested that the female adrenalectomized rat, 21 days after adrenalectomy, was still normally responsive to erythropoietic stimulation. Table 3 shows the results of erythropoietin treatment in intact and adrenalectomized male and female rats.

TABLE 3

ERYTHROPOIETIN TREATMENT OF ADRENALECTOMIZED AND INTACT RATS

Procedure	Days	Hb	Ht	RBC count	Retics	MCV	MCH	MCHC
Males, intact	0	13.3	45	5.86	1.9	77	23.8	29.5
Erythropoietin	7	15.5	54	6.98	5.4	77	22.2	28.5
Males, adrenalectomized	8	15.5	51	7.65	2.0	67	20.2	30.4
	77	11.4	44	6.03	1.9	73	18.9	25.9
Erythropoietin	86	12.2	45	6.78	4.3	66	17.9	27.1
Females, intact	0	13.2	46	5.92	2.3	79	23.0	29.0
Erythropoietin	7	15.1	52	6.67	5.2	80	23.9	28.4
Females,	0	13.5	44	8.23	1.4	54	16.4	30.7
adrenalectomized	21	11.8	43	6.37	2.3	68	18.5	27.4
Erythropoietin	28	14.1	48	7.23	4.7	66	19.5	29.3

Hormonal Replacement with Hydrocortisone :

In male rats, 19 days after adrenalectomy, administration of hydrocortisone in doses of 2 mg per kg of body weight caused a slight increase in hemoglobin, in contrast to a very marked reduction of red cells to almost pre - adrenalectomy levels and considerable increases in red cell indices. The same treatment on the 66th day produced a good elevation of hemoglobin with a moderate increase of erythrocytes. Changes in red cell indices were negligible. The response to hydrocortisone replacement differed in the two stages of postadrenalectomy. The enlargement of the cell under the influence of hydrocortisone on the 19th day was clearly detectable in blood smears (Figure 11). Elevation of hemoglobin on the 66th day following hydrocortisone replacement, though good, was still below that of pre - adrenalectomy values.

In female adrenalectomized rats treatment with the same doses of hydrocortisone resulted in a substantial rise of hemoglobin as in the male, yet caused a further significant fall of the red cell count. This, in turn, was responsible for the appearance of a more hyperchromic and macrocytic cell (Figure 12).

With pharmacologic doses of hydrocortisone in intact rats somewhat different results were obtained. In the male this treatment produced a marked increase of hemoglobin and a significant drop of the red cell count with a very marked increase of red cell size and hemoglobin content. In the female the result of this treat-

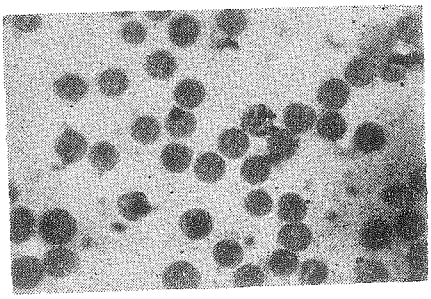

Figure 11. Red cells from the male after treatment with hydrocortisone on the 66th day following adrenalectomy. Cell size is considerably increased.

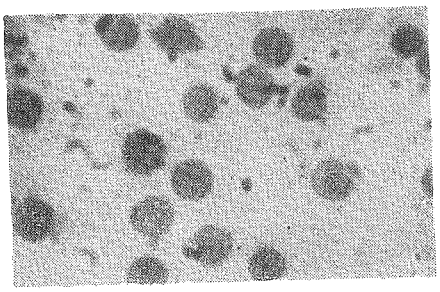

Figure 12. Erythrocytes from the female after treatment with hydrocortisone on the tenth day following adrenalectomy. Red cells are markedly increased in size and hemoglobin content (compare with Figures 7 and 8).

ment consisted of moderate reduction in both hemoglobin and red cell counts with slight enlargement of the cell. Results of hydrocortisone treatment in both groups of rats are shown in Table 4.

TABLE 4

REPLACEMENT WITH HYDROCORTISONE IN ADRENALECTOMIZED RATS

Sex	Days	Hb	Ht	RBC count	Retics	MCV	MCH	MCHC
Male-Adrenalectomized	0	14.5	52	6.92	2.9	75	20.9	27.9
Hydrocortisone	66	11.8	46	6.23	1.2	72	19.0	26.2
	73	13.8	46	6.85	2.6	67	19.4	29.0
Male - intact	0	12.8	43	7.32	1.2	61	18.1	30.0
Hydrocortisone	7	14.1	45	5.68	0.7	80	24.8	31.3
Female	0	13.3	49	6.99	1.6	70	19.0	27.1
Adrenalectomized	10	12.2	47	6.16	0.8	76	20.0	26.0
Hydrocortisone	17	14.1	50	5.75	1.3	87	24.5	82.2
Female - intact	0	14.9	50	8.17	2.1	61	18.2	29.8
Hydrocortisone	7	13.6	50	7.54	1.5	66	18.0	27.2

Combined Treatment with Hydrocortisone and Erythropoietin :

In male rats on the 7th day following adrenalectomy a combined administration of hydrocortisone and erythropoietin for a period of one week caused a moderate increase of hemoglobin while red cell counts decreased to pre-adrenalectomy levels. That the observed effect was not due completely to hydrocortisone, as previous studies suggested, was evidenced by a good rise in reticulocytes. In order to evaluate properly the effect of combined

treatment, animals were followed for an additional seven days. During this period a gradual elevation in hemoglobin and red cell counts was observed. Red cell size increased during injections but later dropped to pretreatment values. Thus, a delay in the development of the erythropoietin effect was apparent.

With the same treatment in females a comparable decrease of red cell counts occurred, but this, in contrast to that in males, was associated with a moderate decline of hemoglobin levels during injections. Enlargement of red cells and a good reticulocytic response were also noticeable during the same period. Following the animals for another week revealed the same pattern of delay in the erythropoietic effect of erythropoietin. Response to combined treatment in females was considerably less than that in males (Table 5).

TABLE 5

COMBINED TREATMENT WITH HYDROCORTISONE AND ERYTHROPOIETIN IN ADRENALECTOMIZED RATS

Sex	Days	Hb	Ht	RBC count	Retics	MCV	MCH	MCHC
Male	0	14.5	47	6.58	3.0	71	22.0	30.8
	7	13.6	48	7.37	3.6	65	18.5	28.3
Hydrocortisone	14	14.1	48	6.58	4.9	73	21.4	29.4
Erythropoietin	21	15.5	49	8.06	3.1	60	19.6	32.3
Female	0	13.3	49	6.99	2.6	70	19.0	27.1
	10	12.2	47	6.16	3.4	76	20.0	26.0
Erythropoietin	17	11.4	47	5.75	5.3	81	20.0	24.3
Hydrocortisone	24	12.8	49	6.89	5.1	72	18.6	26.1

Fe ^{59} Incorporation :

In control rats Fe ^{59} incorporation was 36 per cent, while it was 24 and 17 per cent in male and female adrenalectomized rats, respectively. Data indicated that in the male a 12 per cent and in the female a 19 per cent depression of Fe ^{59} incorporation occurred after adrenalectomy. Residual activity in the plasma after 24 hours was negligible in all three instances and was not indicative of faulty iron removal from the plasma following adrenalectomy.

Stimulation by erythropoietin produced an eight per cent rise in the male adrenalectomized rat in the incorporation, whereas it was five per cent in the female. A corresponding rise in the intact rat was 12 per cent. Thus, adrenalectomized rats seemed responsive to erythropoietin stimulation yet output stayed considerably below

that of controls. Results of Fe^{59} incorporation in adrenalectomized rats agree with hematologic values obtained with the same treatment.

Hydrocortisone treatment resulted in a four per cent decrease of Fe^{59} incorporation in the male and five per cent increase in the female. Reduction observed in the male could only be correlated with the reduction in the red cell count occurring under the influence of hydrocortisone (Table 6).

TABLE 6

FE 59 INCORPORATION IN INTACT AND ADRENALECTOMIZED RATS

	Radioactivity injected microcuries	Activity in whole blood cpm	Plasma activity cpm	Fe^{59} incorporation %
Intact rats	1 microcurie	1319	188	36
Adrenalectomized male rats	2 »	4850	160	24
Female adrenalectomized rats	1 »	1712	248	17
Erythropoietin to intact rats	1 »	2718	84	48
Erythropoietin to adrenalectomized males	1 »	2370	141	32
Erythropoietin to adrenalectomized females	1 »	1812	166	22

Discussion

It was apparent, in this study, that the postadrenalectomy period in both male and female rats was characterized by two different stages with different blood pictures. In males, the only observed effect of adrenalectomy on the blood picture was a gradual increase of the red cell count until the 19th day, while in females a decrease was noted. That the increase of the red cell count was not due to hemoconcentration attending adrenalectomy was evidenced by insignificant changes both in hemoglobin and hematocrit levels, as well as by the results of DOCA treatment (Table 7). The diverse effect of the adrenalectomy in both sexes during the first three weeks upon red cell counts was associated with alterations in erythrocyte characteristics. In males red cells gradually became

TABLE 7

DOCA TREATMENT IN ADRENALECTOMIZED MALE AND FEMALE RATS

Sex		Hb	Ht	Red cell count	Ret	MCV	MCH
MALE	before treatment	14.5	47	7.82	1.3	60	18.5
	after treatment	14.5	48	7.93	1.1	61	18.3
FEMALE	before treatment	12.8	48	7.45	3.2	64	17.1
	after treatment	12.5	50	7.35	3.5	67	17.0

hypochromic and microcytic, while in the female they acquired hyperchromic and macrocytic characteristics. Such a diverse effect of the same endocrine gland in either sex can hardly be explained on the basis of different cortical hormones acting on the bone marrow, since adrenal corticoids have similar actions through the animal kingdom. However, Gordon [19] found a hyperchromic macrocytic anemia with hypocellularity of the marrow in female adrenalectomized rats. In this study also, a hypocellularity was evident in the marrow of female adrenalectomized rats, yet larger red cell precursors were easily demonstrable in marrow smears. In contrast, bone marrow in males showed relative hypercellularity and smaller nucleated red cells. These results indicated that the absence of adrenals affected erythropoiesis differently in each sex. It has been shown that adrenal demedullation exerts no significant influence upon the formed elements of the blood or bone marrow. [19] At this point results of replacement therapy would be of interest. Table 7 shows the ineffectiveness of DOCA treatment in correcting the blood picture in adrenalectomized rats. The same result has been obtained by Gordon in his experiments. In view of the well-known effect of gluco-corticoids upon eosinophils and lymphocytes, hydrocortisone was tested and found capable of reducing the elevated counts in males but further decreased the number of circulating red cells in the female. In Gordon's experiments only whole cortical extract concentrates were found effective in counteracting both the anemia and the marrow changes, while cortisone acetate failed to correct marrow changes. So, it seems probable

that the different blood picture in the early postadrenalectomy period in each sex is brought about by a substance or substances present in whole adrenal cortex extract other than gluco - corticoids or mineralo - corticoids, which readily suggests androgens. Androgens are secreted by the adrenals in considerable quantities in both sexes, and males have another androgenic source in their testicles. In males after adrenalectomy this source would possibly be left intact, while in the female the only source of this activity would be removed. Castration in male rats is associated with a decrease in the red cell count. [11 26] In castrated males prolonged administration of testosterone has been found to increase both red cell count and hemoglobin. [27] In testosterone-treated pullets a significant elevation of red cell count and hematocrit has been demonstrated. [28] In castrated male rats testosterone produces a rise to normal of the reduced red cell count. [29] Administration of testosterone or androgens enhances red cell production in experimental animals. [30] Thus, it seems quite probable that testosterone is responsible for the increase in red cell counts observed in the male adrenalectomized rats early in the postadrenalectomy period.

Nineteen days after adrenalectomy a gradual decline in all of hematologic values in males was observed. This lasted until the day and was associated with the return of the red cell teristics to normal. The same changes were observed in the but earlier than in the male. As a result, both in male and drenalectomized rats a normochromic and normocytic entually developed.

lularity developed in male bone marrow as the omy period lengthened. Eventually hypoplasia was the bone marrow in both sexes. This may reflect ic depression in the marrow due to lack of adrenal e to respond to erythropoietic stimulation by latter possibility was investigated by treating ts after the establishment of anemia. An was induced in the male though considerably ietin - treated intact males (Table 3). In the identical to that of the intact female with ation but increase of the red cell count Because the data suggested an associa- hormones and erythropoietin, we with hydrocortisone and erythro- change except a good reticulocytic

response was noted; however, a week after the completion of the injections a full erythropoietic response developed. The overall response in relation to the red cell count was still inadequate. In view of the conclusion reached for testosterone in promoting red cell production, these results suggested that the factor lacking for full expression of erythropoietin effect may well be testosterone or androgenic activity. The adequacy of hemoglobin response to combined treatment suggested a stimulating effect of hydrocortisone upon hemoglobin formation. All these results were confirmed by Fe^{59} studies.

It may be concluded that hydrocortisone has a stimulating effect upon hemoglobin production while testosterone or androgens have a red cell count increasing activity. How hydrocortisone stimulates hemoglobin synthesis could only be explained by its inhibiting the degeneration of hemoglobin-forming apparatus in nucleated red cells as the cell goes down in the line, since hydrocortisone has been shown to have a mitosis inhibiting action in other tissues.[31-33] However, a metabolic factor may also be partly responsible since it has been demonstrated that the absence of glucose does not affect respiratory activity of bone marrow and bone marrow utilizes fatty acids when glucose is lacking from the medium.[34] In view of the depressive action of gluco-corticoids upon glucose utilization in tissues and fatty acid mobilizing effect, it is not surprising that hemoglobin synthesis would be accelerated by hydrocortisone.

Thus, erythropoiesis in the rat may be considered under the combined control of gluco-corticoids and androgens and a close association with erythropoietin, the former being necessary for optimum hemoglobin production and the latter for adequate numbers of red cells.

Summary

The rate of red blood cell production is studied in adrenalectomized and intact rats with the aid of peripheral hematologic determinations, Fe^{59} incorporation, bone marrow examinations and treatment of the animals with adrenocortical steroids and erythropoietin alone or in combination. The data obtained demonstrated that adrenalectomy produced different blood pictures in male and female rats in the early postadrenalectomy period characterized by an increase in the red cell count with hypochromic and microcytic cells in the male and by a decrease in the red cell count with

hyperchromic and macrocytic cells in the female. In the late post-adrenalectomy stage a normochromic and normocytic anemia eventually developed in both sexes. By erythropoietin stimulation of adrenalectomized and intact rats alone or in combination with hydrocortisone a defect in the marrow of adrenalectomized rats could be demonstrated. This defect was the inability of the marrow to raise both the hemoglobin and red cell counts in the absence of adrenal cortical activity and under erythropoietin stimulation. The defect in hemoglobin production was correctable by hydrocortisone while the defect in increasing red cell count seemed to require androgens. The possible rôle of adrenal gluco-corticoids and androgens in red cell homestasis as well as in the pathogenesis of the anemia of adrenalectomy are discussed. It is concluded that adrenal corticoids and androgens in the male and the female act in concordance to produce cells of normal size and hemoglobin content, as well as in the full expression of erythropoietin effect upon the marrow.

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A Simple Immunologic Method of Urinary Cylic LH Determination for Prediction of Ovulation

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Until recently, only biologic methods have been used for the assay of various protein hormones in human body fluids. These methods are unsatisfactory because of biologic variations, the great number of test animals necessary to obtain reliable results and the time, expense and labor involved.

During the last ten to twelve years, immunology has produced better assay methods for protein hormones, which, under certain circumstances, act as antigens. The breakthrough occurred with insulin by Aguilla and Stavitsky in 1956. Read and Stone, in 1958, used the same technique for the assay of human pituitary growth hormone (HPGH). In 1960, the first reports of immunological detection of luteinizing hormone (LH) were published. Table 1 summarizes recent attempts to detect and titrate human chorionic gonadotrophin (HCG) in urine and serum. [1 - 4] Most of these methods are expensive and require a large research laboratory.

Most laboratories perform assay for total gonadotrophins only, instead of obtaining follicle-stimulating hormone (FSH) and LH separately, due to the laborious biologic procedure involved. Because of the importance of diagnosing endocrine disorders, the measurement of individual hormones is necessary. For these reasons, attempts have been made to develop rapid and convenient methods for urinary assay of LH. Since it is known that LH and HCG have similar immunologic properties, HCG is used for LH testing because, while it is difficult to obtain enough purified LH for use as a component in immunologic assays, large amounts of HCG can be procured from the urine of pregnant women. [5]

The purpose of this paper is to introduce a simple method for LH determination in urine which can be easily, quickly and inexpensively done in any laboratory.

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Materials and Methods

This technique of urinary cyclic LH determination is based on hemagglutination. Latex particles and the gravindex kit were used.

1. About 50 cc of urine was obtained daily from each patient and frozen, if not used immediately.
2. The urine pH was adjusted to 4 using acetic acid.
3. 200 cc of acetone was added to the 50 cc of urine and kept at 4° C for 30 minutes.
4. The total 250 cc was centrifuged at 1500 rpm for 15 minutes. The precipitate was washed with alcohol and ether, recentrifuged and left to dry for 1 hour.
5. The precipitate was resuspended in 2 cc of 6.4 phosphate buffer, centrifuged at 1000 rpm for 10 minutes and the supernatant was used for the test.

The gravindex test is capable of measuring 3500 IU of HCG or LH per liter of 25 times concentrated urine (3500/25). The test is capable of measuring up to 140 IU per liter, either HCG or LH.

Four dilutions of concentrated urine were prepared as follows:

1/1 — to measure 150 IU per liter of HCG in urine
1/2 — to measure 300 IU per liter of HCG in urine
1/4 — to measure 600 IU per liter of HCG in urine
1/8 — to measure 1200 IU per liter of HCG in urine

As long as there is a cross-reaction immunologically and biologically between LH and HCG, their responses are identical.

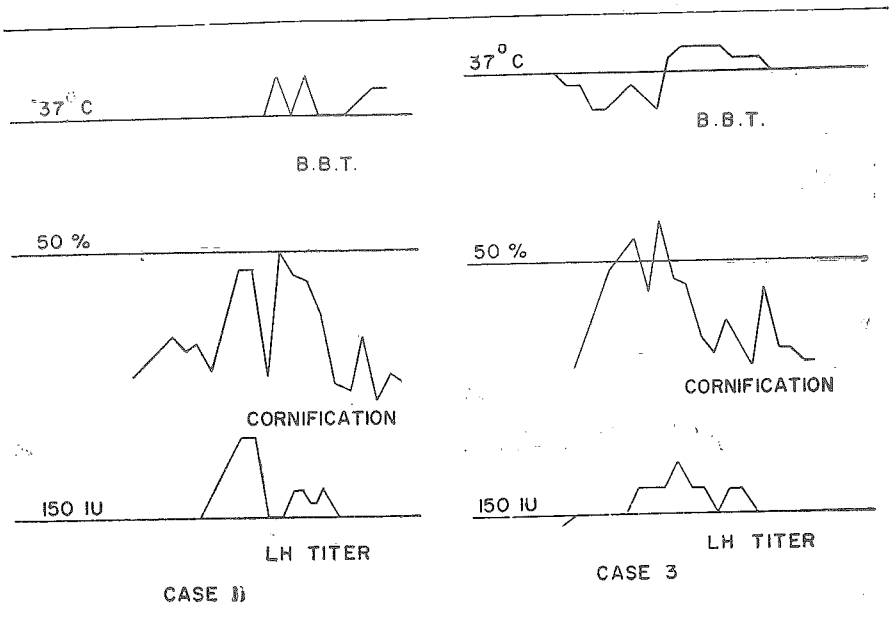
Method of titering was as follows:

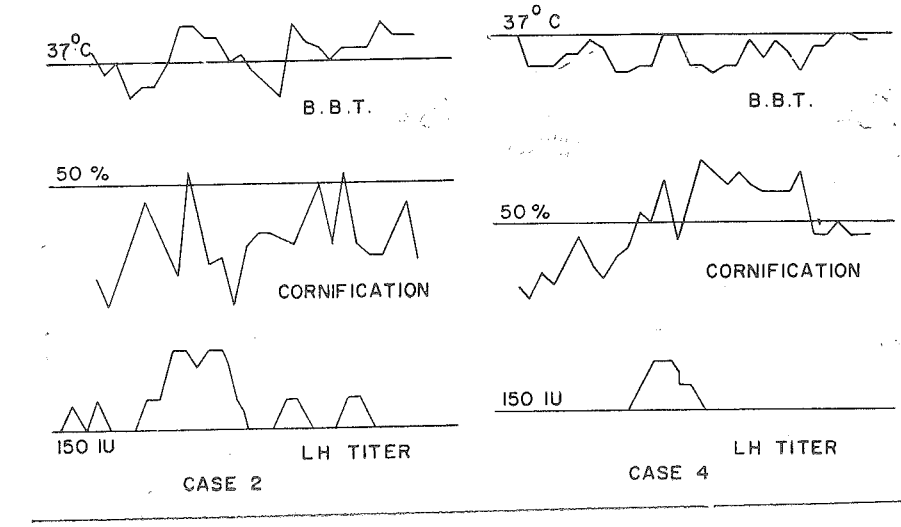
1. Using a clean pipette, one drop of each dilution was placed on a glass slide (2" X 3").
2. One drop of gravindex serum was added, mixed well with an applicator stick and rocked gently for 30 seconds.
3. Two drops of gravindex antigen were added, mixed with an applicator stick, spread over a one inch area and rocked gently for 30 seconds. Agglutinations were expected to occur in 2 minutes.

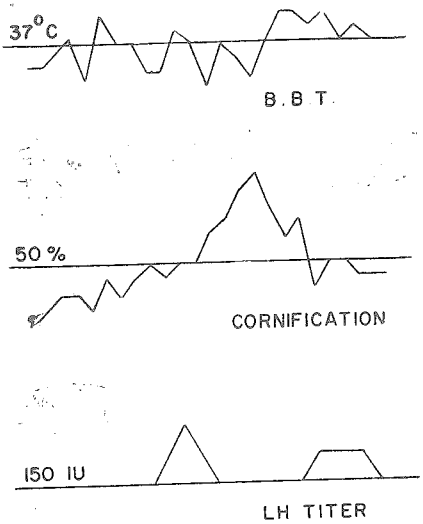
If agglutination occurs, it means that there is not enough LH in the dilution to bind all the antigen. If agglutination does not occur, it means that there is more LH than antigen in this dilution. The smallest dilution showing negative agglutination is the titer of the LH.

Ages and cycles of the seven cases are shown in Table 1. Daily vaginal smears and temperatures were obtained and recorded from all patients. Daily urines from these patients were kept refrigerated at 2° C until tested.

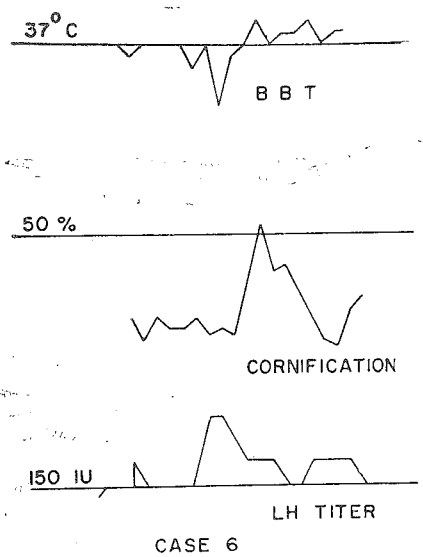
Figures 1- 7 show the results of our findings with comparative drawings of basal body temperature, cornification and LH titer.







CASE 5



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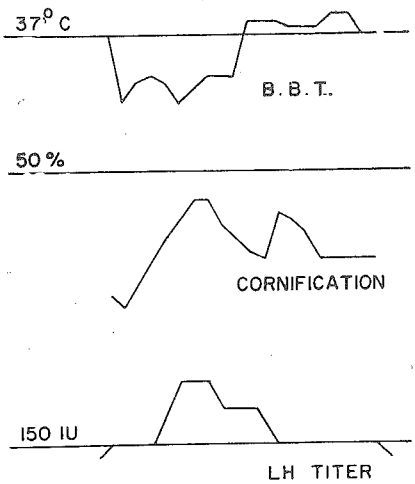

CASE 7

TABLE 1

AGES AND CYCLES OF PATIENTS

Case	Age	Cycle
1	34 years	27 days
2	31 years	25 days
3	27 years	38 days
4	21 years	33 days
5	20 years	32 days
6	23 years	28 days
7	30 years	25 days

Discussion

As has been mentioned above, several different techniques have been used for LH titration in the urine and serum of human females (Table 2). The information obtained from the aforementioned studies indicates that there is almost always a mid-cycle rise in LH excretion in the urine. These results are similar to the pattern of LH excretion found in normal menstrual cycles using biologic assay methods.[6] There is no LH peak in patients taking contraceptive pills containing mestranol and progestins. Patients with polycystic ovary syndrome diagnosed by culdoscopy have a consistently elevated LH. Patients on Clomiphene citrate show an increase in LH secretion when ovulation is induced. Further studies are in progress to determine the response to Clomiphene.

TABLE 2

ATTEMPTS TO DETECT AND TITRATE HCG IN URINE AND SERUM

Date	Investigator	Test
1960	Swierzczynska Samochiwice	Hemagglutination test
1960	Wide, Roos and Gemzell[2]	Hemagglutination test
1960	Brody-Carlstroem	Complement fixation test
1960	McKean	Gel precipitation
1961	Ras-Shaham	Gel precipitation of Ochterlony
1961	Midgley	Immunoelectrophoresis
1962	Keele and Rample	Gel precipitation of Peer
1962	Midgley	Fluorescent antibody technique
1962	Wide[3]	Modified hemagglutination test
1965	Sato and Greenblatt[4]	Modified Gemzell Hemagglutination test
1966	Mishell[1]	Modified Gemzell Hemagglutination test using purified LH as antigen

Conclusion

In our seven patients, LH titer was highest two or three days prior to the estrogen and temperature peaks. Occasionally more than one peak is noted during the cycle, but only the highest peak has any clinical significance. Our purpose was to simplify LH titration in order to make it available to the practicing physician. This test may not be accurate enough to show the actual level of LH during the cycle, but what is significant for the practitioner is the LH peak, and this test, from our point of view, is exact enough to show this peak in 60 to 70 per cent of the cases. We wholly agree that the serum LH titration with radio - immuno - assay is more precise and helpful in general hospital and research use, but the practical point of this test should not be overlooked.

Summary

Cyclic urine titers of LH are determined and compared with cornification and basal body temperatures in seven patients. Grav-index testing of LH, though not so accurate, is simpler than the other methods previously published and helpful to the practicing physician for determining the day of ovulation. For clinical purposes, it is advised that concentration of urine be kept to 10 times instead of 25, because the minor secondary LH peaks will disappear and only the main peak will be detected (10 times concentration is enough to detect a titer of 350 or more IU per liter of urine).

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The Fluorescent Treponemal Antibody Test in the Serodiagnosis of Syphilis

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Serologic tests used in the diagnosis of syphilis can be divided into two groups. The first group includes those using cardiolipin antigen to measure reagins. Tests in this group are two types: flocculation and complement fixation. The VDRL slide test is one of the most frequently used flocculation tests and the standard complement fixation test is the Kolmer test.

The antigens used in nontreponemal tests are now prepared from beef heart lipids with cholesterol and cardiolipin (a phosphatide of beef heart muscle) reinforced by purified lecithin. Although the reagin is nonspecific, not an antibody to treponema, its detection by nontreponemal antigens has been highly valuable in the diagnosis of syphilis. Because the antigens used in the nontreponemal antigen tests are not entirely specific for syphilis, antigens from treponema were prepared in order to produce a specific test. A partial list of tests employing T. pallidum (Nichol's strain) or extracts from this treponema as antigen follows : Treponema pallidum immobilization (TPI), Treponemal pallidum agglutination (TPA), fluorescent treponemal antibody (FTA) and Treponema pallidum complement fixation tests.

The fluorescent treponemal antibody test was first described in 1957 by Deacon [1] as a simple and specific serologic test for syphilis. Initially, 1:5 dilutions of serum were used and positive results were high with false positive reactions present. Later, the same technique was tested using 1:50, 1:100 and 1:200 dilutions. [2 - 4]

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The treponemal antigen tests employed to date have been difficult and costly to perform and some have lacked the sensitivity of the nontreponemal antigen tests. The purpose of our study was to compare the results of the FTA test with those of other standard serologic tests for syphilis in an attempt to show that the FTA test is as specific as the TPI test and easier to perform.

Materials and Methods

In this study sera submitted for TPI were also examined by the VDRL, quantitative Kolmer and FTA tests. The antigen used for the FTA test was Treponema pallidum, Nichol's strain, extracted from rabbit testicular tissue in saline. The suspension was centrifuged for about five minutes at 1,500 rpm to remove blood corpuscles, fragments of tissue and spermatozoa. The antigen was spread on three different areas of one slide and fixed in acetone for ten minutes. Twenty to 25 treponema seen per microscopic field in each suspension of antigen signified a desirable density.

Dilutions of serum (1:50, 1:100, 1:200) were dropped onto the antigens on the slide and kept for 30 minutes at room temperature. The slide was then washed with buffered saline and a drop of conjugated antiserum, diluted 1:20, was placed on the serum-treated antigen. The slides were incubated in a humidified container at room temperature for 30 minutes. Stained slides were examined under a Reichert Binolux microscope using a light field condenser and BG 12/6 mm, GG 9/1 mm and OG 1/1.5 mm filters. The organisms with typical treponema morphology and clear, strong fluorescence were considered positive while those with very weak or no fluorescence were considered negative. TPI, VDRL and quantitative Kolmer tests were performed according to standard procedures [5] [6] and the quantitative Kolmer test by the microtechnique method. [7] Reagents used in these tests were : VDRL antigen, Dade Reagents, Inc., Miami, Florida, U.S.A., Antigen Lot No. VDA - 23; Kolmer antigen, Difco - Kolmer Cardiolipin Antigen, Cat. No. 0438 - 59; and Conjugated antiserum, Difco, Bacto - F. A. Human Globulin Antiglobulin (Rabbit), Cat. No. 2449 - 56.

Results

In the TPI test, the sera which caused immobilization of more than 50 per cent of the live T. pallidum were considered positive and the sera which caused immobilization of less than 20 per cent

of the active treponema were considered negative. Between these two limits, that is between 20 per cent and 50 per cent, the sera that caused immobilization were considered weakly positive. Twenty - nine of the 303 examined sera showed a toxic effect and produced no results. The results of the other 274 sera were : 99 (36 per cent) positive, 137 (50 per cent) negative, and 38 (14 per cent) weakly positive. Table 1 compares these findings with those of the FTA, VDRA and Kolmer tests.

Of the 99 sera which were positive on TPI, 87 per cent were positive on FTA $_{50}$, 76 per cent on FTA $_{100}$, 58 per cent on FTA $_{200}$, 84 per cent on VDRL and 56 per cent on the Kolmer test. Comparison of these indicates that the results obtained with FTA $_{50}$ correlate best with those of the TPI test results. Of the 137 sera which were negative on TPI, 92 per cent were negative on FTA $_{50}$, 96 per cent on FTA $_{100}$, 98 per cent on FTA $_{200}$, 92 per cent on VDRL and 93 per cent on Kolmer. Twenty - nine sera which produced no results on TPI did react to other tests.

Table 2 compares the FTA $_{50}$, FTA $_{100}$ and FTA $_{200}$ results with those of other tests. TPI $_{50}$ indicates the positive sera showing immobilization of treponema above 50 per cent; TPI $_{20}$ the positive sera showing immobilization above 20 per cent.

When we compare the positive results and the negative ones by considering them as a whole we find that all tests correlate at approximately 80 per cent. When we study the three dilutions we find that FTA $_{50}$ produced the results most correlated with those of the other tests. For example VDRL and FTA $_{50}$ correlated at 89 per cent (34 per cent positive and 55 per cent negative in both tests); TPI $_{50}$ and FTA $_{50}$ at 88 per cent, Kolmer and FTA $_{50}$ and TPI $_{20}$ and FTA $_{50}$ at 84 per cent each. These comparisons also show that FTA $_{50}$ results correlate best with those of the other tests, and the TPI $_{50}$ results are more consistent with the results of the other tests than TPI $_{20}$.

Discussion

Although there are many serologic tests for syphilis in use today, negative results are still obtained in some syphilitic patients and positive results in non - syphilitic patients. VDRL, Kahn, Wassermann and Kolmer tests are employed by many laboratories because of simplicity of technique and availability of antigen. These tests are not specific for the T. pallidum organisms,

but, approximately three weeks after the syphilitic chancre appears, they become positive and continue to reveal a high titer for six months, that is during the second stage of syphilis. In treated patients the titers decrease, but in untreated cases the titers remain high for a number of years.

FTA gives positive results as early as or earlier than cardiolipin tests and continues to show the high titer throughout the second stage of the disease.[3 8] This test shows a five to six times higher titer than the other tests, as well as an early negative result; for example in the late stage of syphilis the FTA test is negative if the patient has been treated.

TPI, although very difficult to perform and although toxic reactions do occur, is recognized to be the most efficient test to differentiate the false positives produced by the cardiolipin tests.[9]

When the FTA test was first tried, low dilutions (1:5, 1:10) of serum samples were used and a high percentage of positives were obtained through the detection of the natural antibodies related to the saprophitic treponema normally present in the oral cavity. Individual reports on higher dilutions are available, but to our knowledge, no one has compared the three dilutions in one study. Some authors found FTA_{200} and TPI to correlate at 9 per cent,[10] 88.1 per cent[3] and 90 per cent.[3] In one study FTA_{100} and TPI correlated at 98 per cent and FTA_{50} and TPI at 85 per cent.[3] In our study correlation of FTA and TPI tests was more than 80 per cent (Table 1).

Generally, in the FTA test when 1:100 and 1:200 dilutions were used negative results increased. It is obvious that the titers of the antibodies in the sera affect these results. The results we obtained using FTA and cardiolipin tests correlate closely with those in the literature.

In our study the FTA_{50} and the other tests showed an 11 to 12 per cent discrepancy. This has also occurred in the literature and has been attributed to technical error.[2] In addition, a difference existed in the type of reactant antibodies in the two tests. Although some authors suggest that immobilizin and antibodies in the FTA tests are similar, they are different as far as the time of detection is concerned. Compared with TPI, FTA gives positive results and turns negative earlier.[3]

Some investigators[11] suggest that antibodies in FTA tests act against the group and strain specific antigens of treponema. Tu-

TABLE 1

COMPARISON OF RESULTS (PERCENTAGES) OF TPI TEST WITH THOSE OF FTA, VDRL AND KOLMER TESTS

TPI				FTA 50		FTA 100		FTA 200		VDRL		Kolmer	
Classification	Percentage of immobilization	Sera		+	—	+	—	+	—	+	—	+	—
		Number	Per cent										
Positive	50 - 100	99	36	87	12	76	23	58	41	84	15	73	27
Weakly Positive	30 - 50	14	5	36	64	29	71	7	93	36	64	40	60
	20 - 30	24	9	29	71	25	75	17	83	29	71	6	94
Negative	0 - 20	137	50	8	92	4	96	2	98	8	92	7	93
Toxic		29	—	38	62	28	72	14	86	34	66	41	59

fanelli,[11] using the FTA-absorption test with Teiter strain, found more specific results in the differentiation of false positives. Deacon[12] found a 90.4 per cent correlation between FTA - absorption and TPI tests. The FTA - absorption test is said to be more sensitive and to produce earlier results than TPI and there is no indication of its being less specific.[13 – 15]

In conclusion we can say that FTA_{50} is as specific as TPI, can be used to differentiate biologic false positives, and is especially useful in those cases which either produce toxicity or cannot be examined with TPI because of the lack of technical possibilities. Another advantage is that the slide prepared for the FTA test can be kept in a frozen state up to three weeks to be used when necessary.

TABLE 2

COMPARISON OF RESULTS (PERCENTAGES) OF FTA_{50} FTA_{100} AND FTA_{200} WITH THOSE OF VDRL, KOLMER AND TPI TESTS

		VDRL		Kolmer		TPI_{50}		TPI_{20}	
		+	—	+	—	+	—	+	—
FTA_{50}	+	34	6	25	8	32	7	37	3
	—	5	55	8	59	5	56	13	47
FTA_{100}	+	30	3	22	5	28	5	33	20
	—	9	58	11	62	9	58	17	48
FTA_{200}	+	22	2	16	2	22	2	24	0
	—	16	60	17	65	15	61	26	50

Summary

Three hundred and three sera submitted for TPI testing were also examined by the FTA (treponemal) and VDRL and Kolmer (nontreponemal) tests. For FTA testing three dilutions (1:50, 1:100 and 1:200) were used. Comparison of the TPI test results with those of the three separate dilutions of the FTA test showed correlations of 88, 86 and 83 per cent respectively; VDRL results

correlated with those of FTA tests at 89, 88 and 82 per cent respectively. There was an 11 to 12 per cent discrepancy between TPI and FTA test results attributed to technical error and/or a possible difference in the reactant antibodies in the two tests. The highest correlation with all other tests were obtained through FTA when a 1:50 dilution was used. Our results indicate that FTA test is as specific as TPI and can be used to differentiate biologic false positives and to examine cases when TPI cannot be applied.

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Bacteriostatic Characteristics of Amalgams in Root Canal Therapy

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In dentistry the most important aim is to maintain proper function of the dental pulp and the apical tissues in order to prevent disease. This can be done through caries prophylaxis and the materials and techniques used in therapy.

According to Grossman, [1] it is essential to use bacteriostatic materials in root canal treatment. Soft and absorbent materials are considered preferable for root canal filling. In order to keep the canals sterile, filling materials such as gold foil, copper-paraffin, gold and silver amalgam and cement have been used. Miller [2] proved by filling a tooth with copper amalgam and then extracting it that the dentine layer of the tooth was sterile due to the bactericidal effect of the copper amalgam.

We use bactericidal canal filling because microörganisms can be left in the root canals and their growth must be prevented. We present here a discussion of bactericides, the oligodynamic effects of amalgam and the results of our experiments with copper amalgam in canal filling.

Materials and Methods

The experimental amalgam discs were divided into two groups, copper and silver, prepared according to standard proportions and obtained by filling steel moulds (Figures 1 and 2) designed and manufactured in our laboratory with the amalgam. The amalgam discs were placed in nutrient media used for microbiologic experiments.

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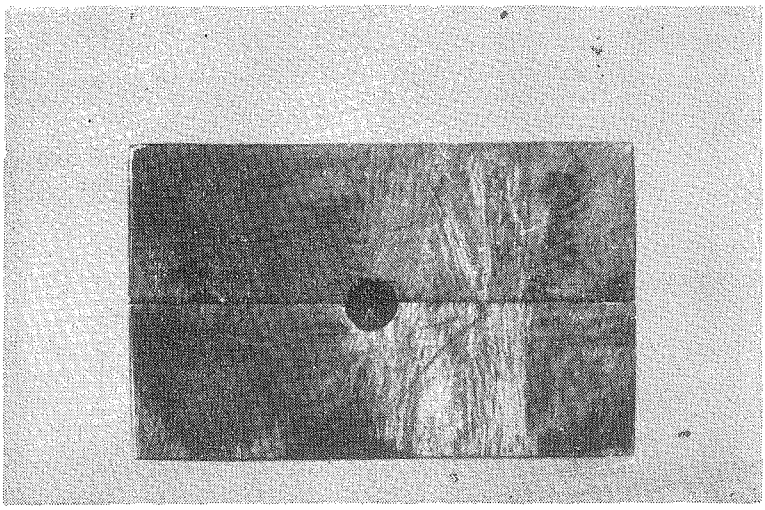

Figure 1. Steel cavity used in preparation of discs.

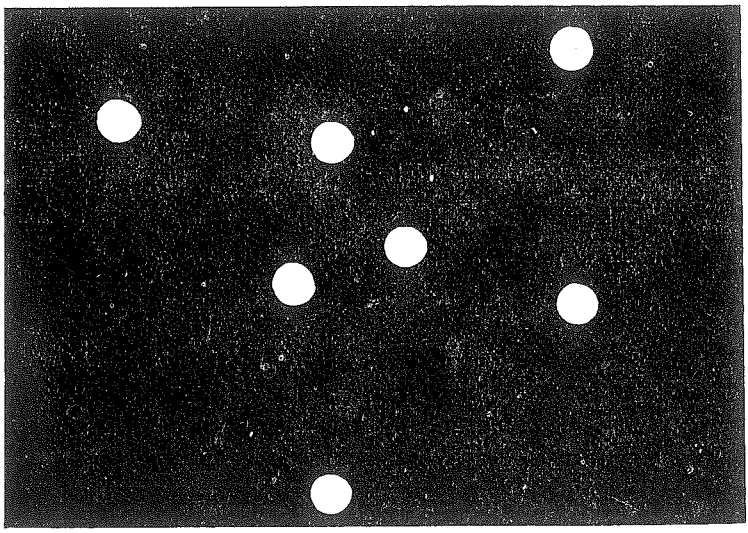

Figure 2. Discs used in experiments.

Cultures were obtained from 46 patients. In this study, only obligate aerobic and facultative anaerobic microörganisms were considered. Microörganisms were isolated from the extracted teeth canals using a sterile loop and were inoculated into Tarozzi broth, thioglycollate broth and blood-agar media.

It was possible to grow aerobic, anaerobic and facultative anaerobic microörganisms on Tarozzi media and thioglycollate broth. After inoculation, media were kept at 37° C from 18 hours to one week. When a growth was observed, transfer was made to a new agar plate to obtain single colonies. The microörganisms isolated are shown in Table 1.

TABLE 1

NUMBER OF MICROÖRGANISMS ISOLATED FROM 46 TEETH

Microörganism	Number
Diplococcus pneumoniae	15
Staphylococcus aureus	9
Gram (—) bacilli (Coliforms)	7
Neisseriae	7
Non-hemolytic streptococcus	6
Staphylococcus albus	5
Alpha hemolytic streptococcus	3
Gram (+) cocci (unidentified)	3
Gram (+) bacilli (unidentified)	2
Beta hemolytic streptococcus	1
Candida and pneumococcus	1

The identified microörganisms were inoculated onto slant agar tubes and kept sterile. From each a loopful of culture was inoculated onto five blood agar plates and spread evenly over the surface. Silver and copper amalgam discs were placed on these plates with sterile forceps within ten minutes of their preparation and plates were incubated for 18 to 24 hours at 37° C. After this period, the inhibition zones formed around the discs were measured by compass and recorded (Figures 3a and 3b). For every microörganism five plates were examined and an average calculated for the inhibition zone diameters (Table 2).

Results

Forty-six teeth were examined by the method described above. As seen in Table 2, the average diameter of the inhibition zones of copper amalgam discs was larger than that of silver. If the copper amalgam discs were left on the plate for more than 24 hours, diffu-

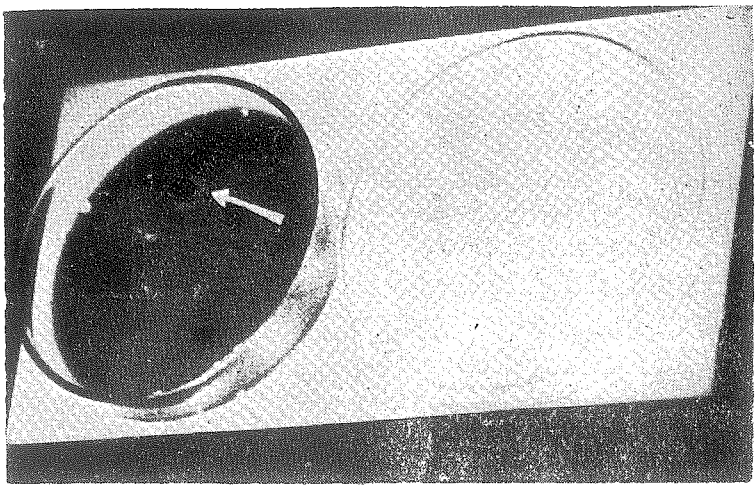

Figure 3a. The inhibition zone formed around the disc is shown with an arrow.

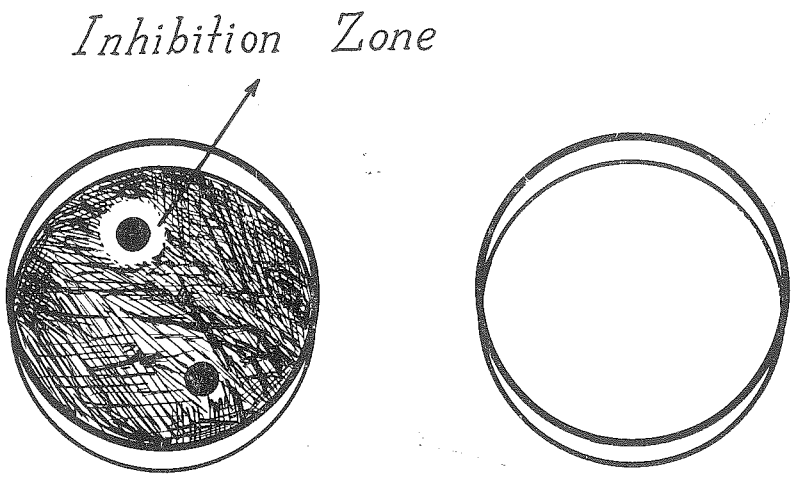

Figure 3b. A schematic drawing of the inhibition zones.

sion of the copper salts in the nutrient media increased and the zones became wider. This indicated that copper amalgam is capable of a bactericidal effect. When a new bacteria was inoculated onto this spreading inhibition zone, it did not grow.

Since the bactericidal effect of copper amalgam was more powerful than that of silver, we applied copper amalgam root canal therapy to 32 patients and followed them by radiologic control of the peridontium and its vicinity. We used the Fischer method [3] of

TABLE 2
INHIBITION ZONES PRODUCED BY AMALGAM ALLOYS

Microörganisms	Inhibition zone*	
	Copper amalgam	Silver amalgam
Staphylococcus aureus	10.8 mm	4.2 mm
Coliform bacilli	8.0 mm	2.4 mm
Staphylococcus albus	12.2 mm	4.2 mm
Beta hemolytic streptococcus	17.2 mm	3.3 mm
D. pneumoniae	8.8 mm	1.1 mm
Neisseriae	18.0 mm	6.2 mm
Candida + pneumococcus	12.0 mm	3.0 mm
Non-hemolytic streptococcus	8.0 mm	1.8 mm
Alpha hemolytic streptococcus	13.6 mm	3.3 mm

* Results are the average of five parallel experiments.

amalgam root canal filling; that is, filling half the canal with copper amalgam and the rest with cement as far as the pulp chamber (Figure 4).

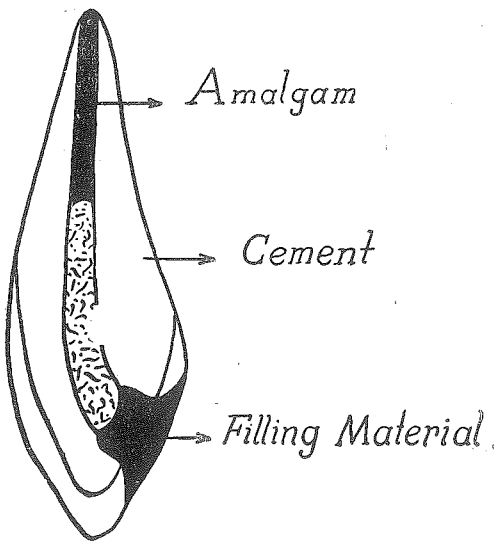

Figure 4. The filled root canal (schematic drawing).

For two years these 32 patients were observed. Of these only four required postoperative treatment. No reaction was observed in

the others. Moreover radiologic examinations revealed that there was general improvement in the periapical tissues.

The two following cases confirm our findings :

Case 1 : Twelve-year-old P. M. came to our clinic with pain in the region of the lower front teeth. Two years before he had suffered trauma of the lower jaw with no immediate consequent reactions. The pain was first noticed one week prior to our examination and had become progressively worse.

On clinical examination the vitality test showed the two lower central incisors and the right lower lateral incisor to be nonvital. The x-ray films showed that around the apical third of the lower lateral there was a wide and diffuse process (an acute exacerbation of the chronic apical parodontitis) and in the roots of the other teeth pathologic periodontal gaps were observed (Figure 5).

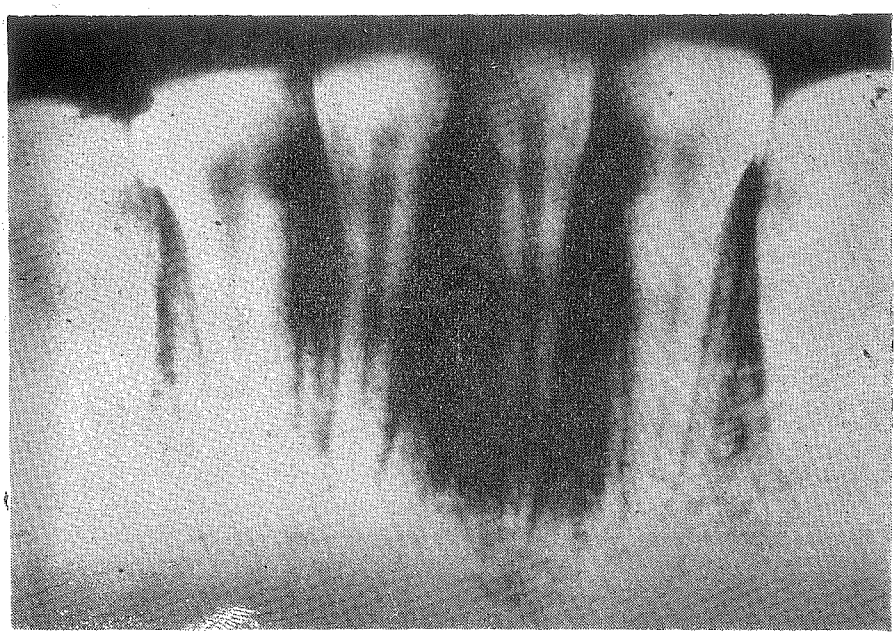

Figure 5. X-ray of the lower jaw incisor and lateral prior to canal therapy. The right lateral incisor clearly shows the existence of a diffuse process in the apex.

Without any intermediate treatment we filled the canals with copper amalgam. Since no postoperative reactions were observed further therapy was not considered. X-rays taken after four months

showed that the pathologic process in the apex of the lateral had decreased and despite the fact that the canals were not filled so far as the apex, the bone in that area had started to ossify (Figure 6). When the patient was examined six months later, the teeth required no clinical interference. Furthermore, the new x-ray films showed that the pathologic process in the apex of the lower lateral incisor had decreased even more and the periodontal gaps had contracted (Figure 7).

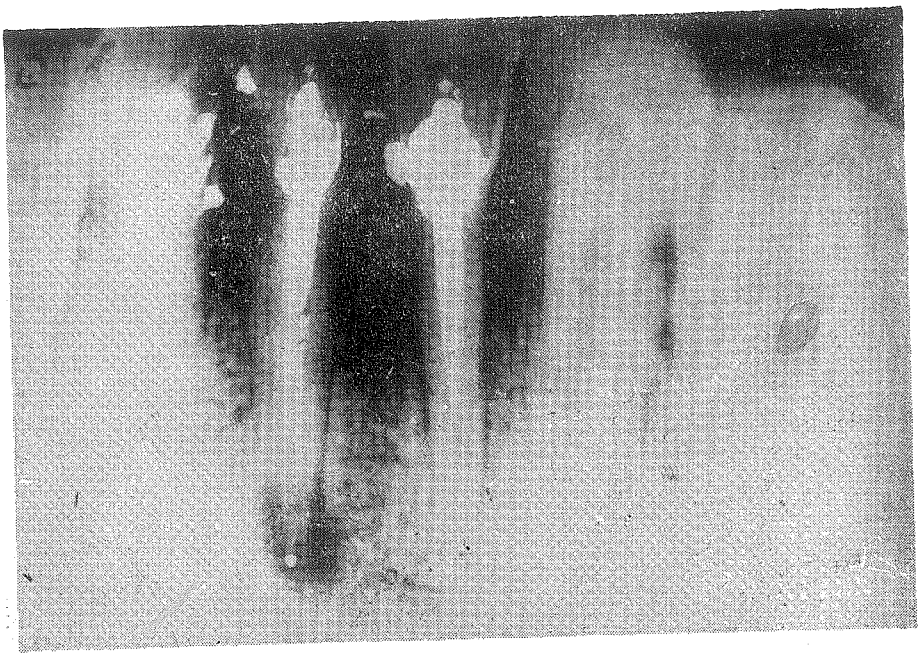

Figure 6. Control x-ray taken four months after the amalgam canal therapy showing the size of the process to be decreased.

Case 2 : Fourteen-year-old J. J. came to our clinic complaining of pain in the region of the upper left central incisor. Three years before he had suffered trauma resulting in fracture of the palatal section of the same incisor, as well as edema in the vestibulum. The edema had subsided and had produced no complications. The vitality test proved the tooth to be nonvital. X-ray films showed a diffuse process around the apex and gaps in the periodontal tissues, particularly of the mesial section (Figure 8). The canal was filled with copper amalgam. Two days after therapy the patient came back to our clinic suffering from postoperative edema. For psychological reasons surgery was not possible. The patient was given analgesics and followed daily. The edema disappeared in a short time.

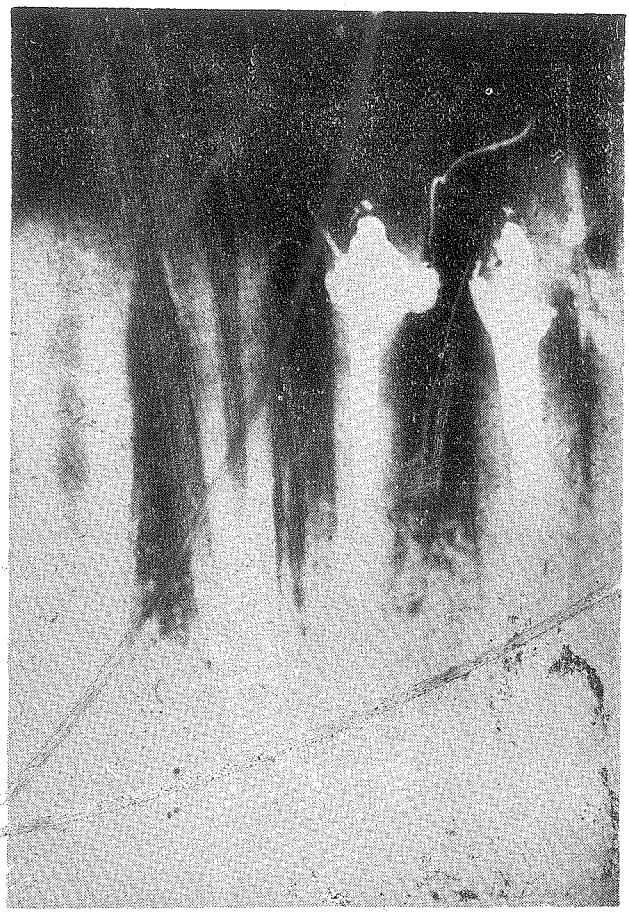

Figure 7. Control x-ray taken after six months. A considerable decrease of the apical process exists in the upper jaw incisor and a decrease in the periodontal space is observed.

When the patient was examined a year later, no reactions were observed. It was also noted on x-ray films that the apical process had disappeared. There were, however, some gaps in the apical region (Figure 9).

Discussion

As a result of our research it was found that copper amalgam produced wider inhibition zones than silver. In our opinion, the insufficiency of the bactericidal effects of silver amalgam result from the covering of the amalgam by undissolved metal salts (Ag_2S) which prevent internal dissolution of the silver ions. A green halo

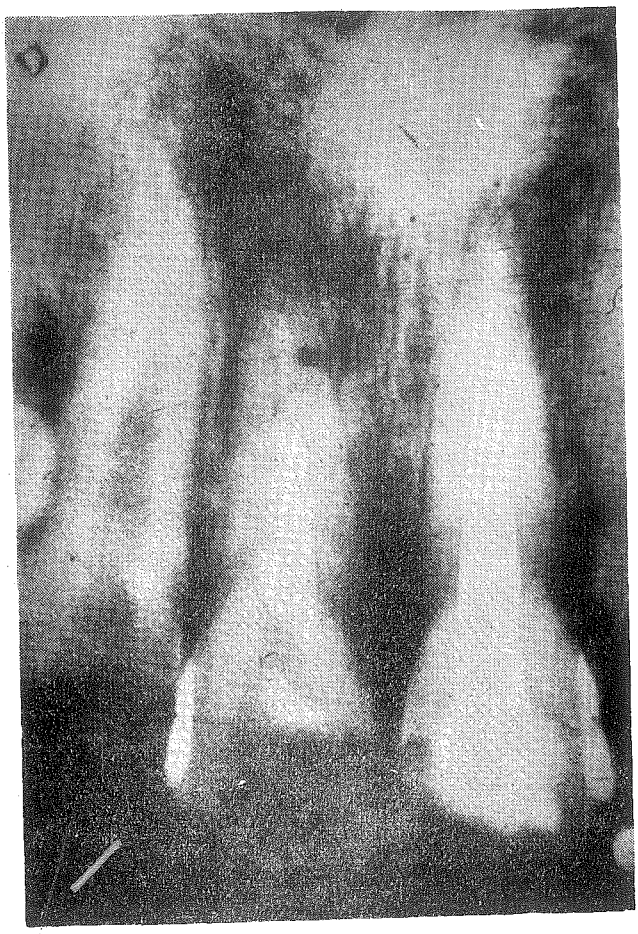

Figure 8. X-ray taken immediately after the canal of the upper jaw incisor was filled with copper amalgam.

is observed around the inhibition zone in the nutrient media [4] formed by a diffusion of copper salts, which continues, increasing the inhibition zone. When new microörganisms are inoculated in these zones, no growth results. In order to obtain an effective bactericide there must be a concentration of copper salts. The possibility of the penetration of copper ions into the dentine canals and the positive bactericidal effect of this metal must be kept in mind.

According to our results, when silver amalgam is used as root canal filling material, it shows little bactericidal or oligodynamic effect. If our observation is correct and bacteria do remain in the canals prepared for therapy, then when the canal is filled with silver

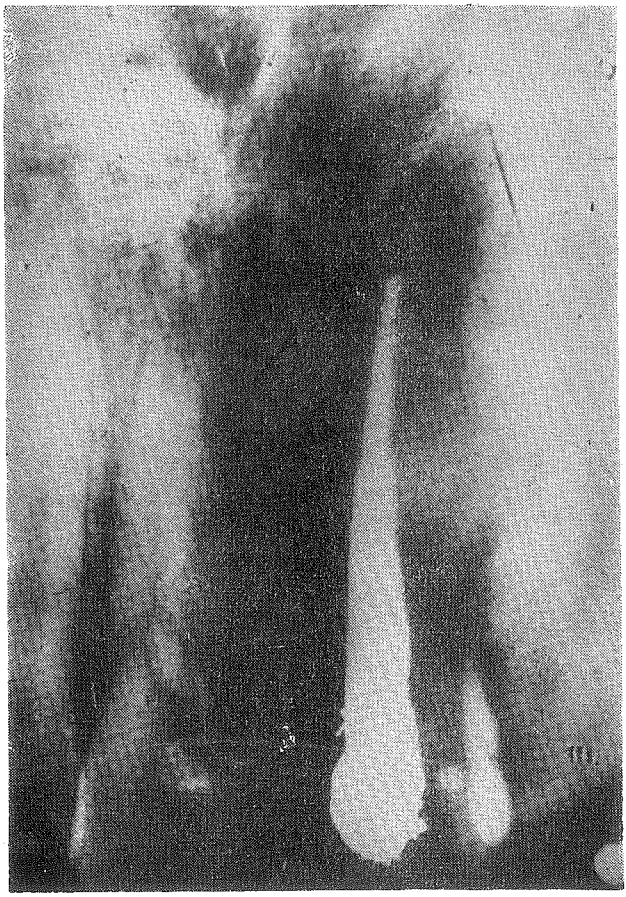

Figure 9. X-ray taken after one year shows the apical process has disappeared.

amalgam the slight oligodynamic effect of this material and its imperfect adaption to the canal walls should be considered.

In spite of the diffuse infections observed in some of our patients, a postoperative reaction was not observed; this indicated the bactericidal effect of copper amalgam. The process of tissue regeneration in all periapical parts of the teeth observed on random x-rays confirmed the results obtained in the laboratory.

According to Weiss and Münch, [5] teeth filled with copper amalgam show some degree of discoloration from the copper sulfates. No change in color has been observed in our patients treated with copper amalgam. In our opinion, this is because only the apical thirds were filled with copper amalgam and the rest with cement.

Summary

Bactericidal and oligodynamic effects of amalgam in canal filling material are examined. Copper and silver amalgams made by various commercial firms were used. The pulp and periapical tissues of 46 teeth were removed, cultures prepared and microörganisms isolated. Prepared amalgam discs were applied to the microörganisms and inhibition zones determined. Copper amalgam inhibition zones were wider than those of silver. Only four of the 32 teeth filled with copper amalgam showed a postoperative reaction. On periodic radiologic control the apical processes and the periapical areas of all teeth were observed to be regenerated. The results of our experiments show that copper amalgam due to its bactericidal effects is preferable as canal filling material.

Acknowledgments

The author expresses his appreciation to the staff of the Microbiology Department of Hacettepe University Faculty of Medicine who carried out the microbiologic experiments of this research.

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Bacilluria in Turkish Women

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Many recent studies have focused upon the detection of bacilluria. Kass, [1-3] Turner [4] and others [5-7] emphasize the importance of diagnosing asymptomatic urinary tract infection before further complications develop.

Our purpose here is to report the incidence of bacilluria from a sample of women attending a gynecologic and obstetric out-patient clinic of Hacettepe and to select a simple method for routine examination.

Materials and Methods

The subjects were 356 unselected married females studied from October 1967 to February 1968. Special inquiries were made of each patient regarding age, date of marriage, duration and number of previous pregnancies, present or past history of urinary complaints or examinations and history of abdominal or vaginal operations. Instructions were given for the collection of a mid-stream[8-10] sample of urine and a sterile container provided.

Within an hour of collection the Griess-Ilosvay nitrate test was done on each urine specimen. Routine cultures were then made directly from the sample on both blood-agar and MacConkey plates by the 0.01 ml drop [4] and the standardized 2 mm internal diameter loop methods. [11] A colony count of 1000 or more colonies by the drop method or 176 or more colonies by the platinum loop technique (Figure 1) constituted significant bacilluria (the equivalent of 100,000 organisms per ml of the uncentrifuged urine).

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A gram-stained preparation was made directly from each urine sample and examined microscopically for the presence of organisms and/or pus cells (in a wet preparation the presence of five or more pus cells per high power field constitutes pyuria [12 13]).

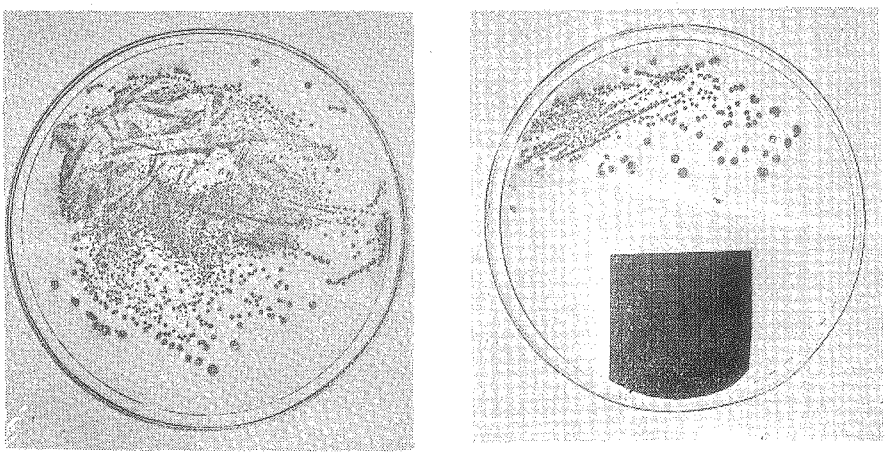

Figure 1. MacConkey plates showing significant bacilluria, drop method (right) and loop method (left).

Results

Of the 356 women, significant bacilluria was detected in 29, an incidence of 8.1 per cent. The influence of pregnancy, parity and the number of years of marriage on the incidence of bacilluria are illustrated in Tables 1, 2 and 3 respectively. Table 4 shows the distribution of the different types of organisms causing bacilluria encountered in this investigation and the results of sensitivity tests to Furadantin and terramycin.

TABLE 1

BACILLURIA RELATED TO PREGNANCY

Condition	Total	Positives	Per cent
Pregnant	193	11	5.6
Non-pregnant	163	18	11.0
Total	356	29	8.1

$X^2 = 2,666$ $\quad 2,666 < 3,841 \quad P > 0.05$

TABLE 2

BACILLURIA RELATED TO PARITY

Condition	Total	Positives	Per cent
Primigravida	58	6	10.3
Multipara	239	17	7.1
Never pregnant	59	6	10.0
Total	356	29	8.1

$X^2 = 1{,}067$ $1{,}067 < 5{,}991$ $P > 0.05$

TABLE 3

BACILLURIA RELATED TO THE NUMBER OF YEARS OF MARRIAGE

Number of years of Marriage	Total	Positives	Per cent
0 to 5	151	16	10.6
6 or more	205	13	6.3
Total	356	29	8.1

$X^2 = 1{,}574$ $1{,}574 < 3{,}841$ $P > 0.05$

TABLE 4

TYPE AND SENSITIVITY RESULTS OF ORGANISMS CAUSING BACILLURIA

Organism	Total	Furadantin Sensitive	Terramycin Sensitive
B. coli	23 (79.3 %)	23 (100 %)	6 (26.1 %)
Proteus	4 (13.8 %)	4 (100 %)	None
Strep. faecalis B. coli Staph. aureus	2 (6.9 %)	2 (100 %)	2 (100 %)
Total	29 (100 %)	29 (100 %)	8 (27.6 %)

Griess-Ilosvay test was found to be positive in 19 cases (65.5 per cent). Proteinuria accompanied by pyuria (Figure 2) was detected in four females with bacilluria (13.7 per cent). Gram negative bacilli were discovered by direct film on 14 (48.2 per cent) of the 29 bacilluria cases.

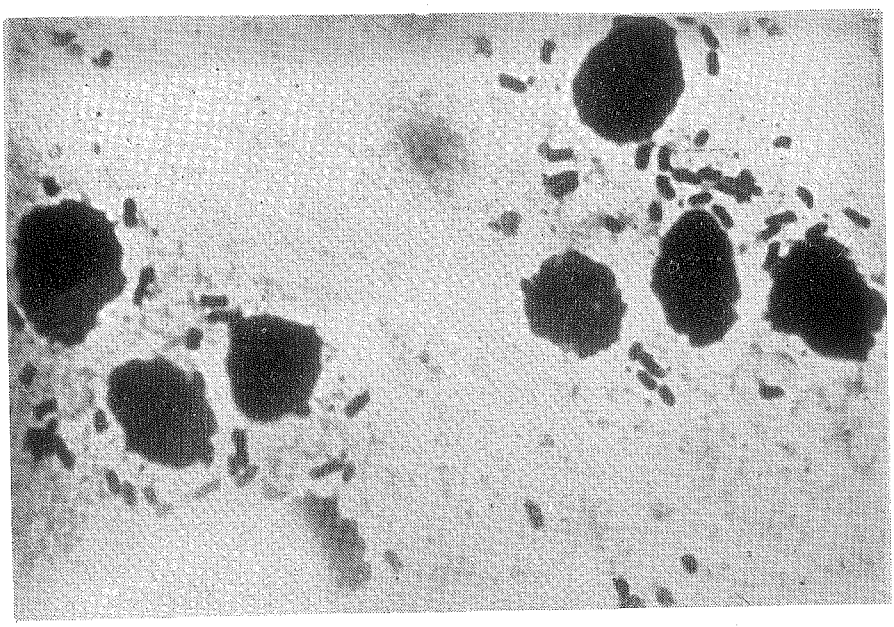

Figure 2. Gram-stained film showing pus cells and gram negative bacilli.

A comparison between the history of urinary tract disease and the presence of bacilluria revealed : 12 (41.4 per cent) had urinary tract disease; of these, seven (24 per cent) had a history of polyuria, dysuria and nocturia, three (10.3 per cent) had dysuria and two (6 per cent) had only nocturia. After three weeks repeat examinations were positive.

Only 27 specimens of urine gave a colony count of less than 500 suggesting contamination, an incidence of 7.6 per cent. The organisms causing contamination in their order of frequency are shown in Table 5.

Discussion

The overall rate of significant bacilluria among the 356 married females examined in this study was 8.1 per cent, somewhat higher than those reported by Kass, [2] Turner, [4] Switzer [5] and Mou and Feldman [14] (6 per cent, 3.2 per cent, 7 per cent and 4.1 per cent respectively). This may be due to a socio-environmental difference.

In this study bacilluria was higher among the primigravida (10.3 per cent) than the multipara (7.1 per cent) and also among those married from zero to five years (10.6 per cent) as opposed

TABLE 5

PROPORTION AND TYPE OF ORGANISM RESPONSIBLE FOR CONTAMINATION

Organism	Number Isolated	Per cent
B. coli	6	22.2
B. coli + Strep. faecalis	4	14.8
B. coli + Staph. aureus	4	14.8
Staph. albus	4	14.8
Staph. aureus	3	11.1
Strep. faecalis	3	11.1
Strep. faecalis + Staph. aureus	2	7.4
B. coli + Proteus	1	3.7

to those married more than six years (6.3 per cent). The decrease in the incidence of bacilluria with the advance of years of marriage or parity might be attributed to the longer period sufficient for detection and treatment.

Bacilluria occurred less frequently in pregnant women (5.6 per cent compared to 11 per cent in the non - pregnant group); however, evidence suggests that the incidence of bacilluria is related to personal hygiene rather than pregnancy. We found a surprisingly high number of cases of bacilluria among the group of married women who have never been pregnant (10 per cent). This may be explained by the frequent gynecological examinations necessary for determining the cause of their sterility.

Furadantin tablets in adequate doses have been recommended for the treatment of bacilluria [15] and all of our cases were sensitive to this drug (Figure 3).

Follow - up of the positive cases of bacilluria with pyuria and albuminuria included only two non - pregnant females. Intravenous pyelography revealed caliectasis in the first (Figure 4) and cessation of excretion of the contrast material in the second (Figure 5). Such findings favor theories that serious renal complications may follow undetected and therefore untreated cases of bacilluria.[4 6 16 17]

From previous studies [1-4 18] concerning detection of bacilluria, we propose to select a method for use in out - patient clinics, gen-

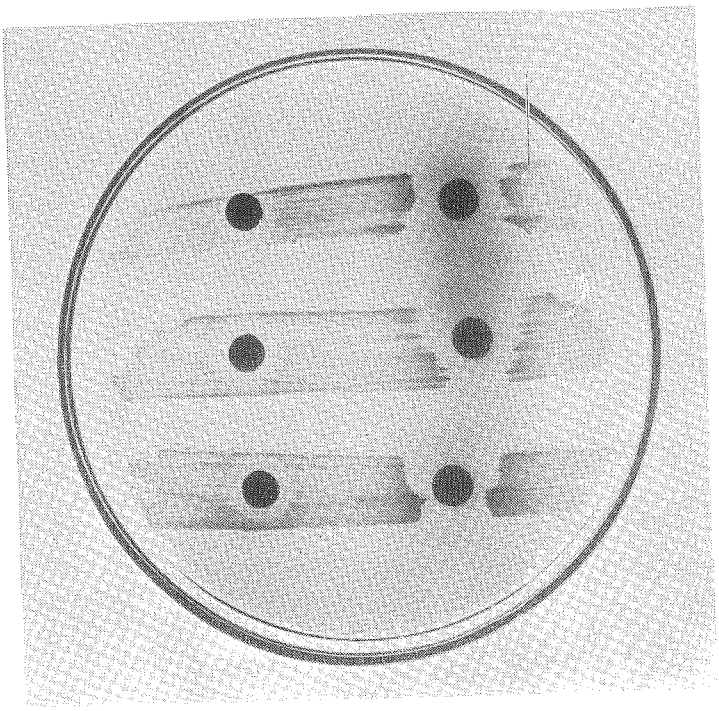

Figure 3. Agar plate showing Furadantin (right) and terramycin (left) sensitivity test results.

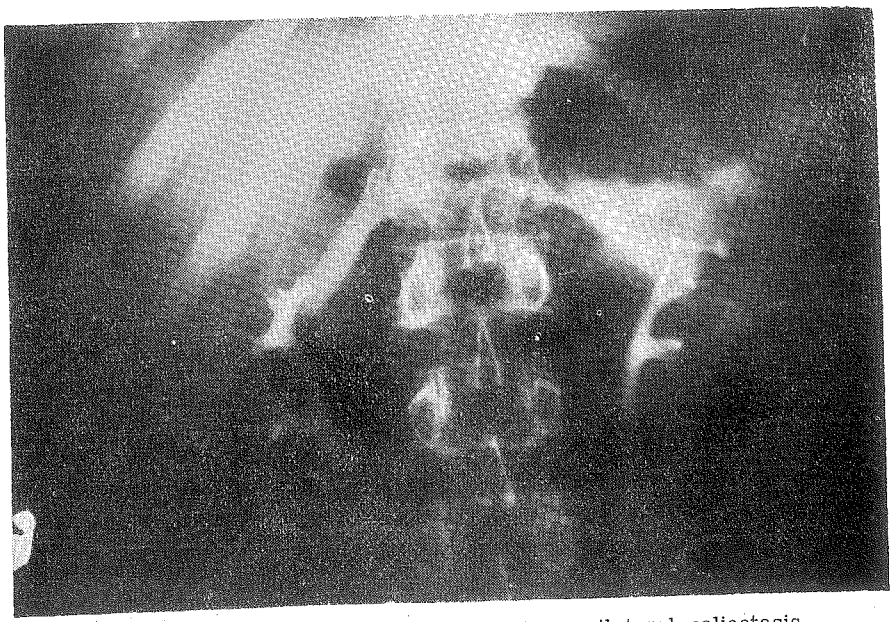

Figure 4. Intravenous urogram showing unilateral caliectasis.

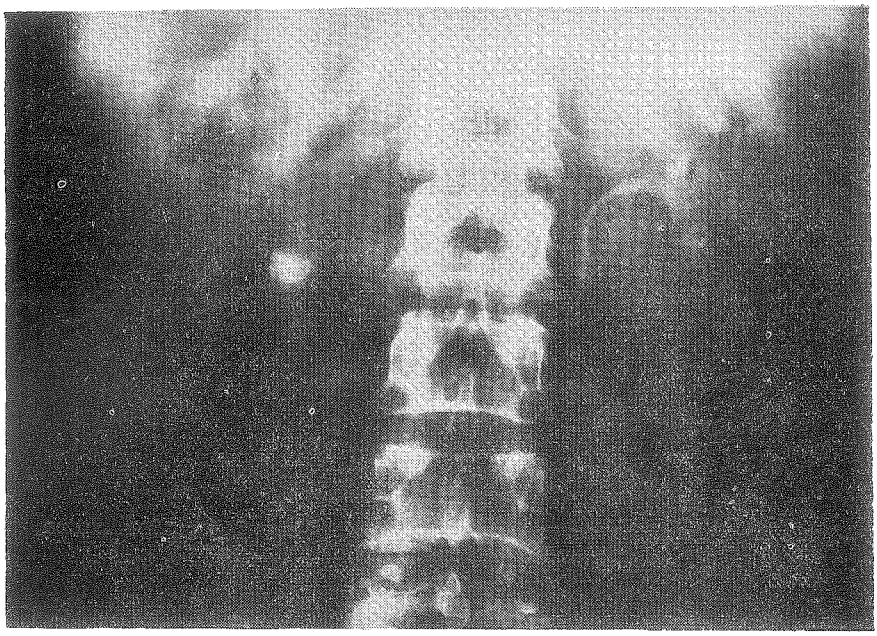

Figure 5. Intravenous urogram showing unilateral stone shadow and no excretion of the dye.

eral practice and in mass - screening. The slide method of Cohen and Kass [18] for quantitative culture was tested in this study (Figure 6) but was of limited value.

The test-tube MacConkey slope method used in this investigation consisted of pouring a sufficient amount of urine into the tube to cover the surface of the MacConkey media. After 24 hours incubation the density of the colony growth was noted (Figure 7). Significant bacilluria produced such growth that colonies were difficult to count, while contaminated urine yielded only slight growth and sterile urine, no growth. We think that the test-tube MacConkey slope method in terms of applicability and cost gives the most satisfactory results.

In view of the results of this study, it can be concluded that routine urine examination for the detection of presymptomatic urinary tract infections should be performed in all obstetric and gynecologic clinics.

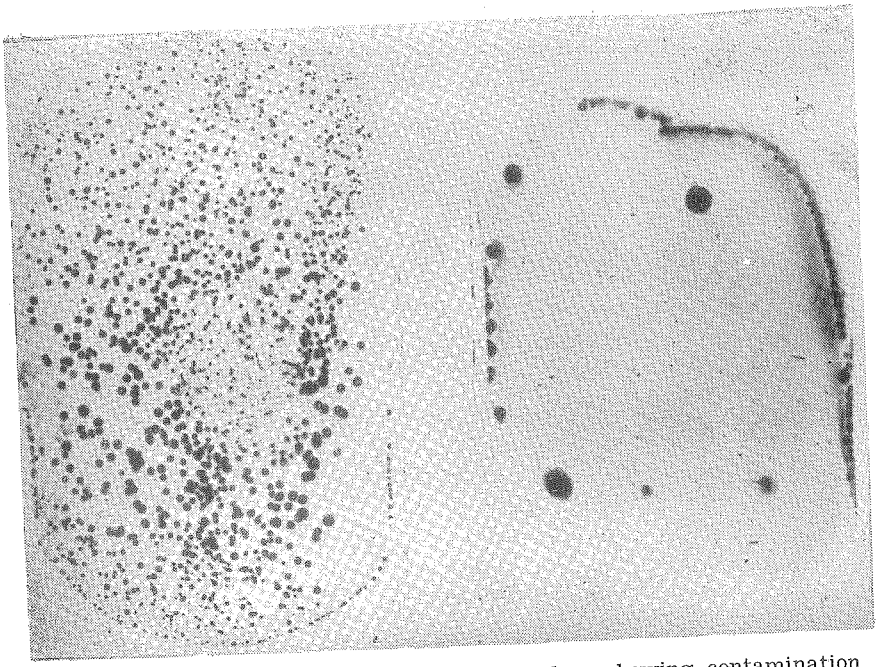

Figure 6. Two slides covered with MacConkey showing contamination (**right**) and significant bacilluria (**left**).

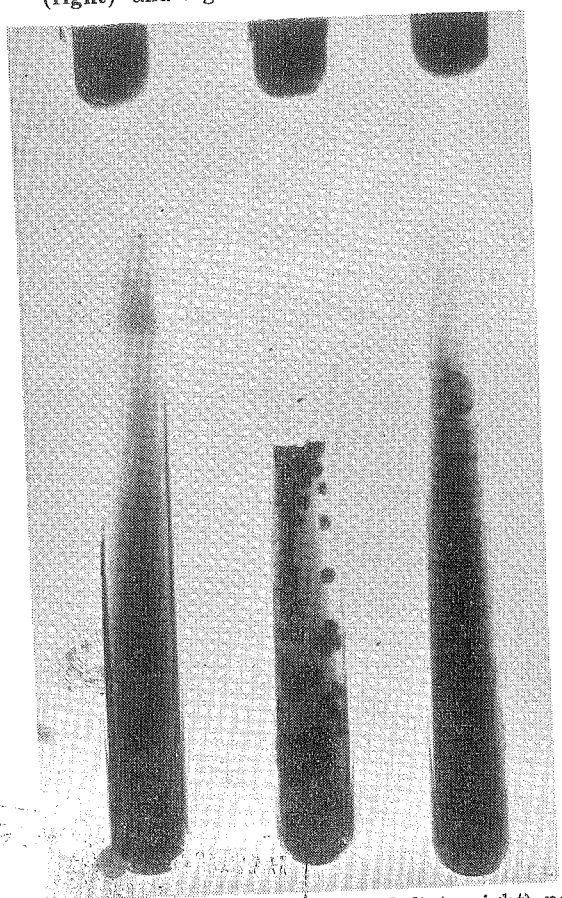

Figure 7. MacConkey slopes showing (left to right) no growth, contamination and significant bacilluria.

Summary

The overall incidence rate of significant bacilluria was found to be 8.1 per cent among the 356 married women examined in one of the gynecologic and obstetric out-patient clinics of Hacettepe. The incidence of bacilluria was found to be 10.3 per cent in primigravida, 7.1 per cent in multipara, 5.6 per cent in pregnant women, 11 per cent in non-pregnant women and 10 per cent among those with no history of pregnancy. Bacilluria was found to be 10.6 per cent among those who had been married recently (up to five years) compared to 6.3 per cent among those married longer. Follow-up study of two cases with significant bacilluria by intravenous pyelography revealed urinary tract abnormalities. The type of organisms responsible for bacilluria and their sensitivity to Furadantin and terramycin were studied. Detection of significant bacilluria by the test-tube MacConkey slope method was discussed.

Acknowledgments

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Intracranial Hemorrhage in Pregnancy

A CASE REPORT

Mithat Erdoğan, M. D.*

Subarachnoid hemorrhage in pregnancy is fortunately rare. When it does occur, however, it usually results either in severe neurological damage to the mother or her death. The incidence has been reported variously as one intracranial hemorrhage in 7172 pregnancies, [1] one in 2000 [2] and one in 5139.[3] The three types of intracranial aneurysms are congenital, mycotic and arteriosclerotic, the most important of these being congenital with bleeding occurring at the bifurcation point or in the branches of the circle of Willis.

The purpose of reporting one of these cases is to discuss their management and treatment.

Case Report

A 40-year-old, married, multigravida female was first seen in the Out-patient Department on February 15, 1966. Physical and laboratory examinations were normal. Obstetric examination revealed a six months' pregnancy, blood pressure 120/70 and pulse 86. The expected date of confinement was May 27, 1966. Antepartum control was normal.

The patient came to the clinic with labor pains on May 21. Examination showed vertex presentation, 1 cm cervical dilatation and — 2 station. Fetal heart sounds were 146 and regular. There was no albumin in the mother's urine nor edema of the feet. She was taken to the labor room.

A short time later the patient complained of sudden pain in the frontal region. A speech difficulty was noted. Pulse was 98 and blood pressure 170/90. Paralysis of the left side of the face was observed.

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Shortly thereafter convulsions started and she became cyanotic. At that time neurologic consultation was called. Laboratory examination revealed a hemoglobin of 12.7 and normal urine. Spinal tap showed bloody cerebrospinal fluid with a pressure of 600 mm of water. The patient was given oxygen, I. V. dextrose in water and sodium pentothal 0.25 gm for the convulsions. One hour later the neurologic examination showed hyperactivity of tendon reflexes and positive Hoffman and Babinski on the left.

From these findings a diagnosis of subarachnoid hemorrhage was made. Arteriogram was unsuccessful. The neurosurgeons suggested conservative treatment of the hemorrhage until the patient came out of coma. Tracheotomy was performed and the patient was taken to the intensive care unit. Three days after this episode the mother's heart sounds were unobtainable and artificial respiration and injection of adrenalin were not beneficial. Fetal heart sounds could not be heard upon careful auscultation and for this reason a postmortem cesarean section was not performed. Permission for autopsy was not granted.

Discussion

The most important sign of rupture of an intracranial aneurysm is a sudden and severe headache usually located in the occipital region. Other symptoms are nausea, vomiting, convulsions, vertigo and vision disturbances. After bleeding has occurred, ptosis and dilatation of the ipsilateral pupil is seen in most cases. Blood in the cerebrospinal fluid is characteristic. In the acute phase, the pressure of the spinal fluid is high.

Differential diagnosis must be made between toxemia, brain tumor, meningitis, hypertensive cardiovascular disease, traumatic intracranial hemorrhage and cerebral embolus. If the angiogram is noncontributory to the diagnosis, physical findings and examination of the cerebrospinal fluid should be conclusive. From postmortem examinations and angiograms we have learned that in 80 per cent of the cases intracranial hemorrhage is seen following an aneurysm. Second to aneurysm, the most common cause of subarachnoid bleeding is arterio-venous malformation.

Greenhill [4] reports that pulse rate, which is increased during pregnancy, is even higher prior to delivery. In the third trimester, it is believed that blood volume is also increased. Blood pressure is usually low until the ninth month but rises during the tenth month

and during labor increases in almost every case. According to these physiologic principles, labor should be a dangerous condition for intracranial aneurysms; however, some investigators do not believe that labor affects an aneurysm. [5-8]

Subarachnoid bleeding during pregnancy causes a high percentage of maternal deaths. Rhoads [9] believes that the incidence of maternal death is probably 50 per cent. According to Hamby [10] death will occur in 45 per cent of the cases during the first crisis. Walton [5] claims that in those patients who survive the first episode there is a 10.5 per cent possibility that death will occur during the second and within six months. In these cases coma, hypertension, papilledema and convulsions usually develop.

We do not yet know which treatment is most helpful to the patient. According to some, [2 5 9 11 12] the main principle is to do nothing until the patient's condition is stable, thus allowing a coagulum to form to repair the tear and stop the bleeding. Another group [10 13 15] believes that an attempt must be made to stop the intracranial bleeding by ligation of the common or internal carotid artery. Another way is ligation or clipping of the intracranial artery.

Aneurysm in subarachnoid bleeding can be seen by arteriogram in only 50 per cent of the cases. Multiple aneurysms cannot always be demonstrated by arteriogram. These patients together with those in coma or with serious neurologic damage, are poor risks for surgery.

Pregnancy itself is not a contraindication for surgery. [2 5 8 11 12] From the obstetric viewpoint, most authors prefer elective cesarean section in approximately the 36th week of gestation. [7 8 11 13 16] C-section performed during coma has produced a maternal mortality rate of 78 per cent and a fetal mortality rate of 38.5 per cent. Others perform C-section only in obstetric indications, [2 5 6 17] Inducing labor with oxytocin does not appear to increase the risk of rupture of an aneurysm. When an oxytocic is used in conjunction with anesthetic, labor progresses at a normal rate, straining in the second stage may be avoided and delivery can be accomplished by outlet forceps. [18] Recently it has been suggested that the two procedures, C-section and intracranial surgery, be done at the same time. [19]

Summary

A case of intracranial aneurysm is presented and the incidence of and literature pertaining to intracranial aneurysm are discussed along with its obstetric management.

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Congenital Uterine Anomaly with Endometriosis

A CASE REPORT

Muammer Alpay, M.D.*

The etiology of endometriosis has not yet been clarified. One of the most standard explanations is Sampson's [1] regurgitation theory, which suggests that during menstruation uterine fragments are regurgitated through the tubes and implanted in the peritoneum. This theory accounts for direct implantation of endometriosis in the pelvis but not in the extrapelvic regions. Meyer and Ivanoff [2] and later Novak [3] support the coelomic metaplasia theory to explain the mechanism of endometrial implant outside the pelvic organs, as in the case of umbilicus and inguinal lymph node implantations. Halban [4] and recently Javert [5 6] support this «benign metastasis theory» saying that the endometrial fragments are transported into the extrapelvic organs by the lymphatic and/or vascular systems, thereby clarifying the rare cases of endometriosis in the arm, leg, lung and kidney. Novak [7] believes that a single theory cannot define the etiology of endometriosis.

The following case is an example supporting Sampon's regurgitation theory and the rôle of retrograde menstruation in the formation of endometriosis in a patient with congenital uterine anomaly.

Case Report

Z. O. (67/11816), a 20-year-old, married female, came to our clinic complaining of difficulty during coitus, primary amenorrhea and progressive cyclic pain in the lower quadrant. Physical examination revealed the breasts to be normal and the distribution of pubic hair typically female. On pelvic examination the vagina permitted the insertion of only one finger, stenosis was noted in the upper third and the depth measured 6 cm. On rectal examination a 4 to 5 cm, apparently symmetrical, bi-lobed mass, joined at the

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midline, was felt. Intravenous pyelogram showed a depression on the upper contour of the urinary bladder thought to be caused by the mass felt on rectal examination (Figure 1). A spina bifida was also seen on the first sacral segment. On laporatomy, the uterus was hypoplastic, the adnexa were palpable on both sides and slightly enlarged foci of endometriosis were discernible grossly on the posterior surface of the uterus and the sigmoid. The cervix could not be palpated. Hysterectomy, bilateral salpingectomy and wedge resection of both ovaries were performed.

The pathology report stated that the endometrium was normal in thickness and covered by columnar epithelium (Figure 2). One or two endometrial glands were noted near the serosa in the endometrium (Figure 3), along with hematosalpinx (Figure 4), endometrial islands in the wall of the tubes (Figure 5) and corpus albicans in the right ovary (Figure 6).

Discussion

Pelvic implantation following the regurgitation of uterine fragments through the tubes during menstruation is the basis of Sampson's regurgitation theory. Sampson also put forth an implantation theory suggesting a relationship between endometriosis and retrograde menstruation resulting from congenital or acquired obstruction.

Te Linde and Scott [8] experimentally produced pelvic endometriosis in monkeys by directing the menstrual flow into the pelvic peritoneum. Kimball and Reeves [9] published two cases of endometriosis which occurred near the area accidently perforated during a curettage which was perfomed six weeks following a normal delivery. In these two cases the endometrial fragments were directly implanted by passing through the perforated area. Ridley and Edwards [10] were the first to succed in experimentally producing endometriosis in humans. Ridley, [11] on 15 patients who were to have laporatomy for myoma, injected their menstrual blood into the abdominal cavity and, at laporatomy, found endometriosis in two of the patients. Sutton [12] examining young girls with endometriosis found one to have congenital imperforate hymen, hematocolpos, hematometria and bilateral hematosalpinx. A case of endometriosis associated with uterus didelphys was diagnosed by McDonald. [13] Fallas [14] discussed endometriosis and its relation to retrograde menstruation in a patient with an uterine anomaly. These experiments show that in order to be implanted after regurgitation, the endometrial fragments have to be viable, transportable by the tubes and capable of growing after implantation.

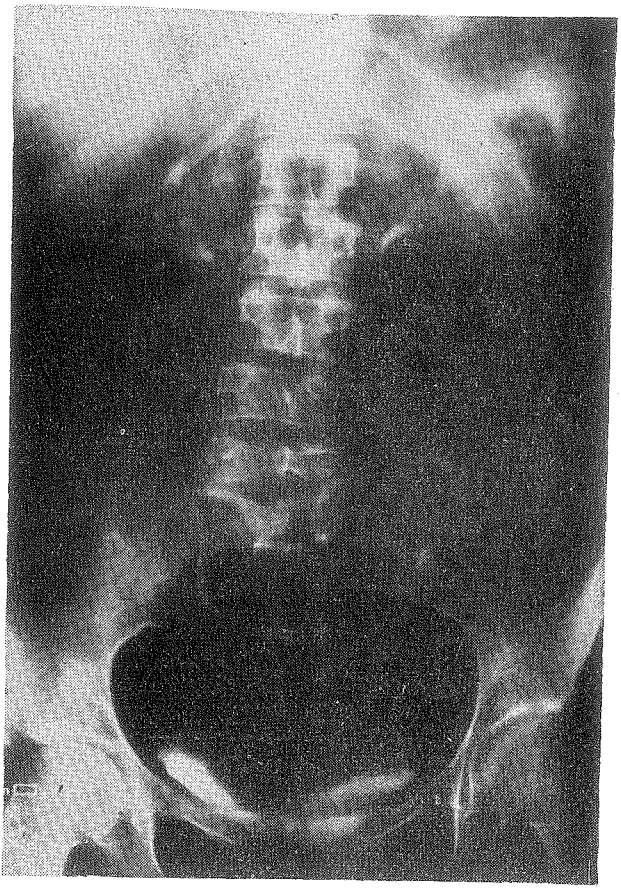

Figure 1. Pyelogram showing filling defect in the urinary bladder due to the uterine anomaly.

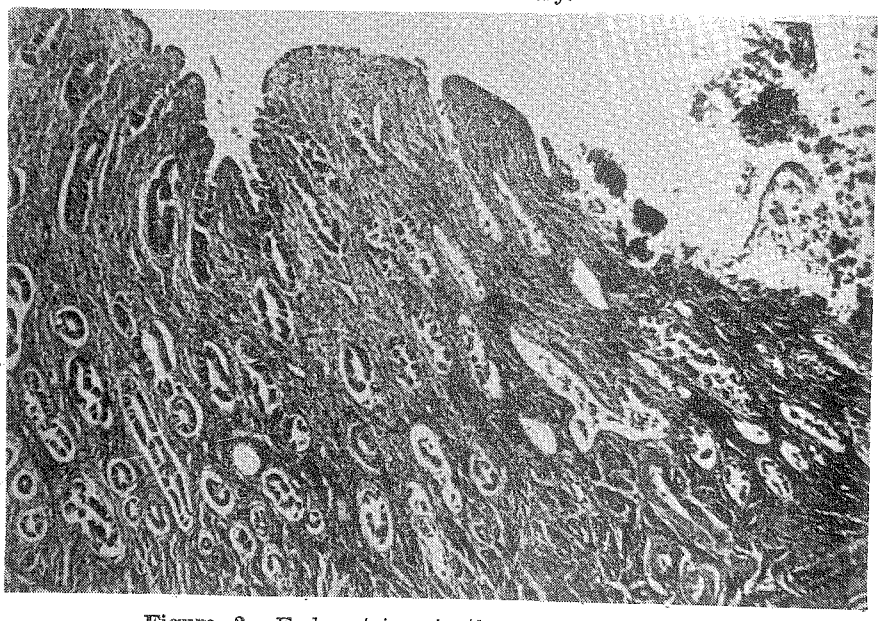

Figure 2. Endometrium in the proliferative phase.

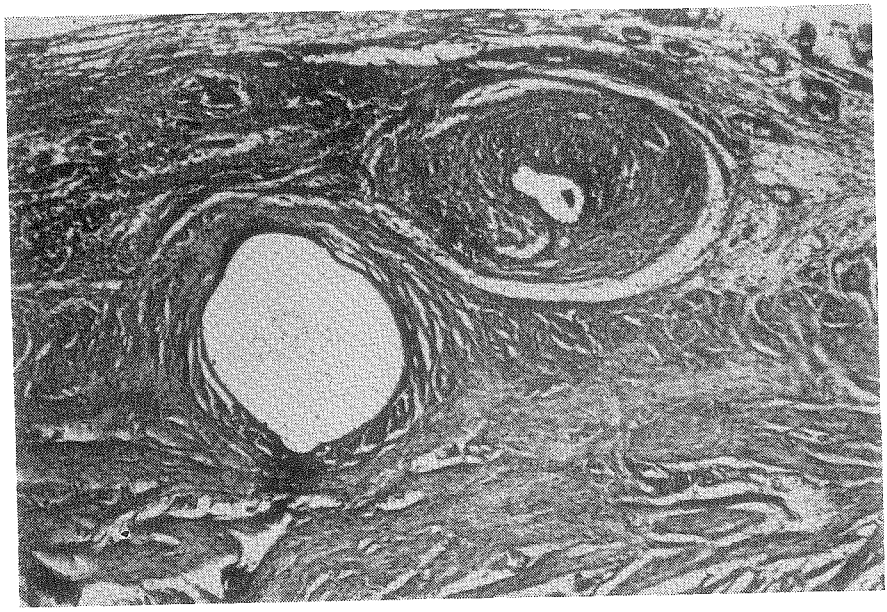

Figure 3. Foci of endometriosis in the uterine serosa.

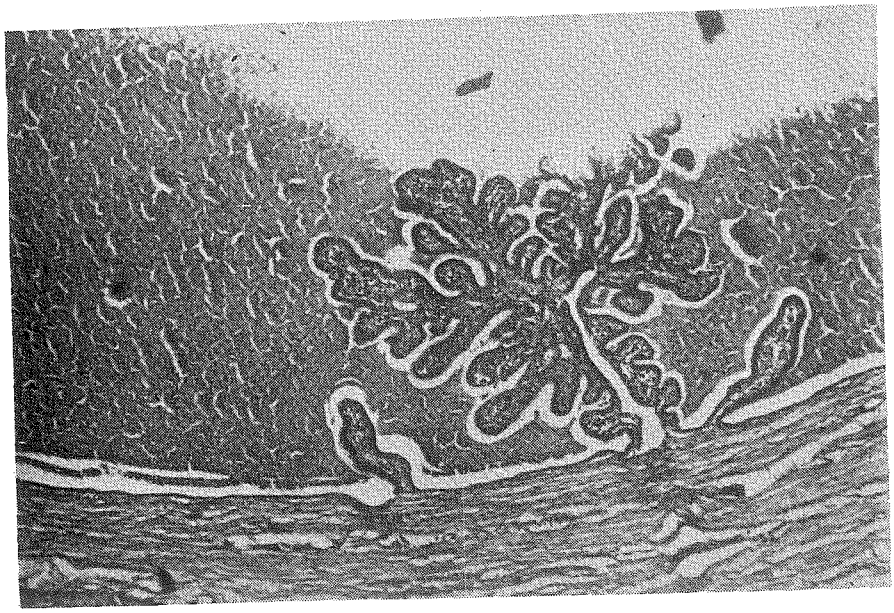

Figure 4. Section of tube showing hematosalpinx.

Figure 5. A focus of endometriosis in the tubal wall.

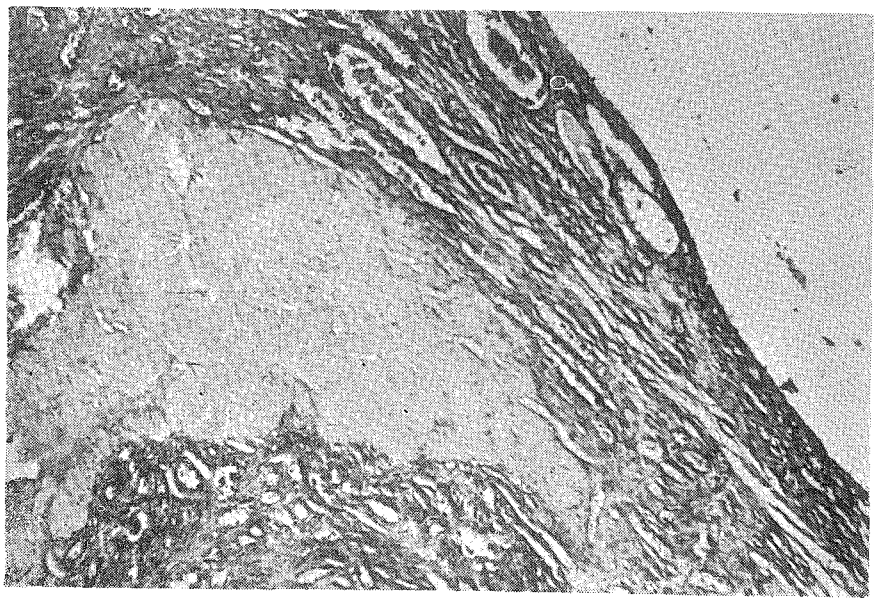

Figure 6. Corpus albicans in the ovary.

The secondary sex characteristics of our patient were normal and the presence of a corpus albicans in the ovary indicated that she was ovulating. However, because of the vaginal atresia, the menstrual flow was retrograde producing cyclic pain. The gross and microscopic bilateral hematosalpinx was due to regurgitation as were the ectopic endometrial foci.

Summary

A case of pelvic endometriosis with congenital uterine anomaly is presented and the relationship between the endometriosis and the anomaly is discussed from an etiologic point of view.

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Dean of the Ghana University
Medical School

□ Professor C. O. Esmond, Dean of the Ghana University Medical School, visited Hacettepe University Medical School on April 30 and May 1, 1968, during his second trip to Turkey. As a surgeon and Head of the Department of Surgery at Ghana Medical School, Professor Esmond is interested in the activities of surgical departments throughout the world, especially in ways of getting teaching staff and routine workers for the basic science branches of the medical school. He visited the Microbiology, Pathology, Biochemistry, Anatomy, Histology and Physiology Departments of Hacettepe and discussed the shortage of staff in his home country with the faculty members of these departments.

At the close of his visit he stated that he was impressed by the spirit he felt at Hacettepe and hoped that in the near future he would be able to welcome some of our faculty members to his University as visiting teaching staff.

□ Dr. L. E. Glynn, Member of the Scientific Staff Medical Research Council, Rheumatism Research Unit, Taplow, and Director of Pathology, Canadian Red Cross Memorial Hospital, visited Ankara from May 21 to 25, 1968.

Doctor Glynn is one of the authors of the book «Autoimmunity and Disease» and is an expert in this field. He gave two lectures while in Ankara, one at Hacettepe University and the other at Ankara University, on the subjects of «Autoimmunity and Disease» and «Autoimmune Mechanism of Cell Damage» Both were most

informative and gave details of the recent advances in these very interesting fields.

□ Prof. Dr. Bernard Greenberg from the University of Illinois, U.S.A., and Instituto Superiore di Sanita, Rome, visited Hacettepe University in May and delivered two lectures in the Biology Institute of the University. The lectures were entitled «Biology of Domestic Flies» and «Fly-Microbe Interactions.»

Congress

□ The Thirteenth Turkish Microbiology Congress will be held in Istanbul in September, 1968. Some of the papers listed in the preliminary program are: «Leptospirosis in Turkey,» «Milk Microbiology,» «The Microbiology of Milk Products,» «Food Standards,» «The Effects of Harmful Gases in the Air on Human Beings, Animals and Plants» and «The Survival Time of Brucella Bacteria in Milk, Cream and Derivatives.»

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HACETTEPE BULLETIN OF
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EDITORIAL

As was explained in the first issue of this journal, the need arose for Hacettepe University to print articles outside the field of pediatrics. As a result of this need, the Hacettepe Bulletin of Medicine/Surgery is now being published.

This issue of the Bulletin represents at least six fields of medicine and surgery, including physiology, microbiology, histology, neurosurgery, plastic surgery and internal medicine. We are proud to have the opportunity of publishing the valuable works of Bor, Karacadağ, Kerse, Erhan, Gürsu and El-Garhy, and many more papers of equal importance are in preparation for future issues. We hope that Hacettepe University will continue contributing to the world of medicine by presenting the studies of some of Turkey's most outstanding researchers, and that in future issues our Bulletin will continue to grow in scope and interest. We feel sure that in the near future the journal will become more satisfactory with the inclusion of a variety of features such as abstracts, letters to the Editor, book reviews, etc., as well as papers from institutions outside of Hacettepe both in Turkey and abroad.

May I remind our readers once again that their interest and assistance will facilitate the development and improvement of this very young journal, and we would therefore welcome any criticism, ideas and recommendations they may have to offer.

Muvaffak Akman, M.D.

Editor

Contribution of Fetal Skin to Transfer of Sodium from Amniotic Fluid

Naci M. Bor, M.D.* / Tuncer Karpuzoğlu, M.D.**

Since it has been demonstrated that amniotic fluid circulates, interest has been increasing in identifying the organs responsible for this function.[1-3] Most authors who have written on this subject believe that amniotic fluid is composed of urine, secretions from the respiratory tract, excretions from the gastrointestinal system and transudation from the skin and the amnion.[3-8] Disposal of this fluid, however, is effected via the alveolar membrane of the lungs, the intestinal wall, the amnion and the skin.[6 8-11]

Review of the literature on this topic shows that before the interest stimulated by the investigations made by Flexner et al[1 2] and Plentl et al[4 8 12] most authors accepted theories having their origin in those of past centuries, which sprang from general impressions rather than from well-documented investigations. This is best expressed by Makepeace et al[13] who stated that, «the source and nature of amniotic fluid, its rate of formation, the mode of reabsorption, if reabsorption occurs, the factors which govern its volume are all matters of conjecture concerning which but little data exits.» Several well-planned studies conducted within the last few years have, however, provided us with valuable information.[5-16]

It is interesting that the contributions of several organs apparently participating in the circulation of amniotic fluid have never been accurately determined. This function has been studied in several publications in which one or other of the organs presumed to contribute was considered responsible to the exclusion of other possibilities.[9 17-19] We therefore set up an experiment eliminating

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all but one of the organs which may participate in the circulation of amniotic fluid.[20] This enables the investigator to study each one separately without interference from the others. Using this experiment, absorption by the amnion of Na^{24} contained in the amniotic fluid was shown.[20] [21] The same type of experiment can also be used singling out the skin to detect its contribution, if any, to the circulation of amniotic fluid. The results of such an experiment are presented in this paper.

Materials and Methods

Eleven ewes in the last month of pregnancy were used for this investigation. Each animal was anesthetized with nembutal (25 mg/kg) and atropine sulfate (0.30 mg). After tracheal intubation and catheterization of one of the peripheral veins, the abdomen was opened at the midline and the uterus exposed under sterile conditions. In a relatively avascular area away from the palpable cotyledons, a longitudinal incision was made and the uterine layers were individually dissected and incized. When the chorion was visualized, the position of the fetus was determined, and the largest pocket of fluid was located. Over this area a small incision was made, a number 12 French catheter was inserted, and approximately 300 ml of amniotic fluid were drained into a container. The incision on the uterine wall and the fetal membranes was extended without further loss of amniotic fluid. The head of the fetus was then slowly delivered, and to prevent respiration its muzzle was immediately placed into a tightly fitting rubber glove. The whole fetus was extracted from the uterine cavity, placed in a plastic bag containing Ringer's solution at 37°C, and held at the same level as the uterus. Tension on the cord was carefully avoided to ensure unimpaired circulation (Figure 1).

The evacuated uterine cavity was examined and the fetal membranes restored to their anatomical positions, and the amniotic fluid was then slowly poured back into the amniotic cavity.

Through a small incision, the right cervical vein of the fetus was catheterized; 25 microcuries of $Na^{22}Cl$ were added to the Ringer solution surrounding the fetus and stirred until completely mixed. Samples of fetal and maternal bloods were taken at ten minute intervals for two hours, following which the umbilical cord was ligated and severed. The surgical wounds were closed and proper care was given to permit survival of the mother sheep. Fetal organ

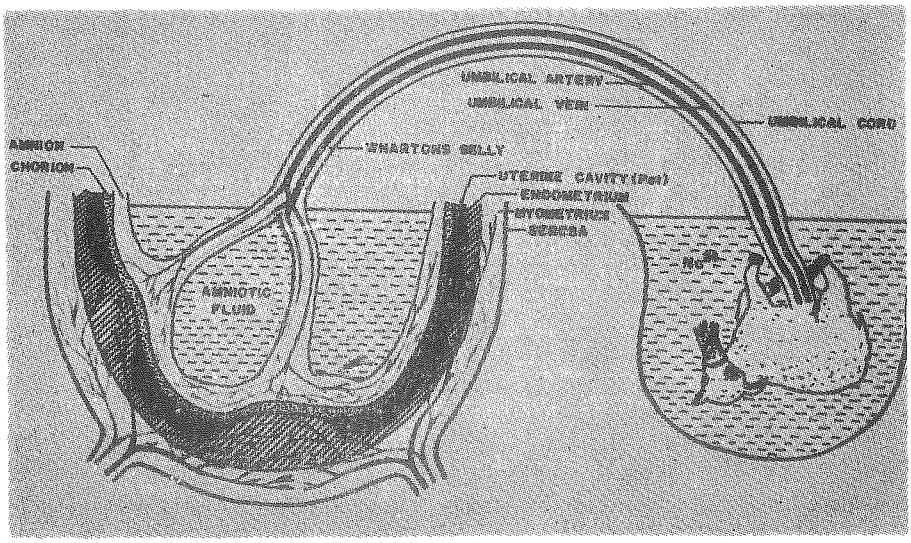

Figure 1

samples were taken and their radionuclide concentrations determined using a Packard liquid scintillation spectrometer.[20]

In order to compare the results of these experiments with those of our former studies, the tracer was placed in the amniotic cavity instead of in the plastic container in two additional cases. Samples of maternal and fetal bloods and organs were obtained as in the other experiments. The results were tabulated and statistically evaluated.

Results

The net counts of blood samples taken at scheduled time intervals after bringing the tracer into contact with the fetal skin in the bathing solution are seen in Table 1. Means of the net counts of samples taken at each step of the experiment ranged from -1.875 (standard error $\pm$ 2.795) at 5 minutes to + 2.277 ($\pm$ 2.345) at 90 minutes. The mean of the entire group was + 0.377 ($\pm$ 184). Variance analyses of these means revealed no significant difference in concentration of the tracer from one stage of the experiment to the other (intergroup $f = 0.358$).

The maternal blood samples also failed to show a rising concentration of the tracer as a function of time (Table 2). The mean of the net counts seen in the entire table was 0.781 ($\pm$ 0.79). Means of the results obtained at different stages of the experiment ranged from + 1.888 ($\pm$ 1.45) at five minutes to — 5.625 ($\pm$ 2.50) at the

end of the first hour. Variance analysis of these groups of figures at different stages of the experiment gave no significant difference ($f = 0.407$). These results confirm the findings of the fetal blood samples.

Tables 3 and 4 present the net counts obtained from two experiments in which the radio tracer was placed in the amniotic cavity. Here the concentration of Na^{24} in the fetal and maternal bloods rose steadily throughout the experiment. This observation corresponds with our former findings of salt absorption from amniotic fluid by the amnion.[20] A comparison was made between the findings in Table 1 and Table 3. Intergroup variance analyses of these two sets of figures revealed a significant difference ($t=13.147$). A parallel study of maternal bloods presented in Tables 2 and 4 also yielded highly significant results ($t=9.33$).

It must be remembered that all the fetal animals used in this series were in the last month of intra-uterine life and therefore rather large. Their skin was keratinized and covered with thick hair. The average fetal weight was 2356 grams (range 1800 to 3275 grams). The average fronto-coccigeal length was 39.8 cm (range 38 to 43 cm).

That fetal skin does not absorb sodium from the bathing solution (amniotic fluid) during the last month of gestation is a valid conclusion drawn from these experiments.

Discussion

Although authors interested in the circulation of amniotic fluid agree that fetal skin plays an important part,[3 5 8 9] there are very few experimental studies in the literature on this subject. One was conducted on humans by Diczfalusy et al[22] who, in cases of planned abortion, injected intravenously labelled estrone sulfate or estriol. Thirty to 35 minutes after injection the pregnancy was terminated, samples of the organs of these human fetuses were taken and their radioactive contents determined. It was found that of all the organs studied, next to the fetal membranes, the skin contained the highest concentration of the tracer. From these results they concluded that the skin was one of the entrance routes of amniotic contents into the fetus. The relative importance of skin, lungs and the gastrointestinal tract as ingresses to the fetus from the amniotic fluid, however, remains unestablished.

Using similar methods we conducted a series of experiments on gravid ewes in the past,[23] in which we opened the abdomen and injected Na^{22} into the amniotic fluid. It was found that only a minute

TABLE 1

NET COUNTS OF NA^{24} FROM FETAL BLOOD SAMPLES

Date of Experiment	Minutes following exposure to tracer placed in bathing solution											
	5	10	20	30	40	50	60	70	80	90	100	110	120
10.2.968	−6	+3	+7	+4	+5	0	+6	+3	+3	+5	—	—	—
16.2.968	+1	+9	−6	+6	−4	−10	−10	+4	+10	+10	+10	−8	—
17.2.968	−17	−4	+2	−2	−3	−10	−3	−8	−4	−9	+2	+5	+5
22.2.968	−1	0	+6	+10	+10	+10	+7	+2	−4	−4	0	+2	−1
23.2.968	+4	−2	+4	+2	+6	+3	+1	+1	+3	+10	+5	+6	+2
29.2.968	−4	0	+6	−5	+2	−8	−11	−10	+2	−1	−8	−6	—
1.3.968	−1	−14	+2	−14	0	+2	+4	+6	−2	−2	+2	−6	+4
7.3.968	—	+6	+8	−1	+9	−1	−5	−5	+9	+10	+5	+21	−3
8.3.968	+10	+1	−13	−8	−6	−3	+2	−1	−1	+6	−5	−5	−4

TABLE 2

NET COUNTS OF NA^{24} FROM MATERNAL BLOOD SAMPLES

Date of Experiment	Minutes following exposure to tracer placed in bathing solution												
	5	10	20	30	40	50	60	70	80	90	100	110	120
10.2.968	+4	−4	+4	−9	—	+2	−8	−12	—	—	—	—	—
16.2.968	−2	−2	+5	+6	+6	−3	−5	+8	−11	−6	+7	+3	0
17.2.968	−4	−9	−9	−9	+2	−7	−6	−4	−2	−5	−7	−7	−4
22.2.968	+11	+12	+12	+4	+15	+4	+9	+1	+10	+2	+2	—	+5
23.2.968	+3	—	—	—	—	—	—	+16	+25	+25	+21	+13	+6
29.2.968	+4	−4	−12	−9	+4	+2	−8	−8	—	—	—	—	—
1.3.968	+2	0	+2	+6	−10	−12	−14	+6	−2	−8	−4	−8	0
7.3.968	0	0	−7	−16	0	+4	0	+1	+1	−1	−12	−16	−9
8.3.968	−1	+7	−1	+12	−9	−11	−13	−4	−14	−2	−4	—	−5

TABLE 3

CONCENTRATION OF NA^{24} IN FETAL BLOOD SAMPLES (Net counts)

Date of Experiment	Minutes following exposure to radio tracer placed in amniotic cavity												
	5	10	20	30	40	50	60	70	80	90	100	110	120
8.2.968	0	201	378	591	560	663	809	961	1197	—	—	—	—
15.2.968	11	82	166	178	209	224	291	286	280	320	303	304	213

TABLE 4

CONCENTRATION OF NA^{24} IN MATERNAL BLOOD SAMPLES

Date of Experiment	Minutes following exposure to radio tracer placed in amniotic cavity												
	5	10	20	30	40	50	60	70	80	90	100	110	120
8.2.968	0	30	113	106	113	168	178	181	107	—	—	—	—
15.2.968	24	71	134	140	110	160	128	150	153	121	124	168	187

amount of the radionuclide could be recovered from the skin; however, this was still a higher concentration than that of the other organs. A far greater quantity of the tracer was recovered from the fetal membranes, which we believed were primarily responsible for the disposal of amniotic fluid. It appeared that the skin probably played a negligible rôle in the transfer of electrolites, while the fetal membranes were primarily responsible for this important function.

Wislocki[9] injected trypan blue into the amniotic cavity of cat and guinea pig fetuses. After the death of the pregnant animals a few minutes to several hours later, it was found that the amnion, the umbilical cord and the skin were stained blue. The dye was also recovered from the fetal stomach, bronchial mucosa and skin.

There are two possible sources of error in all three of these studies. One is that the tracer could have been attached to the skin surface physically rather than being actively absorbed by it. In our studies we were particularly careful to avoid errors from physical adhesion, and we assume that Diczfalusy also considered this point to be of importance. A second possible source of error is that the tracer could enter the fetal bloodstream via other organs and be deposited in the skin. In order to clarify this particular point, we shortened the period of observation to five minutes,[24] and in other experiments extracted the fetus from the amniotic cavity before adding the tracer to the amniotic fluid.[20,21] The latter series of experiments, however, exclude even the most minute rôle of the skin in the disposal of amniotic fluid. For this reason, despite the disclosed valuable information concerning the rôle of the amnion, these experiments could not illuminate the problem of absorption from the skin. In the present study, therefore, we injected the tracer into the container where it would be in contact with the skin and also eliminate all the other possible routes of absorption. It is important to note that none of the animals in these series developed blood radioactivity significantly above background. From these studies we have to conclude that during the last month of gestation the skin of the fetal lamb does not absorb sodium from amniotic fluid.

In a preliminary series of experiments conducted on ewes in the early stages of pregnancy prepared exactly as in the present series, we observed that some of the tracer could be recovered from the fetal blood.[25] Since absorption from other organs was also excluded, it appears that the fetal skin may contribute to the circulation of amniotic fluid in early pregnancy. The extent of this contribution, however, remains to be determined. Lister,[26] studying the ultra-

structure of fetal skin in early gestation demonstrated that there was no keratinization and that the cells of the periderm displayed microvilli. From these observations she condluded that the skin also may have absorptive functions. These findings correspond with the above observations, as do the studies of Diczfalusy et al.

In conclusion, it seems unlikely from the studies reported here that the fetal skin absorbs sodium from amniotic fluid during the last month of pregnancy. In the early stages of gestation, however, the skin probably does contribute to the transport of solutes and perhaps water.

Summary

Pregnant ewes at near term were opened by a midline incision, the fetus was extracted from the amniotic cavity, and placed into an equitherm bath. The umbilical cord remained intact permitting support of the fetus by the mother during the entire course of the experiment. Na^{24} was added to the solution bathing the fetus, and its concentration was determined in fetal and maternal blood samples taken at time intervals of up to two hours. It was found that the radionuclide content of these samples did not rise above background levels throughout the experiment. Thus it was shown that during the last month of pregnancy the fetal skin does not absorb sodium from the amniotic fluid.

Acknowledgment

We acknowledge our gratitude to the staff of the Turkish Atomic Energy Commission, Çekmece Nuclear Training and Investigation Center and especially to Dr. Fikret Tömek, the Head of the Radio Isotope Production Laboratories, who kindly supplied the radiosodium used in these experiments. Our thanks are also due to Dr. Erfüz Edgüer of Hacettepe University who has been very generous with his time and energy.

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Titers of Brucella Agglutinins In Blood Donors

Elmas Tunçel, M.D.* / M. Talâat El-Garhy, Ph.D.**
Muvaffak Akman, M.D., M.P.H.***

Although much has been published concerning the extent of human brucellosis, adequate data does not exist in many parts of the world. Cases of brucellosis may be undiagnosed because of the lack of signs or symptoms. Serological techniques play an important role in the diagnosis. [1 - 5]

In this investigation our purpose was to analyze the titers of brucella agglutinins in a random sample of blood donors.

Materials and Methods

The subjects examined were 252 apparently healthy males aged 20 to 22 years who were blood donors at the Red Crescent Blood Bank in Ankara during January 1968.

Each patient was questioned concerning occupation, consumption of raw milk, connection with cattle, sheep or goats and previous history of any symptoms suggestive of brucellosis.

A 4 cc sample of blood was taken from each subject and kept in a sterile tube until examined. After centrifugation, the sera were subjected to the agglutinin test in parallel dilutions ranging from 1:10, 1:20, 1:40, 1:80, 1:160 and over, using the Refik Saydam Central Institute of Hygiene's Brucella antigen and saline control.

Results

Of the 252 blood samples, only 83 (34.9 per cent) showed brucella agglutinins in their sera at a level of 1:10 dilution or greater (Table 1).

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TABLE 1

RANGE OF BRUCELLA AGGLUTININ TITER IN SERA OF 252 DONORS

Titer	Number of sera reacting	Per cent
1:10	26	10.3
1:20	42	16.7
1:40	11	4.4
1:80*	3	1.2
1:160	—	—
1:320	1	0.4
Total	83	34.9

* Using previous experience in Turkey as a criterion, titers higher than 1:80 are taken as a serological indication of active infection.

Of the 83 positive cases, 26 (31.3 per cent) had an occupational connection with agriculture and 16 (19.3 per cent) owned sheep and/or goats and kept them in their houses. A definite history of consumption of raw or unboiled milk was obtained from 32 of the cases with brucella agglutinins in their sera, an incidence of 38.5 per cent, Only one case (1.2 per cent) gave a history of rheumatoid pains in the joints. This patient had a positive titer of 1:80. Cases of human brucellosis detected in Turkey during the years 1963 to 1966 reported by the Ministry of Health [6] are shown in Table 2.

Table 3 shows the prevalence of brucellosis among examined cattle, sheep and goats and infected milk.

TABLE 2

RECORDED CASES OF HUMAN BRUCELLOSIS IN TURKEY 1963 - 66

Years	Number of Cases
1963	149
1964	61
1965	69
1966	62

Discussion

Brucella agglutinins were found in 34.9 per cent at titers of 1:10 or over and in 1.6 per cent at titers of 1:80 or over of the examined sera. Table 4 shows the results of other investigators.

TABLE 3

CATTLE, SHEEP, GOATS AND MILK SPECIMENS INFECTED WITH BRUCELLOSIS IN TURKEY 1963 - 66

Years	Specification	Number Examined	Number Positive	Per Cent
1963	Cattle	26871	2172	8.0
	Cattle's milk	317	36	11.3
	Sheep and Goats	22275	465	2.1
	Sheep and Goat's milk	—	—	—
1964	Cattle	26322	2092	7.9
	Cattle's milk	149	6	4.0
	Sheep and Goats	29313	727	2.4
	Sheep and Goat's milk	—	—	—
1965	Cattle	23389	1688	7.0
	Cattle's milk	81	—	—
	Sheep and Goat's	39321	1720	4.0
	Sheep and goat's milk	195	—	—
1966	Cattle	17265	1393	8.0
	Cattle's milk	82	4	4.1
	Sheep and Goats	33772	1132	3.3
	Sheep and Goat's milk	195	—	—

TABLE 4

RESULTS OF OTHER INVESTIGATORS

Years	Country	Author	Per Cent
1966	Brazil	Prata et al [8]	3.7
1961	England	Brodigan [1]	3.0
1963	England	Bartram [2]	1.02
1963	Switzerland	Haberli [3]	1.22
1964	Italy	Guerra and Fanci [9]	6.0
1950	U.S.A.	Spink and Anderson [6]	18.56
1951	South Africa	Wilson and Merrifield [5]	56.6
1962	South Africa	Schire [10]	61.2
1947	Turkey	Akyay [11]	14.0
1950	Turkey	Golem [12]	5.9
1957	Turkey	Gürsel [13]	23.1
1960	Turkey	Babaoğlu [14]	7.0
1965	Turkey	Sözen [15]	2.2
1966	Turkey	Tezok et al [16]	0.16

The discrepancy noted in the results might be attributed to different techniques, antigens [17] or the subjects examined.

Wood [18] and Dalrymple-Champneys [19] mention the danger of interhuman brucella infection via blood transfusion and definite transmission by this route was recorded in Mexico and confirmed by blood culture.[20]

The human cases of brucellosis reported in Turkey during the years 1963 to 1966 appear to be low. Our patients with brucella agglutinins in their sera indicated a past exposure to brucellosis.

From the point of view of public health, more emphasis should be placed on the epidemiology of brucellosis in both men and animals. Complete eradication of the disease in man depends upon effective control of animal brucellosis and elimination of possible vectors of human infection by such means as compulsory pasteurization of milk and efficient precautions on the part of all those exposed occupationally. Fleas (Xenopsylla)) may act as a reservoir of infection for brucellosis,[21] a finding worth consideration as a means for future control measures.

In view of our results and the abundant evidence from previous studies, an extensive serological survey of humans and animals for detection of brucella agglutinins is essential.

Summary

Brucella agglutinins were detected in 83 patients (34.9 per cent) at titers of 1:10 to 1:320, four of which (1.6 percent) had titers of 1:180 or over, of the 252 male blood donors subjected to this study. The number and percentage of the reacting sera at different dilutions from 1:10 to 1:320 were discussed. Of the 83 positive cases, 31.3 per cent had an occupational connection with agriculture and 38.5 per cent gave a history of consuming raw or unboiled milk. It is proposed that extensive serological surveys for the detection of brucella agglutinins in the sera of both man and animals should be adopted as a valuable procedure for the detection of reservoirs and vectors [22] of the infection.

Acknowledgments

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Treatment of Caustic Burns of The Esophagus

A CASE REPORT

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Caustic burns of the esophagus are generally encountered as a result of ingestion of alkalies such as sodium hydroxide or potassium hydroxide and acids such as nitric, sulfuric or hydrochloric. If the patient recovers from the acute stage, stricture may form.

We present here a case of corrosive esophagitis, its therapy and results, in a patient who presented symptoms of difficulty in swallowing.

Case Report

B. I., (66/43201), a 27-year-old, white male, on December 24 1964, accidently drank a glass of hydrochloric acid of unknown concentration. Immediately he was taken to a hospital where the treatment consisted of an emetic, antiacid and liquid diet. He was kept in the hospital for 24 hours for control, and when discharged was able to take food in small pieces or liquid form. During the following months he had occasional mild dysphagia, usually associated with the ingestion of solid food, however, he neglected to consult a physician. On September 2, 1966, he experienced retrosternal burning and a feeling of suffocation while drinking an alcoholic beverage and had severe difficulty even on swallowing clear liquids. The next day, for the first time, x-ray of the esophagus was taken (Figure 1) which showed complete obstruction in the upper one-third of the esophagus and mild dilatation proximally. The patient was referred to Hacettepe University Medical Center for further evaluation.

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On admission, physical examination revealed blood pressure to be 100/70, pulse 80/min, respirations 16/min, and temperature 37.5°C. It took the patient five to ten minutes to drink one mouthful of water. The mucous membranes were dry and skin turgor was poor. The remaining physical examination was within normal limits.

Figure 1. X-ray of the esophagus before dilatation.

The patient was placed on antiacids and given aluminum hydroxide suspension every hour and oxytetracycline, 100 mg every eight hours. Within 48 hours the patient was able to swallow liquids. On the third day an esophagoscopy was performed which showed hyperemia, edema and superficial ulceration of the proximal one-third of the esophagus. Because of stricture, the lower esophagus could not be examined. Dilatation with a Hurst dilator was

begun and in a month the patient was able to swallow a number 50 Hurst dilator. On the control esophagogram on October 15, 1966 barium passed easily into the stomach and the diameter of the esophagus measured 1.5 cm (Figure 2). He was asked to come in for follow-up twice during the first month and thereafter once a month.

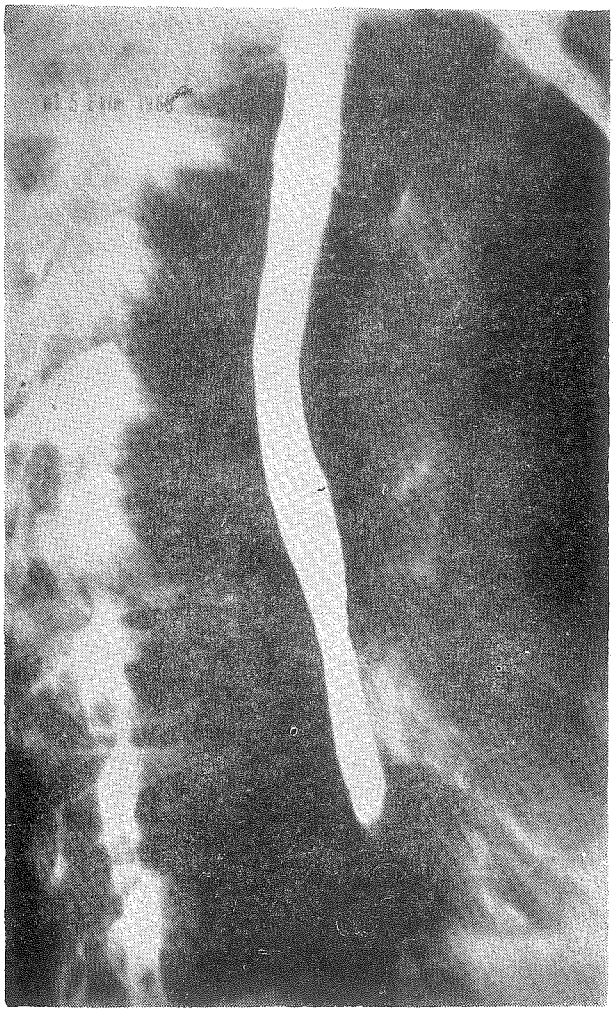

Figure 2. X-ray of the esophagus after dilatation.

Discussion

The degree of burns in the esophagus depend upon the amount and concentration of the corrosive fluid ingested. Corrosive burns in the esophagus fall into three groups: first, second and third

degree.[1] The mucosa and submucosa are affected in first degree burns. There is edema, hyperemia and superficial ulceration, but the layer of muscularis is undamaged. In second degree burns, in addition to the mucosa and submucosa, the muscularis layer is involved and deep ulcerations form, followed by granulation, scarring and stricture. Third degree burns involve all of the layers of the esophagus and periesophaegal tissue. This degree can be fatal because of complications such as perforation, infection and shock.

In order to treat the patient properly, it is important to know the sequence of the pathologic changes in the esophagus. Edema forms in the mucosa shortly after the corrosive material is swallowed; ulcerations form during the first 24 hours and are followed by necrosis of the tissues. In first degree burns the edema disappears during the first three to four days and the esophageal mucosa returns to normal. In second and third degree burns fibroblastic activity starts and two or three weeks later strictures begin to form; in some cases this process occurs later. Palmer[2] states that in 40 to 80 per cent of cases strictures form after ingestion of a corrosive material; 58 per cent of these during the first month, 80 per cent during the second and 99 per cent from the third to the eighth month.

Before treatment can be administered, the type of corrosive material consumed must be ascertained. If the patient has taken acid, magnesium oxide, sodium bicarbonate, calcium carbonate or aluminum hydroxide should be given. If he has taken an alkali, it can be neutralized by giving an acid such as hydrochloric (100 to 200 ml, 0.5 per cent), diluted vinegar or lemon juice. The patient should be hospitalized. Induced vomiting or insertion of a catheter is not recommended because reflux of gastric contents might cause further burning. If there are burns on the lips and/or oral mucosa, therapy must be initiated immediately owing to the high probability of burns in the esophagus.

There are three elements in therapy: antibiotics, steroids and dilatation.[3,4] To inhibit secondary infection penicillin or broad spectrum antibiotics are given. Along with this, steroids are given to prevent inflammation and fibroblastic activity, thus reducing stricture formation.[5] Treatment begins with 30 to 60 mg of prednisolone, a high dosage being administered during the first one to two weeks and gradually reduced, ending during the fourth week. Experimental and clinical studies have affirmed the positive

effect of steroids and antibiotics in therapy. Johnson et al[3] artificially induced esophagitis in dogs by giving 10 per cent sodium hydroxide. No medication was given to one group, antibiotics were given to a second, steroids to a third and antibiotics with steroids to a fourth group. A significantly decreased number of strictures of the esophagus were noted in the group which had been given steroids with antibiotics. In another experimental study performed by Knox et al, [7] a deep segmental esophageal caustic burn was produced in a series of 68 dogs and all were given antibiotic therapy. A comparison was made between a control group and animals treated by bouginage, a low dose of steroids, a high dose of steroids and a combination of bouginage and a low dose of steroids. The lowest incidence of stricture following a deep, segmental esophageal caustic burn occurred in those animals treated with a low dose (0.1 mg/kg/day) of prednisolone and bouginage.

Dilatation should begin 48 to 72 hours after the corrosive substance has been taken, and should be repeated every day during the first two weeks and once every two days during the third and fourth weeks. After the diameter of the esophagus has returned to normal and the patient is able to swallow a Hurst dilator number 50, he should be checked once every two weeks and then once a month. If no narrowing is noted, the patient is then followed every six months and later once a year. Of course, this regime varies from patient to patient. If peroral dilatation is not successful, gastrostomy should be performed and the esophagus dilated in a retrograde fashion. Carver et al [8] in a study of 233 cases found that 206 developed strictures and 91 per cent of these responded to esophageal dilatation. In our patient, if dilatation had been initiated earlier, the probability of a stricture forming would have been greatly decreased.

Summary

A case of corrosive esophagitis with stricture is presented with a brief review of the literature and discussion of therapy.

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The Value of Routine Nose, Throat and Hair Cultures

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Despite the advances made in techniques of disinfection and isolation and the extensive use of chemotherapeutics and antibiotics, cross infections still pose a serious problem and represent a supplementary morbidity in hospitals throughout the world. It is necessary, therefore, to direct attention to the detection of the carriers of staphylococcus and other pathogens, especially among those who are in direct contact with patients and therefore play a significant rôle in the pathogenesis of hospital acquired infection.

Materials and Methods

With the help of the staff of the School of Nursing, Hacettepe University Faculty of Medicine, all nursing students were subjected to routine nose, throat and hair culture three times a week during the months of February and March, 1968.

Each examined subject was asked about any previous history of tonsillitis, sore throat, rheumatic fever, nephritis, diphtheria or diphtheria vaccination. The condition of the tonsils, if present, whether enlarged or congested, was reported during the examination of the oropharynx.

Cotton swabs were moistened with sterile broth and cultures were taken from the nose, throat and hair of each student nurse. Specimens were taken directly to the Microbiology Department and inoculated onto the following media : blood-agar, gentian violet blood-agar and tellurite blood-agar. The plates were then incubated

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at 37°C for 24 hours, following which colonies of pathogenic organisms were recorded. A tube coagulase test for identification of pathogenic staphylococci, Lancefield classification for beta hemolytic streptococci and antibiotic sensitivity tests were performed.

Results

Of the 366 nursing students interviewed, 345 cooperated (94.2 per cent). The subjects' ages ranged from 16 to 23 years.

Staphylococcus aureus was detected in 105 of the nasal cultures, an overall prevalence rate of 30.4 per cent and in 23 of these (21.9 per cent) furunculosis was observed on the face. Phage grouping and typing was carried out on 40 of the isolated strains by the Pasteur Institute, Paris (Table 1). Six (1.7 per cent) of the examined group were found to be hair Staphylococcus aureus carriers and three of these (50 per cent) were also nasal carriers of staphylococcus.

TABLE 1
PHAGE GROUPING AND TYPING

Phage Group*	Number	Percent
I	4	10.0
II	2	5.0
III	11	27.5
IV	1	2.5
V	1	2.5
Mixed	3	7.5
Non-groupable	18	45.0
Total	40	100.0

* Phage types :
Group I : (1) 29, (3) 52.
Group II : (1) 3A/3C /55 /71, (1) 71.
Group III : (1) 54, (3) 77/83 A, (1) 77/85, (1) 53/54 /77 /84 /85, (1) 85, (2) 54/ 77/ 84, (1) 54/ 77/ 83A, (1) 53/ 54/ 75/ 84/ 85.
Group IV : (1) 42 D.
Group V : (1) 187
Mixed : (1) 3A/3C — 47/54, (1) 3A-54, (1) 52/52A-54.

Beta hemolytic streptococci were isolated from the throats of 112 of the examined students (32.5 per cent) and 106 (94.6 per cent) of all the strains detected were sensitive to ten units of bacitracin.

Twenty-one (67.7 per cent) of the strains examined belonged to Group A of the Lancefield classification.

The results of the antibiotic sensitivity tests on both Staphylococcus aureus and beta hemolytic streptococci strains isolated are shown in Table. 2. The relationship between beta hemolytic streptococci in the throat and the condition of the tonsils and oropharynx is illustrated in Tables 3 and 4.

Twenty-four nasal Staphylococcus aureus carriers were treated for one week; half of the patients were given neomycin in the form of Neodrin spray, the other half gramicidin in the form of Graneodin[R] nasal drops. Ten of the beta hemolytic streptococcus throat carriers were also treated for one week with oral penicillin, 300,000 units (Calcipen-Leo[R]). Negative cultures were obtained following completion of treatment in each case.

TABLE 2

ANTIBIOTIC SENSITIVITY TEST RESULTS

Strains	Total Number	Penicillin resistant	Penicillin sensitive	Terramycin resistant	Terramycin sensitive
Staphylococcus aureus (nose)	105	99 (94.3 %)	6 (5.7 %)	51 (48.6 %)	54 (51.4 %)
Beta hemolytic streptococcus (throat)	112	29 (25.9 %)	83 (74.1 %)	54 (48.2 %)	58 (51.8 %)

TABLE 3

RELATIONSHIP BETWEEN BETA HEMOLYTIC STREPTOCOCCUS AND CONDITION OF TONSILS

Condition of tonsils	Total number	Number of beta hemolytic streptococcus carriers	Percent
Removed	36	12	33.3
Enlarged	27	6	22.2
Not enlarged	282	94	33.3
Total	309	100	32.4

TABLE 4

CORRELATION BETWEEN BETA HEMOLYTIC STREPTOCOCCUS IN THE THROAT AND PREVIOUS HISTORY

	Total number	Number of Beta hemolytic streptococcus carriers	Percent
History of tonsillitis or sore throat	81	29	35.8
No history of tonsillitis or sore throat	264	83	31.4
Total	345	112	32.5
History of congestion of oropharynx	20	6	30.0
No history of congestion of oropharynx	325	106	32.6
Total	345	112	32.5

Diphtheria bacilli were detected in the throat of one 17-year-old student nurse (0.3 per cent) with no previous history of the disease and a normal oropharynx. She was considered a carrier. Hemophilus influenzae were isolated from the throats of two subjects, an incidence of 0.6 per cent.

Discussion

Our Staphylococcus aureus nasal carrier rate was 30.4 per cent. Previous investigations on groups of hospital personnel [1-6] gave rates between 34.8 and 88.2 per cent.

The results of phage grouping (Table 1) of our 40 strains of Staphylococcus aureus according to their order of frequency are of epidemiologic interest : the non-groupable strain (45 per cent) having the highest prevalence rate, followed by Type III (27.5 per cent), Type I (10 per cent), mixed type (7,5 per cent), Type II (5 per cent) and Types IV and V (2.5 per cent each). However, these findings differ from those given by Williams et al [7] and Wahdan et al.[6] The discrepancies in results with regard to the rate of Staphylococcus aureus nasal carriers and the phage typing might be due to environmental as well as technical differences.

Our penicillin and terramycin resistant staphylococcus rates were 94.3 and 51 per cent respectively, higher than in some other studies. [5 6 8]

Hair Staphylococcus aureus carriers were 1.7 per cent, 80 per cent of these were also nasal carriers. The hair is an unfavorable site for such organisms and its contamination might be a mechanical one. Higher hair carrier rates were reported by Summers et al,[9] Noble[10] and Wahdan et al.[6]

There is substantial evidence that nasal carriers in the nursing staff are sources of sporadic infections in all maternity hospitals. This could be kept to a minimum by careful nursing techniques and a cleaner hospital environment.[11 12]

Beta hemolytic streptococcus throat carriers were 32.5 per cent in this study, however, the normal carrier rate has been estimated by Türet [13] to be 26 per cent in Turkish schoolchildren. Rather surprisingly he found no association between the carrier rate of beta hemolytic streptococci and the condition of the tonsils or oropharynx. It seems that the carrier state depends more or less on the degree and frequency of exposure to infection.

This investigation also focuses attention on prophylactic measures which could be applied by adequate antibiotic treatment to those who harbor pathogenic organisms in the upper respiratory tract. Our results in treatment indicate that nasal application of antibiotics in the form of neomycin and gramicidin are effective and should be taken into consideration in any community control measures for Staphylococcus aureus carriage.[14] Such prophylactic treatment might also prevent post-staphylococcal complications among carriers such as skin sepsis.[15–17] In addition, oral penicillin seems to be effective in suppressing beta hemolytic streptococcal throat infections, reducing the risk of such streptoccoccal complications as glomerulonephritis [18–20] and preventing rheumatic fever. [21–25]

Our diphtheria carrier was treated with long-acting penicillin and followed up. Compulsory vaccination would minimize the morbidity rate of diphtheria.[26] Hemophilus influenzae were found in 0.6 per cent of our subjects, a lower rate than those reported by others,[27–31] however, the significance of the carriage of such organisms by hospital personnel should be assessed by further studies.

Our results indicate a need for more careful attention to the health of hospital personnel.

Summary

Nose, throat and hair cultures from 345 nursing students revealed a Staphylococcus aureus nasal carrier rate of 30.4 per cent and

a hair carriage rate of 1.7 per cent. A beta hemolytic streptococcus throat carriage rate of 32.5 per cent was found. Phage grouping and typing of the 40 strains of Staphylococcus aureus and results of antibiotic sensitivity tests on both the Staphylococcus aureus and beta hemolytic streptococcus isolated in this investigation are discussed. Study of treatment with neomycin and gramicidin in cases of Staphylococcus aureus nasal carriers and oral penicillin to beta hemolytic streptococcus throat carriers revealed a cure. One diphtheria carrier (0.3 per cent) was detected among the examined group and hemophilus influenzae were isolated from the throats of two subjects (0.6 per cent). It is proposed that further work be devoted to detection of carriers among hospital personnel in order to minimize the risk of cross infection.

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Cranial Subdural Empyemas and Their Treatment

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This clinical entity, also called internal pachymeningitis, subdural empyema and subdural abscess, is not so rare as is generally thought. It is possible that the first published case of subdural empyema was Schultz's in 1812, which was proved by postmortem.

We present four cases of subdural empyema which were treated in our hospital during a one-year period to emphasize the relative frequency of this entity among the space-occupying inflammatory intracranial lesions and the importance of its early diagnosis and treatment as well as the treatment of the primary focus.

Case Reports

Case 1: B. Ş., an eleven-year-old male, was admitted with a history of earache, fever, stupor and difficulty in speech and gait of 15 days' duration.

On admission he had fever; his general condition was poor. There was nuchal rigidity, left-sided facial weakness and a purulent discharge from the left ear. Laboratory examination revealed leukocytosis; x-rays of the skull showed evidence of bilateral mastoiditis; and on E.E.G. generalized dysrhythmia and slowing, especially over the posterior part of the left hemisphere, were seen. C.S.F. was slightly turbulent and 100 polys per mm^3 were counted. The total protein was elevated and sugar content reduced. The patient was put on antibiotics and sulfonamide. In spite of this, his condition continued to deteriorate and convulsions began.

Fifteen days later he lost consciousness, anisocoria was noted and breathing ceased. While under artificial respiration a right carotid angiogram was done which showed evidence of a space-occupying lesion in the right subdural space. The patient was im-

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mediately taken to surgery. Through a left frontal burr hole 60 cc of pus were evacuated.

After the operation he became alert and his general condition improved. Fifteen days after surgery his condition again worsened. His mastoiditis was treated surgically on the 43rd day following admission. The same evening he became unconscious and anisocoria and decerebrate rigidity were noted. A right carotid angiogram revealed hydrocephaly and when the patient was operated upon a cerebellar abscess was found and 15 cc of pus were aspirated from the right cerebellar hemisphere. A ventricular tap was done to reduce intracranial pressure. He expired after surgery in spite of resussitative measures.

This case is an example of a subdural empyema secondary to middle ear and mastoid infection. Initial treatment was successful, but delay in the treatment of the primary foci resulted in a dangerous complication, the cerebellar abscess, whose symptoms merged into those of the primary foci and though its diagnosis and treatment were correct, it was too late to save the patient's life.

Case 2 : V. A., a three-month-old male, was admitted with a history of enlargement of his head over a two-month period.

His head circumference was 46.5 cm and the anterior fontanella was under pressure. He presented dehydration, spastic extremities, convulsions, high fever and leukocytosis. Bilateral subdural taps were done and a total of 40 cc of pus aspirated, mostly from the left side. He was put on antibiotics, sulfonamide and other supportive therapy. Four days later he was operated upon and the left subdural space was washed and drained. His general condition improved and he was discharged upon the request of the family.

Twenty days after discharge he was brought back in poor general condition with fever, convulsions and nuchal rigidity. A left subdural tap yielded 25 cc of pus. A left parieto-temporal craniotomy was performed and the subdural empyema and membranes were removed. Under the cortex of the left hemisphere, subcortical abscesses were palpated, opened and evacuated. The patient's condition improved during the following days and he was discharged on anticonvulsive medication. In this case no primary foci could be found.

Case 3 : I. C., a seven-year-old male, was hit on the head with a stone one month prior to his admission.

He had an infected wound over the left frontal region, slight fever, bilateral papilledema, nuchal rigidity, left-sided hemiparesis and moderate leukocytosis. A carotid angiogram showed changes due to a right subdural space-occupying lesion. After surgical intervention, temperature and white cell count returned to normal, his left hemiparesis diminished and E.E.G. showed improvement. He was discharged in good general condition.

This case is a good example of post-traumatic subdural empyema. There were no fractures or osteomyelitis under the infected scalp wound, infection was spread apparently by emissary veins. This case also shows that a simple scalp infection may result in a severe complication threatening the life of the patient.

Case 4 : A. S., an eleven-year-old male, was admitted with a history of right-sided frontal headache, fever and vomiting which started eight days prior to admission.

On examination slight fever, stupor, restlessness, nuchal rigidity, right-sided hemiparesis and early bilateral papilledema were noted, and shortly thereafter convulsions began. He had leukocytosis with a high percentage of polymorphonuclear cells. E.E.G. revealed generalized dysrhythmia and slow waves over the left parieto-occipital region. A carotid angiogram showed a space-occupying lesion in the left subdural space (Figures 1 and 2). He was treated surgically.

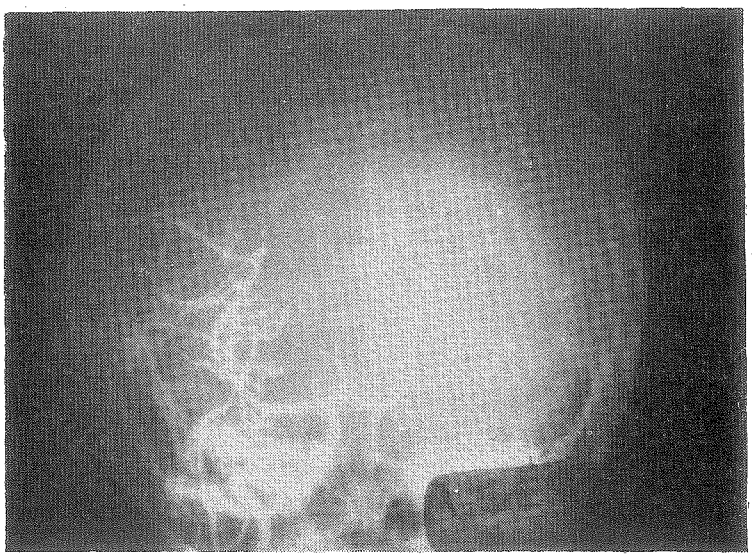

Figure 1. Pre-operative angiogram, antero-posterior view

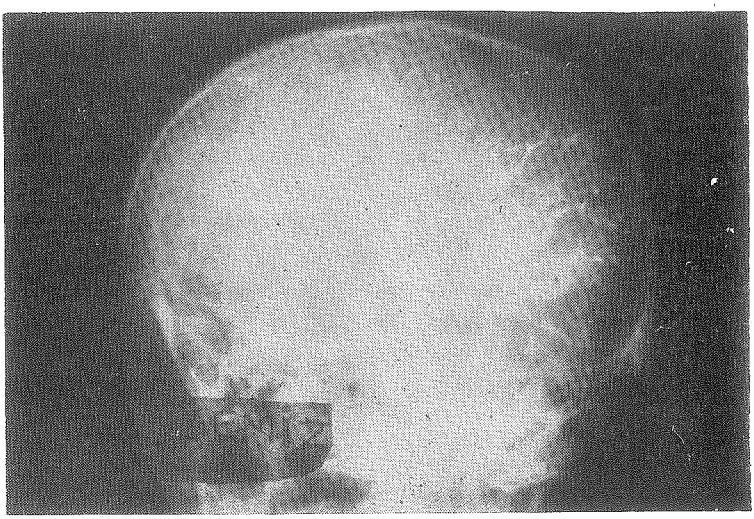

Figure 2. Pre-operative angiogram, oblique view.

After surgery he became alert, convulsions ceased, hemiparesis gradually cleared and temperature and white cell count returned to normal. He had residual aphasia. The control E.E.G. showed improvement; the control carotid angiogram was normal. Forty days after admission he was discharged with only moderate aphasia.

In this case no primary focus was found. Early diagnosis and proper medical and surgical treatment produced very good results.

Discussion

Two mechanisms usually play important rôles in the development of subdural empyemas: 1) metastatic spread of the infection to the dura from distant foci and 2) spread of the infection directly from neighboring tissues.

Metastatic subdural abscesses are rather rare complications secondary to bronchiectasis of the lung, empyema of the pleura, abscess of the lung, bacterial endocarditis, osteomyelitis of the long bones, septicemia and typhoid fever.[1–13] The infection spreads to the dural layers via, for example, emissary veins, Haversian canals and veins, cranial sutures, bone defects due to skull abnormalities, the channels and foramen through which cranial nerves emerge, cochlear ducts, tegmen tympani and fracture lines.[14]

Direct infection can be seen after penetrating wounds involving the scalp, skull and dural layers. The most frequent causes of

direct spread of infection occur after inflammation of the cranial sinuses (frontal,[4] ethmoidal and sphenoidal), infection of the middle ear,[10] mastoiditis, thrombophlebitis of the dural sinuses, traumatic and nontraumatic infectious involvement of the scalp and skull.

The internal layer of the cranial dura is fibrous and covered by mesothelial cells. During an infectious process exudation and formation of granulation tissue are seen over the internal layer of the hyperemic dura.[2] Later changes take place over the external surface of the arachnoid. Pus may collect in the form of localized small abscesses [7] but usually occupies the subdural space and especially on the convexity may reach a large volume (50 to 250 cc) by depressing the cerebral hemisphere. Due to the presence of large vessels and cranial nerves and close contact between the brain and skull because of gravity, collection of a large amount of pus over the base of the skull is not possible. Subdural empyemas may be located in the interhemispheric fissure and in the sylvian and other sulci. [2 7 10]

Clinically the patient may show signs and symptoms of the primary lesion which caused the subdural empyema, but if the primary lesion is not evident its existence may be found in the patient's history. Leptomeningeal irritation causes headache, nausea, vomiting, photophobia and nuchal rigidity; cortical irritation results in restlessness, impairment of mental function, disorientation and convulsions. Hemiparesis or hemiplegia can be seen as a result of pressure on the hemispheres or due to thrombophlebitis of the cortical veins. Choked discs, impairment of the level of consciousness, paresis and paralysis, elevation of the arterial pressure and the slowing of the pulse and respiratory rate are indications of severe intracranial pressure and impending herniation.

If the subdural abscess is not evacuated at this stage the uncus will herniate through the hiatus and compress the third cranial nerve and peduncle causing ipsilateral dilation of the pupil, impairment of motor function of the third nerve, hemiplegia and later decerebrate rigidity on the contralateral side. If the patient is not treated promptly and properly, death will result from respiratory and cardiovascular failure.

Laboratory studies show an elevated white cell count and a rise in the percentage of polymorphonuclear leukocytes, elevated red cell sedimentation rate and slight to severe anemia. Direct x-rays of the skull will show any fractures, osteomyelitis or infected si-

nuses. E.E.G. is usually helpful by showing generalized and localized changes and may indicate lateralization and location of the empyema. Chest x-rays should also be done.

A lumbar puncture is advisable only when there is no evidence of increased intracranial pressure and in the absence of papilledema. Cerebrospinal fluid is usually clear and the pressure increased if there is no meningitis. If not contraindicated, a Tobey-Ayer test should be done and the possibility of the thrombophlebitis of the lateral sinuses ruled out. CSF may show a moderate increase in cell count (20 to 700 leukocytes) and a slight elevation of total protein (75 to 150 mg per cent). [11] Sugar and sodium chloride content may be decreased.

Carotid angiogram will show evidence of a space-occupying lesion and its location. Possibilities of epidural empyema, thrombophlebitis of the dural sinuses,[11] cerebral and cerebellar abscess,[1] purulent meningitis, subdural effusion and, in traumatic cases, subdural, epidural and intracerebral hematoma should be kept in mind in the differential diagnosis.

The treatment of the subdural empyema is surgical. It is necessary to make burr holes in the skull, to evacuate the pus, [5 8 9] to wash the subdural space with normal saline and to instill 10,000 to 20,000 u of crystalline penicillin in one to two cc normal saline into the subdural space. If there is a primary focus it must be treated. The general nursing care is very important. Fever and restlessness must be treated symptomatically and convulsions controlled. The infectious process should be treated first with broad-spectrum antibiotics, sulfonamide and, after receiving the results of the culture and sensitivity tests, specific antibiotics and sulfonamides should be administered. [3 6 9]

After initial treatment the patient should be observed closely and white cell count, sedimentation rate, EEG and studies of the CSF should be repeated at frequent intervals. In a patient with paresis and aphasia, physical, occupational and speech therapy will be of great help.

The prognosis is good if the diagnosis is made early and the patient treated properly. Mortality and morbidity decrease as good nursing care, antibiotics and physiotherapy become more available.

These four cases of subdural empyema treated in one year show that this is not so rare an entity as is generally believed. Al-

though the series is small it proves that if diagnosed early and treated properly the results will be gratifying.

We should like to emphasize the importance of resorting to radical diagnostic methods as early as possible and the value of treating the primary focus, if present, as well as the subdural empyema. It should always be kept in mind that in such patients thrombosis of the dural sinuses, cerebral or cerebellar abscesses, hydrocephaly and epilepsy may be seen as complications.

Summary

Four cases of subdural empyema, their signs, symptoms, treatment and results are presented. The importance of treating the primary focus as well as the resulting subdural empyema is emphasized.

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Median Cleft Deformity *

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Median cleft of the lip is a congenital abnormality believed to occur when the central mesodermal lip mass is absent. The degree of the deformity depends upon the time and the intensity of the mesodermal insult. If the deformity occurs during the fourth week of intrauterine life, a bifid nose and hyperthelorism may be the only results. Median clefts of the upper lip appear either as «true» or «pseudo» median clefts. Pseudo median clefts are usually associated with several other severe deformities such as aplasia or hypoplasia of the premaxilla, eye, ear or nose deformities or congenital absence of the skin of the vertex. Some cases are associated with other abnormalities such as «oral-facial-digital syndrome» and the most severe cases, which are associated with arhinencephalia, are usually fatal. Most of the cases are shown to have chromosome anomalies but the incidence rate is unknown.[1-6] Fogh-Andersen reported that in a 30-year period, during which a series of 3988 facial cleft cases occurred, 3940 were typical cleft lip and palate deformities and 48 were rare atypical clefts of the face; of the 48, 15 were median.[7]

Although Turkish literature, to the best of our knowledge, contains no reports of such a deformity, we had the opportunity of examining four patients with median cleft deformity during the period between March 1965 and September 1966, two per cent of the 174 patients with clefts of the lip or palate seen in the same period. In March 1968 two more cases were examined. None of the earlier patients had a history of either cleft lip or palate in the family; none of the mothers gave a history of taking drugs during pregnancy. We had a positive family history in the sixth case only: an aunt on the father's side had unilateral complete cleft lip and palate.

The purpose of this paper is to present six cases of median cleft showing their different degrees of deformity and to demonstrate one case which was operated upon for cosmetic reasons.

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* Part of this paper was presented at the Fourth International Congress of Plastic Surgery, October 8, 1967, Rome.

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Case Report

Case 1 : (Pseudo Median Cleft) A three-day-old white female was admitted with a flat unsupported nose, no spetum and no columella. As a result, there was a large hole in place of two nostrils. The midline defect of the maxilla was two centimeters wide due to the absent premaxilla and the defect extended into the mouth forming a complete bilateral cleft palate. The child was seen in consultation at the Pediatric Department where she was admitted because of feeding difficulties and after discharge was lost to follow-up.

Case 2 : (True Median Cleft) An 11-year-old white male had a one cm cleft of the upper lip and the alveolus at midline. The rest of the palate and the nose were normal and there were no other congenital anomalies. The parents objected to both photographs and surgical intervention.

Case 3 : (True Median Cleft) A five-year-old white female, whose parents complained that «her upper lip was tied to her teeth», had a one centimeter cleft of the maxillary alveolus at midline with the frenulum of the upper lip stuck into this groove (Figure 1). After several interrogations, the parents confessed that she had been operated upon for this deformity twice before and each time the frenulum had become thicker and the lip shorter. Apparently

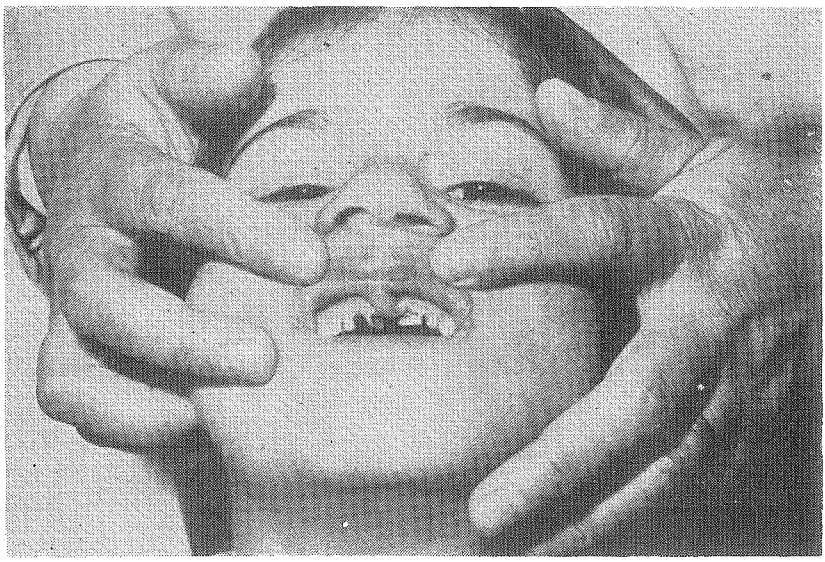

Figure 1

as a result of these operations, the frenulum became a thick mass of scar tissue which contracted and pulled the lip down, while the bony defect remained untreated. The child did not have a median cleft of the lip (Figure 2), but close examination revealed a whitish scar-like tissue at the base of the right nostril with asymmetry of the nostrils which gave the child a right-sided, postoperative cleft lip appearance. There was also a small indentation of the free vermilion border at midline. The parents refused a surgical intervention because of past experiences.

Figure 2

Case 4: (True Median Cleft) A seven-year-old white female was first seen in August 1966 (Figures 3 and 4). The child had a very flat nose, almost like a bifid nose. The nasal bones were quite far apart, with no septal support and no columella. The tip of the nose emerged directly from the upper lip. In addition to her nasal deformity, she had bilateral congenital epicanthal folds, slightly depressed malar bones and there were two very pronounced red spots at the base of both nostrils which were soft, slightly raised from the skin and ran bilaterally down to the upper lip, giving the child an appearance of a rather unsatisfactory postoperative bilateral cleft lip. Closer examination revealed these to be misplaced nasal mucosa due to a congenital developmental error. The upper lip was unusually wide and prominent. The inside of the mouth revealed a midline maxillary cleft 1.5 cm long and 1 cm wide

Figure 3

Figure 4

(Figure 5). The rest of the palate was intact and normal, as was the speech. X-rays of the facial bones were also normal with the exception of the above mentioned midline maxillary cleft (Figure 6).

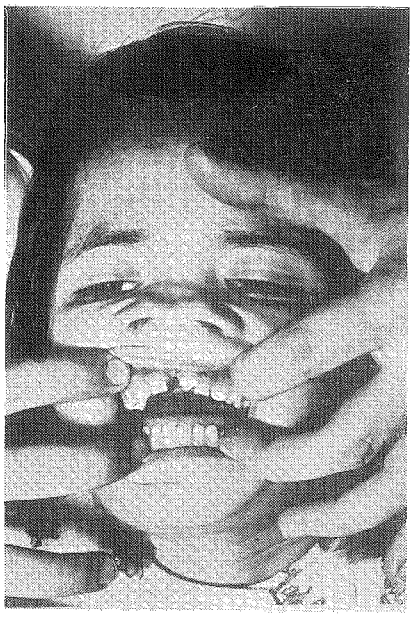

Figure 5

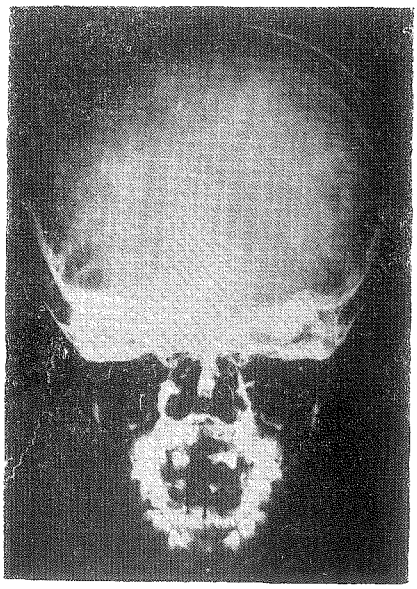

Figure 6

The parents of this child were more conscious of her nasal deformity than her maxillary defect and wanted augmentation of the nose only. Due to the child's age, bone graft was ruled out and a silastic implant was agreed upon. In November 1966 the child was operated upon under general anesthesia using the following technique.

A large fork-flap was prepared with which four procedures were accomplished: 1) the excision of bilateral, unsightly, red scar-like tissue; 2) the reconstruction and lengthening of the columella; 3) the narrowing of the unusually wide upper lip; and 4) the insertion of the silastic implant into the dorsum of the nose.

This implant, which was made of two different densities, was L-shaped. The outer frame was made from the most durable silastic and some spongy silastic was added under the longer arm of the L in order to give more lift to the dorsum in profile. Harder silastic could not be used instead of this spongy part because the skin of the dorsum was very tight and could not be stretched any further. After the insertion of the implant into the columella, the base of the columella was closed in two layers and the defect of the upper lip was sutured together (Figures 7 and 8). The operation was satisfactory, leaving the scars on the upper lip almost invisible.

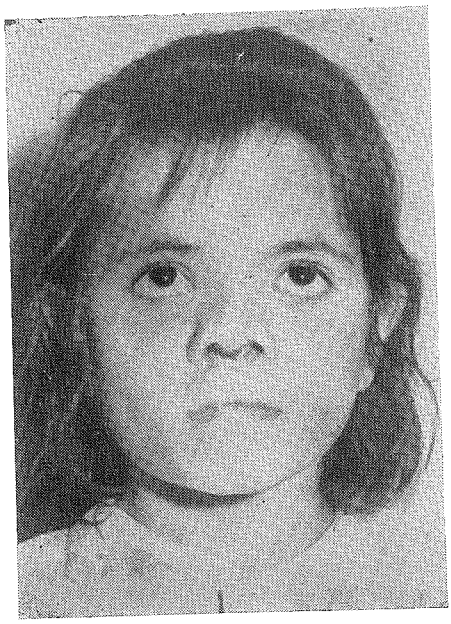

Figure 7

Figure 8

The patient was discharged from the hospital on the twelfth postoperative day and progressed well until seven months after the operation she returned with a discharge from the base of the columella. After the staphylococcus infection was controlled, the sponge-like part of the implant had to be removed, but the durable L-shaped frame seemed clean and attached to the underlying tissue, so this was left undisturbed. Figure 9 shows her as she was in 1966 and as she is today.

Figure 9

Case 5 : (Pseudo Median Cleft) A five-day-old female, who was examined on April 3, 1968, had a total median cleft with columella and septum atresia and complete absence of the premaxilla. This patient had no positive family history except for a brother with a suggestion of first brachial syndrome and a very mild unilateral facial paralysis with no obvious mandibular hypoplasia or macrostomia (Figures 10, 11 and 12). This child's appearance is exactly like Case 1 for whom we have no photographic records.

Case 6 : (Pseudo Median Cleft) A three-and-a-half-month-old female who was operated upon for cleft lip at the age of two days was admitted to the Pediatric Department due to feeding difficulties. She was seen by our department for the first time on March 8, 1968. She also demonstrated a typical pseudo median cleft deformity with columellar septal atresia, absent premaxilla and

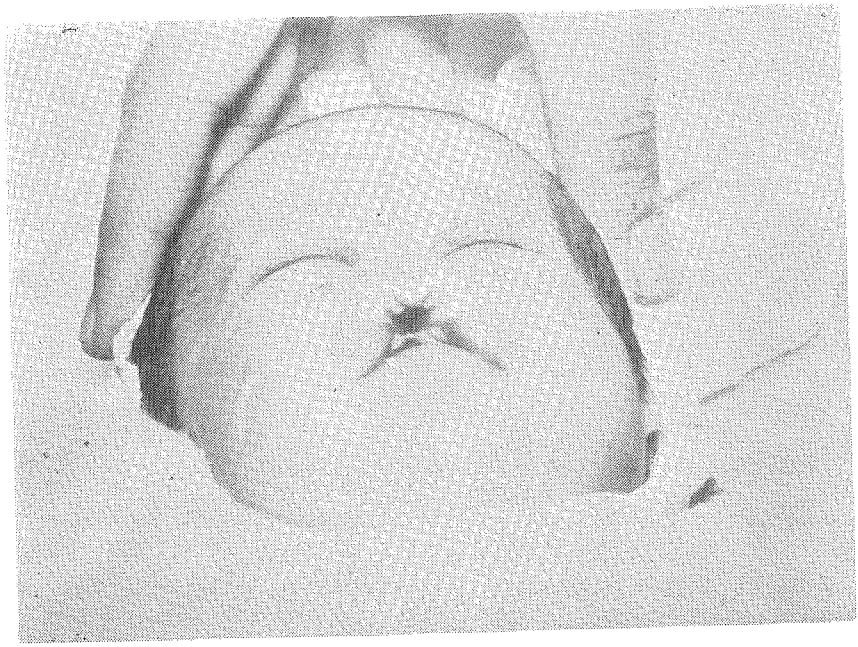

Figure 10

Figure 11

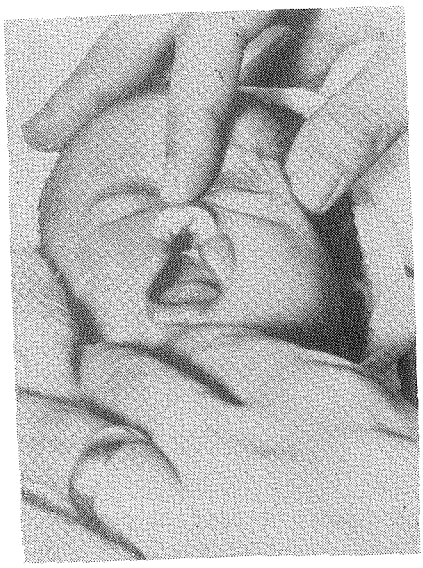

Figure 12

wide-set eyes. Figures 13 illustrates a midline postoperative scar and the septal and columellar defect.

[These last two cases are not included in our earlier statistics of two per cent incidence rate for median clefts.]

Figure 13

Summary

A short history and etiology of median cleft deformity (true and pseudo) is given and six cases are presented. One of these was operated upon for cosmetic reasons by means of an augmentation nasoplasty with silastic implant.

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Conjunctival and Nasal Carriers of Pathogenic Organisms in Turkish Schoolchildren

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Detection of the carrier state among normal children can be considered an index for determining their general health status.[1 2] The aim of this study is to learn the prevalence rate of conjunctival and nasal carriers of pathogenic organisms in a sample of Turkish schoolchildren; to suggest efficent prophylactic measures; and, in addition, to assess the influence of nasal flora on conjunctival bacteria, for if a relation could be found, both infections could be cleared with a single treatment.

Materials and Methods

The group studied was a random sample of apparently healthy schoolchildren, aged 8 to 12 years, of both sexes, attending primary schools in the Etimesğut area, Ankara, Turkey. A medical history was obtained through a questionnaire which each child completed with the help of his parents, one day prior to physical examination. Those children who had had injuries, surgery or illnesses effecting the nose and/or eye were eliminated from the study.

After clinical examination, cultures were taken from the lower fornix of each eye and from each nostril. The swabs were immediately placed in sterile broth-filled tubes and taken to the Microbiology Department, Hacettepe University Faculty of Medicine, to be inoculated into blood-agar and tellurite blood-agar media. The plates

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were incubated overnight and the results read on the following day. Furthermore, a tube coagulase test for identification of pathogenic staphylococci was performed.

Results

Of the 324 apparently healthy schoolchildren examined during the months of May and June, 1968, pathogenic organisms were detected in the eyes of 24, a prevalence of 7.4 per cent, and 89 children were nasal carriers, an overall prevalence of 27.5 per cent. The distribution of pathogenic organisms found in the eyes and noses of the children is shown in Table 1.

TABLE 1
THE DISTRIBUTION OF PATHOGENIC ORGANISMS

	Staph. Aureus coag. +		Beta hemolytic strept.		Staph. albus coag. +		Diptheroid	
	Nose	Eyes	Nose	Eyes	Nose	Eyes	Nose	Eyes
Females	29 (18.3 %)	6 (3.8 %)	5 (3.2 %)	3 (1.9 %)	5 (3.2 %)	2 (1.3 %)	5 (3.2 %)	—
Males	27 (16.2 %)	6 (3.6 %)	3 (1.8 %)	1 (0.6 %)	7 (4.2 %)	6 (3.6 %)	8 (4.8 %)	— —
Both Sexes	56 (17.3 %)	12 (3.7 %)	8 (2.5 %)	4 (1.2 %)	12 (3.7 %)	8 (2.5 %)	13 (4 %)	—

Fifteen children were double carriers, that is they had pathogenic organisms in both the eyes and the nose, an incidence of 13.3 per cent of all carriers.

Congested conjunctivae with positive nasal bacterial cultures were found in four children, a prevalence of 12 per cent, while congested conjunctivae with negative cultures were detected in 21, a prevalence of 6.5 per cent of all the children.

Discussion

Overall prevalence rates of 7.4 per cent conjunctival carriers and 27.5 per cent nasal carriers of pathogenic organisms detected in this study coincide with figures reported by other writers.[3–5] The slight discrepancies in results may be attributed to the different types of populations studied, as well as to the technical and environmental differences. The rates are sufficiently high in this study to suggest the need for broad prophylactic measures in Turkey.

TABLE 2

TYPES OF ORGANISMS RESPONSIBLE FOR DOUBLE INFECTIONS

Types of organisms	Females	Males	Total
Staph. aureus coag. + in both eyes and nose	1	5	6
Staph, albus coag. + in both eyes and nose	1	—	1
Beta hemolytic strept. in both eyes and nose	1	1	2
Staph aureus coag. + in eyes and Staph. albus coag. + in nose	1	2	3
Beta hemolytic strept in eyes and staph. aureus coag. + in nose	1	—	1
Staph. aureus coag. + in eyes and diptheroid in nose	—	1	1
Staph. albus coag. + in eyes and diptheroid in nose	1	1	1
TOTAL	5	10	15

The significance of the conjunctival carrier of pathogenic organisms has been reported,[6–7] and staphylococcus aureus, which is frequently found in normal eyes, has been found to be the etiologic agent of ocular infections in the great majority of cases. It has also been stated that an individual usually carries only a single strain (determined by phage typing) which is often present in the nose and throat, and on the skin, as well as in the eyes.

It is of interest to consider the relation of the nasal flora to the conjunctival findings, for the nose and eyes may carry the same type of organisms. Nolan[8] claims, however, that nasal flora is not significant in conjunctival infection, and is considered only in cases of recurrent conjunctivitis.

In this study also, there appeared to be no association between congestion of the conjunctivae and the carrier state: the incidence of conjunctivitis with *negative* nasal bacterial cultures was higher (6.5 per cent) than the rate of conjunctivitis with *positive* cultures (1.2 per cent).

Since staphylococcus aureus is one of the most frequent pathogenic organisms isolated from the nose and/or eyes, and since it

is usually resistant to penicillin,[11–14] a shift to a broad spectrum of antibiotics seems necessary for treatment.

From the results of this study, the value of adequate antibiotic prophylactic measures to control the carrier state among the Turkish school population should be evident.

Summary

The prevalence rate of pathogenic organisms detected in the eyes and noses of 324 apparently healthy Turkish primary schoolchildren were found to be 7.4 per cent and 27.5 per cent respectively.

The distribution, proportion and type of pathogenic organisms isolated are shown in Tables 1 and 2.

Carriers of pathogenic organisms in both eyes and nose of the same person were detected in 9.1 per cent of females, 17.2 per cent of males, and 13.3 per cent of both sexes; no significant relation was found, however, between conjunctivitis and the nasal flora.

Broad spectrum antibiotics were recommended to treat nasal and conjunctival infections.

Acknowledgments

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Comportement des Granulosités des Cellules Entérochromaffines de Kultschitsky Après L'Hypophysectomie

Jozef Staszyc, M.D. */ İlhan Kerse, M.D. **/ Refik Soylu ***

Introduction

Dans les cellules de Kultschitsky - Schmidt-Ciaccio se trouvent des granulations diazopositives qui sont identifées avec le sérotonine (5HT).[1 2] Ces cellules forment un organe endocrine spécifique du tube digestif.[3 4 5] Les cellules entérochromaffines de Kultschitsky restent en rapport génétique et fonctionnel avec le système nerveux autonome,[6] la secrétion des appareils surrénaux[7] et de l'hypophyse cérébrale.[8]

Ainsi, on a décidé de faire l'analyse du comportement des cellules entérochromaffines de Kultschitsky du duodénum du grenouille après résection de l'hypophyse cérébrale.

Matériéls et Méthodes

On a examiné les grenouilles (femelles de Rana temporaria L.) d'un poids de 300 gr environ. Pendant les quatre jours avant les expériences, et après l'opération, les grenouilles n'étaient pas nourries; ces animaux ont été maintenues dans un milieu avec une température constante de 20° - 22° C à pleine lumière du jour. Pour éviter les effets d'extérieur, tous les examens ont été faits à la même heure de midi.

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Les grenouilles ont été divisées en quatre groupes de trois femelles chacun. Les expériences sont fait sur les trois premiers groupes, et le quatrième servait de contrôle.

L'hypophysectomie a été fermée par suture continue. Du premier groupe on a pris le matière à examiner le troisième jour, du deuxième groupe, le sixième jour, et du troisième groupe, le douzième jour après l'opération. Les arenouilles étaient décapitées. Les dissection des grenouilles expérimentales demontraient en chaque cas que l'hypophyséctomie avait été totale.

Les coupes du duodénum étaient fixées pendant 18 heures dans le fixateur de Baker (formaline de calcium de 10 %). Après déshydratation, les fragments étaient inclus dans la paraffine et divisés en tranches à un micron d'épaisseur. Ils étaient colorés à l'aide du sel de diazonium Fast Red B Gurr's (George T. Gurr, Ltd. London S.W.6, England) dans une température 18° - 20° pendant 15 minutes à pH = 7.2.

Résultats

Groupe de Contrôle

Dans le matériel de contrôle, les cellules de Kultschitsky, colorés par le sel de diazonium Fast Red B, étaient situées souvent sur le fond des glandes de Lieberkühn près de la lumière. Elles apparaissaient aussi aux sommets des villosités (Figure 1), étant plus nombreuses dans les cryptes de Lieberkühn. Les cellules de Kultschitsky avaient la forme caractéristique de bouteille ou d'un cône plutôt allongé (Figure 2). Les cellules prenaient la couleur de une brique brune. Le noyau cellulaire était assez grand, ovale ou rond, et situé dans la partie plus raprochée de la base ou du centre. Des granules diazopositifs (la sérotonine) étaient presents dans tout le cytoplasme (Figure 3), intensivement colorées de brun - rouge.

Groupe I

Trois jours après l'hypophysectomie, le nombre des cellules de Kultschitsky n'avait pas subi de changement net. Aussi, toutes les cellules entérochromaffines ont recouvré les vellosités intestinales, et le nombre, la couleur et la dislocation des granules spécifiques apparaissaient dans les cryptes de Lieberkühn. La sérotonine semblait d'être identique (Figure 4).

Groupe II

La matière a été pris six jours après l'hypophysectomie. Les substances entérochromaffines se présentaient sous la forme de granules peu nombreuses dans le cytoplasme. Les granules spécifiques dans plusieurs cas remplissaient la zone supranucléaire (Figure 5), et les granules formaient des grandes conglomérations. Entre le noyau et la base se trouvaient de fines granulosités de sérotonine (Figure 6). Leur nombre dans diverses cellules n'était pas identique. Il y avait des changements morphologiques indiquant une sécrétion augmentée.

Groupe III

Dans l'épithélium recouvré des sommets de villosités du duodénum des grenouilles, il y avait des cellules de Kultschitsky où les granules diazopositifs étaient disposés de façon chaotique (Figure 7). Aussi, dans les glandes de Lieberkühn le nombre de granules spécifiques semblait être identique. En comparaison avec le groupe de contrôle, les cellules de Kultschitsky étaient plus nombreuses. Les capillaires des villosités démonstraient une dilatation de la lumière.

Discussion et Conclusion

A base des expériences, on a établi que les granulosités spécifiques dans les cellules de Kultschitsky-Schmidt-Ciaccio contiennent l'entéramine identifiée avec la sérotonine (5HT).[9] L'activité enzymatique dans les cellules de Kultschitsky de l'homme et des animaux a été examinée par Pearce et Pepler et ses collègues.[10] I isont examiné les cellules de Kultschitsky dans les intestins des hommes sains et malades. L'existence de ces cellules dans les grenouilles a été signalée par Jakovleva et Sahnazarova[12] et par Czerny.[13,14]

Chez les grenouilles du groupe de contrôle qu'on a examiné, les granulosités spécifiques étaient presents dans tout le cytoplasme. Aprés l'hypophysèctomie, les granules de sérotonine étaient localisés surtout du noyau. Ces granulosités sont probablement liées avec le cycle du travail cellulaire. Cela peut souligner le rôle important de l'hypophyse cérébrale dans le métabolisme des cellules de Kultschitsky. Nos examens des cellules entérochromaffines ont démontré que le changement de leur activité dépend de l'état physiologique de l'hypophyse cérébrale. Ces observations étaient les plus intéressantes dans le duexième et troisième groupes, grâce à l'apparition des cellules.

Les changements observés dans la disposition et dans le nombre de granules spécifiques aprés l'hypophysèctomie démontraient qu'elles

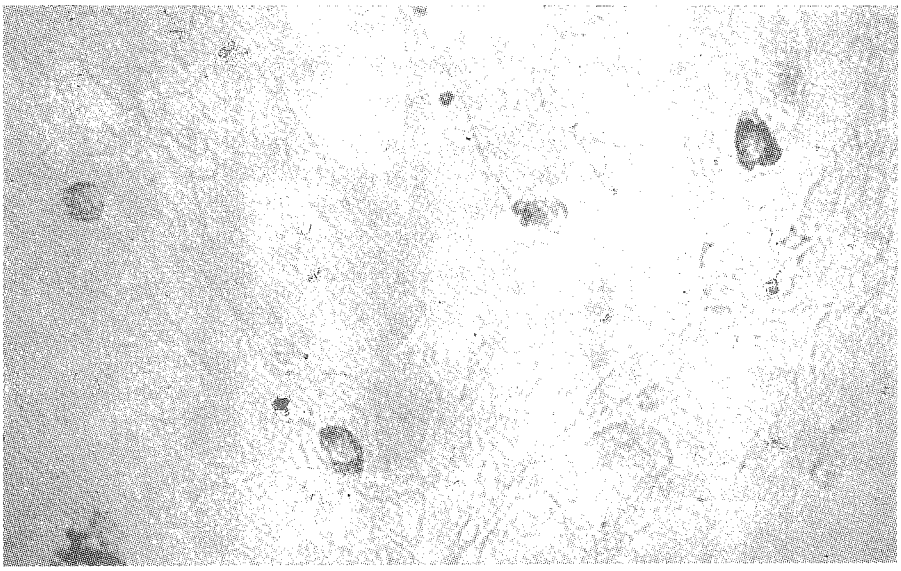

Figure 1

Duodénum de la grenouille. Groupe de contrôle. Les cellules de Kultschitsky situées dans les glandes de Lieberkühn et aux sommets des villosités. Coloration Fast Red B. Augment. ca 400 x.

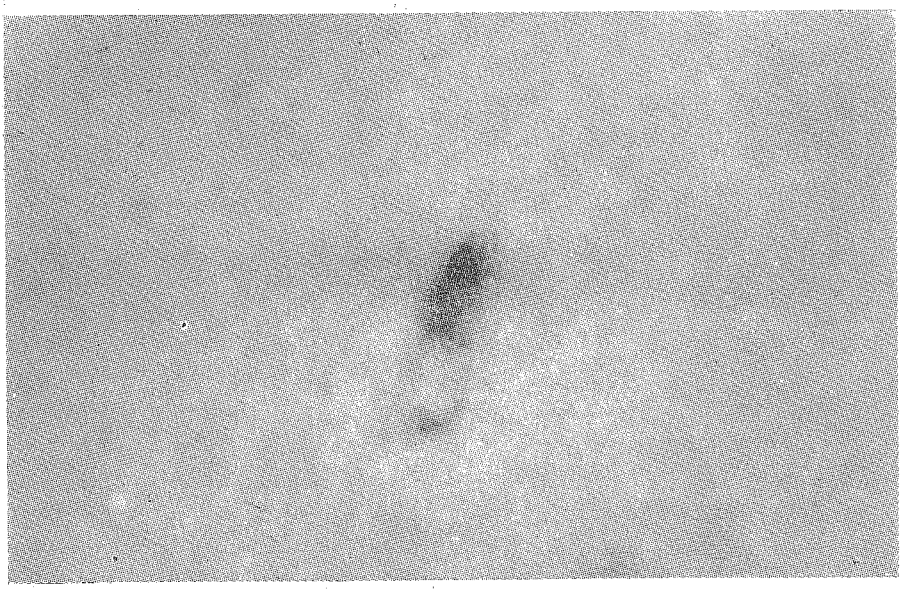

Figure 2

Duodénum de la grenouille. Groupe de contrôle. La cellule de Kultschitsky en forme de bouteille allongée. Coloration Fast Red B. Augment. ca. 1600 x.

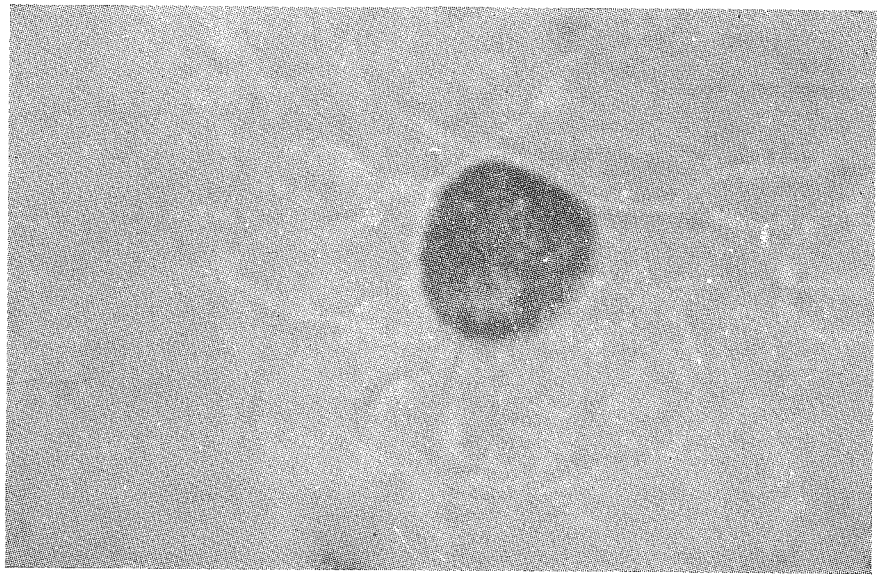

Figure 3

Duodénum de la grenouille. Groupe de contrôle. Granulosités diazopositives colorées spécifiques dans tout le cytoplasme. Coloration Fast Red B. Augment. ca. 1600 x.

Figure 4

Duodénum de la grenouille. Premier groupe expérimental. Dans les cellules de Kultschitsky les granules diazopositifs remplissent presques tout le cytoplasme. Coloration Fast Red B. Augment. ca. 1600 x.

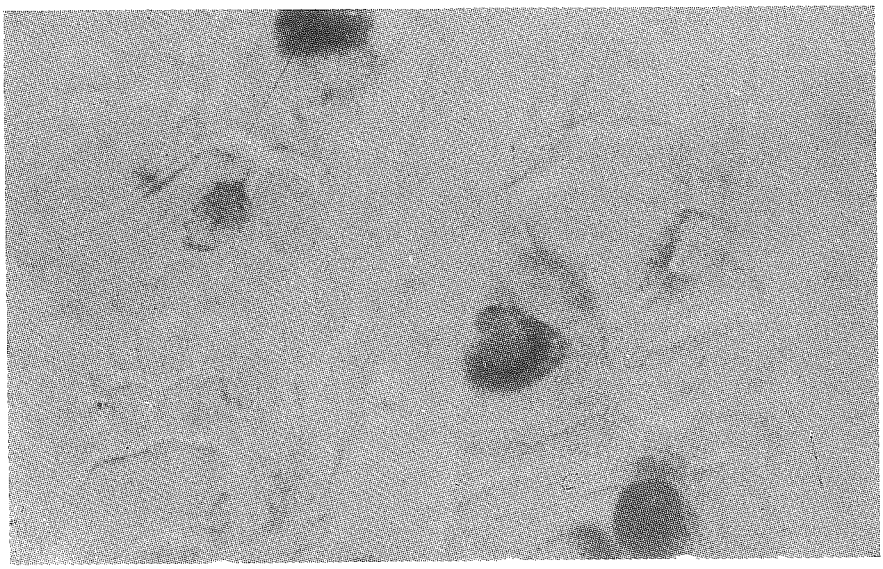

Figure 5

Duodénum de la grenouille. Deuxième groupe expérimental. Diminution du nombre des granulosités diazopositives dans la partie supranucléaire des cellules de Kultschitsky. Coloration Fast Red. B. Augment. ca. 1600 x.

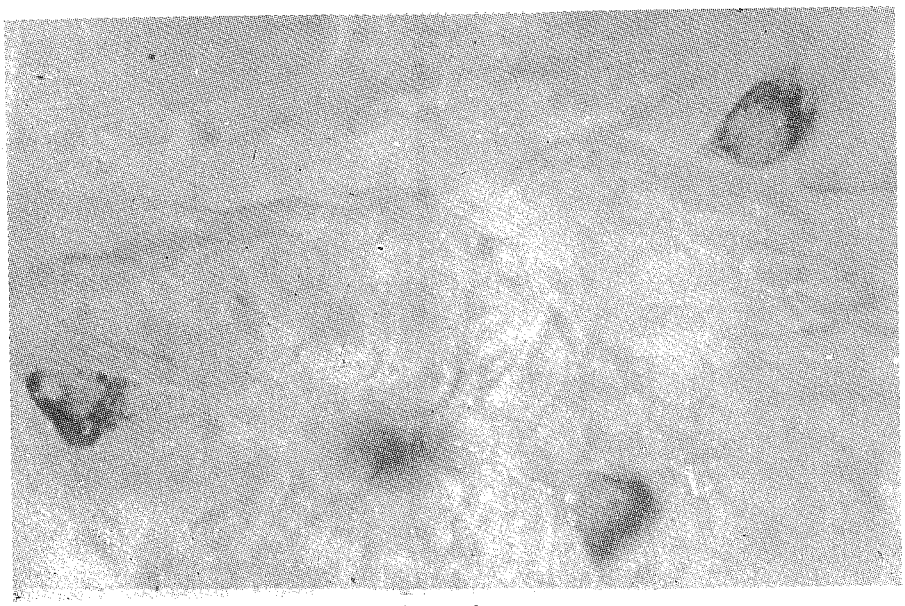

Figure 6

Duodénum de la grenouille. Deuxième groupe expérimental. Changement de la localisation du noyau et des granulosités diazopositives dans les cellules de Kultschitsky. A la base de la cellule et au dessous du noyau on voit des granulosités spécifiques. Coloration Fast Red B. Augment. ca. 1600 x.

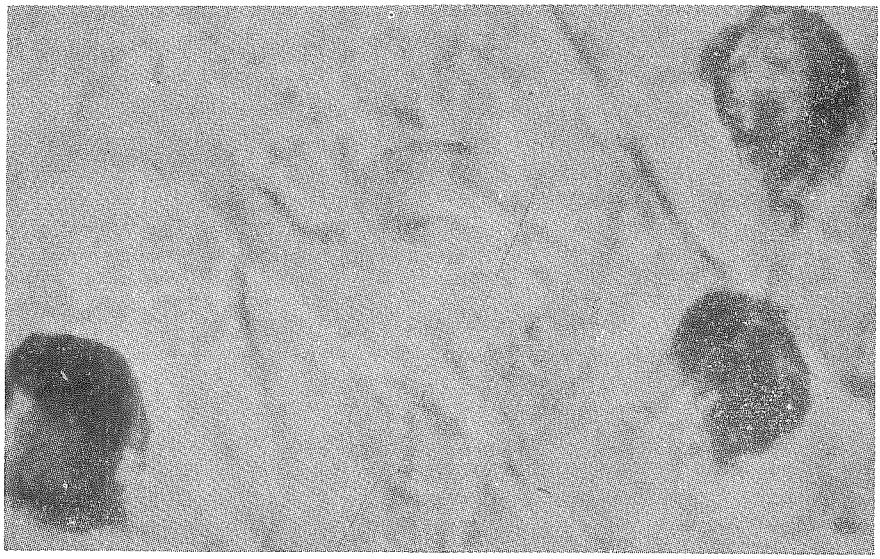

Figure 7

Duodénum de la grenouille. Troisième groupe expérimental. Granulosités diazopositives démontrent la localisation diverse dans le cytoplasme des cellules de Kultschitsky. Coloration Fast Red. B. Augment. ca. 2000 x.

sont liées avec le cycle du travail de la cellule de Kultschitsky. Les granules étaient relâchés surtout par les voies nerveuses et humorales, comme s'est demontré des expériences précèdentes.[67] Selon Czerny [14] cet état n'est pas à cause de la diminution du nombre des cellules entérochromaffines, mais résulte du manque des granules de sérotonine. On peut, sans aucun doute, considérer la présence des granules spécifiques dans la zone du noyau comme changement de l'état fonctionnel des cellules de Kultschitsky provoqué par l'hypophyséctomie. Des pareilles résultats étaient observées par Grzycki et ses collègues [8] aprés l'hypophysèctomie des rats blancs mâles. Nous croyons que l'hypophysectomie à provoqué surtout les changements entre les hormones, comme se faisait voir de nos préparatifs, sous la forme des changements de localisation des granulosités spécifiques (la sérotonine) dans les cellules entérochromaffines.

Le phénomène d'adaptation chez les grenouilles est lié principalement avec l'action des hormones de l'hypophyse cérébrale.[16 17] Dans nos expériences, l'adaptation a été rendue plus difficile par l'hypophysectomie. A cause des changements observés, nous croyons que les cellules de Kultschitsky du duodénum du grenouille de Rana Temporaria L. restent en rapport fonctionnel avec l'hypophyse cérébrale.

Résumé

Les auteurs observaient le comportement des granulosités argentophiles dans les cellules de Kultschitsky du duodénum des grenouilles après l'hypophyséctomie. Les résultats des examens cytochimiques demontraient la variabilité morphologique et physiologique des cellules de Kultschitsky formant la glande d'Erspamer. Les cellules de Kultschitsky restent en rapport fonctionnel avec l'hypophyse cérébrale.

Streszczenie

Po chirurgicznym usunieciu przysadki mozgowej u autorzy obserwowali zmiany ilosciowe oraz rozmieszczenie ziarniztosci diazopozytyvych (seratoniny) w komorkach Kulczyckiego dwunastnicy. Na podstawie otrzymanych wynikow rochodza do wniosku, ze komorki te tworzace narzad Erspamera znajduja sie pod wplywem normonow przysadki môzgowej.

Summary

The granules of enterochromaffin Kultschitsky cells in the Lieberkühn crypts of the duodenum of frogs (Rana temporaria L.). were examined after hypophysectomy. The experimental animals were divided into three groups and compared with a fourth normal control group. The diazonium Fast Red Blue staining method was applied to the paraffin sections.

In the normal animals, the shape of the Kultschitsky cells was round or oval, stained brown - red with diazonium Fast Red B staining, and they were located in the base of the Lieberkühn crypts (Figures 1, 2, 3).

Three days after hypophysectomy, there was no difference between Group I and the control group.

Six days after hypophysectomy, the specific granules of the Kultschitsky cells were located in the supranuclear zone, and formed conglomerations (Figures 5, 6).

In Group III, twelve days after hypophysectomy, the diazopositive granules of the Kultschitsky cells were irregular both in the epithelium and in the Lieberkhün crypts (Figure 7). The number of Kultschitsky cells was found to have increased. The lumen of the villi cappillaries were dilated.

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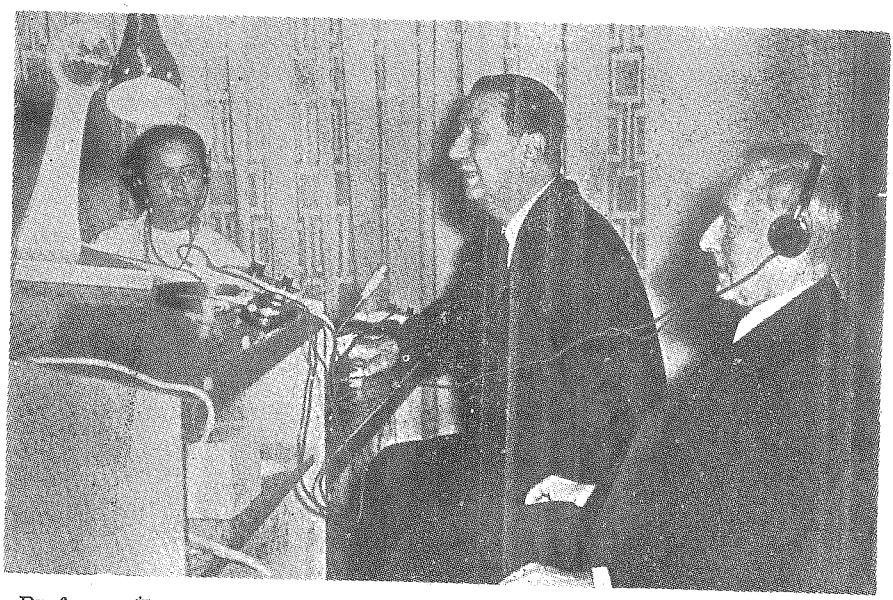

Professor İhsan Doğramacı, President of Hacettepe University introduces Professor Debré to staff members of Hacettepe Faculty of Medicine.

□ Professor Robert Debré of the International Children's Center, Paris, visited Hacettepe University on October 23, 1968 and delivered a lecture on «The New Trends in Medical Education» to the teaching staff members of the Faculty of Medicine.

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