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Ultrastructural Observations on a Case of Chronic Rheumatic Carditis*

I. Alterations of Myocardial Cells

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Introduction

Few authors until now have been inclined to make electron microscopical studies on chronic rheumatic carditis, particularly the pathological alterations displayed by the myocardial cells. The Aschoff nodule, the specific pathological structure found in the interstitial cardiac tissue, is a means by which the illness can be identified at light microscopical level. However, in higher magnifications, at electron microscopical level, the term "nodule" cannot be treated similarly to that at light microscopy level. The Aschoff lesions at electron microscopical level consist of Aschoff cells closely related with the fibrinoid aggregates, pathologically altered collagenous fibers and normal collagenous fibers. It may positively be expected that the myocardial cells may also have some specific ultrastructural features related to the rheumatic process in the myocardium.

In view of these facts, a typical case of chronic rheumatic carditis was taken up and a specimen from the auricular region of the right atrium was used, thinking that it would contain fewer specific Aschoff lesions in the interstitium.

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The aim of this study is to establish the pathological ultrastructural alterations in the myocardial cells and intermyocardial tissue of the myocardium other than the Aschoff lesions and identify any changes that might be specifically related to the rheumatic process in question.

Material and Method

The biopsy obtained from the anterior wall of the right atrium during an open heart operation was used as material in this study. The patient was an eighteen year old female, with a definite diagnosis of chronic rheumatic heart disease based on previous clinical and laboratory findings. The rheumatic inflammatory process in the myocardium dated back nine years.

The biopsy specimen taken was immediately placed in a solution of 1% osmium tetroxyde, buffered with phosphate to pH. 7.1. It was then bisected into tiny blocks of approximately 1 cu. mm. each and fixed for three hours at 0-4°C in the above solution, which was renewed three times. Following the fixation, the tiny blocks were taken into a solution of 7.5% cold sucrose, buffered with phosphate to pH. 7.1 and kept for the whole night at 0-4°C. They were then dehydrated in graded alcohols. Meanwhile, the blocks were stained for one hour in 70° alcohol saturated with uranyl acetate during the process of dehydration. Tissue blocks were then embedded in 00 gelatine capsules in a mixture of Araldite 502 and Dodecenyl succinic anhydre (DDSA). The silver-tone ultrathin sections were obtained, using Porter-Blum MTI ultramicrotome and stained for the second time in an alkaline solution of lead citrate. Carl Zeiss E.M. 9A type electron microscope was used for the examination and photography of the sections.

Observations

Myocardium in general :

In low-power magnifications, it was observed that the myocardial cells were rarely normal both in their interrelations and internal structures and displayed varying degrees of pathological alterations in almost all the areas examined. Even in the areas with a rather normal appearance, the myocardial cells seemed separated from each other with numerous indifferentiated connective tissue elements, particularly, the indifferentiated and other types of cells, which will be treated in another paper to follow this study.

Myocardial cells :

The myocardial cells were irregular in shape and varied in size. (Figure 1). The bodies of cells seemed almost fragmented with deep invaginations, both narrow and wide. In the interior of all these cells, there was a striking increase in the number of mitochondria. In areas displaying severe pathological alterations, certain parts of the sarcoplasm of myocardial cells were liquefied and free from organelles. They appeared as small or large cystic formations with somewhat granular like contents (Figures 2, 3). The intracellular lytic process was noted as small or large cystic formations to take place both in the central and peripheral parts of the cells. In some instances, it appeared large enough to look like a peripheral cystic formation (Figure 4).

Bundles of myofilaments were damaged either locally or extensively in the cells. The myofilaments were packaged in between abundant distorted mitochondria. In many areas, the sarcomeres were distorted. Consequently, the banding pattern could not be traced, but some irregular dark bands appeared along the myofilament bundles. It was also frequently observed that myofilaments fused together, in a homogenous pattern.

Alterations in the intercellular relations and the sarcolemma:

Alterations in the intercellular relations and the sarcolemma: The myocardial cells having continuous basal laminae were interrelated with each other along the highly indented surfaces (Figure 5). The cells seemed nearly fragmented with deep infoldings of the sarcolemma and the basal lamina. Intercellular elements were also traced in these infoldings, branching out into the cell with numerous micropinocytotic vesicles along their walls (Figures 6 and 7). At times, these infoldings seemed to extend across the cell almost dividing it into two parts, save a narrow bridge. It was observed that the formation of these invaginations was distinctly different from that of the transverse elements of the sarcoplamic reticula and the altered intercalated discs (Figure 7 and 8). In the sarcoplasmic area adjacent to the invaginations, a clear open space free from cellular organelles, but rich in micropinocytotic vesicles, was visible (Figures 6 and 8).

Alterations in the interior of the myocardial cell:

The nuclei were noted to have indented contours, at times so much so that the sectionsof the infoldings could easily be seen (Figure 9). Their extreme polar edges were often tapered. The nuclei were rarely

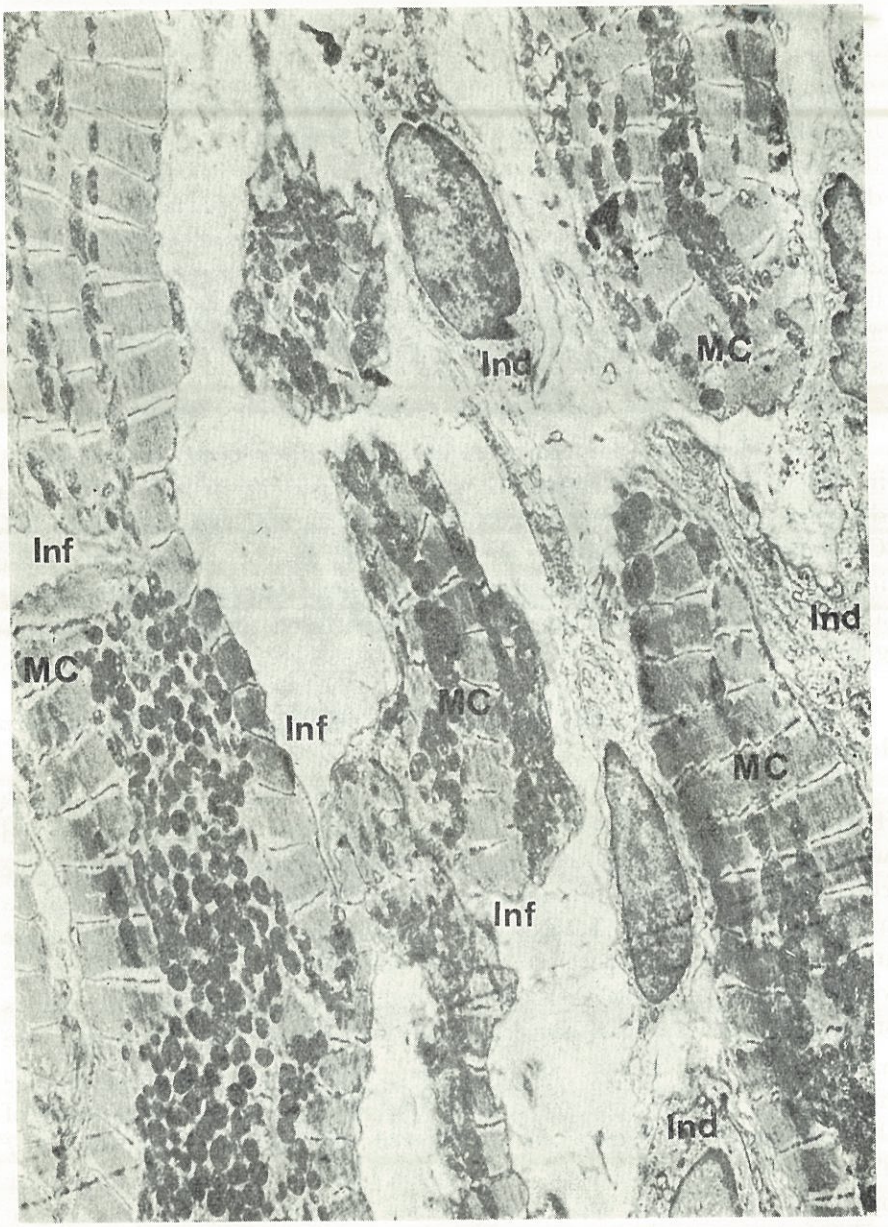

Figure 1

Panoramic view of a region of the myocardium slightly altered. Myocardial cells (MC) are widely separated from each other and nearly fragmented with deep sarcolemmal infoldings (Inf). Indifferentiated connective tissue cells (Ind) observed in between the myocardial cells are larger and more numerous in number. Osmium-Araldite-Uranyl-Lead. X 6600.

Figure 2

Myocardial cells (MC) having advanced pathological alterations are seen. Many of the myofilament bundles do not have normal banding pattern (X). Abnormal dense areas (DA) take place along the myofilaments. Mitochondria (M) having abnormal morphology have increased in number. In the right half of the micrograph large lytic areas (LA) are observed in the interior of the myocardial cell free from cell organelles. ID, Intercalated disc. Osmium-Araldite-Uranyl-Lead X 6600.

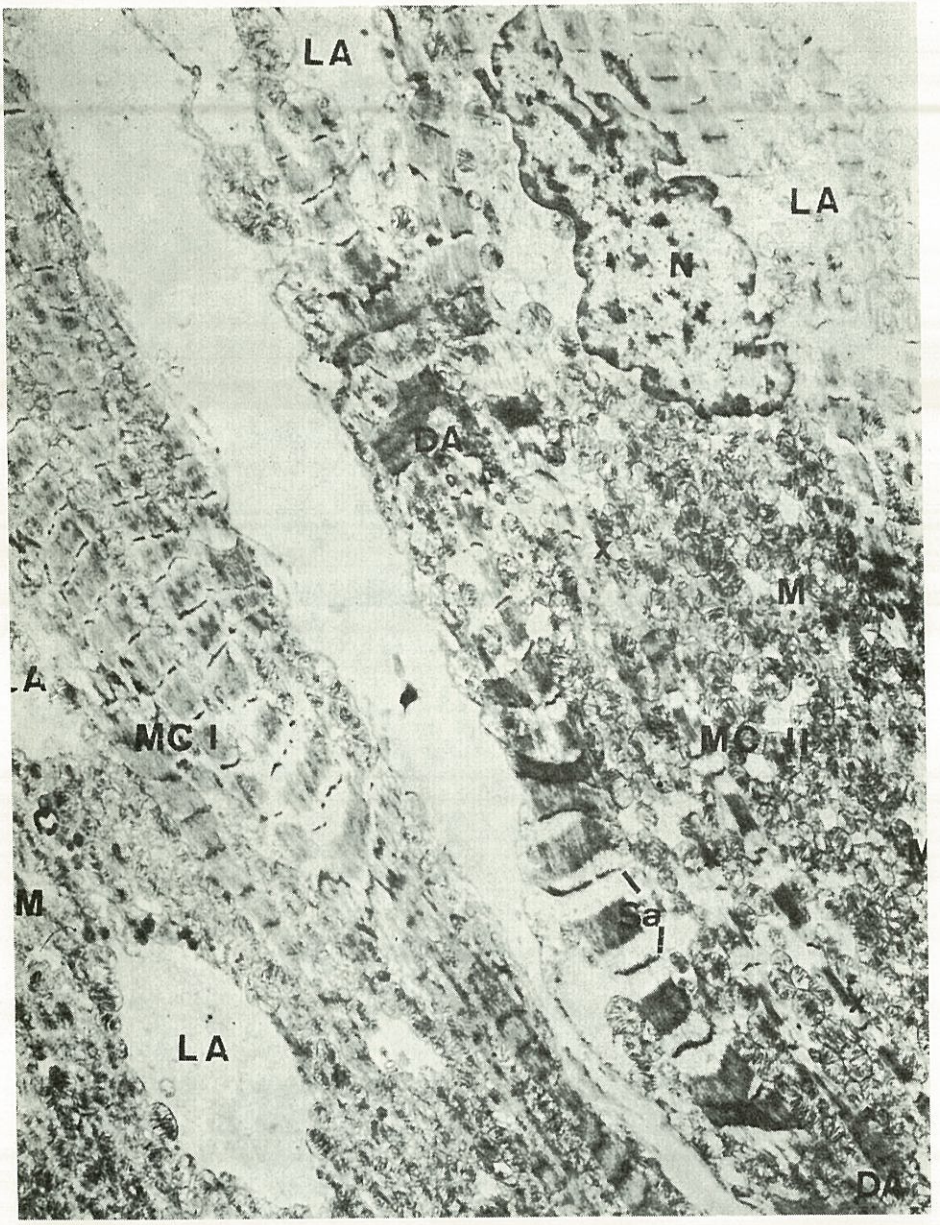

Figure 3

Two other myocardial cells (MCI, MCII) are seen with advanced intracellular alterations : Myofilament bundles are disorganized. Contracted and relaxed sarcomeres (Sa) are seen along the bundles together within a cell. Small and large lytic areas (LA) are noticed in different parts of the cells. Numerous mitochondria (M) fill up the central part of the cells and the myofilament bundles are sandwiched in between them (X). DA, dense, areas; N, nucleus. Osmium-Araldite-Uranyl-Lead. X 7000.

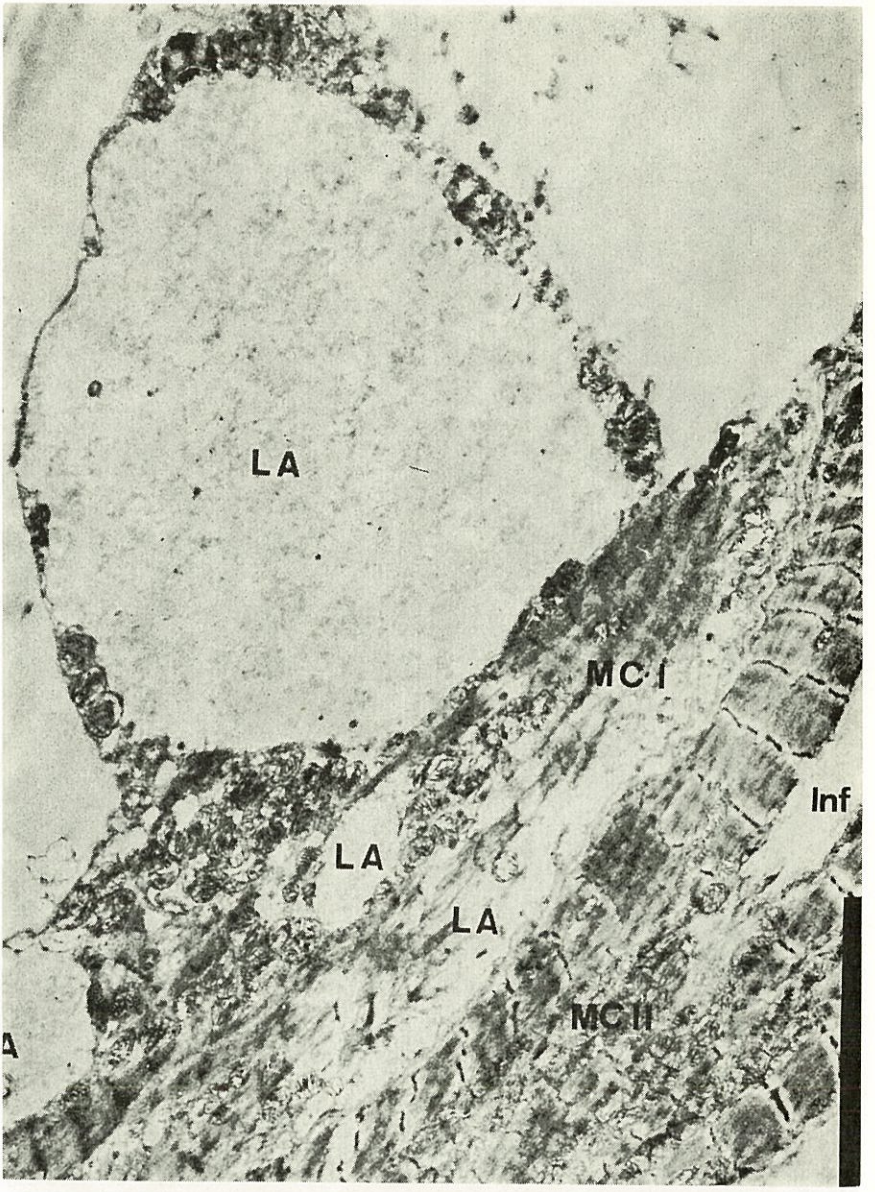

Figure 4

Two myocardial cells (MCI, MCII) showing severe pathological manifestations : Cell I has almost no normal intracellular structure. Bundles of myofilaments are observed in a necrotic state having a homogenous appearance. Focal lysis of the organelles (LA) is remarkable in different regions of the sarcoplasm, the largest being observed at the periphery of the cell with a granular content in it. Cell II is rather normal compared with cell I, but has a deep and indented sarcolemmal infolding (Inf) with intercellular material in it. Osmium-Araldite-Uranyl-Lead. X 7000.

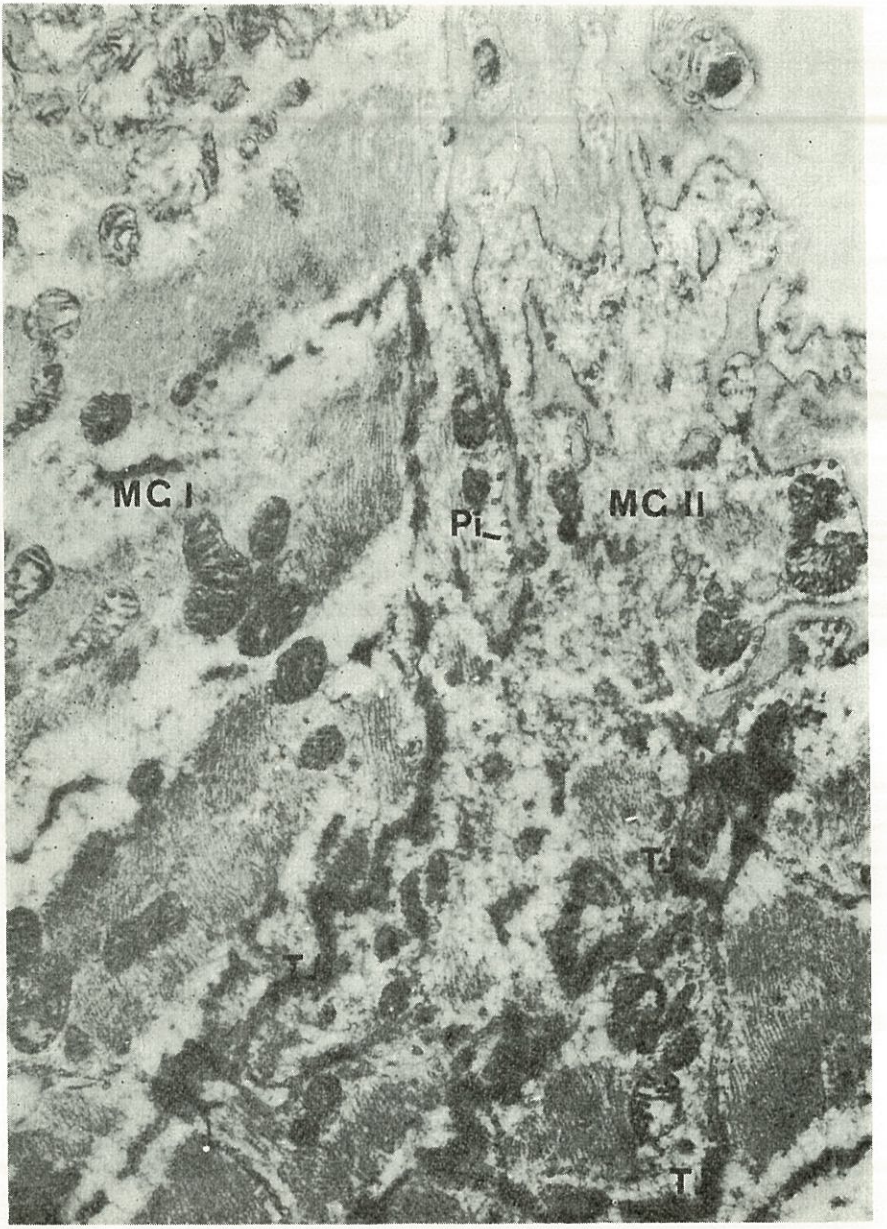

Figure 5

Interrelation between two myocardial cells (MCI, MCII) are observed. The cells are deeply indented with each other having numerous tight junctions (TJ) and micropinocytotic vesicles (Pi) along the course of their membranes. Cell I has a rather normal morphology compared with Cell II. Cell II seems to be a part of a fragmented myocardial cells with very irregular contours and disorganized myofilament content. Osmium-Araldite-Uranyl-Lead. X 27000.

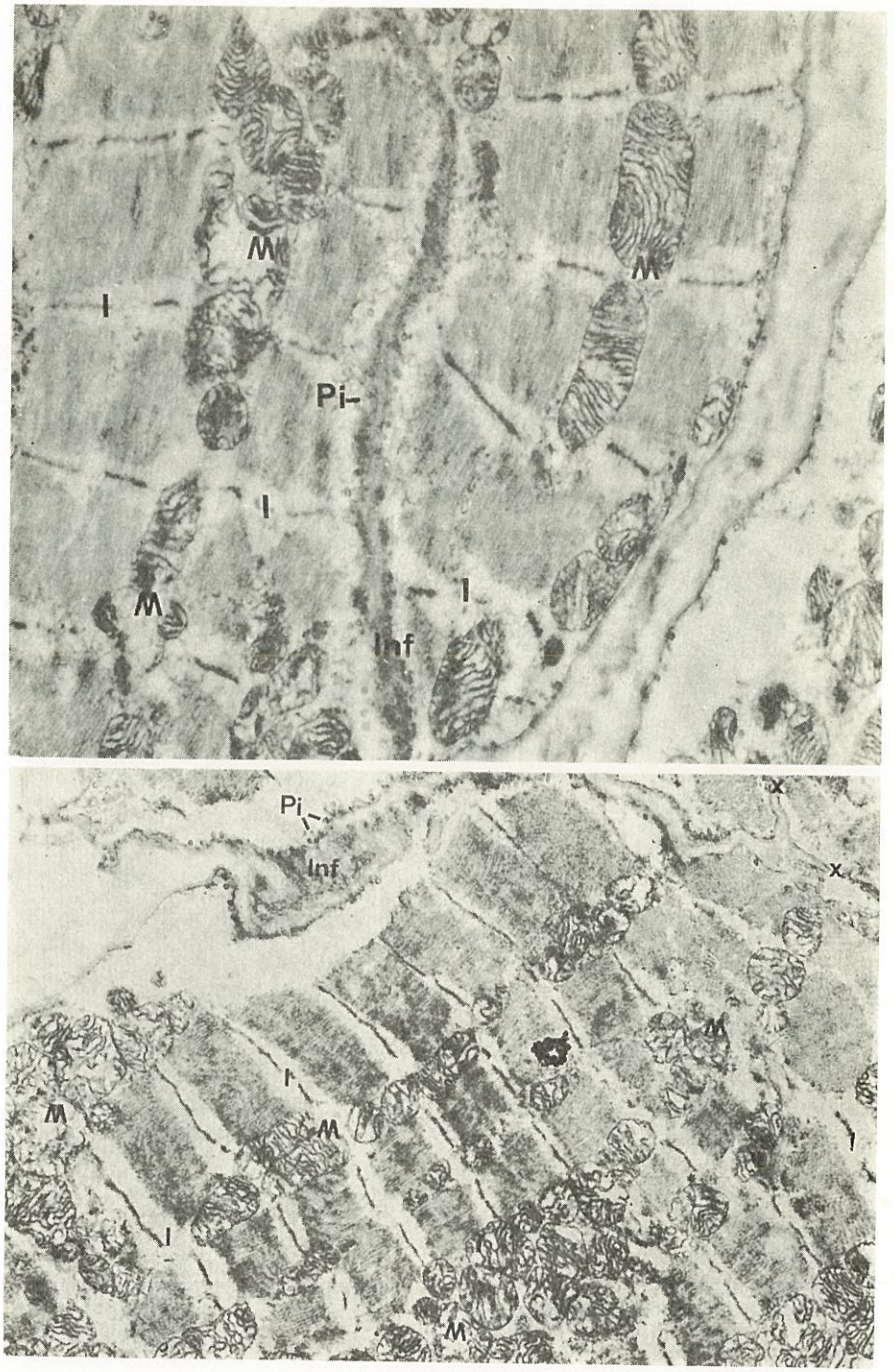

Figure 6 (a and b)
Two distinct myocardial cells are seen having deep sarcolemmal infoldings (Inf) with intercellular material branching into the cell (X marks). A clear zone of the sarcoplasm extends to the vicinity of the invaginations. Numerous micropinocytotic vesicles (Pi) are seen along the sarcolemma. Note that the I bands (I) and mitochondria (M) are disorganized. Osmium-Araldite-Uranyl-Lead. X 27000. X 24.500.

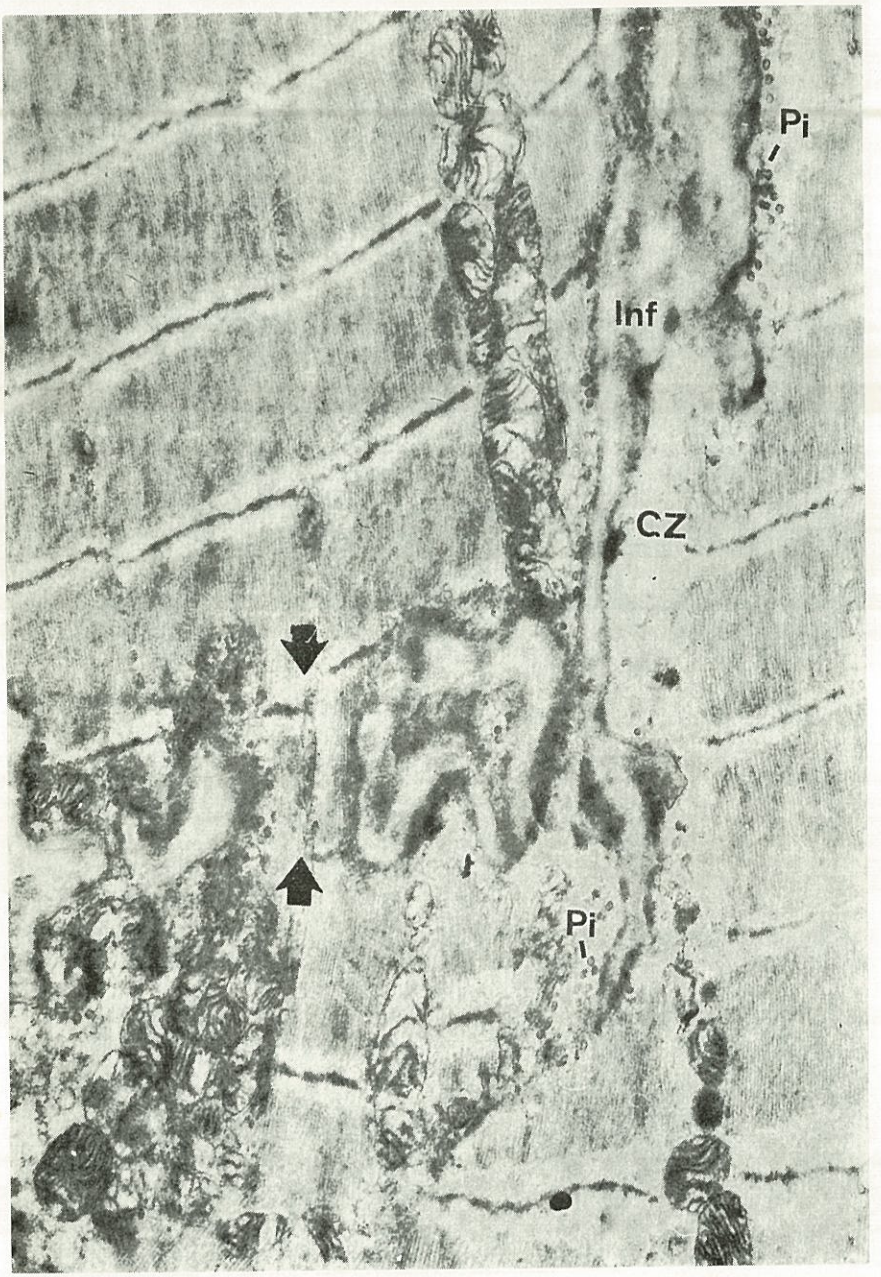

Figure 7

Part of a myocardial cell with deep sarcolemmal indentations (Inf). Only a narrow bridge connects the two divided portions of the cell (arrows). Note the clear zone (CZ) and numerous micropinocytotic vesicles (Pi) inside the sarcolemmal infoldings. Osmium-Araldite-Uranyl-Lead. X 27000.

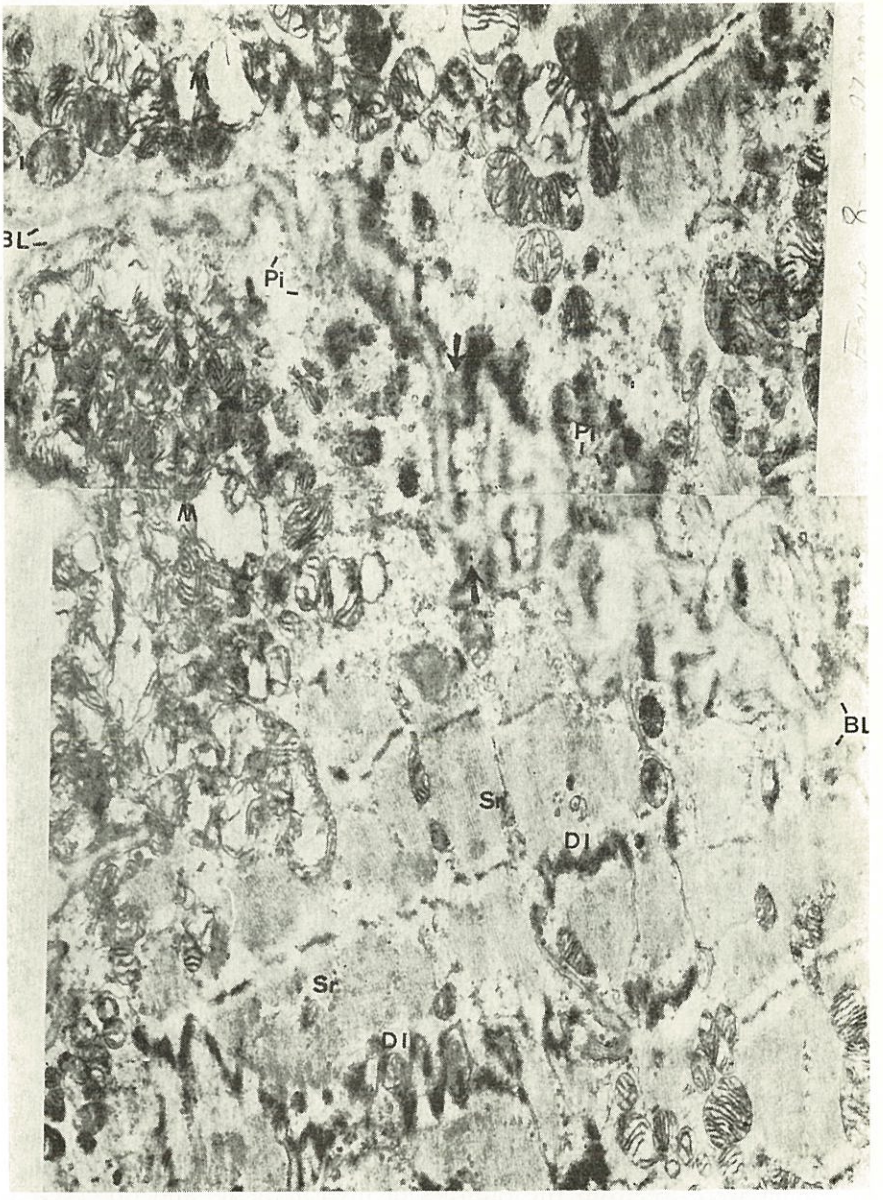

Figure 8

A myocardial cell nearly fragmented into two distinct portions except a narrow bridge connecting the two (arrows) is observed. Note that the fragmentation of the cell does not occur in the region of the intercalated disc (DI) also normally seen in this cell. Basal Lamina (BL) are traced indenting into the cell together with the sarcolemma. Clusters of distorted and cristolytic mitochondria (M) and numerous micropinocytotic vesicles (Pi) are located in the enlarged clear zone under the sarcolemma of the deeply indented region. Fine tubular profiles of the longitudinal sarcoplasmic reticulum (Sr) are seen between the myofilament bundles. Osmium-Araldite-Uranyl Lead. X 27000.

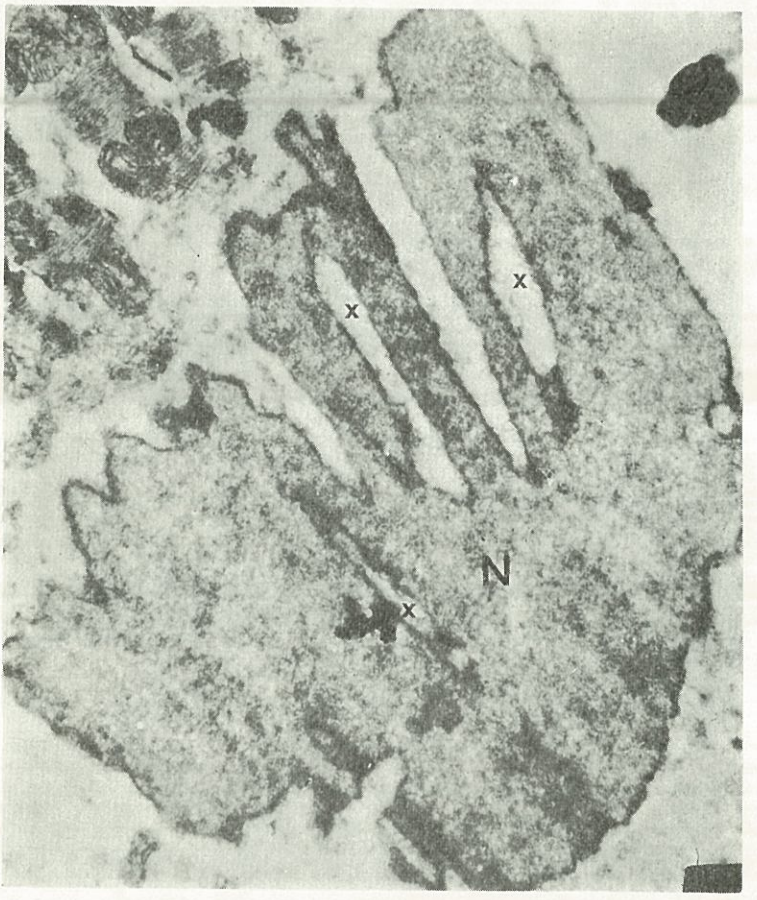

Figure 9

Nucleus (N) of a heart muscle cell with very indented contours and a dense and homogenous chromatin content. Profiles of deep nuclear infoldings are observed in the center of the nucleus (X). Osmium-Araldite-Uranyl-Lead. X 27000.

observed to be oval. The areas found in the lower and upper poles of the nuclei were freefrom the bundles of myofilaments and observed to be larger than nor-mal (Figures 10 and 11).

The mitochondria highly increased in both the areas adjacent to the nuclei and those in between the bundles of myofilaments. They were then observed to form random aggregates within the cell, containing normal as well as abnormal or distorted mitochondria. Such abnormal mitochondria seemed to be either larger or smaller than the normal ones and crystolysis was clearly visible (Figure 12). In addition to these, lipid particles and lysosomal bodies invested with the unit

membrane and having a dense granular content were also encountered. The mitochondria, lipids and the lysosomal bodies were closely located to one another with fine granular material between them within the cell. It was possible to observe these both in areas by the nuclei (Figures 9 and 10) and the peripheral parts (Figures 13 and 14) of the cells. No glycogen particles are encountered within the myocardial cells.

In some cells, it was observed that the myofilament bundles preserved their normal structure, having the usual banding pattern. In a

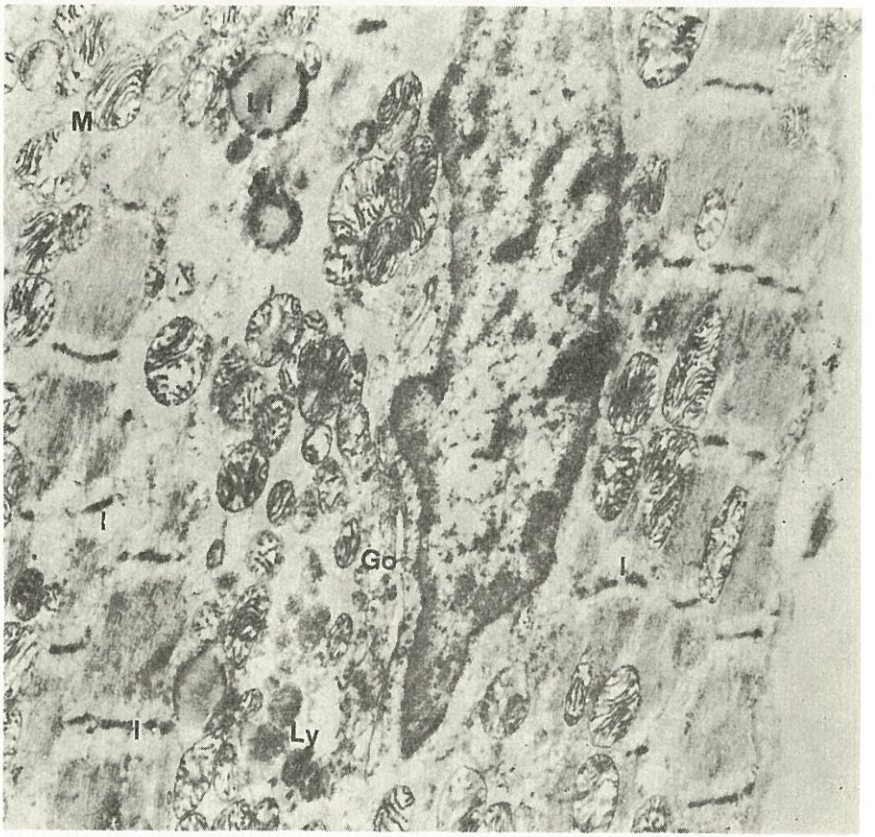

Figure 10

Part of a myocardial cell having the nucleus is seen above. The nucleus is elongated with a peripheral chromatin content and tapered at the polar ends. A noticeable Golgi Complex (Go) takes place near the lower end of the nucleus. Lipid inclusions (Li), dense lysosomal bodies (Ly) and distorted mitochondria (M) are observed in the enlarged perinuclear area. Note that the actin filaments are damaged and seem to have disappeared in the I band regions (I). Osmium-Araldite-Uranyl-Lead. X 27000.

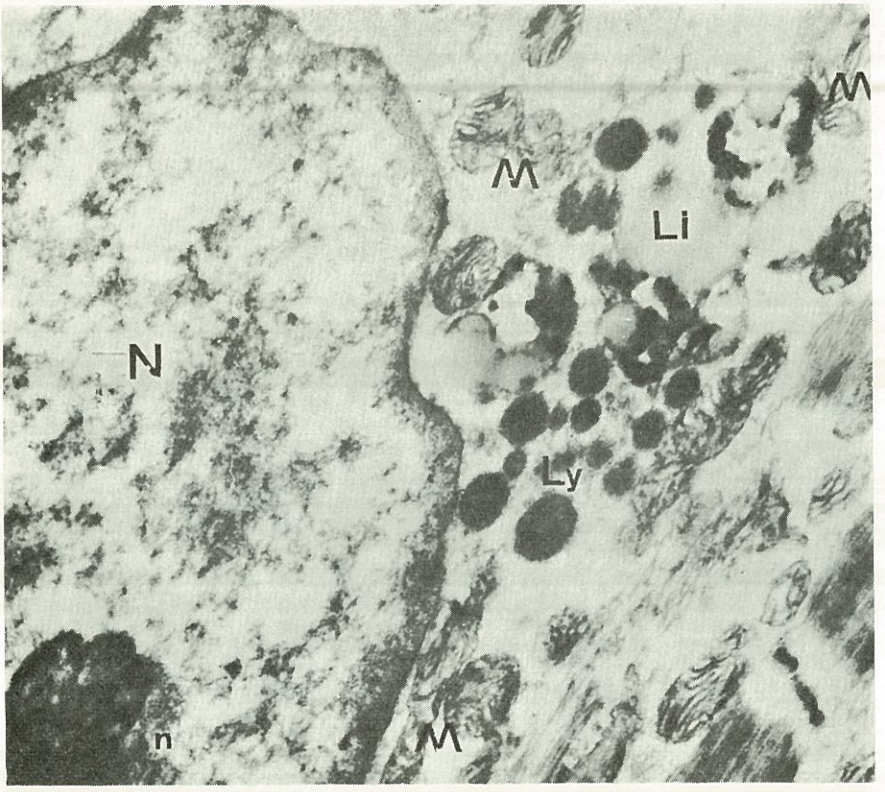

Figure 11

Perinuclear zone of a myocardial cell is seen having lipid granules (Li), dense lysosomal bodies (Ly) closely related to distorted mitochondria (M). It is also noticeable that the nucleus (N) is not indented, has a rather oval shape, a prominent nucleolus (n) and a dense peripheral chromatin band. Osmium-Araldite-Uranyl-Lead. X 33000.

greater majority of cells, these bundles displayed pathological changes varying from loss of the banding pattern to complete necrosis (Figure 15). The myofilament bundles in the cells with deep invaginations of the sarcolemma appeared normal or nearly normal, but in the cells where these infoldings disappeared, total fragmentation and complete necrosis of myofilament bundles attracted attention. (Compare Figure 7 and 15). The pathological alterations were particularly observed in the I bands and Z line regions of the sarcomeres along the myofilament bundles. The actin filaments almost disappeared and dense structures were also encountered along the course of normal sarcomeres (Figures

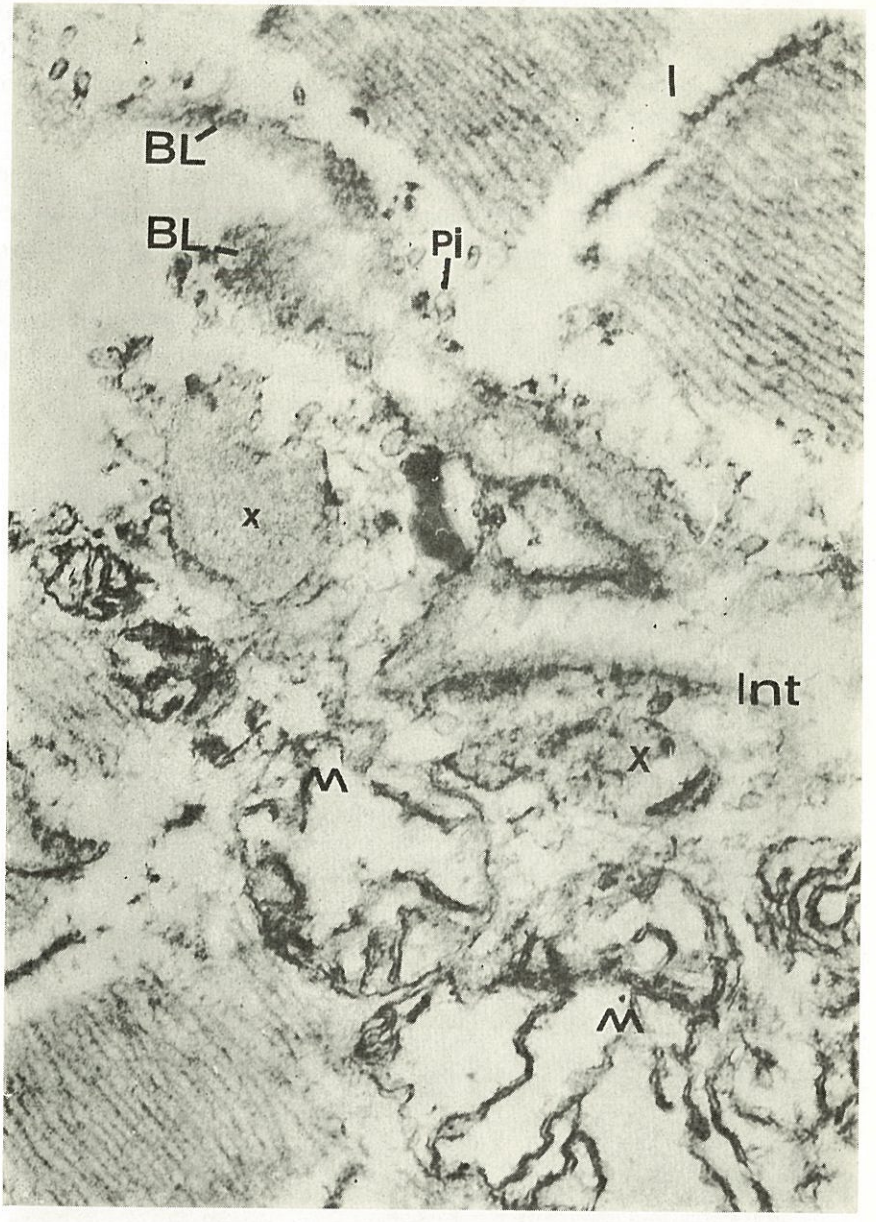

Figure 12

High power micrograph of the intercellular region of two adjacent myocardial cells (Int). Each cell has its own basal lamina (BL) on the sarcolemmal surfaces. Micropinocytotic vesicles (Pi) take place just beneath the sarcolemma. Two distented structures possibly belonging to the sarcoplasmic reticulum elements are observed (X). Crystolysis is obvious in the peripheral mitochondria (M). Actin filaments again seem to have disappeared in the I band (I). Osmium-Araldite-Uranyl-Lead. X 72000.

2, 3, 16). Cystic formations invested with a membrane and lytic areas free from organelles, with or without any content in both cases, were frequently encountered in the severely affected cells containing necrotic myofilament bundles and numerous distorted mitochondria (Figure 15).

The longitudinal tubuli of the sarcoplasmic reticula were noticed in between the bundles of myofilaments in the myocardial cells, undergoing rather minor pathological alterations. Few vesicles of the transverse system along the Z lines were noted (Figures 7, 8). On the other hand, no structures belonging to the transverse and longitudinal elements of the sarcoplasmic reticulum were seen in the highly damaged myocardial cells (Figure 15).

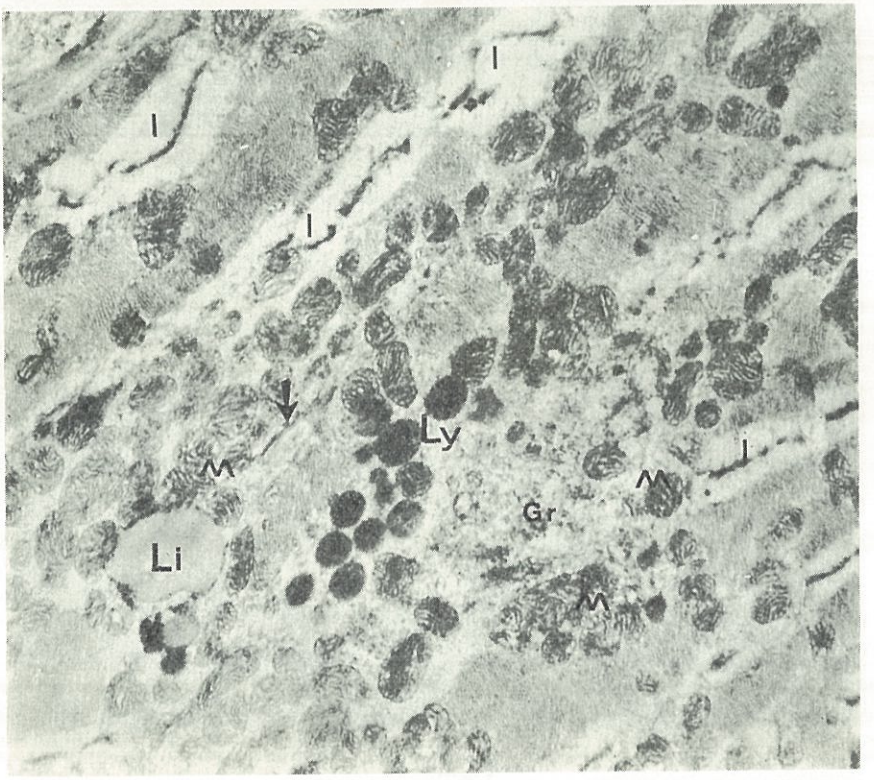

Figure 13

Peripheral part of a myocardial cell. Mitochondria (M), dense lysosomal bodies (Ly) and lipid granules (Li) are observed altogether in close approximity, encircling a granular material (Gr.) Profile of a deep infolding is noticed (arrow) in the cell. I bands (I) are damaged. Osmium-Araldite-Uranyl-Lead. x 27000

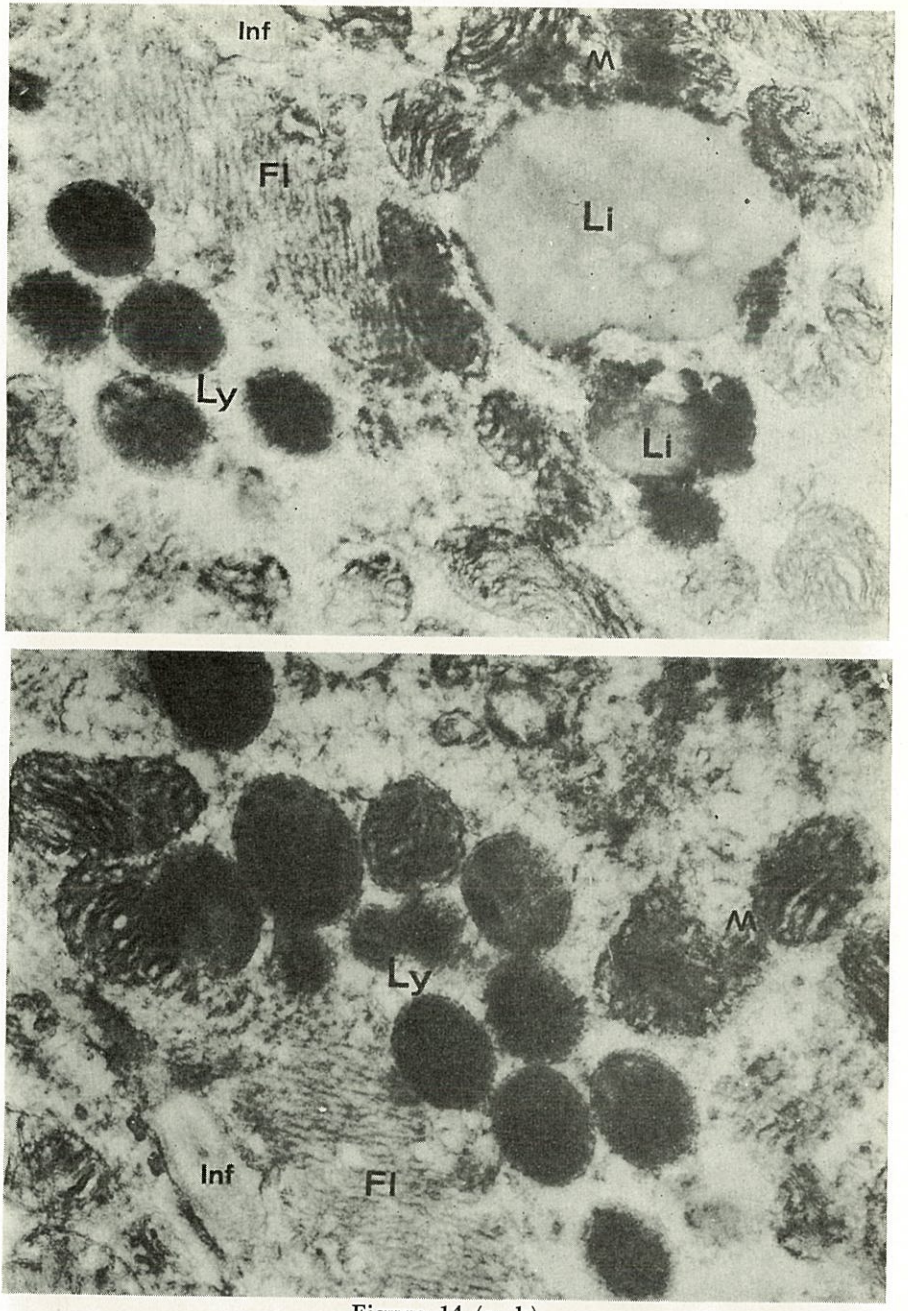

Figure 14 (a, b)

Higher magnifications of two parts of Figure 13, showing close interrelations in between the mitochondria (M), lipid granulas (Li) and dense lysosomal bodies (Ly). Slightly distorted mitochondria are located in the periphery of a large lipid granule and their outer membranes seem to have fused with the lipid substance. Dense lysosomal bodies seem to be invested with a membrane and have heterogenous content. Note the profile of a sarcolemmal infolding (Inf) and disorganized myofilament bundles (Fi), Osmium-Araldite-Uranyl-Lead. X 72000.

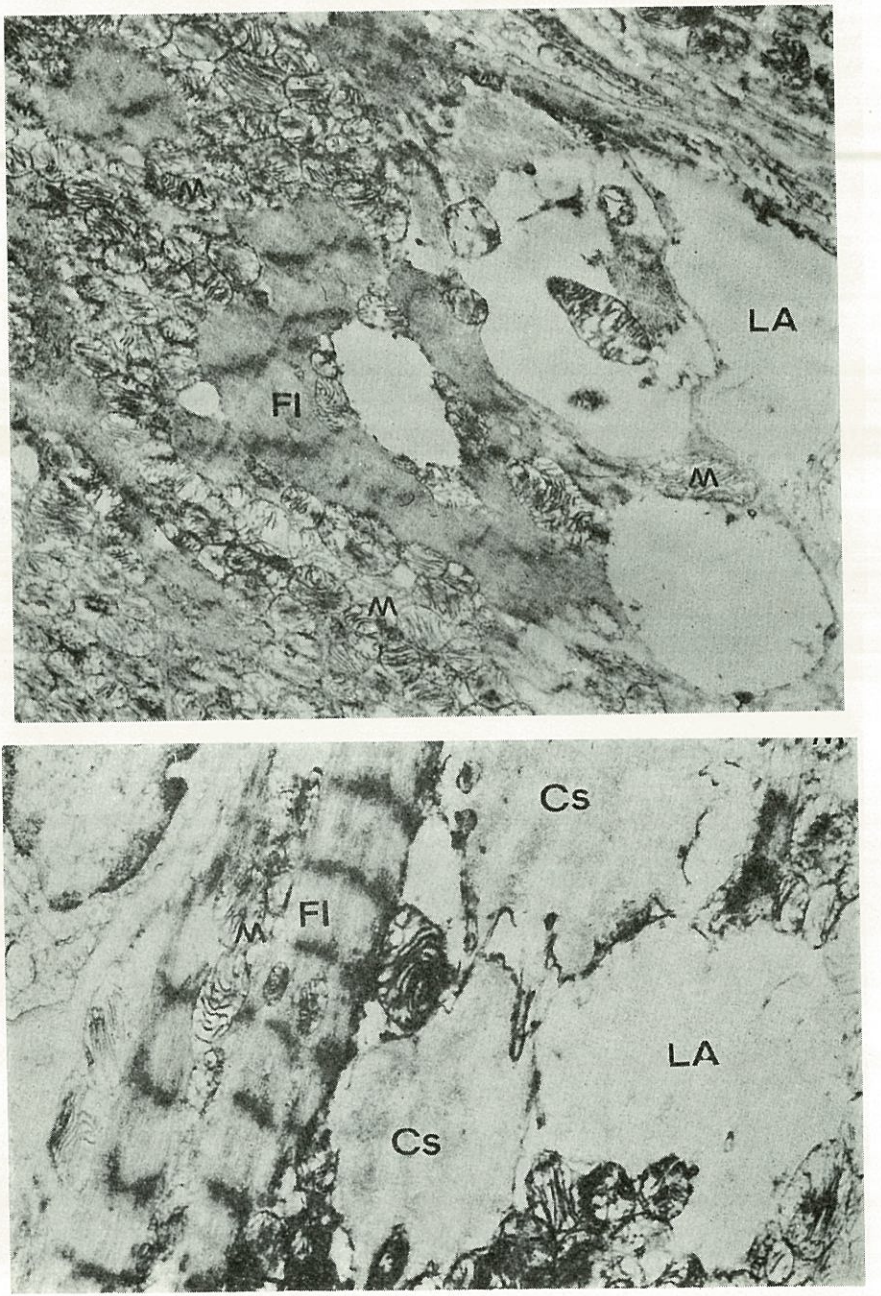

Figure 15 (a, b)

Interiors of two severely damaged myocardial cells are observed. Both small and large lytic areas (LA) as well as cystic structures (CS) with a content attract attention. Mitochondria (M) are disorganized and show crystolysis. Bundles of myofilaments (Fi) have nearly lost the characteristic banding pattern and seem homogenous as a result of fusion and packed up in between numerous mitochondria. Osmium-Araldite-Uranyl-Lead. X 24 500.

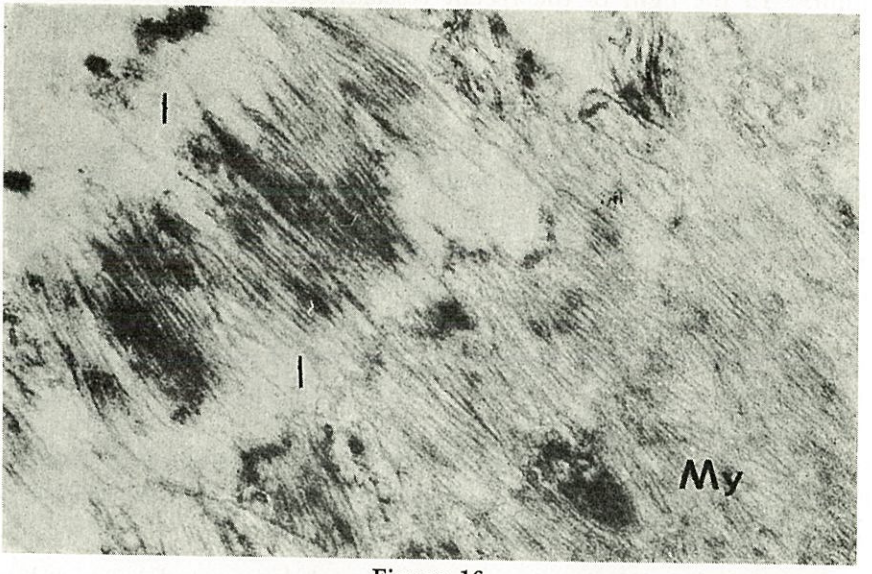

Figure 16

High-power micrograph of disorganized myofilament bundles. In the upper left, the actin filaments in the I band (I) have almost been lost as a result of intracellular damage. Down below, No I band is visible. A dense elongated area seemingly consisting of only myosine filaments (My) is noticed. Osmium-Araldite-Uranyl-Lead. X 72000.

Discussion

The pathology of rheumatic carditis at the electron microscopy level has, until now, been studied by only Lannigan and Zaki. These researchers investigated the ultrastructure of the normal and rheumatic heart in a series of articles during the last decade and defined the alterations in the endocardium and myocardium.[1-5] Studies on the definition of normal mammalian and human hearts at the electron microscopy level are still continuing. As the methods used in electron microscopy are more highly developed it is possible to observe further details in the ultrastructure of the myocardium, as well as structural variations which could not so far be identified. Recent researches have brought to light some structural features concerning the interior of the cells and the cellular interrelations in the myocardium. With the excellent techniques employed, it has been possible to define the ultrastructural details of the myocardium[6-10] and especially the interior of myocardial cells.[11-15] A better analysis of the myocardial ultrastructure has led to a better interpretation of the changes in various pathological conditions[16,17] and certainly a better accuracy in the identification of interrelations between the ultrastructure and function of the myocar-

dium.[18] In the light of the recent researches on the normal ultrastructure of the myocardium, it should undoubtedly be useful to review and evaluate the ultrastructure of the rheumatic alterations in the myocardium.

The first original observation made in this study on the myocardium generally has been the separation of the myocardial cells, which in turn, caused a widening of the intercellular spaces. In these spaces, intercellular material i.e. altered collagenous fibers, ground substance and numerous indifferentiated cells, was observed. The myocardial cells were usually fragmented with deep infoldings of the sarcolemma together with the basal lamina. The formation of these sarcolemmal invaginations had clearly nothing to do with the transverse tubuli of the sarcoplasmic reticula. It has also been reported that similar structures are found in the myocardial cells of normal bovin myocardium.[11] However, these were narrow and did not go deep into the cell, as seen in the affected cells observed in this study.

A close interrelation has been reported between the intercalated discs and transverse tubuli of the sarcoplasmic reticula in the normal mammalian myocardial cells[12]. In this study, the intercalated discs in between the myocardial cells had no communication with the elements of transverse tubuli and showed a minute alteration from normal even in the most severely damaged cells. However, it was occasionally observed that the intercellular spaces of the intercalated discs enlarged and the junctional complexes disappeared. In an electron microscopical study made on the non-rheumatic hypertrophy of the ventricule of a human heart, finger-like protrusions filled by mitochodria were observed along the lateral faces of myocardial cells. In other words, the sarcoplemma displayed indentations at regular intervals.[19]

An increasing number of researches analysing the effects of various experimental pathological conditions on the mammalian myocardium at the electron microscopy level have recently been published. The ultrastructural alterations in the myocardial cells have been investigated in experimentally-produced chronic compensated myocardial hypertrophy,[20,21] animals subjected to hyperexercises,[23] acute toxic carditis,[24] general deep hypothermia,[25] acute hypoxy[26,27], ischemia,hypercapnia and hyperkalemia[28-31], acute failure and kidney pathology leading to chronic hypertrophy of the heart.[33] The main aim of all the researches mentioned above is to determine the structural response of myocardial cells when the heart is experimentally subjected to acute and chronic overloading. The following facts may be derived from these researches:

1. The sarcolemma shows an indented and irregular course with invaginations or finger-like protrusions.
2. The mitochondria increase in number, are distorted, become smaller or larger than normal, display crystolysis and disappearance of matrix granules.
3. The size of myofilament bundles increases at the beginning. However, with the persistence of pathological status, they decrease in size and appear packed up in between abundant mitochondria.
4. Particularly the transverse tubuli of the sarcoplasmic reticula are extremely enlarged forming intracellular cystic structures within the myocardial cells.

It would be of considerable interest to note here that similar changes have also been observed in the myocardial cells of the myocardia of virus-infected mice.[34,35]

The fundamental ultrastructural changes displayed by the myocardial cells, as mentioned above, were noted to be parallel to the findings of Lannigan and Zaki in rheumatic carditis. In addition, the findings in this research on chronic rheumatic carditis, generally confirmed those of Lannigan and Zaki. Moreover, the separation of myocardial cells fragmented with deep infoldings of the sarcoplemma and basal lamina and the increase in the intermyocardial connective tissue elements, especially the indifferentiated-type cells observed in this study, have been considered by the authors as features particular to rheumatic process itself. It accordingly seems logical to conclude that the separation and irregular fragmentation of the myocardial cells arise from the morphological distortion caused by the rheumatic process, rather than a compensation activity of the myocardial cells when the heart is overloaded. With the progress of rheumatic process, the myocardial cells are completely disintegrated. In addition, occasional lysis causing organelle-free regions, a complete loss of the sarcoplasmic reticulum, necrosis of the myofilament bundles, occur within the cell. The mitochondria grow abundant, particularly those which are not normal. With the exception of acute toxicosis, no widespread necrosis of the myofilament bundles owing to various experimental pathological conditions have been mentioned in literature. Furthermore, the integrity of the myocardial cell has not been affected as much as that in rheumatic carditis.

In the case studied, the alterations in the mitochondria are not particular. Because the increase or decrease in size, crystolysis, and loss of

granules in the matrix are also noted in the mitochondria of the myocardial cells subjected to experimental overloading. Sarcoplasmic reticulum elements were nearly eliminated in the severely affected myocardial cells. It is possible that the contents of the intracellular cystic formations originate from the tubuli of the transverse system. Although several authors have reported the existence of a developed granular endoplasmic reticulum in normal and hypertrophic myocardial cell, [9,10,14,20] almost no granular endoplasmic reticulum was encountered in the rhuematic myocardial cells during the course of this research.

Lysosomes and lysosome-like granules were investigated in the myocardium of the atrial region. Lysosomal structures usually occur in the vicinities of the nuclei, particularly closely related to the Golgi Complex[13,15]. In this study, the lysosomal structures were noted to be closely interrelated with the lipid granules and mitochondria, both located adjacent to the nuclei and the periphery of the cells. As for the myofilament bundles, the morphological damages starting with the loss of the normal banding pattern of sarcomeres, displaying dense and elongated regions along their courses, were noted to lead to a complete necrosis.

Summary

The pathological alterations in the myocardial cells in a case of chronic rheumatic carditis have been studied at electron microscopy level. It has been attempted to determine the particular morphological aspects of the rheumatic process in the myocardial cells. Features interpreted to be related to rheumatic process in the myocardium may be outlined thus:

Some of the myocardial cells with a rather normal internal structure were separated from each other nearly fragmented with deep infoldinsg of the sarcolemma and basal lamina and had increasing connective tissue contents in between them. The normal banding pattern along the myofilament bundles was lost and dense elongated regions formed. With the progress of pathological process, the myocardial cells were completely disintegrated. Lysis causing organelle-free regions, intracellular cystic formations and a complete necrosis of the myofilament bundles ensued within the cell.

Acknowledgement

We are thankful to Professor A. Yüksel Bozer, M.D., Director of the Department of Cardiovascular Surgery and Specialized Assistan. Yurdakul Yurdakul, M.D. in the same department, for their kindness in supplying us with suitable specimens during open-heart operationst

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Studies in Antihistamines with Schultz-Dale Apparatus

Kemal Özkaragöz, M.D. / Yıldız Saraçlar, M.D.

Antihistamines are effective experimentally in combatting histamine shock in vivo and in vitro. The most probable explanation for the action of antihistamines, is that they compete with histamines for the cellular receptors, and they block the action of histamines. There is a prevention of contraction of the isolated sensitized guinea pig smooth muscle in the Schultz-Dale bath. The minimum antihistamine dose required to neutralize the effect of histamine directly depends on the quantity of free histamine present; above a certain anti-histaminic dose; histamine, whose receivers are entirely blocked, is unable to exert effect. Once the receptor is blocked by acertain amount of antihistamines, the increased amount of histamine cannot produce contraction of the organs.

There is no criteria in literature about the potent power of antihistamines. Generally speaking, the more potent the agents, the more likely they cause drowsiness and sleepiness. This study is done in an attempt to investigate the potency among three main groups of antihistamines. By selecting one compound belonging to each representing group, the minimum amount of antihistamine is investigated to prevent a fixed amount of histamine contraction for a certain period of time.

Materials and Methods

1. Guinea pigs: 100 female virgin short-hair guinea pigs of the same stock, ten to twelve weeks old and weighing 300-350 grams were used in this study. They were all in a non-estrus state. The animals

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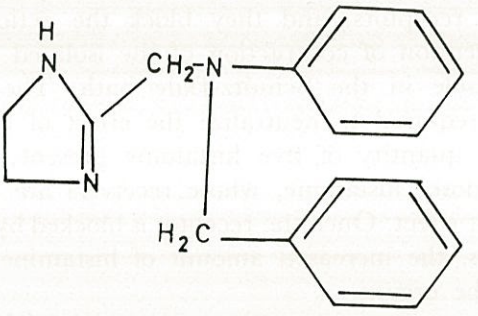
were sacrificed by ether anesthesia. Two pieces of ileum were attached to the transducer and two other pieces stored in separate baths filled with tyrodes solution and easily controlled conditions of 37° C, oxygenation with 95 percent oxygen and five percent carbon dioxide and pH of 7.4. In this bath, the reserved ileum pieces were stored for 2-4 hours without any sign of degeneration.

2. Histamine : A stock solution of histamine hydrochloride merck was made by dissolving 10 mgm in 10 cc of tyrode's solution, and was kept at 4° C. One ml of this stock solution was diluted to 100 ml with tyrode's solution immediately before the tests, to obtoin a concentration of 10γ histamine per ml.

3. Antihistamines:

a) Ethylenediamine group.

An antazoline (Antazolidene) compound is used for this group. (Figure 1)



Ethylenediamine group
(antazoline)

Figure 1

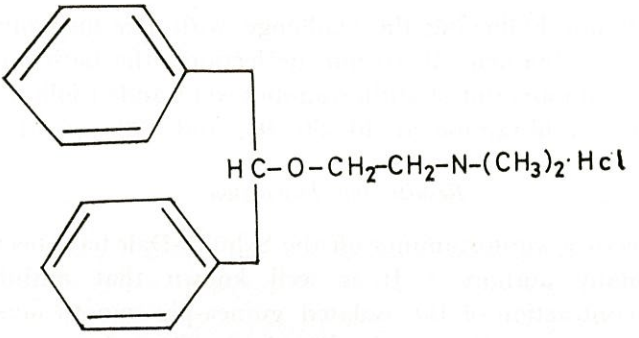
A stock solution of antihistamine was prepared by dissolving 100 mgm in 100 cc of tyrode's solution. One ml of this stock solution was diluted, so that 1 ml could contain 10γ of antihistamine. This dilution was prepared daily, immediately prior to the test.

b) Ethanolamine group, A (Figure 2)

Diphenhydramine compound was used for this group. The dilution was prepared as described above.

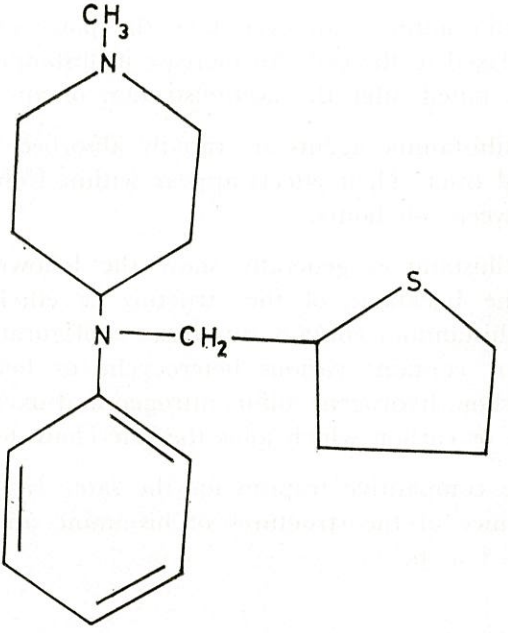
c) Monamine group.

A thenalidine was used for this group. (Figure 3). The dilution was prepared as described above.



Ethanolamine group
(diphenhydramine)

Figure 2



Monamine group
(thenalidine tartrate)

Figure 3

4. Operation of the physiograph: Before beginning each experiment the instrument was calibrated, so that the weight of a particular metal mass (0.68 gr) would cause a deflection of the recording needles

of exactly 30 mm. Following the challenge with 10γ histamine, a full contraction was obtained (40-70 mm deflection), the bath was flushed and the minimum amount of antihistamines were added followed by re-administration of histamine at 30, 60, 90, and 120 second intervals.

Results and Discussion

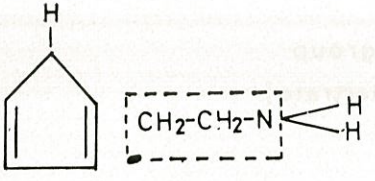
The effects of antihistamines on the Schultz-Dale have been investigated by many authors.[1 2] It is well known that antihistamines prevent the contraction of the isolated guinea-pig smooth muscle produced by histamine in the Schultz-Dale bath. These drugs are effective also in combatting histamine shock in vivo and vitro. Antihistamines compete successfully against histamine for affinity of the affected cells, they do not neutralize histamine in the tissues or in the blood. Some antihistamines also produce anticholinergic (atropine-like) effect and some of them potentialized epinephrine.

Antihistamines can even take the place of an histamine that is already fixed to the cell. An increase in histamine concentration in the blood is noted after the administration of an antihistamine agent.

Antihistamine agents are rapidly absorbed by the skin and gastro-intestinal tract. Their effects appear within 15-60 minutes and usually last between 3-6 hours.

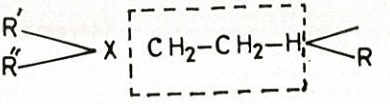
Antihistamines generally show the following structure: R-X-C-C-N. The backbone of the structure is ethylamine. Almost all of the antihistamines contain this basic configuration. R is the nucleus. This part contains various heterocyclic or benzene structure which have carbon, hydrogen, sulfur, nitrogen and oxygen; X may be oxygen, nitrogen or carbon which joins the side chain to the nucleus.

The competitive tropism for the same receptors results from the resemblance of the structures of histamine and the anti-histaminics. (Figures 4 a, b).



Histamine

Figure 4 a



Antihistamine

Figure 4 b

TABLE 1

Trade name	Generic name	Each tablet
Ethylene diamine group	(x = nitrogen)	mg
Antistine	Antazoline	100 mgm
Longifene	Buclizine	25 mgm
Diparalene	Chlorcyclizine	50 mgm
Synopen	Chloropyramine	25 mgm
Allercur	Clemizole	20 mgm
Phenergan	Promethazine	25 mgm
Pyribenzamin	Tripelennamine	25 mgm
Dimitronal	Cinnarizine	
Ethanolamine group	(x = oxygen)	
Benadryl	Diphenhydramine	25 mgm
Dramamine	Dimenhydrinate	80 mgm
Monamine group	(x = carbon)	
Soventol	Bamipine	50 mgm
Chlorotrimeton	Chlorpheniramine	4 mgm
Indical	Mebhydroline	50 mgm
Doxergan	Oxomemazine	10 mgm
Sandostene	Thenalidine	25 mgm
Avil	Pheniramine	25 mgm

Under the X groups, antihistamines can be classified into three groups. The first is ethylenediamine group in which x is nitrogen, second is ethamolamine group in which x is oxygen and third is the monamine group, in which x is carbon. The trade and generic names of some antihistamines in the market are listed in Table 1. Antazoline for ethylenediamine group, diphenhydramine for ethanolamine group and thenalidine for monamine groups have been selected for this study.

Experiment I

Results of quantitative studies with ethylenediamine group antihistamines on excised guinea pig ileum are summarized in Table II.

150 pieces of ileum excised from 40 guinea pigs were used in this experiment. All ileum was first tested with 10γ histamine which gave a complete contraction (40-70 mm deflection). 12 (40 %) of 16 ileum reacted to 10γ histamine 60 seconds after administration of 2.5γ antihistamine. When antihistamine was increased to 7.5γ the time lapse increased to 120 seconds. The amount of antihistamine did not effect the duration of inhibition time after 7.5γ. When the amount was doubled or tripled, there seemed to be no difference. (Figure 5).

TABLE 2

RESULTS OF INHIBITION EFFECT OF ANTIHISTAMINE (ETHYLENEDIAMINE GROUP) ON THE EXCISED ILEUM OF GUINEA PIGS

Guinea pigs		Ethylene diamine (Antazoline)	Challenge dose of Histamine	Interval between antihistamine and Histamine in second			
Number	Number of ileum			30 Sec	60 Sec	90 Sec	120
40	30	2.5 γ	10 γ	–	12(40%)	18(60%)	
	30	5 γ	10 γ	–	10(33%)	14(46.6%)	6(20%)
	30	7.5 γ	10 γ	–	–	–	30
	30	10 γ	10 γ	–	–	–	30
	30	20 γ	10 γ	–	–	–	30

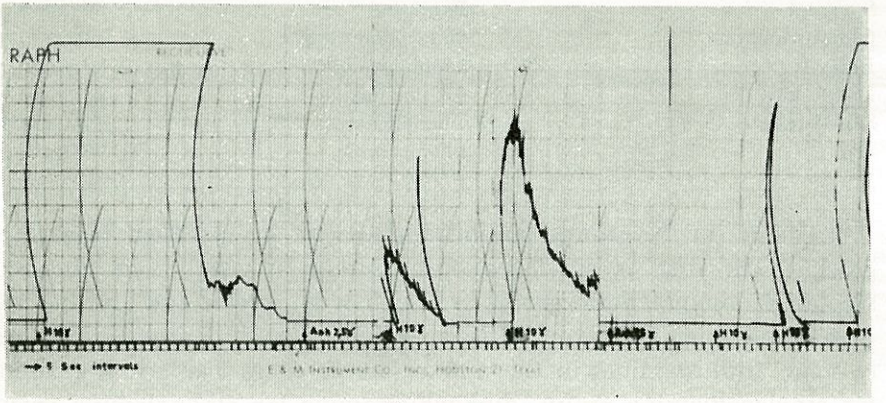


Figure 5

Response of ileum to Histamine (increase of recovery time of ileum with increase in amounts of ethylenediamine group of antihistamine)

Experiments II, III

Similar experiments were repeated with the ethanolamine and monamine groups, as seen in Tables III and IV. The optimum amount of antihistamines, in order to suppress 10γ histamine, the effects are similar.

With ethanolamine group, 5γ antihistamine suppressed the histamine effect, for only 60 seconds. With 7.5γ, 50 % of the ileum did not show contraction until 90 seconds, but when 10γ ethanolamine group antihistamine is added to the bath none of the ileum gave any contraction for 120 second duration. (Figure 6).

TABLE 3

RESULTS OF INHIBITION EFFECT OF ANTIHISTAMINE (ETHANOLAMINE GROUP) ON THE EXCISED ILEUM OF GUINEA PIGS

Guinea pisg		Ethano-lamine (Diphendy-dramine)	Histamine	Interval between antihistamine and histamine administration (in seconds)			
Number	Number of ileum			30 Sec	60 Sec	90 Sec	120 Sec
30	20	5 γ	10 γ	–	–	20	
	20	7.5 γ	10 γ	–	–	10	10
	20	10 γ	10 γ	–	–	–	20
	20	10 γ	10 γ	–	–	–	20

TABLE 4

RESULTS OF INHIBITION EFFECT OF ANTIHISTAMINES (MONAMINE GROUP) ON THE EXCISED ILEUM OF GUINEA PIGS

Guinea pigs		Monamine of group (thenalidine)	histamine 10 γ	Interval between antihistamines and histamine administration (in seconds)			
Number	Number of ileum			30 Sec	60 Sec	90 Sec	120 Sec
30	20	2.5 γ	10 γ	–	5	10	5
	20	5 γ	10 γ	–	–	15	5
	20	10 γ	10 γ	–	–	–	20
	20	10 γ	10 γ	–	–	–	20

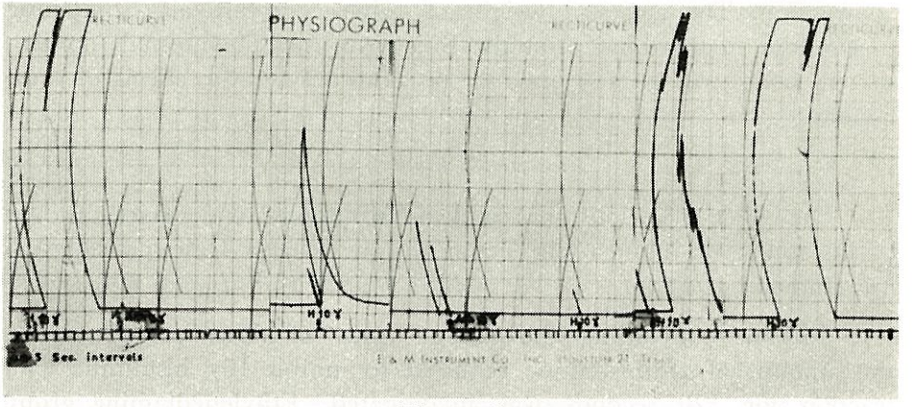


Figure 6

Response of ileum to Histamine (increase of recovery time of ileum with increase in amount of ethanolamine group of antihistamine)

The results with monamine group were very much the same as those with the ethanolamine group. As 5γ monamine antihistamine inhibits 15 (75 percent) of ileum for 90 seconds, 10γ inhibited all the ileum for 120 seconds, and double dose seemed to give similar results. (Figure 7).

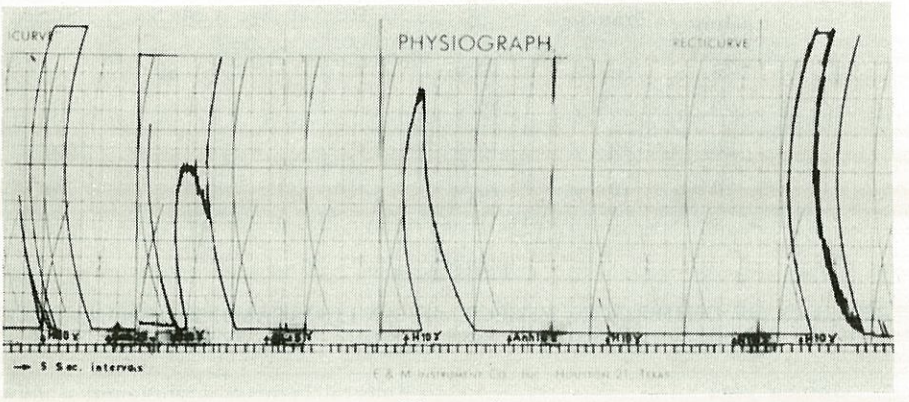


Figure 7

Response of ileum to Histamine (increase of recovery time of ileum with increase in amount of monamine group of antihistamine).

7.5γ of ethylenediamine group, 10γ of ethanolamine and monamine group completely abolished 10γ histamine contraction for 120 second duration in those experiments. The dosage of these antihistamines in clinic use are different. These also proved the different effect of antihistamines in vitro and in vivo. This also partly explained why we accept the 40-70 mm histamine deflection as a complete and full contraction. The age and weight of guinea pigs are also something to consider in this kind of study. Since we used the normal, nonsensitized, non-estrus guinea pigs, we do not consider the effects of these factors.

Conclusion

The effect of various groups of antihistamines to the fixed amount of histamine on guinea pig ileum in the Schultz-Dale physiograph are studied.

Without concluding the potent value of these antihistamines, the amount of antihistamine required to inhibit 10γ histamine contraction for 120 seconds was investigated. Ethylenediamine group antihistamines seemed to be somewhat more powerful than ethanolamine and monamine group.

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Nasopharyngeal Hairy Polyp: Report of a Case

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Nasopharyngeal tumors resulting from a lack of embryonic growth are generally classified in three categories: dermoid cyst, teratoid, and epignathus.[1,2]

Nasopharyngeal tumors existing since birth usually fall into one of the above three categories. They originate from the midline or lateral wall of the nasopharynx, but very rarely occur in autolaryngology.[3,4]

In 1838, in his works concerning anomalies, Geoffrey St. Hilare mentioned epignathus, for the first time in medical literature. However, an extensive analysis on the epignathi was carried out by Ahfeld in 1875.[15,5,7,13] Five years later, the same author reported 40 cases. In 1888 Ahfeld divided the teratomatous tumors into four groups. Articles relating to teratoid tumors of the pharynx were published by Marchand in 1897, Schawalbe in 1907, and Enrich in 1945.[8,9,10,13]

The four groups Ahfeld proposed for the teratomatous were: 1) Dermoid, 2) Teratoid, 3) Teratoma, 4) Epignathi.

Dermoid Tumors : Nasopharyngeal tumors generally belong to this group. These tumors originate from the epidural and mesodermal germinal layers. They are lobulated and have pedicles. They originate from the basisphenoid area and the vicinities of the midline. These are covered with skin and have hair follicles, sweat and sebaceous glands. They therefore are called hairy polyps.[6,16]

Teratoid and Teratomatous Tumors: Both of these groups originate from the three germinal layers. Teratoid tumors are less differentiated than teratomas. Real teratomas are differentiated as a result which also makes a clear histological identification of the

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organs possible. Such tumors are accompanied by palatal fissures, hemicrania, and unencephalie.[12,14]

There is 30% malignancy in the teratoid type. However, no malignancy has ever been established in the nasopharyngeal teratomas.

Epignathi which are trigerminal in origin are generally differentiated and have fetal organs.[14]

In 1940, Ewing reclassified these tumors by placing them into somewhat similar groups to those of Ahfeld. No definite finding has yet been obtained on the etiopathogenesis of these tumors. However, as these tumors originate from the midline, this has been accepted as a finding indicating a lack of fusion.

According to a theory, these tumors originate from ectopic tissue of primitive strike.[14] All of these tumors occur in the vicinities of the Rathke pouche, oral membrane and the anterior of the nothocord.[5] In embryogenesis, it is believed that these tumors develop from the embryonic tissue which is not influenced by the primary organizer (primordial chorda mesoderm).[11]

Furthermore, there are certain authors who accept that the tumor may be caused by heterotopic growth owing to a stationary area during blastula phase, early cell dislocation during formation and abnormal conditions during blastula and gastrula phase.[7,8,9]

Hairy polyp cases are often seen among these tumors with high incidence. The first case was reported in 1784. Kesson published 89 cases in 1953.[15,16] These tumors are seen only in the fetus or the newborn on rare occasions.[5] The ages of patients range from a few days to months. In the literature the age of the second group, following the newborn, is between 7-19 years. It is understood that in these cases the tumor which existed since birth gave no symptoms until the patient reached these ages.[4] Enrich reported a case of about 66 years old.[13] This type of tumor is generally observed in the female newborn. It is not known whether or not familial tendencies have any relation to these tumors.[4]

These tumors cause acute respiratory diseases, respiratory distress and some disturbances in feeding of the newborn. In some cases tracheostomy and gastrostomy may become necessary.[3,9] These tumors occurring in the nasopharyngeal area of the newborn should be differentiated from polyps, chondromas, glioms, dermoid cysts, neurofibromas, chordomas, sphenopharyngeal cephaloceles and mixed tumors of salivary glands.[3] Radiological examination provides no definite results (Figures 1 and 2). The usual therapy is the excision of the mass. A definite diagnosis

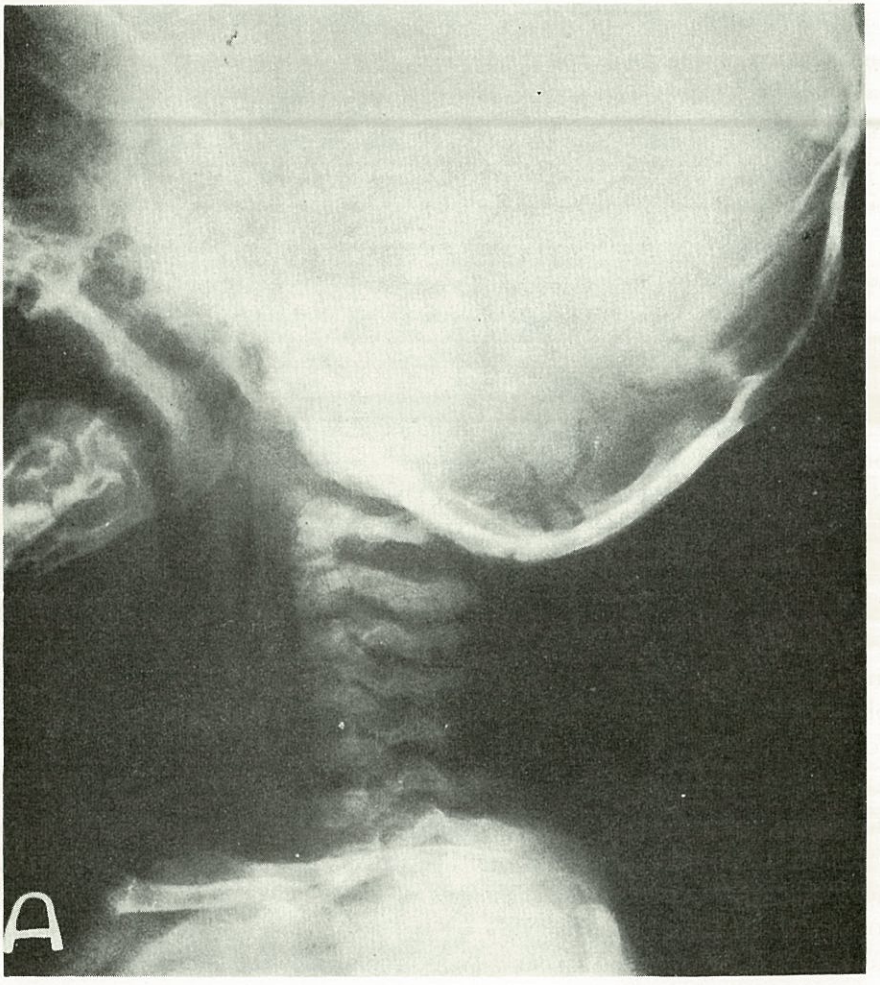

Figure 1
Lateral cervical graphy prior to the operation. x1/3

is made with the help of a histologic examination. The histologic section of the hairy polyps are composed of skin and its adnexa, connective tissue and cartilage. Bone, muscle, respiratory epithelia and neural elements are rarely encountered.[3,6] These tumors have not any potentiality of malignant degeneration.[4,5]

Case Report : E.Y., 3 month old female baby (protocol no. 45442) admitted to hospital on August 16, 1968 with complaints of snarling while breathing heavily, coughing cyanosis during suckling and a mass protruding down the throat.

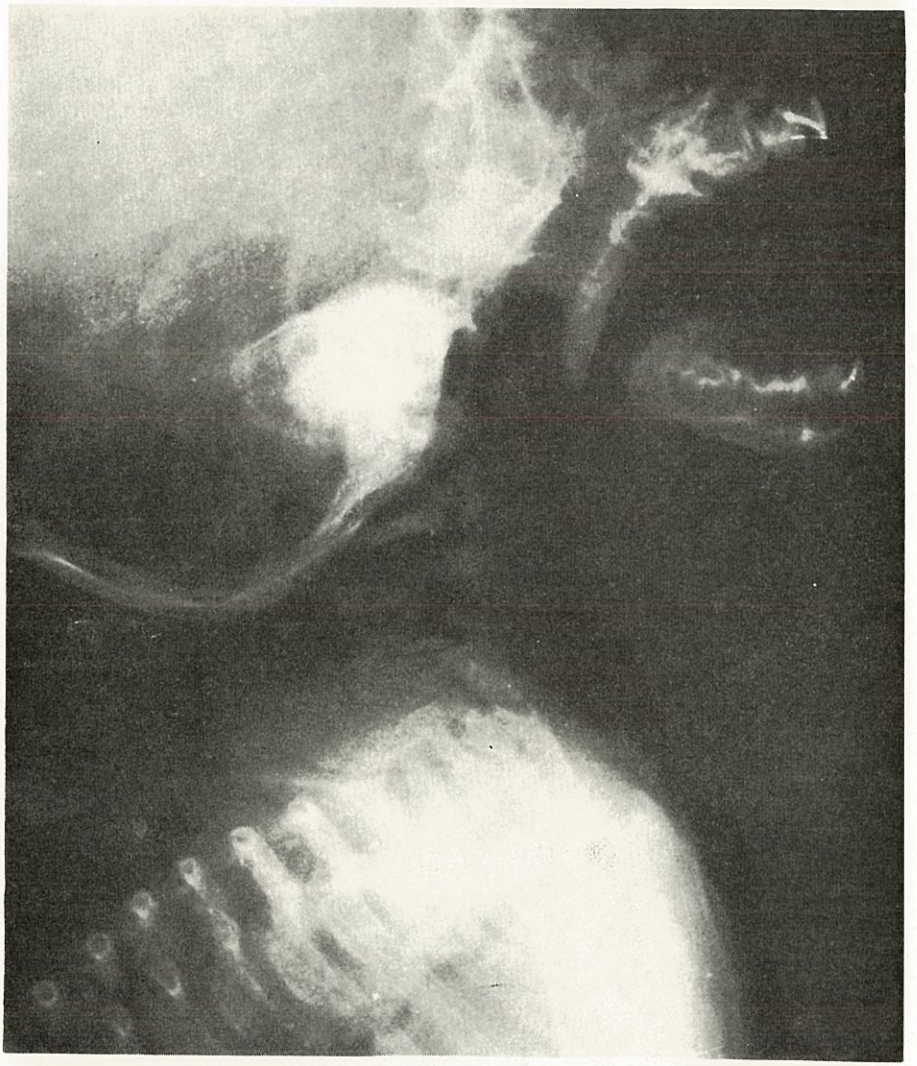

Figure 2

Lateral cervical graphy after the operation. x 1/3

It was learned from her anamnesis that she had been suffering from snarling during breathing since birth. Previously She was taken to a hospital and her ailment was diagnosed as bronchitis and was treated accordingly. The patient, havever, continued to suffer from cyanosis, particularly while coughing and suckling and a mass protruding toward her tongue was observed by her mother while coughing and vomiting. She was taken to an autolaryngologist who referred the patient to our hospital.

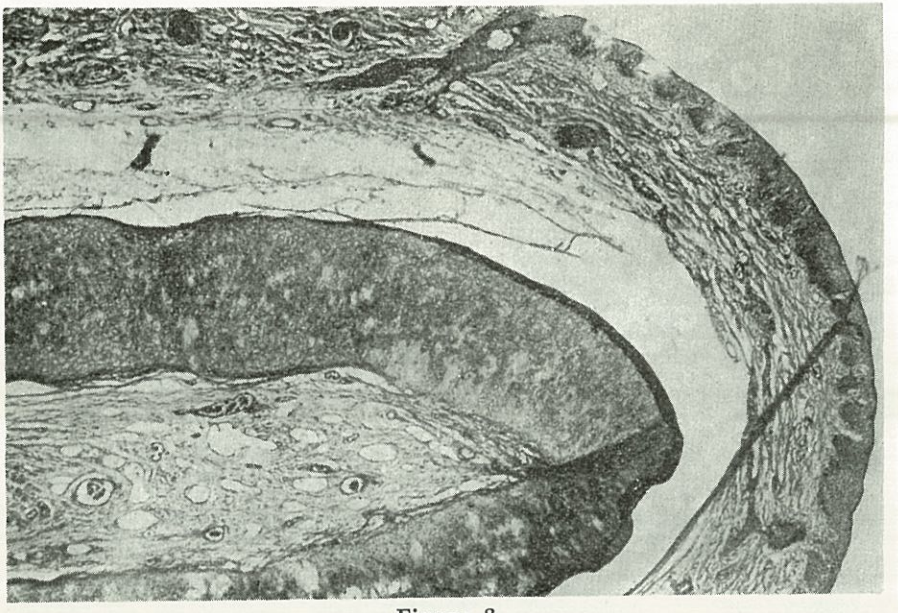

Figure 3

The cartilage and connective tissues together with the hair follicles are seen in the figure above. x 30

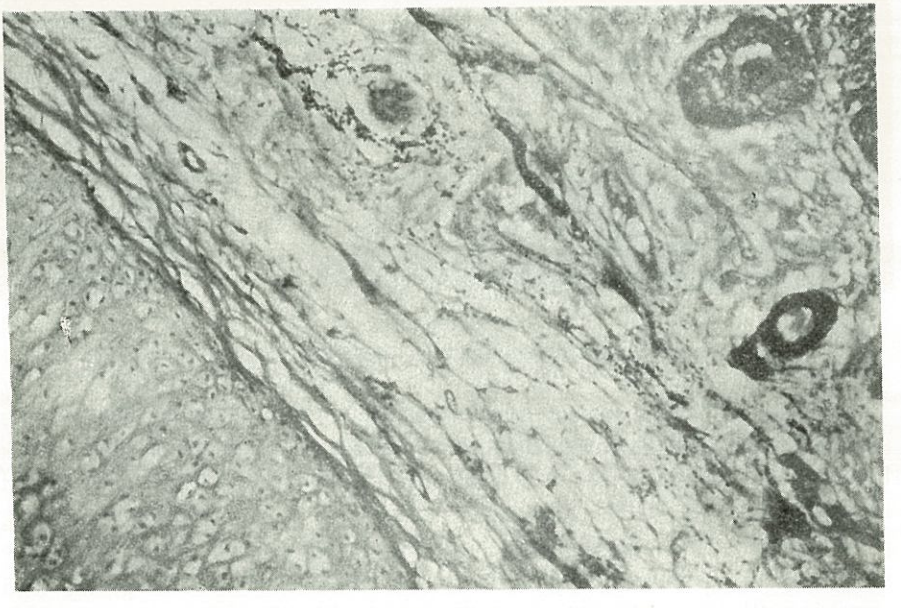

Figure 4

High magnification of the same section. x 75

Physical Examination : Active, conscious, weight: 4,338 gms., height: 62 cms. The patient was observed to be occasionally suffering from cyanosis. Transmittants ralles were noted in her lungs. In the autolaryngological examination, the ears and nose were found normal. In the examination of the throat, a mobile and smooth-surfaced mass was identified protruding from the nasopharynx to the oropharynx. The mass was hard by palpation and light pink in color.

Laboratory Findings : Hb 10% gr., leucocyte 6,200 mm^3, urinal findings: in normal ranges. The chest graphy indicated normal results. In the lateral graphy of the neck, a 3.5 cm. soft tissue mass was established, protruding from the palatum molle towards the oropharynx and partly obstructing the breathing passage. The patient was taken into the operating room on August 16. She was entubated and her mouth was opened. Following the identification, the above-mentioned mass was completely extirpated. Bleeding was checked and controlled and the operation completed.

The specimen extirpated was 0.5 x 2 x 3.5 cm. in size and showed elastic consistency of tissue with a fine granular structure, and a color of pinkish-gray showing occasional changes to brownish pink. The surface of the section was pinkish-gray and a large area which was hard, whitish, and pearl-like was observed in the middle.

Microscopical Examination : It was observed that polypoid tissue was covered with epidermis and dermis containing skin adnexa similar to those of the sweat glands and hair follicles. Cartilage was noted in the center of the tissue (Figures 3 and 4). The case was diagnosed to be nasopharyngeal epignathi (Biopsy number: 3384-68). Following the operation, the patient recovered completely. The lateral cervical graphy (Figure 2) indicated no remnants of the mass and the breathing passage seemed to be free of obstacles. The patient was requested to report for periodic medical control and was discharged on August 23. The medical examination carried out a year after the operation showed that the patient was completely normal.

Summary

A hairy polyp was diagnosed in a three month old female infant. Since the incidence of such tumors is very rare, we decided to report the case. The pathogenesis, clinical and pathological aspects of the epignathies were considered and the literature was reviewed in connection with it.

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Allergy to Laboratory Animals among Laboratory Workers: Report of Acute Anaphylactic Reaction to Guinea Pig Dander, with a Review of the Literature*

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Although most textbooks of allergy[1,2,3] cite the fact that sensitivity to various laboratory animals is relatively common. Tufts[2] states that sensitivity to mouse and rat dander is exceedingly rare. A careful search of the literature revealed few cases of sensitivity to rat, guinea-pig or mouse dander and or serum. Lund and Hunt[4] state that sensitivity to guinea pig protein is not common. We believe that the scarcity of reported cases is probably not the true picture of incidence of this type of sensitivity which, in some instances, is hazardous and disabling for research or laboratory workers.

Recently, we reported a case of anaphylactic reaction due to a rat bite in a laboratory technician, with resultant coagulation defects.[5] We have now observed an acute anaphylactic reaction to guinea pig dander

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in another technician, although coagulation changes could not be found. Our interest was stimulated to correlate data from similar cases available in the literature, to alert research and laboratory workers to this hazard and to report the symptoms and physical findings exhibited by this technician.

Case Report

A 25 year old female laboratory technician was seen in an acute anaphylactic reaction precipitated by guinea pig dander which she accidentally rubbed into her left eye. Her past history revealed that for five years she had marked eczema of the eyelids due to eye make-up. She was highly sensitive to poison ivy and for several years had suffered from perennial allergic rhinitis. Although a sister had asthma and urticaria occasionally, there was no other family history of allergy.

For three weeks before the accident, when working with guinea pigs, she noted increased nasal stuffiness and discharge with watering of the eyes. She complained of dryness of the throat, itching and tearing of the eyes and nasal blockage.

The day of the episode she had assisted in clipping several guinea pigs for passive cutaneous anaphylaxis (P.C.A.) testing. By the time she came to the last guinea pig which she held, she was complaining of stuffiness of her nose, itching of her eyelids and dryness of her throat. As she prepared to leave the laboratory she rubbed her left eye and instantaneously became weak and dizzy, her vision blurred and the left eye became massively swollen.

Upon examination, there was marked respiratory distress, she complained of burning and itching of the entire face, especially of the left eye. Breathing was rapid and gasping; pulse, when first checked, was 82 and rapidly rose to 140, becoming weak and thready. An erythematous rash appeared on the sides of her neck, which soon spread to the chin and became urticarial. Hair on the neck raised to an erect position. The left eyelids turned out completely due to massive edema of periorbital tissue. There was marked redness of the conjunctiva. Blood pressure was not taken at the time. The technician was given 5 minims of 1/1,000 aqueous epinephrine intramuscularly within two minutes after reaction began.

Laboratory Findings: Blood studies showed 10,600 white blood cells per cubic millimeter, with a differential count of 56 per cent polymorphonuclear leukocytes, 1 per cent band form polymorphonuclear leukocytes and 43 per cent lymphocytes; 3,570,000 red blood cells

per cubic millimeter, with hematocrit of 35 per cent; and platelet count of 456,000 per cubic millimeter of blood. Coagulation time was two minutes by capillary tube method; bleeding time was one minute. Prothrombin time, prothrombin consumption, Rumpel-Leede test for capillary fragility and fibrinogen levels using Fibrindex method were normal. Clot retraction was good in 24 hours. Fibrinolytic studies and proteolytic studies showed no change in activity of these particular enzyme systems.

Course of Illness: Blood pressure, taken five minutes after injection of epinephrine, was 100 systolic and 60 diastolic. Dyspnea, skin rash, erection of hair on nape of neck and swelling of the eyelids responded rapidly. She was kept resting in bed for 18 hours and was given Triaminic tablets every six hours.

Following complete recovery direct intradermal skin tests showed 2+reaction to $1:1x10^6$ concentration of guinea pig dander and immediate flare and large wheal with pseudopods to $1:1x10^5$.

Subsequent to the technician's severe reaction, all contact with guinea pigs was eliminated. Three months later, she still sneezed and her eyes watered when she entered a room where, two hours before, guinea pigs had been under study.

Discussion

Only five cases of sensitivity to mice, rats, or guinea pigs could be found in a careful review on the literature (see Table 1). Two of these were research or laboratory workers; one was a hospital worker who had received guinea pig blood intracutaneously, instigating acute anaphylactic reaction which resulted in death despite heroic efforts to save her. Three cases showed hay fever symptoms when exposed to a particular animal dander. Three cases had local urticaria when the animal was handled and one suffered generalized itching of the skin for three months. One developed asthma upon contact with animal dander. Our previously reported case of rat bite in a laboratory worker is the only one which showed coagulation defects associated with the anaphylactic reactions. Three cases were sensitive by skin testing to the specific animal dander; a fourth was sensitive to the specific animal dander as demonstrated by provocation and the fifth showed sensitivity to guinea pig serum by Prausnits-Kustner reaction. Either direct and/or indirect (Prausnits-Kustner) skin tests were carried out in all but one case, in which provocation of symptoms was instituted by using the suspected animal. Epinephrine was used for the treatment of acute reactions. Avoidance

TABLE 1

Author	Age, Sex Race	Occupation	Prior Allergic Symptoms	Type of Material	Reaction	Tested Used	Treatment
Lintz, W. 1923	36 Female	Housewife	Severe asthma for 7 yrs. Pruritus from Dec. to Mar. Yrly.	Mouse dander	Severe asthma	Provocative test using "little white mouse in box" +	Avoidance measures. Extermination of Rodents.
Romanoff, A. 1940	27 Female	Laboratory Technician	Rhinitis, urticaria when handling rats 1 yr.	Rat dander	Same as prior Allergy Symptoms	2+ 1 C reaction to ext. of rat dander PK testing neg. in recipients	Avoidance measures
Lund, H., Hunt, E. L. 1941	21 Female White	Hospital worker	Asthma for 8 yrs. No known contact with guinea pigs	0.2 cc of g. p. blood I. C.	Acute anaphylaxis with death	Positive passive transfer test (PK) to guinea pig blood	Epinephrine supportive measures
Sorrell, A. et al. 1957	22 Female	Research worker	Rhinitis, urticaria while working with mice 3 months	C, 58 Mouse dander	Same–as prior allergic symptoms	Scratch tests 4+ to C-58 dander. Stock mouse extract +. Direct I. C. tests with C-58 extract 1/1000, 1/1000 +, 1/10 4+. PK with C-58 ext conc. 4+	Hyposensitization resulting in no rhinitis but urticaria on contact with mice
Özkaragöz, K. et al. 1970	24 Female White	Research worker	Penicillin "serum sickness" 8 years ago. Hay fever 6 years-Rhinitis and local urticaria if handles rats 15 months	Rat dander	**Prolonged** shock symptoms with increased coag. time	Rat extract. Scratch 4+PK-2+ 1 : 1x104 conc. of rat dander extract 4+ 1 : 1x104 Direct I. C 3+ 1 : 1x 106 4+ 1 : 1x105	Strict avoidance measures

measures were instituted in three cases and one case received hyposensitization with the specific mouse dander.

The safety of instillation of a specific antigen into the conjunctival sac of a sensitive person already under heavy exposure to the antigen by other routes can certainly be questioned. Under these conditions it is a hazardous procedure, as has been demonstrated. We have found one case of extreme sensitivity to rats and one to guinea pigs in laboratory workers within a nine-month period. When it is considered that both cases were limited to one isolated area and both occurred during the limited period of nine months, laboratory-animal hypersensitivity, though possibly not widespread, is, at least more common than the evidence in the literature. It is pertinent, we feel, to alert research workers to the possibility of a severe and acute reaction to the dander of laboratory animals, with the suggestion that minimal testing be instituted as a reasonable precautionary measure. More important, the reaction can be such that immediate administration of epinephrine is paramount for survival. A ready source of epinephrine and suitable syringes should be maintained by laboratories using experimental animals.

Summary

A case of anaphylactic reaction to guinea pig dander proved, by skin tests, in a laboratory worker is reported. Immediate administration of epinephrine may be lifesaving in such a reaction. A review of the literature showed only five reported cases of any type of sensitivity to rat, mouse or guinea pig dander and/or serum. Sensitivity reaction in laboratory workers to animals on which they conduct studies probably is more common than is evidenced in the literature.

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CONTENTS

Observations

The rheumatic myocardial cells, the ultrastructure of which had been studied in the first part of this work, have been found in a rheumatic intermyocardial connective tissue that has undergone a series of characteristic alterations, particularly through its cells and intercellular substance. The intermyocardial connective tissue has been observed to increase. In all the areas examined, it was noted that the spaces in between the myocardial cells expanded more than normal and were filled with a connective tissue very rich in capillaries. In many places the connective tissue replaced the myocardial cells which had been destroyed. The intermyocardial connective tissue displayed the characteristics of a loose connective tissue which was highly active in producing new cells and intercellular substance. The capillaries increased in number prominently. They were large in diameters and seemed as if to surround the myocardial cells all the way round. The myocardial cells appeared as though embedded in a capillary mesh. (Figures 1, 2). Besides the pericytes or adventitial cells investing the capillary, endothelium were frequently observed to detach from the capillary wall so as to form new indifferentiated cells as seen in the intermyocardium (Figures 1, 2 and 3). The capillaries were closely invested by bundles of very fine collagen fibrils. (Figure 4).

In the intermyocardium, advanced structural disorders were encountered in the connective tissue alongside the normal areas. The intermyocardial cells, the phagocytic macrophages, indifferentiated cells together with the ruins of myocardial cells were noted as large masses. The cells making up such masses displayed structural disorders of varying degrees (Figure 5).

The active collagenogenesis occurred in the intermyocardial connective tissue. Active fibroblasts producing collagen were found to be frequent. Those fibroblasts were located at the end of the newly-formed large bundles of collagen fibrils (Figure 6a and b). Our attention was also drawn to the fact that the newly-formed bundles of collagen fibrils were frequently encountered adjacent to the pericytes already detached from the capillary walls. However, the detached pericytes adjacent to the bundles of collagen fibrils did not exhibit the structural characteristics of active fibroblasts producing collagen (Figure 7). They had the features of indifferentiated cells. The new bundles of collagen fibrils were thick, sometimes displaying a multilayered structural order when arranged in different angles (Figure 8). The young fibroblasts which had not yet begun to produce collagen were encoun-

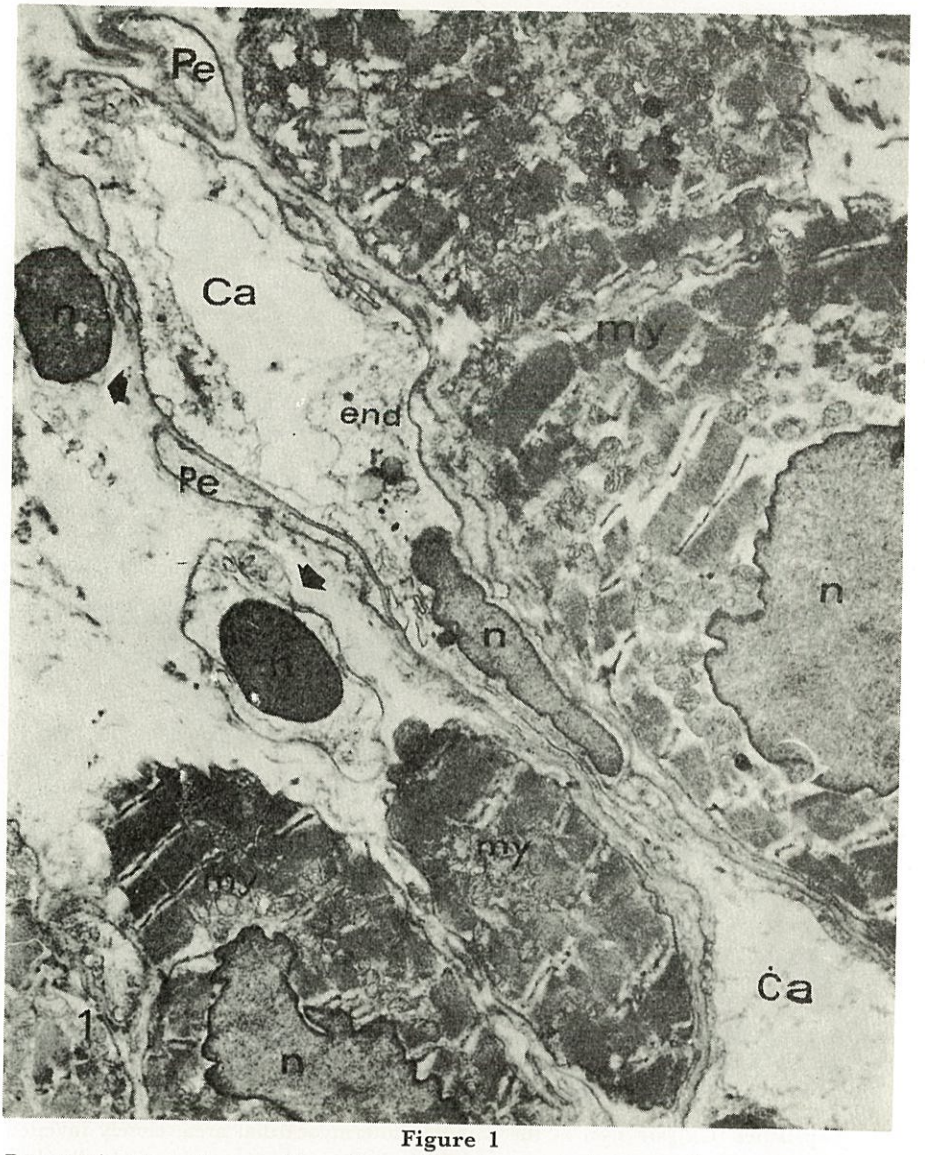

Figure 1

Panoramic view of mycardium. A capillary is seen in between the myocardial cells (my) encircled by pericytes (Pe). Two pericytes (arrows) are detached from the capillary wall forming new indifferentiated cells in the intermyocardial connective tissue. (Ca), capillary; (end), capillary endothelium; (n), nucleus. Osmium-araldite-uranyl-lead. x 6.600.

tered in the intermyocardial connective tissue. These fibroblasts the cytoplasms of which were very poor in organelles looked like indifferentiated cells but were identified by their tubuli of endoplasmic reticula

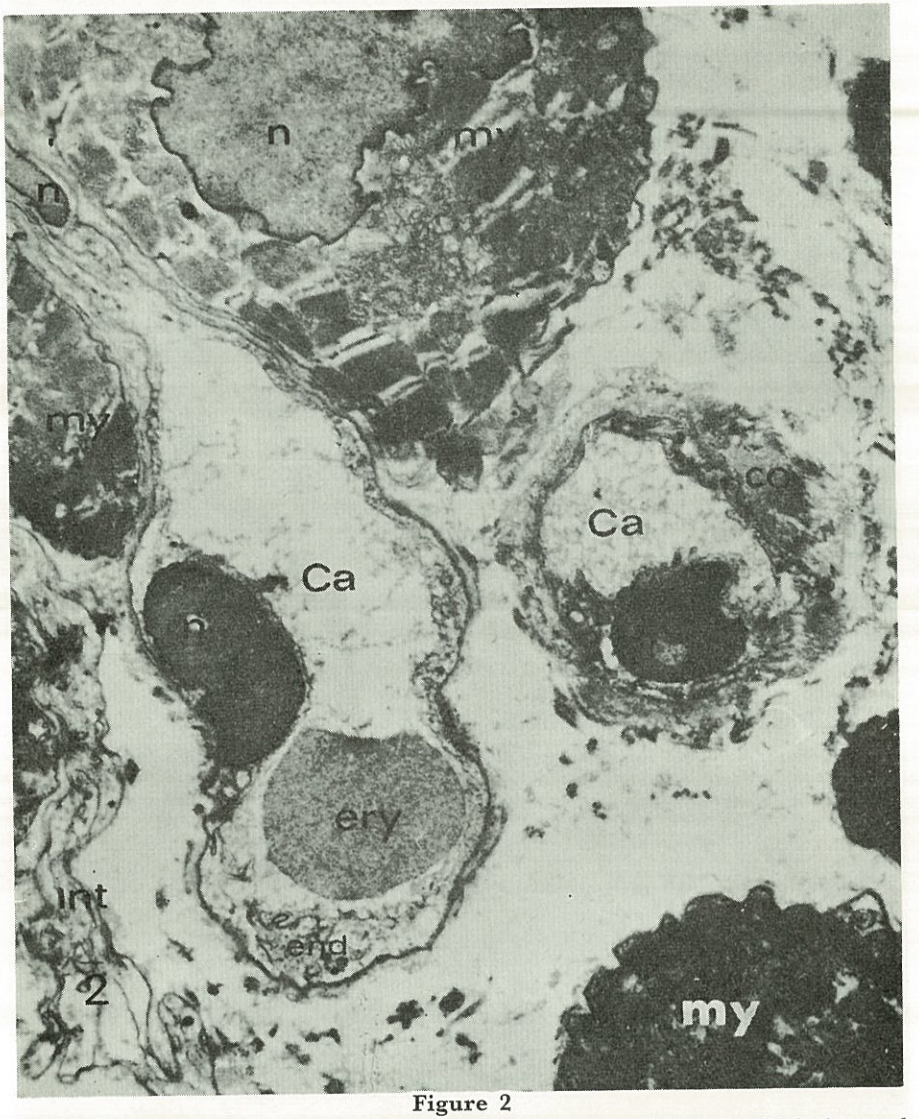

Figure 2

Two capillaries (Ca) are seen in the enlarged intermyocardial area, closely invested with the collagen fibrils (Co). At lower-left, extensions of intermyocardial cells (Int) are detected. (My), myocardial cell; (n), nucleus; (ery), erythrocyte. Osmium-araldite-uranyl-lead. x 6.600.

located between the ecto-and-endoplasmic regions. In the highly active fibroblasts, the peripherial barriers of endoplasmic reticula were seen to disappear. The nuclei and endoplasms of these cells seemed embedded in the masses of collagen fibrils which they had produced themselves (Figure 9 a, b). When examined in further detail, the collagen

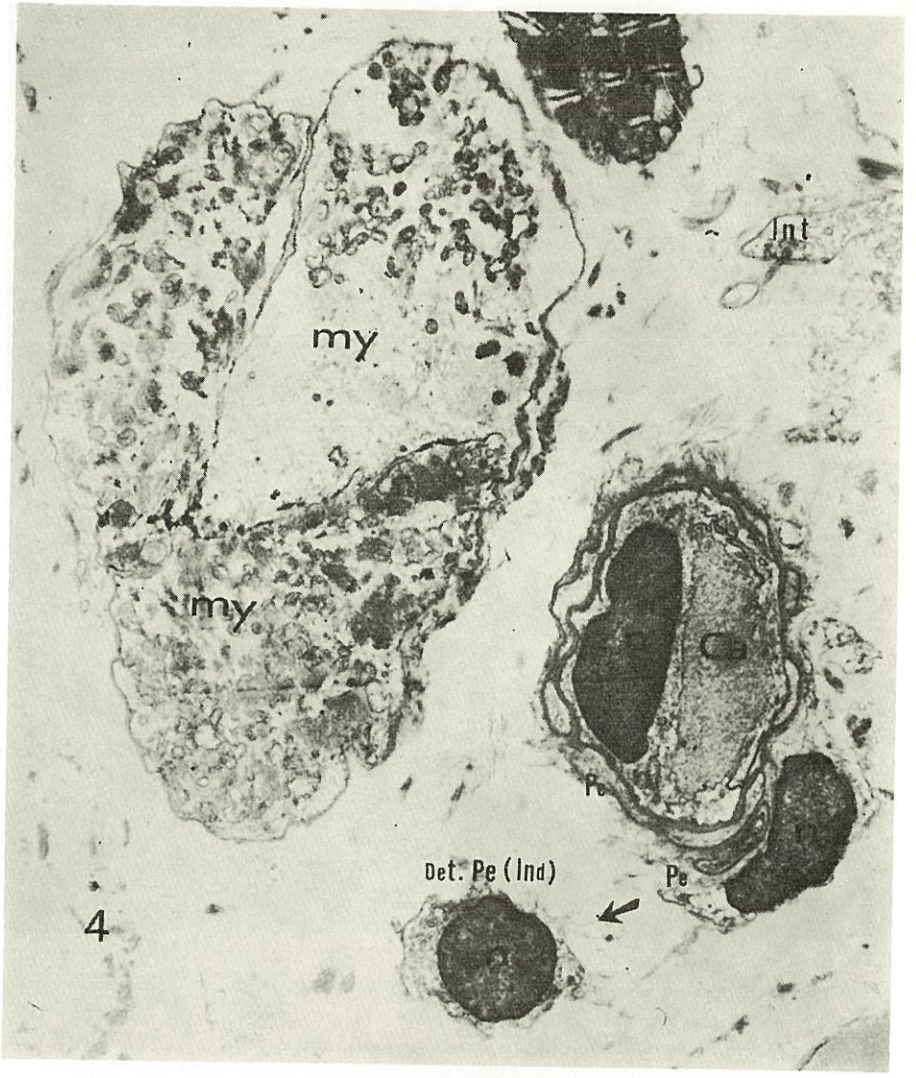

Figure 3

A capillary (Ca) with its detaching pericytes (Pe) is observed towards the myocardial cells (my). Note that one pericyte is fully detached forming an indifferentiated cell [Det. Pe (Ind)] and the other is still going to be detached from the capillary wall. Collagenous fibers investing the capillary wall are detectable. (Int), intermyocardial cell; (n), nucleus. Osmium-araldite-uranyl-lead. x 6.600.

units seemed to sprout out from the ectoplasm towards the exterior of the cells. In these areas, the cell membranes completely disappeared (Figure 10 a, b). The newly-formed collagen was found to be normal in its structural order.

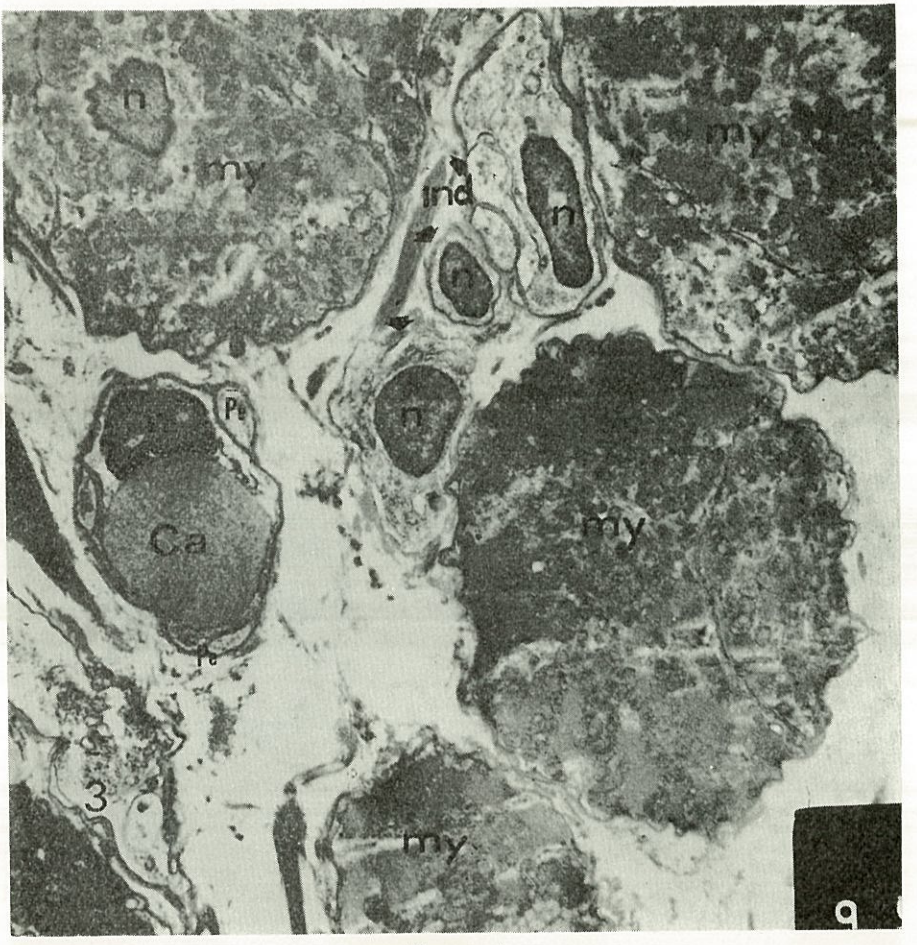

Figure 4

Indifferentiated connective tissue cells (Ind) are noted towards the capillary (Ca) in between the myocardial cells (my), possibly originated from the pericytes (Pe) encircling the capillary endothelium. (n), nucleus. Osmium-araldite-uranyl-lead. x 6.600.

The intermyocardial cells were encountered both in between the myocardial cells and the intermyocardial connective tissue. They were extremely large and irregular in contours, with long and thick extensions embedded in intercellular substance. Cells having more than one nucleus were also frequent. The nuclear contours were either smooth or indented. A system of internal tubular labyrinth existed within the cell. The intracellular tubuli enlarged in cisternal forms, occasionally forming compartments in the cellular body. There were numerous micropinocytotic vesicles in the cytoplasm and on the walls of intracellular tubuli (Figures 11, 12, and 13). Other organelles in the

Figure 5

A mass of different degenerated cells and cellular remnants in the intermyocardial area is shown, limited by the arrows. Ruins of myocardial cells (r. my) are loosely invested by the extentions of intermyocardial cells (Int), indifferentiated cells (Ind) and closely packed phagocytic cells (phag) degenerated in morphological sense. (my), myocardial cell. Osmium-araldite-uranyl-lead. x 6.600.

cytoplasm were not abundant. Lysosomes could be found. There were few mitochondria. Every intermyocardial cell together with its extensions was invested by a continuous basal lamina and a thin layer of bundles of collagen fibrils.

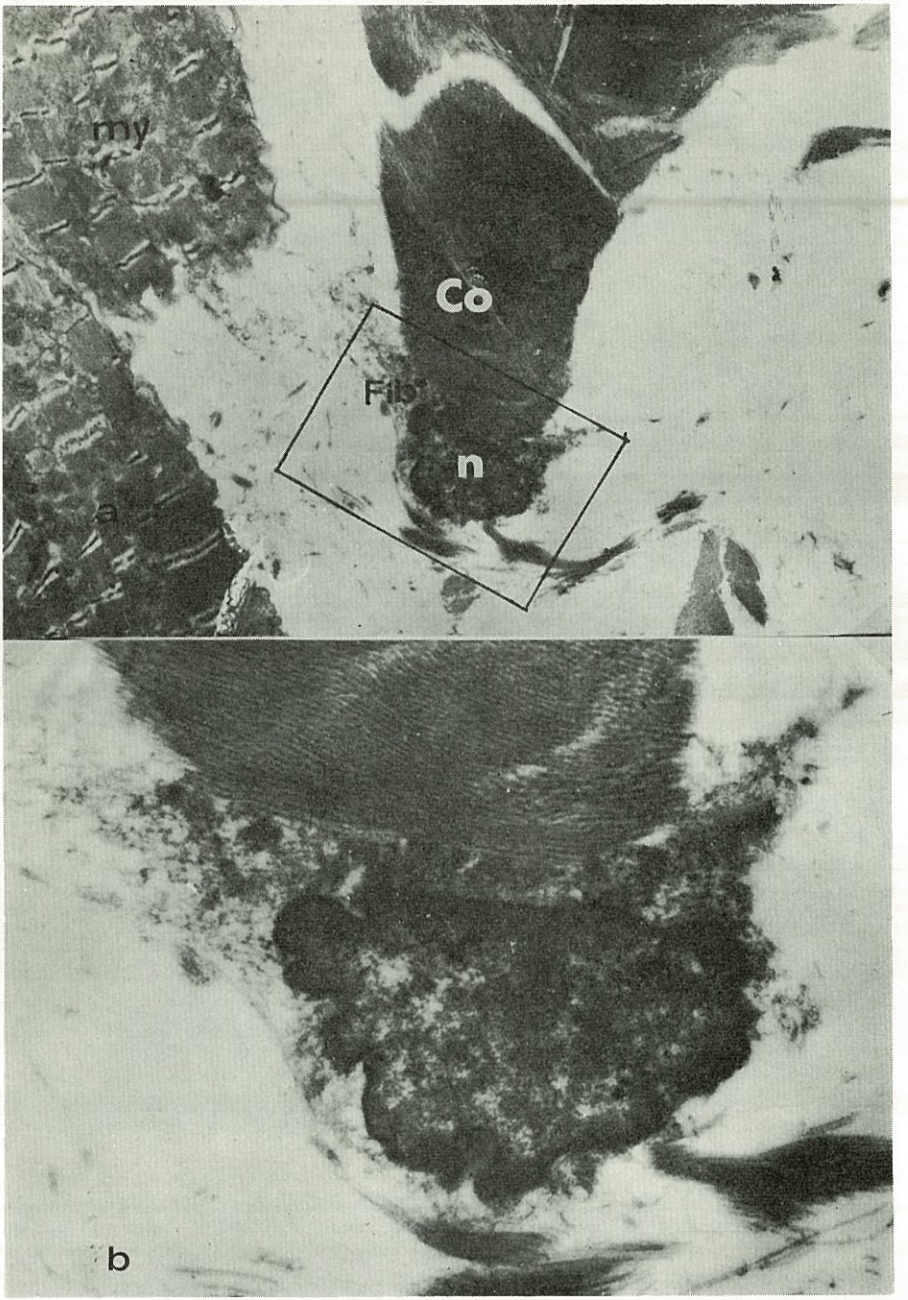

Figure 6 a and b

Large masses of newly formed bundles of collagen fibrils (Co) are seen in the enlarged intermyocardial area. In 6a, a collagen producing fibroblast (Fib) is detected at the lower end of the collagenous mass (inset). 6b shows the detailed structure of active fibroblast, inset in 6a. The cell membrane is completely discontinuous and collagen fibrils are secreted from the ectoplasm of the cell so as to be packed in the extra cellular space. Osmium-araldite-uranyl-lead. 7a x 6.600, 7b 24.500.

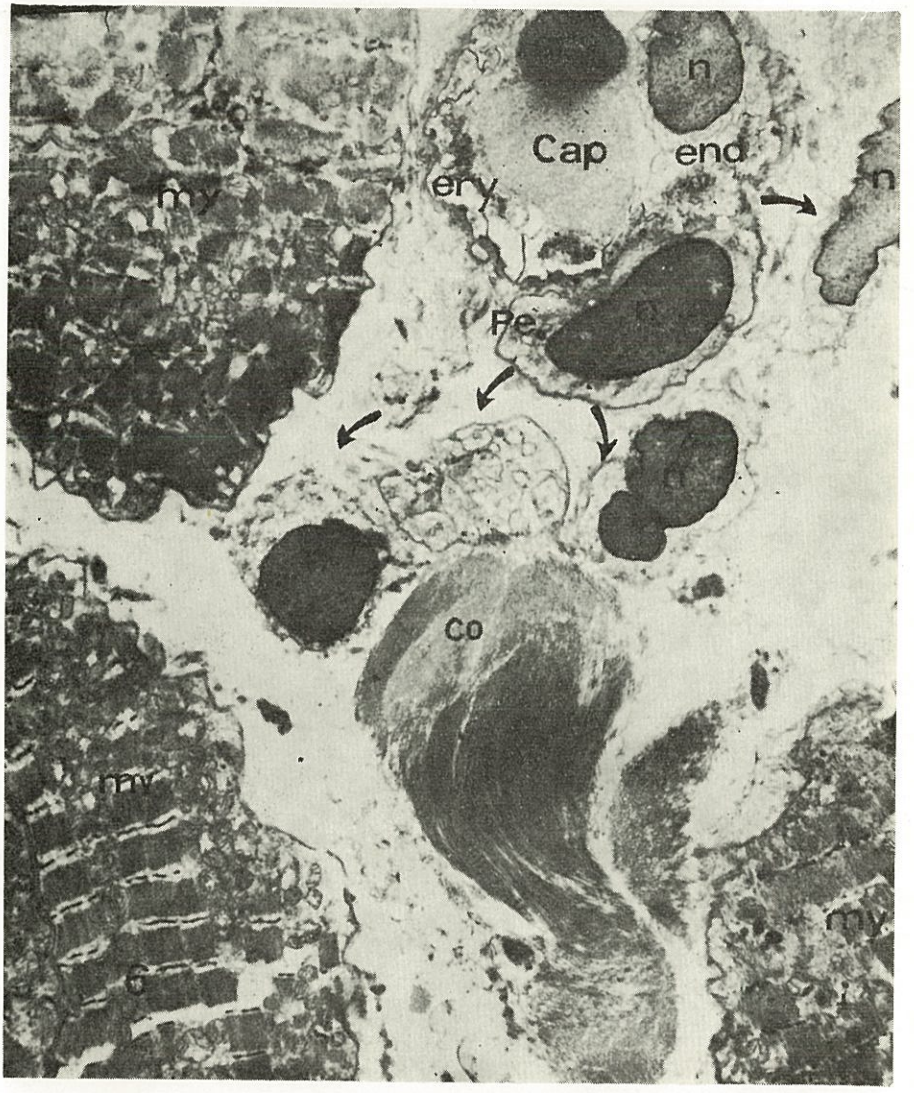

Figure 7

Another enlarged intermyocardial area containing a capillary (Cap) and detached pericytes (Pe) adjacent to it. Indifferentiated cells originating from the detachment of the capillary pericytes are indicated by the arrows. In addition, thick bundles of collagen fibrils (Co) are detected in the lower half of the micrograph close to the detached cells. (my), myocardial cell; (n), nucleus; ery, capillary endothelium; end, erythrocyte. Osmium-araldite-uranyl-lead. x 6.600.

The intermyocardial cells were usually embedded in fine bundles of collagen fibrils. Some of them were found to be phagocytic. In their cytoplasms erythrocytes were often swallowed. (Figure 14a, b).

Figure 8

Newly-formed bundles of collagen fibrils having somewhat regular arrangement in the intermyocardium, are observed. Note that transections of the collagen fibrils taking place in between the longitudinal bundles indicating the different bundles to be perpendicular to each other in their arrangements. Osmium-araldite-uranyl-lead. x 24.500.

Numerous extensions of the intermyocardial cells were observed to be embedded in the intercellular substance. In areas where the structure had not been highly damaged, a net formed by the normal periodic collagen was observed in the amorph ground substance. As for the places with advanced degeneration in the intermyocardium, the

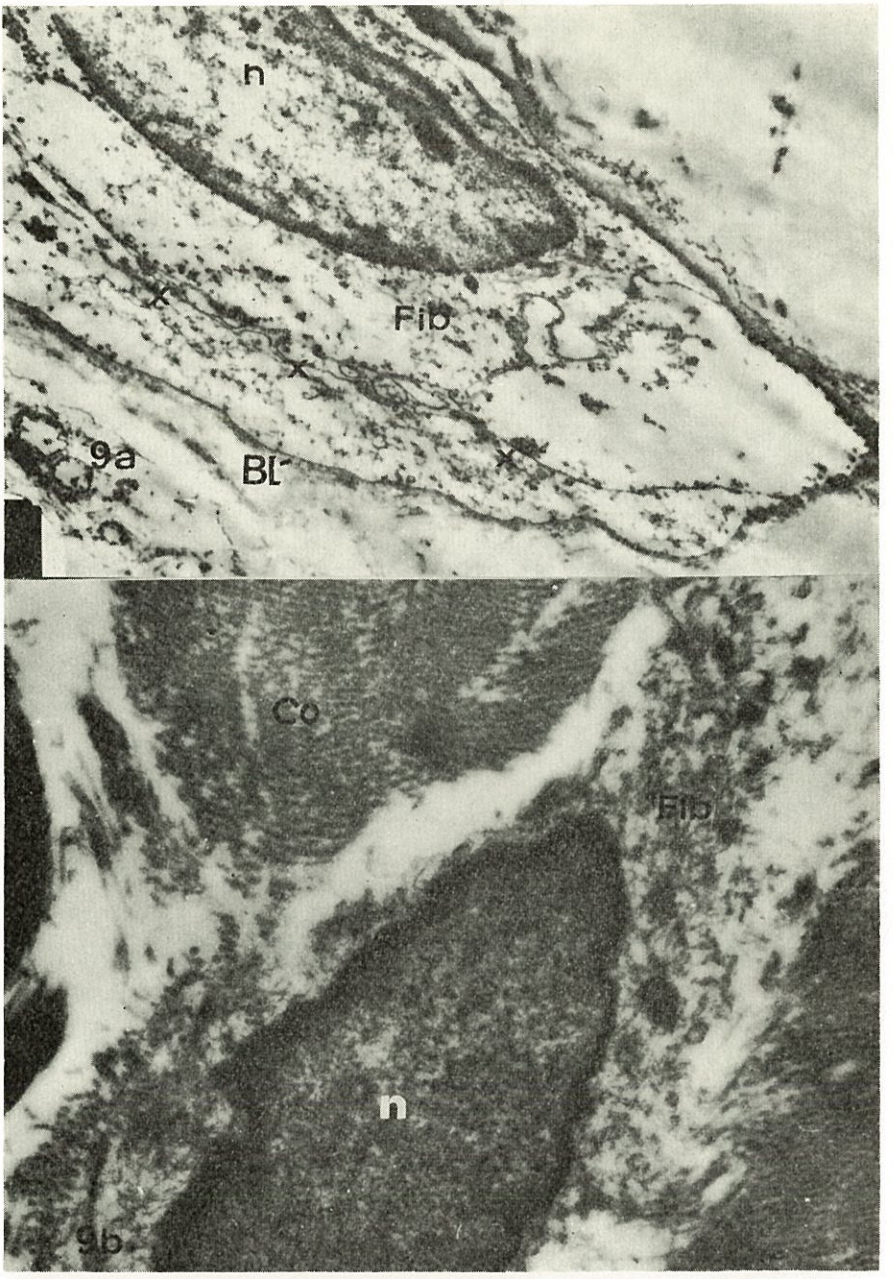

Figure 9

Inactive (a) and active (b) fibroblasts (Fib) are observed. The cell boundry of the inactive fibroblast is clear and invested with a continuous basal lamina (Bl). The typical localization of tubuli of the endoplasmic reticulum (x) is detected between endo- and ectoplasmic zones. The tubuli do not still contain ribosomes. In the active fibroblast the cell membrane is completely discontinuous. Collagen units seem to originate from the ectoplasm to extend and be packed in the extracellular space. (n), nucleus. Osmium-araldite-uranyl-lead. 9a x 24.500, 9b x 36.000.

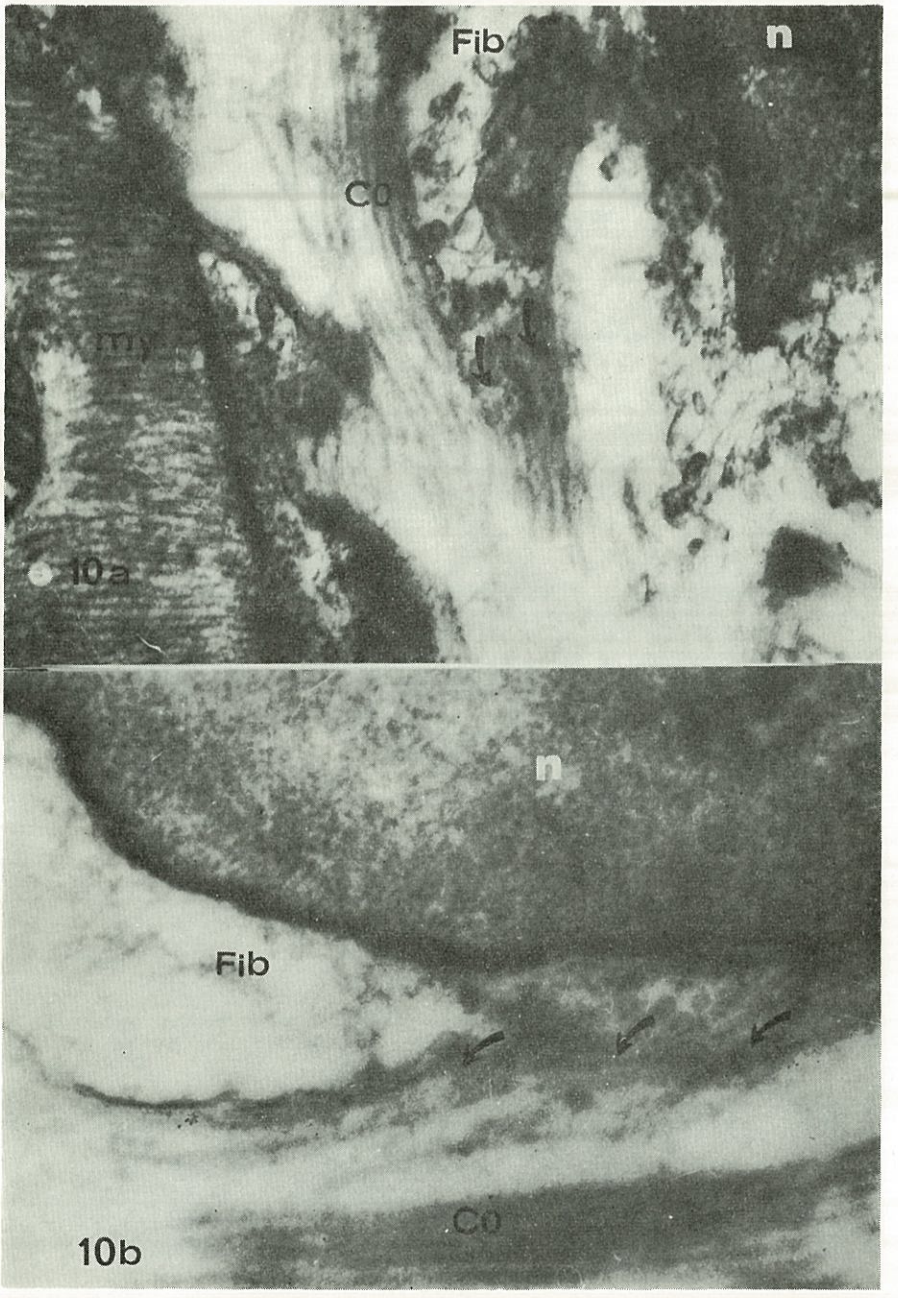

Figure 10

Detailed ultrastructures of two active fibroblasts (Fib) are observed. One (a) is closely located to the myocardial cell (my) and the other (b) is in the intermyocardial connective tissue. Both of the cells have partially discontinued plasma membranes and secrete (arrows) collagen units (Co) through the ectoplasms which are thrown forward toward the exterior of the cells. (n), Nucleus. Osmium-araldite-uranyl-lead. a and b x 72.000.

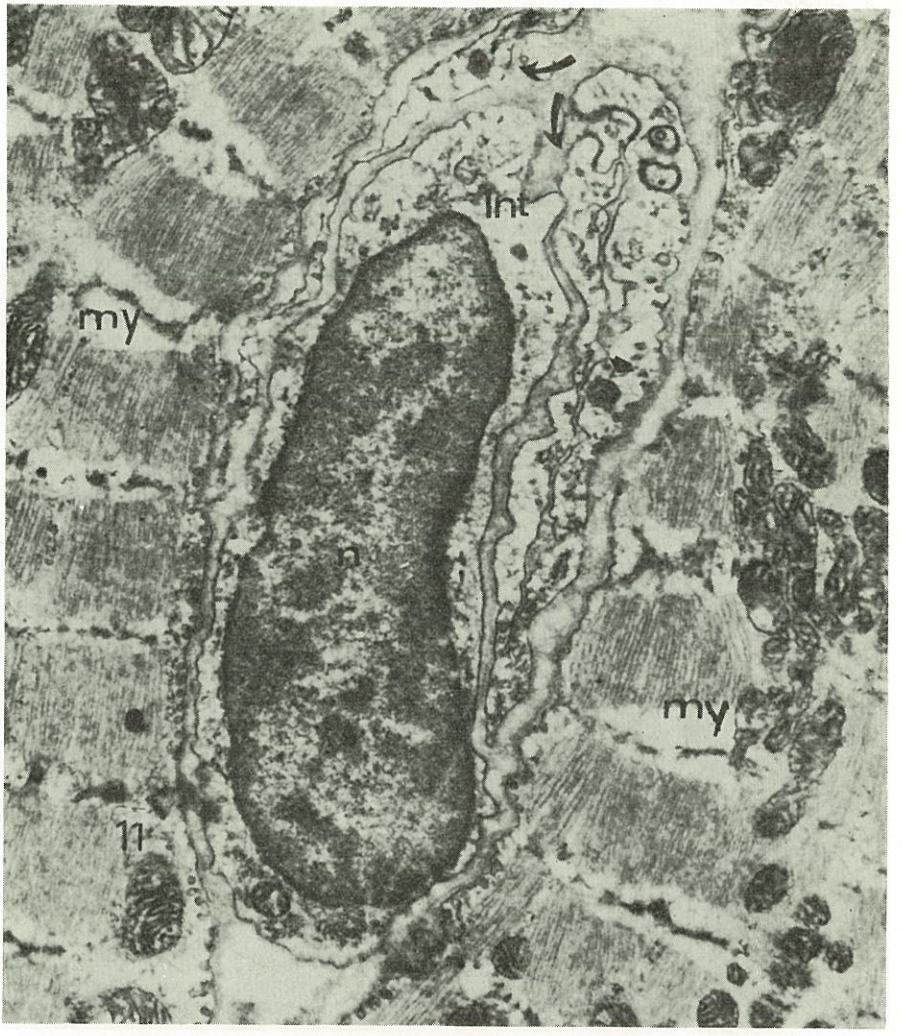

Figure 11

Intermyocardial cell (Int) and extensions (arrows) in between two myocardial cells (my) are seen. The cell has its own continuous basal lamina and a normal internal structure. Extensions detected adjacent to the cell could belong to the cell itself or vice versa. Osmium-araldite-uranyl-lead. x 24.500.

normal fibrillar collagen fused together in the amorph ground-substance. The intermyocardial cell extensions were seen embedded in a rather dense and homogenous ground-substance (Figure 15a, b).

The structure of the endocardium in all the areas examined were found normal. The endothelial cells lining the internal face of the endocardium were rather high. Their nuclei displayed an irregular

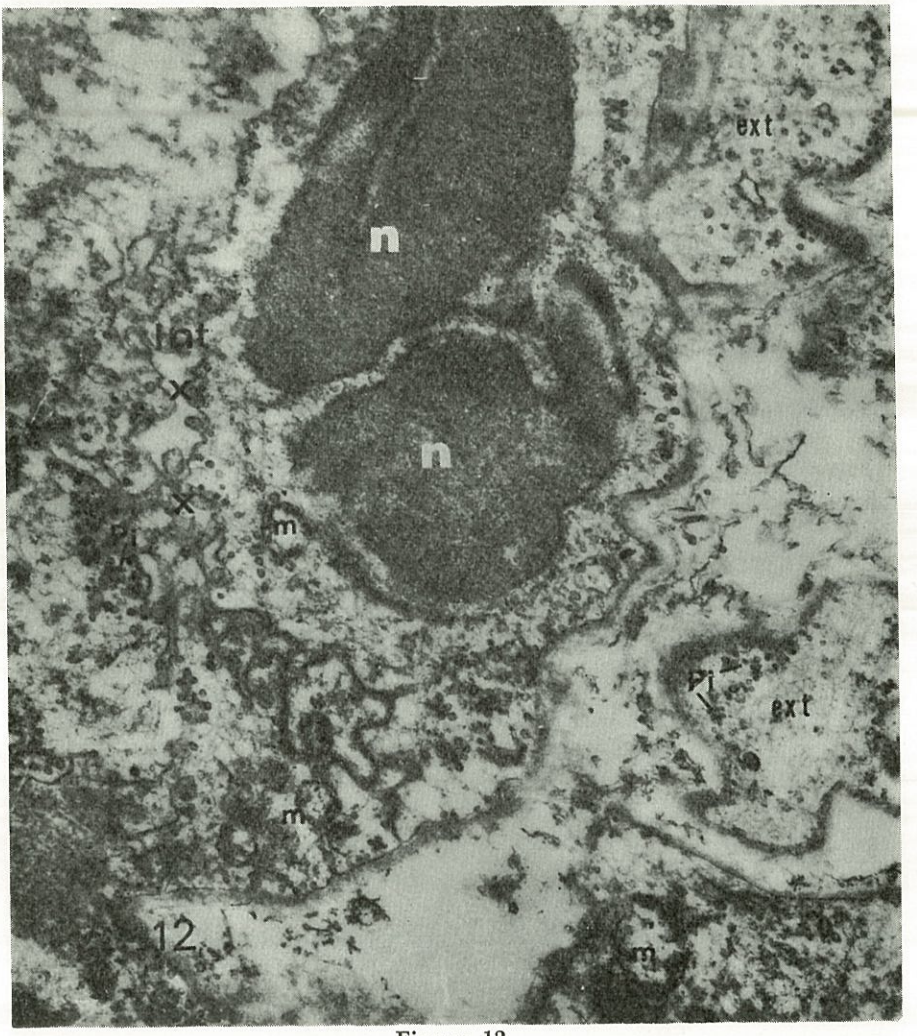

Figure 12

An intermyocardial cell (Int) is seen above showing further development with irregular cell contours, two indented nuclei (n) and a complex and, contorted intracellular canalicular system. The canaliculi are enlarged in some places in the cell (x) and the cell membrane have numerous micropinocytotic vesicles (Pi). The cell membrane also have so many micropinocytotic vesicles on it at the cell body and extensions. Note the large extensions of the intermyocardial cells taking place adjacent to the cell body. Mitochondrion, (m). Osmium-araldite-uranyl-lead. x 24.500.

contour. A continuous basal lamina and a dense collagenous layer were observed beneath the endothelium. Further below, a thick layer of smooth muscle cells was noted (Figure 16). Tonofilaments occurred in the cytoplasms of the endocardial cells together with lipidic granules.

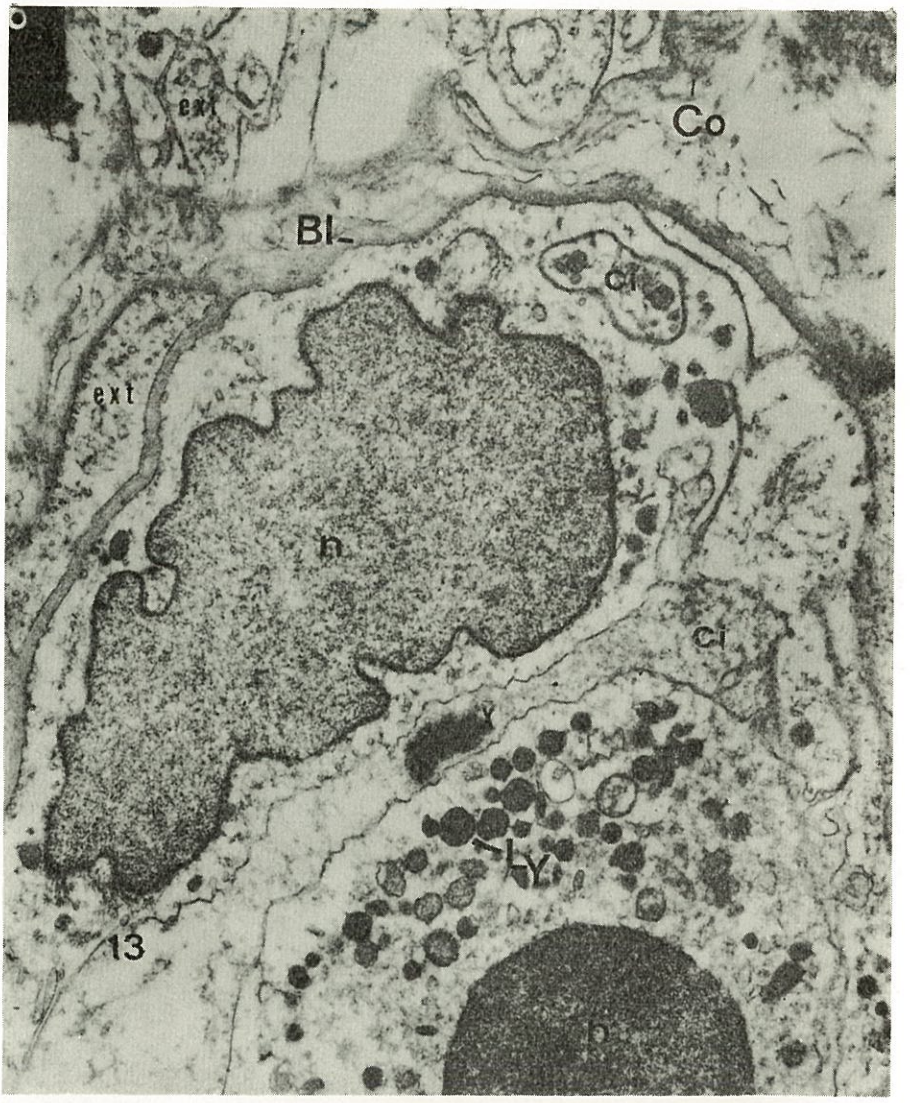

Figure 13

Developed intermyocardial cell. The cell has its own continuous basal lamina (Bl) and invested with the extensions (ext) of itself or other intermyocardial cells and collagen fibrils (Co). A part of the intracellular canaliculi is enlarged in cisternal from (Ci) at the center of the cell between the two nuclei (n) having a somewhat granular content and a dense structure (x) in it. Lysosomal granules (Ly) in varying densities are detected at the perinuclear zone. Osmium-araldite-uranyl-lead. x 24.500.

Desmosomes in between the lateral surfaces of the endothelial cells as well as semi-desmosomes at the basal surfaces were detected. No periodicity could be determined in the collagenous layer beneath the endothelium (Figures 17, 18).

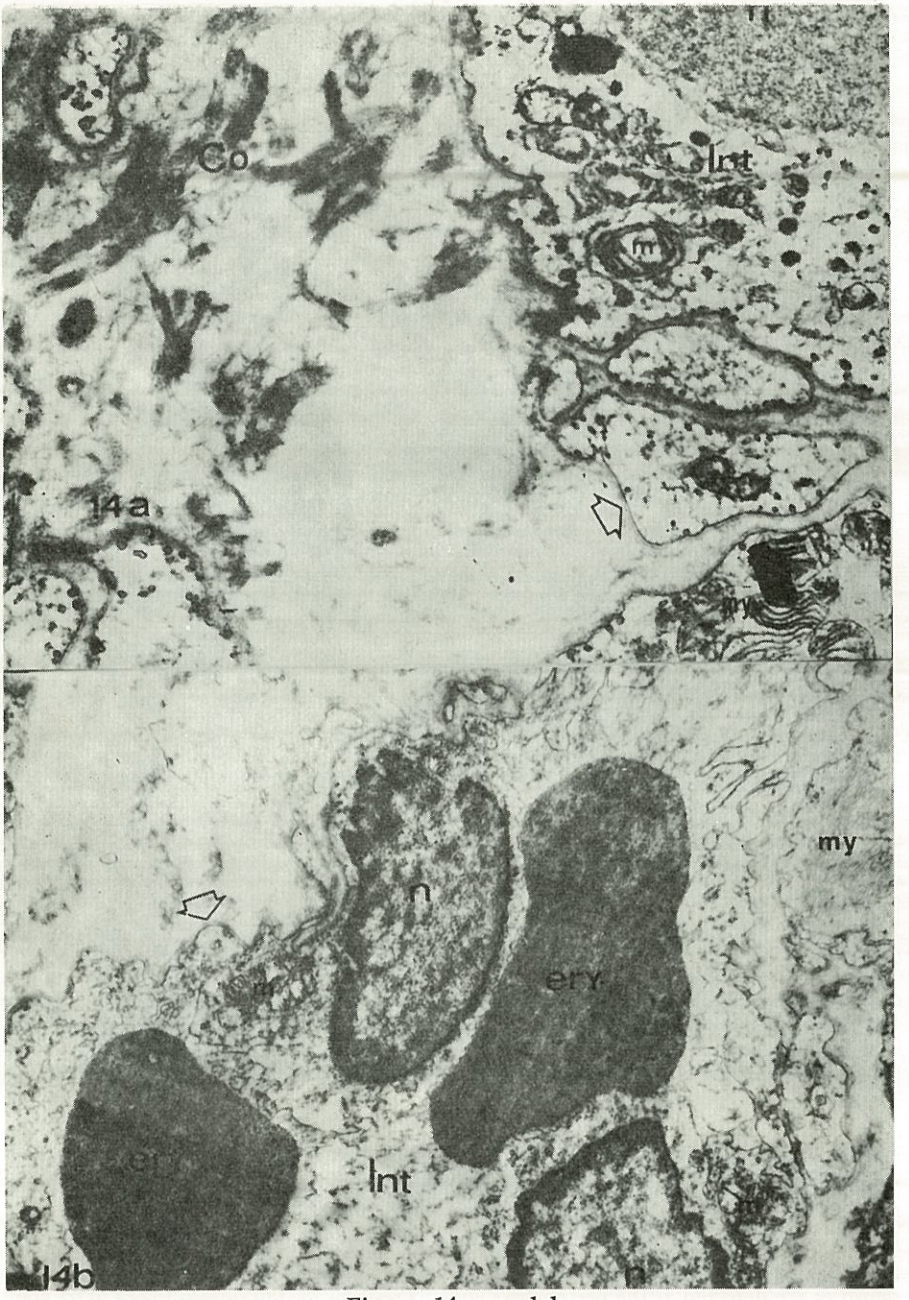

Figure 14 a and b

Two developed intermyocardial cells (Int) adjacent to the myocardial cells (my) are observed. At the upper one (a), only a part of the cell and extensions are seen embedded in disorganized collagenous elements (Co). Lower cell (b) shows phagocytotic activity having swallowed erythrocytes (ery) in its cytoplasm. Both of the cells and extentions have continuous basal lamina (open arrows). Mitochondrion, (m). Osmium-araldite-uranyl-lead. a and b x 24.500.

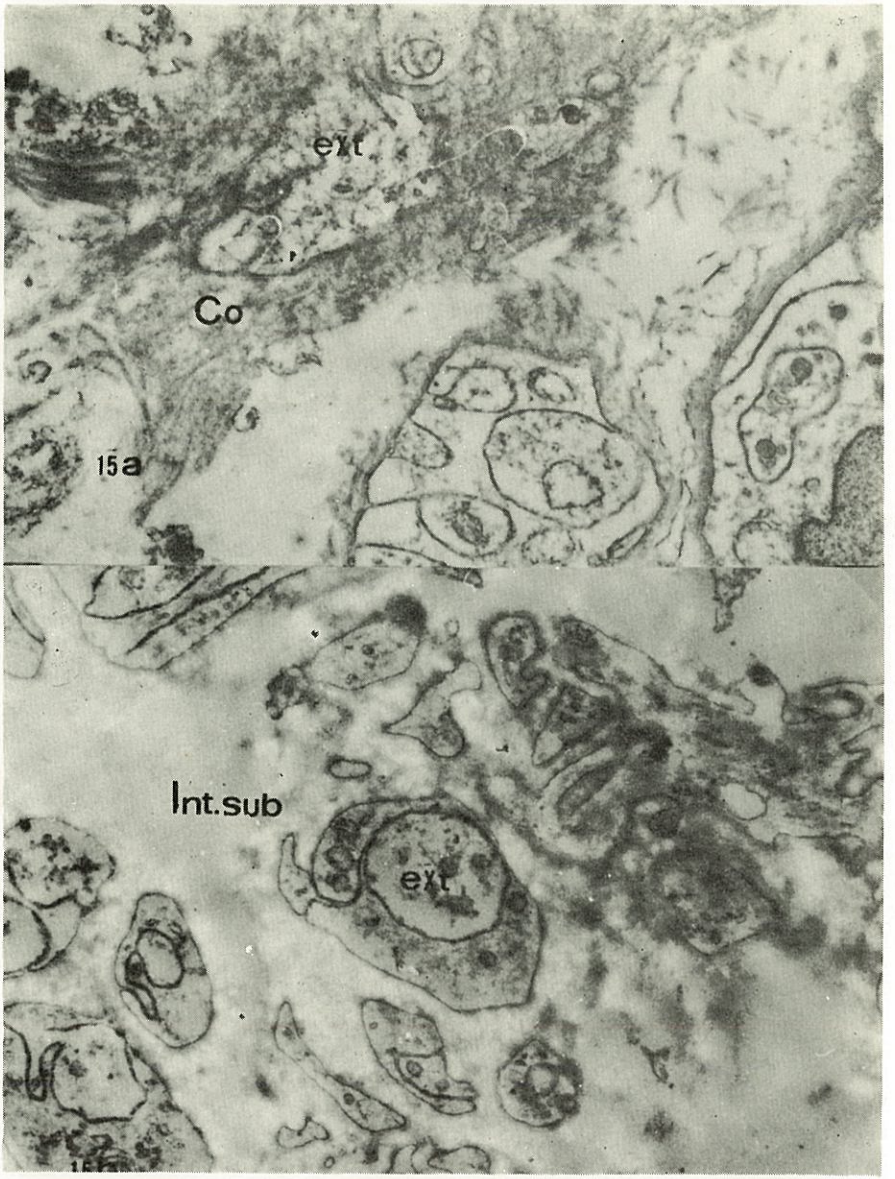

Figure 15 a and b

Two distinct areas of intermyocardium are observed having extensions (ext) of intermyocardial cells and intercellular elements. At the top (a), cell extensions are embedded in a collagenous mesh and seem to have more or less normal collagen fibrils (Co) with typical banding patterns and are clearly detectable in the amorph substance. The cell extensions in the lower micrograph (b) are embedded in a dense and homogenous intercellular substance (Int. sub), the existence of which is possibly due to the complete disorganization of the collagen fibrils (later stage). Fibrillar collagenous elements have disappeared fusing together with the amorph ground substance so as to produce a matrix, seem to be somewhat dense and uniform. Osmium-araldite-uranyl-lead. a and b x 24.500.

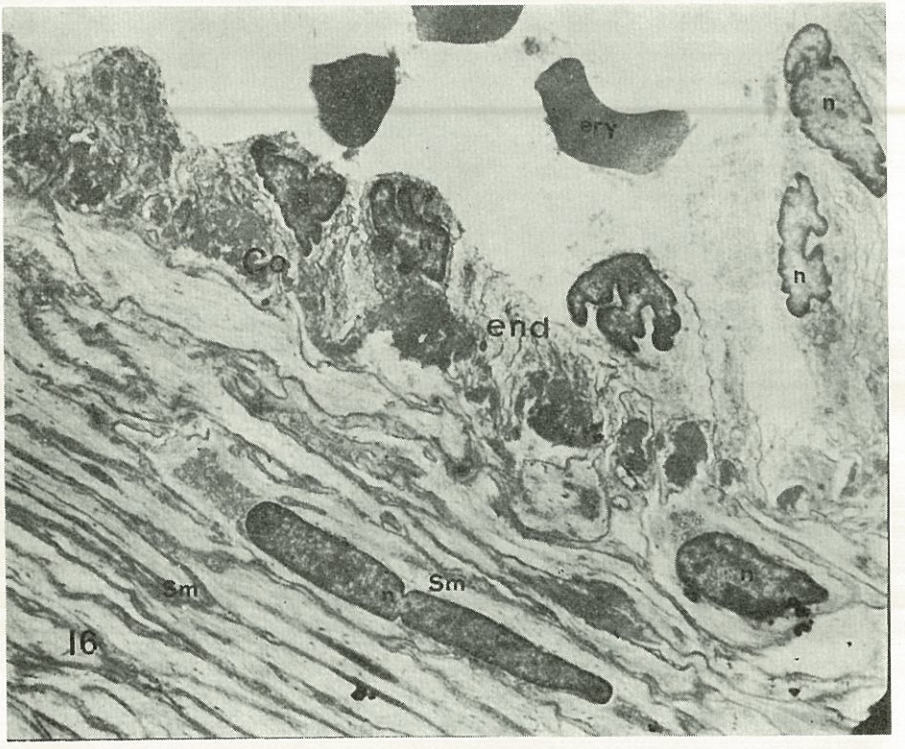

Figure 16

Panoramic view of the endocardium. A dense collagenous mass (Co) is observed just beneath the endocardial endothelium (and), arranged in a single layer. The smooth muscle cells (Sm) are closely packed under the collagenous layer, all quite normal in their structural characteristics. nucleus; (n), erythrocyte, (ery). Osmium-araldite-uranyl-lead. x 6.600.

Smooth muscle cells displayed no pathological alteration. These cells were filled up with actomyosin filaments, except their nuclei and the narrow perinuclear area. On the cell membranes, numerous micropinocytotic vesicles typical to the smooth muscle cells were noticed. It was also observed that fine collagen fibrils filled up the spaces in between the smooth muscle cells (Figure 19).

Discussion

The pathological changes occurring in the intermyocardial connective tissue in rheumatic carditis have not so far clearly been defined in the morphological sense. In the studies made at light and electron-microscopical levels, the Aschoff nodules and related elements which are characteristic pathological formations were taken up. According

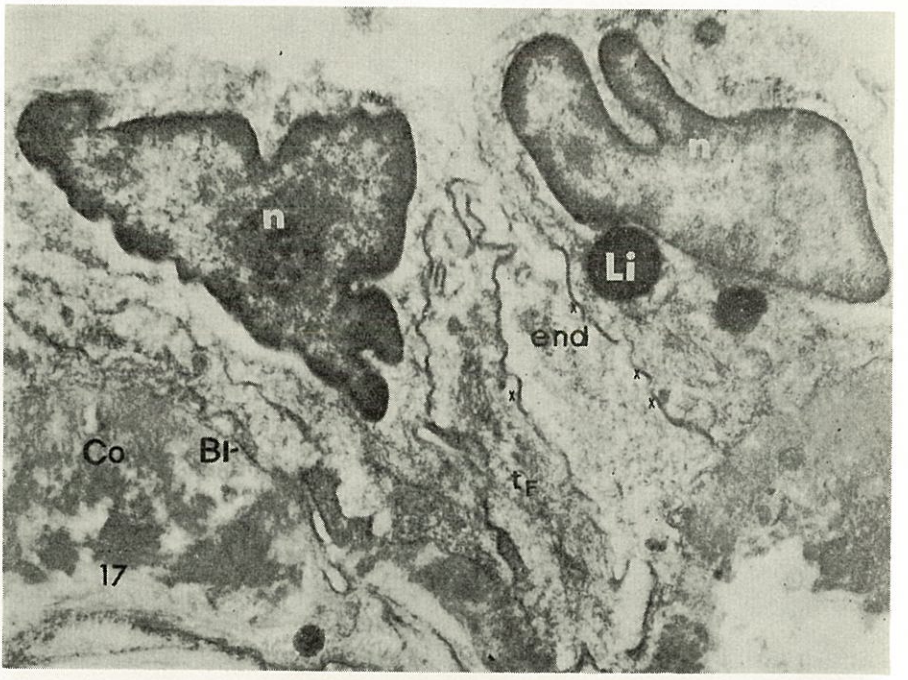

Figure 17

Further structural details are observed in the endothelial cells in higher magnification of a limited area of Figure 16. The endothelium (end), is high and based upon a continuous basal lamina (Bl). Numerous junctional complexes (x), are detectable between the lateral surfaces of the cell. In the interior of the cells, tonofilaments (Tf) exist. Nuclei (n) are dense and indented. Lipid inclusions (Li), take place at the perinuclear area. Collagenous layer (Co), seem to consist of fibrillar collagenous material. Osmium-araldite-uranyl-lead. x 24.500.

to Lannigan and Zaki,[1 2] an Aschoff nodule consists of typical Aschoff cells embedded in degenerated collagenous material. These are large and sometimes irregular basophilic cells with large and indented nuclei and display highly-developed granular endoplasmic reticulum. They are often observed having more than one nucleus and numerous irregular extensions. Phagocytic material was encountered in their cytoplasms and these were thought to be either degenerated collagenous remnants or fragments of myocardial cells.

As for Boyd[3] he has identified four different pathological components in rheumatic lesions:

1. A centre of fibrinoid necrosis,
2. Large multinucleated cells looking like epithelial cells suggested to have originated from the reticulo-endothelial system. He

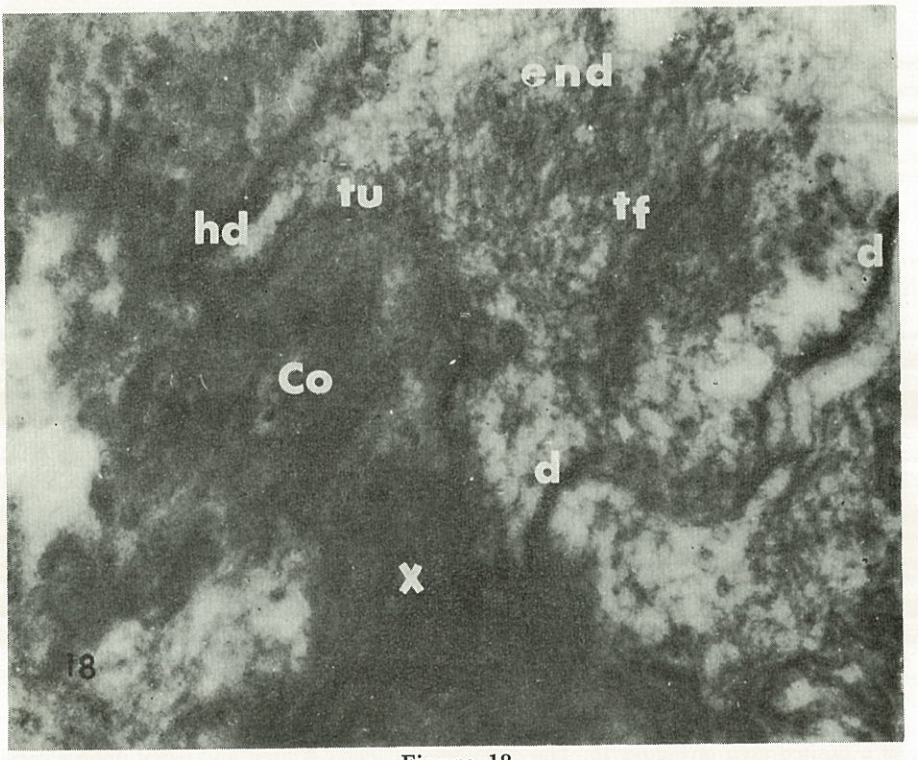

Figure 18

Detailed ultrastructure of the interrelations between endocardial cells (end) and collagenous layer (Co). Tonofilaments (tf) and microtubular structures (ta) are observed in the basal part of the endocardial cells. Desmosomes (d) exist between the lateral surfaces of the cells. Half desmosomes are also seen at the basal plasma membrane. A lower cellular extension (x) with a vesicular content is noted in the collagenous layer. Osmium-araldite-uranyl-lead. x 72.000.

described these cells, similar to large Hodgkin cells, as Aschoff cells. Under the conventional light microscope, he identified the Aschoff cells to be identical with the epitheloid cells.

3. Inflammatory cells i.e. neutrophilic leukocytes, lymphocytes and plasma cells.
4. The increase in fibroblasts followed by fibrosis and intercellular oedema together with the swelling of the vascular endothelium are observed. In addition, Boyd has also stressed that the basic pathological procedure takes place in collagenous elements. Fibrinoid degeneration followed by necrosis is detected at light microscopy level in the bundles of collagen fibrils. He also pointed out that at least some of the fibrinoid material came by way of blood and accumulated in the tissues. The experiments

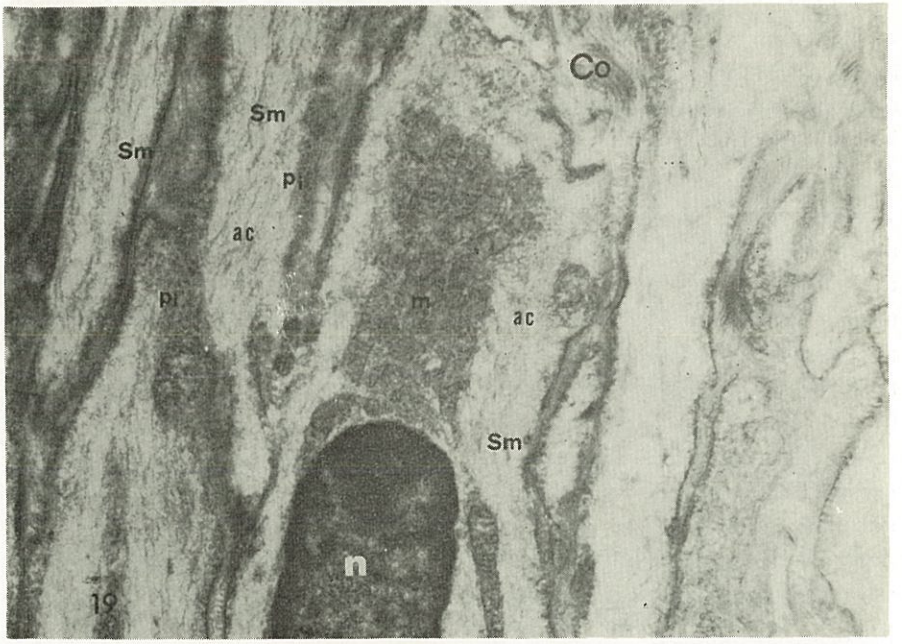

Figure 19

Endocardial smooth muscle layer as observed in a higher magnification of a limited area of Figure 16. Smooth muscle cells (Sm) are quite normal by their structural characteristics, completely filled up by the actomyosine (ac) filaments except the perinuclear zone occupied by the mitochondria (m) and the other organelles. Numerous micropinocytotic vesicles (Pi) exist along the cell membranes. Normal collagen fibrils (Co) are seen to occupy the intercellular spaces. Osmium-araldite-uranyl-lead. x 24.500.

made using human antisera labelled with fluorescent material have confirmed this.

Lannigan and Zaki[4] have pointed out that only some of the abnormal material in the areas where collagen degeneration occurs originate from degenerated collagen fibrils. Parts of contractile myofilaments from fragmental myocardial cells were also traced in the same structure. However, in none of the studies were the structural alterations in the myorcardium examined as a whole. The pathology of the intercellular connective tissue was not dwelled upon, except the nature of Aschoff lesions.

According to the findings obtained from this research, the intermyocardial connective tissue alterations observed in chronic carditis may be considered in two groups.

1. A considerable increase in the number of capillaries so much so that myocardial cells were observed to be embedded within a capillary mesh. Most often, the myocardial cells were seen in very close proximity to the capillary wall save only the basal lamina in between the both sides. Increase in capillaries has been confirmed.[5]

2. Active cell proliferation. The proliferation of cells seemed to originate from two sources. The first of these is the pericytes which surround the capillary endothelium but detach from the capillary wall, thus forming indifferentiated cells in the intermyocardial connective tissue. Indifferentiated cells may transform into other cell types when necessary. The detachment of pericytes from the capillary wall so as to form macrophages and other connective tissue cells usually occurs in the case of the capillaries of the organism under physiological conditions. The pericytes surround the capillaries almost as a continuous layer. The capillary endothelium and pericytes have their own basal laminae. The pericytes are not accepted as perivascular cells but a part of the endothelial wall itself.[6] [7] In this case studied, there is strong morphological evidence to believe that the numerous macrophages observed in the intermyocardial connective tissue formed as a result of the transformation of the pericytes that had detached from the vascular wall.

As for the second source for the active cell increase, these are the intermyocardial cells. These cells are rather long and oval in shape and have numerous extensions. Their cytoplasms are extremely poor in organelles with smooth-surfaced endoplasmic reticula having a few ribosomes and displaying cisternal enlargements. There are a few mitochrondria in the cytoplasms. The intermyocardial cells constitute a significant cell reserve of the intermyocardium. In pathological cases where new cells are needed for the tissue, intermyocardial cells respond first. While these cells proliferate by successive mitosis on one hand, they transform into other types of cells on the other. The intermyocardial cells are identical with the indifferentiated connective tissue cells which are found throughout the whole loose connective tissue of the organism. These look like inactive fibroblasts. Nevertheless, they are really multipotential cells having the ability to transform into various types of cells. They maintain the same characteristics in the connective tissue as from the embryological stages.[8]

The intermyocardial cells were often embedded in an intercellular substance displaying advanced pathological disorders, as also described by other workers.[9] [10] The normal banding pattern of the collagen fibrils found in the intercellular substance had deterio-

rated. Generally the fibrillar organization was damaged so much that it fused up with the amorph ground substance, looking like a dense and homogenous matrix in between the cells. Numerous extensions from intermyocardial cells were traced in this dense matrix. They were primitive cells, having only a few organelles in their cytoplasms. It is difficult basing ourselves on the morphological evidence, to make any interpretation about the interrelationship between the extensions of the primitive intermyocardial cells and degenerated intercellular substance in which they were embedded. However, in such degenerated foci, the acid mucopolysaccarides were found to occur both in the periphery of disorganized collagenous material and in the cytoplasms of the endothelial cells, indifferentiated and macrophage cells following the use of the dialized iron method.[11]

The fibroblasts increased most of all, as observed in this study. The young fibroblasts displayed a rather indifferentiated structure with cytoplasms poor in organelles. They, however, were identified with the barrier of the tubuli of endoplasmic reticulum in between their endo- and ectoplasms. In the active collagen producing fibroblasts the barrier disappeared. The ectoplasm was seen full of fine filaments. The plasma membrane is discontinued from place to place or as a whole. Filaments are directed out into the intercellular space so as to form periodic collagen fibrils.[12] Active fibroblasts were usually seen in this case in the intermyocardium embedded in a collagenous material produced by themselves, forming active collagen producing foci. The collagenogenesis was quite normal in such foci.

As conclusion the dominant cells in the intermyocardium observed in this case were fibroblasts, indifferentiated intermyocardial cells and indifferentiated pericytes detached from the capillary wall and multinucleated macrophages. Various swallowed materials, particularly erythrocytes were observed in the cytoplasms of macrophages. Leucocytes and plasma cells were encountered at random. Almost no typical Aschoff cell was observed. In all the regions examined, the structure of the endocardium was found normal despite the distinctive pathological manifestations in the myocardium. The smooth muscle layer of the endocardium has been reported to be involved in the pathological process of rheumatic carditis.[13] In this case studied, the smooth muscle cells were quite normal in their ultrastructure, a fact which contradicted the conventional description.

Summary

Regeneration together with degeneration was observed in the morphology of the intermyocardial connective tissue in the chronic

rheumatic carditis case examined in this work. The myocardial tissue elements were damaged and fragmented due to the effect of rheumatic process. Degeneration at varying degrees, was observed in the intercellular substance found in between the damaged and fragmented myocardial cells and cell remnants. The active intermyocardial connective tissue highly endeavoured to clear away the disorganized foci and form a new repair tissue. In consequence, indifferentiated cells proliferate in the connective tissue in response to rheumatic damage. Numerous macrophages together with fibroblasts which form as a result of the transformation of intermyocardial cells and pericytes detached from the vascular walls try to secure regeneration. While the macrophages swallow up the degenerated tissue elements, the fibroblasts try to fill up these spaces by active collagen formation.

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Reflux into the Common Bile Duct and Pancreatic Canal in Duodenal Closed Loop Syndrome

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In 1908 Williams and Bush[1] claimed that the reflux of duodenal contents could produce pancreatitis. This theory was not confirmed by Archibald[2] who observed that ampulla of water did not permit passage from the duodenum into the common bile duct or into the pancreatic canal despite luminal pressures of 1,000 mm water. Depending on experiments performed on cadavers, he claimed that reflux from the duodenum into the pancreatic canal was impossible.

Pfeffer, Stasior and Hinton[3] observed pancreatitis in their experiments on dogs whose duodenum was occluded at both ends. McCuttcheon and Rice[4] used the same experimental preparation but infused barium into the loop. They recovered the radio-opaque material within the pancreatic canal as a result of which claimed that reflux had occurred. This, in some cases, resulted in pancreatitis. When the pancreatic canal was tied, however, pancreatitis was prevented.

The general character of the above and similar studies appear to be pressure oriented. The above investigators were trying to find out whether reflux would appear under increased intraduodenal pressures.

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The answer to this question, however, is not yet clear, because it is possible that the pancreatic canal wall musculature and the sphincter of the papilla, as well as the pressure of the flow of pancreatic juice, may contract and induce some independent changes on the pancreatic canal pressure. We therefore believe that pressure changes in the pancreatic canal cannot be accepted as entirely reflective of intraduodenal pressure. Under these conditions increasing pressure in the duodenum does not necessarily prove that reflux occurs. We therefore decided to study this problem radiologically in experiments where reflux if it occurred, could visually be demonstrated by x-ray films at different intraduodenal pressures.

Methods

The experiments were conducted on 15 mongrel dogs, their average weight being 16 kg. Tracheal intubation was performed immediately after anesthesia with intravenous pentobarbital 30 mg/kg. After preoperative preparation and under sterile conditions, the abdomen was opened at the midline and intraduodenal pressure was measured using a water manometer.

The duodenum was mobilized by incising the ligament and was tied at both ends, thus forming a closed loop. At about 4 cm proximal to the Treitz ligament, a firm plastic catheter was introduced into the duodenum around which a purse string was placed. A three-way stopcock was placed at the outer end of the catheter.

Because of differences in anatomical positions (Figure 1) of the pancreas and its canals between man and dogs, it was very difficult to evaluate the results of the preliminary experiments[5]. The borders of the pancreas, gallbladder and ducts were therefore marked by radioopaque wire thus making them visible on the roentgen film. The abdominal wall was then closed leaving the free end of the intraduodenal catheter outside. The pressure changes in the duodenum were recorded on a Gillson Direct Writer. Because of the respiratory motions reflected to the intraduodenal pressure, contractions of the pectoral muscles were simultaneously recorded using a Grass Force Displacement Transducer (F. T. 10 C).

After recording the intraduodenal pressure under these conditions, gastrografin diluted by equal amounts of saline, was injected into the duodenum to increase the intraduodenal pressure by 10 mm Hg steps. Allowing 10 minutes for stabilization, radiograms were taken to show the duodenum, pancreas and the canal systems by stepwise increase of

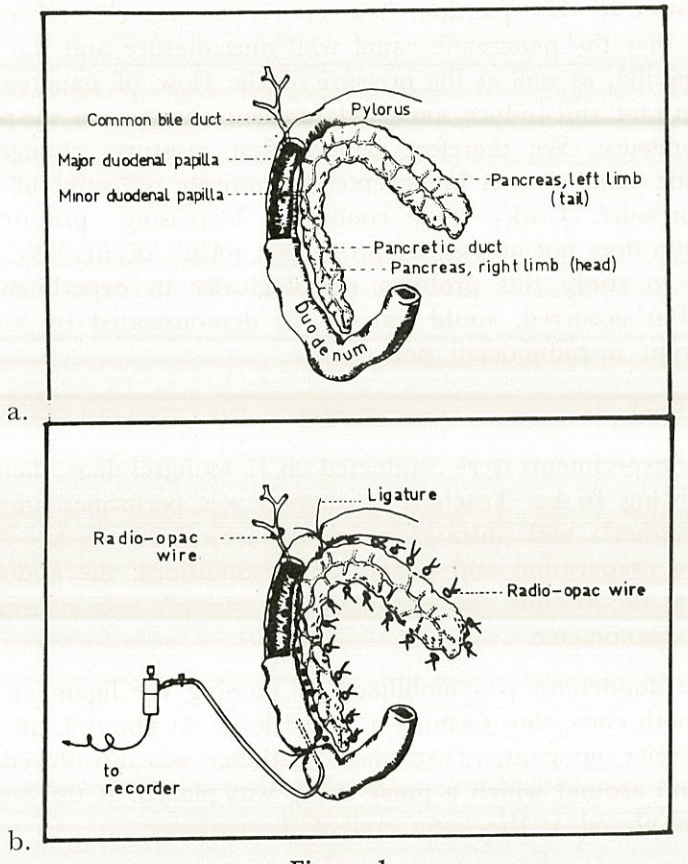

Figure 1

a) Anatomy and relationship of pancreas, its canal and common bile duct in dogs.
b) Our experimental preparation.

the duodenal pressure to 80 mm Hg. Repeating the studies at each step it was possible to detect reflux, if it occurred; and simultaneously study the intraduodenal pressure. Their correlation therefore became possible. At the end of the experiments the abdomen was opened, the ligatures at both ends of the duodenum and the catheter were removed and the animal was given proper care to permit survival.

Results

Out of 15 experiments the radio-opaque material was recovered in the pancreatic canal or the common bile duct in three instances. In one of these cases gastrografin entered the biliary canal at 20 mm Hg. pressure (Figure 2) and into the pancreatic canal at 30 mm Hg. pressure (Figure 3). In another instance the radiograms taken at 70 mm Hg. intraduodenal

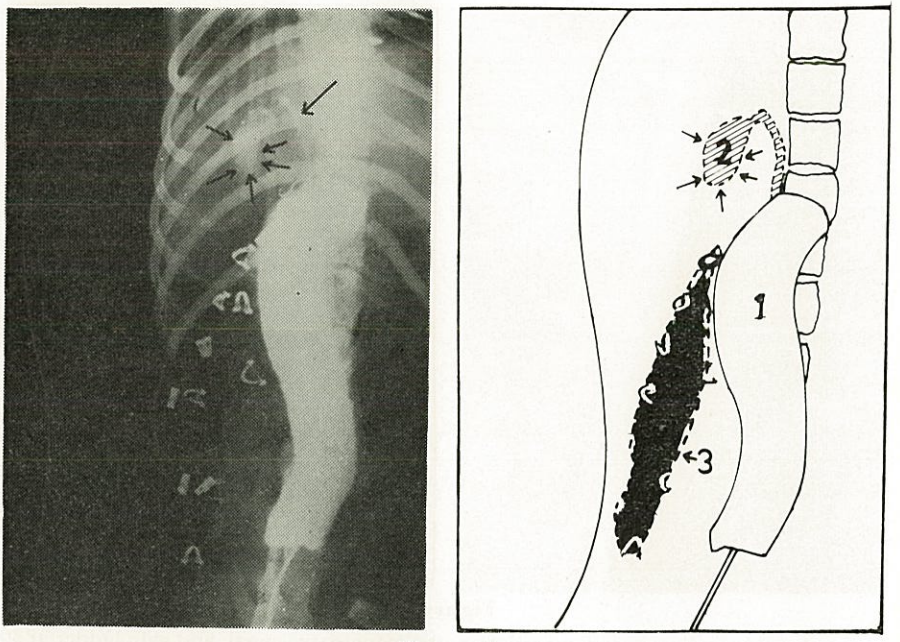

Figure 2

X-ray on the left and schematic representation on the right showing radio-opaque material into the common bile duct and gallbladder. Note that reflux into pancreatic canal has not occurred at this stage of the experiment (I. D. pressure 20 mm Hg).

1. Depicts closed loop of duodenum.
2. Gallbladder.
3. Pancreas.
4. Pancreatic canal.

pressure revealed the dye in the gallbladder. It was not present, however, in the pancreatic canal. In the third case the biliary ducts contained gastrografin beginning at 60 mm Hg. intraduodenal pressure and the pancreatic canal at 80 mm Hg. pressure (Figure 4). It is interesting that in the latter two experiments the pressures obtained at and above reflux levels were found unstable: a gradual reduction of the intraduodenal pressure seemed to accompany incompetence of the valve mechanisms. This is a preliminary observation and should be statistically evaluated.

In the remaining 12 experiments the radioopaque material did not appear in the bile duct systems nor in the pancreatic canals. In these cases the pressure fall referred to in the above paragraph was not found.

Alterations of intraduodenal pressure: Following the opening of the abdominal wall, the intraduodenal pressure was found very close to the atmospheric pressure. In our experiments three different types of

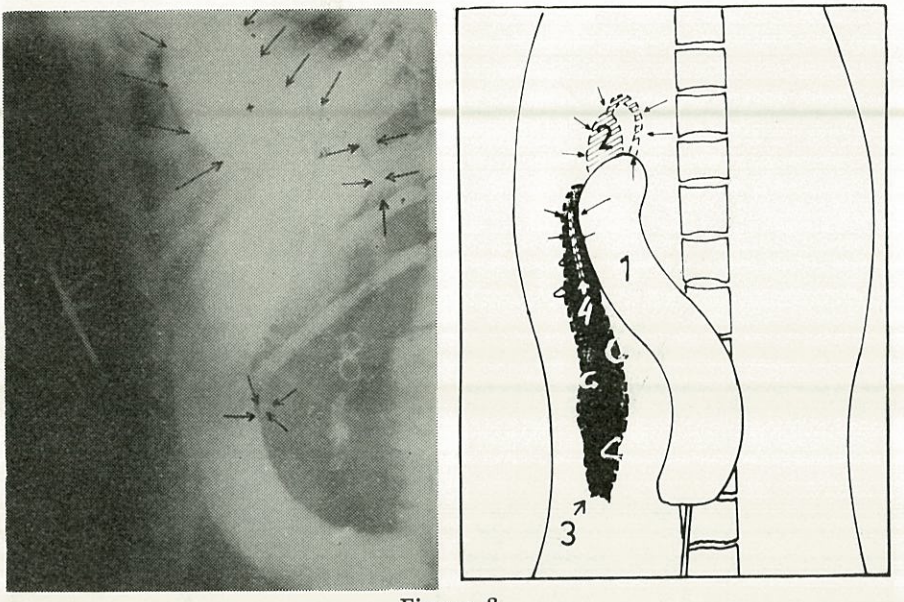

Figure 3

X-ray showing radio-opaque material in the pancreatic canal and gallbladder at 30 mm Hg. in intraduodenal pressure.

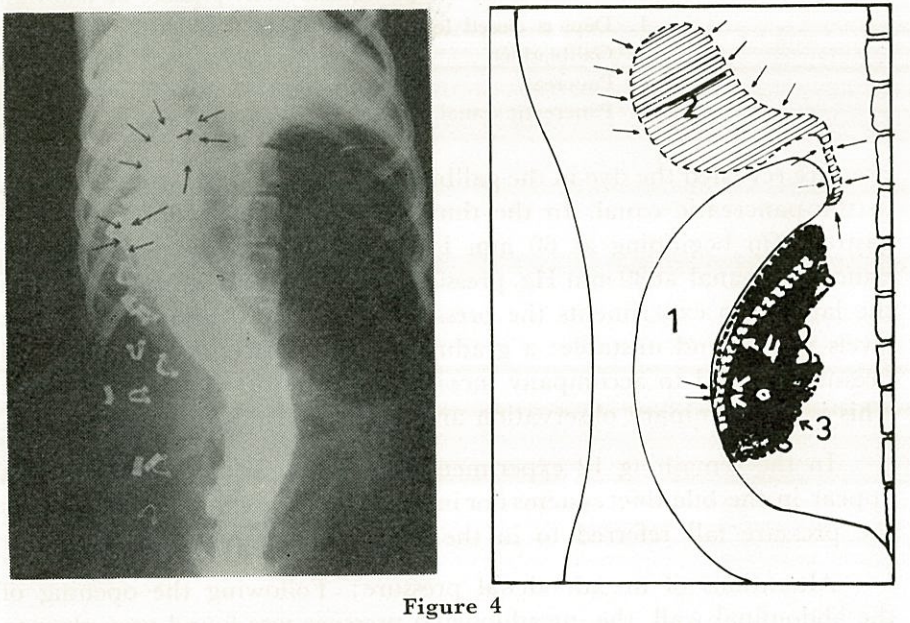

Figure 4

X-ray showing radio-opaque material in the pancreatic canal and gallbladder at 80 mm Hg. intraduodenal pressure.

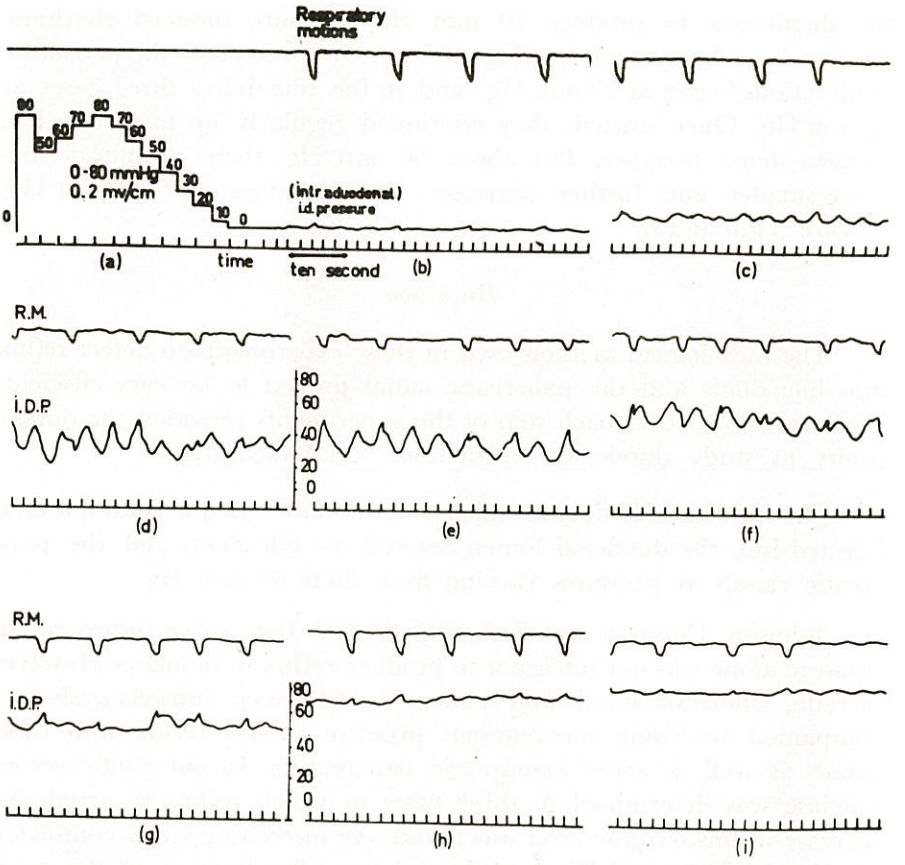

Figure 5

Reproduction of an experimental record.

a) Calibration of intraduodenal pressure.

b) Higher record shows respiratory motions, lower record intraduodenal pressure changes at 10 mm Hg.

c-i) Intraduodenal pressure was stepwise increased from 20 to 80 mm Hg. Note the differences in duodenal wall contractions.

changes were detected in the duodenal pressure. The first of these was reflective of respiratory motions and was conducted through the diaphragm to the duodenum. A close parallelism of intensity and of rhythm to the respiratory contractions of pectoral muscles was shown in each case (Figure 5 b).

Duodenal Contractions : Prolonged observation of intraluminal pressure immediately after opening the abdominal wall did not reveal any alterations. In five experiments infusion of radio-opaque material into

the duodenum to produce 10 mm Hg. pressure induced rhythmic duodenal wall contractions (Figure 5 c). In seven cases the peristaltic contractions began at 20 mm Hg. and in the remaining three cases at 30 mm Hg. Once started, they continued regularly up to 40 mm Hg. intraduodenal pressure. But above 50 mm Hg. their amplitude became smaller and further decreased at a pressure of 70-80 mm Hg. (Figure 5 g,h and i).

Discussion

The radiological method used in these experiments to detect reflux into bile ducts and the pancreatic canal proved to be very efficient. Pressures recorded at each step of the experiments provided the opportunity to study duodenal contractions simultaneously.

Our studies of 15 dogs revealed that the radio-opaque fluid material injected into the duodenal lumen entered the bile ducts and the pancreatic canals at pressures varying from 20 to 80 mm Hg.

Johnson, Dopman and Bethesda[6] showed that rising intraluminal pressure alone was not sufficient to produce reflux in monkeys. However secretin, cholecystokinin and pancreozymin given intravenously accompanied by rising intraluminal pressure caused reflux into these canals as well as acute hemorragic pancreatitis. In our studies serum amylase was determined in three cases in which reflux occurred. No increase in this enzyme level was found. An interesting study conducted on dogs by Hiatt and Warner[7] showed that after ligation of the main pancreatic canal serum amylase was twice the normal level on the second day. It reached the maximum on the third and returned to normal value on the ninth day.

In our experiments it is observed that reflux into the bile ducts and the pancreatic canal occurred under increased intraduodenal pressures. It is also interesting that the dye first entered into the bile ducts and then at higher levels of intraluminal pressure into the pancreatic canal. This seems logical, because a study of Menguy et. al.[8] has disclosed that pressure in the pancreatic canal was higher than that of the bile ducts under normal conditions.

It can be thought that because of the anatomical differences between dogs and human beings the results may not be applicable to human pathology. It is known that pancreatic canals and bile ducts have separate endings in dogs. However they do have a generally combined opening at the papilla of water in human beings. Statistical studies reveal

that in 43-72 % of cases the bile ducts and Wirsung canal have separate endings in human beings. The results obtained in our studies are therefore applicable to a large percentage of human beings.

Summary

In 15 dogs the abdomen was opened and the duodenum was tied at both ends forming a closed loop. Into this, a radio-opaque dye (gastrografin) was injected under pressure via a plastic catheter. Roentgen films were taken at 10-20-30-.....80 mm Hg. intraduodenal pressure. It was found that in three cases the radio-opaque material entered into the bile ducts and in two into the pancreatic canal.

Spontaneous and periodical alterations of the intraduodenal pressures were observed beginning at 10 mm Hg. in some cases and at higher pressures in the remaining experiments. They were regular and 3-5 per minutes apart. Their amplitude, however, became smaller after 50 mm Hg. intraduodenal pressure.

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Results

The major findings in the first rheumatic attack of the patients was as follows: arthritis 69.3 %, carditis 28 %, chorea 2 %, subcutaneus nodules 0.7 %. The mean age of the patients in their first attack was 8.5 years (3 to 15 years); 78 of them (52 %) experienced one or more recurrences before starting penicillin prophylaxis. The duration of the prophylactic regiment ranged from 3 months to 8 years. One hundred and twenty four patients (82.7 %) were stated to be in the first category described in *Materials and Methods*. Sixteen (10.6 %) were in the second and 10 (6.6 %) were in the third category.

Among patients maintained in prophylaxis, 30 experienced rheumatic fever recurrences. Of them 15 belonged in Group 1, 9 in Group 2 and 6 in Group 3. Thus the recurrence rate among the patients in the first group was 12.1 % and 56 % and 60 % in the other two groups respectively (Table I).

TABLE I

	Group 1	Group 2	Group 3
Number of cases	124 (82.7 %)	16 (10.6 %)	10 (6.6 %)
Recurrences	15 (12.1 %)	9 (56 %)	6 (60 %)

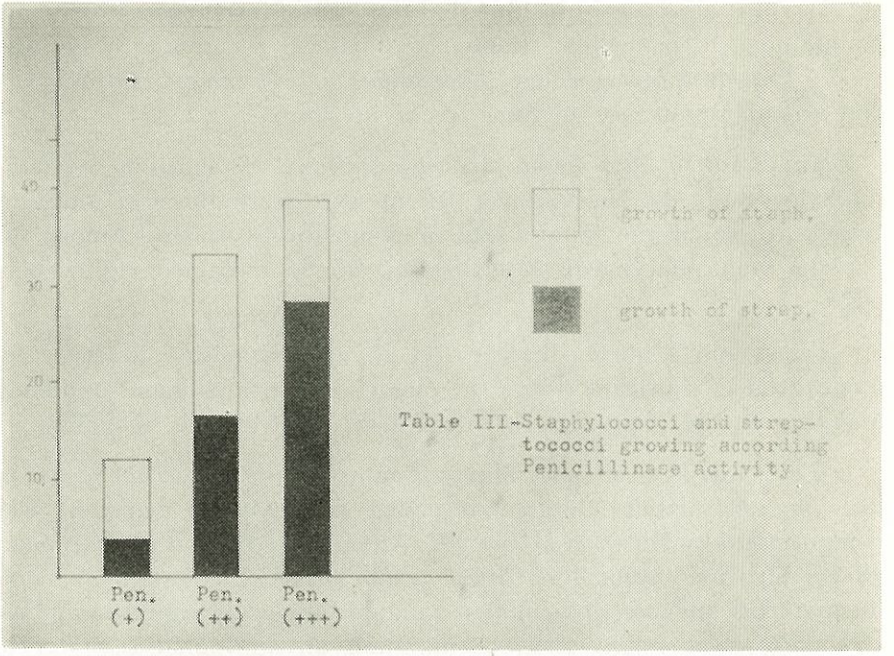
Throat cultures in 67 subjects (44.5%) revealed micro-organisms like candida, diphtheroid bacilli, coagulase negative staphylococci and gram negative organisms and they were diagnosed as "normal throat flora".

Penicillinase producing, coagulase-positive staphylococci was present in 83 cultures (55.5 %). In addition in 47 cultures (31.3 %) among these patients group A streptococci was isolated. Note should be taken that streptococci could not be shown in the absence of staphylococci. All the patients were free of symptoms. In the control group 2 patients harbored penicillinase-producing staphylococci (7 %) and 1 patient group A streptococci (3.3 %). (Table II.).

TABLE II

	Normal flora	Staphyloccoci	Streptococci
Number of cases	67 (44 %)	83 (55 %)	47 (31.3 %)
Control cases	27 (92.7 %)	2 (7 %)	1 (3.3 %)

TABLE III



Penicillinase activity was one plus (+) in 12 cases (14.5 %), two plus (++) in 33 cases (39.7 %) and three plus (+++) in 38 cases (45.6 %). The relationship between the growth of streptococci and the degree of penicillinase activity of the staphylococci is demonstrated in Table III. In cases with (+) penicillinase-producing staphylococci, streptococci isolation was 33.3 %, in (++) penicillinase production it was 51.1 % and in (+++) penicillinase production 71 %. (Table IV).

TABLE IV

Penicillinase activity	(-)	(+)	(++)	(+++)
Staphylococci		12 (14.5 %)	33 (39.7 %)	38 (45.6 %)
Streptococci		4 (33.3 %)	16 (51.1 %)	27 (71 %)
Recurrences	3 (4.4 %)	5 (41.1 %)	8 (24.2 %)	14 (36 %)

The antibiotic testing revealed that 98 % of the streptococci were sensitive to oxacillin, dicloxacillin and the cephalosporin. There were no penicillin sensitive staphylococci strain.

Comment

Wood et al[6] reported in 1964 that the risk of recurrences in patients maintained in penicilllin prophylaxis against rheumatic fever was 0.4 %. Our data reveal a high percentage, but we can explain it according to our results and to those of the literature:

Tunewall[7] in 1955 found that the presence of penicillin resistant staphylococci in the pharynges of patients with scarlet fever or tonsillitis greatly increased the failure rate of penicillin treatment. Among 16 patients with pharyngeal staphylococci, 56 % remained streptococci positive.

Frank and Muller[4], Bernstein et al[8] and Kundsin[9] in 1964 demonstrated a significant rise in the incidence of penicillinase producing organism in the pharynges of the patients treated with penicillin.

That carriage of penicillinase-producing staphylococci does in fact increase among children receiving prophylactic penicillin was demonstrated by Harris et al[2] in 1962. Among children on this regiment 48 % harbored penicillin-resistant staphylococci. The authors commented that the use of penicillin in a prophylactic regiment against rheumatic fever might serve to maintain an additional reservoir of penicillin resistant staphylococci in the community.

Michael et Michael[3] in 1964 determined the incidence of staphylococci in the throat of a large series of subjects and they revealed that the patients on daily prophylactic penicillin harbored substantially more penicillinase-producing staphylococci than those who were not taking penicillin. They found that cultures from 56.5 % of the patients on prophylaxis were positive for penicillinase-producing staphylococci. The authors concluded that in many instances staphyloccoci from the throat flora may produce sufficient amounts of penicillinase to inactivate amounts of penicillin which are commonly used in bacterial therapy.

Our data revealed that 55.5 % of the patients in Benzathine penicillin prophylaxis harbored penicillinase producing staphylococci in their throat. This incidence is in accordance with Michael et Michael's[4] study and not greatly in variance from the value reported by Harris et al.[2] The authors do not comment on the presence of streptococci. We isolated this organism in 31.3 % of the patients in prophylactic penicillin regiment. The relationship between penicillinase activity and the growth of streptococci is significant ($0.02 < p < 0.05$). Recurrences were detected in a higher degree among patients

harboring group A streptococci and the relationship between recurrences and the presence of streptococci is also significant ($0.02 < p < 0.01$). Although penicillin prophylaxis reduce the recurrence rate from 52 % to 12.1 % ($p < 0.01$) the incidence is still too high.

The growth of streptococci despite penicillin prophylaxis may be explained by the destruction of the antibiotic by the penicillinase produced by staphylococci before it can exert its antibacterial effect. The augmentation of the dosage of penicillin will not be the solution as demonstrated by Simon and Sakai[10] who studied 12 patients whose streptococcal pharyngitis had repeatedly failed to respond to penicillin G despite a high dosage of the antibiotic; penicillin resistant staphylococci in addition to hemolitic streptococci were recovered from all of his patients.

Since 98 % of the staphylococci of our patients were sensitive to oxacillin, cephalosporin and dicloxacillin, the use of one of these agents will be of great value in the prevention of recurrences of rheumatic fever. Among these antibiotics dicloxacillin having both antistaphylococcal and antistreptococcal activities may be specifically indicated. The problem rises in the cost of this regiment. Thus we propose to take throat cultures at least once a month, in the presence of staphylococci to treat patients for 10 days with this antibiotic, and to continue the course with Benzathine penicillin if subsequent cultures reveal absence of penicillinase-producing organisms.

Summary

In this study throat cultures from 150 children with a mean age of 12.4 years were investigated for the presence of coagulase-positive, penicillinase-producing staphylococci. In 55.5 % of the cases this organism was present and among cultures positive for staphylococci in 31.3 %, in addition, group A streptococci was grown. The recurrence rate of rheumatic subjects in continued prophylaxis was found as 12.1 % and this high percentage was explained by the destruction of penicillin by penicillinase produced by staphylococci. The role of penicillinase resistant penicillin in the prophylaxis of rheumatic fever attacks was discussed.

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A Study Showing the Toxic Effect of Pig Pituitary Extract on Rats*

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The hypocalcemic effect of pituitary extract has been demonstrated in rabbits[1-3] and rats.[4] Zileli et al[5] have observed that intravenous injection of pituitary extract has caused death in seventeen out of sixty rats (28.3 per cent), while no rats died after the injection of extracts prepared from brain and hypothalamus.[6] The purpose of thisstudy was to investigate the arterial blood pressure and histological changes in the tissues of rats which received guinea pig pituitary extract intravenously.

Material and Method

Thirty-six albino rats weighing 150 to 200 gm each and 260 guinea pigs were used in the study. The guinea pigs were killed by decapitation, and the pituitary glands were removed immediately and frozen on dry ice. The same day, the glands were homogenized in 0.5 ml of chilled normal saline in a glass homogenizer in a cold room. The crude homogenate was then centrifuged at 11.000 g for 5 minutes at 4° C in an ultracentrifuge. The supernatant was separated and the precipitate was mixed with 0.5 ml of normal saline for 3 minutes and recentrifuged as before. The supernatant was added to the previously collected specimen and was immediately frozen to -20° C and used within two days. One ml of clear extract obtained from the pituitary glands of ten guinea pigs was used for one rat.

Tracheostomy was performed on rats after pentobarbital anesthesia. A thin polyethylene catheter was inserted into the right jugular vein for infusion. Each rat received 100 units of heparin in 0.5 ml of 5 per cent dextrose to prevent clot formation. Arterial blood pressure was recorded on a smoked kymograph paper by a Condon mercury manometer connected to the left carotid artery by a tube filled with heparinized saline. Continuous blood pressure recording was obtained for 3 hours, before and after the intravenous administration of pituitary extract, prednisolone and saline. The adrenal glands, lungs, brains, kidneys and livers of the rats that died during the experiment were immediately removed and put into 10 per cent formalin. The rats that survived the experiment were followed up for 3 hours and then killed with a high dose of nembutal injected into the peritoneal cavity. The above mentioned organs were saved for investigation.

The rats used in the study were separated into 3 groups :

Group I : To each of the sixteen rats in this group, 1 ml of pituitary extract, obtained from ten guinea pigs, was given by a rapid jugular vein injection and this was followed by infusion of 3 ml of normal saline for 3 hours.

Group II : Ten rats were used as controls. They were first injected with 1 ml of normal saline intravenously and then infused 3 ml of normal saline over 3 hours.

Group III : Ten rats were given a rapid intravenous injection of 1 mg of Prednisolone in 1 ml of normal saline. Within 5 minutes, pituitary extract, the same dose used in Group I, was administered, then 1 mg of Prednisolone was infused in 2 ml of normal saline over 3 hours.

Findings

Twelve rats out of sixteen in Group I died and only four survived until the end of the experiment, giving a mortality rate of 75 per cent. Blood pressure of the four rats which survived dropped to 65, 60, 60 and 30 mm Hg, respectively. The remaining 12 rats developed tachypnea 3 or 5 minutes after the injection of pituitary extract, then pink-colored fluid came from the tracheostomy cannulae, blood pressure dropped to zero within 5 minutes and the animals died of apnea within an hour (Table I). The adrenal, brain, lung, liver and kidney of these animals showed marked congestion and in some of the specimens hemorrhage was observed. There was no marked congestion in the organs of the four rats which survived the experiment.

TABLE I

BLOOD PRESSURE LEVELS IN RATS BEFORE AND AFTER GUINEA PIG PITUITARY EXTRACT INJECTION

Number of rats	Control B. P. (mm Hg)	Post-injection B. P. (mm Hg)
1	110	0
2	95	0
3	110	0
4	95	0
5	85	0
6	90	30
7	80	0
8	125	0
9	75	0
10	95	0
11	90	60
12	120	0
13	95	65
14	110	0
15	120	60
16	75	0

The animals in Group II showed no change in general condition and in blood pressure during the entire period of observation (Table II). Their organs were histologically normal.

TABLE II

BLOOD PRESSURE LEVELS IN RATS BEFORE AND AFTER NORMAL SALINE INJECTION

Number of rats	Control B. P. mm(Hg)	Post-injection B. P. (mm Hg)
1	90	100
2	93	90
3	85	85
4	100	85
5	85	90
6	110	90
7	90	86
8	95	115
9	80	100
10	95	100

The clinical condition and blood pressure of the Group III rats remained stable until the end of the experiment (Table III). Histological examination revealed no abnormality in the organs of these animals.

TABLE III

BLOOD PRESSURE LEVELS IN RATS BEFORE AND AFTER PREDNISOLONE AND GUINEA PIG PITUITARY EXTRACT INJECTION

Number of rats	Control B. P. (mm Hg)	Post-injection B. P. (mm Hg)
1	110	97
2	110	101
3	108	96
4	105	108
5	95	115
6	130	122
7	129	124
8	125	142
9	135	112
10	111	115

Discussion

Seventy-five per cent of rats given pituitary extract died within one hour. Among the groups of rats which received normal saline, and prednisolone with pituitary extract no death was recorded. Since all rats received the same amount of fluid intravenously, fluid overload cannot be held responsible for death. In a previous study reported by us, the mortality rate was found to be 28.3 per cent. A mortality rate of 75 per cent in the present study may be explained by the time factor. The rats were followed for 3 hours in the present study whereas the test period was only 30 minutes in the previous one.

The fact that the intravenous injections of extracts prepared from cerebral cortex and hypothalamus have not produced death, suggests that one or more factors present in the pituitary gland may be responsible for the outcome. Some substances apart from corticotropin, chorionic gonadotropin, pitressin, thyrotropin and insulin might account for death since single intravenous administration of the above hormones have not caused death in the experimental animals.[4]

Friesen et al[7] isolated two substances, peptide I and II, from a crude extract obtained from the anterior pituitary gland of hogs. Following the subcutaneous injection of these purified peptides to rabbits the animals became lethargic, unresponsive and weak and some of them died within one to two hours. A similar phenomenon in rabbits had been described earlier by Keller et al[8] which they attributed to lipemia produced by the crude pituitary extract. Hyperlipemia would not be responsible for death because of the protective effect of prednisolone, a lypolytic substance, in the present study.

Recently Solcia[9] claimed that the pituitary factor, which kills the animals, is a tromboplastin-like substance. He obtained full protection of the animals by using heparin. Each of our experimental rats received 100 U. of heparin which did not protect the animals from shock and death. We have yet no idea about the killing substance or substances of the pituitary gland.

Glucocorticoids (Prednisolone) in the present study protected the rats from death; blood pressure remained stable and no pathological changes have been observed in the tissues. To us the mechanism of this protection remains unknown. This study has not revealed the cause of death and we have not detected any clue beyond the drop in blood pressure and marked tissue congestion in the rats which expired.

Summary

Guinea pig pituitary extract was injected into rats intravenously. Seventy-five per cent of the rats died within one hour. In the animals which expired, the blood pressure dropped to zero within 5 minutes and tissues showed marked congestion. Normal saline injected rats and prednisolone and pituitary extract injected rats showed no death and their blood pressures remained normal and there was no evidence of congestion in their tissues. Prednisolone protected the animals from death. The mechanism of this protection remains unknown.

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CONTENTS

cyanosis on exertion. Cyanosis was present since his birth. Dyspnea was obvious especially during the last three months.

Physical examination of the patient revealed clubbing of his fingers. No murmur was heard during auscultation of the heart and lungs. Patient was severely cyanotic. On inspection, he resembled a case of Tetralogy of Fallot.

Laboratory Findings: Blood analysis showed hemoglobin to be : 23.42 gr %, 4,000 leucocytes and hematocrit: 74 %, Sedimentation rate was 1 mm/hour. ECG revealed left ventricular hypertrophy and left axis deviation. Direct postero-anterior chest X ray displayed a suspicious shadow near the left contour of the heart in the left lower lobe of the lung, resembling bronchiectasis or some vascular malformation (Figure 1). Communications due to pulmonary arteriovenous fistulae in the left lower lobe of the lung, excluding the superior segment, were detected in right heart catheterization and angiography (Figures 2 and 3).

Patient underwent a classic left posterolateral thoracotomy on 21st of April, 1971. On inspection, arteriovenous communications in the basal segments of left lower lobe of the lung were observed (Figure 4). All segments of the lower lobe were removed, except the superior segment.

Postoperative course was uneventful. Chest X ray was normal (Figure 5). Blood analysis displayed hemoglobin: 11.85 gr %, and hematocrit: 50 %. Cyanosis disappeared completely. He was discharged from hospital on 4th of May, 1971 with complete recovery.

Case 2: A. I. (Hospital number: 67/60937), this 9 year-old boy, entered the hospital on 12th of June, 1969, because of cyanosis, dyspnea, palpitation and sputum production. Cyanosis was present since birth.

On physical examination cyanosis and clubbing of the fingers were observed. No murmur was present on auscultation of the heart and lungs.

Laboratory Findings: Blood analysis showed hemoglobin: 18.91 gr %, 7800 leucocytes and hematocrite: 61 %. ECG displayed left ventricular hypertrophy. Direct posteroanterior chest X ray called attention to some diffuse vascular shadows on the right lung field (Figure 6). Diffuse arteriovenous communications were detected in the capillary level of the right lung in right heart catheterization and angiography. Because of these communications, aorta was filled with contrast medium early in the series of films while arteriovenous communications were still obviously seen (Figures 7, 8).

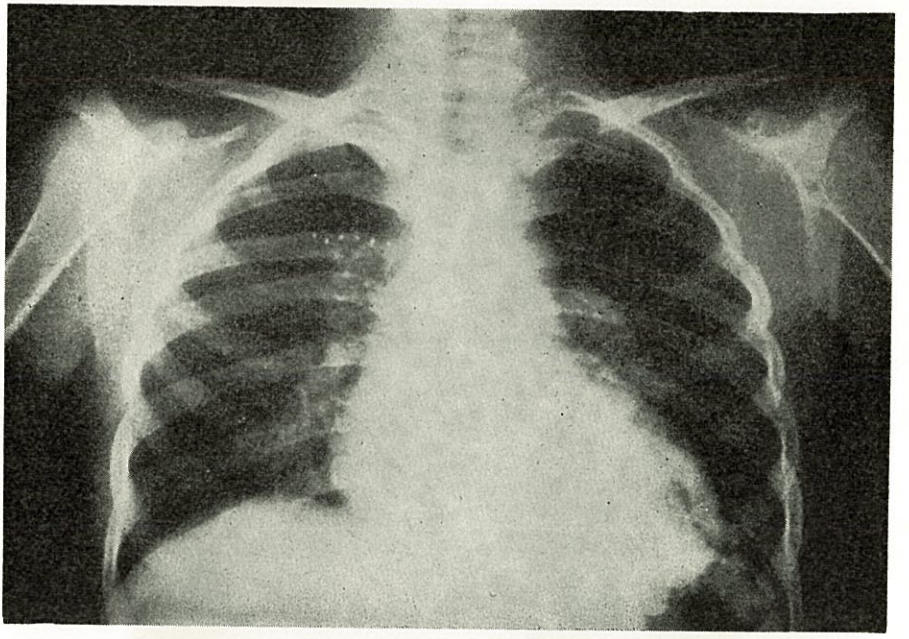

Figure 1

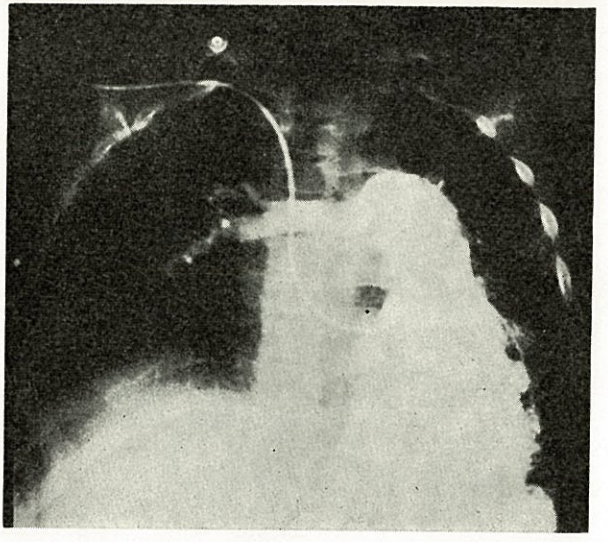

Figure 2

The patient was taken to operating room on 30th of June, 1969. He underwent a classic right posterolateral thoracotomy. Increased vascularity was observed on the right lung. Right pneumonectomy was done.

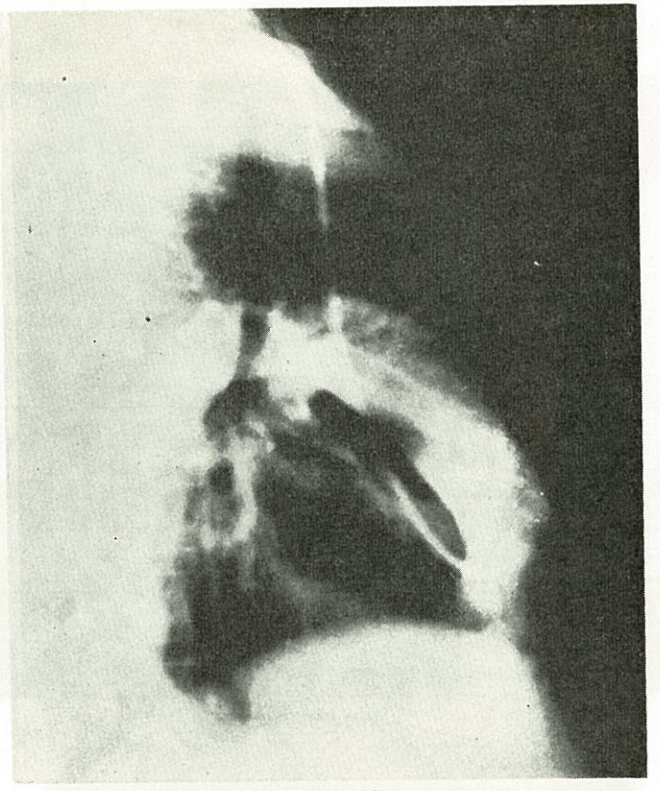

Figure 3

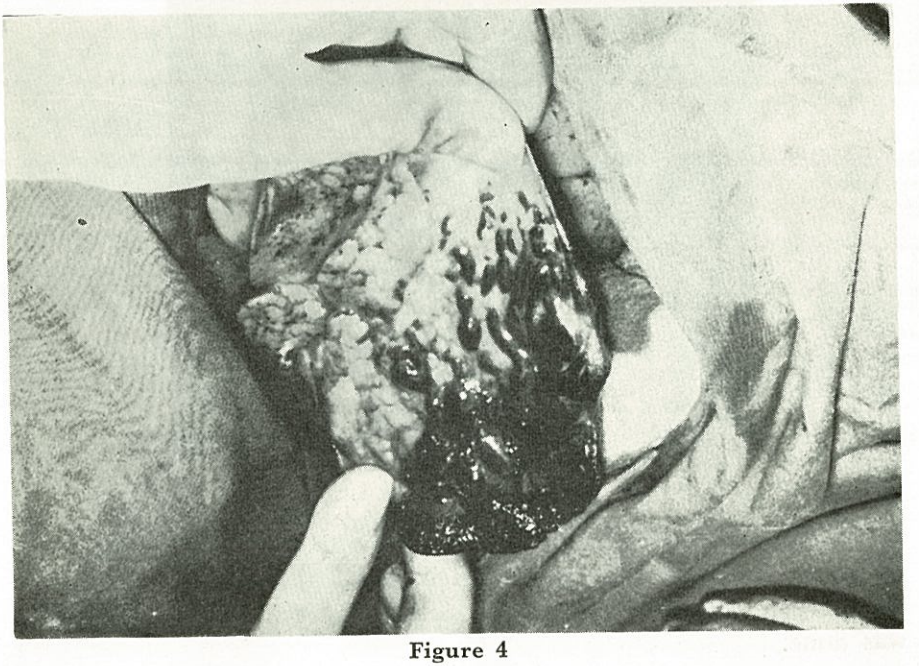

Figure 4

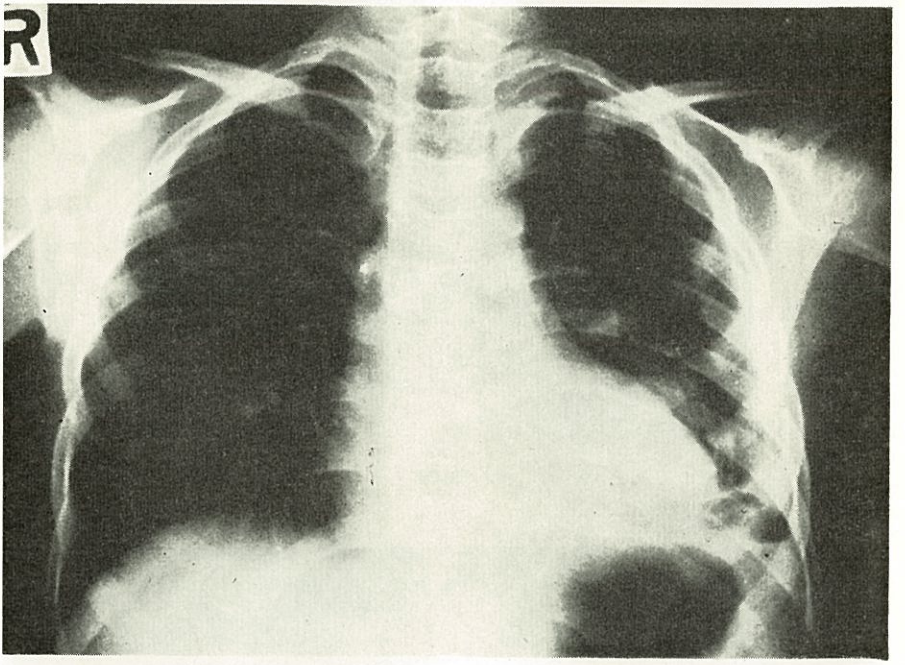

Figure 5

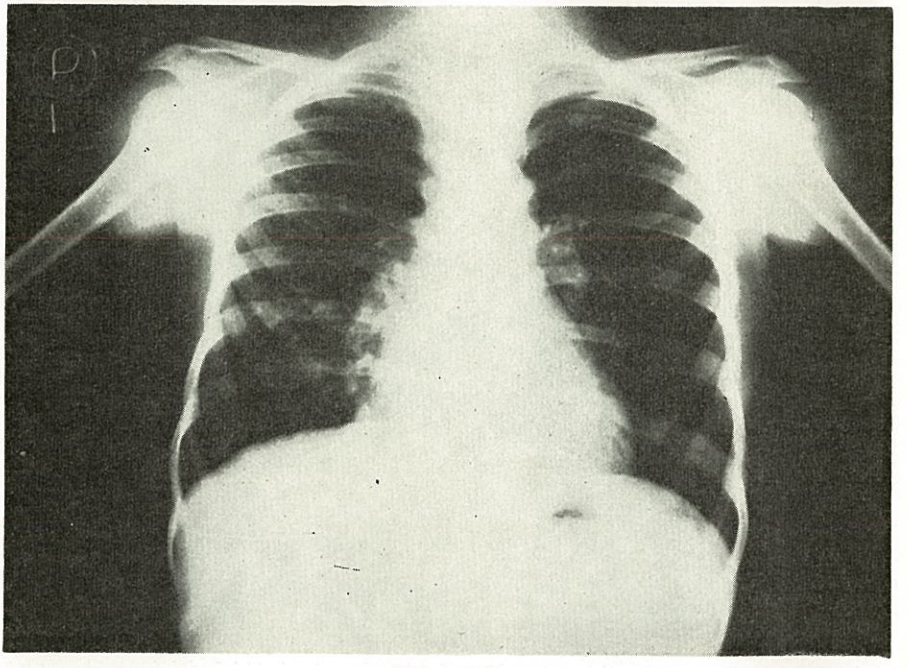

Figure 6

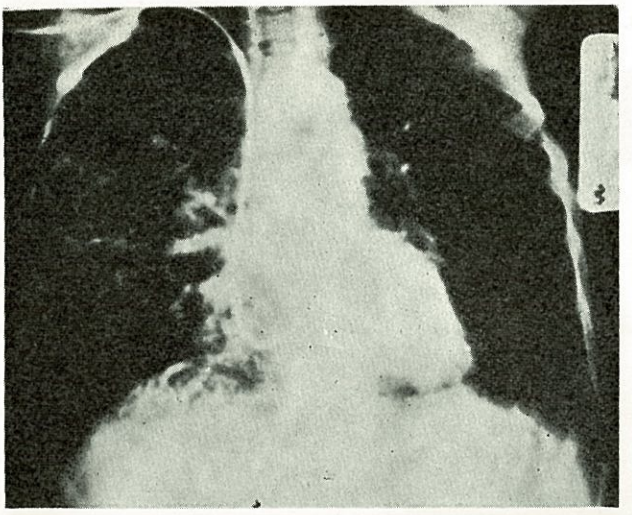

Figure 7

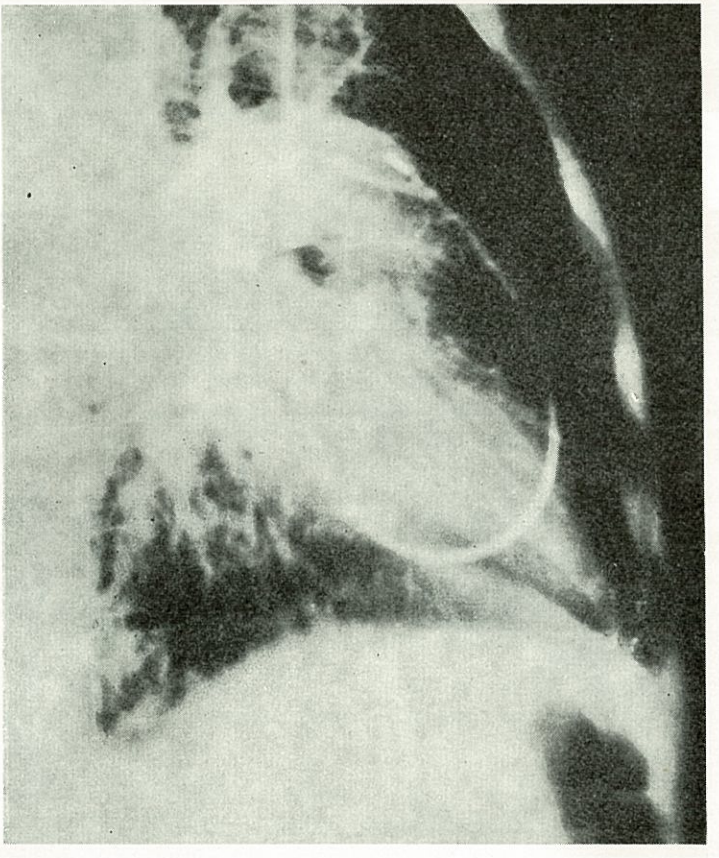

Figure 8

The patient did well during the postoperative period. Cyanosis disappeared. Dyspnea was no more present. Patient was discharged from hospital on 14th of July, 1969 with complete recovery.

The patient came for control on 20th of October, 1970. Control chest X ray showed a slight deviation of the mediastinum to the right due to right pneumonectomy. Left lung was completely normal (Figure 9).

Pathological examination confirmed the diagnosis in both cases.

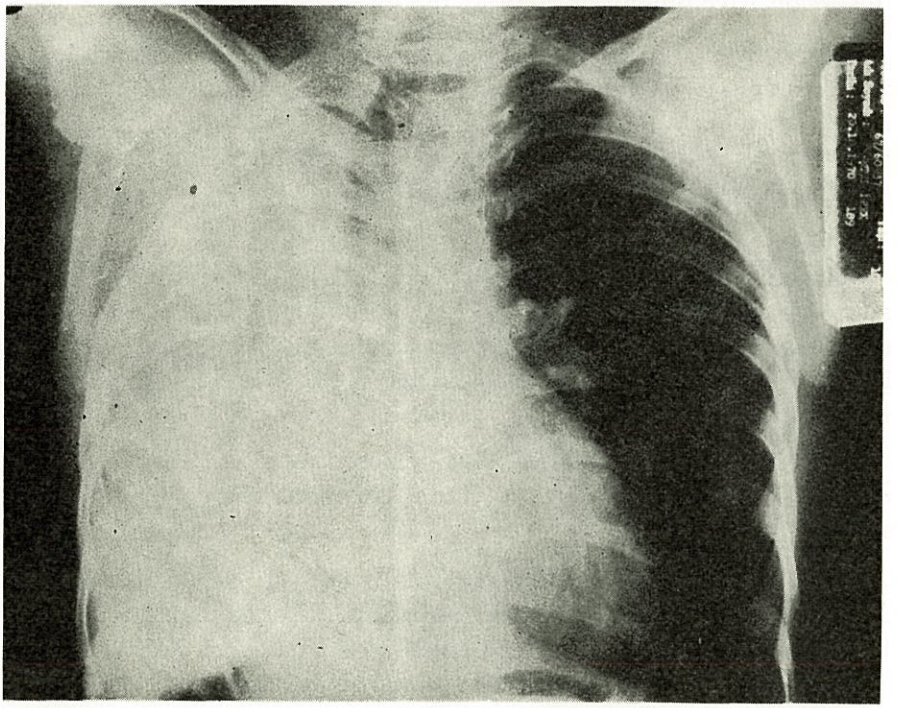

Figure 9

Discussion

Pulmonary arteriovenous fistulae were reported by Churton[3] in 1897 and Wilkens[4] in 1917, as autopsy findings. First living patient with pulmonary a-v fistulae was presented by Smith and Horton[5] in 1939. This patient had polycythemia and he was treated with multiple venesections. Successful surgical treatment was first performed in 1940 by Shenstone[7] in a 23 year-old patient, previously diagnosed by Hepburn and Dauphinee.[6] The patient had diffuse arteriovenous fistulae and pneumonectomy was done.

Pulmonary arteriovenous fistula is associated with hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber disease) in 53 % of the cases.[8] It is sometimes encountered in siblings[9 10 11] and even in the father and son at the same time.[12] Therefore it has been assumed that familial hereditary factor might play a role in the etiology of this disease. Goldman[9] stated that this lesion is a variant of hereditary hemorrhagic telangectasia and inherited as a dominant character. Usually no sex difference is present[9] but some reported that male/female ratio is 3/2.[8] As understood from the review of the literature, this lesion is commonly located at the lower lobes of the lung.

Arteriovenous communication is usually between pulmonary artery and vein. But, it has been also reported that a direct branch from thoracic aorta, bronchial arteries, pericardiophrenic artery and a. mammalia interna may also be a component of the fistula.[1] About 12 such rare cases have been reported in the literature.[1]

Hemorrhagic telangiectasia encountered in cases of pulmonary arteriovenous fistulae was explained by Sparks and Tombridge[13] with serotonin metabolism. They stated that serotonin, which can not be detoxicated in lungs because of short circuit due to fistulae, circulates in blood and creates a tendency for telangectasia in skin and mucous membranes.

Gross pathology of this lesion was demonstrated in 1950 by Lindskog et al[14] as bronchovascular vinlylite casts prepared from pathologic specimens.

Secondary polycythemia and clubbing of the fingers due to hypoxia are present in the majority of the cases. Small fistulae may be symptomless. Increase in pulmonary vascular resistance and cardiac output is directly proportional to the size of the shunt and results in cardiac hypertrophy. Even in small shunts, pulmonary vascular resistance may increase due to hypoxia and multiple capillary thrombi, as seen in cases of Tetralogy of Fallot.[8] Blood volume usually remains normal contrary to peripheric arteriovenous fistulae, where blood volume increases.[5] Dyspnea is the most commonly seen symptom. Symtoms of central nervous system, such as headache, dizziness, speech difficulties, temporary hemiparesis and convulsions may be seen in cases where a high degree of polycythemia occurs.[8 14] Epixtaxis, hemoptysis and chest pain are some other syptoms. Haemothorax may develop due to rupture of the fistulae. In some cases, a systolodiastolic murmur may be heard on auscultation of the lungs. Laboratory findings are helpful in diagnosis. ECG usually shows normal pattern, but sometimes right

ventricular hypertrophy and right axis deviation may be present. In our cases we have detected hypertrophy of left ventricle and left axis deviation. Direct chest x-rays may show some suspicious shadows due to fistulae. These shadows decrease in size during deep expiration against closed glottis (Valsalva's maneuver), and increase in size during deep inspiration against closed glottis (Müller's maneuver). Calcification may be seen in chest X rays. Most reliable method of diagnosis is the right heart catheterization and angiography.

Treatment of this malformation is surgical. Degree of surgical excision depends on the location and size of the lesion. Pneumonectomy, lobectomy or segmentectomy may be performed. Local excision may be done if fistula is small1. Treatment of diffuse bilateral lesions is still a difficult problem.

Summary

Two cases of pulmonary arteriovenous fistulae in 12 and 9 year-old boys are presented. Left lower basal segmentectomy was performed in the first case, and pneumonectomy in the second. Literature concerning pulmonary arteriovenous fistulae is reviewed. The effectiveness of surgical therapy as a radical method as shown in our cases is emphasized.

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The Effect of Extracorporeal Circulation on Magnesium Metabolism

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A great number of investigations have been done in various diseases since analytic detection of serum Magnesium was improved. In later years, Sheinman et al.[1 2] have called attention to variations of Magnesium metabolism during extracorporeal circulation. The presence of Magnesium in intracellulary compartment of human organism and its relationship to cardiovascular pathophysiology is the basic factor in this subject. In this report, the effect of extracorporeal circulation on Magnesium metabolism was investigated and the results were analyzed accordingly.

Material and Method

Twenty-three patients, underwent open heart surgery and 8 cases of closed mitral commissurotomy are included in this investigation. In 23 cases of open heart surgery, 12 were females and 11 were males. The ages of these patients varied from 18 to 54, making an average age of 32. The following operations were performed in these patients:

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Type of Operation	Number of Patients
Mitral valve replacement	13
Mitral and aortic valve replacements	7
Mitral valve replacement and Tricuspid plasty	1
Open mitral commissurotomy	1
Pulmonary valvulotomy	1

Cases of closed mitral commissurotomy were included in this series to understand better the relationship between extracorporeal circulation, anesthesia, the type of operation performed and Magnesium metabolism. In this series, male and female ratio was equal and average age of the patients was 26, ranging from 22 to 30.

All patients were on law salt diet and digitalis preparations. Diuretics were not given to these patients for at least last 3 weeks before surgery. Rygg-Kyvsgaard disposable oxygenator and Pemco Heart-Lung Machine with DeBakey pumps were used for extracorporeal circulation. Heart-Lung machines were primed with Ringer's Lactate (20 ml/kg), 80 ml of % 20 Manitol, 20 mEq $NaHCO_3$ and 50 ml of % 5 Dextrose for hemodilution. Electrolyte values were checked during operation and deficits replaced. In 10 cases of open heart surgery Magnesium was added into the circuit in amount of 2 mEq per 1000 ml Ringer's Lactate. In 13 cases, no Magnesium was added into the priming volume. Average time of extracorporeal circulation was 44 minutes, changing from 12 to 71. In cases of closed mitral commissurotomy blood was taken for Magnesium analysis just prior to operation, immediately after the operation and during the 24th hour postoperatively. In cases of open heart surgery, it was taken in the same manner and also during extracorporeal circulation. Magnesium in blood serum was analyzed depending on the photometric method, originally described by Hill[3] and Schachter.[4]

Results

In cases of closed mitral commissurotomy, the average value of serum Magnesium in the preoperative period was m: 2.12 $\pm$ 0.03 mEq/lt. In the early postoperative period this value was m: 2.09 $\pm$ 0.05 mEq/lt, and m: 2.06 $\pm$ 0.04 mEq/lt in the 24 th hour postoperatively. In this group of patients comparison between preoperative, and both postoperative serum Magnesium levels revealed no significance ($p < 0.35$ and p: 0.15). These values are shown in Tables I and II.

TABLE I

SERUM MAGNESIUM LEVELS IN CASES OF CLOSED MITRAL COMMISSUROTOMY

Patient	Age	Serum Mg^{++} Levels mEq/lt Preoperative*	Early** postoperative	Late*** postoperative
H.K.	30	2.30	2.35	2.25
H.G.	23	2.00	2.20	1.90
N.A.	25	2.10	2.10	2.20
A.P.	22	2.25	2.00	2.10
A.A.	23	2.15	2.10	2.10
H.Ç.	27	2.05	2.10	1.90
S.P.	28	2.10	2.05	2.10
A.A.	28	2.00	1.85	1.95

* m: 2.12 $\mp$ 0.03
** m: 2.09 $\mp$ 0.05
*** m: 2.06 $\mp$ 0.04

TABLE II

SERUM MAGNESIUM LEVELS IN CASES OF CLOSED MITRAL COMMISSUROTOMY

A Comparison of the Values Obtained Mg^{++} mEq/lt		
Preoperative Period I	Early Postoperative Period II	Late Postoperative Period III (24th Hour)
n : 8	n : 8	n : 8
m : 2.12 $\pm$ 0.03	m : 2.09 $\pm$ 0.05	m : 2.06 $\pm$ 0.04
	I–II $p < 0.35$	
	I–III p: 0.15	

In 13 cases of open heart surgery, containing no Magnesium in the priming volume, average preoperative control value of Magnesium was found m: 2.40 $\pm$ 0.13 mEq/lt. In all cases serum Magnesium values decreased during operation (m: 1.68 $\pm$ 0.04 mEq/lt). Comparison of these values proved a significant fall in serum Magnesium levels during operation ($p < 0.0005$). Just after operation, average serum Magnesium value was m: 1.91 $\pm$ 0.06 mEq/lt. This was also found less when compared to control values ($p < 0.0005$). During the 24th hour postoperatively, serum Magnesium level increased to m: 2.37 $\pm$ 0.11 mEq/lt. This amount, when compared with preoperative values, displayed no significance (p: 0.30). These values are seen in Tables III and IV.

TABLE III

SERUM MAGNESIUM VALUES IN CASES OF OPEN HEART SURGERY CONTAINING NO MAGNESIUM IN THE EXTRACORPOREAL CIRCUIT

Patient	AGE	Type of Operation	Duration of by-pass (minutes)	Serum Mg^{++} mEq/lt Preoperative*	Operative**	Postoperative Early***	Late****
E.G.	30	M V R	18	2.35	1.60	2.00	2.80
A.S.	50	M V R	44	3.15	1.70	1.95	2.40
A.Ç.	30	A V R–M V R	71	2.85	1.60	2.40	2.10
B.A.	34	A V R–M V R	68	2.80	1.75	2.10	2.35
E.G.	54	M V R	44	2.35	2.15	1.80	2.70
A.A.	35	M V R	35	1.95	1.50	1.95	2.75
H.Ö.	25	M V R	40	1.95	1.65	1.90	3.00
A.A.	20	A V R–M V R	72	2.40	1.65	1.90	2.05
F.M.	22	M V R–T P	65	1.90	1.60	1.45	1.35
D.A.	43	M V R	39	2.35	1.60	1.70	2.50
Y.A.	31	M V R	39	2.20	1.70	1.80	2.10
S.A.	18	P V	13	2.35	1.55	1.85	2.20
Z.A.	25	A V R–M V R	62	3.45	1.80	2.05	2.60

M V R : Mitral valve replacement
A V R : Aortic valve replacement
T P : Tricuspid plasty
P V : Pulmonary valvulotomy

* m: 2.40 ∓ 0.13
** m: 1.68 ∓ 0.14
*** m: 1.91 ∓ 0.06
**** m: 2.37 ∓ 0.11

TABLE IV

SERUM MAGNESIUM LEVELS IN CASES OF OPEN HEART SURGERY CONTAINING NO MAGNESIUM IN THE EXTRACORPOREAL CIRCUIT

A Comparison of the Values Obtained
Mg^{++} mEq/lt

Preoperative Period I	Operative Period II	Early Postoperative Period III	Late Postoperative Period IV (24th Hour)
n: 13	n: 13	n: 13	n: 13
m: 2.40 ∓ 0.13	m: 1.68 ∓ 0.04	m: 1.91 ∓ 0.06	m: 2.37 ∓ 0.11

R 2 1–11 $p < 0.0005$
I–III $p < 0.0005$
I–IV $p < 0.030$
II–III $p < 0.005$

In 10 cases, containing Magnesium in the priming volume of the extracorporeal circuit in amount of 2 mEq Magnesium per 1000 ml Ringer's Lactate, the average value of preoperative serum Magnesium was m: 2.33 $\pm$ 0.12 mEq/lt. In all cases, serum Magnesium levels fell to m: 1.54 $\pm$ 0.05 mEq/lt. during operation. Operative values of serum Magnesium compared to control values were found significant ($p < 0.0005$). During the early postoperative period serum Magnesium level was m: 2.06 $\pm$ 0.14 mEq/lt. This was insignificant when related to preoperative values ($p < 0.10$).

In the 24th hour postoperatively, serum Magnesium level increased m: 2.17 $\pm$ 0.15 mEq/lt. This was not significant either, when compared with control values (p: 0.20). A comparison was made between serum Magnesium values during cardiopulmonary by-pass and early post-operative phase.T he result obtained was significant ($p < 0.025$). These values are shown in Tables V and VI.

TABLE V

SERUM MAGNESIUM LEVELS IN CASES OF OPEN HEART SURGERY CONTAINING MAGNESIUM IN THE EXTRACORPOREAL CIRCUIT

Patient	Age	Type of Operation	Duration of by-pass (minutes)	Serum Mg^{++} levels mEq/lt Preoperative*	Operative**	Postoperative Early***	Late***
E.S.	36	O M C	12	2.70	1.58	2.85	2.80
U.G.	18	M V R	38	2.20	1.65	1.95	2.15
Ş.A.	32	A V R–M V R	55	1.90	1.60	2.15	2.10
Ö.K.	35	M V R	49	2.80	1.75	2.75	2.70
H.S.	33	M V R	35	2.70	1.45	1.95	2.00
E.B.	37	M V R	41	2.80	1.60	2.15	2.00
M.E.	32	A V R–M V R	68	2.20	1.55	1.90	1.60
S.T.	45	A V R–M V R	67	2.15	1.50	1.85	2.85
H.T.	35	M V R	45	2.15	1.65	1.85	2.00
H.A.	25	M V R	32	1.70	1.15	1.25	1.50

M V R : Mitral valve replacement
A V R : Aortic valve replacement
O M C : Open mitral commissurotomy

* m: 2.33 $\mp$ 0.12
** m: 1.54 $\mp$ 0.05
*** m: 2.06 $\mp$ 0.14
**** m: 2.17 $\mp$ 0.15

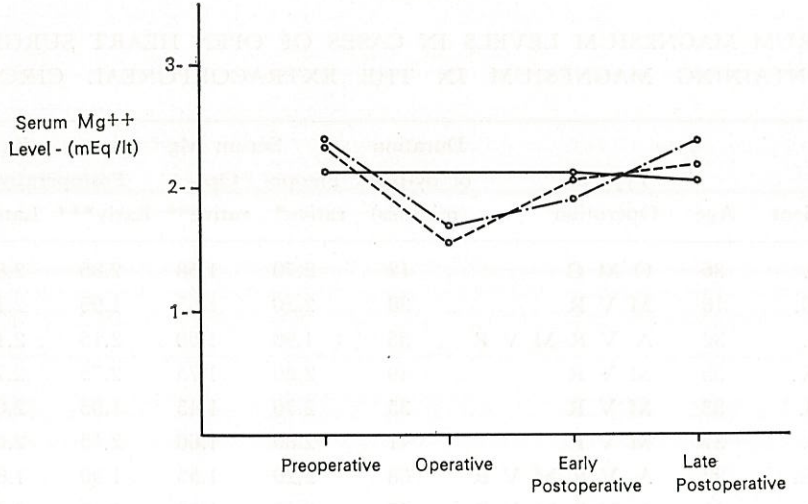
TABLE VI

SERUM MAGNESIUM LEVELS IN CASES OF OPEN HEART SURGERY CONTAINING MAGNESIUM IN THE EXTRACORPOREAL CIRCUIT

A Comparison of the Values Obtained Mg^{++} mEq/lt			
Preoperative Period I	Operative Period I	Early Postoperative Period III	Late Postoperative Period IV (24th Hour)
n: 10	n:10	n:10	n: 10
m: 2.33 ∓ 0.12	m: 1.54 ∓ 0.05	m: 2.06 ∓ 0.14	m: 2.17 ∓ 0.15

I–II	$p < 0.0005$
I–III	$p < 0.10$
I–IV	$p < 0.20$
II–III	$p < 0.25$

Differences in serum Magnesium values in this investigation are shown schematically in Figure 1.



——— Cases of closed mitral commissurotomy

—.— Cases of open heart surgery containing no Mg++ in the extracorporeal circuit

- - - - - Cases of open heart surgery containing Mg++ in the extracorporeal circuit

Discussion

The effect of extracorporeal circulation on Magnesium metabolism was investigated by Sheinman et al.[1] in 1969. They found that serum

Magnesium levels decreased significantly during cardiopulmonary by-pass, where Magnesium was not added into the priming volume. Dieter et al[5] came to the same results in 1970. In 1971, Sheinman et al[2] observed low serum Magnesium levels during the postoperative period in 5 of the 8 cases, though Magnesium was added into the extracorporeal circuit. Investigators proposed that this decrease in serum Magnesium values might be due to hemodilution and increase in renal clearance. Some other theories concerning this subject may also be proposed.

The role of renal clearance reminds us that there are relations between serum K, Magnesium levels and aldosterone activity. Hyperpotassemia and hypermagnesemia occur in nephrectomized animals.[6-8] Hypopotassemia and hypomagnesemia take place in hyperaldosterinism and malnutrition.[9-13] Hypopotassemia during extracorporeal circulation was also observed by some investigators.[5 14-17] Hyperaldosterinism has been mentioned to play a role in hypopotassemia during extracorporeal circulation.[5] Aldosterone causes magnesuria and has an effect on intestinal metabolism of Magnesium.[13] Also it is said that hypomagnesemia activates reninangiotensine II system, thus causing hyperaldosterinism.[18] Taking the above points into consideration it can be proposed that hyperaldosterinism, due to hypoperfusion of body tissues, causes hypomagnesemia during cardiopulmonary by-pass by excretion of Magnesium in urine and stool. It is also reasonable that hypomagnesemia stimulates renin-angiotensine II system and causes the continuity of hypomagnesemia and hypopotassemia, thus creating a *circulus viciosus.*

Hypocalcemia is also encountered during extracorporeal circulation.[5 18] Intracellulary Magnesium levels fall in cases of hyperparothyroidism.[19] The relationship between Magnesium and parathormone has not yet been clearly understood, but it has been put forth that parathormone increases Magnesium absorption in renal tubuli and intestines and causes Magnesium excretion from bones, thus making a negative balance of Magnesium metabolism.[20 21] As these points are considered, one can think, that secondary hyperparathyroidism caused by hypocalcemia may be responsible for hypomagnesemia during open-heart surgery.

Diuretics may also produce hypomagnesemia by increasing the excretion of Magnesium in urine.[22 23] In our cases, Mannitol added into the extracorporeal circuit might have played a role in hypomagnesemia by osmotic diuresis.

Increased tendency for digitalis intoxication in hypomagnesemia is a known fact today.[23-25] Hence, hypomagnesemia may be accountable in the production of postoperative arrythmias. Decreased incidence of postoperative arrythmias was reported by Scheinman et al[2] in the presence of Magnesium in the priming volume of the heart-lung machine. Our observation also supports the same findings.

The function of hypomagnesemia in early thromboembolic complications is also a question to be replied. It has already been reported that Magnesium activates fibrinolysis[26] and latent thrombine generation.[27] Hughes and Tonks[28] have reported hypomagnesemia in cases of acute myocardial infarction. In 1967, Durlach[29] presented Magnesium as an agent, having influence in thrombus formation, and observed good theurapeutic results of Magnesium administered orally in physiologic doses. In 1969, Dupont et al[30] mentioned the use of Magnesium in a case thrombophlebitis, possibly due to hypomagnesemia. Therefore, administration of Magnesium in cases of postoperative and postpartum thromboembolism is still a matter to be discussed.[19] We also think that the participation of hypomagnesemia in thrombo embolic complications after open heart surgery is worth invetisgation although we have not encountered such complications in our cases during the 24 hour postoperative period.

Consequently, we think that Magnesium added into the extracorporeal circuit or given to patient during the postoperative period has practically importance especially in the protection of postoperative arrythmias, no matter what the real cause of hypomagnesemia is. The exact cause of hypomagnesemia during cardiopulmonary by-pass needs further investigations depending on the determination of Magnesium in intracellulary space and urine.

Summary

Serum Magnesium levels were determined in 23 patients undergoing open heart surgery and in 8 patients of closed mitral commissurotomy. In cases of open heart surgery, serum Magnesium determinations were made preoperatively, during extracorporeal circulation, immediately after operation and during the 24th hour postoperatively. Hemodilution was made with Ringer's Lactate, a Magnesium free solution. In 10 of these cases 2 mEq of Magnesium Sulphate was added to each liter of Ringer's Lactate. During cardiopulmonary by-pass, a significant fall in serum Magnesium values was noted in all patients ($p < 0.0005$). Serum Magnesium values just after

operation were significantly low in 13 cases, where Magnesium was not added into the extracorporeal circuit ($p < 0.0005$), but close to normal in 10 cases, containing Magnesium in the priming volume of the heart lung machine ($p < 0.10$). In both groups, values of serum Magnesium during the late postoperative period were not significant compared to preoperative control values (p: 0.30 and p: 0.20).

In 8 patients in whom closed mitral commissurotomy was performed no fall in serum Magnesium levels was found during the postoperative period ($p < 0.35$). We believe that the fall in serum Magnesium levels is not directly related to anesthesia and surgery but particularly due to the use of cardiopulmonary by-pass. Hemodilution, hyperaldosterinism, hyperparathyroidism and administration of diuretics were discussed as possible factors for hypomagnesemia. The role of hypomagnesemia in the production of early postoperative complications such as arrythmias and thromboembolic phenomenon were also discussed.

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Intractable Angina Pectoris Will Subside with Electric Stimulation of Carotid Sinus Nerves

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Implantation of a specially designed electric stimulator on carotid sinus nerve for the treatment of essential hypertension was reported by Schwartz et al, and for paroxysmal tachycardia, intractable angina pectoris by other authors.[1-7] Three patients with intractable angina pectoris had operations for implantation of carotid sinus nerve stimulator to control the pain. The selection of the patients, surgical techniques and the results will be the subject of this presentation.

Material

A total of 269 patients were catheterized and coronary angiographies were done between June 1969, and August 1971, at Edgewater Hospital in Chicago, for diagnosis of coronary arterial insufficiency. Out of 269 patients, 188 were found to have some degree of coronary arterial insufficiency. Among these, 151 (18.0 %) were operated on. Of the 34 cases,11 had ventricular aneurysm resected, but 2 of these also had coronary artery by-pass, and 12 cases out of the 34 received aorta to coronary by-pass only, single or double, and 8 cases had epicardiolysis (by the other surgical group), and 3 cases (1.6 %) had implantations of carotid sinus nerve stimulator. The selection and evaluation of these cases were based on the degree of increasing intractable pain, which means the far advanced coronary artery disease. All three were taking nitroglycerin and 2 were placed on Propranolol, the other one was unable to take Propranolol. All three took some kind of analgesics or narcotics for pain. Their activities were strictly limited in their homes.

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Case 1: S. I. was a 67-year-old white male and also a known diabetic for more than 20 years. He had not worked since he had been hospitalized for the first time in 1968, for chest pain. Since the first heart attack, he had been hospitalized 8 times for recurrent attacks of severe angina pectoris and shortness of breath. On May 25, 1970, he was admitted through the emergency room because of chest pain and shortness of breath. The pain was relieved with narcotics only. Coronary angiography was carried out and it showed complete occlusion of the left anterior descending coronary artery. The circumflex artery had several segments of atherosclerotic narrowing. The right coronary artery was extremely narrowed from the origin to the distal branches. Ventriculogram was normal. Electrocardiogram revealed abnormal ST-T changes. Surgical management was considered, and on June 18, 1970, implantation of angistat (Carotid Sinus Nerve Stimulator) was done successfully.

Case 2: L. V. was a 56-year-old, white female referred to us on June 18, 1970, for further evaluation of her chest pain. The pain was felt in the midsternal region and was gripping in character. In the earlier phase of her illness, pain started with exertion and was relieved by rest, but later on she got pain even at rest and at night. The pain was not relieved even with nitroglycerin, and it recurred as frequent as 3-4 times a day and each time it lasted for about two hours. It radiated through the left arm to the elbow and subsided with analgesics. For the previous eight years, she had had shortness of breath quite often. The physical findings and laboratory tests were all normal except an elevation of the LDH. Electrocardiogram showed subendocardial ischemia and digitalis effects. The chest x-ray revealed enlarged heart to the left with prominence of the left ventricle. On July 20, 1970, coronary angiography was performed. The results were as follows: The pressure in the left ventricle and aorta were normal. Cineangiograms revealed excellent results. The left anterior descending coronary artery was occluded at its origin, possibly with some retrograde filling, the left main being open. A diagonal branch was noted, again with severe occlusive picture. There was a thread left of the circumflex coronary artery which disappeared at about the usual level of the bifurcation. The right coronary artery was quite large. However, it was occluded in two places, at the level of the bifurcation and just above it, there was approximately 80 % obstruction. Tremendous collateralization of the left side was noted with a complete filling of the sinus including the left coronary vein. Both the circumflex and left anterior descending systems were led up on the right coronary

shots. The left ventriculogram showed fairly good contraction. However, there was a small area of dysynergy at the apex. On September 1970, the angistat was implanted.

Case 3: C. R. was a 49-year-old white male patient, who was admitted on October 7, 1970. He had been complaining of sharp pain in the left side of his chest. The pain was radiating to his left shoulder and left arm and also was felt in his back sometimes. The pain was precipitated by any physical activities and subsided with rest, and also it was tolerable for 5-6 months. He had had cardiac surgery (cardiopexy) in Cleveland in February, 1968. Six months later he had intercostal neurectomy because of development of steady sharp pain in the incision site. The physical and laboratory examinations revaled no abnormality. Electrocardiogram was abnormal with myocardial ischemia involving the antero-septal and lateral wall. A coronary angiography was performed on October 10, 1970. The results were as follows: The pressure in the left ventricle and aorta was normal. Cineangiograms were of excellent quality. The main left coronary artery was patent with some indentation of the wall. A long segment of the proximal portion of the circumflex artery was occluded about 90 %. The left anterior descending coronary artery was completely occluded at its origin. The first diagonal was open, but showed 80 % occlusion in the distal third of the descending portion. The conus branch was quite large and had a wide open collateral into the left anterior descending system, which was visualized very well on the right coronary injections. The left ventriculogram showed a relatively small asynergic apical aneurysm. An angistat implantation was performed on October 20, 1970.

Method

Carotid sinus nerve stimulator (CSNS) consists of an external transmitter and implantable receiver (Figure 1). The transmitter antenna transmits radio frequency signals transcutaneously to the receiver. The received pulses are conducted to the carotid sinus nerve via the attached electrodes (Figure 2). Implantable parts are coated with transparent silicone rubber. The transmitter contains an on-off switch and rate-amplitude-regulator, which a physician has to regulate while patient's BP and ECG are monitored a month after implantation, or further re-adjustments later on. The transmitter is carried in a belt outside of the shirt and the antenna is taped on the skin at the region of the receiver.

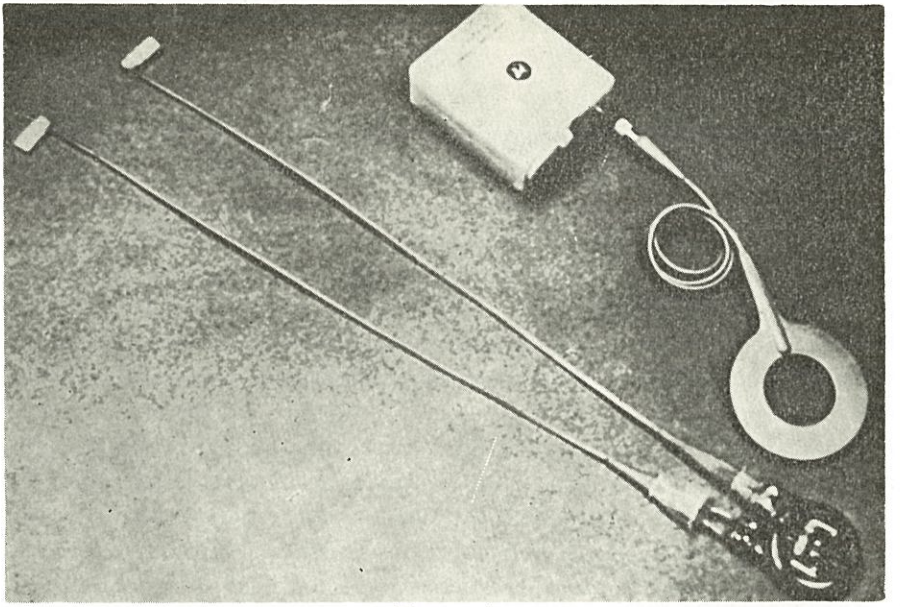

Figure 1
A picture of Carotid Sinus Nerve Stimulator (CSNS). It consists of an external transmitter and implantable receiver.

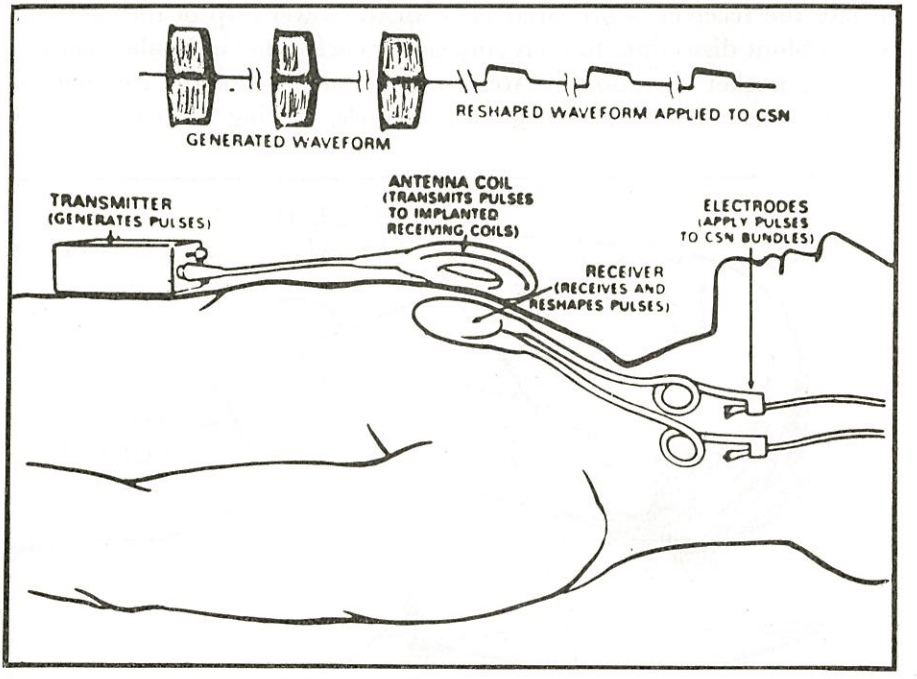

Figure 2
The transmitter antenna coil transmits radio-frequency signals transcutaneously to the receiver. The received and reshaped pulses by receiver are transmitted to the carotid sinus nerve via the electrodes.

Surgical Technique

The surgical technique now available was adopted by Schwartz et al (Figure 3). General anesthesia, atropine premedication, thiopental, N_2O+O_2 induction, endotracheal intubation and maintained halothane anesthetic are done as usual. Intra-arterial pressure and ECG are monitored throughout and during 24 hours following surgery. A transverse neck incision is made following Langer's lines at the level of the hyoid bone about 5 cm. in length towards the mastoid bone process, at the submandibular region. Sterno-cleido-mastoideus is retracted laterally and that is followed by incision of carotid fascia and the dissection of internal and external carotid arteries just 1 cm. above the bifurcation, and a narrow penrose drain is put around them for retraction. Between two carotid arteries, there are the carotid sinus nerve, blood vessels to nerve and carotid sinus and connective tissues. Platinum electrodes are placed around this bundle of tissue and fixed in position (Figure 4). There is no need for further dissection for nerve identification. After doing this on both sides of the neck, a transverse incision is made 5 cm. in length, 5 cm. below the clavicle, and a pocket large enough to accommodate the receiver is prepared beneath the lower flap of the incision. With a blunt dissection, tunnels connecting each submandibular incision with the pocket are also prepared on each side. Then the free end of the electrodes is pulled through the tunnels, leaving a small loop of

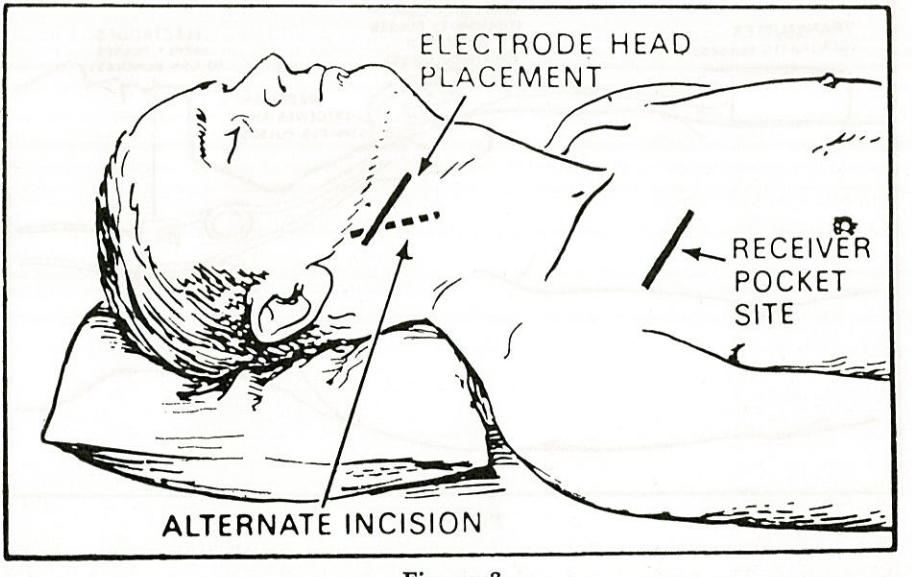

Figure 3
The suggested skin incisions for the CSN isolation and for the receiver pocket are shown.

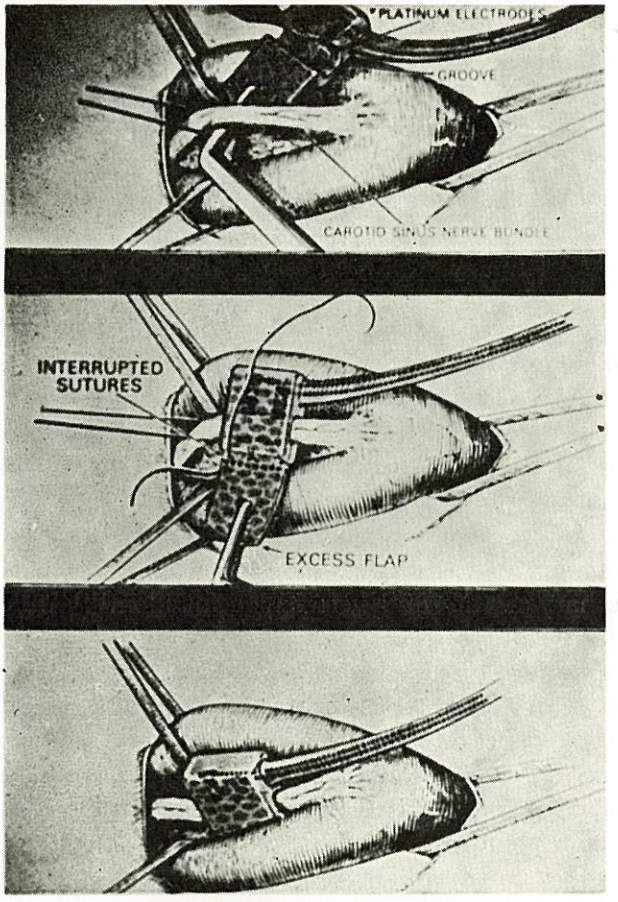

Figure 4
The technique of placing of the platinum electrodes around the carotid sinus nerve is illustrated stepwise.

extra part of catheter in the neck. Nerve stimulator tested separately on each side demonstrates that it slows the heart rate and lowers the blood pressure. It may be tested again after both catheters are connected to the receiver by applying antenna on the top of the receiver. We found it is necessary to be ready to use IV atropine, Lidocaine bolus and in solution, Levophed solution at the time of anesthesia and surgery.

Result

For the first and third patients, CSNS were turned on at the end of the 4th week of surgery. They responded to carotid sinus nerve stimulation very well. Heart rate slowed down by 10% and blood pressure down

by 10-15 mm. Hg in duration of less than 3 seconds of stimulation with 1.5 volts and 50 frequencies in second. Follow up to six months showed that the patients were much pleased with the results. Both patients did not have to take nitroglycerin any more and their activities were doubled. Unfortunately, the second patient expired on the 5th day of surgery following chest pain. Postmortem examination was not permitted.

Discussion

When the angina pectoris is intractable with medical measures, surgery is mandatory. In earlier days of this century, in order to cut-off the pain pathway, low cervical and upper thoracic bilateral sympathectomy was the surgical method of choice for intractable angina pectoris, and later on, only upper thoracic bilateral sympathectomy.[7 8] Epicardiectomy was also proved to be effective by some surgeons.[9 10] Since diagnostic coronary angiography became a routine procedure for the evaluation of chest pain, the treatment has depended on the status of coronary arteries and ventricular function.[11 12] Those patients with extensively diseased coronary arteries and intractable angina pectoris in spite of medical measures should be considered for surgical management with CSNS.[13-15] Carotid sinus nerve stimulation leads to an increase in afferant impulse traffic to central autonomic centers and results in reflex diminution of nor-epinephrine discharges to the entire cardiovascular system. Thus, arteriolar dilatation is produced, myocardial contractility is reduced, and the heart rate is slowed, all of which reduce myocardial oxygen requirements[16-19] (Figure 5). It has been known for a long time that angina pectoris can be relieved by external carotid massage, which was thought to be a method to differentiate angina pectoris from chest pain of other origins.[20 22] The carotid sinus massage is not free from complications and even death can occur.[23 24] Catecholamines were blamed for the initiation of angina pectoris for those patients who had poor blood flow in their coronaries. At the onset of angina pectoris, we can see the high level of catecholamines in blood.[25] Relationship between baroreceptor and adrenal secretion of catecholamines has been clearly demonstrated, and also control of vascular resistance and ventricular contractability by carotid sinus and chemoreceptor was proved.[26 28] Of medical measures, nitroglycerin reduces venous resistance and also causes venous pooling, decreased end-diastolic and end-systolic dimension, which lowers oxygen demands in myocardium.[29] The beta-adrenergic blocking agents decreases the arterial resistance and slows the velocity of myocardial contraction.[30 31] Dilatation of atherosclerosed coronary

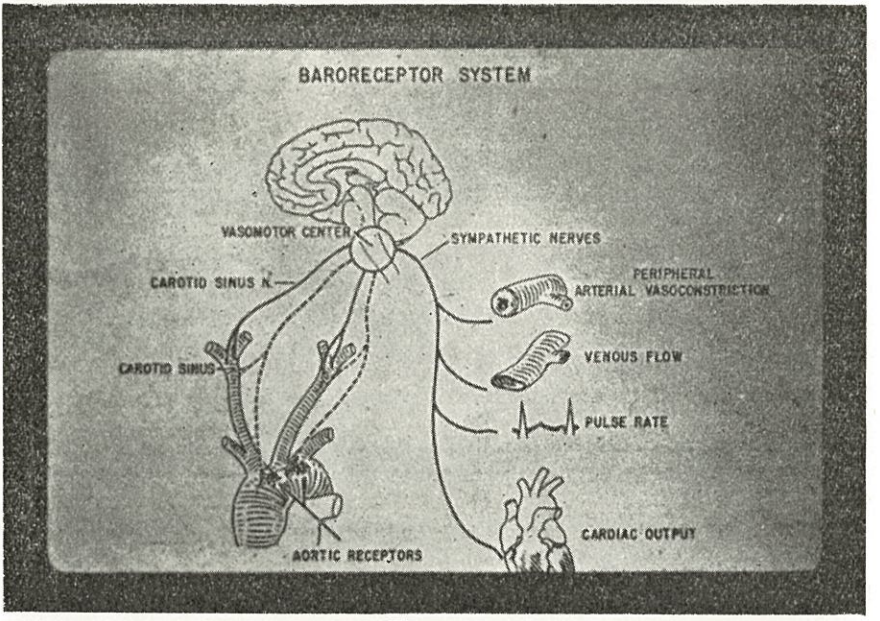

Figure 5

Baroreceptor system, which controls the cardio-vascular function is shown. Electric stimulation of CSN will reduce vascular resistance. Ventricular velocity of contractility and rate by reflex mechanism.

artery is not possible with any medical measures. Electric stimulation of carotid sinus nerve is preferred because its faster action as a reliever of pain builds up a confidence and gives the patient an increased activity and relaxation. The promising function of the Carotid Sinus Nerve Stimulation needs more time for clinical evaluation before routine use in the treatment of intractable angina pectoris.

Summary

Three patients with intractable angina pectoris were presented and discussed the results of intermittent electric stimulation of carotid sinus nerve. Incidents of implantation of carotid sinus nerve stimulator was 1.6 % among the 188 cases of angina pectoris, and they were treated one way or the other. The action of angistat was fast and reliable and it relieved the pain. The patients were able to increase their activities almost twofold.

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Whipple's Disease Associated with Multiple Myeloma: A Possible Immunologic Relationship*

Autopsy Study of a Case

Bedri Uzunâlimoğlu, M.D. **

In 1907 Dr. Whipple published an article reporting morphological as well as clinical features of a "hitherto undescribed disease" which bears his name. In his original article he described the presence of rodshaped organisms in the sections and clearly stated that their distribution was suggestive of an etiological factor.[1] His observations were confirmed eventually after employment of the electronmicroscope. Yardley and Hendrix, and Chears and Ashworth have demonstrated the bacillus-like organisms in the macrophages, as well as in the extra cellular spaces of the small intestinal tissues.[2] [3] Today, it has been generally accepted that bacteria are an essential component of the disease; however the way by which bacteria affect individuals remains to be settled.

In recent years, attention has been focused on a possible host-related abnormality which may be responsible for bacterial invasion into the intestinal mucosa and other tissues of patients who acquire Whipple's disease.[4] Although this focus has mainly been concerned with altered cellular immunity, the possible role of humoral immunity has also been studied, but less extensively.

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While multiple myeloma is considered one of the relatively rare diseases in medicine, only 150-200 Whipple's cases have been reported in medical literature.[5] [6] (It is said that there are more articles on Whipple's disease than patients reported.[6])

In the present report, a case of Whipple's disease associated with multiple myeloma will be presented and the possible underlying immunologic relationships will be discussed in the light of such an association.

To our knowledge, it is the first case of such a combination in the medical literature.

Report of A Case

A 65-year-old house-wife was admitted to the Hacettepe University Hospital with the chief complaints of constant pain in her right hip, fever, and loss of weight and strength. Five months prior to her admission she had experienced this pain in her right hip which had gradually increased in intensity. She was febrile a month before her admission. A loss of 18 kg. had occurred in the preceding 3 months. She had developed a diarrheal disease which occurred approximately 2 times a month lasting 3-4 days each time, for a year. The stool was noted to be loosetextured, pale and free of excess mucus or blood.

Examination revealed a chronicly ill, thin, pale white female with a temperature of 37.3°C and blood pressure 120/70 mmHg. Examination of the systems revealed normal findings except for a barely palpable liver, a grade 1/6 systolic murmur over the apex of the heart and a few crepitan rales over the bases of both lung fields. Palpation and percussion of the costae and sternum had been painful. The patient was complaining of the severe constant pain in her right hip; however, there had been no limitations in its active and passive motions in any direction.

She had normochromic normocytic anemia with a Hb 4.80 gr/100 ml. and albumin 5.2 % gm and globulin 2.4 % gm. Urinalysis revealed trace amount of proteinuria. E.C.G. was noted to be normal. The X-Ray examination of the bony structures was reported to show diffuse and severe osteoporosis. A purified protein derivative (P.P.D.) skin test was positive.

She became hypotensive on the thirth hospital day. Chest X-ray revealed bilateral diffuse pulmonary infiltration. She had developed mild congestive heart failure. She was treated symptomaticly. Clinical status gradually deteriorated and the patient died four days after admission.

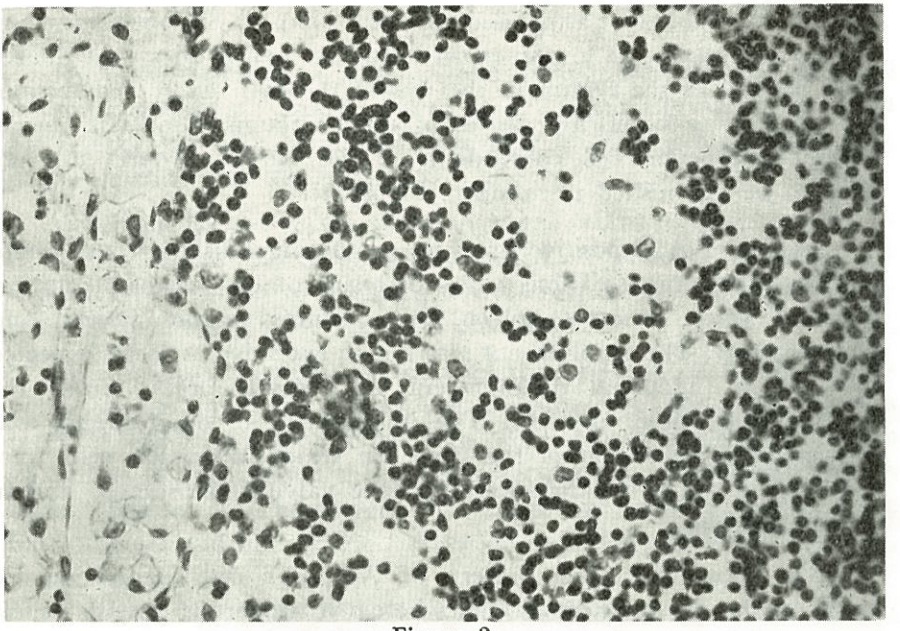

Figure 2
The same cells in and around the lymph follicule. Mesenteric lymph node. H+E Stain (x 210)

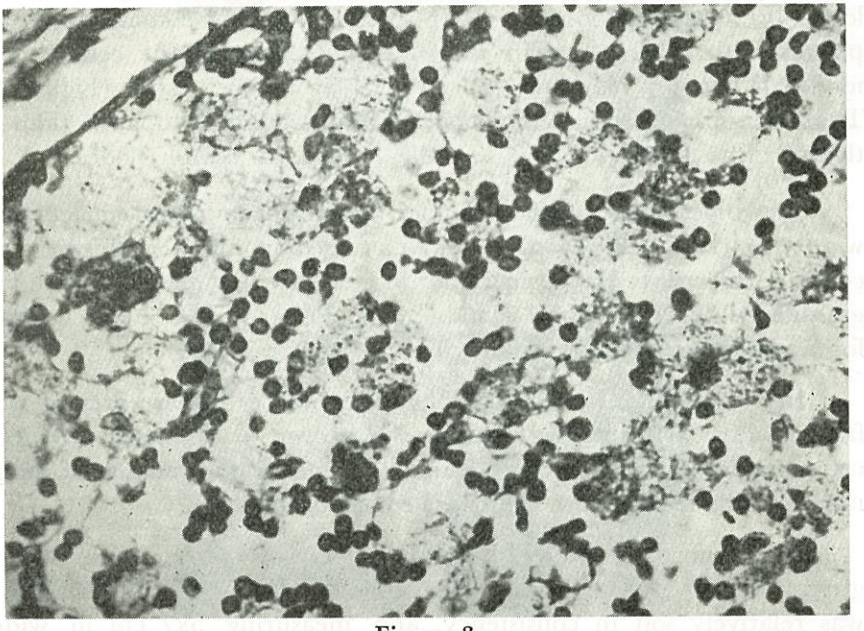

Figure 3
The P.A.S. (Periodic acid Schiff) positive granules within the macrophages. Mesenteric lymph node. P.A.S. Stain (x 336)

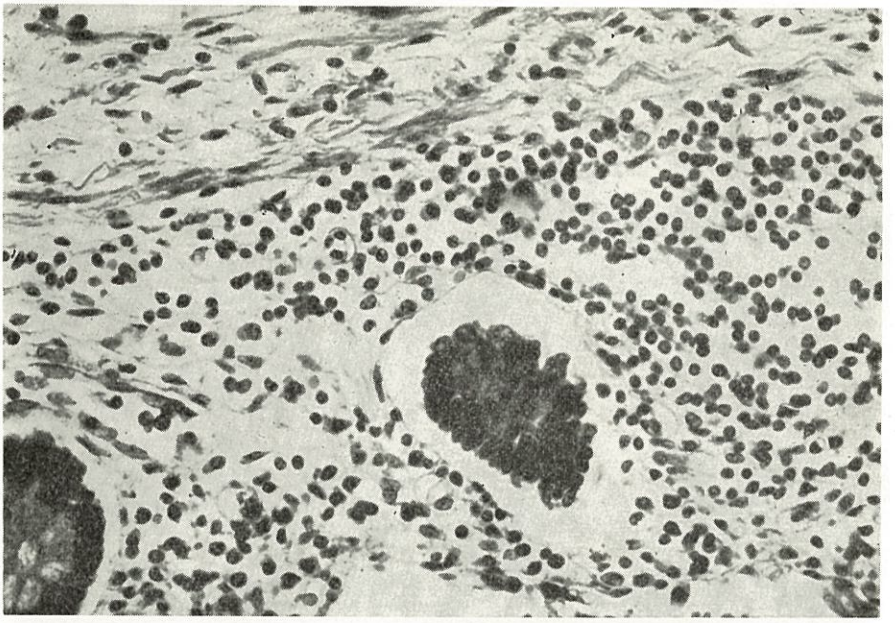

Figure 4
Plasma-lymphocytic cells in the lamina propria. H+E Stain (x 210)

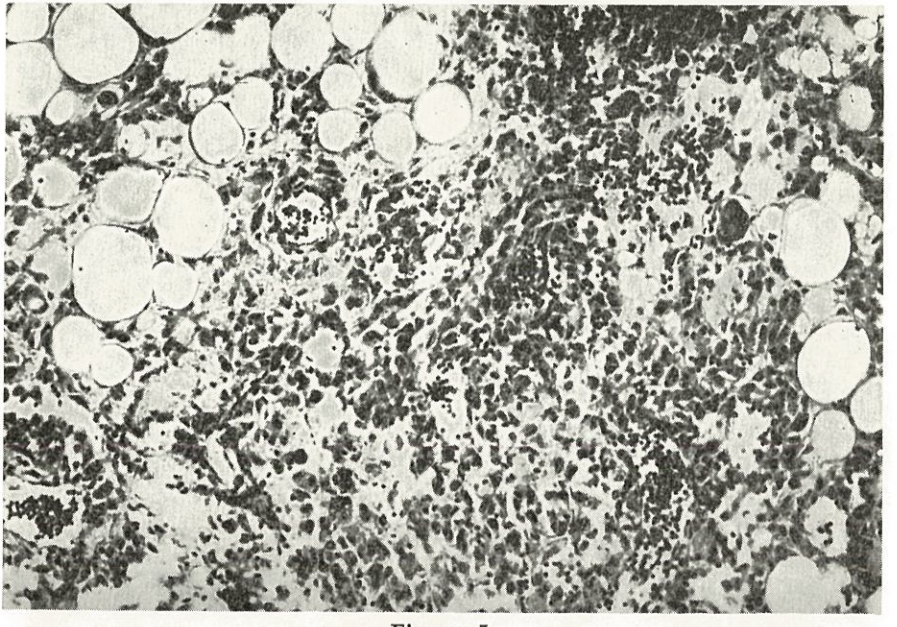

Figure 5
Histological appearance of the perirenal tumorous mass. Note the presence of macrophages with giant cells and moderate amounts of mononuclear cells in a fatty tissue. H.E. Stain (x 84)

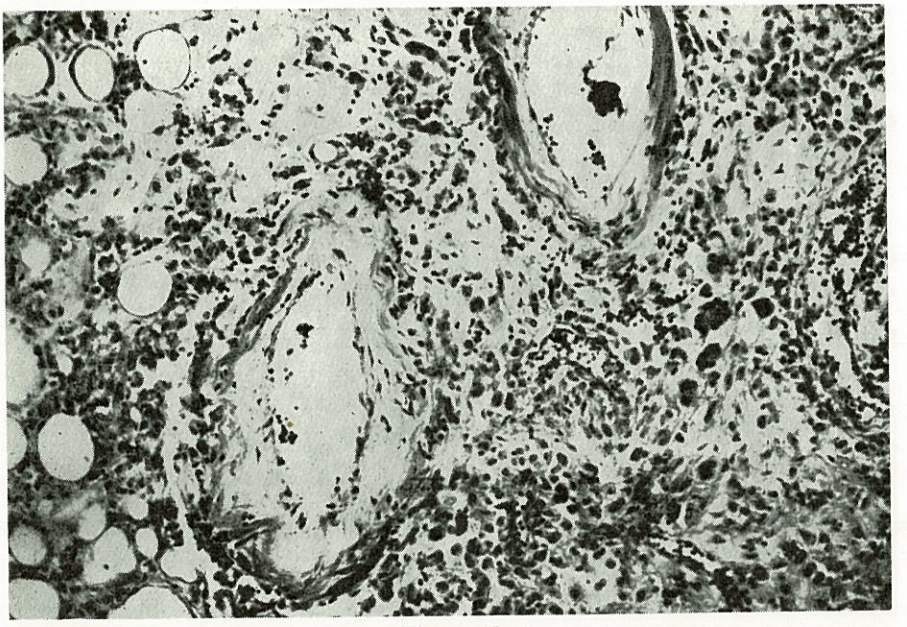

Figure 6
An another area of the same mass. Thick walled blood vessels are shown. H+E Stain (x 84)

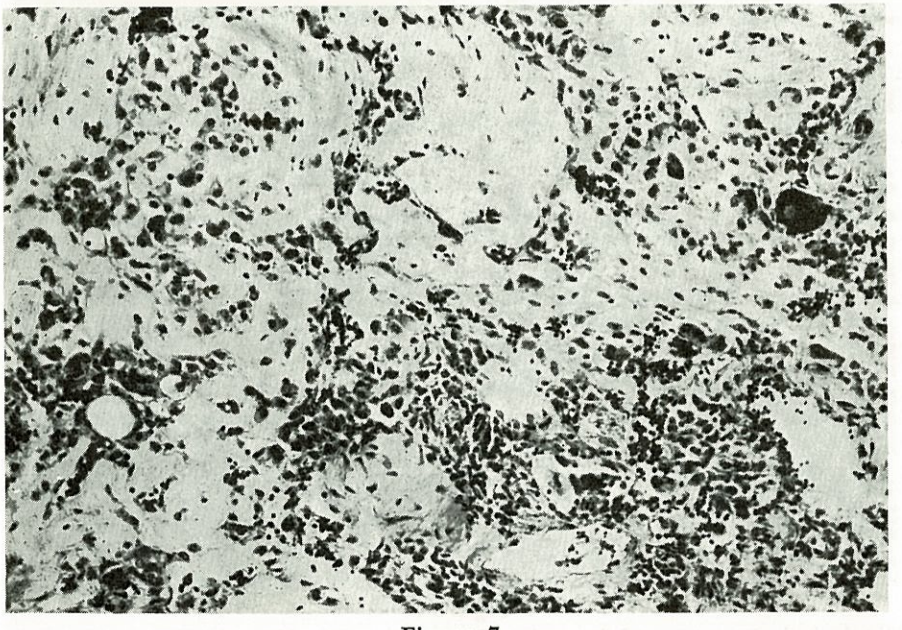

Figure 7
Homogenous slightly fibrillar material accumulated between the cells and blood vessels. Perirenal tumorous mass. H+E Stain (x 84)

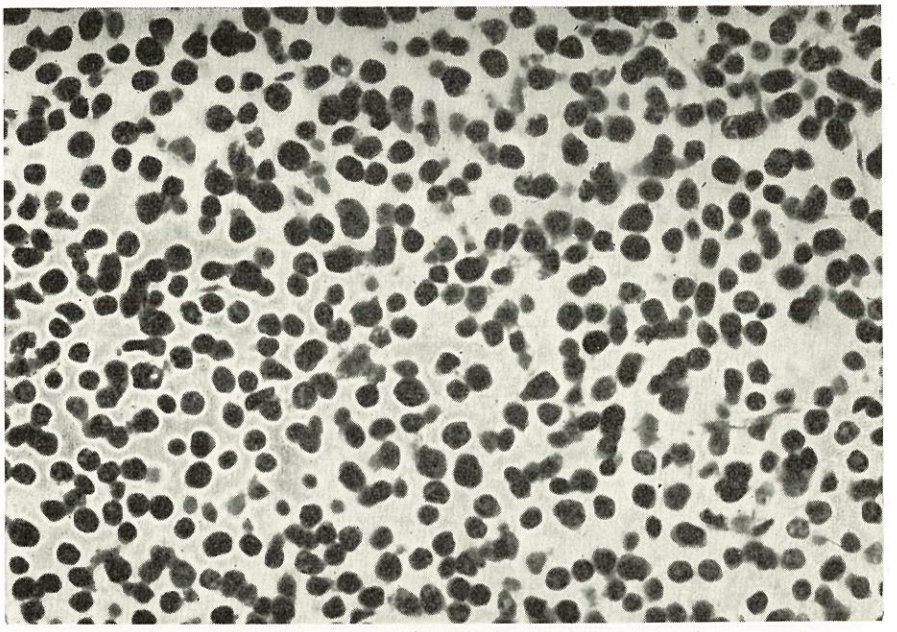

Figure 8

Histological picture of the tumor located in the right pelvis. Note the homogenous structure consisting of atypical plasma cells. H+E Stain (x 210)

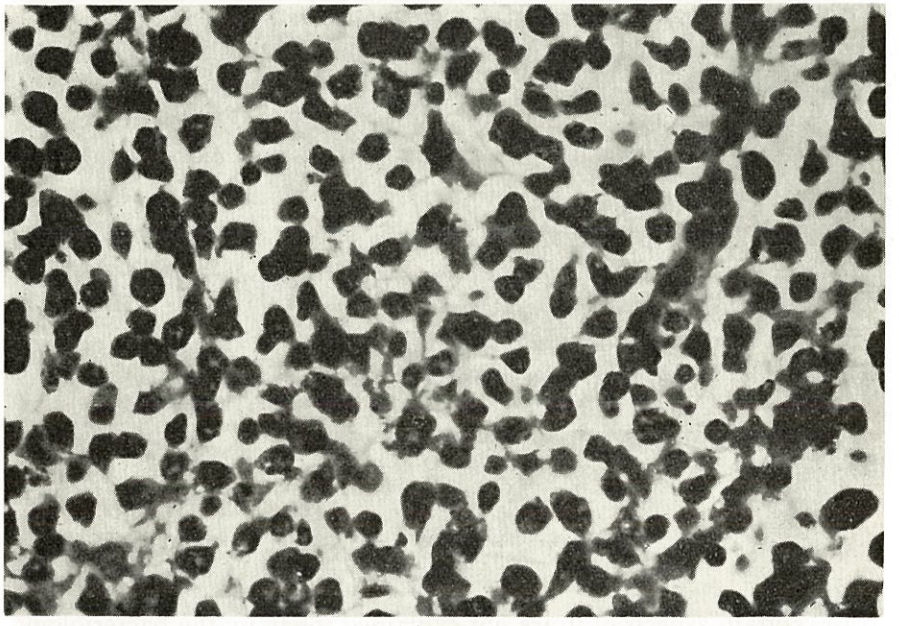

Figure 9

High magnification of the same tumor. H+E Stain (x 336)

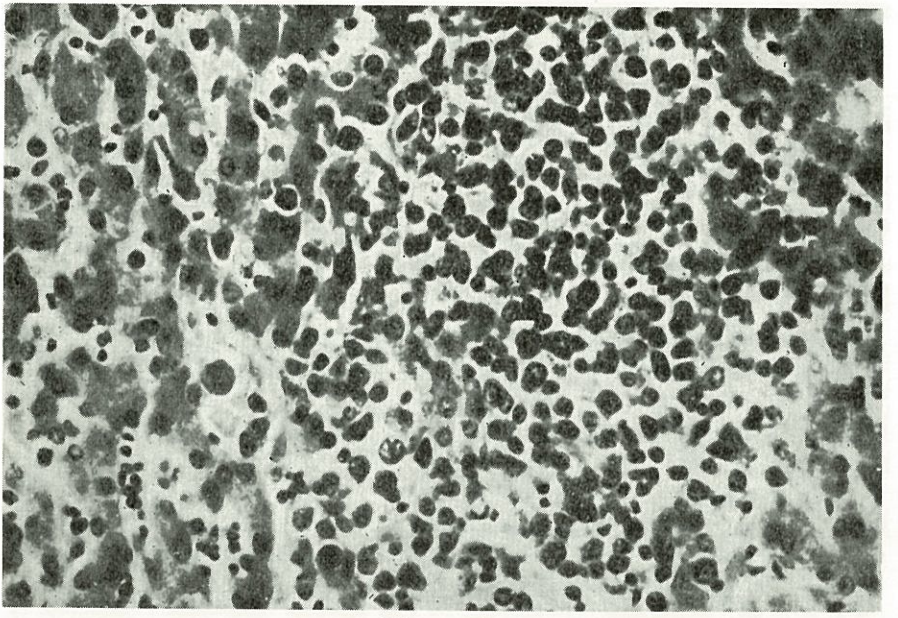

Figure 10

Myeloma cells in the liver paranchyma. H+E Stain (x 210)

Histologically, it consisted of a strikingly homogenous mass of atypical cells closely resembling plasma cells (Figures 8, 9). Generally the cells were round and oval in shape with distinct cytoplasm, eccentrically located nuclei with clumped chromatin, and sometimes more than one prominent nucleoli. While the majority showed a moderate degree of pleomorphism, occasional giant forms with two or more nuclei were noted.

Within the ribs and vertebral bones the same cells were found forming nodules with necrosis of the bony trabeculae presenting the morphologic picture of multiple myeloma. Infiltration in the liver and spleen was also demonstrated (Figure 10). The cytoplasm of these cells stained pinkish red with methyl green pyronine stain.

The immediate cause of death was attributed to the bilateral diffuse pneumonia.

Comment

Despite the good evidence for involvement of bacteria in Whipple's disease, certain aspects of it are not explained solely by the bacteriologic hypothesis. The presence of an abundant amount of structures elec-

tronmicroscopically similar to known bacteria in the tissues infiltrated by P.A.S. (Periodic-acid-Shiff) positive macrophages in the active stages of the disease and their disappearance during the clinical remission, following effective antibiotic treatment, strongly suggests an etiologic relationship.[7 8] On the other hand, its extreme rarity, and prediliction for Caucasian males weaken such an assumption.[4 6] Furthermore, the morphological features are not those of an ordinary bacterial disease. The picture has been further complicated by the failure to isolate a single common bacterial species from all patients.[1 9 10 11] Therefore, it has been suggested that the host factors might be as important as the microorganism itself.[4]

Yardley and Hendrix have suggested the abnormality of one or more enzymes in the macrophages to be responsible for destruction of the ingested bacteria.[2] However, subsequent histochemical studies failed to show abnormalities of a variety of lytic and other enzymes in P.A.S. positive macrophages.[12 13]

The striking decrease observed in plasma-lymphocytic constituents in the lamina propria in patients with Whipple's disease has raised the possibility of an underlying immunologic deficit.[14 15 16] This idea has been supported by the demonstration of impaired lymphocyte response to phytohaemagglutinin stimulation in vitro in one case. On the basis of this observation, Maxwell et al have suggested that altered cellular immunity might well account for such a deficit.[4] Diminished response of lymphocytes to antigenic stimuli may well be taken as an evidence of altered cellular immunity; however the possible significance of decrease in number of plasma cells which are known to produce immunoglobulins, mainly IgA, is not well understood.[17 18] It should be recalled at this moment that the plasma-lymphocytic cells in the small intestine was normal in number in the case under discussion. It seems reasonable to assume that if they are involved in some way in the etiology of Whipple's disease, their decisive or altered function might well be as responsible for the immunologic deficit as their diminished numbers, in reported cases. It is interesting to note that circulating lymphocytes have also been shown to fail in synthesizing nucleic acids after phytohaemagglutinin stimulation in myeloma-patients.[18] In a recent report lymphocyte transformation test was normal or nearly so in three cases in whom lymphocytes in the small intestine were also noted to be decreased in number as were mainly the IgA producing plasmocytes.[18] This observation is in accord with a previous one.[20]

It was demonstrated that when using a lymphocyte culture of patients with celiac disease, the patient's plasma was replaced with homologous plasma from a healthy donor, the development of desoxyribonucleic acid (D.N.A.) synthesizing blast cells was increased and other features of a normal P. H.A. culture returned. At the completion of the cross-exchange, the placement of the control cells in the patient's plasma, retarded the normal response of the control cells to phytohaemagglutinin.[21] This observation indicates that there have been humoral factors in the plasma influencing the lymphocyte response. This also clearly shows the fact that the immunologic mechanisms involved in host response to antigenic stimuli might be much more complex than to allow establishment on the basis of observations made on a limited number of patients or by the few tests available.

On the other hand, it has been shown that infections were more frequent in patients with multiple myeloma than in a total clinical center population. Antibody response to antigen administration was found to be impaired in all patients and impaired antibody response was related significantly to susceptibility to infections.[22] The time at which immune impairment occurs in this disease is not established, but there is evidence to suggest that it often occurs by time the other symptoms appear. Studies with experimental myeloma in mice indicated that tumors in previously normal mice were capable of producing an impairment in antibody response to antigen administration.[23 24] It has been also demonstrated that the quantity of tumors is a factor in determining the degree of immune impairment.[23 24]

Impaired antibody response and low levels of normal gamma globulin components have indicated that reduction of normal plasma cell function is a prominent feature in multiple myeloma.[22] Quantitative studies revealed that mean levels of polyclonal Igs were reduced to 10-30 % of the normal in multiple myeloma.[25] In subsequent larger series, subnormal levels of one or other polyclonal Igs occurred in about 90 % of myeloma patients.[26]

In a recent study, a deficiency of one or more classes of polyclonal immunoglobulins measured occurred in 82 % of a total of 80 subjects, (72 with multiple myeloma 8 with macroglobulinemia).[27] The incidence of immunoglobulin deficiency appeared to be similar in myeloma associated with monoclonal IgG, IgA or Bence Jones protein only.[27]

While accumulated evidence in medical literature has suggested some form of immunologic deficiency in the etio-pathogenesis of Whipple's disease, the exact nature of the abnormality is far from

being settled. On the other hand, immunologic deficiency has been clearly established as accompanying multiple myeloma as outlined above.

It is suggested that immunologic impairment in multiple myeloma could be responsible for the development of Whipple's disease in this presented combination. However the study of many more patients seems to be essential to understand the nature of the assumed basic immunologic defect.

Summary

A case of Whipple's disease associated with multiple myeloma is presented. It is the first report of such a combination in medical literature. Current concepts concerning etio-patogenesis of Whipple's disease are discussed. A possible basic immunologic relationship is suggested in the light of such an association.

Acknowledgment

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The Value of Diagnostic Laparotomy in Hodgkin's Disease*

Ekrem Müftüoğlu, M.D. / Özen Arat, M.D.*****

The determination of the correct stage of Hodgkin's disease is of utmost importance with regard to the treatment and the prognosis of the disease.[1] A number of tools have been employed to determine the stage of this disease; however, the conclusions regarding clinical stages may be inaccurate because of the inherent limitations of staging techniques. For this reason, staging laparotomy has been performed in certain centers since 1968. However, there is no general agreement as to which patients should be subjected to laparotomy, at the present time.[2-9]

In this paper two cases with Hodgkin's disease who have been subjected to diagnostic laparotomy in 1971 at Hacettepe Hospital will be reported, and the importance of laparotomy for accurate staging will be discussed.

Case Reports

Case 1: İ. K. (292502), a 41-year-old man was admitted to the hospital because of masses at both sides of his neck. He noticed these masses about one year ago. Three months before his admission, masses of 3-4 cm. diameter developed in the left axillary region. He had had pruritus for two years.

On physical examination, the temperature was 36.7°C, the pulse 90, and the blood pressure was 130 systolic, 90 diastolic. A firm, non-tender, fixed mass of 5 by 8 cm. in the left cervical region and three

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lymph nodes of 3 by 3 cm., 4 by 3 cm., 2 by 1 cm. in the right cervical region were felt. There was a firm, mobile, tender mass of 4 by 5 cm. in the left axillary region and a lymph node of 2 by 2 cm. in the right axillary region.

The urine was normal. The hemoglobin was 13.5 gr. per 100 ml; the hematocrit was 38 per cent; the white-cell count was 3.000-9.200, with a normal differential count. The sedimentation rate was 90 mm. per hour. The blood urea nitrogen was 10 mg., the fasting glucose 59 mg. and the serum protein 6.2 gr. (the albumin 4 gr., and the globulin 2.2 gr.) per 100 ml. The glutamic oxalacetic transaminase (SGOT) was 25 U; the glutamic pyruvic transaminase (SGPT) was 12 U; the alkaline phosphatase was 4-5.4 Bodansky U. The bromsulfalein retention was 1.5 per cent at 45 minutes. The skeletal survey was negative for metastasis. An upper gastrointestinal series and barium enema examination were normal. An intravenous pyelographic examination was negative. A liver scan showed multiple infiltrative or space-occupying suspicious defects. A needle biopsy of the liver revealed normal histology. A biopsy of one of the enlarged lymph nodes was reported as disclosing histological features consistent with Hodgkin's granuloma (mixed cellularity type of modified Lukes and Butler classification). A lower extremity lymphangiogram revealed enlargement of inguinal lymph nodes, and a micronodular architecture and central filling defects in the lymph nodes of the left para-aortic chain at the level of the 2nd and 3rd lumbar segments and of the right para-aortic chain at the level of the 2nd and 5th lumbar segments. These changes were reported as compatible with either lymphoma or chronic granulomatous inflammation.

An abdominal laparotomy was performed to determine the correct stage of the disease. The laparotomy disclosed a grossly normal liver and para-aortic lymph nodes. A liver biopsy was negative with regard to lymphamatous infiltration on histological examination. A proliferation of reticuloendothelial cells in the histological examination of para-aortic lymph nodes was noted and was reported as sinus histiocytosis. Splenectomy was not performed in this case because of technical problems.

Case 2: S. Y. (120098), a 28-year-old man was admitted to the hospital because of a mass on the right side of his neck. Nine years ago, he noticed movable masses of 1-2 cm. diameter at the right posterior cervical region. These masses reached diameters of 3-4 cm. in two years and 7-8 cm. in four years. More masses developed at the same

region and were matted together. A diagnosis of Hodgkin's granuloma was established at Hacettepe Hospital 3 years ago, based on a lymph node biopsy and the patient was regarded as stage I. He was treated with high-dose radiation therapy. One month before his second admission, masses of 1-2 cm. diameter developed at the left side of his neck. At the same time, a hard, non-mobile mass developed at the right side of his neck, extending to the right submaxillary and postauricular regions.

On physical examination, the temperature was 36.5°C, the pulse 72, and the blood pressure 100 systolic, 70 diastolic. Multiple firm, mobile, non-tender lymph nodes were palpable in the right supraclavicular, right anterior and posterior cervical regions. There was a hard, tender, fixed mass of 15 by 20 cm. extending from the right preauricular and postauricular regions to the submaxillary and upper cervical regions. Lymph nodes of various diameters were felt in the left preauricular and postauricular, left supraclavicular, left anterior and posterior cervical, and both axillary regions. The edge of the liver was felt just below the right costal margin. The spleen was palpable 2 cm. below the left costal margin.

The hemoglobin was 15.75 gr. per 100 ml. The hematocrit was 48 per cent. The white-cell count was 3,600-12,000, with a normal differential count. The urine was normal. The blood urea nitrogen was 18.5 mg., the fasting glucose 73 mg., the serum protein 6.7 gr. (the albumin 4.7 gr. and the globulin 2 gr.), the uric acid 6.3 mg. per 100 ml. The glutamic oxalacetic transaminase (SGOT) was 20 U and the glutamic pyruvic transaminase (SGPT) 12 U. A barium enema examination and an intravenous pyelographic examination were normal. A liver scan revealed no abnormality. A needle biopsy of the liver did not show involvement with the disease. A lower extremity lymphangiography disclosed bilateral inflammatory changes in the inguinal lymph nodes, and central filling defects and a reticular pattern in the right inferior para-aortic lymph nodes and in one lymph node of the left para-aortic chain at the level of the 2nd lumbar segment. These changes were reported as possibly consistent with lymphoma or chronic granulomatous inflammation.

An abdominal laparotomy was performed in order to determine the extent of the disease. On gross examination, the spleen was enlarged and the liver showed multiple nodules. Biopsies of the liver and one of the para-aortic lymph nodes, and splenectomy were performed. Histological examination revealed involvement of the liver, spleen and para-aortic lymph node with Hodgkin's disease.

Results

Although the lymphangiogram was positive in the first case, the laparotomy did not reveal involvement of the para-aortic lymph nodes with Hodgkin's disease and this patient was concluded as stage II B. However, because splenectomy was not performed in this case, the exact determination of the correct stage of this patient has not been possible.

In the second case, the laparotomy confirmed the lymphangiographic findings. However, although the liver biopsy was negative preoperatively, it was positive postoperatively. Thus, the stage of this patient was changed from III A to IV A, as the result of surgical findings.

Discussion

Accurate staging is the most important ingredient to the proper planning of treatment in Hodgkin's disease, since treatment depends on the stage and since rational therapy may cure this disease.

Classically, Hodgkin's disease is classified into four clinical stages.[10*] In order to determine the correct stage of this disease, a number of staging techniques must be employed.[2] In addition to the history and complete physical examination, extensive hematologic, biochemical and radiographic procedures are performed. Posterioanterior and lateral chest roentgenograms, chest tomography, metastatic skeletal surveys and bone scans, liver function tests, renal function tests, liver and spleen scans are done. If abnormalities of the liver are found on scan or biochemically (if a single liver function test is abnormal), percutaneous needle biopsies of the liver are performed. Bone marrow aspiration is generally done but is inadequate, because foci of Hodgkin's disease cannot easily be aspirated and requires bone marrow biopsy. Using multiple needle biopsy sites seems more adequate. In addition to these

* Stage I – Disease limited to a single anatomical site or to two contiguous anatomical sites on the same side of the diaphragm.

Stage II – Disease that involves more than two anatomical sites or two non-contiguous anatomical sites, but still limited to one side of the diaphragm.

Stage III – Disease on both sides of the diaphragm but limited to the lymphatic system, including the spleen and the Waldeyer ring.

Stage IV – Disease with involvement of some extralymphatic tissue (liver, bone marrow, or any of the other organ systems such as the gastrointestinal or genitourinary tracts) in addition to the lymphatic involvement.

Stages II, III, IV are further classified into A and B categories depending on absence or presence of constitutional symptoms (fever, night sweats, pruritus).

procedures, cutaneous anergy should be documented. Upper gastrointestinal series, barium enema, and intravenous pyelographic examinations should be performed.

Bilateral lower extremity lymphangiography is used to determine the involvement of the retroperitoneal lymph nodes below the level of the second lumbar segment. Inferior vena cavogram may prove useful to determine the involvement of the lymph nodes above this segment. The pattern in various types of lymphoma as demonstrated by lymphangiography has been described as characteristic features.[1] In addition, post-lymphangiography graphies and repeat lymphangiographies provide valuable information about regression, recurrence or extension of the disease. In one study, lymphatico-venous communications on lymphangiography have been detected, and it has been pointed out that the prognosis is poor in such cases.[11]

The staging techniques described are frequently inadequate for the determination of the correct stage of the disease, as were in our cases, and surgical abdominal exploration should be considered as a staging procedure in such patients. During laparotomy, splenectomy, liver biopsies (wedge and needle), para-aortic lymph node biopsies and bone marrow biopsy should be performed. In young females, oophoropexy is performed during laparotomy, in order to preserve ovarian function after X ray treatment.[2]

Since 1968, Kaplan et al have performed the staging laparotomy on all patients with Hodgkin's diseases. Among 100 patients evaluated, 14 patients had false positive liver evaluations and 2 had false negatives. Eight patients had false positive spleen evaluations and 20 had false negatives. The lymphangiogram was accurate in over 80 % of the cases. However, routine lymphangiography does not give information about the involvement of the splenic hilar lymph nodes.[3]

A group of 65 selected patients with Hodgkin's disease, subjected to laparotomy, was reported by Gladstein et al.[4] A correlation was noted between the presence of constitutional symptoms and the extension of the disease below the diaphragm. No involvement of the liver was found without involvement of the spleen. In more than half the cases with splenic disease, the liver was also involved. Splenic disease was demonstrated in 76 % of patients with palpable spleens and in 50 % of patients with non-palpable spleens. Even if the liver biopsy is negative, microscopical foci cannot be eliminated; thus, these investigators suggest that a positive histology in the spleen may indicate the involvement of the liver.

The same investigators performed diagnostic laparotomy on 50 unselected patients with Hodgkin's disease.[5] Of 5 patients whose liver function tests were abnormal, only one had a positive biopsy. Of 45 patients whose liver function tests were normal, 3 had positive biopsies. Splenic disease was detected in 7 of 12 patients with splenomegaly, and in 10 of 38 patients without splenomegaly. For this reason, splenomegaly is not necessarily an indication of splenic disease, and non-palpable spleens may harbor the disease. Although intra-abdominal involvement with the disease was suspected in 23 patients preoperatively, it was proved in only 15, postoperatively. Of 13 patients whose liver, spleen and abdominal lymph node evaluations were regarded as normal at the result of all available diagnostic modalities before surgical exploration, 5 patients revealed histological evidence of the disease after laparotomy. Of 17 patients with negative lymphangiograms, non had positive para-aortic lymph node biopsies; however, 2 of these patients had involvement of the splenic hilar lymph nodes. In summary, of 27 patients with no apparent clinical disease below the diaphragm, 8 patients (30 %) had involvement of the spleen and splenic hilar lymph nodes. Gladstein et al suggest diagnostic laparotomy as a routine staging investigation.

Johnson's opinion, however, is that surgical exploration of the abdomen is not routinely justified for disease clinically limited to lymph nodes above the diaphragm.[8] Exploratory laparotomy is routinely indicated only for patients presenting with clinical evidence of disease in the upper abdomen. A positive lymphangiogram or splenomegaly or both, implies a high risk of unrecognized disease in either the liver or atypically located lymph nodes, such as the porta hepatis or mesentery lymph nodes, outside the standard radiation fields. In these cases, routine laparotomy is justified because it frequently alters the therapeutic approach. In addition, there are two reasons laparotomy should be routinely performed when splenomegaly is noted on pre-treatment evaluation. The first is that excessive radiation exposure to the left kidney and to lower lobe of the left lung is technically unavoidable when the spleen is grossly enlarged. And second, these patients frequently have involvement of the liver with the disease. A number of other advantages can be added to these about the value of splenectomy in patients with Hodgkin's disease.[12] These patients tolerate chemotherapy better if splenectomy is performed previously. Splenectomy acts favorably on the autoimmune hemolytical anemia and thrombocytopenia, which are quite frequent in patients with Hodgkin's disease. Also, the risk of hypersplenism is discarded.

Since Johnson suggests prophylactic abdominal irradiation, he claims that surgical exploration of the abdomen is not routinely justified for disease clinically limited to lymph nodes above the diaphragm. This conclusion is based on the observation that unsuspected disease in sites outside the standard prophylactic treatment fields is extremely uncommon and with proper field localization, irradiation of non-enlarged spleens is an effective and safe alternative to splenectomy.

In another study, 9 patients with Hodgkin's disease had positive lymphangiograms preoperatively, but in 4 of these 9 patients, surgical biopsies did not confirm the lymphangiographic findings. In one patient with a positive vena cavogram, laparotomy findings were negative. Of 6 patients who were evaluated as having splenic involvement, only 4 patients revealed histological evidence of splenic disease. On the basis of laparotomy findings, the stage of the disease was changed in 3 patients, and the extent of lymphomatous involvement in 7.[6]

Previously, Aisenberg suggested the staging laparotomy for only selected patients and stated the following indications for the basis of selection:[12]

1. Presence of constitutional symptoms.
2. Positive lymphangiography.
3. Palpable spleen.
4. Two of the following minor signs:
    a. Splenomegaly roentgenologically.
    b. Equivocal lymphangiogram.
    c. Palpable liver.
    d. Bromsulfalein retention.
    e. High alkaline phosphatase.
    f. Mixt cellularity or lymphocytic depletion type histology.

Recently, the same investigator has stated that he has altered his view, and suggests laparotomy for almost all cases with Hodgkin's disease.[7]

Although laparotomy findings have changed the stages of both of our patients, it seems difficult to reach a definite conclusion based on just two cases. However, we believe that performing the staging laparotomy routinely on all patients with Hodgkin's disease would be logical, except for cases evaluated definitely as stage IV.

Summary

In this paper, 2 patients with Hodgkin's disease who were subjected to diagnostic laparotomy have been reported. The first patient was evaluated as stage III B preoperatively, but the laparotomy findings revealed stage II B disease. However, the evaluation of this patient may be considered inadequate, since splenectomy was not performed in this case. The second patient was regarded as stage III A before laparotomy, but the postoperative histological findings disclosed stage IV A disease. The value of diagnostic laparotomy in Hodgkin's disease has been discussed.

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Bioengineering: A Better Intrauterine Device Design, according to the Cellular Basis of Contraception*

Nuri Sağıroğlu, M.D., F.I.A.C.**

It was demonstrated that an intrauterine device acts as a contraceptive through macrophages which are the mobile and phagocytic cells of the reticuloendothelial system. This means that, an intrauterine device, as a giant foreign body or antigen, stimulates the defensive power of the host. As a specific foreign body reaction, the precursors of macrophages and other blood leukocytes enter the endometrial cavity and encircle the foreign body. This reaction commences immediately, following the insertion of the device. During the first week of the device use, polymorphonuclear leukocytes subside, but mononuclear cells, the precurcors of macrophages, increase. On the eight day following insertion of the device, the cell population in the uterine cavity is almost entirely dominated by well developed macrophages. From the number of cells observed in one single slide (up to 100,000) it was easily estimated that there are many millions of macrophages encircling the device (Figure 1). The intrauterine device thus becomes like a giant living structure with many millions mouths.[1 4]

It was also demonsrated that macrophages phagocytize advancing spermatozoa and destroy them within 18 hours following intercourse. According to their phagocytic and destructive power (one macrophage may engulf and kill 41 spermatozoa in one operation), there are always

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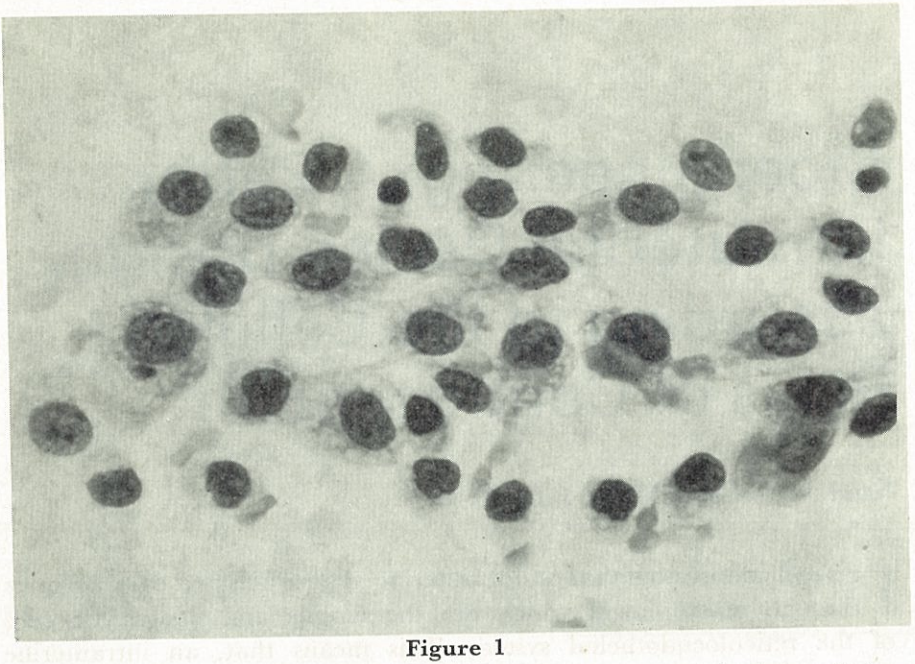

Figure 1

Macrophages are observed in the close vicinity of an intrauterine polyethylene device, Lippes loop, Loopal smear 1000X. (IUD mechanism explained by Turks, Science Journal, Vol. 6, No. 7, pp: 12-13, July, 1970).

enough macrophages in number and in capacity to destroy all of the spermatozoa ejaculated into the vagina.[5 7] Thus it is woman's own defensive power, the heavy brigades of macrophages which prevent pregnancy by the destruction of spermatozoa in the uterine cavity (Figure 2).

Theoretically, it may be accepted that some spermatozoa pass the deadly barrier in the uterine cavity, which can be compared to a piranha infested pool, and may reach the ovum. In such a case if the spermatozoa is not defected with the lysosomal enzymes poured into the endometrial fluid[8] a fertilization may take place, but it must still come into the endometrial cavity for implantation.

The fertilized ovum itself is now also a foreign body having part of the father's genetic material introduced by the spermatozoa. Therefore, it is also the subject of encirclement and phagocytosis by the macrophages whenever it enters into the endometrial cavity (collective phagocytosis), (Figure 3).

In the literature it was shown that even the best intrauterine device which has been used presently results in at least 2 percent of pregnancy, device in situ.[9–13]

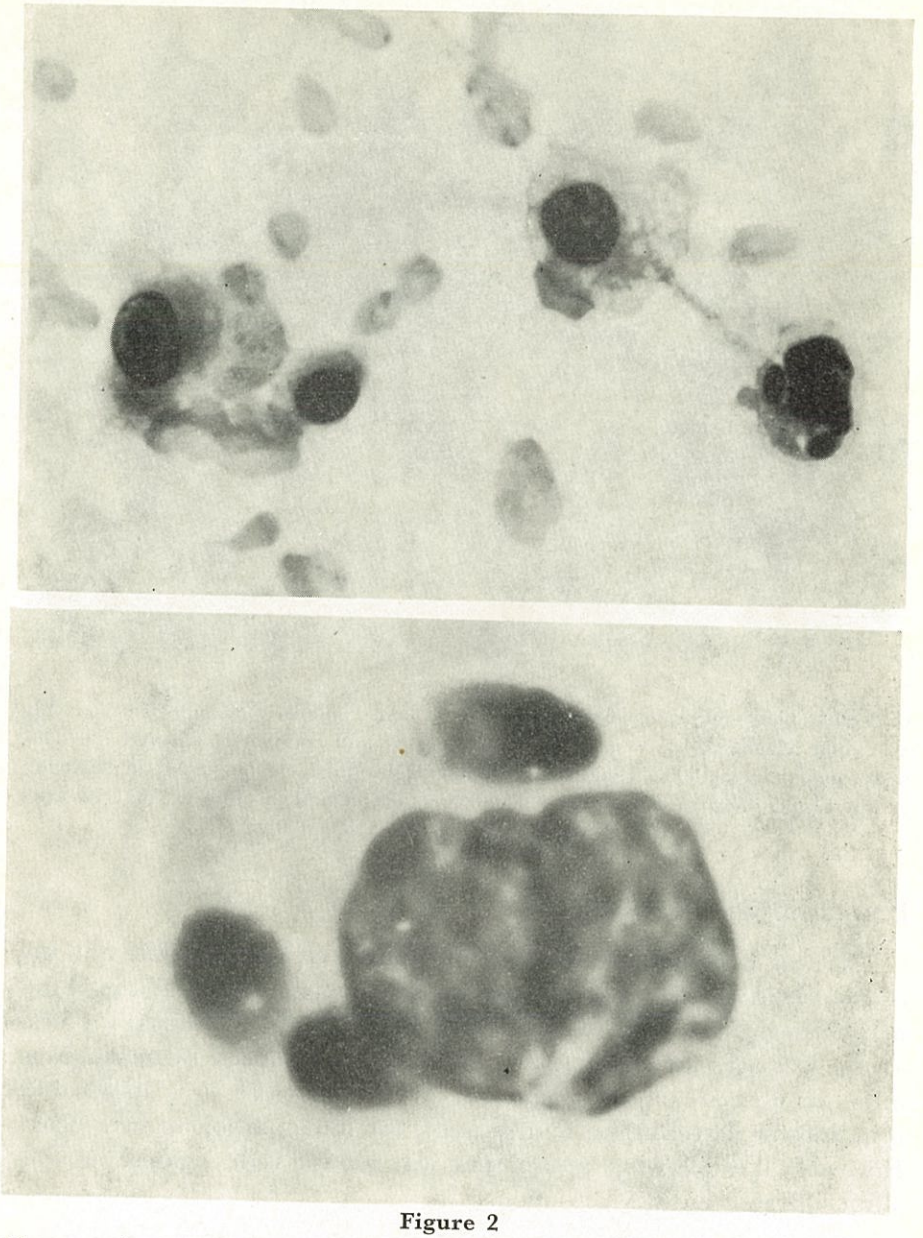

Figure 2

Phagocytosis of spermatozoa in the uterine cavity of woman (Intrauterine device was removed 8 hours following the intercourse). Loopal smear top, four macrophages and erythrocytes, 1000X. Bottom, the macrophage at extreme right of the top figure is magnified. Three well preserved heads of spermatozoa are observed phagocytized within the cytoplasm of this relatively small macrophage, 6000X (Sağıroğlu, N., Phagocytosis of spermatozoa in the uterine cavity of woman using intrauterine device. International Journal of Fertility, 16: 1-14, 1971).

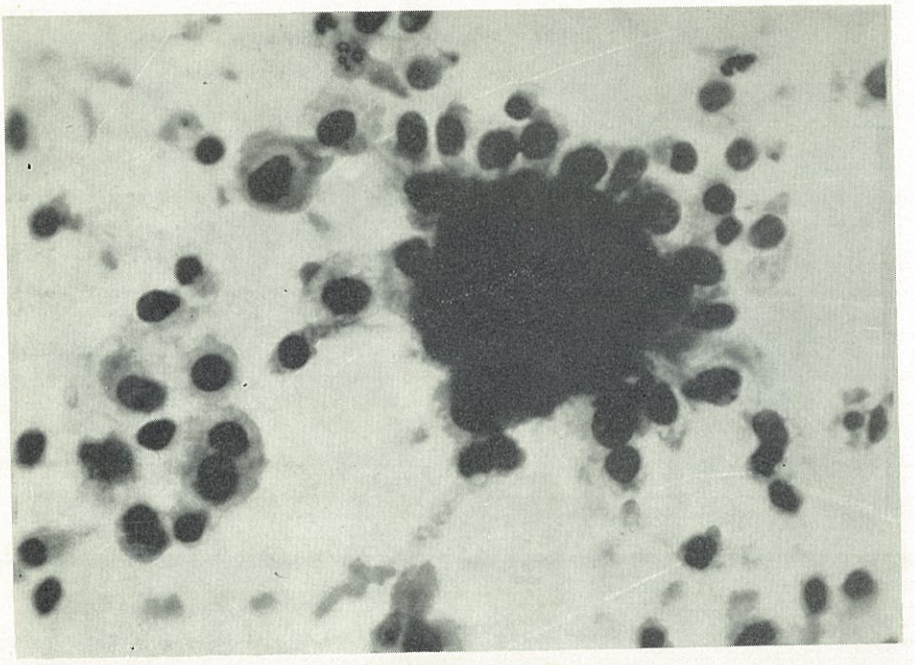

Figure 3

Collective phagocytosis of macrophages of a large microscopical globule, possibly an ovum. Macrophages seem to have surrounded and completely covered the dark body, loopal smear 900X (Sağıroğlu, N. and Sağıroğlu, E.: Biologic Mode of Action of the Lippes loop in Intrauterine Contraception, American Journal of Obstetrics and Gynecology, 106: 506-515, 1970).

How then, does this happen?

It was demonstrated that the protective cells accumulate and are concentrated in the close vicinity of the foreign matter. If the intrauterine device has been well-fitted, it means that it practically blocks all the passages for spermatozoa and also for fertilized ovum entering into the uterine lumen (Figure 4, WF). Therefore it is impossible, primarily for fertilization, and secondly for implantation to take place. However, the following possibilities will forever fight against an idealistic intrauterine device action.

Usually, when an intrauterine device is inserted, the uterine cavity cannot be accurately measured to ensure a perfect fit and as all women vary slightly in this respect, there will always remain some free space for the passage of spermatozoa. In addition to this, the uterus is an active and functional organ which contracts and relaxes under physiological (hormonal) and psychological stimuli. During these contrac-

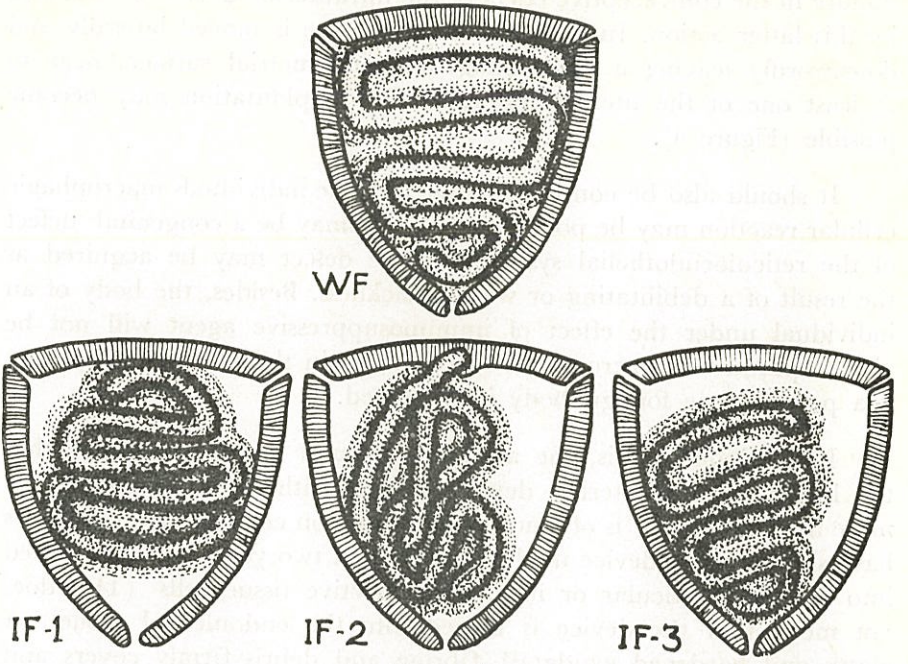

Figure 4

Polyethylene intrauterine device and the uterine cavity. Stripped frame represents the endometrial tissue from a frontal section. WF shows a well-fit device in the uterine lumen. The cavity is completely covered by the device. The macrophages shown by dots cover the entire surface of the endometrium. The upswimming of spermatozoa seems to be impossible. Pregnancy is thus prevented. IF-1, IF-2 and IF-3 point to the ill-fit device, the first one turned down, the second entangled and the third in small size for the cavity. In all cases of these three ill-fit applications, large endometrial surface (shown in white) are deprived of the coverage and protection of macrophages. Therefore spermatozoa pass into the cavity freely causing occurance of fertilization of an ovum and its implantation despite the presence of a protective device.

tions, the device, although it may have been well-fitted initially, may become pushed to one side of the cavity or its shape could be slightly distorted, thus making a free space for the passage of spermatozoa, (Figure 4, IF-1, IF-2 and IF-3).

The author therefore believes that in the majority of device users, spermatozoa find a free way between the device and the endometrial surface. Therefore fertilization may occur frequently in women using the device.

However, encirclement and isolation of the fertilized ovum in a manner of collective phagocytosis by the macrophages is another important face and probably the basis of intrauterine contraception.

Failure in the contraceptive effect of the intrauterine device is overruled by this latter action. In such a case the device is moved laterally and downwordly leaving a macrophage-free endometrial surface, near to at least one of the uterine horns, so that implantation may become possible (Figure 4).

It should also be considered that in some individuals macrophagic cellular reaction may be poor. Such a case may be a congenital defect of the reticuloendothelial system. Or the defect may be acquired as the result of a debilitating or wasting sickness. Besides, the body of an individual under the effect of immunosuppressive agent will not be able to produce a macrophagic reaction within the uterine cavity even if a polyethylene foreign body is employed.

In addition to this, the author has found in his experience that the longer an intrauterine device is used without replacement, the more likelihood there is of macrophagic reaction ceasing. Loopal smears have shown that a device used for more than two years becomes buried into the fixed reticular or inactive connective tissue cells (This does not mean that the device is buried into the endometrial tissue). A glossy and hardened exudate[14] fibrine and debris firmly covers and isolates it, while it is in the uterine lumen. In such a condition, the foreign body is almost covered by the cells and cellular production of the host so that it can no longer stimulate enough foreign body reaction to fill and control the whole endometrial cavity. In the cases where the device had been used three or four years, few macrophages showing functional activities (phagocytosis and mitosis) were seen in the loopal smears. The reactive cells observed were mostly fibroblastic, fibrocytic types and frequent multinucleated giant cells, in the form of large sheets firmly covering the device. This condition, namely, permanent isolation of the stimulant, reduces the number and function of the macrophages, perhaps resulting in pregnancy in spite of a well-fitted device in uterus.

An intrauterine device can also produce some side effects which may make its usage difficult or even impossible. Pain and bleeding occur mostly when bulky devices are fitted which distend the uterine wall and break the fragile superficial endometrial blood vessels. Therefore a device should be of such a shape and size to enable it to fill the uterine cavity without distention. It should be large enough to cover as wide an area as possible of the endometrial surface without exerting pressure but at the same time it should be as light as possible. Its shape should also enable easy insertion and removal.

Taking all these clinical, mechanical, biological and immunological factors into account, an intrauterine contraceptive device should carry the following characteristics for a near-idealistic action.

For maximum contraceptive effectiveness, an intrauterine device should has a large surface as possible in its construction. Therefore, because the macrophagic reaction is to cover and isolate the surface of a foreign body, the larger the surface of the device, the larger the number of macrophages in the uterine cavity. The most effective results may be achieved simply by making a ciliated surface on the device. All the devices which are in use for practice of contraception may be made ciliated. It is the firm belief of this author that, the "S" shaped small backbone of our polyethylene device section of which is a square-like shape originating long soft cilia will serve as one of the ideal forms in the bioengineering of IUD's (Figure 6). The cilia should be as thin, as long as pliable and, as many as possible. Because of the reduction in the thickness of the backbone, it will still be inserted easily by the same size of inserter-tubes presently used, in spite of the crowded cilia. Long and soft cilia, will enwrap the body of the IUD's similar to the backbone of a fish. In a vertical or cross section of the "SSD" the cilia which form a tall and narrow "X" on the main body of IUD will bounce on the mucous surface (Figure 6). and produce very little distention and pressure effects on the endometrium. Because of its pliability, there will be reduced irritation of the endometrium and therefore very little myometrial contraction. This may result, therefore in very little pain and bleeding if none at all.

With this ciliated SSD practically all the surface of the endometrium will be covered with the web of foreign material, and therefore all the surface will be lined with the macrophages surrounding the cilia. Depending on the frequency of the cilia on the backbone there will be from 100 to 1000 fold more macrophages than with the older IUD without cilia waiting to attack spermatozoa and ovum in the endometrial cavity.

The ciliated "SSD," if it should become, shows a deplaced in the uterine cavity the cilia take care of the area from which the body of the device is partially withdrawn.

Such a ciliated device, because of a thinner backbone and space between the cilia, will cause less blockade for the menstrual bleeding during menstruation (Figure 7).

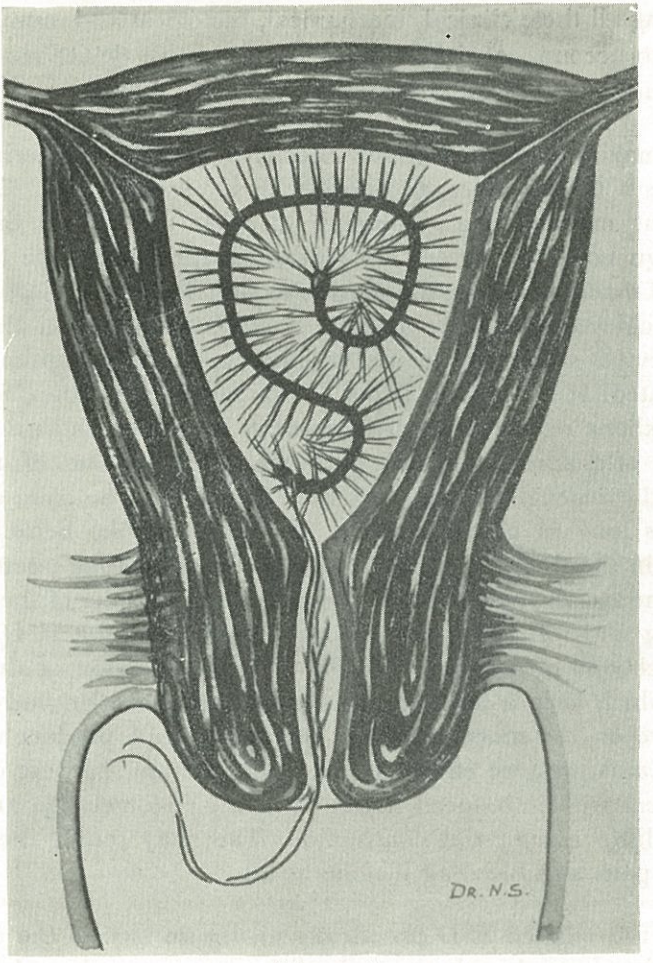

Figure 5

An ideal structure of an intrauterine contraceptive device made of polyethylene in the shape of a capital "S" with the upper part being larger than the lower part. This device is covered completely by thin and long cilia. Such a device may easily fit nearly all sizes of uterine cavities because of its soft springlike construction in all directions. Very few side effect such as pain and bleeding, if any at all, could be expected and one hundred per cent of protection against pregnancy is hoped for.

The Period of Time to Remove and Replace :

According to present experience, ideally, the device should be changed annually. It is absolutely necessary for the device to be removed and replaced following two years of use after this time, even with well fitting devices, pregnancies may occur due to the isolation of the surface and the inactivation of foreign-body character of the device.

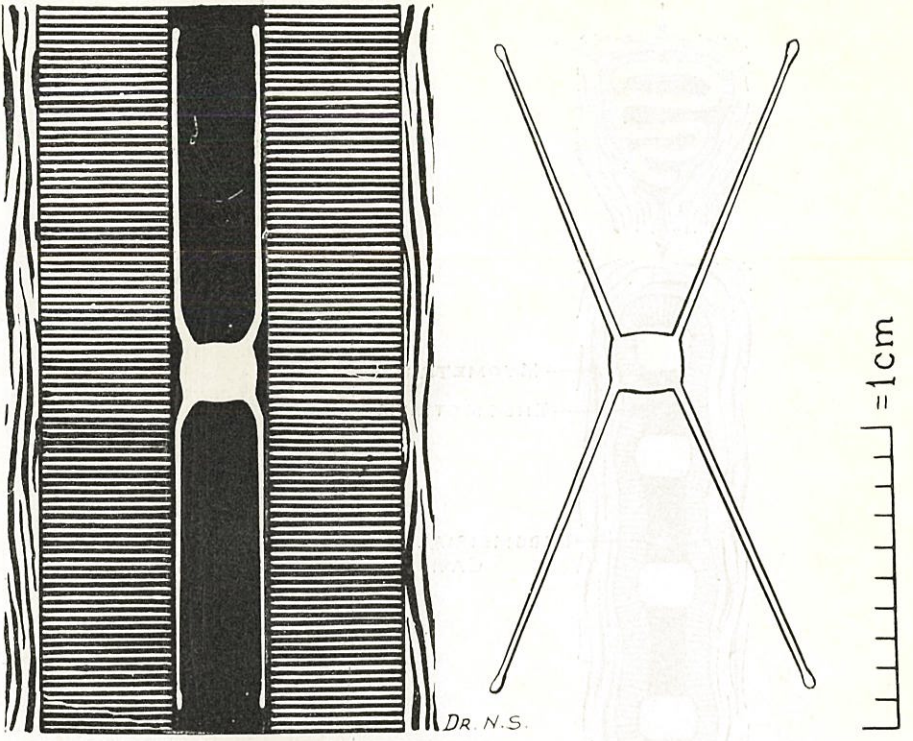

Figure 6

Cross-section of ciliated SSD (soft spring device). The body of SSD is almost square in section. Thin, long and pliable polyethylene cilia originate from all four rounded corners of this square, creating a tall and narrow letter of "X" (at right). In the uterine lumen, between the anterior and posterior walls of the endometrium, long cilia of the letter "X" changed in to the shape of a tall and narrow "H". In the figure, the block areas seen is uterine lumen, horizontal stripes show the endometrial tissue and vertical ones the myometrium.

Similar to a booster shot given in certain vaccinations the replacement of the IUD in every one or two years will stimulate the macrophagic reaction and resulting in a more effective prevention of pregnancy.

Material to be Employed in the Manufacture of IUD's :

One more comment should be made on the material from which the devices are manufactured. Polyethylene appears to be best according to the experimental study of this author. Silk surgical threads, nylon, stainless steel, magnesium, and copper wires all have been studied under the same condition. A larger number of macrophages, with better functional activities, were found in the use of polyethylene.[15] Copper is

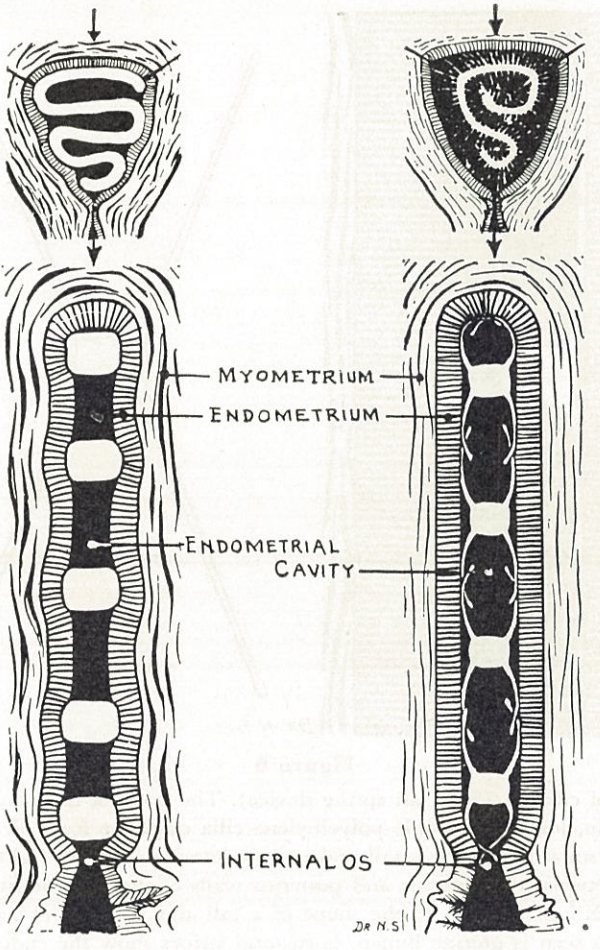

Figure 7

Comparative frontal (top) and sagital (bottom) sections of uterus containing a well-fit Lippes loop (left) and a ciliated SSD (right). Best protection against pregnancy is obtained by the use of Loop-D, the largest, thickest and the bulkiest Lippes loop. This device streches the endometrial tissue forcefully and therefore creates deep grooves on its surface. Because of this pressure, endometrium and myometrium are irritiated resulting uterine constructions which promotes pain and bleeding. These symptomes, in most cases continue and end with the removal of the device. Since the ciliated SSD will not touch the endometrium directly but through its long and pliable cilia, the endometrium will not be irritiated but will bounce gently in all circumstances. In case of bouncing hundreds of cilia will prevent the harmful effect upon and the stretching of the endometrial tissue. No pain or bleeding will therefore occur. In the meantime the SSD will cover the entire surface of the endometrium with the help of its cilia which create a fine network attracting billions of macrophages, the army of defenders of human health.

claimed to be more active in experimental animals. We found the least number of macrophages, surrounding the copper. In addition to this, the endometrial glandular cells and connective tissue elements of rabbits were found destroyed at the contact-site of copper wire, after 45 days of use. It has quite possibly been affected because of the cytolitic toxicity of copper salts products in animal.[16] [17] Because of the strong probbility of toxic effect, the use of this low quality metal in the human body should be prohibited.

Summary

In an attempt to improve the birth control effectiveness and reduce the unwanted side effects of intrauterine contraceptive devices, a new model was developed and presented. This new device is engineered according to its biological mode of action. In the previous report the mechanism of how an intrauterine device acts as contraceptive in women was demonstrated. Intrauterine devices, especially those made of polyethylene, stimulate woman's own cellular defensive mechanism and cause mobilisation, attraction, and accumulation of the macrophages in the uterine cavity. Macrophages primarily enwrap the colossal foreign body and try to phagocytize it (collective phagocytosis). Meanwhile, they attack and phagocytize all foreign body entering into the endometrial lumen. Thus, spermatozoa and possibly fertilized ovum are also phagocytized and destroyed by macrophages resulting contraception in a most natural or biological way.

The antifertility effect of intrauterine device therefore depends on the number of the macrophages in the uterine cavity. Macrophages attracted by the IUD to cover its surface. In this new model of IUD (the SSD : Soft-Spring-Device) presented, the surface is maximally enlarged adding as many as possible cilia which are thin, long and pliable.

Unwanted side effects of intrauterine device are, most importantly, pain and bleeding after the insertion and during the use. These are possibly minimalized, with small and light construction of the body of IUD, and the placement of the cilia at the surface.

The new device, therefore, will not irritiate the endometrium and myometrium which are the cause of bleeding and pain.

All other type of devices presently in use in the practice of birth control may be improved on the similar bioengineering basis.

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Paroxysmal Nocturnal Hemoglobinuria: Report of a Case with Features of Intravascular Consumption Coagulopathy*

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Paroxysmal nocturnal hemoglobinuria is a chronic hemolytic anemia characterized by intermittent hemoglobinuria usually occurring during the night and continuous hemosiderinuria. Thrombotic phenomena following hemolytic episodes occur rather frequently during the course of this disease. Thromboplastic material liberated into the blood stream from hemolyzed erythrocytes is thought to be responsible for initiating the process.[1]

In this paper we describe a case of paroxysmal nocturnal hemoglobinuria which presented laboratory findings suggestive of intravascularconsumption coagulopathy during an episode of cerebro-vascular accident.

Methods

Routine hematologic investigations were carried out by standard techniques.[2] [3] Plasma hemoglobin was measured as described by Crosby,[4] hemosiderinuria was determined as described by Wintrobe[5] and urine hemoglobin was measured by the benzidine method.[3] Acidified serum test was performed by Ham's[6] and thrombin test was carried out by Crosby's methods.[7]

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The coagulation studies revealed an essentially normal picture: the bleeding time (Ivy) 5 min., clotting time (Lee-White) 8-10 min. and the tourniquet test was negative. Prothrombin time was 76 %, and plasma thrombin time was 13 sec. Fibrinogen was 320 mg %, euglobulin lysis time was 120 min.

Platelet factor 3 availability tests were normal. Platelet aggregation with ADP, thrombin, adrenalin and noradrenalin were also normal (Table II).

Clinical Course - The first episode of acute intravascular hemolysis in the hospital lasted about 10 days. At the end of this period his plasma hemoglobin fell to 45 mg %, his total bilirubin to 0.6 mg % with 0.4 mg % indirect reacting type, his urinary benzidine test turned negative but slight hemosiderinuria continued.

On the seventh day in hospital as the signs and symptoms of acute hemolytic episode were regressing, the patient started complaining of headache, nausea and vomiting. The throbbing occipital headache became worse during the following days. He spiked a temperature up to 39.4°C and complained of blurring of vision. A neurological examination was negative except for a slight neck rigidity. These symptoms and signs were interpreted as meningeal irritation. A lumbar puncture revealed grossly hemorrhagic liquor. This was considered to be a sign of subarachnoid hemorrhage. Fundoscopic examination showed mild bilateral papilledema and fresh hemorrhages in the right fundus.

The signs and symptoms of CNS involvement lasted about 7 days and subsided spontaneously. Coagulation studies performed during this episode revealed definite abnormalities in several tests. The laboratory evidence of hemostatic disturbance lasted longer than the clinical episode and finally all tests turned to normal in 2 weeks (Table II).

After a period of 50 days in the hospital without hemoglobinuria, the patient complained of backache and weakness upon exposure to cold weather. His urine turned black and his sclerae became icteric. The laboratory investigations performed during this hemolytic crisis revealed serum bilirubin to be 1.3 mg % with 0.8 mg % indirect reacting type, plasma hemoglobin 170 mg %, hemosiderinuria strongly positive and hemoglobinuria 4 plus. At the end of the four days attack of hemoglobinuria the patient's blood values fell slightly. After 4 days icterus and hemoglobinuria subsided. Plasma hemoglobin and serum bilirubins decreased.

In the hospital the patient received 4 units of washed red cells without any complications. He was also put on prednisolone 30 mg/day

TABLE II

COAGULATION STUDIES

	13.1.1970	19.1.1970	28.1.1790	4.2.1970	12.2.1970	27.2.1970	3.3.1970
Bleeding time	5 min.	12 min.	–	–	–	–	3.5 min.
Tourniquet test	neg	neg	–	–	–	–	neg
Platelet count	150.000	60.000	40.000	–	80.000	–	130.000
Clotting time	8–10 min.	12–15 min.	–	–	–	–	5.5–7.5 min.
Prothrombin time	76%	56%	39%	70%	–	78%	77%
Thrombin time	13" (13")	–	22" (10")	–	–	–	12"(12.5")
Fibrinogen	320 mg%	420 mg%	90 mg%	80 mg%	310 mg%	300 mg%	280 mg%
Euglobulin lysis time	120 min.	120 min.	55 min.	50 min.	85 min.	80 min.	130 min.
Factor VIII	–	–	85 %	–	–	–	98%
Platelet F. 3 (Inceman-Tangün)	18"	–	–	–	–	–	–
Platelet aggregation	normal	–	–	–	–	–	–

and sublingual testis hormone 50 mg/day and was maintained on this regimen for three months. He has been followed in the outpatient department since his discharge. He reported an attack of icterus with questionable hemoglobinuria following a day of sea-bathing in the summer of 1970. He has been taking 5 mg of prednisolone daily and is being followed at 3-month intervals since.

Discussion

The abnormal predisposition to thromboses in PNH has been the subject of several investigations.[19-23] Crosby[19] attributed this phenomenon to an abnormality in the platelets rendering them susceptible to destruction in the circulation liberating thromboplastic material into the blood stream. He also found these platelets to have increased speed of aggregation. However, these findings have not been confirmed by other workers.[22-24] In this case we were unable to show a functional abnormality of platelets. The platelet factor 3 availability tests and the platelet aggregation by ADP, thrombin, adrenalin and noradrenalin were normal.

The thromboplastic activity of erythrocytes in PNH was first shown by McKellar and Dacie.[21] It was suggested that this phenomenon might play a part in the genesis of a hypercoagulable state in in PNH.

Besides these studies a number of authors have published accounts of deficiency or hyperactivity of certain of the clotting factors in PNH.[18 24-26] Some of these changes are probably the result of disseminated intravascular coagulation rather than the cause of it. The clinical and laboratory findings in the present case support the latter point of view. At the beginning of the hospitalisation, no abnormalities could be demonstrated in the routine clotting tests except for a slightly reduced platelet count. During the episode of cerebro-vascular accident shown to be associated with subarachnoid and retinal hemorrhages, his platelet count fell markedly, his bleeding time was prolonged, his prothrombin activity was reduced and even his clotting time was slightly prolonged. His fibrinogen level remained high at the beginning but fell markedly in a few days and remained low longer than the clinical episode. Plasma thrombin time was also prolonged and the euglobulin lysis time was considerably shortened. The Factor VIII activity determined towards the end of the episode was within normal limits but was found to be higher later. Factor V and fibrinogen split products were not measured.

The diagnosis of consumption coagulopathy rests on demonstrating the depletion of certain labile clotting factors such as fibrinogen, prothrombin, Factors V and VIII and platelets. In addition, due to secondary fibrinolysis, fibrinogen split products are usually found in the serum. The shortening of the euglobulin lysis time is an indirect evidence of fibrinolysis, although fibrinogenopenia may contribute to it in this case. The chain of events was probably started by the liberation of thromboplastic material from the erythrocytes during the hemolytic crisis.

Headache is a frequent complaint of PNH patients. In this case headache was the first symptom of a cerebro-vascular accident which was associated with certain features of intravascular consumption coagulopathy. It will be interesting to look for the incidence of these abnormalities in PNH patients during thrombotic episodes.

Summary

A 37-year-old man with paroxysmal nocturnal hemoglobinuria experienced an episode of cerebro-vascular accident following a hemolytic crisis. Laboratory investigations at this time revealed certain features of intravascular consumption coagulopathy, i. e. fibrinogenopenia, hypoprothrombinemia and thrombocytopenia. The shortening of the euglobulin lysis time was also taken as an indirect evidence of secondary fibrinolysis following disseminated intravascular coagulation. The chain of events was thought to be started by the liberation of thromboplastic material from the erythrocytes during the hemolytic crisis.

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Primary Carcinoma of the Fallopian Tube: Two Case Presentations and the Review of the World Literature

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Primary carcinoma of the Fallopian tube is a rare clinical entity. The preoperative diagnosis is quite difficult and the diagnosis is generally made post-operatively. Approximately 800 cases of primary carcinoma of the Fallopian tube have been reported in the world literature.[1-3] One of the largest series published in last years was from the Mayo Clinic.[4 5] Tubal carcinoma consists of about 0.02-0.25 percent of all genital tract cancers. The incidence of this disease has been reported differently by the various authors: In Hu et al's series, only one primary tubal cancer was found in 19,439 cases,[4] and 12 cases of carcinoma of the Fallopian tube were reported from the Free Hospital for Women.[4]

The purpose of this paper is to report the 2 primary carcinoma cases of the Fallopian tubes which have occurred within the last year. During the period of 1965-1971, the number of the patients who were seen at our out-patient clinic was 11,350 of which 3,850 had undergone either major or minor operations.

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Case 1: This (Prot. No. 215187) is a case of 42-year-old white female (Gra: 0, Para: 0), married for 20 years, who was seen in the out-patient clinic with a history of continuous vaginal discharge and of lower abdominal pain which became worse during the last 2-3 months. There was no history which suggested pelvic tuberculosis.

Her past surgical history revealed that she had undergone an abdominal operation 2 years previously for a pelvic mass. According to the non-official information which was brought to us by her husband, nothing was taken out at the time of laparotomy, because her tubes were found to be swollen and inflamed. Her abdomen was closed and she was placed on antibiotics since the condition was thought to be due to P.I.D.

Her physical examination revealed her B.P. to be 120/80 kg. 47... Her Hg: 11.50 gm., her lungs and heart revealed negative findings. Abdominal examination showed a soft non-tender mass filling the lower quadrants of her abdomen.

The vaginal examination revealed a hard irregular tumor mass which filled almost all of the pelvis. The cervix was nulliparous and the uterus could not be differentiated from the tumor mass. The pap smear was taken and was reported as Class II. Examination under anesthesia confirmed the above findings. A diagnostic curettage and Douglas aspiration were carried out cells which revealed some suspicious cells. A laparotomy was decided upon. At the time of operation, the uterus was normal in shape, slightly bigger than normal. The Fallopian tubes were swollen, sausage shaped and elongated. The ovaries could not be identified. There was very small amount of fluid in the abdominal cavity. The omentum was free. Exploration of the abdominal cavity revealed nothing important. No visible metastases to the other pelvic organs were present.

About 80 percent of the tumor mass was removed. The uterus was purposely left inside.

Gross examination of the tumor revealed both tubes to be 4 cm. in diameter with friable cauliflower-like tissue inside. The rest of the tumor tissue which was removed from the broad ligament was around 8 cms in diameter and showed the same pathological structure. The ovaries were not differentiated on gross examination.

Microscopical examination of the tumor tissues revealed the carcinoma of the Fallopian tubes. Tubal wall was thickened and the serosa was intact (Figures 1 and 2). Tumoral growth was protruding

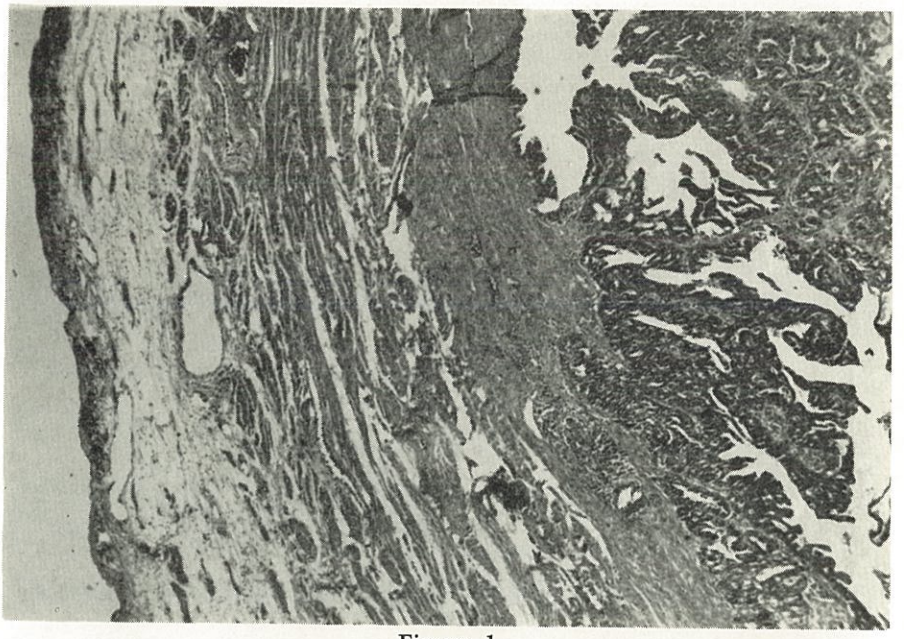

Figure 1

Intact tubal seroza (outer), thickened muscular wall, and tumoral growth in the tubal lumen. Tumoral invasion is seen (H + E, 2.5 X 10). Biopsy no: 4907/70.

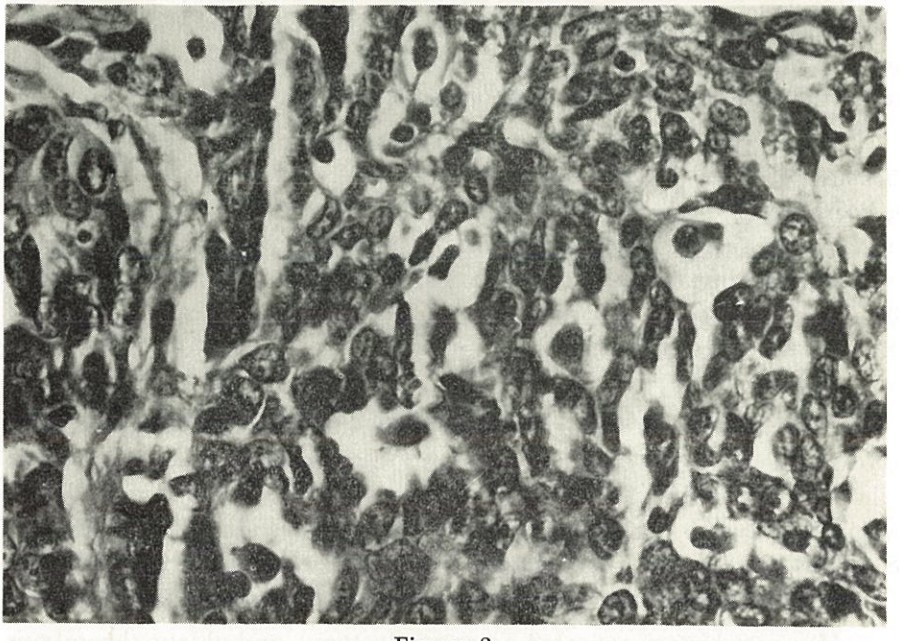

Figure 2

Tumor tissue, high magnification (H + E) 40 X 10.

into the lumen. Ovarian tissue appeared to be normal, but some tumoral implantation on the surface of the ovary was noted (Figure 3). The endometrium appeared to be normal.

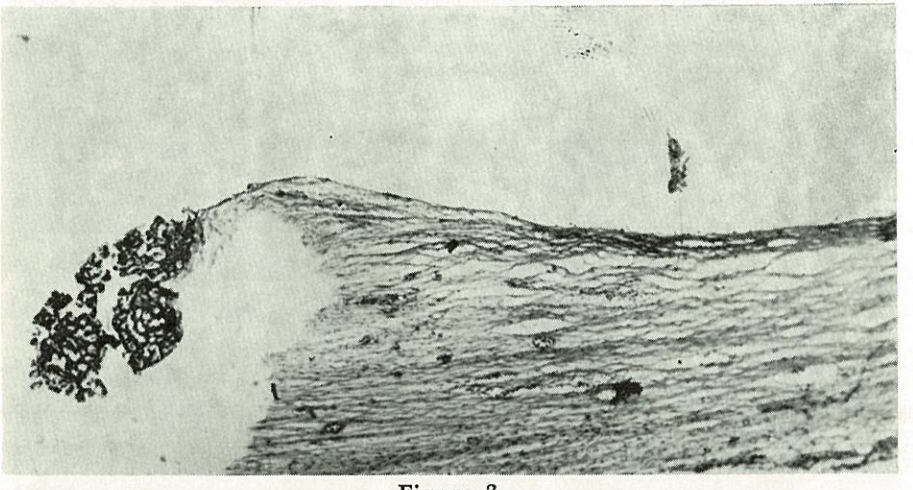

Figure 3

Tumoral implantation on the ovarial surface (H + E, 2.5X10).

On the tenth post-operative day, following the recommendations of our cancer committee, the patient was given 100 mgm. radioactive gold intra-abdominally and 2800 r. external radiation. She was discharged from the hospital on 27th post-operative day.

Seven months later, the patient was readmitted to the hospital because of severe back pain, and of bilateral paraplegias of the lower extremities. She was transferred to the Department of Neurosurgery. An exploratory surgery was performed. Unfortunately, metastatic tumor had already invaded the anterior portion of the spine. Nothing could be done. Her condition deteriorated rapidly. She became cachectic and finally died of generalized metastases eight months after first admission.

Case 2: M. A. (Prot. No. 297910), 52-year-old white female, (Gra. III, P. I. 2 criminal abortus,) was seen in the out-patient clinic on November 3, 1971, with a history of continuous right lower quadrant pain for last 15 days. Her last period was about 6 months ago. She was complaining of yellowish vaginal discharge, without itching. Climacteric disturbances were also noted since menopause.

Her past history revealed an appendectomy, and phlebo thrombosis of the right leg about 30 years ago.

Her menstrual history indicated that her menarche had started at the age of 13 and her menses had been 30 d/4d/ normal amounts. Her child was delivered by breech extraction 16 years ago. Since then she had 2 criminal abortions carried out at durations of 4 and 2 months respectively.

Her physical examination revealed a well-built middle-aged lady with a BP: 140/90., Hgb 11.70 gm., Hct. 40 p., blood sugar % 78 mgm. BUN % 10 mgm. Her blood group was ARh (+). Cardio-respiratory system was normal and no palpable mass was felt with abdominal palpation.

Vaginal examination indicated a relaxed vagina with a small amount of discharge cervix multipara, clean uterus retroverted, mobile and normal in size. There was a tender, mobile cyst on the right adnexae. Nothing was felt on the left side. The pap smear was taken and reported as Class II. With a preoperative diagnosis of possible right ovarian tumor, she was recommended to have a laparotomy.

She was then admitted to the hospital on November 27, 1971 and the repeated vaginal examinations revealed the same findings mentioned above. With the possibility of the removal of uterus and the mobile adnexal mass via relaxed vagina, she was scheduled for vaginal hysterec-

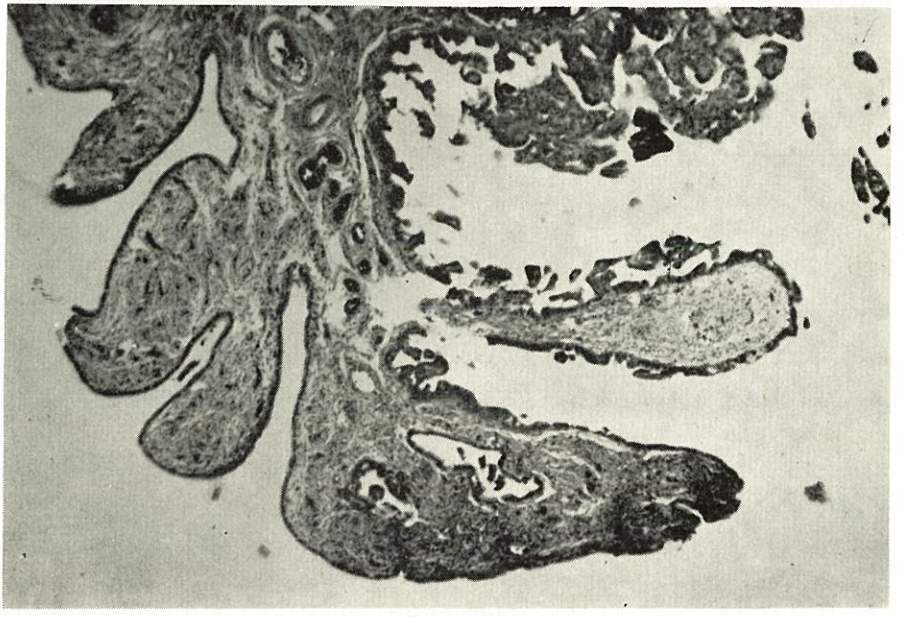

Figure 4

Carcinoma of the left Fallopian tube (Case 2).

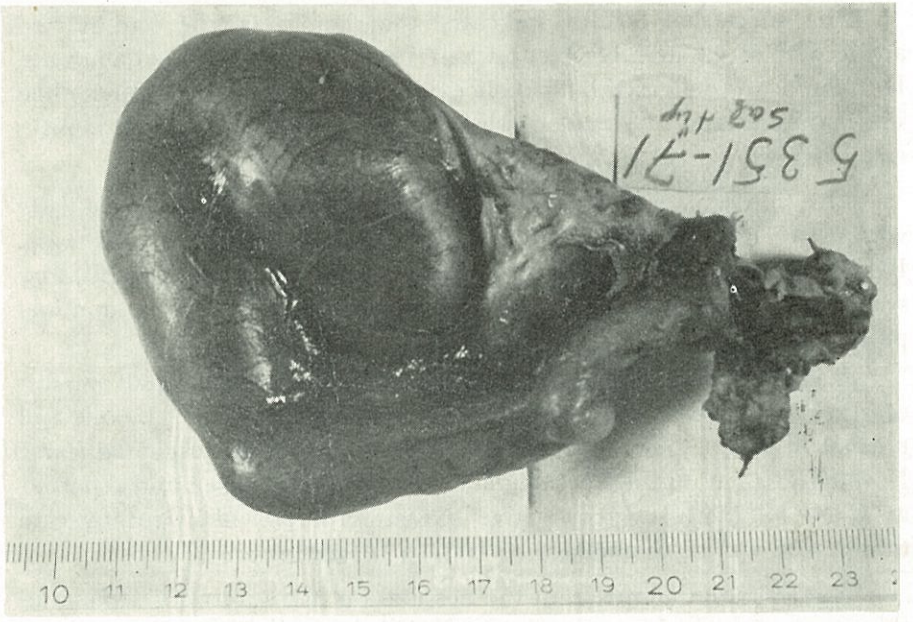

Figure 5

Gross picture of the carcinoma of the right tube (Case 2).

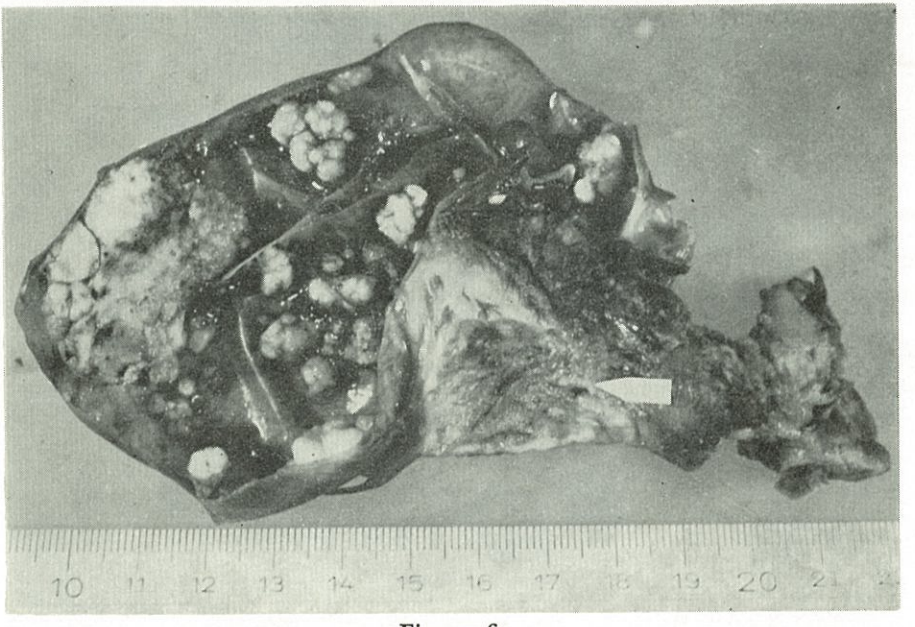

Figure 6

Inner view of the right tube (Case 2).

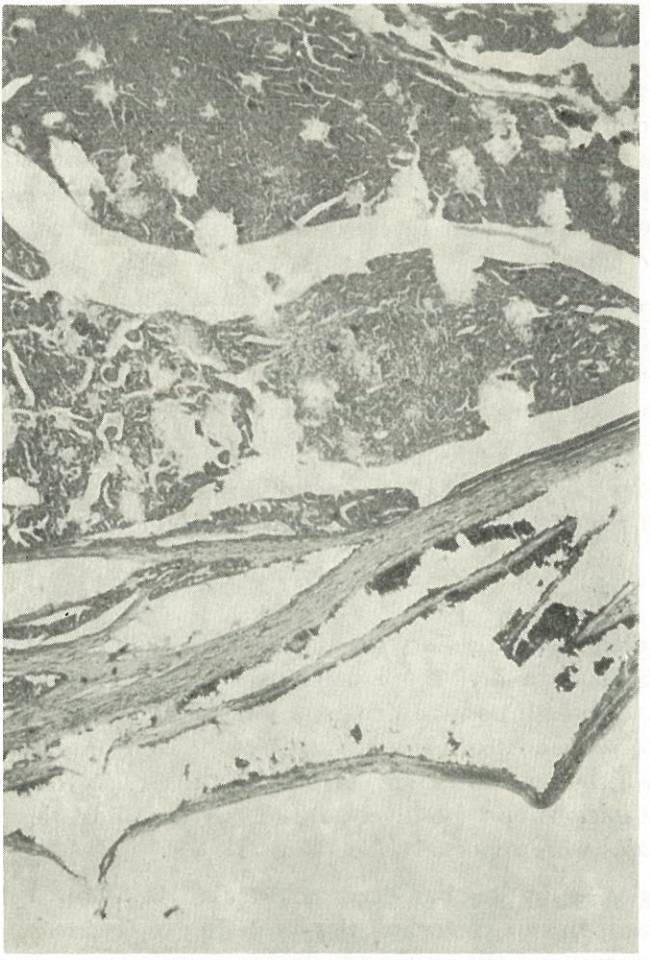

Figure 7

The right tube (Case 2). Tumor mass filling the lumen, interchanging zone, invasion to the tubal muscularis and the intact seroza (H+E, 2.5X10).

tomy. First, a D+C was carried out. The cavity was found to be 8 cm. in depth and no material was obtained. The mass on the right side was felt to be around 6x6 cm. in diameter.

The uterus was removed without any difficulty. It was then observed a green coloured, sausage shaped tumor mass on left side filled with a papillar growth. There was some spillage of the serous fluid from inside of the tube during removal of the tumor. A quick frozen section aroused some suspicion of adenocarcinoma of the tube. The right tube appeared to be soft and swollen. Both ovaries looked rather atrophic.

Histopathological report confirmed the presumptive diagnosis of the carcinoma of the Fallopian tube (Figure 4).

She was then rescheduled for laparotomy at which the right tube was found to be swollen, elongated and inflamed (Figures 5 and 6), the ovary was atrophic. There was no adhesions of this adnexal mass to the surrounding organs. The tube and the ovary were removed. Histological sections also revealed the carcinomatous changes on the right tube (Figure 7).

There was a small friable mass on left side probably consisted of granulation tissue due to previous operations on left ovarium. Biopsy was taken which revealed granulation tissue. The palpation of the abdominal organs revealed nothing important.

The immediate poot-oparative course of the patient was uneventful. On her 12th post-oparative day, external radiation therapy was started on an out-patient basis and she was given 5500 r. totally.

Discussion

The diagnosis of primary carcinoma of the Fallopian tube is seldom made before laparotomy.[1 3-6] Vaginal smears are not great of help in early diagnosis. Although positive smears of some isolated cases have been reported, correct incidence is not very high.[3-6] In Hanton's series of 27 patients which were all diagnosed postoperatively, cervical smears were neither suspicious nor positive.[4] In our present study, the pap smears of both cases were reported to be Class II.

Douglas aspiration has been reported to be positive in 12 tubal carcinomas.[7] In our first case, this procedure was carried out for research purpose, and the smear which was prepared from the aspiration material has revealed some suspicious cells for malignancy.

Preoperative diagnosis was not made in either of our 2 cases. It is definite that the reason for poor preoperative diagnostic accuracy was the lack of suspicion. It was especially easy to make a diagnosis in our second case retrospectively when one looks for the diagnostic triad of pain, vaginal discharge and palpable adnexal mass.[4 10]

The low fertility index associated with this neoplasm[2 4 10 11] was also noted in our first case (Gravida 0, Para 0).

The recommended optimal treatment for primary carcinoma of the Fallopian tube is total hysterectomy and bilateral salpingo-oophorectomy.[1 4 6 8] As a rule, extensive resection of the pelvic lymph nodes does not appear to be necessary.[1 5 6]

Post operative external radiation of the pelvis increases the five years survival rate. There are very few reports about the experience with chemotherapy in the treatment of this disease.[1 4] Two patients in this study were treated differently because of lack of preoperative diagnosis. In the first case, removal of the bilateral tumor mass, followed by intra-abdominal installation of radioactive gold and external irradiation of the abdomen were carried out.

In the second case, a vaginal hysterectomy and vaginal removal of the adnexal mass were performed. After pathological report of the tumor was confirmed, a laparotomy was performed and the rest of the pelvic organs was removed. She was then given external radiation. It is interesting to note that, in this case, carcinoma of the Fallopian tube was found on both Fallopian tubes.

With the experience we have gotten from the cases presented above and through the world literature that was reviewed, we can say that the most important factor in the diagnosis of carcinoma of the Fallopian tube is the high index of suspicion. With the probability of the disease in mind, the diagnosis should be made early and the patient should be treated according to the findings at the time of laparotomy.

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