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EFFECT OF LONG PERIOD OF UNDERNUTRITION ON THE STRUCTURE AND HISTOCHEMISTRY OF RAT TESTIS

(With 6 Figures)

By

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(Received at 9/9/1995)

تأثير فترة طويله من نقص كمية الغذاء على تركيب وهستوكيمائية خصية الفأر الأبيض

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عشرين من ذكر الفأر الأبيض بمتوسط وزن حواى ٣٥٠ جرام قد تعرضوا لفتره طويله من سوء التغذيه بواسطة تغذيتهم بكميات محدوده (٣٠٪ من الغذاء الطبيعى) لمدة أربعون يوما. تم فحص الخصى هستولوجيا وهستوكيميائيا ل (الكربوهيدرات – الدهون – وكذلك الفسفاتيز القاعدى) من الناحيه الهستولوجيه ، كان هناك نقص فىنصف قطر الأنيبيبات المنويه مع عدم الانتظام فى حدودها. فى بعض الأنيبيبات ظهر نقص فى النسيج الخلوى مع خلايا عاديه وأنبيبيبات أخرى كانت قد أضيرت بشده وظهر تليف واضح فى صورة سمك فى قشرة الخصيه والغلاف القاعدى حول الأنيبيبيات مع زياده فى الألياف الشبكيه لنسيج البينى الضام ، وحول الأوعيه الدمويه التى زادت فى العدد بوضوح. وباستخدام الطرق الهستوكيميائيه كانت الكربوهيدرات صعبه فى تتبعها ولكن بعض الرؤوس المضطربه للطلائع المنويه القديمه أظهرت تفاعل قوى. المحتوى الدهنى كان زائد فى الخلايا البنيه ولكن كان هناك حبيبات دهون قليله فى السيتوبلازم المجوف لأمهات المنى وخلايا سرتولى. كان هناك زياده فى نشاط الفوسفاتيز القاعدى للطلائع المنويه الصغيره والخلايا المنويه الأوليه وهذا النشاط يشبه من قليل أو من بعيد هذا الذى بالمجموعه الطبيعيه. ومن الممكن التعليق بأن سوء التغذيه لمدة طويله قد تسبب فى تغيرات هستولوجيه وهستوكيميائيه فى خصية الفأر وهذه التغيرات كانت العوامل المسببه التى نتج عنها توقف الوظائف المنويه الطبيعيه.

SUMMARY

20 adult male albino rats with an average weight 35 gm were subjected to a long period of undernutrition by feeding them in restricted amount (30% normal food intake) for 40 days. The testes were then examined histologically and histochemically for carbohydrates, lipid contents and also for alkaline phosphatase activity. Histologically there was diminution in the diameters of the seminiferous tubules with irregularities of their outlines. Some tubules showed hypoplasia with normal cells and other tubules were heavily damaged.

Fibrosis was appeared in the form of thickening of the testicular capsule, basement membranes of the tubules with an increase in the reticular fibers of the interstitial connective tissue and around the blood capillaries which showed a marked increase in number. Histochemically, carbohydrates were difficult to be demonstrated except some disrupted heads of the old spermatids which showed intense reaction. Lipid contents were increased in the interstitial cells. But there was few lipid droplets in the grossly vacuolated cytoplasm of the spermatogonia and Sertoli cells. There was an increase in the alkaline phasphatase activity of young spermatids and spermatocytes and this activity resembled more or less that of the control group. It could be concluded that undernutrition for a long time produced histological and histochemical changes in the rat testes such changes were the causative factors which produce inhibition in the normal spermatogenic functions.

Keywords: *Undernutrition, Structure, Histochemistry, Rattestis.*

INTRODUCTION

Undernutrition was known to have a wide variety of effects on endocrine systems in animals and man. The defects seen with undernutrition could be explained by abnormaltities of the hypothalamic pituitary unit rather than defects in endocrine end organs *(MULINOS et al, 1940). KULIN et al (1982)* proposed a direct effect of malnutrition on sexual growth and development, but the exact nature of the connection bewteen physical growth and sexual maturation remains elusive. In this study the histological and histochemical changes in the testis subjected to undernutrition were discussed, in a trial to clarify the expected morphological alterations.

MATERIAL AND METHODS

20 Adult male rats weighing about 350 grams were chosen for this study. The animals were divided into groups, each group was housed separately in individual cage

One group received a normal diet (Control group) and the other group feed in restricted amounts (30% normal intake). All animals were given free access of water, fed a diet of defined composition *(GLASS et al, 1979)*, and exposed to a 14-h light, 10-h dark cycle.

Both grroups were left so for 40 days before being killed. All animals were anesthised. From the same animal one testis was fixed in Bouin's solution and subsequently sectioned and stained for histological examination and the other testis was taken fresh for cryostat sectioning of 10 microns to demonstrate alkaline phosphatase, lipids by Sudan Black stain, and carbohydrates by PAS technique. The histological stains were, Haematoxylin and eosin, van Gieson's stain, and silver stain *(DRURY and WALLINGTON, 1980).*

Morphometric studies of the seminiferous tubular diameter of both control and undernourished group were

done using a micrometer eye piece *(ALLAN et al, 1979).*

RESULTS

The Control Group.

Histological observations

The seminiferous tubules were surrounded by basement membranes which appeared thick and deeply stained, and were lined by spermatogonia (type A and B), spermatocytes and Sertoli cells formed more than one layer. Their nuclei had no distinct muclear outlines. The spermatids were smaller cells with nuclei having distinct nuclear membrane and deeply stained nucleoli. The spermatozoa appeared in the lumen of the seminiferous tubules. In the connective tissue between the seminiferous tubules there were clusters of interstitial cells which lie in compact groups blood capillaries. They are large polyhedral plae stained cells (Fig.1). Using Van Gieson's stain the testiular capsule was heavily stained but the basement membranes of the tubules appeared lighter (Fig.2). By the use of silver stain the retiular fibers of the basement membranes around the blood capillaries and in the interstitial connective tissue appeared brown to black short wavy fibers (Fig.3).

Histochemical Stains

PAS technique showed that the positive reaction (carbohydrates) was principaly observed in the developing stages of spermatids mainly attached to their nuclei (the acrosomic system). The boundary tissue of the tubules had a moderate PAS. positive reaction, the Sertoli cell cytoplasm contained few fine stained graunles but the spermatogonia and spermatocytes showed negative reaction (Fig. 4) Lipids appeared in the cytoplasm of spermatogenia as large droplets and in the cytoplasm of sertoli cells as small granules mainly in their basal parts. The more centrally located spermatocytes contained a moderate amount of lipid granules. The young spermatids contained little amount of lipids, while the residual bodies appeared to have a large amount of lipid droplets. The interstitial cells also had small lipid granules (Fig. 5&5a).

Alkaline phosphatase activity was tense in the boundary tissue and in the spermatids. The nuclei and the nuclear membranes of spermatogonia and spermatocytes showed a moderate activity, but the reaction of the nuclear membranes was greater. The nuclei of the Sertoli cells showed a moderate activity but this was variable in different seminiferous tubules. The interstitial cells and the boundary tissue of the interstitial blood copillaries showed intense activity (Fig. 6).

The undernourished animals.

Histological observations

The seminiferous tubules appeared smaller in diameters and their outlines were somewhat irreggular. Some tubules showed hypoplasia with normal spermatogonia, Sertoli cells and primary spermatocytes. Other tubules were heavily damaged with atrophied cells and the spermatogonia usually formed of one raw with few degenerated primary spermatocytes. The cells appeared vacuolated and widely separated from each other. The Leydig cells were masked by a vast amount of interstitial connective tissue.

The cytoplasm of these cells appeared deeely stained with dense nuclei. There was a noticable increase in the number of interstitial blood capillaries (Fig.1a). Van Geison's stain showed marked thickening of the testicular capsule and the basement membranes around the seminiferous tubules appeared also densely stained and thick (Fig.2a). Silver stain showed increase of the reticuler fibers of the basement membranes of the tubules. The reticular fibers of the interstitial connective tissue and blood capillaries were markedly increased and deeply stained (Fig.3a).

Histochemical Stains

PAS stain showed that the carbohydrates were difficult to be demonstrated due to disturbance of the normal histological structure of the tubules. The intense reaction was only noticed in some disrupted heads of the old spermatids which still attached to the normal Sertoli cells (Fig. 4a).

Lipids was observed as few droplets in the grossly vacuolated cytoplasm of the remaining spermatogonia and Sertoli cells, this is because most of the germ cells were exfoliated outside the seminiferous tubules. The interstitial cells showed an increase in the lipid contents if compared with those of the control group (Fig.5b). Alkaline phosphatase activity of the spermatogonia and interstitial cells resembled more or less that of the control group, but there was a denser enzyme activity in young spermatids and spermatocytes (Fig.6a).

Morphometric Study

The mean diameter of the seminiferous tubules of the undernourished group (110 ± 10 [sd] Um) was decreased if compared with this of the control group (260 ± 16 [sd] Um) sd = standard deviation.

DISCUSSION

Previous studies had reported not only decreased serum testosterone, sexual accessory gland weight, and testosterone output in experimental undernutrition *(TRIPATHI et al, 1968, GREWEL et al, 1971 and NEGRO-VILAR et al, 1971)* but also FSH output *(STEWART et al, 1975)*. Reduction of the anterior pituitary hormones was also reported even in short term starvation *(PERGENDAHL et al, 1989)*. *NELSON (1987)* reported that the underfed animals appeared to be more susceptible to the effect of the pineal gland.

In the present study the prolonged period of undernutrition had a damaging effect on the testis including decreased tubular diameter with irregular outlines. This is in accordance with *CAMPBELL et al (1977)*. Who found that the weight and size of the testis and accessory reproductive organs were reduced in rats starved for seven days and this reduction increased in chronic starved animals. *RIDDEL (1982)* found that shrinkage of the seminiferous tubules was due to thinning of the germinal epithelium as a result of degeneration and atrophy of germ cells.

In this study there was atrophied germ cells and degenerated spermatocytes of the seminiferous tubules. This is in the

same line of *RATNOFF et al (1942) and FOUQUET et al (1989a)* who found that there was marked reduction in the number of germ cells and loss of the more mature germ cells of the tubules of the testis in cases of liver cirrhosis which in turn imparis food metabolism. The increase in the thickness of the basement membranes and its surrounding fibrillar material and the interstitial connective tissue and around blood vessels observed in this study was also observed by *MOHAMED (1975)* who attributed it to the irritative action of tubuler degeneration. This led to the formation of more reticular and collagen fibers to compensate the marked diminution in the diameters of the tubules. This was in agree with *CLERMONT et al (1990)*. The increase in the number of blood vessels distributed per tubule was present in this study, this may be a trial for repair. *JACKSON et al (1984)* observed the same finding in a state of diabetes of relatively short duration. They attributed this to be due to altered response to testosterone or its accessibility to target tissue seen in adult male wistar rats after 30 days of artificial diabetes.

In the histochemical study of this work, it was found that the amount of lipids in the most severely damaged tubules was more or less reduced. The decrease in the amount of lipids was found to be related to the exfoliation of most germ cells, inhibition of the spermatogenic activity and atrophy of the testis. These findings are in accordance with *DAVIS et al (1966) and JOHNSON (1970)* who found that the total lipids of the tubules decreased as the testis weight decreased. The presence of few lipid droplets in the cytoplasm of Sertoli cells of undernourished animals observed in this study because the Sertoli cells are the major energy source for sperm movement and generally for spermatogenic process. This was in agree with *CLERMONT et al (1991)*.

The increase in the lipid contents of interstitial cells observed in this study may be due to failure of the cells to secrete testosterone and this in turn lead to accumulation of lipids within the cytoplasm of the interstitial cells. This was in the same line with *EL-HOSSINY et al (1988)* who found that there was increase in the amount of lipids in the interstitial cells of leydig due to the decrease in their activities and consequent accumulation of lipids within their cytoplasm.

In this study it was found a large amount of carbohydrates in the acrosomic system of spermatids this finding is in agreement with *ARPIHAGOPIAN et al (1976)*. The decrease in the amount of carbohydrates due to undernutrition of albino rat testis may be the result of the change in the structure and activity of Golgi apparatus which is closely associated with the formation of acrosomic system *(MOUSSA et al, 1983)*. The accumulation of carbohydrates in the testis due to the defect in carbohydrate metabolism was noticed by *KOCEN and CAVAZOS (1958)* due to the effect of scurvy on the guinea pig testis.

The localization of alkaline phosphatase at the wall of the seminiferous tubules may play a role in spermatogenesis, intermediate

carbohydrate metabolism and synthesis of the testicular hormone *(MONESI, 1965 and JEHAN et al, 1970). TICE et al, (1963)* attributed locolization of alkaline phosphtaase at the wall of the seminiferous tubules to its role in the active transport of phosphate group. These findings were in the same line of the results obtained in this study.

The increase in the alkaline phosphatase activity due to undernutrition in this study was also noticed by *DIXIT (1977)* following vascular occlusion of the dog testis. He attributed this high activity to an indirect effect resulting from the damge of the spermatogenic activity. Contrary to the results of *DEMPSEY et al (1949)* who observed a maked decrease in the alkaline phosphatase activity following hypophysectomy of the rat. However, *KOCEN and CAVAZOS (1958)* found that scurvy had a little or no effect on the enzyme activity in the guinea Pig testis.

It could be concluded that the atrophy and degenerative changes in the testis was due to the destructive effect of prolonged periods of malnutrition which lead to fibrosis of the organ with subsequent impairment of the permeability of its blood vessels. The histochemical changes that occur in the undernourished group were mainly due to failure of the process of spermatogenesis.

The observed relative resistance of spermatogonia to the effect of prolonged period of undernutrition makes it possible to suggest that the potentiality of spermatogenesis is still kept wheather they will regain the power of cellular divison after a period of rehabilitation is yet to be investigated.

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LEGENDS OF FIGURES

Fig.(1): Photomicrograph of the testis of an adult control rat showing seminiferous tubules and interstitial cells lie near blood capillaries (arrow). (Hx.E x 400).

1a): Photomicrograph of the testis of a underourished rat showing semintiterous tubules with smaller dameters and irreguler outlines. The cells of some tubules appear vacuolated and widly separated from each other (arrow). There is a noticable increase in the number of interstitital blood capilleries (double arrows).(Hx.E x 400).

Fig.(2): Photomicrograph of the testis of an adult control rat showing. The testiculer capsule heavily stained, but the basement membranes of the tubules appear lighter (arrow). (Van Gieson Stain x 150).

2a): Photmicrograph of the testis of a underourished rat showing marked thickening of the testicular capsule. The basement membranes around the tubules appear densely stained and thick (arrow). (Van Gieson stain x 150).

Fig.(3): Photomicrograph of the testis of an adult control rat showing the reticular fibers of the basement membranes around seminifereus tabules, around blood

capillaries and in the interstitial cennective tissue appear as brwn to black short wavy fibers (Silver stain x150).

3a): Photomicrograph of the testis of underourished rat showing an increase of the reticular fibers of the basement membranes of the tubules. Notice that the reticular fibers around blood copillaries are markedly increased and deeply stained (arrow). (Silver stain x150).

Fig.(4): Photomicrograph of the testis of an adult control rat showing+ve reaction principaly observed in the developing stages of spermatids mainly attached to their nuclei (arrow). The Sertoli cell cytoplasm contain few fine granules (arrow head), but the spermatagonia and spermatotocytes showed-ve reaction (PAS stain x400).

4a): Photomicrograph of the testis of a underourished rat showing that the carbohydrates are difficult to be followed. The intense reaction is only noticed in some disrupted heads of old spermatids attached to normal Sertoli cells (arrows) (PAS stain x400)

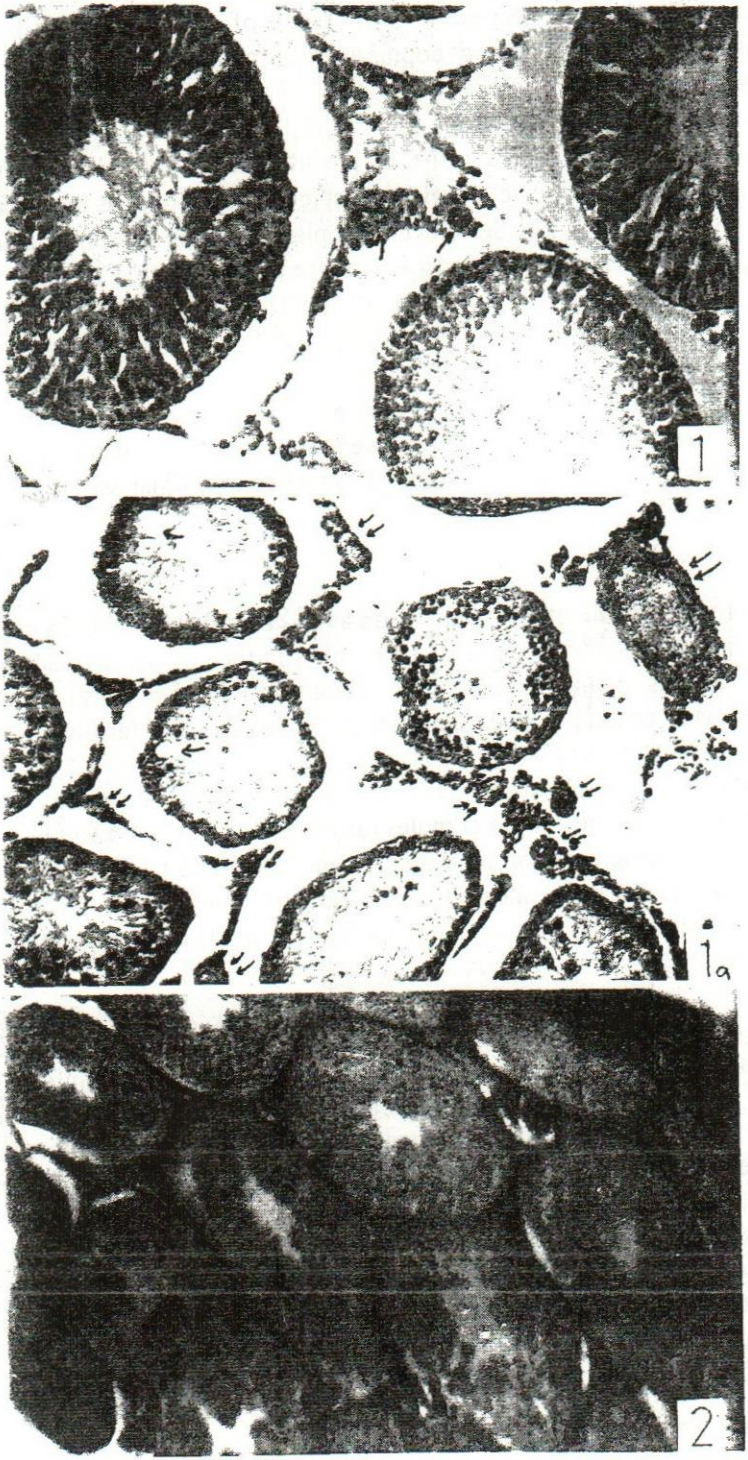
Fig.(5): Photomicrograph of the testis of an adult-control rat showing a seminiferous tubule in which lipids appear as large droplets in the sytoplasm of spermatogonia (arrow) and as small granulles in the cytoplasm of sertoli celis (double arrows) Spematocytes contain a moderate amount of lipid granules (arrow heads) (Sudan black x 1000).

5a): Photomicrograph of the testis of an adult Control rat showing that the interstitial cells has small lipid granules (arrows) (Sudan black x1000).

5b): Photomicrogramph of the testis of a underourished rat. lipids appear as few droplets in the remaining spermatogonia and Sertoli cells (arrow). Notice that the interstitial cells show an increase in the lipid contents if compared with these of the previous figure (double arrows). (Sudan black x1000).

Fig.(6): Photomicrograph of the testis of an adult control rat showing that the activity of alkaline phosphatase is tense in the boundary tissue of the seminiferous tubules (double arrows). The nuclei and membranes of spermatogonia and spermatacytes showed a moderate activity, but the reaction of the nuclear membranes is greater. The interstitial cells and the boudary tissue of blood capllaries show intease activity (arrows) (ALK.ph. x400).

6a): Photomicrograph of the testis of a underourished rat Showing that the alkaline phospatase activity of the spermatogonia and interstitial cells resembles more or less that of the Control group. There is a denser activity in young spermatids and spermatocytes (arrows) (ALK. ph. x400).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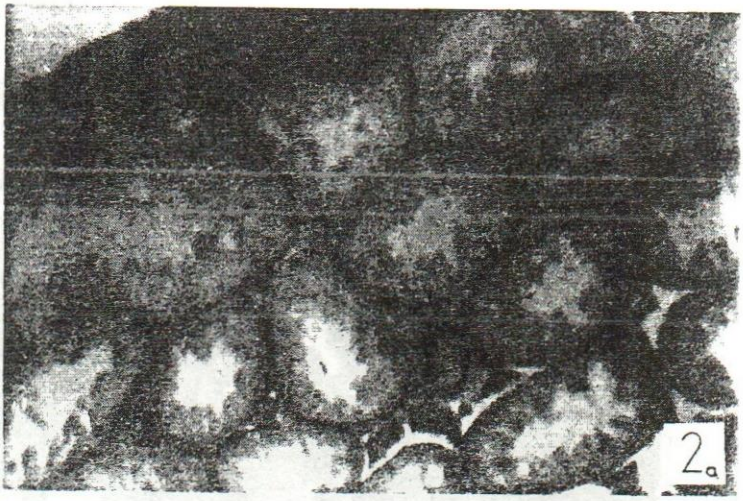
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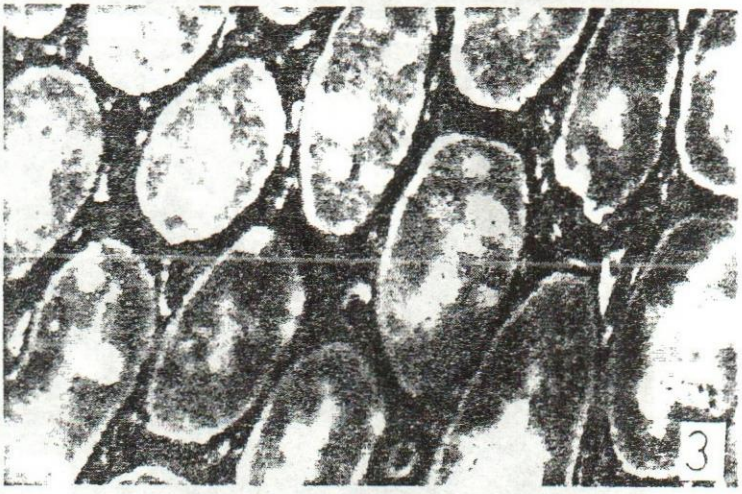
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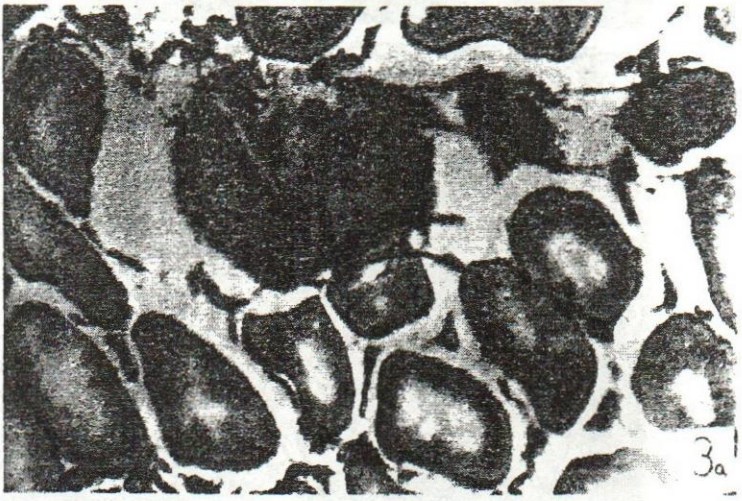
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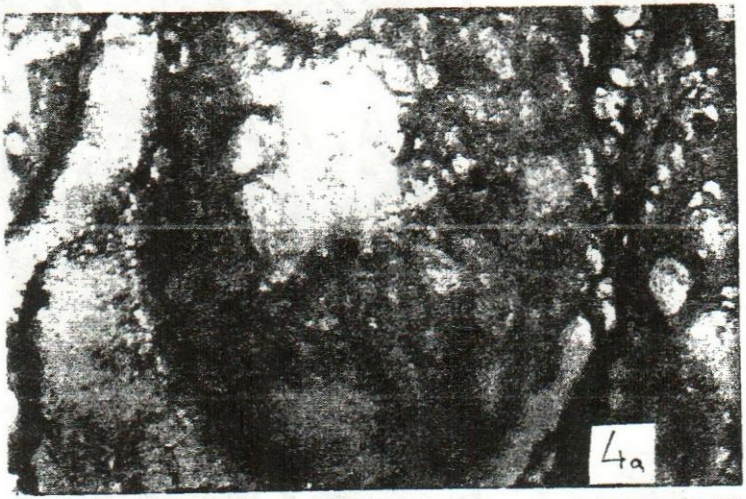
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