PATHOLOGICAL CHANGES IN THE TISSUES OF RATS (ALBINO) AND MONKEYS (*MACACA RADIATA*) IN FLUORINE TOXICOSIS

By T. K. Wadhwani, B.Pharm., F.I.I.Sc.
and A. S. Ramaswamy, B.Sc., M.B.B.S.
(*Section of Pharmacology, Department of Biochemistry, Indian Institute of Science, Bangalore-3*)

Summary

1. Stomach, duodenum, small intestine, kidney, liver, spleen, skin, heart, aorta, lungs, brain, pancreas, adrenals, thyroid and parathyroid of rats and monkeys suffering from chronic fluorosis have been histologically examined.

2. Fluorine has not been found to have any effect on the heart muscle, aorta, skin and parathyroids, whereas it has been found to adversely affect the histological structure of the remaining tissues, though in a varying degree.

3. The pathological changes observed in these tissues have been discussed.

Introduction

The effect of fluorine on the different tissues of the body is not precisely known. Sollmann *et al.* (1921) reported that they were unable to find histological lesions in rats fed 8 mg. of NaF per kg. of body weight daily for 9 weeks. Latterly, fluorine has been shown to cause degenerative changes in the kidney (Kick *et al.*, 1933, 1935; Phillips *et al.*, 1934; Roholm, 1937), liver (McClure and Mitchell, 1931; Phillips *et al.*, 1934; Velu and Zottner, 1934; Roholm, 1937) of pigs, cattle and sheep, and in the supra-renals, heart muscle and central nervous system of cattle (Phillips *et al.*, 1934; Roholm, 1937). Fluorine and fluorine compounds have been found to be antagonistic to the proper functioning of thyroids (Litzka, 1936; Kraft, 1936; May, 1935, 1937). Fluorine particularly has been reported to cause an increase in the size of the glands of dogs and rats (Goldemberg, 1927). Though, this has not been confirmed by Channels (1929), and Tolle and Maynard (1931), Phillips *et al.* (1935) have shown that, in the growing chick, the non-toxic levels of desiccated thyroid are made distinctly toxic by chronic fluorine poisoning produced by the ingestion of NaF. Feeding of F has been reported to alter the structure and functions of parathyroids (Bergara, 1927; Channels, 1929; Pavlovic and Tihomirov, 1932; Kochman, 1934);

but this has not been confirmed by Hauck *et al.* (1933, 1933 *a*, 1934) and Kick *et al.* (1935).

Thus, it can be seen that, except for isolated and limited studies on few tissues of the body, no comprehensive histological study of almost all the tissues has so far been made under the conditions of fluorine toxicosis, and what has been done, besides being not commensurate with the importance of endemic fluorosis, does not seem to be very well established. The latter circumstance is mainly due to the fact that, in the studies on the effect of fluorine on the various tissues carried out hitherto, some or all of the following points have either been ignored or overlooked.

(i) The toxicity of F is determined by the nature of the fluorine compound, its quantum and mode of ingestion, the age and the species of the experimental animals, and the composition of the diet fed.

(ii) Fluorine, in large doses, behaves like a corrosive poison, and, in small doses, like a systemic poison. The toxic effects of F as a corrosive poison are necessarily to be different from those of F as a systemic poison.

(iii) Keeping all the other factors constant, the toxicity of a definite concentration of F increases with its period of administration. The effect of F on the various tissues of any animal in the initial and intermediate stages of F administration is likely to be, besides quantitatively, also qualitatively different from that in the final stages of F intoxication. To acquire complete knowledge about the effect of F on the various tissues, it is therefore necessary to study such an effect of F when the experimental animal is in the final stage of fluorosis.

Bearing the above points in mind, studies have been carried out on the effect of F on the various tissues of rats and monkeys, when these animals have been placed on a normal diet, and have been administered the dose of F, which, over a period of time, has been found to result in the syndrome of chronic fluorosis.

MATERIALS AND METHODS

12 Albino rats, five to six weeks old, and 4 male monkeys (*Macaca radiata*), nearly of equal weight, were equally divided into two groups of experimental and control. The rats were placed on the diet, consisting of cottonseed globulin 9%, gelatin 4%, nitrogen-free starch 65%, salts 4%, choline hydrochloride in sugar (1 : 9) 1%, cystine in sugar (1 : 19) 1%, vegetable fat 16%, and the requisite amounts of vitamins A, D and of B complex. Each of the rats in the experimental group was given 2 mgm. of NaF daily for a period of 20 weeks.

The monkeys were placed on the following diet. In the morning at 9 A.M., each animal was given 50 gm. of bread with 2 mgm. of ascorbic acid and sufficient shark liver oil to supply approximately 100 units of vitamin A and 30 units of vitamin D. At noon, at 12, each animal was given a cake prepared from 50 gm. of the mixture, consisting of wheat flour 61%, casein 15%, sugar 5%, salts 4% and fat 10%, and, in the evening at 4 P.M., 25 gm. of groundnuts. Water was given *ad libitum*. To each experimental animal, before the morning bread was offered, F as NaF was daily administered in the concentration of 10 mgm. of NaF per kg. of body weight for a period of 24 weeks.

In the case of both the rats and monkeys, sodium fluoride in solution was given per os, delivered from a suitable pipette, about an hour before the food was offered. By directly giving the dose of fluoride dissolved in water, and not admixed with the diet, the ingestion of the above quantity of fluoride was thus ensured.

When the experimental animals were in the last stage of fluorosis, *i.e.*, at the end of the period given above, the animals were sacrificed, and the necessary tissues were taken out for histological examination.

Selection of the tissues

The tissues were selected on the basis of the fact that F is a systemic poison, and F toxicosis, a generalised systemic reaction. An attempt has therefore been made to examine the tissues from nearly all the systems of the body. The following tissues have been histologically examined:—

1. *From the digestive tract.*—Stomach, duodenum and small intestine.
2. *From the urinary system.*—Kidney.
3. *From the circulatory system.*—Heart and aorta.
4. *From the respiratory system.*—Lungs.
5. *From the nervous system.*—Cerebral cortex.
6. *From among the ductless glands.*—Thyroid, parathyroid, pancreas and adrenals.
7. *From among the other tissues.*—Liver, spleen and skin.

The sections of these tissues were prepared according to the methods described by Carlton (1938). The sections were stained with Heidenhain's iron-hæmatoxylin.

The photo-micrographs given here are for the tissues of monkeys only. No photomicrographs of the tissues of rats are given here as the changes observed in these are similar to those observed in the tissues of monkeys,

excepting that these changes are less pronounced in the tissues of rats th in the tissues of monkeys.

Description of the photo-micrographs

1. *Section of the stomach of monkey.*—There is marked degeneration of the epithelium and irregularity of the glands together with the atrophy of the glandular tissue. The sub-mucous coat is intact. × 73.

2. *Section of the duodenum of monkey.*—Destruction of the epithelium and lymphocytic infiltration are marked. × 153.

3. *Section of the small intestine of monkey.*—Mucous and sub-mucous coats are considerably destroyed. There is patchy infiltration of lymphocytes. × 153.

4. *Section of the kidney of monkey.*—There is marked degeneration and necrosis of the tubular cells. The glomerulus is considerably atrophied. × 306.

5. *Section of the liver of monkey.*—Save for general pigmentation, no significant change has been observed around the portal tract. There is pronounced central necrosis. The cells around the central vein are pale and vacuolated. ×306.

6. *Section of the spleen of monkey.*—Fibrosis is present with patchy areas of cellular destruction. × 306.

7. *Section of the spleen of monkey.*—Periarterial thickening is marked. × 306.

8. *Section of the lung of monkey.*—Alveoli are ruptured. Epithelium shows signs of degeneration. × 73.

9. *Section of the lung of monkey.*—Fibrosis is present with indications of regeneration of alveolar epithelium. × 73.

10. *Section of the cerebral cortex of monkey.*—There is chromatolysis of the cells. × 306.

11. *Section of the cerebral cortex of monkey.*—There is chromatolysis of the cells, with replacement with fibrous tissue. × 306.

12. *Section of the pancreas of monkey.*—The arteries have been observed to be thickened. There is slight degeneration of the glandular cells. × 306.

13. *Section of the pancreas of monkey.*—There is slight degeneration of the islet cells. × 306.

14. *Section of the supra-renal of monkey.*—Pigmentation has been observed both in the cortex as well as in the medulla. There is patchy degeneration of the cortical cells. × 306.

15. *Section of the supra-renal of monkey.*—There is marked degeneration of the medullary tissue. × 306.

16. *Section of the thyroid of monkey.*—Epithelium is atrophied. The colloid is abundant, and stains deeply. × 153.

No changes have been observed in the heart muscle, aorta, parathyroid and skin of rats and monkeys.

Discussion

From the photo-micrographs given here, it can be seen that fluorine seems to cause a selective destruction of the epithelium. The epithelium has been found to be considerably damaged in the stomach,[1] duodenum,[2] small intestine,[3] lungs[8] and thyroid.[16] The loss or damage of the epithelium of the digestive tract would undoubtedly have an adverse effect on the functions of the tract. Such a circumstance has rather already been demonstrated (Wadhwani, 1953). It has been shown that, as a result of F ingestion, the primary and secondary absorption of nitrogen, calcium and phosphorus is markedly reduced in rats, and that there is also the reduced retention of these elements in the system, or, in other words, their increased excretion in the urine, indicating either lesser utilisation of these elements at the site of the cell or their uncontrolled passage through the kidney or both. Pathological changes brought about in the kidney of the monkey[4] in fluorine toxicosis would suggest that the increased urinary excretion of nitrogen, calcium and phosphorus in fluorosis, may, in part, be due to the disfunctioning of the kidney. In humans with severe chronic fluorosis, Shortt *et al.* (1937) observed that, as judged by filtration rate and blood urea clearance values, in the majority of the cases, kidney function was impaired, and in some, markedly so. In experimental monkeys receiving 10 mgm. of NaF per kg. of body weight daily, Pandit and Rao (1940) made a similar observation, and found the presence of albumin in the urine.

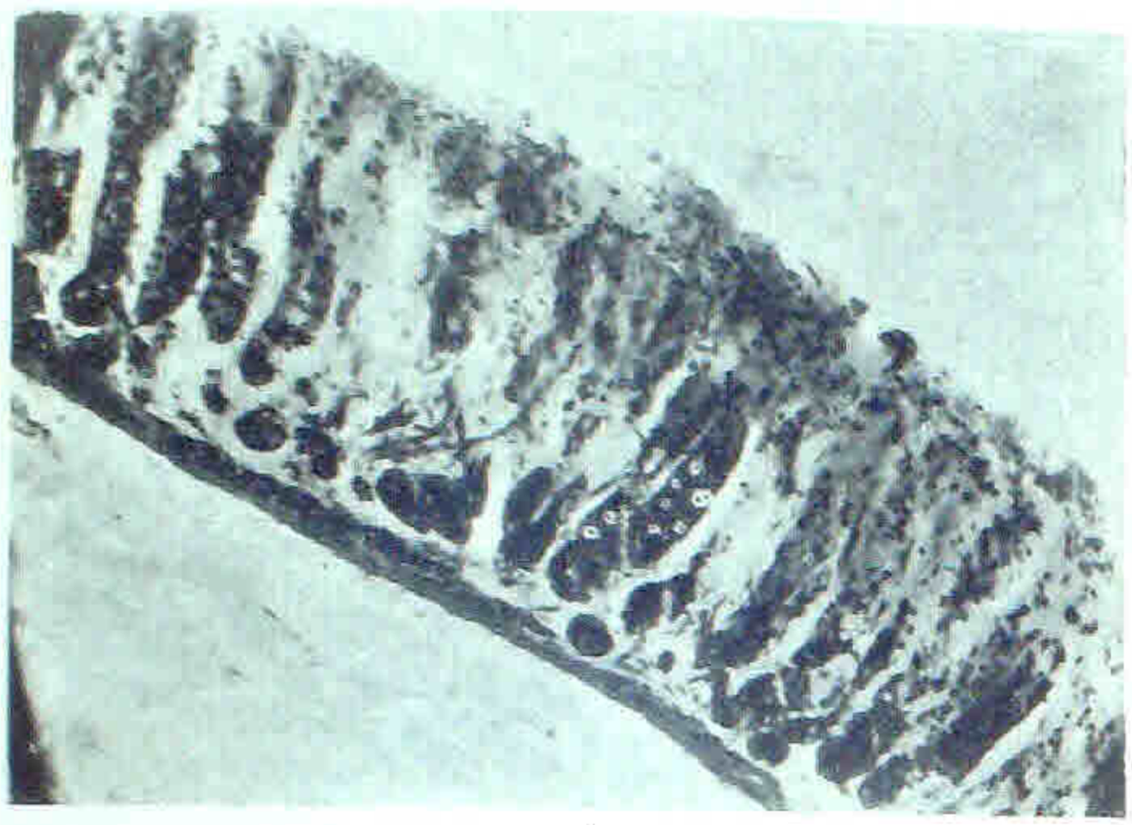
Among the tissues of the circulatory system, F has not been found to have any adverse effect on the histological structure of heart muscle and aorta. The arteries, however, have been found to be thickened.[7, 12] Similarly, F has not been observed to affect the structure of the skin. The changes observed in the liver[5] and spleen[6] are of degenerative type, with fibrosis present in the case of the latter.[6] Such a state of the liver and spleen will necessarily be reflected in the picture of the blood. It has been reported that chronic fluorine poisoning brings about a condition of anæmia. This has been shown by Leake and Ritchie (1926), and Risi (1931) in dogs, by Valjavec (1932) in rabbits, by Slagsvold (1934) in sheep, by Mazumdar and Ray (1946) in bulls, and by Shortt *et al.* (1937) in humans.

The effect of F on the cerebral cortex is much pronounced. There is considerable chromatolysis of the cells,[10] and the formation of the fibrous tissue.[11] In the final stages of chronic fluorosis, the experimental monkeys did not conduct themselves with intelligence and agility of mind normally associated with them. There was a significant lack of co-ordination in their behaviour. In humans with chronic fluorosis, Shortt *et al.* (1937) have made a similar observation. They found that " there was some diminution in pain and thermal sensation in several cases; thermal sensation was lost over the lower extremities in two most severe cases; tactile and vibration sense was also lost over the same area, and, in these two cases, there was also loss of sphincter control ".

Among the ductless glands examined, F seems to have no effect on the parathyroid, and has the most deleterious effect on the adrenals, thyroid and pancreas coming next in the order. The data on the effect of F on the parathyroid have so far been conflicting (*vide* Introduction). It is now shown that, under normal dietetic conditions, fluorine, given in the concentration required to produce chronic fluorosis over a reasonably long period, has no effect on the parathyroid of monkeys. The administration of parathyroid harmone has not been found to arrest the development of F intoxication in growing rats (Munoz, 1936).

The effect of F on the pancreas[12, 13] has been found to be rather slight. Except for slight degeneration of the glandular and islet cells, no other change has been observed in the histological structure of pancreas of monkeys suffering from chronic fluorosis. However, Handler *et al.* (1946) showed that fatal F poisoning in rats was accompanied by markedly elevated blood glucose and lactic acid concentrations, and severely diminished liver and muscle glycogen concentrations, and that the administration of insulin 30 minutes before the ingestion of fluoride prevented the increase in blood glucose and decrease in muscle glycogen but did not affect the accumulation of lactic acid or the depletion of liver glycogen.

The toxic action of F on the thyroid of the monkeys has resulted in a condition similar to colloid goiter. The epithelium is considerably degenerated, and the acini are filled with a large amount of colloid which stains deeply.[16] According to Boyd (1940), colloid goiter is a compensatory hypertrophy wherein the gland, after meeting the demands of the tissues, is unable to return to its former size. Such a condition of the thyroid has been observed in endemic goiter also, and Wilson (1941) has suggested that endemic goiter is due to F compounds present in excess in soil, diet and water, and that the distribution of endemic goiter in Punjab and England is related to the



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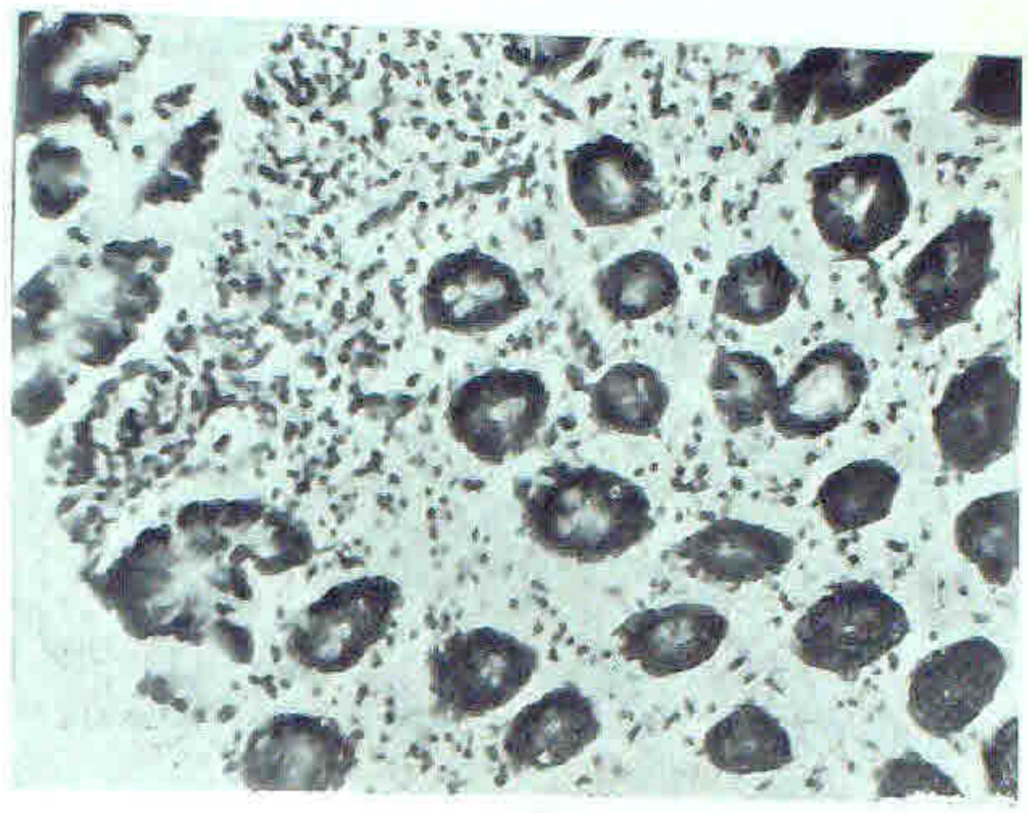

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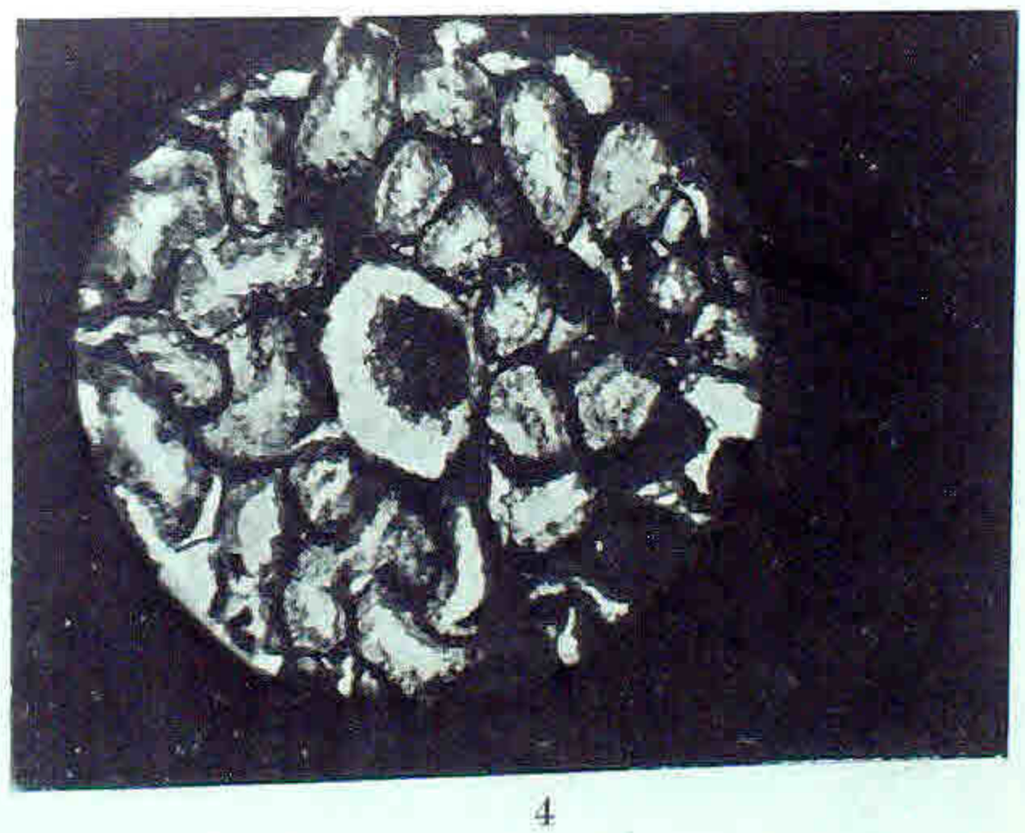

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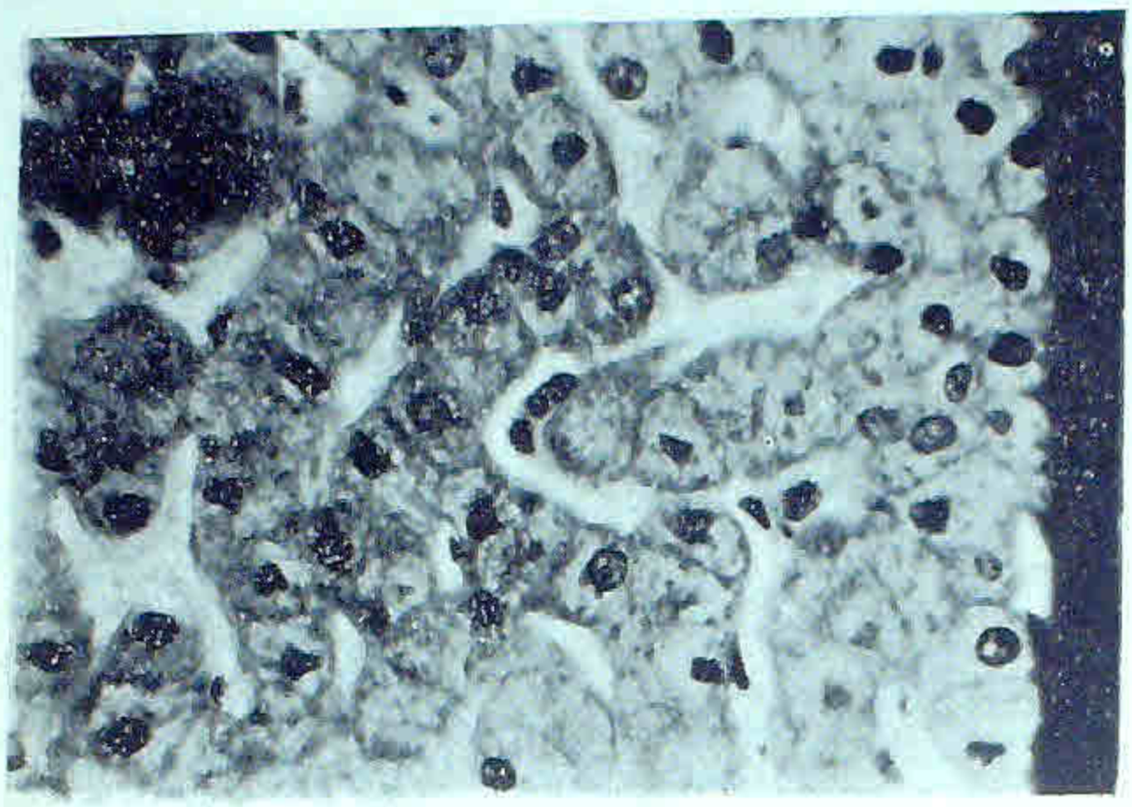

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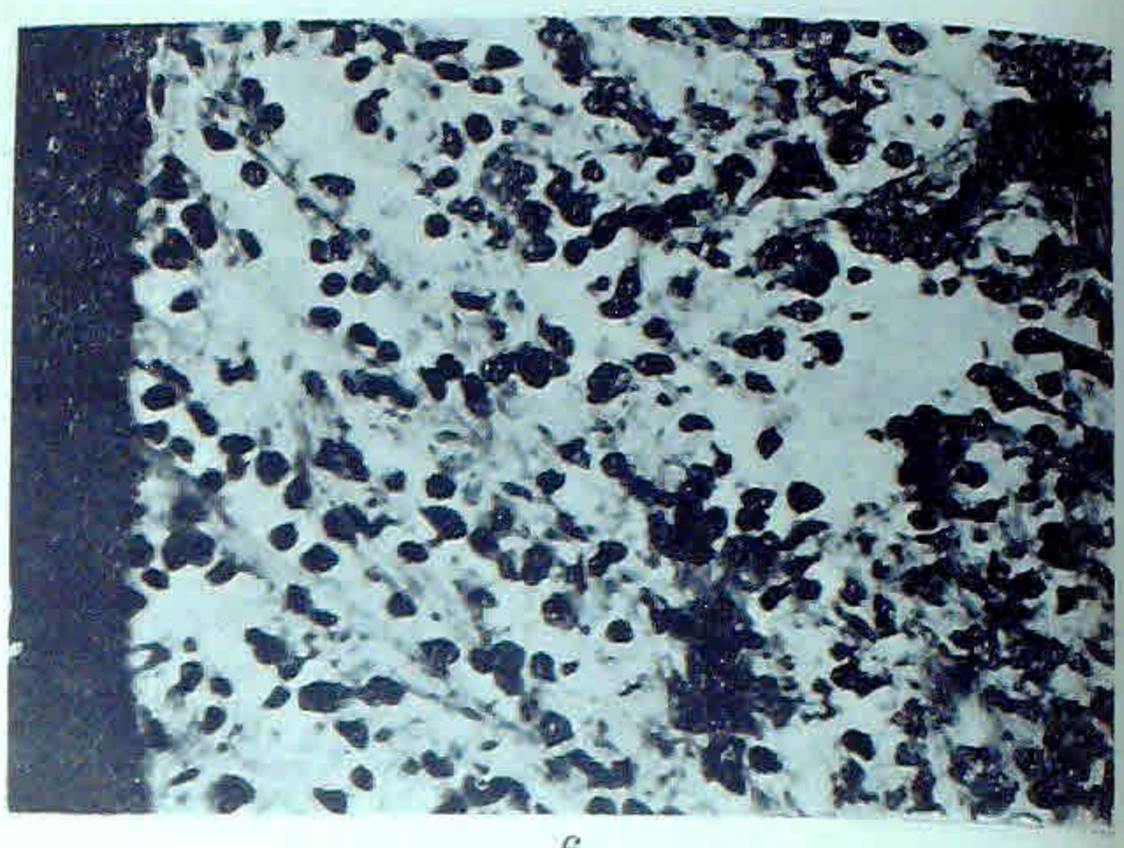

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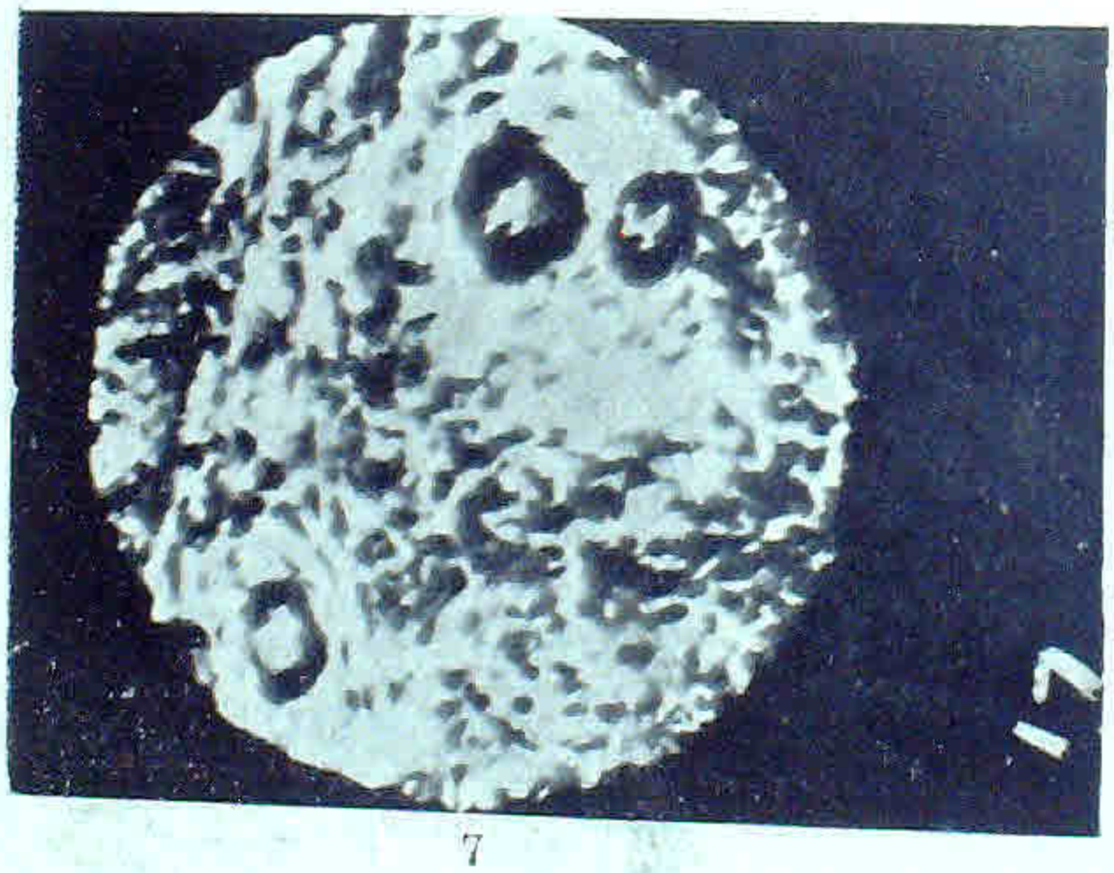

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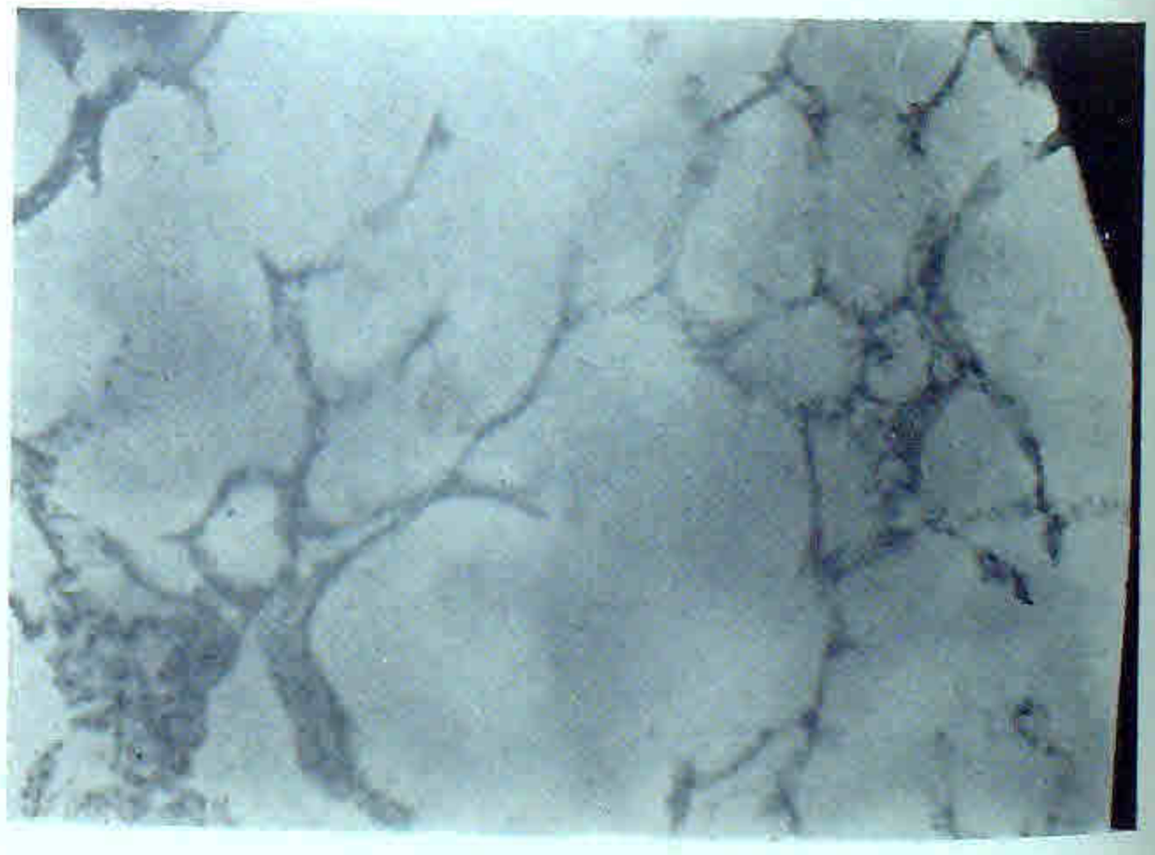

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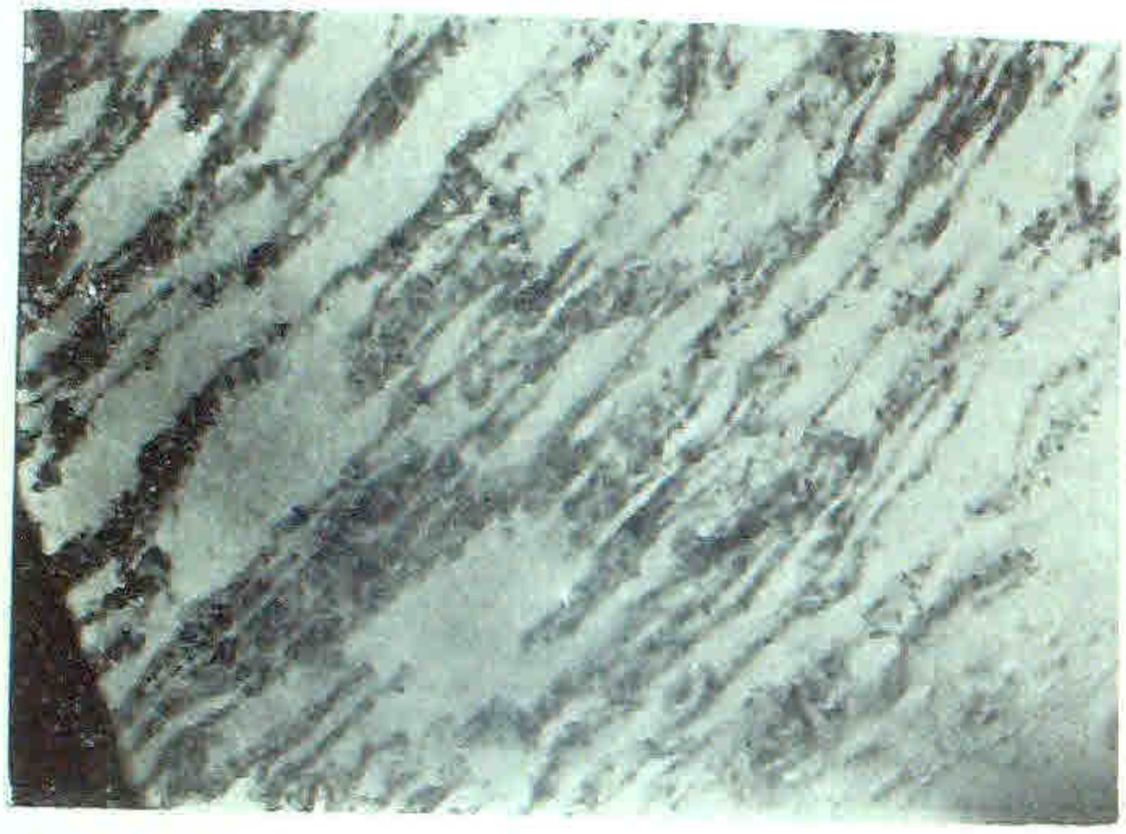

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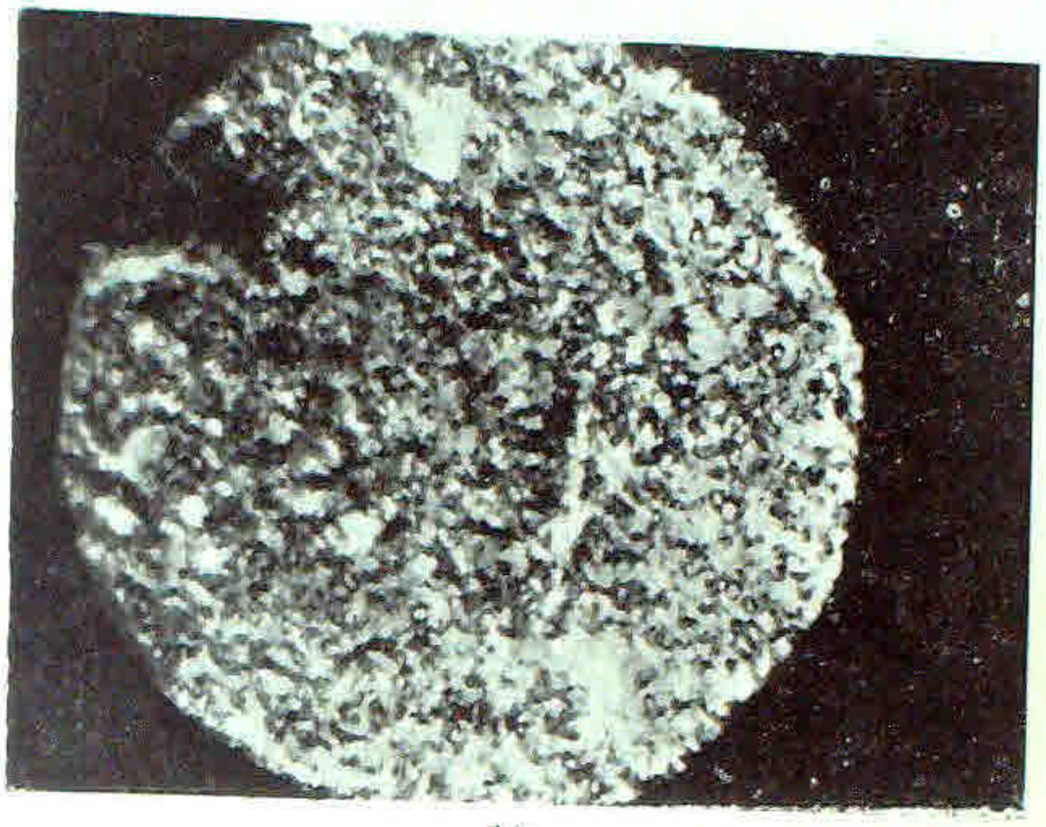

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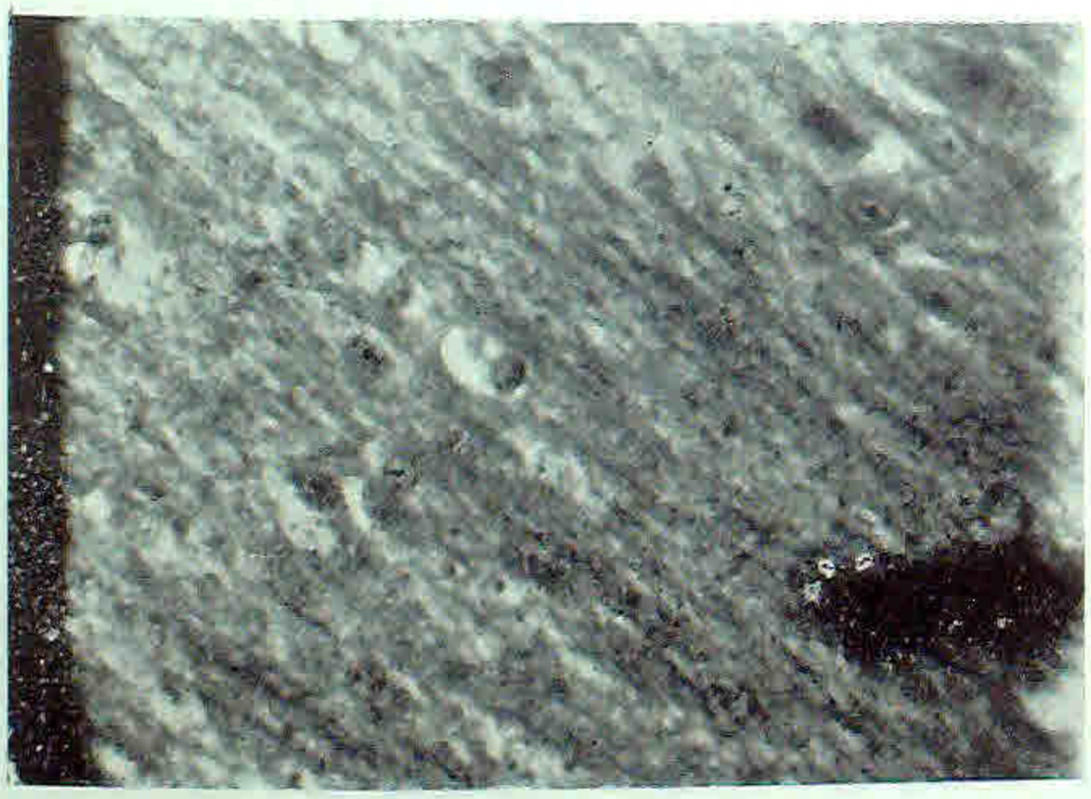

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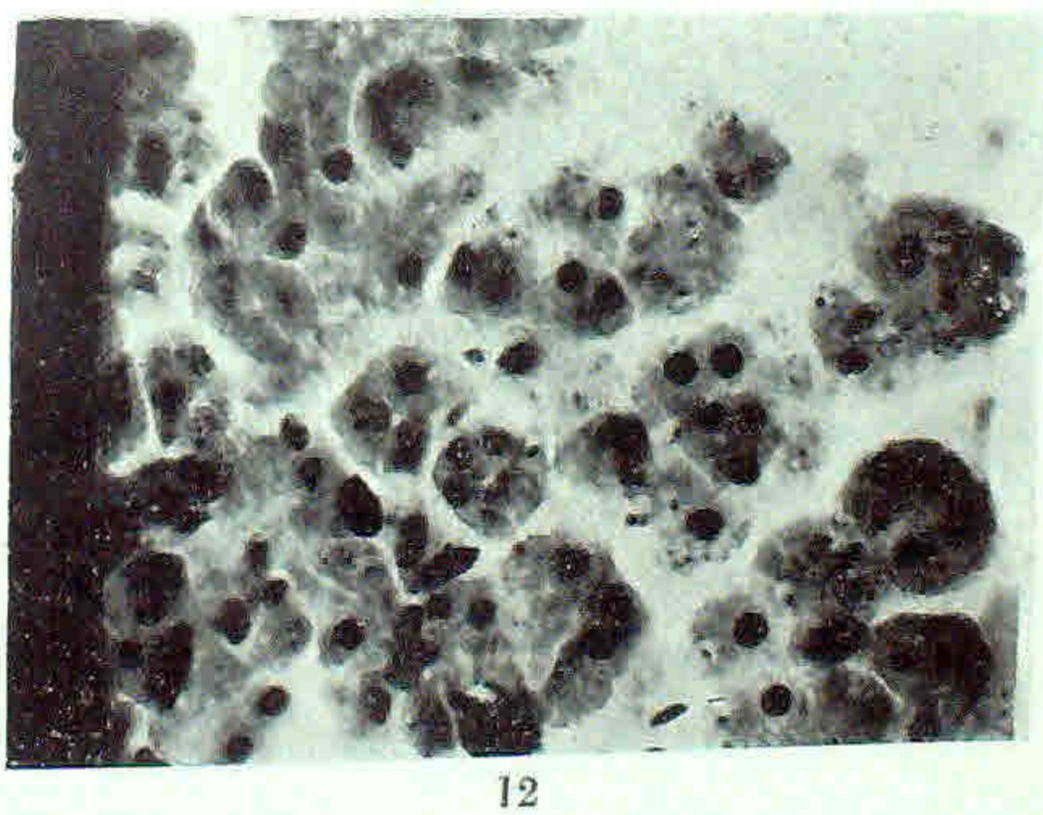

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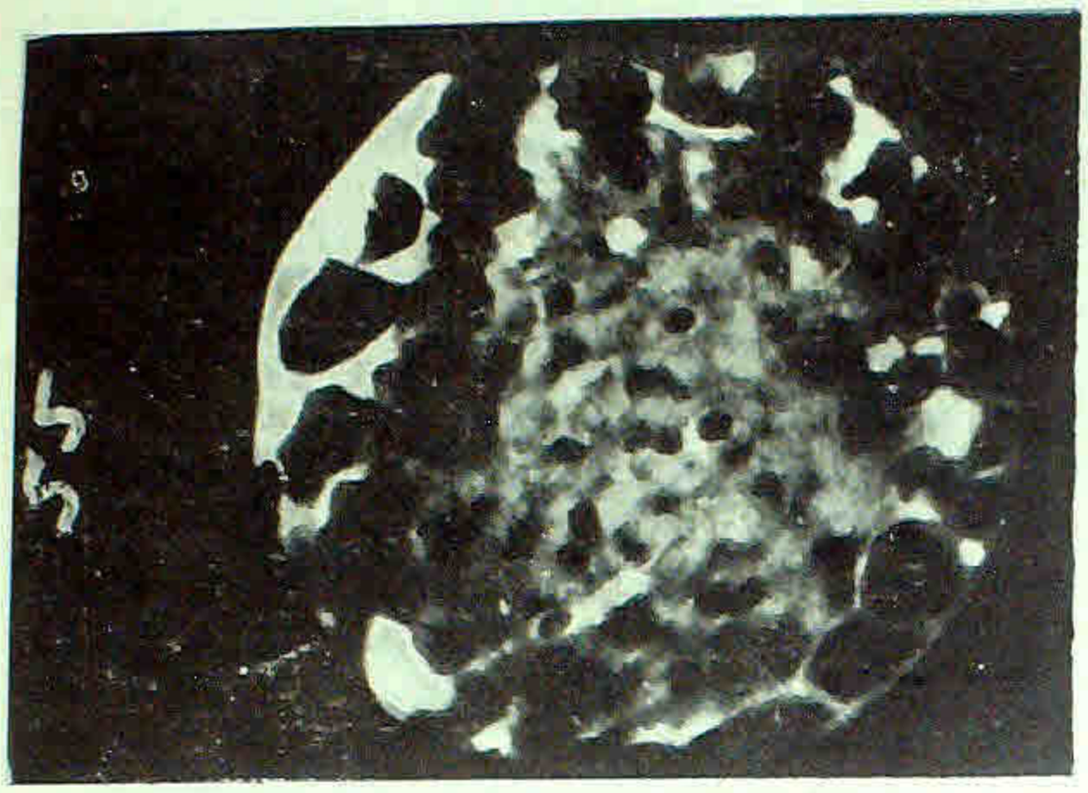

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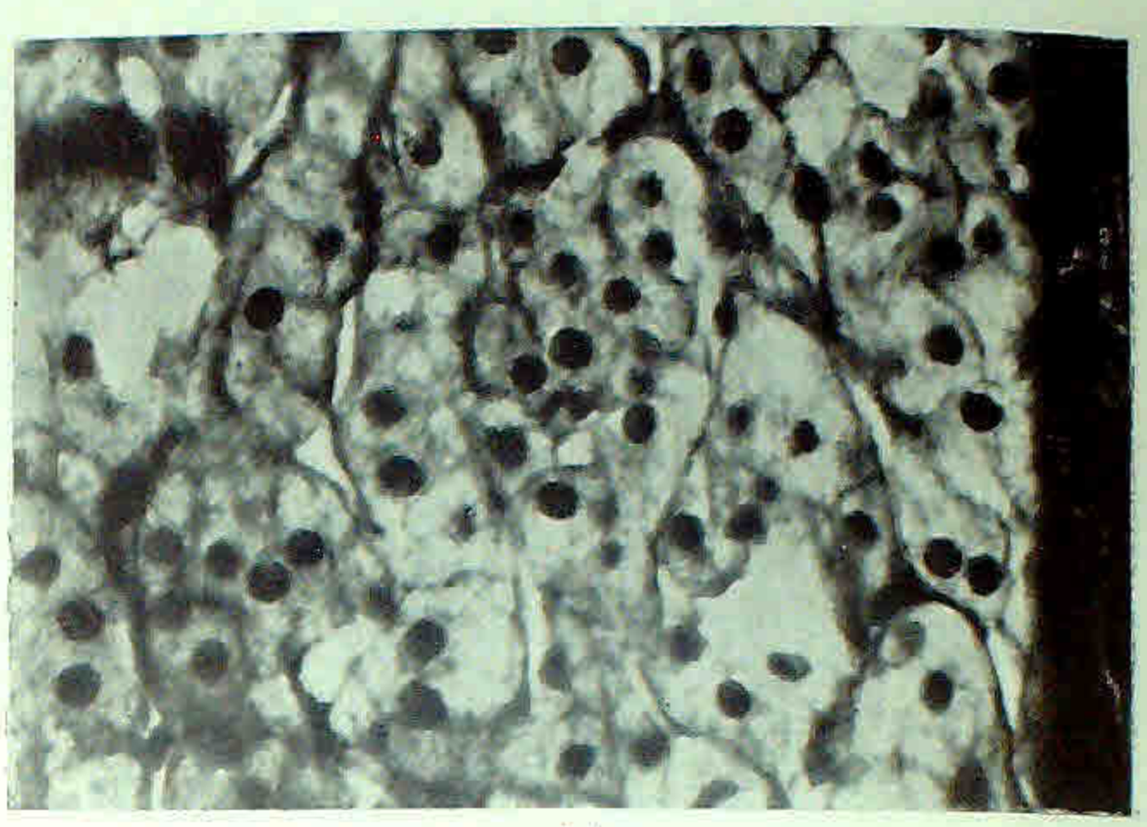

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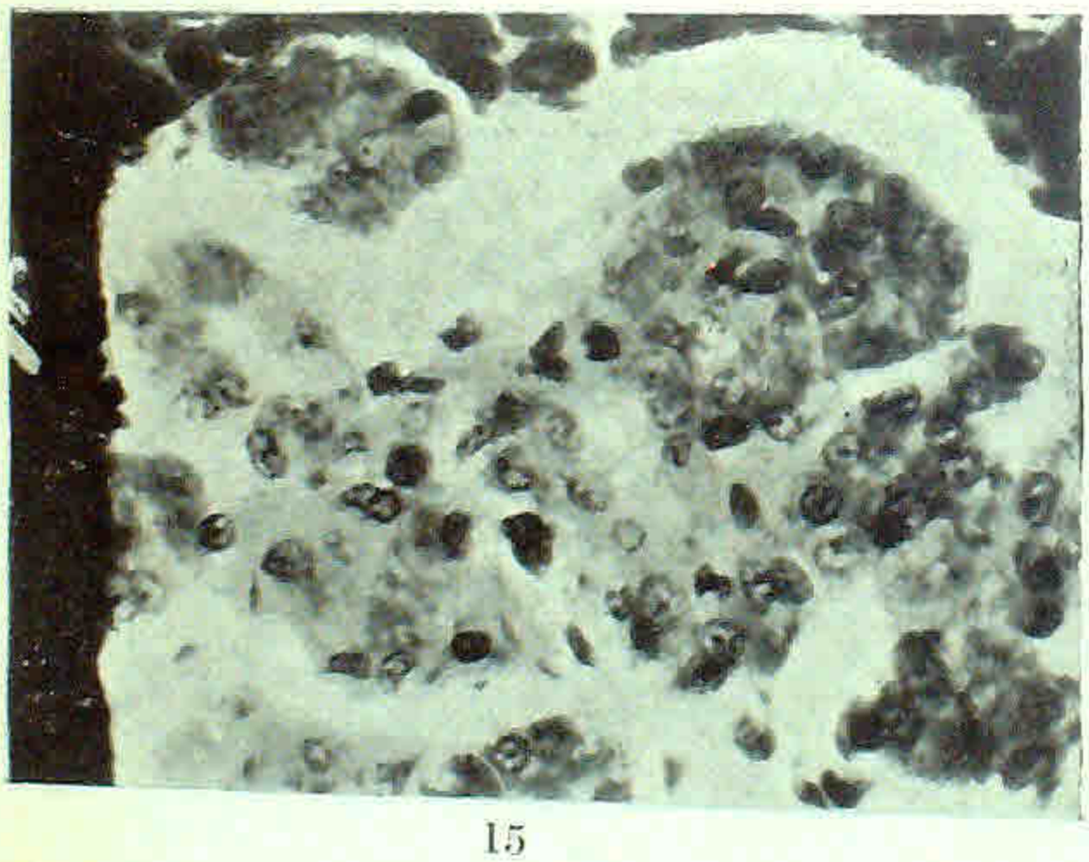

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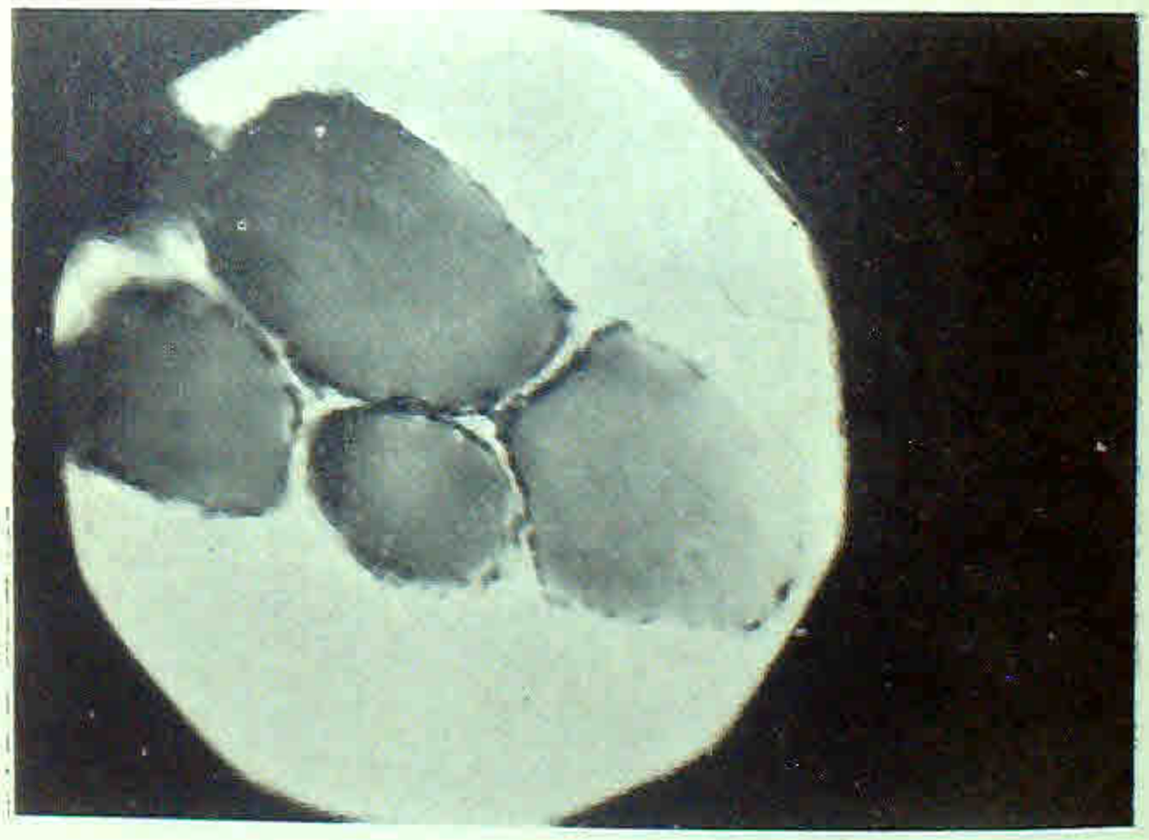

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geological distribution of F, and to the distribution of human dental fluorosis.

As the suprarenal cortex controls sexual development, and the medulla stimulates all structures innervated by the sympathetic nervous system, the toxic action of F on the adrenals, causing the degeneration of the cortical cells[14] and the medullary tissue,[15] will have a very adverse effect on these functions of the glands.

Though F is known as a systemic poison, and F toxicosis, as a generalised systemic reaction, it cannot be said that F affects all the tissues of the system. In chronic F toxicosis, produced as a result of ingestion of small quantities over a long period, under normal dietetic conditions, F has not been found to have any effect on the heart muscle, aorta, skin and parathyroid of rats and monkeys, and its effect on the other tissues has not been observed to be equally adverse.

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