# EFFECTS OF CORTISONE AND EPINEPHRINE ON HEPATIC AND MYOCARDIAL GLYCOGEN OF MICE AND RATS[1]

W. LANE WILLIAMS, RICHARD L. DAVIS
AND CHARLES U. LOWE

*Department of Anatomy, University of Minnesota, Minneapolis, and the Statler Research Laboratories, Children's Hospital, Department of Pediatrics, University of Buffalo School of Medicine, Buffalo, N.Y.*

EIGHTEEN FIGURES

## INTRODUCTION

That administration of cortisone results in an increase of hepatic glycogen has been demonstrated in rabbits and rats by chemical analyses and by histochemical technics that permit intracellular localization of this carbohydrate (Lowe, Williams and Thomas, '51; Timiras and Koch, '52; Williams, Lowe and Thomas, '53). In the present study mice and rats were injected with large amounts of cortisone to increase the glycogen content of hepatic parenchyma and of myocardial fibres (Williams, '54; Williams and Lowe, '54). A localization-staining method, the periodic acid-Schiff's reagent (PA-S) technic, was used to demonstrate glycogen. Some of the mice were injected with epinephrine to determine the activity of this hormone in depleting this quantitatively atypical content of glycogen within the liver and myocardium (Davis and Williams, '55).

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## MATERIALS AND METHODS

Male and female rats (Wistar stock) and mice (Strong A x Bagg albino hybrids) were used. Tissues from 10 rats and 30 mice served as control material for the experiments described below.

Ten rats (100 to 200 gm in weight) and 50 mice (22 to 24 gm in weight) were injected with cortisone acetate.[2] The rats were injected intramuscularly with 25 mg of cortisone daily for 5 days; the mice, subcutaneously with 2.5 mg daily for 7 days. These animals were killed 24 hours after the last injection of cortisone.

Fourteen mice were injected with 2.5 mg, and 10 mice with 0.625 mg of cortisone daily for 7 days. Twenty-four hours after the last injection of cortisone all of these mice were injected with epinephrine [3] (0.5 mg in saline intraperitoneally) and were killed 1, 2 and 5 hours subsequently. Twenty of these mice were killed one hour after injection of epinephrine.

Twelve mice (not previously injected with cortisone) received epinephrine as above and were killed 1, 2 and 5 hours later.

Animals were killed between 10 and 11 A.M., the rats by exsanguination through the portal vein during light ether anesthesia, and the mice by digital compression of the cervical spinal column. The rats that had been injected with cortisone were fasted for 24 hours before death. Food was available to control rats and to all of the mice until the time of death. All animals were fed Purina Fox Chow.

Tissues were fixed immediately in Lavdowsky's solution. The histologic and histochemical procedures including the PA-S technic and saliva digestion of carbohydrate were used as described previously (Williams, Lowe and Thomas, '53; Lowe and Williams, '53). Relative quantitation of the amount of glycogen was accomplished by "grading" the amount of

[2] Epinephrine solution, Burroughs Wellcome and Co.

[3] Generously supplied by the Schering Corporation through the courtesy of Dr. Edward Henderson.

PA-S staining as seen with a comparison ocular fitted to two identical microscopes with a single source of illumination.

## OBSERVATIONS

The results are summarized in table 1.

### *Controls*

*Livers.* In both mice and rats granules of glycogen (PA-S positive material digested by saliva) were abundant in the cytoplasm of parenchymal cells (figs. 1, 2, 9). The intralobular distribution of glycogen was relatively uniform.

TABLE 1

| | STAINING REACTIONS | |
|---|---|---|
| | PA-S technic | Saliva-digestion (30 minutes) followed by PA-S technic |
| I. Cytoplasm of hepatic parenchymal cells in: | | |
| A. Controls: | | |
| Rats | 4 + | 0 |
| Mice | 4 + | 0 |
| B. Epinephrine-injected [1]: | | |
| Mice | 0–1 + | 0 |
| C. Cortisone-injected: | | |
| Rats [2] | 6 + | 0 |
| Mice [3] | 6 + | 0 |
| D. Mice injected with cortisone [3] and epinephrine [2] | 0–1 + | 0 |
| II. Myocardial fibres in: | | |
| A. Controls: | | |
| Rats | trace | 0 |
| Mice | trace–1 + | 0 |
| B. Epinephrine-injected [1]: | | |
| Mice | trace–1 + | 0 |
| C. Cortisone-injected: | | |
| Rats [2] | 6 + | 0 |
| Mice [3] | 4 +–6 + | 0 |
| D. Mice injected with cortisone [3] and epinephrine [2] | 4 +–6 + | 0 |

[1] 0.5 mg of epinephrine 1–5 hours before death.

[2] 25 mg of cortisone daily for 5 days.

[3] 2.5 or 0.625 mg of cortisone daily for 7 days.

*Myocardium.* Evidence of localization of glycogen was limited to trace amounts of PA-S positive material at the sarcolemmic borders of the muscle fibers (figs. 11 and 12). Incubation of sections in saliva eliminated this small amount of staining. The PA-S positivity of the connective tissue of the heart was well demonstrated and this staining was not reduced following exposure of the sections to saliva.

### *Epinephrine-treated mice*

In mice injected with epinephrine PA-S positive material was absent from hepatic parenchymal cells except in cells with a predominantly portal (zone) location in the lobules (figs. 5 and 6). These remaining granules were not PA-S positive in sections that had been incubated in saliva. Since the amount of residual glycogen was constant at one to 5 hours after injection of epinephrine it does not seem likely that it represents repletion.

*Myocardium.* The amount and distribution of PA-S positive material was identical with that observed in mice that had not received epinephrine.

### *Cortisone-treated mice and rats*

*Livers.* An increase in cytoplasmic glycogen represents only one of the reactions of hepatic parenchyma to excessive amounts of glucocorticoids. Others including depletion of cytoplasmic acidophilia and basophilia have been described previously (Lowe, Williams and Thomas, '51; Williams, Lowe and Thomas, '53; Lowe and Williams, '53). In rats and mice the cytoplasm of parenchymal cells was slightly enlarged and was completely filled with PA-S positive granules which were not present in sections that had been incubated in saliva (figs. 3, 4, 10). In size and number the granules of glycogen were greatly increased in relation to those observed in mice and rats that had not been injected with cortisone (figs. 1, 2, 9). The appearance of the cytoplasm of liver cells was identical in mice and rats except that in the latter the "fixa-

tion'' of glycogen seemed superior since the granules were more uniform in size and distribution.

*Myocardium.* The cardiac muscle fibres of rats injected with cortisone were completely filled with glycogen (figs. 11–14). In similarly treated mice the myocardial glycogen was less evenly distributed within individual fibres (figs. 15 and 16). Mice that had received 0.625 mg of cortisone (daily) showed as much glycogen in hearts and livers as did those injected daily with 2.5 mg for the same period of time (7 days).

*Other effects of cortisone.* The total number (74) of mice injected with cortisone was sufficient to demonstrate several actions of a glucocorticoid in this species. Body weight decreased approximately 12% after 7 days of injection at either dose level. In addition to glycogenosis the hepatic parenchyma showed the cytoplasmic depletion of acidophilia and basophilia mentioned earlier in this report. There was moderate atrophy of lymphoid tissue. Diffuse pneumonia was common.

### *Mice injected with cortisone and epinephrine*

*Livers.* These mice showed depletion of hepatic glycogen identical with that described in mice receiving epinephrine, but no cortisone (figs. 7 and 8).

*Myocardium.* The epinephrine did not reduce the characteristic cortisone-induced glycopexis of the myocardium (figs. 17 and 18). The PA-S positivity of the cardiac muscle fibres was equal to that seen in hearts of mice injected with cortisone alone.

### *Effects of adrenal hormones on skeletal muscle*

No attempt was made to survey individual muscles of the rats and mice. In normal rats and mice and in those receiving cortisone the demonstration of glycogen in anterior abdominal and in thigh muscles was limited to very weak reactions at the borders of some of the fibres. Neither cortisone nor epinephrine increased or decreased this staining. The dia-

phragm was studied in control mice and in those injected with one or both of the adrenal hormones. In the controls positive staining for glycogen was more evident in muscle fibres of the diaphragm than in abdominal or thigh muscles. Injection of cortisone for 7 days increased the intensity of the reaction for glycogen in the diaphragm. However, the increase in glycogen in the muscle fibres of the diaphragm was insignificant when compared with the increment produced by cortisone in the myocardium (which also showed only trace amounts of glycogen in normal mice). Injection of epinephrine to mice previously treated (for 7 days) with cortisone produced no significant reduction of the staining reaction for glycogen in the diaphragm.

## DISCUSSION

Early studies demonstrated that injection of adrenocorticotropic hormone or of certain adrenal cortical extracts is followed by increase in the glycogen content of hepatic parenchyma and of voluntary muscle (Long, '42). This glycostasis or glycopexis represents a major activity of compounds classified as glucocorticoids. (Ingle, '50.)

The mobilizing or glycogenolytic action of epinephrine upon carbohydrate stores, particularly those of the liver and of skeletal muscle, is well known (Cori, '31 and '40; Britton and Silvette, '37). The present histochemical findings demonstrating depletion of hepatic glycogen by epinephrine in normal and cortisone-injected mice are in general agreement with studies of the actions of epinephrine on glycogen stores of rats. Strand and Gordon ('53) observed that within an hour epinephrine produced a decrease in hepatic glycogen which was followed three hours later by a rise to levels exceeding those existing prior to injection of the hormone. Although the mouse livers studied here showed the same decrease in glycogen at one, two and 5 hours after injection of epinephrine the number of animals killed at 5 hours was too small to determine whether or not some replacement immediately followed the initial depletion.

The plan of the present experiment does not permit consideration of the activity of glucocorticoids in the depletion of glycogen stores subsequent to epinephrine-induced glycogenolysis (Winternitz and Long, '52). Understanding of reciprocal relationships of organs and tissues in the storage and loss of glycogen as reactions to epinephrine requires extensive studies utilizing chemical analysis and histochemical localization applied not only to liver, muscle, blood and excreted material, but also to such depots as lymphoid and adipose tissue (Engel and Scott, '50; Strand and Gordon, '53).

The glycopexic activity of glucocorticoids on skeletal muscle has been studied extensively (Russell and Wilhemi, '50; Leonard, '53). Epinephrine exerts its glycogenolytic action on the cortisone-induced glycogenosis in such muscles, but not to the same degree quantitatively in individual muscles (Illingworth and Russell, '51, Leonard and Ringler, '54; Kostyo and Leonard, '55). In the present study the failure of the PA-S technic to demonstrate significant amounts of carbohydrate in skeletal muscles of either control or cortisone-injected animals permits no conclusions concerning the actions of either of the adrenal hormones upon the glycogen of this tissue.

The resistance to epinephrine of the myocardial glycogen of cortisone-injected mice is in substantial agreement with the studies of Evans ('34) demonstrating in fasting rats that the amount of cardiac glycogen was essentially unchanged by injection of epinephrine, exercise or changes in acid-base reaction of the blood. Injection of epinephrine decreased the glycogen content of the gastrocnemius. In rats an increment in cardiac glycogen is a characteristic response to fasting (24–48 hours), but in contrast to skeletal muscle the glycogen of the heart shows relative independence of the supply of insulin under several experimental conditions (Evans, '34 and '41; Russell and Bloom, '56).

The rat has been used almost exclusively for the studies of the actions of adrenal hormones upon muscle glycogen. Here most of the experiments with cortisone and all of those with

epinephrine were done in mice. The data do not suggest significant differences in the responses of this species to glucocorticoids. In mice hepatic glycopexis is one of the most striking results of injection of cortisone. The amount of liver glycogen per 100 gm body weight is used to bioassay glucocorticoids by the "mouse glycogen" method (see Nissim, '53).

The absence of clearly positive PA-S reactions for glycogen in normal skeletal muscle of mice has been reported previously (Swigart and Williams, '52 and '54). The same lack of positivity observed here in skeletal and cardiac muscle of normal mice might suggest that this histochemical method fails to show small amounts of glycogen, but does reveal large amounts as in the hearts of mice and rats injected with cortisone. The PA-S technic does show very small amounts of glycogen in livers of starved and of polyhalogen-treated mice (Williams, '51). The equivocal positivity of the PA-S reaction of striated muscle, both skeletal (in control and in cortisone-injected animals) and cardiac (in control animals), may be an expression of the same variations in glycogen content shown by chemical analyses of various muscles (abdominal, appendicular and the diaphragm) or rats which had been subjected to a variety of experimental conditions (Illingworth and Russell, '51; Leonard, '53; Leonard and Ringler, '54).

## SUMMARY

1. In rats and mice injection of large amounts of cortisone increase deposition of glycogen in hepatic parenchymal cells and myocardial fibres. Myocardium of control animals was very faintly PA-S positive while that of cortisone-injected rats and mice was intensely positive. This positivity was absent in sections incubated in saliva. Cortisone produced no glycogenosis in skeletal muscle except for a very slight increase in the mouse diaphragm.

2. Hepatic glycogen was uniformly reduced in mice that had been injected with epinephrine 1–5 hours prior to death.

The increased deposits of glycogen in the liver cells of cortisone-treated mice were as susceptible to the depletive action of epinephrine as were the lesser amounts in the hepatic parenchymal cytoplasm of mice that had not been injected with the glucocorticoid.

3. Epinephrine did not decrease the amount of glycogen in myocardial fibres of normal or of cortisone-injected mice.

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# PLATES

## PLATE 1

### EXPLANATION OF FIGURES

All figures (× 200) in this plate and in plates 2 and 3 were stained by the periodic acid-Schiff's reagent (PA-S) technic.

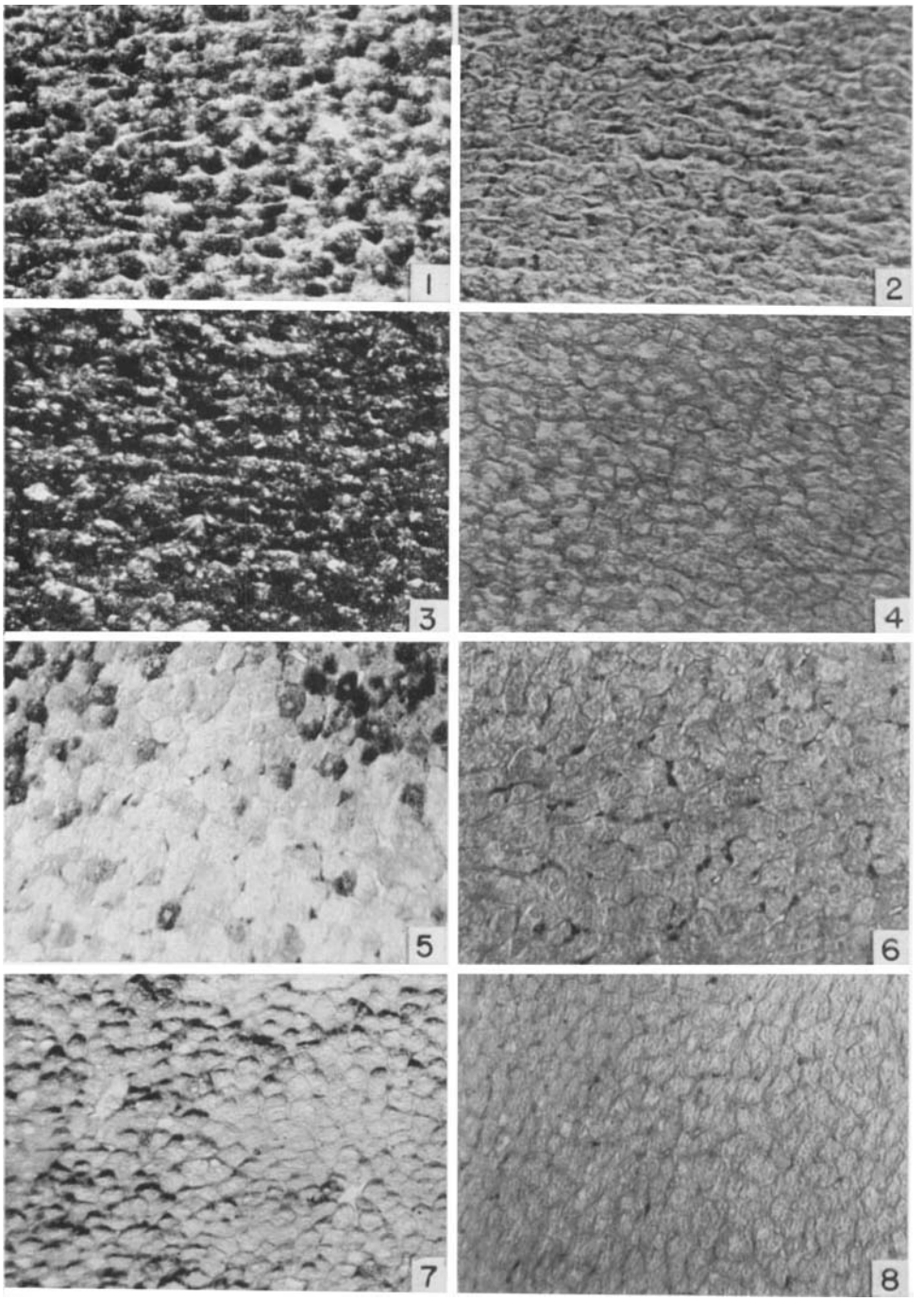
Figures 2, 4, 6 and 8 (below) show sections that were saliva-digested prior to application of the PA-S technic.

1 and 2 Normal mouse liver.

3 and 4 Liver of cortisone-injected mouse.

5 and 6 Liver of mouse injected with epinephrine 24 hours prior to death. Some PA-S positive material remains in section shown in figure 5. This material was removed by saliva-digestion (fig. 6).

7 and 8 Liver of mouse injected with cortisone daily for 7 days and then with epinephrine 24 hours before death. Not all of the glycogen was mobilized by the epinephrine (fig. 7), but was removed from the section by saliva (fig. 8).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## PLATE 2

### EXPLANATION OF FIGURES

9 Liver of normal rat.

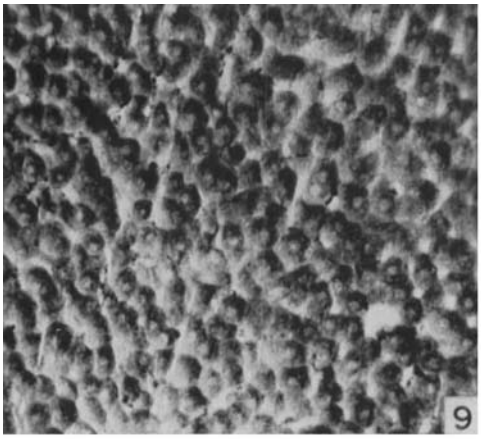
10 Liver of rat injected with cortisone for 5 days. Note the increased glycogen.

11 Myocardium of normal rat. PA-S positivity indicating glycogen is at a trace level.

12 Same as in figure 11, except that section was saliva-digested.

13 Myocardium of rat injected with cortisone for 5 days. PA-S positivity is intense.

14 Saliva-digested section of same heart as shown in figure 13.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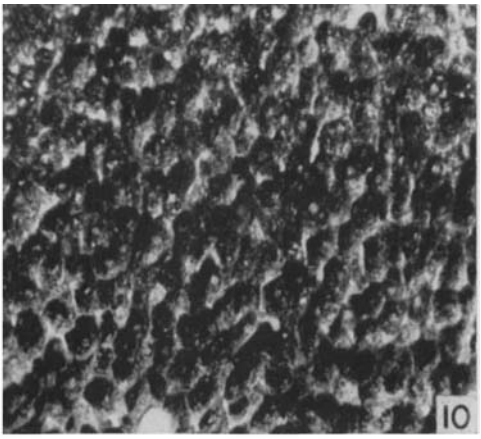
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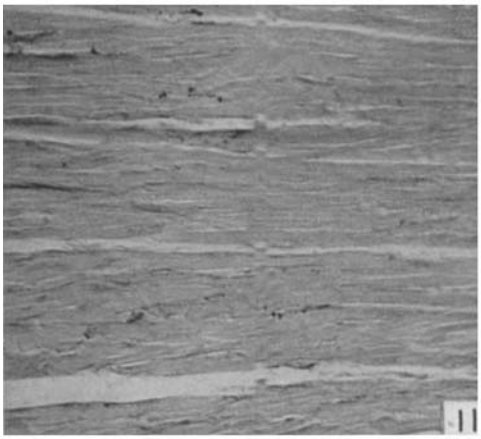
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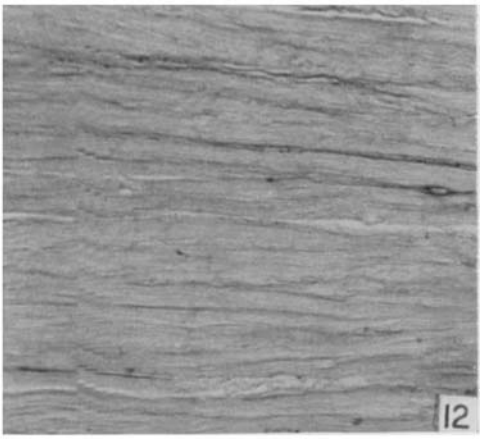
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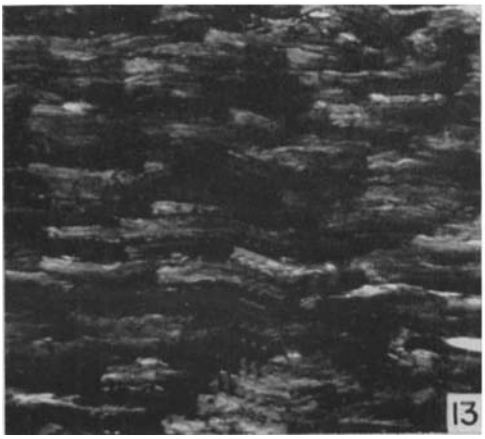
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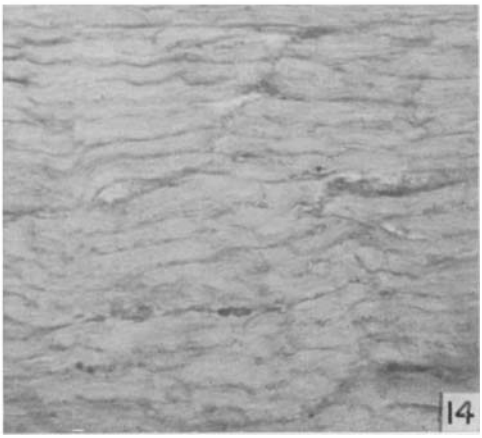
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## PLATE 3

### EXPLANATION OF FIGURES

15 Myocardium of mouse injected with cortisone for 7 days. PA-S positivity is intense.

16 Section of same heart as shown in figure 15. Saliva-digestion has removed essentially all of the PA-S positivity.

17 and 18 Myocardium of mouse injected with cortisone for 7 days and then with epinephrine 24 hours before death. Epinephrine has not reduced the amount of glycogen within the fibres. Muscle fibres are shown in longitudinal, transverse and oblique sections.


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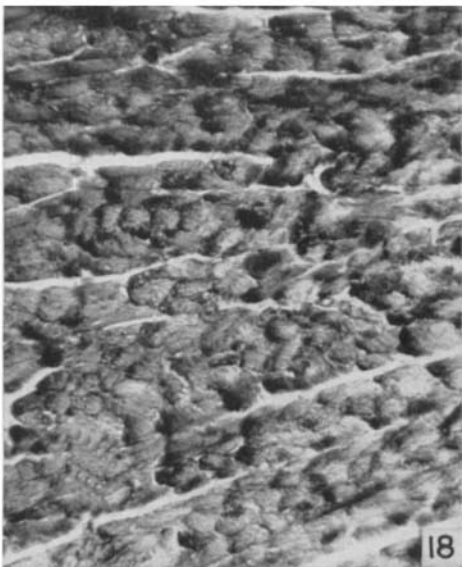
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