

THE DISTURBANCE OF THE CARBOHYDRATE METABOLISM AND THE SPECIFIC RETINAL LESIONS OF RATS FED WITH A HIGH FAT, AND HIGH CALORY DIET

KAZUO KATSUMATA*

*3rd Department of the Internal Medicine, Nagoya University School of Medicine
(Director: Prof. Kouzo Yamada)*

ABSTRACT

Authors fed rats with a high fat, high calory diet for 400 days. Noticeable increase of body weights and disarrangement of carbohydrate metabolism of rats were induced. The pathogenesis of the impaired carbohydrate metabolism was partially due to the decrease of glycolytic system as well as the increase of the gluconeogenic system in livers of rats with the abundant oxidation of fatty acid. It is considered to be one form of the metabolic adaptation.

The alteration of metabolism was also present at some of the peripheral tissues of rats. The disturbance of glycolytic system was observed in retinas of rats fed with a high fat, high calory diet.

In the retinas of rats fed with a high fat, high calory diet, bleeding of the retinal vessels as well as alteration of visual and ganglion cells were apparent. In the trypsin-digested flat preparation of retinas of these rats, the impairment of the blood vessel wall was indicated.

In the pancreas of rats fed with high fat, high calory diet, the hypertrophy of Langerhans island and the fibrosis of exocrine glands were observed. Fibrosis of muscles was noticed in the heart of rats fed with a high fat, high calory diet.

Authors discussed these experimental results in connection with the diabetic retinopathy and the metabolic adaptation of rats to the high fat feeding.

INTRODUCTION

The special attention is converging recently toward the fact that although fat is an essential nutriente, the abundant oxidation of fatty acid can inhibit the glycolytic enzymes. The precise effect of an abundant oxidation of fatty acid on the metabolism must be clarified.

C. Gonzalenz¹⁾ reported the decrease of liver glucokinase activity in rats fed with high fat diet. Authors also have reported the impairment of glucose metabolism and disturbed insulin sensitivity of rats fed with a high fat diet²⁾³⁾. In the present communication authors undertook to clarify the metabolic dis-

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arrangement of rats fed with high fat, high calory diet for a long duration through the examinations of glycolytic and gluconeogenic system of the liver. It was clarified that glycolytic system was decreased and that gluconeogenic system was increased in the liver of rats fed with high fats, high calory diet. A retina is well-known tissue where abundant oxidation of glucose take splace. It is interesting to examine whether at the retinas of rats fed with high fats, high calory diet a decrease of glycolytic system can be observed or not.

Authors found that apparent decrease of glycolytic system at the retina of rats fed with high fat, high calory diet was present, and that specific histological findings of retinal hamorrhage was also present. Authors discussed these experimental results in connection with the diabetic retinopathy as well as the acommodation of the metabolism of rats to the high fat feedings.

MATERIALS AND METHODS

44 rats of Wistar strain, each weighing approximately 70 grams respectively were divided into two groups, 22 rats were fed with normal diet and called the normal diet group, and 22 were fed with high fat diet and called the high fat diet group. Rats were fed ad libitum with diets tabulated in Table 1 and had free access to water. Each rat was confined in a separate metabolic cage and was provided with synthetic diet including vitamin mixture*.

These rats were fed for 100-400 days. On the 100th day of feeding, authors decapitated 5 rats of each group. Retinas of both group were cut off. After weighing each retina, the retinas were put in the small flask containing 2 ml Krebs-Ringer phosphate buffer with 200 mg% glucose, 0.5 μ c pyruvate-U-C¹⁴ or

TABLE 1. Composition of Diet

	Fat ⁱ⁾	Protein ⁱⁱ⁾	Carbohydrate ⁱⁱⁱ⁾	Macculum salt ^{**}
Normal diet	10	24	62	4
High fat diet	48	24	24	4

i) Ratio of fat components.

cotton seed oil : butter = 6 : 4

ii) Milk casein was used for protein

iii) A potato starch and sucrose (50 : 50) was used for carbohydrate.

***) KJ 30 g, Fe citrate 100 g, MgSO₄·7 H₂O 500 g, Ca lactate 1300 g, Na₂HPO₄ 200 g, KH₂PO₄ 1100 g, KCL 150 g, NaCl 50 g, CaHPO₄·2 H₂O 500g.

0.4 grams of the above mixture was added per 10 grams of diet.

Non-nutritive cellulose powder was added to the 2% of the total amount of this diet.

* Vitamin mixture (B₁ 40 r, B₂ 60 r, Nicotinic Acid 200 r, PABA 0.5 mg, Biotin 1 r, B₁₂ 0.1 r, Folic Acid 5 r, Choline 10 mg, Inositol 3 mg, A 300 I.U., D 30 I.U., B₆ 60 r, V.E. 1.2 mg, V.C. 1.0 mg and V.K. 0.1 mg were adjusted to 0.1 ml by distilled water) was added to 10 grams of synthetic diet.

0.5 μc glucose-U-C¹⁴. These flask were gassed with 95% O₂ and 5% CO₂, then were constantly agitated for 2 hours at 37°C. When incubation was finished, 0.2 ml of 1 N H₂SO₄ was added into the flask and then 0.4 ml of 1.8 N NaOH was added to the flask to catch the expired C¹⁴O₂. BaC¹⁴O₃ was counted by the gas flow counter. On the 115th day of feeding, authors decapitated 5 rats of each group and liver was removed quickly. 200 mg liver slices were put in the flask containing 2 ml of Krebs-Ringer phosphate buffer with 200 mg% glucose. Incubation was conducted in the same manner as before with the addition of 0.5 μc glucose-U-C¹⁴ or 0.5 μc sodium acetate-I-C¹⁴. The incorporation of C¹⁴ into expired CO₂ was counted and expressed as cpm/mg CO₂. Abdominal injection of 10 μc alanine-U-C¹⁴ per 100 grams of body weight to 5 rats of each group was conducted on the 110th day. 4 hours later after decapitation the incorporation into liver glycogen was tested as follows.

Authors extracted glycogen by R. M. Gomery's method⁴). Counting incorporated C¹⁴ into the half of the extract of glycogen by the gas flow counter, the glycogen content of the remaining half was determined by M. Dubois' method⁵). The incorporated C¹⁴ into liver glycogen was expressed as cpm/mg glycogen. Glucose tolerance test was done as follows: On the 400th day of feeding, authors made the oral administration of 1 ml of 20% glucose solution per 100 grams of body weight to 7 rats of each group by gastric tubes. 0.1 ml blood was taken from the tail vein, and the content of glucose was measured by Hagedron-Jensen's method.

Authors decapitated 5 rats of each group and quickly removed eyeballs as well as other organs. After stripping off the retinas, the authors put them together with other organs in to bottles containing formalin or pure alcohol for fixation. The flat preparation of retinal blood vessel tree of rats was also conducted by means of Kuwabara-Cogan's trysin digestion method⁶). Dyeing with HE, PAS, SUDAN III of other organs were conducted for the histological examination.

RESULTS

Body Weight

The weights of the rats of the normal diet group increased up to 195 grams on the 50th day, to 220 grams on the 100th day, to 250 grams on the 200th day, and to approximately 250 grams on the 300th day. But there was no increase of body weight after that. Changes of body weight of rats fed with normal diet agreed with the data obtained from rats of this strain fed with the laboratory chows—oriental pellets (purchased from the oriental company). On the otherhand, rats fed with the high fat diet group weighed already 350 grams on the 100th day and approximately 400 grams on the 200th day, maintaining the state of adiposity after that.

Glucose Tolerance Test

G.T.T. of the rats were conducted on the 400th day. G.T.T. of the rats fed with normal diet showed that the fasting blood glucose was 94.5 ± 12.3 mg/dl and that an increase of blood glucose after glucose loading was not apparent. The fasting blood sugar of rats of the high fat diet group increased to 112.3 ± 24.5 mg/dl. The obvious disarrangement of glucose tolerance after glucose loading was observed in G.T.T. of rats fed with high fat, and high calory diet (see Fig. 1).

The Incorporation of Glucose- $U-C^{14}$ or Acetate- $1-C^{14}$ into Expired CO_2 in the Liver Slices

As Fig. 2 indicates, the incorporation of glucose- $U-C^{14}$ into CO_2 in liver slices of rats fed with the high fat diet on the 115th day was 1721 ± 282 cpm/mg CO_2 which was evidently lower than 5580 ± 489 cpm/mg CO_2 in liver slices of rats fed with the normal diet. But the incorporation of acetate- $1-C^{14}$ into

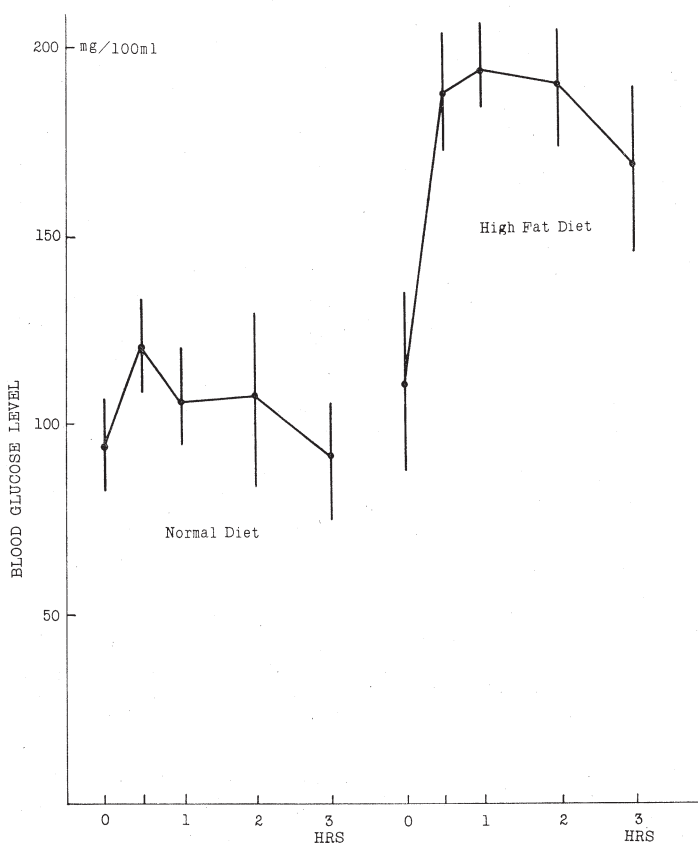


FIG. 1. Glucose tolerance test of rats in both groups. Each point represents mean blood glucose \pm S.E. of 7 rats in each group before and after glucose loading. These test were done on the 400th day.

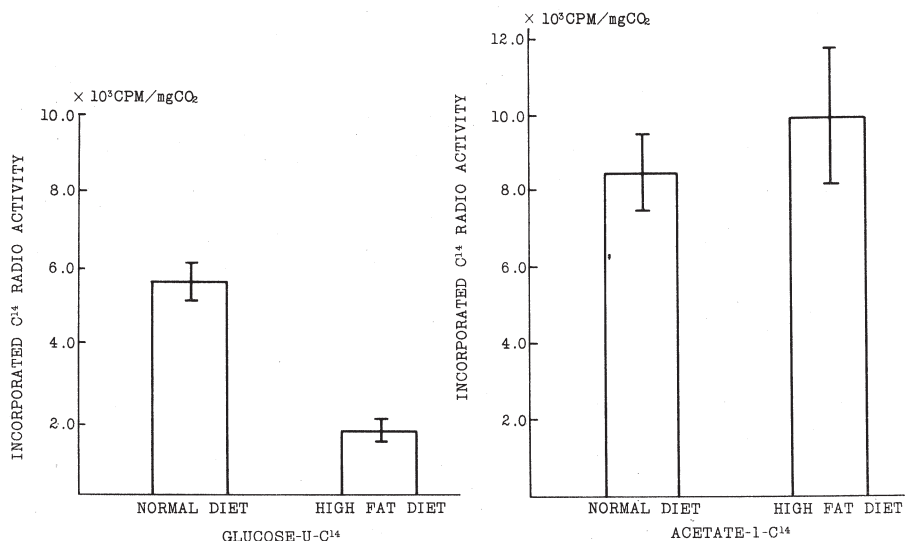


FIG. 2. Incorporation of glucose-U-C¹⁴ or acetate-1-C¹⁴ into expired CO₂ (Liver).

Fig. (left) showed the incorporation of Glucose-U-C¹⁴ into expired CO₂. Each bar represents mean incorporated C¹⁴ radio activity of 5 rats in each group. CO₂ production using 200 mg liver slices of each rats in both group were tested on the 115th day.

Fig. (right) showed the incorporation of Acetate-1-C¹⁴ into expired CO₂. Each bar represent mean incorporated C¹⁴ radio activity of 5 rats in each group. CO₂ production using 200 mg liver slices of each rats in both group were tested on the 115th day.

CO₂ in liver slices of rats fed with high fat diet group was 9989 ± 1487 cpm/mg CO₂ which was higher than 8510 ± 998 cpm/mg CO₂ in liver slices of rats fed with the normal diet.

The Incorporation of Alanine-U-C¹⁴ into Liver Glycogen

The incorporation of alanine-U-C¹⁴ into liver glycogen was conducted on the 110th day. The incorporation of C¹⁴ into liver glycogen increased obviously in liver slices of rats fed with the high fat, high calory diet as shown in Fig. 3.

The Incorporation of Glucose-U-C¹⁴ into Expired CO₂ in a Retina

The incorporation of glucose-U-C¹⁴ into CO₂ in retinas of rats fed with high fat, high calory diet on the 100th day of feeding were 2462 ± 583 cpm, which were lower than 4981 ± 683 cpm/mg CO₂ in retinas of rats fed with the normal diet (Fig. 4). The incorporated C¹⁴ radio activity was expressed as cpm/mg CO₂ per one retina of each rat. The retinal wet weight in rats fed with the normal diet was 10 ± 2.1 mg while the retinal wet weight in rats of the high fat diet group was 14 ± 1.6 mg which was higher than the former. Accordingly, the incorporation of C¹⁴O₂ per retinal weight of rats fed with high fat diet was lower than rats fed with control diet.

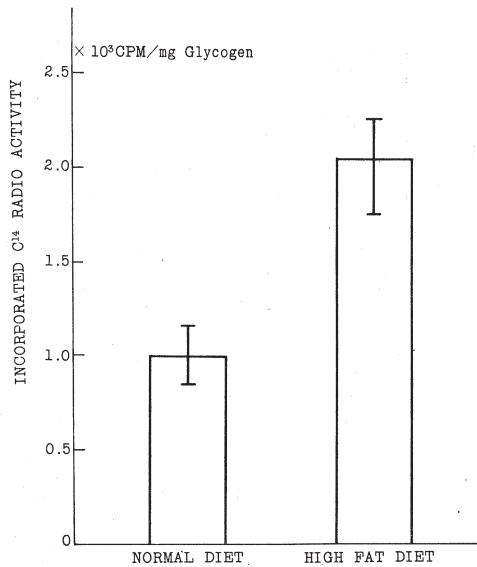


FIG. 3. Incorporation of alanine-U-C¹⁴ into liver glycogen. Each bar represents mean incorporated C¹⁴ radio activity \pm S.E. of 5 rats in each group. The experiment was performed on the 110th day.

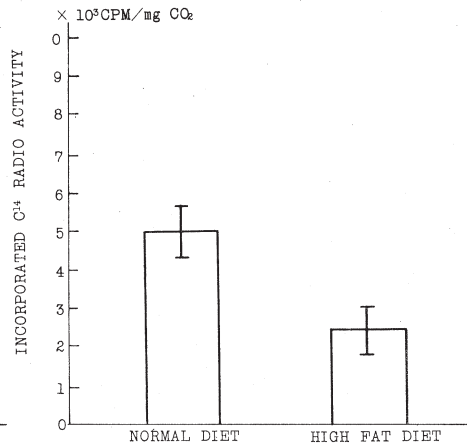


FIG. 4. Incorporation of glucose-U-C¹⁴ into expired CO₂ (retina). Each bar represents mean incorporated C¹⁴ radio activity of retinas of 5 rats of both group. Each vessel contains one retina of rats in each group with 2 ml buffer containing 200 mg glucose and 0.5 μ c glucose-U-C¹⁴. This experiment was performed on the 100th day.

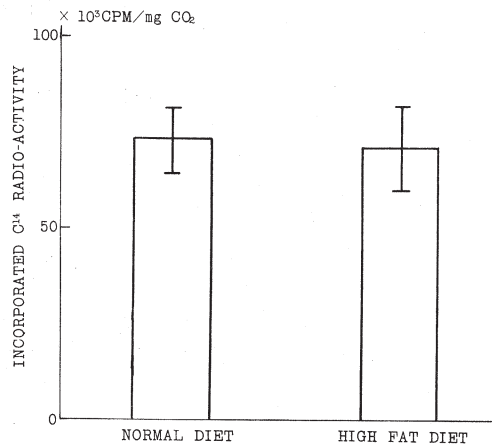


FIG. 5. Incorporation of pyruvate-U-C¹⁴ into expired CO₂ (retina).

Experimental condition was the same of Fig. 4 except pyruvate U-C¹⁴ in stead of glucose-U-C¹⁴,

The Incorporation of Pyruvate-U-C¹⁴ into Expired CO₂ in Retina

The incorporation of pyruvate-U-C¹⁴ into expired CO₂ in retinas of rats in high fat diet group on the 100th day was 72313±894 cpm/mg CO₂. That of retinas in rats of the normal diet group was 72414±901 cpm/mg CO₂. No noticeable difference between the two groups were observed. (See Fig. 5).

Histological Observations of Retina

The various significant alteration were observed in retinas of rats in the high fat diet group fed for 400 days, as shown in Table 2, but no disarrangement appeared in retinas of rats in the normal diet group (Fig. 6). Bleeding of the retinal vessels and the visual and ganglion cell degeneration in rats of the high fat diet group were apparent (Fig. 7 and Fig. 8). In the flat preparation of trypsin digestion, retinal blood vessels of the high fat diet rats showed an obvious decrease of mural cells (Fig. 10) compared to that of rats fed with normal diet (Fig. 9).

TABLE 2. Observations on the Retinal Tissue

	Rat No.	Degeneration of visual cells	Bleeding of retina	Expansion of capillary	Hydropic changes of ganglion cells	Flat preparation (trypsin digestion method)			
						Decrease of mural cells	Micro aneurysma	Increase of intra-capillary strand	Increase of endothelial cell
High fat diet	1	+	+	+	+	##	-	+	-
	2	+	+	+	+	##	-	+	-
	3	+	-	-	+	##	-	+	-
	4	-	-	-	-	##	-	-	-
	5	-	-	-	+	##	-	-	-
	6	+	-	-	+	##	-	-	-
	7	-	-	-	-	##	-	-	-
Normal diet	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-

Histological Observations on the Liver, the Pancreas and the Heart of Rats

The obvious histological alterations in the tissues other than the retina were liver, pancreas and heart in rats fed with the high fat diet group on the 400th day. The deposition of the diffuse lipid was seen in a liver of rats in the high fat diet group, but fibrosis in a liver did not happen. Fibrosis in the exocrine gland and the hypertrophy of Langerhans island were apparent in the pancreas of rats fed with high fat, high calory diet. Fibrosis was also noticed in parts of hearts of these rats. On the other hand, in rats of the normal diet group, a disarrangement of these organ was not noticed anywhere except in the liver where a little fat deposition was observed. These histological findings were indicated in Table 3.

TABLE 3. Histological Observation on the Liver, the Pancreas and the Heart

	Rat No.	Liver			Pancreas		Heart
		Fat deposition	Increase of biliary ducts	Fibrosis	Fibrosis of exocrine gland	Hypertrophy of Langerhans island	Fibrosis of heart muscles
High fat diet	1	##	+	-	+	+	+
	2	##	##	-	##	##	-
	3	###	###	-	+	+	##
	4	##	##	-	-	+	+
	5	##	+	-	-	##	-
	6	##	+	-	-	+	-
	7	##	+	-	-	+	-
Normal diet	1	+	+	-	-	-	-
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	4	+	-	-	-	-	-
	5	-	-	-	-	-	-
	6	-	-	-	-	-	-
	7	-	-	-	-	-	-

DISCUSSION

In the present experiments rats fed with the high fat diet had very high calory and low glucose in the diet compared with the rats fed with normal diet. Therefore, one of the significant factors in the metabolic impairment of rats in the high fat group seems to be the high rate of fat in diet, the high calory and the low rate of glucose in diet. Many data concerning the high fat feeding have been reported, but there are few papers showing the distinct impairment of carbohydrate metabolism of rats fed with high fat diet for a long duration. In the present communication, current efforts were directed toward the clarification of the obvious disarrangement of carbohydrate metabolism in rats fed with high fat diet. The obvious impairment of glycolytic system, the increased activity of the gluconeogenic system and the increase of acetate oxidation in the liver of rats in the high fat diet group were observed. As the rate of fat in diet was abundant in rats fed with a high fat diet, the increased oxidation of fat and acetic acid were rational from the standpoint of adaptation of the metabolism.

Recently, the increased oxidation of fatty acid at the liver of rats fed with high fat diet has been reported to decrease the glycolytic enzyme activity of liver¹⁾⁷⁾. On the other hand, it has been also reported, that the increase of acetyl CoA was induced by the abundant oxidation of fatty acid, and this increased acetyl CoA potentiated the activity of pyruvate carboxylase through the allosteric effect⁸⁾. The present finding on the metabolic disarrangement of the liver in rats fed with high fat diet may be interpreted in a manner that because the rate of carbohydrate in diet was small, the oxidation of fatty acid became increasing with the increase of gluconeogenic system of liver and the concomitant saving of the oxidation of glucose.

Whether these metabolic adaptation of rats in high fat diet group is observed in the tissue other than a liver or not, is an interesting problem. Authors already have reported the existence of the impaired glycolytic system in fat pads and muscles of rats fed with high fat diet⁹.

In the present paper the impairment of glycolytic system was observed in retina of rats in high fat group, and it is natural to suppose that because the oxidation of glucose was abundant in the retina of rats in the normal diet group the decreased oxidation of glucose at the retina must evoke important damage on retinal metabolism. As the incorporation of pyruvate-U-C¹⁴ into expired CO₂ of the retina fed with high fat diet was not different from those of the retina in the control group, the impairment of glycolytic system at the retina of rats in high fat diet group must happen somewhere between glucose and pyruvate. Though the mechanism of the disturbed glycolytic system in a retina of the high fat diet group was not clear, the degeneration of visual and ganglion cells and the retinal bleeding were due to the impaired utilization of glucose in the retina. The decrease of mural cells in the flat preparation of trypsin digestion of retinas of rats in high fat diet group was interesting. The decrease of mural cells has been reported by several researchers to be the primary feature of the diabetic retinopathy¹⁰.

The pathogenesis of fibrosis in a part of the heart muscle and in a part of the exocrine gland and the Langerhans island of pancreas observed in rats on a high fat diet was not clear. The hypertrophy of Langerhans island may be secondary to the disturbance of glycolytic system and to the increased secretion of insulin corresponding to the increased gluconeogenesis and high calory intake.

In the case that Langerhans island is healthy insulin must be secreted enough to overcome the metabolic disarrangement due to high fat feeding in a manner that diabetic state was not induced. The pathogenesis of the metabolic alteration of rats in high fat group is not entirely clear. Experiments to clarify these pathogenesis are now under study.

SUMMARY

Rats were fed with high fat, high calory diet for 400 days. Noticeable disturbance of carbohydrate metabolism was found. The biochemical and pathological disturbances were observed in retinas of rats. In the retinas of rats fed with high fat, high calory diet, bleeding of the retinal vessels was apparent. In pancreas and heart muscles of these rats, pathological findings were also observed. Authors discussed these experimental results in connection with the diabetic retinopathy and the metabolic adaptation of rats to the high fat feeding.

ACKNOWLEDGEMENTS

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LEGENDS TO FIGURES

- FIG. 6. Retina of rat fed with normal diet for 400 days. PAS \times 100.
- FIG. 7. Retina of rat fed with high fat diet for 400 days. Azan \times 100. Bleeding at the retina was apparent.
- FIG. 8. Retina of rats fed with high fat diet for 400 days. H.E. \times 200. Degenerations of visual and ganglion cells were observed.
- FIG. 9. Trypsin-digested blood vessel tree of retina of rat fed with normal diet for 400 days. PAS, H.E. \times 400.
- Fig. 10. Trypsin-digested blood vessel tree of retina of rat fed with high fat diet for 400 days. PAS, H.E. \times 400. Numbers of mural cells were decreased.

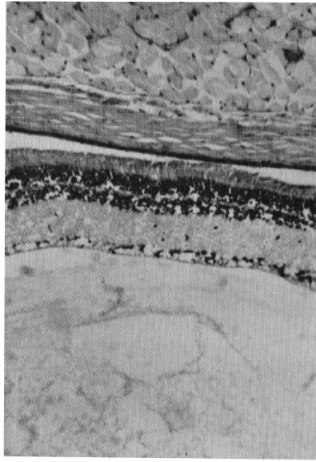


FIG. 6

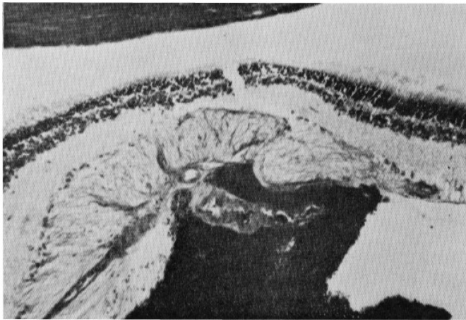


FIG. 7

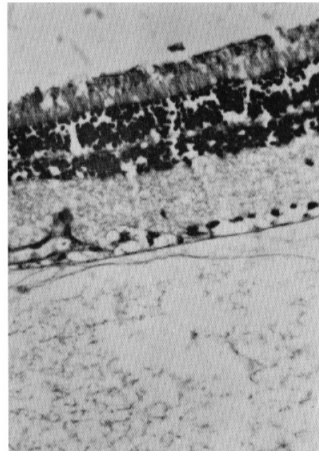


FIG. 8

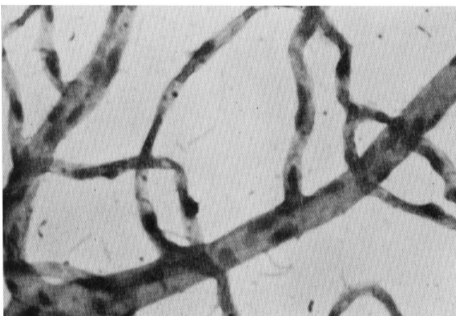


FIG. 9

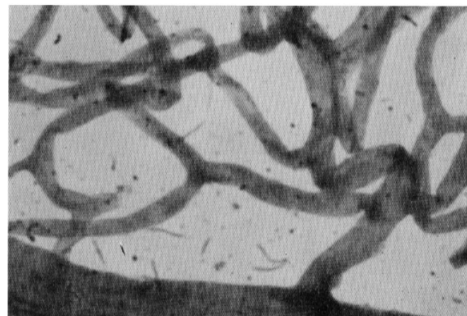


FIG. 10