

DIETETIC HEPATIC LESIONS

Influence of Protein Level and Vitamin B₂ Complex Deficiency
on the Liver of Rats

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NUMBER of publications have appeared on the production of experimental hepatic lesions in various species of animals by dietary means (Rich and Hamilton, 1940; Lillie *et al.*, 1941, 1942; Blumberg and Grady, 1942; Webster, 1942; Gyorgy and Goldblatt, 1942; Himsworth and Glynn, 1944, 1944 *a* and *b*; Handler and Dubin, 1946; Jeff *et al.*, 1950). These lesions are preventable in many cases by an adequate addition of yeast, choline or other lipotropic agents to the basal diet, and in the case of hepatic necrosis by cystine, tocopherol (Gyorgy, 1949; Gyorgy and Goldblatt, 1949 and Himsworth and Lindon, 1949) and antibiotics (Gyorgy *et al.*, 1950). It has been stressed by many workers that the particular dietetic deficiency does not appear to affect the ultimate fibrosis and in fact most clinical cases of liver cirrhosis have a history not of a single isolated deficiency of a 'B' vitamin, or of protein or of a particular amino-acid but of longstanding and cumulative deficiencies of various dietary factors including vitamins of the 'B' complex and proteins.

The present investigations were designed to study the interrelationships between proteins and vitamins of the 'B' complex as they affect the liver.

MATERIAL AND METHODS

One hundred and sixty-eight healthy albino rats (Haffkine Inbred Strain) weighing between 35–48 gm. were distributed equally with reference to age, weight, sex and litter mates into eight groups. The composition of the diets is given in Table I.

In addition, all the animals of every group received daily 2 drops of cod-liver oil containing 1,000 I.U. of vitamin A and 100 I.U. of vitamin D per gm.

* An enquiry on the 'Role of Nutritional Factors in Hepatic Cirrhosis' under the auspices of the Indian Council of Medical Research, at the Haffkine Institute, Bombay, under Dr. M. V. Radhakrishna Rao.

TABLE I
Composition of the diets with supplements

Group No.	Casein %	Starch %	Sucorse %	Clarified butter %	Salt mixture* %	Supplement per rat per day
I	18	35	35	8	4	20 μ g. of Thiamine
II	18	35	35	8	4	0.5 gm. of Brewer's yeast
III	10	39	39	8	4	20 μ g. of Thiamine
IV	10	39	39	8	4	0.5 gm. of Brewer's yeast
V	8	40	40	8	4	20 μ g. of Thiamine
VI	8	40	40	8	4	0.5 gm of Brewer's yeast
VII	5	41.5	41.5	8	4	20 μ g. of Thiamine
VIII	5	41.5	41.5	8	4	0.5 gm. of Brewer's yeast

* Osborne and Mendell's Salt Mixture No. 185.

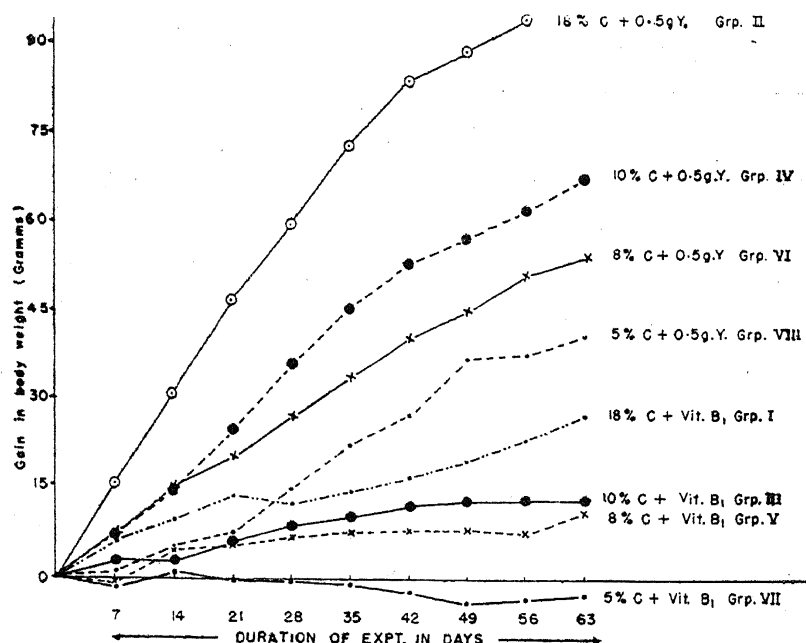
The food was supplied *ad libitum*, and the feeding of the diets was generally extended over many days, in certain cases up to 450 days. A number of animals from the deficient group however died early. In all cases, liver weight, its fat, protein and moisture contents were determined, following standard procedures.

Small portions of liver and kidney were taken for histological examination. All sections were cut to 6-7 μ thickness and stained by Ehrlich's acid hæmatoxylin and counterstained by 1 per cent. acloholic eosin. For collagen fibres Weigert's iron hæmatoxylin counterstained by Van Giesson's picric acid fuchsin was used. Reticulum fibres were studied by the silver impregnation method of Gomori.

RESULTS AND DISCUSSION

Graph I shows the growth curves of the animals.

Comparison of the growth curves (Graph I) of groups I, III, V and VII shows that animals of Group VII did not grow at all. They maintained their initial weight for some time and then gradually declined in weight. The



Graph I. Showing growth curves.

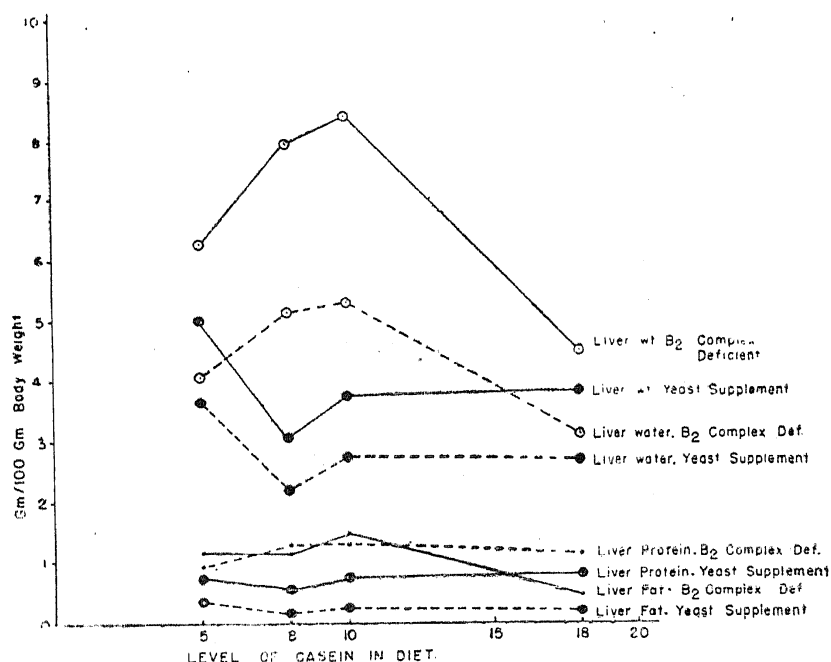
animals of the other three groups, viz., V, III and I gained in weight and their growth was proportional to the level of casein in the diet. It is evident that at 5 per cent. casein (Group VII) level in the diet and in the absence of vitamin B₂ complex, the animals failed to grow. They also did not survive more than 100 days. Whereas, even when the diet is deficient in vitamin B₂ complex, increasing the level of protein in the diet brought about corresponding increase in the growth rate.

If Group VII and Group VIII are compared, it can be seen that though the animals of both the groups received protein at the same level (5 per cent. casein) in the diet, supplementation of the diet of Group VIII with vitamin B₂ complex brought about not only growth but also a remarkable increase in the growth rate. The animals of Group VIII survived as long as 400 days at the end of which period they were killed. Thus, it is observed that a very low protein diet when supplemented with vitamin B₂ complex promotes growth. Similar growth differences are also evident in other corresponding groups with and without yeast supplement.

Although the animals of Groups VIII, VI IV and II received the same quantum of vitamin B₂ complex, they showed a progressive increase in the growth rate as the level of protein (casein) in the diet was increased. This is so, for as the level of protein in the diet is increased greater quantum of protein is available from the diet for isogramme quantities of the different

diets for the growth of the animals. It has already been pointed out that for the same level of protein in the diet, the animals receiving vitamin B₂ complex showed marked increase in growth as compared to the animals deficient in vitamin B₂ complex. Hence it is clear that the rate of growth depends on the level of protein supplied and the adequacy of the vitamin B₂ complex in the diet.

From Graph II and Table II it can be seen that liver weight (g./100 g. body weight) of animals of Groups II, IV and VI, *i.e.*, those receiving dietary



Graph II. Showing liver analysis values.

protein at 8 per cent. and higher levels with vitamin B₂ complex was within the normal range of 3 to 5 g./100 g. body weight. In Group VIII (casein 5 per cent. + B₂ complex), however, the liver weight was found to be slightly above the normal range. This could be attributed to the low protein level of the diet and consequent low protein intake resulting in higher moisture and lipid content.

In the vitamin B₂ complex deficient groups (Groups I, III, V and VII) the liver weight of the animals varied with the level of casein in the diet. It can be seen from Table II and Graph II that even though the animals of Group I were deficient in vitamin B₂ complex, yet as their intake of protein was at a higher level (18 per cent. in the diet), their liver weight was only 4.54 g./100 g. body weight, which is within the normal range. At 10 and 8 per cent. level of protein intake (Groups III and V respectively) the liver weight increased considerably. However when the protein intake was at

Liver weight, moisture, lipids and protein

TABLE II

Liver analysis for moisture, total lipids and protein

(Average values are expressed in g./100 g. body weight)

	Group No.	Liver			
		Weight	Moisture	Total lipids	Protein
Deficient in B ₂ Complex ..	I	4.54	3.10	0.47	0.87
	III	8.45	5.32	1.45	1.30
	V	8.00	5.15	1.16	1.25
	VII	6.31	4.05	1.15	0.94
Receiving yeast supplement	II	3.85	2.75	0.17	0.79
	IV	3.82	2.81	0.19	0.73
	VI	3.10	2.22	0.14	0.61
	VIII	5.09	3.72	0.36	0.77

5 per cent. level (Group VII), the liver weight showed a decrease as compared to the 10 and 8 per cent. protein level groups. This may probably be due to inanition and the earlier death of the animals in this group. No significance can therefore be attached to the values obtained for this group for purposes of comparison. From the above findings it is observed that on a low protein intake, particularly in the absence of the members of the B₂ complex, there is an increase in the liver weight which is largely due to the summation effect of an increase in water and total lipid content of the liver.

Liver moisture and total lipids followed almost the same pattern as the liver weight described above for both the set of animals (*viz.*, Groups II, IV and VI; Groups I, III and V) except in the 5 per cent. casein-fed animals. The animals of Group VIII receiving vitamin B₂ complex showed slightly higher values for liver moisture and total lipid content whereas in animals deficient in vitamin B₂ complex (Group VII) the liver moisture and total lipid content had decreased. This was also corroborated by histological examination and may probably be due to the lowered protein intake in both the groups (Group VII and VIII) and inanition and shorter life-span of animals

particularly in Group VII. Liver protein values did not show much variation in animals of Groups II, IV, VI and VIII which received yeast supplement. Whereas in B₂ complex deficient animals 10 and 8 per cent. (Group III and V) casein-fed animals showed a higher liver protein value than the 18 and 5 per cent. (Groups I and VII) casein-fed groups. The lower liver protein content in 5 per cent. casein (Group VII) may be due to inanition.

A comparison of the liver total lipids of the groups of rats receiving casein at different levels in the diet in the presence and absence of the members of the B₂ complex may be interesting. Even while the dietary protein is as high as 18 per cent. casein, the absence of the members of the B₂ complex (Group I) increases the total lipids of the liver by nearly three times that of the corresponding 18 per cent. casein group receiving the vitamins of the B₂ complex (Group II). Under similar conditions, on a 10 and 8 per cent. casein diets, the difference in lipid deposition is nearly eight times. Addition of the members of the B₂ complex to a 5 per cent. casein diet (Group VIII) definitely decreases the total liver lipid content, but it is still higher than the normal values. These results of the effect of various levels of casein in a B₂ complex deficient diet are in general agreement with the previous reports (Gyorgy and Goldblatt, 1941; Webster, 1941; Handler and Dubin, 1946; Drill and Loomis, 1948) and show that both protein and the vitamins of the B₂ complex are important factors in the prevention of dietary fatty infiltration of the liver.

HISTOLOGICAL FINDINGS

The microscopic examination of liver sections showed that the liver parenchyma retained its normal histological structure in animals fed on 18 and 10 per cent. casein diets (Groups II and IV) with vitamins of the B₂ complex. Slight fatty infiltration of the parenchyma mostly round the central veins was seen in the livers of the animals which received 8 and 5 per cent. casein diets with vitamins of the B₂ complex (Groups VI and VIII). This fatty infiltration of the liver was more predominant in rats receiving the 5 per cent. casein diet (Group VIII). The liver sections of this group also showed a verified appearance of the cytoplasm, which was not seen in the other groups. Hepatic fibrosis was altogether absent in any of the groups of rats and its absence in the liver of the animals which received the low protein diets with the addition of yeast was particularly striking. It appears that yeast exerts an inhibitory effect on the development of hepatic fibrosis by virtue of its lipotropic activity. The observations of Himsworth and Glynn (1944 *a* and *b*) support these findings.

The microscopic examination of the liver sections of the rats from the four B₂ complex deficient groups (Groups I, III, V and VII) showed definite histological changes. Liver sections of rats receiving 18 per cent. casein diet (Group I) showed marked fatty infiltration of the parenchyma, particularly around the central veins. None of the animals of this group showed any signs of fibrosis. The 10 and 8 per cent. casein groups (Groups III and V) showed extensive fatty infiltration more marked around the central veins even as early as 70 days. Moderate replacement fibrosis, patchy in distribution around the central veins, was also common in these groups within 150 days. Necrosis of a few parenchymal cells around the central veins in other areas was seen along with dilated and congested sinusoids. The cells in these areas were more faintly stained than those around the portal veins. The areas showing marked fatty infiltration were represented at a later stage by dilated spaces in which a yellowish brown pigment-ceroid was seen.

Two animals from each of the 10 and 8 per cent. casein groups (Groups III and V) showed marked replacement fibrosis with a tendency for the formation of pseudolobules. Histological evidence favours the opinion that diets low in protein and deficient in the vitamins of the B₂ complex, as in these experiments, were more conducive for an earlier production of cirrhosis of the liver. On the 5 per cent. casein diet (Group VII), the liver sections showed varying degrees of fatty infiltration of the hepatic parenchyma and this was more marked around the central veins. Animals of this group did not show any marked degree of fibrosis. The animals of this group formed an exception as in their case the food intake was low and consequently the animals died at a very early period.

None of the animals in these experiments, which were on the low protein diets (Groups V and VII) however, showed acute massive hepatic necrosis, similar to that described by Himsworth and Glynn (1944 *a, b*).

The influence of yeast in the prevention of fatty livers may be due to the considerable amount of choline it contains (Himsworth *et al.*, 1944 *a, b*). Choline as well as casein possess lipotropic action, the effect of the latter being largely due to its methionine content (Tucker and Eckstein, 1937, 1938), which serves as a precursor of choline (du Vigneaud *et al.*, 1940, 1941). The lipotropic action of choline is particularly emphasised on a comparison of liver fat (*see* Table II) of groups of rats fed 5 per cent. casein with (Group VIII) and without (Group VII) yeast supplements and that of methionine on a comparison of liver fat of groups of rats receiving casein at levels of 18 per cent. (Group I) and 5 per cent. (Group VII) respectively in the absence of the vitamins of B₂ complex,

SUMMARY

1. Attempts have been made to induce hepatic lesions in albino rats by varying the quantity of the protein (casein) and by withholding the supply of the vitamins of the B₂ complex.

2. On the vitamin B₂ complex deficient diets, the average life-span of the rats was largely influenced by the casein content of the diet, while those which received similar casein diets with yeast as supplement lived longer.

3. Fatty infiltration of the liver with progressive fibrosis (akin to human portal cirrhosis) can be more easily produced in rats by prolonged feeding of diets low in protein (casein) content and deficient in the vitamins of the B₂ complex.

4. Increasing the level of protein (casein) in the diet or supplementing low protein diets with yeast has a marked corrective effect on fatty infiltration of the liver and this is attributed to their respective methionine and choline content.

5. In general, the vitamins of the B₂ complex have a marked beneficial effect on the fatty livers when given early enough as a supplement.

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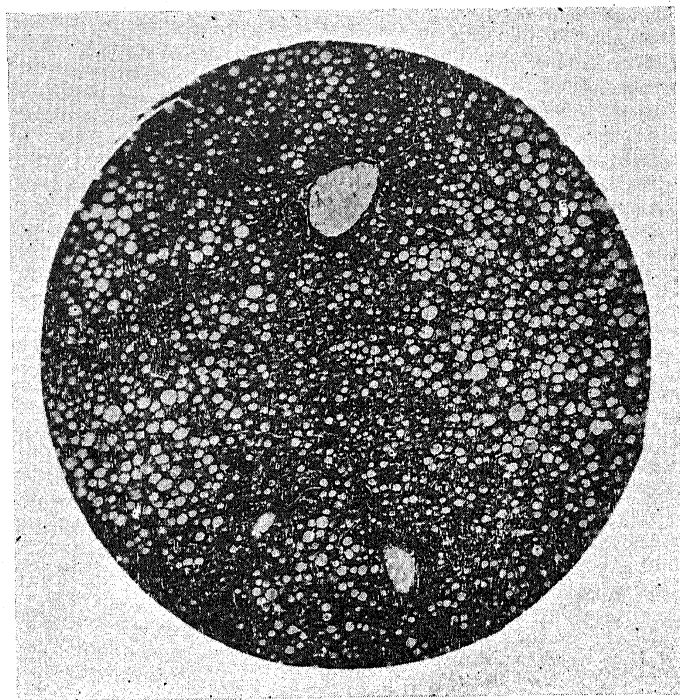
EXPLANATION OF PLATES

PLATE VI

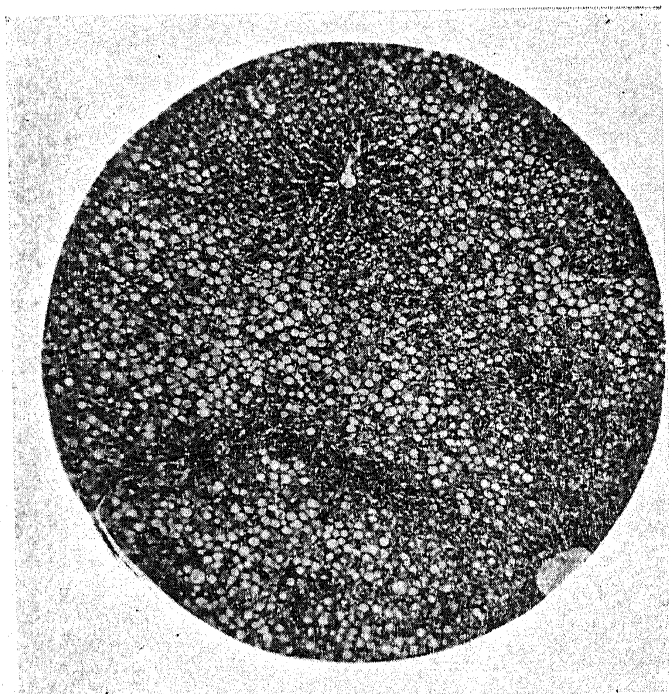
- Rat No. 11.—18 per cent. casein diet with vitamin B₁. Duration 84 days. Shows moderate fatty infiltration (Hæmatoxylin and Eosin, × 70).
- Rat No. 163.—5 per cent. casein diet with vitamin B₁. Duration 63 days. Shows extensive fatty infiltration of the hepatic parenchyma (Hæmatoxylin and Eosin, × 70).
- Rat No. 66.—10 per cent. casein diet with vitamin B₁. Duration 119 days. Shows fatty infiltration with marked ceroid pigmentation, (Hæmatoxylin and Eosin; × 70).

PLATE VII

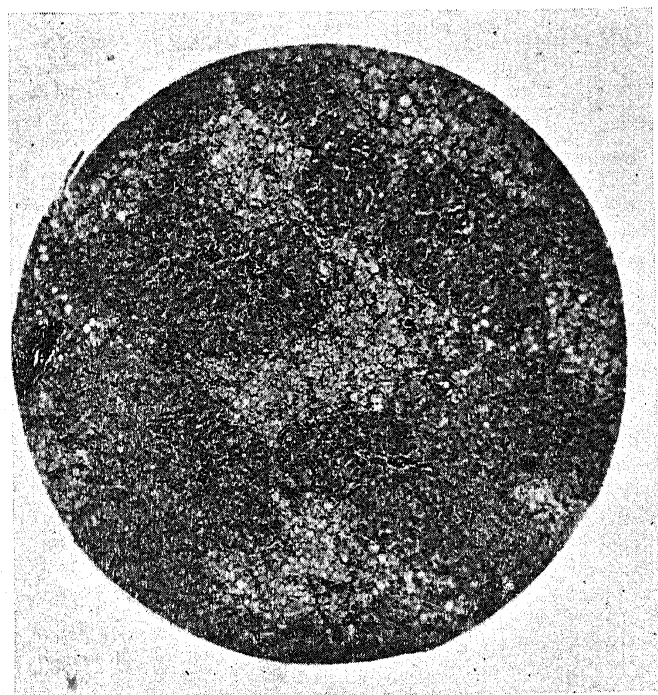
- Rat No. 126.—10 per cent. casein diet with vitamin B₁. Duration 196 days. Shows fatty infiltration with well-marked connective tissue bands (Hæmatoxylin and Eosin, × 70).
- Rat No. 126.—10 per cent. casein diet with vitamin B₁. Duration 196 days. Section stained with Gomori's silver impregnation method to delineate the reticulum fibres (Gomori's silver technique, × 140).
- Rat No. 150.—8 per cent. casein diet with vitamin B₁. Duration 119 days. Shows fatty change and replacement fibrosis well distributed with a tendency for the formation of pseudocystules (Hæmatoxylin and Eosin, × 70).



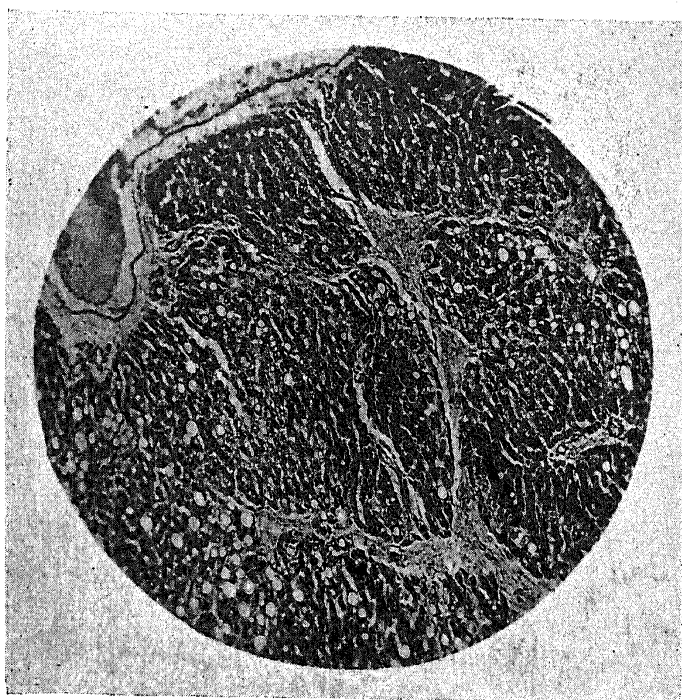
RAT 11



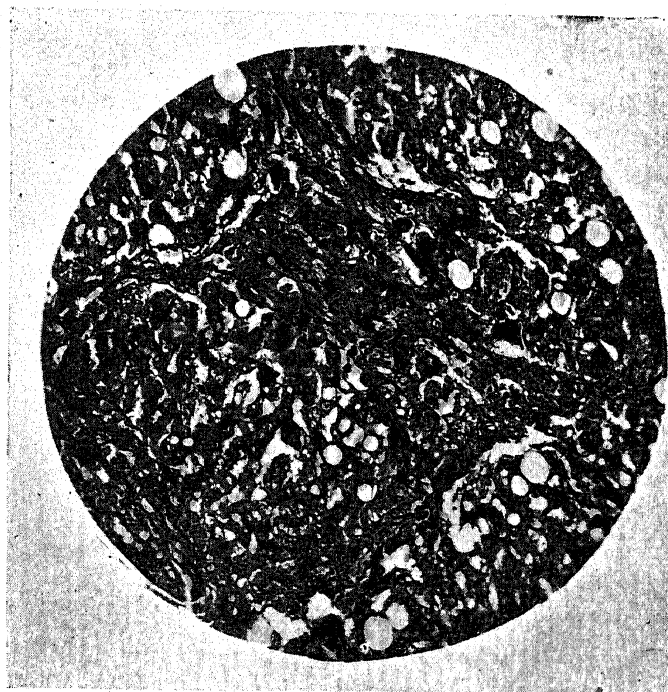
RAT 163



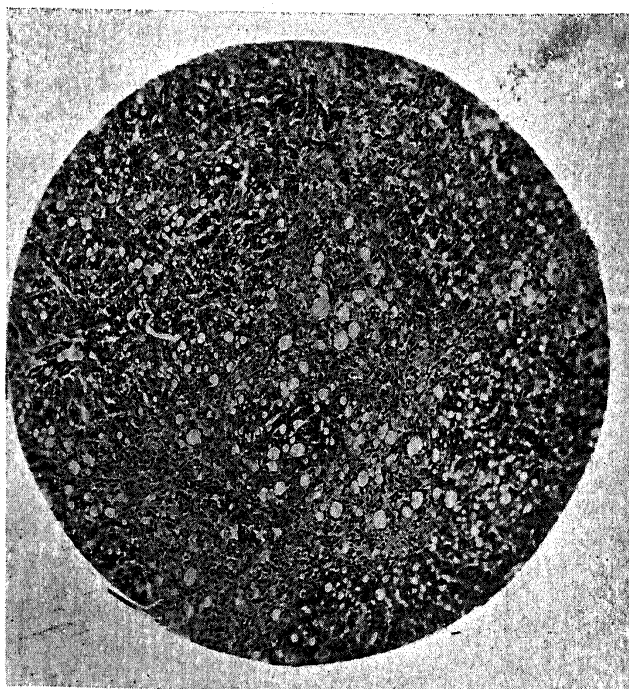
RAT 66



RAT 126



RAT 126



RAT 150