DIET AND TISSUE GROWTH.

I. The Regeneration of Liver Tissue on Various Adequate Diets.*

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Plates 1 and 2.

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A consideration of the problem of growth brings out certain analogies between this phenomenon and tissue repair. Structural increase is common to both processes and it seems reasonable to suppose that the optimal nutritive conditions for the enlargement of the body as a whole are similar to those necessary for the reconstruction of one of its constituent parts. It also seems probable that the processes of repair may be controlled, just as growth is, by certain dietary factors. This is particularly interesting from a practical point of view as the determination of the optimum conditions for the rapid healing of wounds is one of the ideals in surgery. Conversely, a study of diet and tissue regeneration might also throw valuable sidelights on growth. The present investigation is concerned with the effect of various adequate diets on the reparative process in the liver.

It is obvious at the outset that any effect brought about by adjustment of dietary factors will not be a sudden one. The striking characteristic of practically all deficiency lesions or conditions is that the onset is gradual, hence very likely to escape notice. In this type of experiment, therefore, it is desirable to establish the animal definitely in nutritive equilibrium on the ration in question before producing the tissue injury. This involves, in turn, a thorough knowledge not only of what constitutes an adequate diet but also of the optimum relationship of the various factors therein. A proper appreciation of these facts is possible only as a result of contributions to the science of nutrition made during the last decade. It is of value, therefore, to repeat experiments which have been made on this point but

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under controlled conditions using synthetic diets each factor of which can be evaluated.

The effect of diet on healing of skin wounds in dogs was brought out by Clark (1919). In these experiments it was found that changing from a fat to a protein diet increased the rate of healing mainly by decreasing the length of the quiescent period. The diets used were extreme, and in no sense adequate, and the number of cases was small. Opie and Alford (1914) studied the effect of carbohydrate diet (oats and sugar), protein diet (meat), and fat diet (beef suet) on hepatic necrosis in rats, but here, too, the diets were inadequate and the length of time on the various rations was short (4 days). They concluded that the resistance to chloroform poisoning was greater on the carbohydrate than on the fat diet. Davis and Whipple (1919, *a*) (1919, *b*) and Davis, Hall, and Whipple (1919) report that carbohydrate favors regeneration of liver tissue in dogs after chloroform necroses but that starvation and fat diet are decidedly unfavorable to repair. The carbohydrate diet consisted of cane-sugar alone or with rice and potatoes; the fat diet contained butter, lard, fat meat, and cotton seed oil, mixtures which would suffice neither for growth nor probably for maintenance for any length of time. Brain, beef heart, liver, or kidney was fed at different times but in all these experiments no effort seems to have been made to accustom the animal thoroughly to the diet before giving the chloroform. According to these investigators the increased nitrogen loss observed after chloroform necrosis is markedly reduced by feeding sugar and this suggests to them that here is an elite example of the protein-sparing action of carbohydrates, an idea previously proposed by Opie (1914).

EXPERIMENTAL.

The plan of the present experiments was to produce a standard severe tissue destruction in animals which had been fed definite diets. At uniform intervals after the injection the animals were killed and the comparative rate of regeneration of tissue observed histologically. There were four diets: a normal balanced diet, a high fat diet, a high carbohydrate diet, and a high protein diet. The animals (white rats) were placed on these rations soon after weaning and allowed to grow to 80 to 100 gm. when the tissue injury was made. The demand on the organism was thus doubly severe, for tissue repair took place during the period of most rapid growth.

The albino rat was used for two reasons. First, the growth rate of these animals has been standardized through many carefully controlled experiments. The activity of the rat under given conditions can thus be predicted and those individuals not conforming to the usual behavior may be discarded. Second, the small size of the animal enables large numbers of individuals to be cared for in relatively small space and, what is more important, the food intake is small enough to permit feeding materials more or less difficult to prepare, such as

certain pure proteins or amino acids. After some preliminary work using shorter intervals it was decided to examine the livers of the rats at 24 hour intervals from 24 hours after the administration of chloroform to 144 hours after. At least four animals were used at every interval on every diet to rule out the individuality factor. They were killed by asphyxiation with artificial illuminating gas and the livers immediately removed and fixed. The technique of feeding, weighing, and caring for the animals was that outlined by Ferry (1919–20).

The composition of the diets used is as follows:

Percentage composition.		Calories per kilo of food.	Apportionment of total calories.	
Standard balanced diet.				
	per cent			*per cent*
Casein	18	738	Protein	13.8
Starch	51	2,091	Carbohydrate	39.2
Lard (or beef suet)	22	2,046	Fat	47.0
Cod liver oil	5	465		
Salts*	4			
		5,340		
High protein diet.				
Casein	80	3,280	Protein	68.7
Lard (or suet)	12	1,488	Fat	31.3
Cod liver oil	4			
Salts	4			
		4,768		
High fat diet.				
Casein	25	1,025	Protein	13.8
Lard (or suet)	65	6,417	Fat	86.2
Cod liver oil	4			
Salts	6			
		7,442		
High carbohydrate diet.†				
Casein	14	514	Protein	13.8
Starch	79	3,239	Carbohydrate	77.8
Cod liver oil	3.8	353	Fat	8.4
Salts	3.2			
		4,166		

* The salt mixture used was that of Osborne and Mendel (Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, xxxvii, 557).

† The high carbohydrate food was made up as a stiff paste with the weighed amount of starch and boiling water. It was then cooled and the other ingredients stirred in. The moist food was spread in thin layers and dried to a crisp cracker. It was found necessary to do this to prevent scattering of food. Also the high percentage of raw starch induced diarrhea in some of the rats. On the high fat diet the growth was somewhat subnormal but on the other diets normal growth was obtained.

Beef suet which had been melted and freed from water was substituted for lard during the summer in order to maintain a firmer consistency of the paste foods. Vitamin B was provided in 100 mg. tablets of dried yeast fed apart from the food.[1] On diets of such widely different energy value it is desirable to adjust the protein and salts to the total calories so that lack of neither of these food factors will disturb the normal growth (see Smith and Carey (1923)).

In these experiments we have used the liver merely as a tissue in which a fairly standard injury can be produced. We were not interested in this organ *per se.*

The chloroform was dissolved in sterile mineral oil and injected subcutaneously. This method was employed because the quantity could be measured accurately and because the chloroform was slowly absorbed when given with the oil. The dosage was so adjusted that the total volume of injected fluid was always 1 cc. Since we were interested primarily in survivals and, at the same time, maximum liver damage, it was necessary to determine the maximum non-lethal dose of chloroform on each of the various diets. In order to obtain a valid index of the toxicity, it is important to use only fresh chloroform. In most of the present experiments the sample of Squibb's anesthesia chloroform was redistilled and the fraction boiling between 61° and 62°C., was used within an hour.

Results.

Toxicity.—The dose of chloroform after which the majority of rats on the standard balanced ration survived was 1.5 cc. per kilo of body weight. Opie (1914) stated that 2 cc. per kilo failed to kill his rats and in some cases he gave three times that amount, but in the present experiments when 2 cc. were given, too few rats on this diet survived for our purpose. 1.5 cc. per kilo were used for the rats on the high carbohydrate diet likewise, but it seems from our data that on this latter diet the toxicity was distinctly less than on the standard balanced ration. We have not observed a greater toxicity on high protein diet than on high carbohydrate food as stated by Opie and Alford (1915). On the other hand the maximum non-lethal dose administered in our routine experiments was 2.5 cc. per kilo of body weight. It is worthy of notice that in these animals and even in a few on the high protein diet surviving 3 cc. per kilo the extent of initial necrosis is distinctly less than that on other diets after the maximum

[1] This dosage had previously been found to furnish vitamin B adequate for normal growth over the range in size involved in these experiments.

non-lethal dose. On the high fat ration the toxicity was definitely greater and the dose of chloroform had to be reduced to 1.0 cc. per kilo in order to secure enough survivals for subsequent examination. With all the above doses of chloroform definite comparable degrees of liver necrosis were observed when the animals were killed from 24 to 36 hours after the injection, *i.e.*, before repair process had set in. It appears from our experiments that the toxicity of chloroform varies with the diet in the following order of decreasing susceptibility: high fat > standard > high carbohydrate > high protein diets.

Repair of the liver. Standard Diet.—The necrosis appears at its maximum at about 24 hours after the injection, at which period there is an extensive hyaline necrosis around the central veins involving from two-thirds to four-fifths of each lobule (Fig. 1). The necrosis may involve all portions of the liver lobule except circular areas of varying size immediately surrounding the larger portal vessels. The destruction of the parenchymal cells is uniform and complete. There may be a narrow rim of liver cells, with pale vacuolated cytoplasm, indistinct cell outlines and pycnotic nuclei separating the necrotic from the living tissues. In a few instances there is a considerable amount of fat with no evident liver cells in the central three-fourths of each lobule.

The central vessels and the surrounding capillaries are distended with red blood cells and in some instances there are hemorrhages of varying extent in the necrotic areas.

At this period there is no evidence of activity either by infiltration of leucocytes or by regeneration of the liver cells.

In the 48 hour period the picture has changed markedly. The process of repair is initiated by an early mobilization of leucocytes and clearing away of the necrotic cellular debris coincident with active regeneration of the hepatic parenchyma by a proliferation of the uninjured liver cells at the periphery of the lobule. The degree of repair varies considerably and while the most conspicuous feature is in some instances, the clearing away of the cellular debris, in others the predominant finding is active regeneration of the hepatic cells as shown by numerous mitotic figures. In some areas large liver cells containing two nuclei are seen.

The mitotic figures are rarely seen at the margin of the areas of complete necrosis but are much more numerous in the deeper layers of the surviving liver cells.

In some sections in the 72 hour period there is evidence of very active and rapid regeneration (Fig. 2). The sections show very large numbers of mitotic figures and the injury has been repaired to a considerable extent. The largest remaining unhealed areas are about one-third of the size of the liver lobule. The cellular debris has been completely cleared away. There is no evidence of connective tissue proliferation. In some instances only slight remains of the injury are evident.

In the 96 hour sections nothing remains of the necrotic area. The cells are quite granular and their arrangement in columns is somewhat less regular than in the normal liver. There is no evidence of connective tissue proliferation.

In the 120 and 144 hour sections the liver is histologically normal with no evidence whatever of a recent injury. The liver could not be distinguished from a section taken from a normal animal, except for less distinct cell columns and for fatty accumulations in the central zones of some lobules.

In each period there is considerable variation in the activity and degree of repair.

High Carbohydrate Diet.—The extent of the necrosis is somewhat less than is seen on the standard diet. In the 24 and 48 hour sections, hemorrhages into the necrotic areas are conspicuous. The evidence of regeneration, in the 48 hour period, is less marked than that observed on standard diet. At 72 hours the most active regeneration is seen. In one instance in the 96 hour period there is marked evidence of endothelial and connective tissue proliferation with resulting scars presenting a picture suggesting early cirrhosis. The scars are intralobular in character. The other 96 hour sections show only slight remaining evidence of the injury. Except for one instance (Rat 611, 120 hour) showing focal areas of connective tissue and endothelial proliferation, all of the 120 and 144 hour sections show the normal liver with no evidence of scarring or other traces of the injury.

Protein Diet.—The extent of the necrosis is less than is observed on the other diets although a larger amount of chloroform was ad-

ministered. Two series were studied, with dosages of 1.5 and 2.5 cc. per kilo, respectively. The findings in the two series parallel one another. The reparative process is less active from a histological view-point than on the carbohydrate and standard diets. There is little or no leucocytic infiltration and mitoses are few and far between. In the 72 and 96 hour periods the nuclei of the hepatic cells vary greatly in size and chromatin content. In many areas two nuclei are seen within a single cell, suggesting direct cell division. In some instances after the 96 hour period accumulations of fat are seen in the central portions of the lobule. In the 120, 144, and 168 hour sections the repair is complete without resulting scars.

High Fat Diet.—The necrosis is more extensive than on the standard, the high carbohydrate, or high protein diets. There is extensive hemorrhage into the areas of necrosis. The infiltration with phagocytic cells is less obvious and the number of mitotic figures is definitely smaller than is seen on the standard diet. In the 72 hour period, some sections show slight connective tissue and endothelial proliferation in the central portions of the lobule. In others, wide areas of hyaline necrosis persist (Fig. 3). In the 96 and 120 hour sections the reparative process is less advanced than on the standard and carbohydrate diets. The sections, in these periods, show small central areas in which the regeneration of the liver cells is not complete and there is a varying amount of fat around the central vessels. In some cases the connective tissue and endothelial proliferation is very conspicuous (Fig. 4). This is also present in one 144 hour section.

In the 144 and 168 hour sections the liver regeneration is apparently complete although there is a considerable amount of fat in the central zones of some sections.

DISCUSSION.

Various theories have been advanced to account for the peculiar toxicity of chloroform. Opie (1914) suggested that the mutual solubility of fat and chloroform tended to concentrate its effect in the liver. Graham (1915) showed that a similar, though not identical, lesion was produced by intraportal injections of small amounts of hydrochloric acid and postulated that the poisonous effect of chloroform

was due to hydrochloric acid formed in its decomposition.[2] Neither Davis and Whipple (1919) nor Underhill and Kapsinow (1922), attacking the problem from different points of view, have been able to corroborate the suggestion of Graham. All who have worked on this problem recognize the increased toxicity on diets high in fat. Simonds (1919) states that the oxidative powers of the animal are low after chloroform poisoning. The well known difficulty of oxidation of fats in the body may account for this greater toxicity in such diets no matter what theory is invoked to explain the mechanisms of the initial tissue damage.

Our data indicate a relatively marked degree of resistance to the deleterious effects of chloroform in animals fed diets high in protein. This observation taken in connection with the smaller extent of necrosis observed in rats given the maximum non-lethal dose of chloroform seems to show that protein exerts a protective action on the hepatic cells in the presence of the poison or its decomposition products. What the mechanism of this protective action maybe, we are unable to say at present.

We have confirmed other workers in this field in the high toxicity in animals on high fat diet and in the somewhat greater resistance of those on high carbohydrate diet. A comparison of the diets on the basis of the resistance to the toxicity of chloroform conferred by them shows the following: protein > carbohydrate > standard > fat.

A comparative study of the repair of the liver on a standard balanced diet, a high carbohydrate diet, a high protein diet, and a high fat diet shows differences between the toxicity of chloroform and the rate and character of the reparative process under the conditions of these experiments.

The reparative process is most active on the standard balanced diet and there is a complete return of the liver to the normal anatomical picture without evidence of scarring. In the 96 hour sections there is very little remaining evidence of injury, and in the 120 and 144 hour sections the repair is complete.

[2] Schultz, Hall, and Baker (Schultz, E. W., Hall, E. M., and Baker, H. V., *J. Med. Research*, 1923, xliv, 207) have shown that periportal areas of necrosis are obtained when chloroform is injected into the portal vein of dogs. The lesions described by Graham were apparently similarly located.

On the high carbohydrate diet the necrosis is slightly less extensive. The reparative process, judging largely by the numbers of mitotic figures, is less active in the early periods than on the standard diet. In the 96 and 120 hour periods an interesting difference is observed. There is quite marked proliferation of the endothelium and the connective tissue stroma in the injured areas. This is so marked in some sections that a histological picture of cirrhosis of the liver is seen, although it is usually stated that the repair of a chloroform necrosis of the liver takes place without resulting scars or other evidences of the injury (Whipple and Sperry (1909)). A similar picture has been described by Pearce (1906) in the regeneration of necroses in the liver produced by the intravenous injection of hemagglutinative sera. In this case, however, the injury differs from that produced by chloroform in that the hepatic cells and endothelial cells are completely destroyed while in the chloroform injury only the parenchymatous cells are affected. These findings with the results in later periods will be described in detail in another communication.

On the high protein diet, in spite of much larger doses of chloroform, the necrosis is considerably less extensive. The actual time interval after which repair is complete may be slightly earlier than on the other diets, *i.e.*, at 96 hours, although the activity of the repair, as may be judged from the histological picture is less striking than is observed in the standard diet. The difference in the time of healing is very probably due to the less extensive injury and not to a dietary factor. There are no areas of scarring.

On the high fat diet the necrosis is more extensive than is observed on the other diets and the rate of repair is definitely less advanced at a given period. The cicatrization described above on high carbohydrate diet is even more extensive on the high fat food. These scars are observed in 144 hour sections (Rats 650 and 730) but in the remaining 144 and the 168 hour sections the regeneration is complete although a considerable amount of fat is present in the central zones of the lobules in some instances.

In regard to the cicatrization mentioned above it is interesting to note that this finding is most conspicuous on the high fat diet, which shows the slowest rate of repair and is completely absent on the standard diet in which the repair is most active. The scars are not seen in

the high protein diet animals. It is suggested that these scars are indicative of and possibly result from a delayed process of repair. They are not associated with the extent of the necrosis only, as they are observed in the high carbohydrate diet animals showing a less extensive necrosis than the standard diet animals in which scars were not found.

Our results on the slower rate of regeneration on the high fat diet in rats confirm those of Whipple and his collaborators on dogs. On the other hand, the most rapid rate of healing was observed in our experiments on the standard diet and not on the high carbohydrate diet as reported by them on dogs. In comparing the process of repair on the protein and carbohydrate diets we find that they differ chiefly in the absence of any connective tissue and endothelial proliferation on the protein diet. This may be indicative of a slower rate of repair on the carbohydrate diet. Finally, in comparing the activity of the process of repair, the standard diet is first and the high fat diet last, with little if any difference between the carbohydrate and protein diets.

CONCLUSIONS.

1. The toxicity of chloroform varies according to the diets used in these experiments in the following order of decreasing susceptibility of the animals: high fat > standard > high carbohydrate > high protein diets.

2. On the high fat and high carbohydrate diets there may be a more or less marked proliferation of the endothelium and the connective tissue stroma in the necrotic area producing in some instances scars resembling the picture of an early cirrhosis.

3. On the diets studied, standard, high carbohydrate, high protein, and high fat, the most active and rapid repair is observed on the standard balanced diet. On the high fat diet the reparative process is definitely delayed in comparison with the others. There are only slight differences between the high carbohydrate and high protein diets which suggest but do not conclusively show a more rapid repair with the latter diet.

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EXPLANATION OF PLATES.

PLATE 1.

FIG. 1. Rat 429. Standard diet. Chloroform necrosis of liver 24 hours after injection. There is an extensive central necrosis. Only a narrow rim of uninjured liver cells remains at the periphery of each lobule. × 150.

FIG. 2. Rat 309. Standard diet. Chloroform necrosis of the liver 72 hours after injection. The reparative process is strikingly active. There are large numbers of mitotic figures. A large proportion of the injury has been repaired. × 150.

PLATE 2.

FIG. 3. Rat 668. Fat diet. Chloroform necrosis of the liver 72 hours after injection. A large area of hyaline necrosis persists. The reparative process is not active. Mitotic figures are inconspicuous. × 150.

FIG. 4. Rat 656. Fat diet. Chloroform necrosis of the liver 96 hours after injection. The picture resembles a cirrhosis of the liver. There is a striking proliferation of the connective tissue and endothelial cells within the injured area. The scars are intralobular. × 150.

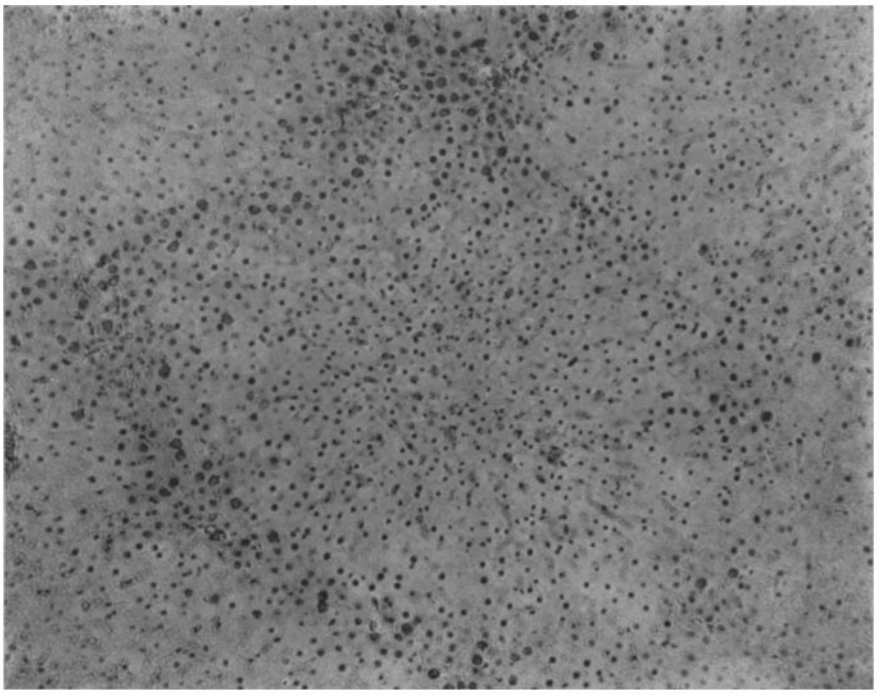

FIG. 1.

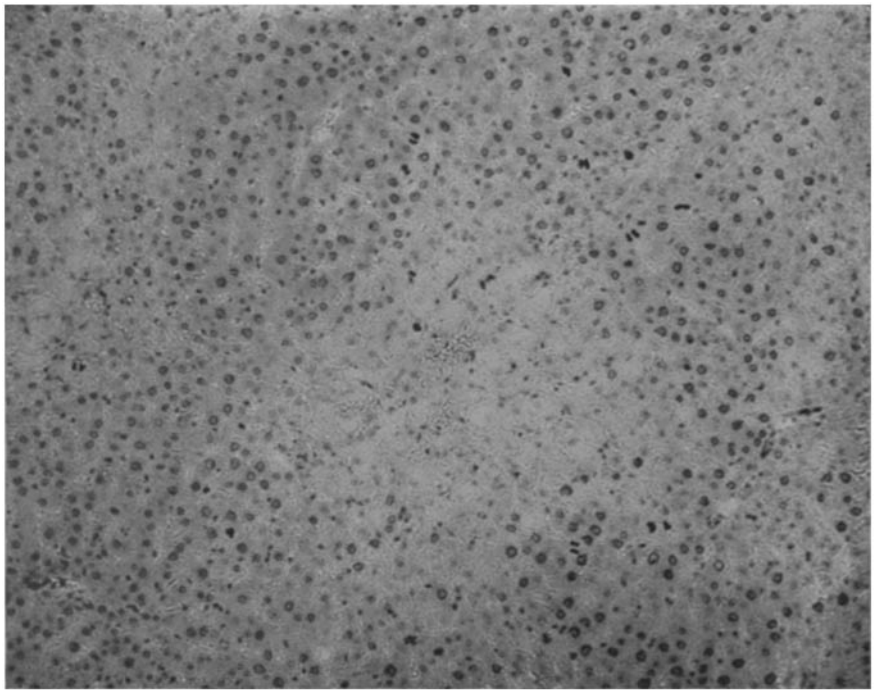

FIG. 2.

(Moise and Smith: Tissue regeneration. I.)

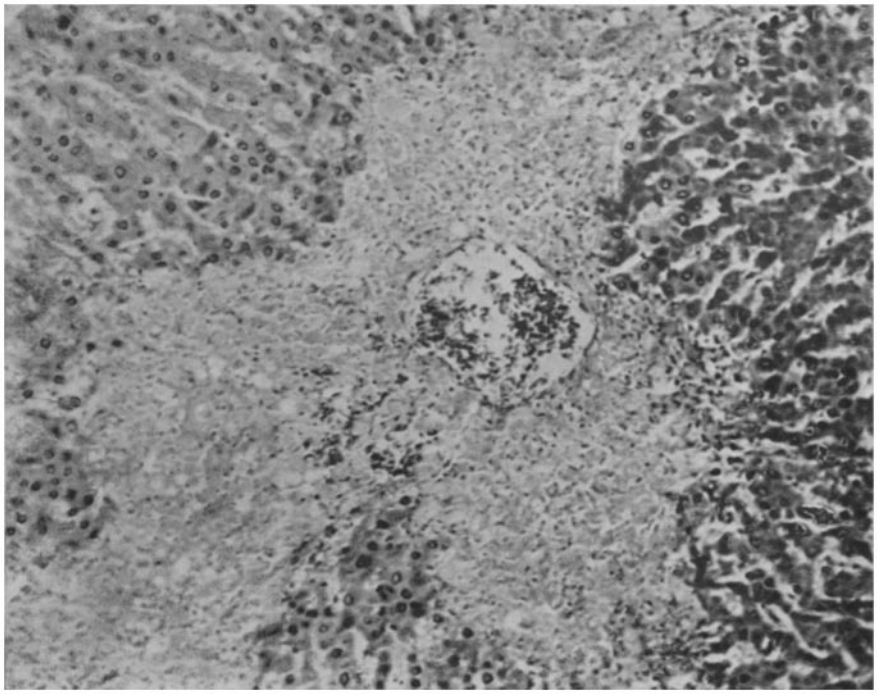

FIG. 3.

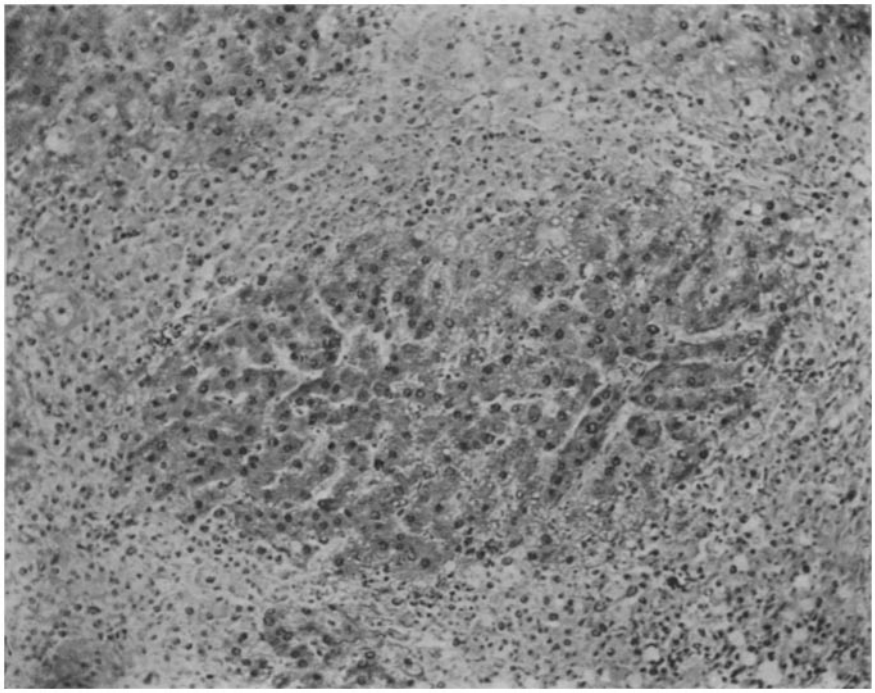

FIG. 4.

(Moise and Smith: Tissue regeneration. I.)