DIET AND TISSUE GROWTH.

II. The Regeneration of Liver Tissue during Nutrition on Inadequate Diets and Fasting.*

By ARTHUR H. SMITH, Ph.D., and THEODORE S. MOISE, M.D.

(From the Laboratory of Physiological Chemistry and the Department of Surgery, Yale University, New Haven.)

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In a previous paper (Moise and Smith (1924)) the postulate was made that nutritive conditions which are ideal for tissue regeneration probably likewise suffice for growth and the experimental results bore out the theory. In the present investigation the aim is to examine the influence of inadequate diets on the rate of tissue regeneration. From our point of view such a diet may be one on which either maintenance of, or increase in body weight is impossible.

The methods available for experimentally inhibiting growth or stunting are numerous. The most obvious is that of underfeeding with an adequate diet. It may be called quantitative stunting, for the animal is prevented from growing because it receives too few calories. Another method that may be called qualitative stunting involves the use of a diet adequate in calories but either lacking entirely, or containing an insufficient amount of, some indispensable constituent such as one of the accessory food factors, essential amino acids, or requisite mineral salts. In the present experiments diets complete in every respect save that they contained a protein which was deficient in one or more of the physiologically necessary amino acids, were used to restrict body growth.

In 1906 Willcock and Hopkins (1906–07) presented data showing that mice declined and died when fed a purified diet in which zein provided all the nitrogen. The decline was delayed but no growth obtained when the amino acid tryptophane was added to the diet. These experiments were repeated by Wheeler (1913) who

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found that, when tryptophane was added to the zein food, her mice maintained their weight for 60 days. It appears that when the lacking tryptophane is added to zein in an otherwise adequate diet, the experimental animal can maintain itself at a more or less uniform weight but cannot grow.

On the other hand, certain proteins can furnish the proper kinds of amino acids for maintenance but not for growth. Gliadin, the prolamine from wheat is one of these. The classic work of Osborne and Mendel (1912) on this protein has indicated the nature of the deficiencies measured by the biological method. They found that if a diet containing gliadin as practically the sole source of protein[1] was fed to rats the animals would remain stationary in weight or grow very slowly if no change was made in the ration. The addition of the amino acid lysine or small amounts of other proteins yielding lysine, resulted in immediate resumption of growth. This information was later applied in experiments (Osborne and Mendel (1914)) with zein with the result that rats fed zein and tryptophane and lysine grew as well as those fed gliadin and lysine and both these groups grew like rats whose diet contained a complete protein, such as lactalbumin, as the source of nitrogen.

A similar, though more severe, inadequacy is involved when gelatin is used as the source of the nitrogen in the food. Early workers were aware that this protein would not suffice for nutritive well-being (von Voit (1881)). Experimental animals maintained in a positive nitrogen balance immediately suffer a negative nitrogen balance when gelatin is included in the diet as the sole protein (von Voit (1881), Örum (1879), Abderhalden and Hirsch (1912)). A glance at the list of amino acids yielded by gelatin on hydrolysis, shows that tryptophane, cystine, and tyrosine are lacking. On the other hand, gelatin which yields lysine can supplement gliadin and so cannot be considered worthless in nutrition, a point early emphasized by von Voit (1881). Kauffmann (1905) showed that gelatin nitrogen could replace as much as one-fifth of the nitrogen of casein in a food mixture without altering the nitrogenous efficacy of the diet. Employing pigs at an endogenous plane of protein metabolism, McCollum (1911–12) demonstrated that more than one-half of the nitrogen of the gelatin was available to the animal, while Murlin (1907) in experiments on man, substituted two-thirds of the nitrogen of meat with gelatin nitrogen without observing a negative nitrogen balance.

The effect of an inadequate diet on the rate of growth of transplanted tumors has been studied by Benedict and Rahe (1917). The ration employed by them was deficient only in vitamin B. Merely enough of a source of this food factor was given to maintain the animal. Under these conditions it appears that the tumor grew though the host could not. Drummond (1917) working with rats used diets containing low percentages of adequate protein as well as diets containing deficient proteins in an effort to influence the growth of tumors in these animals but found that any inadequacy which stopped the growth of the neoplasm prevented even maintenance of the host. He also found that vitamin deficiency

[1] Their diets contained protein-free milk.

inhibits the tumor growth. The effect of gelatin feeding on the regeneration of liver cells after chloroform poisoning in dogs was studied by Davis and Whipple (1919), but no conclusions were drawn from the small number of observations made.

Method.

The plan of the present investigation was to study the rate of regeneration of hepatic cells in the white rat after chloroform poisoning (1) on an otherwise adequate diet containing gliadin as the protein, (2) on a diet containing gelatin as the protein, and (3) in fasting. The liver was used merely because in this organ a uniform type and degree of tissue injury can be produced. The animals were killed at 24 hour intervals up to 168 hours after the administration of the chloroform and the livers sectioned and studied histologically. The technique of the care of the animals was the same as that outlined in the previous paper (Moise and Smith (1924)).

The gliadin food was made up as follows:

	per cent
Gliadin	18
Starch	51
Lard	18
Butter fat	9
Salts*	4

The gelatin food had the following composition:

	per cent
Gelatin (powdered)	18
Starch	51
Lard	18
Butter fat	9
Salts*	4

* Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, xxxvii, 557.

Vitamin B was provided by 100 mg. of dried yeast fed apart from the synthetic food, while the butter therein contained the fat-soluble factor. The energy value was about 5.3 Calories per gm. The diets fed were thus adequate save for the quality of the protein.

The experiments with the gliadin diet were conducted on rats which had grown to at least 70 gm. on a comparable adequate casein diet before being changed to the experimental food. Immediately upon making the change growth stopped and the animals remained at a practically constant weight level. The supplementary

nitrogen of the yeast (7 mg. per day) had no more effect in making good the gliadin deficiencies in these experiments than had the residual nitrogen of the protein-free milk in the experience of Osborne and Mendel. Chloroform was administered only after growth had been inhibited for at least 5 days.

On the gelatin ration the animals did not maintain themselves but lost weight steadily though not so fast as the rats without food. The chloroform was given when the decline on this diet had progressed for at least 2 days.

The loss of weight was more rapid when no food was given and the chloroform was administered usually 2 days after food had been removed from the cages. In order to allow for the rapid decline in weight during the 2 day foreperiod and for the 7 day afterperiod, rats weighing about 200 gm. were employed—animals considerably larger than were used in the other phases of this investigation.

The chloroform used was dissolved in sterile mineral oil and injected subcutaneously. The dosage was so adjusted that the total volume of fluid injected was 1 cc. The chloroform was redistilled, and the fraction 61°–62°C., was used within an hour to obviate the toxic effects of decomposition products due, presumably, to oxidation.

The maximum non-lethal dose of chloroform for the animals on the gliadin food was 1.5 cc. per kilo of body weight. This is the same quantity used for animals on the standard balanced ration and on the high carbohydrate diet.[2] For the rats on gelatin diet the amount of chloroform giving maximum liver injury and minimum number of deaths was 1 cc. per kilo of body weight while the rats receiving no food withstood 1.2 cc. per kilo. The comparative toxicity in these various inadequate dietary conditions is, therefore, as follows: gelatin > fasting > gliadin.

A detailed description of the injury and progressive repair of the liver cells after chloroform poisoning in animals which were fed various adequate diets both of normal and of unusual compositions was presented in a previous paper (Moise and Smith (1924)). In the present account frequent reference and comparison with the findings in animals on the balanced diet is made. A brief description of these results is therefore given.

Repair of the Liver. Standard Diet.—There is an extensive hyaline necrosis involving from two-thirds to four-fifths of the central portion of each liver lobule. After 48 hours there occurs a simultaneous infiltration of leucocytes, a clearing away of the cellular detritus and an active repair of the injury. The process of repair is most active during the 72 hour period, when there are very large numbers of mitotic figures in the uninjured cells at the periphery of the lobule.

[2] See Moise, T. S., and Smith, A. H., *J. Exp. Med.*, 1924, xl, 13.

In the 96 hour sections only slight evidences of the injury or of an active reparative process are seen. After 120 to 148 hours the regeneration is complete and the liver is histologically normal.

Gliadin Diet.—The extent of the injury is about equal to that described on the standard diet. The rate of procedure of the repair is almost identical with that observed on the standard balanced diet. The percentage of the injured area that has been repaired at 48 and 72 hours is practically the same; although the number of mitotic figures is slightly less than in the corresponding periods on standard diet. In the 96, 120, and 144 hour periods only the following slight differences are noted. There is a slightly greater amount of fat in the region about the central vessels and in one instance (gliadin Rat 387, 144 hour period) there are small groups of connective tissue and multinucleated giant cells. These have been described by Pearce (1906) whose interpretation is that these giant cells result from direct division of the slightly injured hepatic cells at the margin of the necrotic area. These differences are so insignificant and so rarely present that they were not mentioned in a preliminary report of this phase of the work (Smith and Moise (1922–23)).

Gelatin Diet.—The necrosis involves from two-thirds to three-fourths of the liver lobule and is no more marked than that observed on the adequate diets (Moise and Smith (1924)). The process of repair is, however, definitely delayed. In the 48 hour period there are very few mitotic figures. There are quite marked variations in the size and the chromatin content of the nuclei of the liver cells. In the 72 hour period, active evidence of regeneration of the liver cells is still absent. In one section there is definite evidence of endothelial and connective tissue proliferation. A few mitotic figures are seen in the proliferating endothelial cells. In the 96 hour period, small necrotic areas persist and there is slight evidence of endothelial proliferation. In the 120 and 144 hour sections there are a few scars and small fatty accumulations in the central portion of some lobules but the greater part of the liver appears normal.

Fasting.—The necrosis involves about two-thirds of the liver lobule. In the 48 and 72 hour periods the reparative process is somewhat less active but is otherwise identical with that described for the standard and gliadin diets. In the 96 hour period there are

small unregenerated areas and occasional scars seen. Some sections still show mitotic figures in the liver cells. In the 120 hour section there is some scarring and slight fatty accumulation in the central zones of the lobules. The 144 hour sections appear normal.

DISCUSSION.

From the data given above it appears that chloroform is no more toxic to animals fed gliadin as the sole protein in an otherwise balanced ration than to those given a complete protein. Actual fasting exerts a definite effect on the resistance of the animal to chloroform, the maximum non-lethal dose being smaller than with the rats on gliadin food. The animals declining on gelatin food were still more sensitive to chloroform although losing weight somewhat less rapidly than those without any food. The more pronounced amino acid deficiency, probable decreased calorie intake due to physical character of the gelatin diet, together with the specific dynamic action of the amino acids in the gelatin may account for the increased toxicity of the poison in this group over that in the fasting group. It is worthy of notice, too, that chloroform is less toxic to animals without any food than to animals fed diets high in fat (Moise and Smith (1924)).

As previously stated the regenerative process is most active on the standard balanced diet. On this ration there is a rapid repair of the injury with a complete return of the liver to the normal anatomical picture without evidence of scarring after 120 to 144 hours.

On the gliadin diet the regeneration is identical with that observed on standard diet with the following insignificant variations. The numbers of mitotic figures are somewhat small on gliadin diet. There is slightly more fat in the central zones of the liver lobules in the 120 and 144 hour sections. In one instance an atypical proliferation of the liver cells resulted in small groups of multinucleated giant hepatic cells (Pearce (1906)). The repair is complete after 120 to 144 hours.

On the gelatin diet the necrosis is no more extensive than was observed on the preceding diet but the process of repair is less advanced at a given period. There is some evidence of cicatrization as was described on high fat and high carbohydrate diets in a previous

paper (Moise and Smith (1924)). These scars are seen in the 72, 96, 120, and 144 hour periods. Except for these small areas of fibrosis and slight fat accumulations in the central zones the repair is complete in the 120 and 144 hour sections.

Under conditions of fasting the necrosis is no more extensive than on the standard, gliadin, and gelatin diets. The process of repair is identical with that described on gelatin diet.

In comparing these inadequate diets, gliadin and gelatin, and starvation, with standard diet, it is seen that the rate of procedure of the repair is practically the same on the standard and gliadin diets. Under conditions of fasting and on a gelatin diet the reparative process is slightly but about equally delayed, while on these latter diets small scars are observed.

In a previous communication (Moise and Smith (1924)) it was suggested that these scars possibly resulted from the delay in repair as they are most conspicuous on a high fat diet, on which the toxicity of the chloroform is greatest and the rate of repair is delayed to a greater extent than on any other diet studied.

In comparing the results of the reparative process in inadequate nutritive conditions, (*i.e.* on gliadin food, gelatin food, and in fasting) with the repair on adequate diets (standard food, high carbohydrate food, high protein food, and high fat food) the most rapid healing is observed on the standard balanced diet and the greatest delay in the repair is observed on the high fat diet. The fasting and the gelatin-fed animals show a somewhat less marked delay than is seen in those on the high fat diet. The animals on carbohydrate diet and on protein diet show a more rapid repair than those fasting or on gelatin food, while the changes in the gliadin-fed animals are almost identical with those on standard diet.

The results of the present investigation indicate that the gliadin diet, one having certain obvious deficiencies, while not sufficing for body growth, does permit repair of damaged liver tissue at the same rate as that in an animal provided with an adequate food. We are dealing with the possibility of growth of some of the component parts while the body as a whole cannot grow. Osborne and Mendel (1912) have cited examples of female rats stunted with gliadin, which became pregnant and gave birth to litters. While these experiments

raise practically the same questions as does the present investigation, they can scarcely be accepted as proof of bodily synthesis of essential amino acids since gliadin does yield a small quantity of lysine on hydrolysis (Osborne (1924)). The protein-free milk may have provided some lysine and the possible contribution of the body tissues is one that has not been adequately evaluated.

In the present series of experiments we find certain tissue—hepatic tissue—not only regenerating on a diet (gliadin) on which body growth is impossible but regenerating as rapidly as the same tissues in animals fed a so called adequate food. It might be argued that the "complete set" of amino acids necessary to rebuild the damaged tissue arises from the breakdown of other tissues such as, for instance, the muscles. We have found that immediately following the chloroform injection the animal appears anesthetized; for 2 or 3 days after the injection the rat is definitely "sick" and eats little, and the curve of body weight drops. However, after the repair is histologically complete and often before that stage is reached, the body weight has regained its original stunted level. These facts seem to show that other body tissue was not breaking down to furnish the amino acids required for hepatic tissue synthesis. Again, Lindsay (1911) and Davis, Hall, and Whipple (1919) have demonstrated that chloroform poisoning is followed by a prompt rise in nitrogen output indicating loss of the hepatic protein nitrogen which, in turn, makes it appear unlikely that all the amino acids necessary for the synthesis of new hepatic tissue come from the necrosed liver cells. One is forced to conclude either that hepatic tissue requires far less lysine than muscle and other body tissues or that correlated growth resulting in an increased body weight is not identical with nor dependent on the same mechanism as individual tissue growth. In the present experiments we are not dealing with an absolute lack of lysine, for this amino acid may be provided (1) in the gliadin to the extent of 0.6 per cent, (2) in the 44 mg. of yeast protein, and (3) possibly by the residue of broken down liver cells. Since we do observe normal liver regeneration under these dietary conditions it appears that there is here a clear demonstration of the quantitative difference between the requirement for the growth of a part and that for the increase of the organism as a whole. It is worthy of note that animals

on the gelatin diet in which the deficiency is more pronounced than in the case of gliadin, are still able to regenerate the liver, though, to be sure, at a slightly less rapid rate. The progressive loss in weight of the rats eating the gelatin food, together with the commonly observed negative nitrogen balance under these conditions indicate that body tissue is being broken down. It is conceivable in this case, that the requisite amino acids for the synthesis of new hepatic cells are provided largely by this endogenous catabolism of proteins.

SUMMARY.

The relative toxicity of chloroform given subcutaneously to rats fed on two diets deficient in respect to their nitrogenous components, the sole sources of protein in which were, respectively, gliadin and gelatin and to rats without food, is as follows: gelatin diet > fasting > gliadin diet.

The rate of regeneration of liver cells after chloroform poisoning in rats fed a diet in which gliadin is the only protein is about as rapid as that in animals fed a diet containing casein as the source of nitrogen. The rate of regeneration on a food containing its nitrogen as gelatin and also during fasting is definitely slower than that in animals fed the gliadin food.

The results of these experiments indicate that the requirement for the essential amino acids for growth of individual organs of the body is less than that for correlated growth of the whole body.

BIBLIOGRAPHY.

Abderhalden, E., and Hirsch, P., *Z. physiol. Chem.*, 1912, lxxxi, 323.
Benedict, S. R., and Rahe, A. H., *J. Cancer Research*, 1917, ii, 159.
Davis, N. C., Hall, C. C., and Whipple, G. H., *Arch. Int. Med.*, 1919, xxiii, 1689.
Davis, N. C., and Whipple, G. H., *Arch. Int. Med.*, 1919, xxiii, 612.
Drummond, J. C., *Biochem. J.*, 1917, xi, 325.
Kauffmann, M., *Arch. ges. Physiol.*, 1905, cix, 440.
Lindsay, D. E., *Biochem. J.*, 1911, v, 407.
McCollum, E. V., *Am. J. Physiol.*, 1911–12, xxix, 215.
Moise, T. S., and Smith, A. H., *J. Exp. Med.*, 1924, xl, 13.
Murlin, J. R., *Am. J. Physiol.*, 1907, xix, 285.
Örum, N. P., *Jahres-ber. Fortschr. Thier-chem.*, 1879, ix, 308.
Osborne, T. B., The vegetable proteins, London, 2nd edition, 1924.

Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1912, xii, 473.
Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1914, xvii, 325.
Pearce, R. M., *J. Med. Research*, 1906, xv, 99.
Smith, A. H., and Moise, T. S., *Proc. Soc. Exp. Biol. and Med.*, 1922–23, xx, 440.
von Voit, C., in Hermann, L., Handbuch der Physiologie, Leipsic, 1881, vi, pt. 1, 396.
Wheeler, R., *J. Exp. Zool.*, 1913, xv, 209.
Willcock, E. G., and Hopkins, F. G., *J. Physiol.*, 1906–07, xxxv, 88.