

LIVER REGENERATION FOLLOWING CHLOROFORM INJURY AS INFLUENCED BY VARIOUS DIETS

MECHANISM OF PROTEIN SPARING ACTION OF FAT *

PAPER IV

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This article cannot be looked on as complete in any sense, yet the data have considerable value in conjunction with the experiments given in the other papers of the series. It is evident that more experiments are needed to determine the exact influence of the cooked parenchymatous organs and organ extracts on liver cell repair. Incomplete proteins, for example, gelatin, should be tested to ascertain if possible what repair can take place on such diets. The lipoids and allied substances may have a part in this reaction and the influence of thyroid and other ductless glands or extractives of these glands should be worked out. Some of this work we hope to carry out in the near future.

From the experiments at hand it is clear that the liver will regenerate at maximum speed following a unit chloroform necrosis if the animal is fed a diet rich in carbohydrate, a bread and milk diet or an ordinary mixed diet. The diets which rank next to this optimum group may be the parenchymatous organs (liver and kidney) and meat, perhaps brain, although this last diet is usually distasteful to dogs. Liver regeneration is rapid on these rich protein diets and more data may show that the optimum regeneration may at times follow the administration of such foods.

Thyroid fed in large amounts is known to favor tissue katabolism, but this does not favor liver regeneration and may even inhibit it. Under such circumstances the liver cannot take advantage of the protein split products and conserve them for use in construction of liver cells. It will be of considerable interest to observe the effect of large doses of thyroid combined with carbohydrate and of small doses of thyroid with and without carbohydrate.

Fat feeding supplies the most interesting observation brought out in this paper. Sufficient data are presented to show that liver injury will be regenerated just as rapidly as during complete fasting on a pure fat diet. In other words, the fat does not contribute in the least

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to the tissue building in the liver. We may make several deductions from this evidence when we recall that pure sugar makes possible a rapid and complete liver repair. Both fat and sugar are burned as fuel in the body and are recognized as "sparing protein" in one way or another. We have suggested in the preceding paper that sugar must "spare protein" by conservation of protein split products which are constructed into liver protoplasm. It is of course possible and even probable that sugar also is capable of "sparing protein" by acting at the source of protein katabolism and preventing this autolysis or tissue break down. Fat obviously acts as a "protein sparer" not by conservation of end-products and reconstruction of new liver tissue, but by some protecting action at the source of tissue katabolism.

These experiments mark out a clean-cut difference in the metabolism of fat and carbohydrate relating to protein construction and destruction. It may be that this reaction is limited to the peculiar conditions of these experiments, but we believe it may be applicable to general body metabolism. Surely the metabolism and cell repair of a large and important organ like the liver must play an important part in the body metabolism and give more than a hint concerning the reaction of other less rapidly functioning cells.

The striking difference between the fat feeding and brain feeding experiments is worthy of further study. It will be of some interest to determine in what way the neutral fats differ from brain extractives (cholesterol, lipins, etc.) when given in diets to influence liver repair.

The values of various foods and combinations of foods for promoting growth and maintaining weight and bodily activities have been extensively studied by Osborne and Mendel,¹ McCollum,² and a host of others. Such studies may have a bearing on our particular problem, although we have used comparatively short feeding periods. The effect of various diets on the growth of tumors has been enthusiastically studied by many investigators³ because of the tremendous practical value of such knowledge.

Hunt,⁴ Janney,⁵ Kendall⁶ and others have suggested that the thyroid has a rôle in regulating the supply of food for the formation

1. Osborne, T. B., and Mendel, L. B.: Feeding Experiments with Isolated Food Substances, Pub. Carnegie Inst., Washington, D. C., 1911; Pub. 156, Part II, *J. Biol. Chem.* **15**:311, 1913; **16**:423, 1913-1914; **20**:351, 1915.

2. McCollum, E. V.: Summarized in: *The Newer Knowledge of Nutrition*, The Macmillan Company, New York, 1918.

3. Sweet, J. E., Carson-White, E. P., and Saxon, G. J.: *J. Biol. Chem.* **15**:181, 1913. Van Alstyne, E., and Beebe, S. P.: *J. M. Res.* **29**:217, 1913-1914. Rous, Peyton: *J. Exper. M.* **20**:433, 1914. Robertson, T. B., and Burnett, T. C.: *J. Exper. M.* **23**:631, 1916.

4. Hunt, Reid: *Hygienic Lab. Bull.*, No. 69, 1910.

5. Janney, N. W.: *J. Biol. Chem.* **24**: 1916, Proc. 30.

6. Kendall, A. C.: *J. A. M. A.* **71**:871, 1918.

of living protoplasm. That other ductless glands have a function in governing growth and development is well recognized; Robertson⁷ has recently emphasized the action of the pituitary gland in this respect. We cannot deny that the activities of the organs of internal secretion may play an important part in cell regeneration and in liver repair.

MacCallum,⁸ Pearce,⁹ Whipple and Sperry¹⁰ have studied regenerative changes in the liver after necrosis, but without especial reference to the influence of diet.

Blood serum protein regeneration has been recently studied by Kerr, Hurwitz and Whipple.¹¹ It was clear that fasting delayed the serum protein regeneration and a heavy protein diet was most favorable for rapid repair. Under optimum conditions after a severe plasma depletion to about 30 or 40 per cent. of normal, the serum protein regeneration required from five to eight days. This slow curve of repair is very like the curve of regeneration noted in liver injuries. It suggests that the serum protein is built up with the same difficulty as the liver protein. The experiments indicate, further, that liver cells may be concerned in serum protein regeneration as a liver injury interferes with the serum protein repair.

METHODS

The chloroform injury has been quite definitely ascertained in each case here reported, usually by operative removal of a small piece of liver for microscopic examination on the second day following anesthesia.

Fluids such as cottonseed oil and suspension of thyroid powder were given by stomach tube; solid foods were left in the cages for consumption. Amounts are indicated in the individual protocols.

Repair has been determined microscopically at the end of a week or more, either by means of another operation or after sacrifice of the animal.

The technic of our experiments has been more fully explained in the other papers of this series. All operative procedures without exception were carried out under ether anesthesia.

EXPERIMENTAL OBSERVATIONS

All observations are given in the form of tables to facilitate a survey of the various groups of experiments. In addition, each experiment is given in the form of a history, including the necessary experimental details, histologic data and necropsy findings. These histories usually follow the proper tables.

7. Robertson, T. B.: *J. Biol. Chem.* **24**:363, 385 and 397, 1916.

8. MacCallum, W. G.: *Johns Hopkins Hosp. Rep.* **10**:375, 1902; *ibid.* *J. A. M. A.* **43**:649, 1904.

9. Pearce, R. M.: *J. M. Res.* **15**:99, 1906.

10. Whipple, G. H., and Sperry, J. A.: *Bull. Johns Hopkins Hosp.* **20**:1, 1909.

11. Kerr, W. J., Hurwitz, S. H., and Whipple, G. H.: *Am. J. Physiol.* **47**:356, 370 and 379, 1918.

TABLE 12.—LIVER REGENERATION ON BREAD AND SKIM MILK DIET

Experiment	Chloroform Hours	Liver Injury	Regeneration Diet	Liver Repair	Remarks
90 (Pup 2a)	1	1/3 necrosis: extensive, diffuse fat	Sacrifice in 2 days (control)	Milk and mixed diet; no preliminary starvation; Wt., 1.94 lbs.
(Pup 2b)	1	Estimated the same as 2a	Bread and skim milk ad lib. for 7 days	8th day: complete repair; no fat; much glycogen	Same diet as 2a; same age; Wt., 1.75 lbs.; sacrificed on 8th day
91 (Dog 18-82)	1½	1/2 to 3/5 necrosis; fat light over 2/5	2d day: bread and skim milk ad lib.	9th day: repair practically complete; trace of fat; much glycogen	Operation on 2d day after chloroform; feeding begun on 3d day; sacrificed on 9th day

EXPERIMENT 90.—*Regeneration on Bread and Skim Milk Diet.*—Pup 2a (control), a black female.

April 23: Pup is 6 weeks old; in good health; wt., 1.94 lbs.; previous milk and mixed diet. *Chloroform anesthesia for one hour* (from 10:55 to 11:55 a. m.).

April 24: Active; no food.

April 25: Sacrificed at 3:30; blood urea, 30 mg.; urea N, 14 mg.

Necropsy Report.—Blood clots slowly; clots are flabby. *Liver:* weight, 38 gm. Lobule centers are hard to discern; about two thirds of each lobule is opaque and white; outer zones of lobules are translucent gray. Intestine contains two round worms and several tape worms.

Microscopic Report.—*Liver:* Necrosis of about one third; extensive, diffuse fat deposits, especially toward lobule centers.

Pup 2b, a tan male.

April 23: Same litter as 2a, and on same diet; wt., 1.75 lbs. *Chloroform anesthesia for one hour* (from 10:55 to 11:55 a. m.); *bread and skim milk diet.*

April 24 to 30: Bread and milk *ad lib.*; eats well; noisy.

May 1: Wt., 1.38 lbs. *Sacrificed* at 10:30 a. m.; blood urea, 30 mg.; urea N, 14 mg.

Necropsy Report.—Blood clots quickly; viscera a little pale. *Liver:* weight, 54 gm.; no evidence of necrosis or fat.

Microscopic Report.—No necrosis or collapse in lobules; no fat; much glycogen; cells around central veins have a dense formation, and stain differently from the peripheries—possibly newly formed tissue.

EXPERIMENT 91.—*Regeneration on Bread and Skim Milk Diet.*—Dog 18-82, a young white female bull.

June 11: Wt., 18.63 lbs. *Chloroform anesthesia for one and a half hours*, with carbonate solution intravenously at the same time.

June 12: Wt., 18.63 lbs. Very lively.

June 13: Wt., 17.56 lbs. *Removed piece of liver* at 2 o'clock. Liver lobules show central necrosis involving from one half to three fifths of all cells. The remaining cells contain much fat. Lost considerable blood; gave epinephrin subcutaneously.

June 14: Wt., 17.2 lbs. Dog is rather weak; feeding begun; bread and skim milk *ad lib.*

June 15: Wt., 17.63 lbs.; June 16, wt., 18.06 lbs.; June 17, wt., 18.38 lbs.; June 18, wt., 18.38 lbs.

June 19: Wt., 18.25 lbs. *Bread and skim milk ad lib.*; dog is very active again.

June 20: Sacrificed at 9 a. m.; blood urea, 38 mg.; urea N, 17.7 mg.

Necropsy Report.—Liver: weight, 286 gm.; necrotic material and fat practically gone, but lobule centers are very prominent. *Spleen:* surface is quite nodular; malpighian bodies are very large and milky. *Kidney cortex* seems to have an unusual streaking with white.

Microscopic Report.—Liver: some lobule centers seem to have a very slight amount of stroma not filled out; a trace of fat, a few wandering cells, and much glycogen present.

The foregoing experiments show an excellent regeneration on bread and skim milk. This diet is rich in carbohydrate, but contains more protein than the sugar and potato diet fed in Experiment 88 (Paper III of this series). Both of these diets are very poor in fat. These mixed diets are, perhaps, somewhat better than pure carbohydrate for liver cell regeneration.

TABLE 13.—LIVER REGENERATION ON FAT DIETS

Experiment	Chloroform Hours	Liver Injury	Regeneration Diet	Liver Repair	Remarks
92 (Dog 19-5)	1½	2d day: 1/4 to 1/3 necrosis; severe cytoplasmic injury up to 1/2; fat throughout	Fat diet: butter, lard, fat meat, cottonseed oil	7th day: 1/5 to 1/4 not repaired; fat over 2/5	Operation on 2d day; operation on 7th day
93 (Dog 19-6)	1½	2d day: 1/4 to 1/3 necrosis; severe cytoplasmic injury up to 1/2; fat throughout	Fat diet: butter, lard, fat meat, cottonseed oil	7th day: 1/5 to 1/4 not repaired; slight scattering of fat	Operation on 2d day; operation on 7th day; almost the same picture throughout as in Exper. 92
94 (Dog 18-124)	1¼	2d day: 1/2 necrosis; some surrounding injury; 1/3 fatty	Fat diet: fat meat, lard, cottonseed oil; fat from beef hearts	7th day: 1/4 not repaired; 3/4 fatty	Operation on 2d day; operation on 7th day
95 (Dog 19-37)	1¼	2d day: about 3/5 necrosis; some fat throughout	Mixed food (control)	Operation on 2d day to find the necrosis obtained under these "standard" conditions
96 (Dog 19-37)	1¼	Estimated 3/5 as in Exper. 95	75 c.c. cottonseed oil daily	7th day: 1/4 to 1/3 not repaired; 3/5 fatty	Metabolism experiment; N rose about 75%, but fell below the pre-anesthetic base line by the 6th day; died under anesthetic on 7th day

EXPERIMENT 92.—*Regeneration on Fat Diet.*—Dog 19-5, an adult male Airdale.

July 17: Wt., 26.75 lbs. Chloroform anesthesia for one and a half hours with carbonate solution intravenously; previous starvation of three days.

July 18: Wt., 26.25 lbs. Apparently as well as usual; butter and lard.

July 19: Wt., 26.13 lbs. Vomited about 2 o'clock. Piece of liver removed at 2:45 p. m.; severe injury of one half, with actual necrosis involving from one fourth to one third of lobule; fat throughout; fat left in cage.

July 20: Wt., 26.2 lbs. Wound in good condition; eats fat all right.

July 21: Wt., 25.94 lbs. Wound in good condition; fat diet *ad lib.*

July 22: Wt., 26.3 lbs. Losing appetite; added scraps of fat meat, and gave 100 c.c. cottonseed oil by stomach tube.

July 23: Wt., 25.9 lbs. Wound is opening; fat and oil as yesterday.

July 24: Wt., 25.56 lbs. Beginning distemper. *Piece of liver removed at 3 o'clock. Sections show scattered fat over central two fifths of lobules; central collapse of one fifth to one fourth (actual necrosis not repaired).*

July 25: Mixed diet.

EXPERIMENT 93.—*Regeneration on Fat Diet.*—Dog 19-6, a yellow adult male mongrel.

Aug. 19: Wt., 26.75 lbs. *Chloroform anesthesia for one and a half hours (from 9:10 to 10:40 a. m.); carbonate solution intravenously during anesthesia; previous starvation of three days.*

Aug. 20: Wt., 25.9 lbs. Quieter than usual; diet of *butter, lard* and a little cracker meal.

Aug. 21: Wt., 25.2 lbs. Eats *fat* well; bright and active. *Piece of liver removed at 1:15 p. m. Sections show necrosis of one fourth to one third, severe cytoplasmic injury up to one half, and scattering of fat to lobule peripheries.*

Aug. 22: Wt., 25.7 lbs. In addition to *fat mixture ad lib.*, gave 100 c.c. *cottonseed oil* by stomach tube.

Aug. 23: Wt., 26 lbs. Eats some *butter, lard and fat meat scraps*; 100 c.c. *cottonseed oil* by stomach tube.

Aug. 24: Wt., 26.2 lbs.; 100 c.c. *cottonseed oil, and fat scraps.*

Aug. 25: Wt., 26.3 lbs.; 100 c.c. *cottonseed oil, and ¼ pound butter.*

Aug. 26: Wt., 26.13 lbs. *Fat scraps ad lib. Piece of liver removed at 3 o'clock. Sections show a moderate central collapse, distinctly greater than after regeneration on meat diet (experiment 99); wandering cells present; slight scattering of fat.*

EXPERIMENT 94.—*Regeneration on Fat Diet.*—Dog 18-124, a young, black and white female.

Oct. 11: Wt., 13.75 lbs. Isolated for starvation before daily feeding, active; previously fed thyroid, chloroformed, and regenerated on carbohydrate diet.

Oct. 12: Wt., 13.44 lbs.; Oct. 13, wt., 13.25 lbs.; *starvation.*

Oct. 14: Wt., 13 lbs. In good condition; fourth day of starvation. *Chloroform anesthesia for one and a quarter hours (from 2 to 3:15 p. m.).*

Oct. 15: Wt., 12.44 lbs. Bright and active; 75 c.c. *cottonseed oil* by stomach tube; also *fat meat* left in cage.

Oct. 16: Wt., 12.38 lbs.; 75 c.c. *cottonseed oil; fat scraps and lard. Piece of liver removed at 3:30 p. m. Sections show one half necrosis; fat involves over one third and is moderate in amount; some vacuolated injured cells surrounding necrosis.*

Oct. 17: Wt., 12.38 lbs. Bright and active; 75 c.c. *cottonseed oil, also fat scraps*; vomited some; *fat* from beef hearts in p. m.

Oct. 18: Wt., 12.63 lbs.; 75 c.c. *cottonseed oil and fat* from beef hearts; vomits a little.

Oct. 19: Wt., 12.13 lbs. Active as usual; wound red and swollen; food as yesterday.

Oct. 20: Wt., 12.44 lbs. Active; wound slightly open; 75 c.c. *cottonseed oil, lard, and fat* from beef hearts.

Oct. 21: Wt., 12.38 lbs. *Fat only. Piece of liver removed at 1:30 p. m. Sections show liver lobules one fourth unrepaired; the remaining liver cells show fatty degeneration.*

EXPERIMENT 95.—*Simple Starvation and Chloroform (Control).*—Dog 19-37, a yellowish-brown male mongrel.

Oct. 2: Wt., 33.5 lbs. Isolated for starvation before daily feeding; healthy and very active.

Oct. 3: Wt., 32.25 lbs.; Oct. 4, wt., 31.7 lbs.; *no food.*

Oct. 5: Wt., 31.75 lbs. *Chloroform for one and one-fourth hours (from 9:25 to 10:40 a. m.).*

Oct. 6: Wt., 30.75 lbs. Quiet.

Oct. 7: Wt., 30.2 lbs. Quiet but fairly bright. *Piece of liver removed at 3 p. m.* Sections show hyaline necrosis involving about three fifths of each liver lobule; some fat to lobule peripheries. Mixed food.

EXPERIMENT 96.—*Liver Regeneration on Cottonseed Oil.*—Dog 19-37, a yellowish-brown male mongrel.

Date	Weight, Lbs.	Urine Cath., C.c.	Cage, C.c.	Nitro- gen, Gm.	Diet	Remarks
Oct. 28	32.94	Water ad lib.	Dog normal
29	31.5	Bladder	washed	250 c.c. water	
30	31.25	22	340	4.25	250 c.c. water	
31	30.56	6	310	4.12	75 c.c. oil; 150 c.c. water	Vomited; Kjeldahl shows no N in vegetable oil
Nov. 1	29.38	22	440	6.10	1½ hours (2:30-3:45 p. m.) 75 c.c. oil; 150 c.c. water	Feces
2	28.9	12	300	7.35	a. m. 75 c.c. oil; 150 c.c. water p. m. 75 c.c. oil; 150 c.c. water	Vomited in a. m.
3	28.5	..	440	6.02	75 c.c. oil; 350 c.c. water	Feces
4	28.5	14	225	5.62	75 c.c. oil; 350 c.c. water	Feces
5	28.25	1	230	4.36	75 c.c. oil; 350 c.c. water	Feces
6	27.75	20	150	3.55	75 c.c. oil; 350 c.c. water	
7	28.2	5	330	3.89	Died under anesthesia

Necropsy Report.—Greatly distended heart and congested viscera. *Liver* is quite distinctly dimpled, and lobule centers are red, giving the impression of unrepaired injury.

Microscopic Report.—*Liver:* about one fourth to one third of each lobule unrepaired; marked capillary stasis; fatty degeneration involves over three fifths of the liver cells. *Kidneys:* a little pyelonephritis.

The experiments listed in Table 13 indicate that the liver repair on a fat diet is very little better than that on starvation. Experiment 96, in which the daily output of nitrogen was determined, shows that the cottonseed oil has a definite sparing action on protein. The fact that repair was very incomplete in this case, and that there was an enormous output of nitrogen immediately following chloroform anesthesia, indicates that the oil did not actively "fix" much of the necrotic liver split products for new protoplasm, but exerted its influence more actively on sparing of bodily katabolism after the liver waste was removed. These phenomena are, therefore, capable of explanation on dynamic grounds alone. We must not forget, however, that a relatively small part of the oil molecule—the glycerol radical—can be made available as sugar in the body, and might play a more or less insignificant part in tissue building. Mendel and Lewis¹² found a delay of nitrogen elimination following ingestion of cottonseed oil. Classical metabolism experiments which may be found cited by Lusk,¹³ Sherman,¹⁴

12. Mendel, L. B., and Lewis, R. C.: J. Biol. Chem. **16**:37, 1913-1914.

13. Lusk, Graham: The Elements of the Science of Nutrition, Ed. 3, W. B. Saunders and Company, 1917.

14. Sherman, H. C.: Chemistry of Food and Nutrition, Ed. 2, The Macmillan Company, 1918.

and in books on nutrition by other authors, prove that carbohydrate cannot be wholly replaced by fat as a protein sparer, when the subject is in nitrogen equilibrium.

TABLE 14.—LIVER REGENERATION ON DIETS OF LIVER, KIDNEY, MUSCLE, BEEF EXTRACT, BRAIN, AND POWDERED THYROID

Experiment	Chloroform Hours	Liver Injury	Regeneration Diet	Liver Repair	Remarks
97 (Pup 19-45)	1	2d day: 1/2 to 3/5 necrosis; moderate fat over 2/5	Ground liver	7th day: repair good; lack of regeneration not exceeding 1/5; trace of fat	Operation on 2d day; sacrifice on 7th day
98 (Pup 19-56)	1	2d day: slight necrosis; vacuolated cells and fat over 1/2	Ground kidney	7th day: normal	Operation on 2d day; operation on 7th day
99 (Dog 19-6)	1½	2d day: 1/4 to 1/3 necrosis; cytoplasmic injury up to 1/2; very little fat	Skeletal muscle	7th day: slight lack of repair; trace of fat	Operation on 2d day; operation on 7th day; repair better than Exper. 93
100 (Dog 19-28)	1¼	2d day; 2/5 necrosis; moderate fat over 2/3	Skeletal muscle	9th day: about 1/4 not repaired; trace of fat; glycogen +	Operation on 2d day; operation on 9th day
101 (Dog 19-74)	1¼	2d day: 1/2 necrosis; moderate fat over 1/3	Beef extract, 10 gm. daily	9th day: 1/3 not repaired; moderate fat over 1/3	Operation on 2d day; operation on 9th day
102 (Dog 19-92)	1	2d day: 2/5 to 1/2 necrosis; moderate fat over 1/3	Brain	7th day: 1/4 not repaired; trace of fat	Operation on 2d day; operation on 7th day
103 (Dog 19-39)	1¼	Estimated 1/2 to 3/5 necrosis; see Exper. 82	Thyroid powder, 3 gm. daily	7th day: 1/3 not repaired; fat in large globules over 2/5	Operation on 7th day

EXPERIMENT 97.—*Regeneration on Liver Diet.*—Dog 19-45, a black mongrel, female pup.

Oct. 8: *Chloroform anesthesia for one hour*; has been fed a diet of alfalfa meal and cracker meal.

Oct. 9: Wt., 8.38 lbs. *Ground liver*, 150 gm.; active; not clinically sick.

Oct. 10: Wt., 8.5 lbs. *Ground liver*, 150 gm. *Piece of liver removed* at 1:45 p. m. Sections show necrosis involving one half or three fifths of each liver lobule, and fatty degeneration of moderate degree involving the remaining cells.

Oct. 11: Wt., 8.44 lbs.; Oct. 12, wt., 8.25 lbs.; Oct. 13, wt., 8.3 lbs.; *ground liver*, 160 gm., daily.

Oct. 14: Wt., 8.25 lbs. *Ground liver*, 140 gm.; wound opening a little.

Oct. 15: Wt., 8.25 lbs. Sacrificed in a. m.

Necropsy Report.—Small walled-off abscess beneath abdominal incision. *Liver*: finely dimpled; normal color; necrosis and fat not apparent. *Kidneys*: left shows a couple of small yellowish-white blotches in cortex suggesting abscesses; other viscera negative.

Microscopic Report.—*Liver*: repair is good in most places; some areas show lobules not completely regenerated (lack of repair probably not exceeding one fifth). *Kidneys*: a few abscesses at juncture of cortex and medulla.

EXPERIMENT 98.—*Regeneration on Kidney Diet.*—Pup 19-56, a female collie pup.

Oct. 16: Wt., 6.63 lbs. *Chloroform anesthesia for one hour* (from 10:30 to 11:30 a. m.); beef extract for four days previously; *kidney*, 150 gm., at 4:30 p. m.

Oct. 17: Wt., 6.75 lbs. A little dull; *kidney*, 150 gm.

Oct. 18: Wt., 6.8 lbs. *Kidney*, 150 gm. *Piece of liver removed* at 3 p. m. Sections show a minimal injury; very little necrosis, but a cytoplasmic disturbance, with vacuolization and deposition of fat over one half of each lobule; dog almost died under anesthesia.

Oct. 19: Wt., 6.8 lbs.; Oct. 20, wt., 6.75 lbs.; Oct. 21, wt., 6.9 lbs.; *kidney*, 150 gm. daily; continues bright and active.

Oct. 22: Wt., 6.8 lbs. No food.

Oct. 23: Wt., 6.63 lbs. *Kidney*, 200 gm., early in morning. *Piece of liver removed* at 3 p. m. Sections show a practically normal liver.

Oct. 24: Pup is up and active; experiment discontinued. Mixed food.

EXPERIMENT 99.—*Regeneration on Lean Meat Diet*.—Dog 19-6, a yellow, adult male mongrel.

July 20: Wt., 28.25 lbs. *Chloroform anesthesia for one and a half hours* (from 9:15 to 10:45 a. m.); saline intravenously during first hour of anesthesia; starvation for three days previous to chloroform.

July 21: Wt., 26.94 lbs. Very active; *lean meat diet*.

July 22: Wt., 27.13 lbs. *Lean meat diet ad lib*. *Piece of liver removed* at 11 a. m. Sections show very little fat; actual necrosis of one fourth to one third, with severe cytoplasmic reaction up to one half (cells swollen, little cytoplasm).

July 23: Wt., 28.06 lbs. Very active; wound in good condition; eats *meat ad lib*.

July 24: Wt., 27.63 lbs.; July 25, wt., 27.7 lbs.; July 26, wt., 27.44 lbs.; *skeletal muscle ad lib*.

July 27: Wt., 27.44 lbs. Operative wound slightly open. *Piece of liver removed* at 10 a. m. Sections show a slight amount of unrepaired injury with central collapse; some vacuolated cells in region of lobule centers; a few fat droplets.

EXPERIMENT 100.—*Regeneration on Lean Meat*.—Dog 19-28, a fox terrier, male adult.

Dec. 21: Wt., 15.8 lbs. Bright and healthy. *Chloroform anesthesia for one and a quarter hours* (from 11:15 to 12:30); casein digest by stomach tube a few minutes before anesthesia; *skeletal muscle ad lib*, in the evening.

Dec. 22: Wt., 16.2 lbs. *Lean meat ad lib*; eats well; clinically not sick.

Dec. 23: Wt., 16 lbs. Appears quite well; *meat diet*. *Piece of liver removed* at 2:30 p. m. Sections show hyaline necrosis involving two fifths of each lobule and moderate fatty degeneration involving the remaining liver cells.

Dec. 24: Wt., 15.13 lbs. A little dull after operation.

Dec. 25 to 27: *Meat ad lib*; eats well.

Dec. 28: Wt., 16.5 lbs. Operative site is swollen and red; chest and pelvic regions show purpuric areas; *meat ad lib*; eats well.

Dec. 29: Wt., 16.13 lbs. Wound looks bad but is not open; *meat* as before.

Dec. 30: Wt., 16.44 lbs. Wound somewhat better; *meat* taken all right. *Piece of liver removed* at 1:30 p. m. Sections show a little fat and central collapse of perhaps one fourth; glycogen ++; experiment discontinued; mixed food.

EXPERIMENT 91.—*Regeneration on Beef Extract*.—Dog 19-74, a young, black and white male terrier.

Dec. 7: Wt., 11.8 lbs. *Chloroform anesthesia for one and a quarter hours*; fourth day of starvation.

Dec. 9: Wt., 11.06 lbs. Active. *Piece of liver removed* in p. m.; bled freely. Sections show necrosis involving one half of each liver lobule and a moderate degree of fatty degeneration involving about one third of the lobule.

Dec. 10: Wt., 10.94 lbs.; Dec. 11., wt., 10.7 lbs.; Dec. 12, wt., 10.5 lbs. *Beef extract*, 10 gm., + kaolin in 100 c.c. water, by stomach tube daily.

Dec. 13: Wt., 10.44 lbs. Wound opening superficially; *beef extract*, 10 gm., as before.

Dec. 14: Wt., 10.25 lbs.; Dec. 15, wt., 10 lbs.; weak and thin; *beef extract*, 10 gm., daily.

Dec. 16: Wt., 9.94 lbs. *Beef extract*, 10 gm., in a. m. *Piece of liver removed* at 2:15 p. m. Sections show a collapse and lack of repair involving the central one third of each liver lobule; there is moderate fatty degeneration involving one third of the lobule. Mixed food and recovery.

EXPERIMENT 102.—*Regeneration on Brain*.—Dog 19-92, an adult male terrier.

Dec. 25: Isolated for starvation; active.

Dec. 26 to 27: Fasting.

Dec. 28: Wt., 18.63 lbs. Somewhat thin, but active. *Chloroform anesthesia* for one hour (from 11 to 12); *brain*, 200 gm. in p. m.

Dec. 29: Wt., 18.63 lbs. Active; *brain*, 200 gm.

Dec. 30: Wt., 17.9 lbs. Weak. *Piece of liver removed* at 3:45 p. m. Sections show hyaline necrosis involving from two fifths to one half of each liver lobule, and fatty degeneration involving the midzone (one third in amount); ate 300 gm. *brain* late in evening.

Dec. 31: Wt., 17.9 lbs. Sick; *brain*, 200 gm., eaten.

Jan. 1, 1919: Wt., 17.38 lbs. Brighter than yesterday; ate 300 gm. *brain*.

Jan. 2: Wt., 17 lbs. *Brain ad lib.*; ate 330 gm.

Jan. 3: Wt., 16.5 lbs. *Brain ad lib.*; ate about 250 gm.

Jan. 4: Wt., 15.75 lbs. Mixed food. *Piece of liver removed* at 11:30 a. m. Sections show collapse and lack of repair involving the central one fourth of each liver lobule. There is a trace of fatty degeneration.

EXPERIMENT 103.—*Liver Regeneration on Thyroid Feeding*.—Dog 19-39, a female Airdale.

Date	Weight, Lbs.	Urine—		Nitro- gen, Gm.	Diet	Remarks
		Cath., C.c.	Cage, C.c.			
Nov. 23	27.38	Water ad lib.	Dog normal
24	26.25	Bladder washed	300 c.c. water	Feces ++
25	25.63	96	400	2.41	250 c.c. water	
26	25.06	2	375	2.06	300 c.c. water	
26	Chloroform anesthesia for 1½ hours				(10:00-11:15 a. m.)	
27	23.63	83	450	3.50	300 c.c. water; 3 gm. powdered thyroid	Feces +; thyroid contains 126 mg. N per gram
28	23.0	22	385	4.48	300 c.c. water; 3 gm. powdered thyroid	See control Exper. 82, same dog; 1/2 to 3/5 necrosis
29	22.56	18	355	3.86	300 c.c. water; 3 gm. powdered thyroid	
30	22.2	10	300	3.09	300 c.c. water; 3 gm. powdered thyroid	
Dec. 1	21.56	18	340	3.37	300 c.c. water; 3 gm. powdered thyroid	
2	21.38	4	290	3.68	300 c.c. water; 3 gm. powdered thyroid	
3	21.13	1	275	3.71	Water ad lib.	Metabolism discontinued; mixed food
3	Piece of liver removed at 3 p. m.; 1/3 unrepaired; fat over 2/5					

Experiment 97 shows a very good regeneration on liver diet and the initial injury was almost maximal. Experiment 98 shows complete repair on kidney diet, but in this case the initial injury was slight. Both of these experiments were on pups, and age may be a definite factor in repair.

Experiments 99 and 100, using skeletal muscle as reparative diet, indicate a good regeneration, although probably not as thorough as would have occurred with a high carbohydrate or a mixed diet intake (such as bread and skim milk).

Experiment 101 shows very imperfect repair on beef extract feeding, which would indicate that fairly liberal amounts of extractives alone are not conducive to active repair. It will be recalled (Paper I of this series) that beef extract gave very good protection against chloroform injury when fed before the anesthetic.

Brain diet (Experiment 102) seems to equal skeletal muscle in reparative efficiency and to be distinctly better than the fats. The striking difference between brain and fat diets deserves further investigation.

Thyroid feeding alone (Experiment 103, Dog 19-39) certainly does not stimulate liver repair. Reference to the nitrogen curve of this animal reveals a sustained high output during the experiment. This seems to indicate a stimulated katabolic activity, and probably a deleterious or destructive rather than a reparative action on the injured organ. The thyroid used was an Armour preparation, and had previously been shown by Dr. Rohde to increase greatly the nitrogen metabolism in normal animals in the dosage here employed.¹⁵

GENERAL DISCUSSION

It is clear from the experimental data given that liver regeneration can be completed more rapidly on a diet rich in carbohydrate than on a very rich protein diet. This applies to mixed diets like bread and skim milk as contrasted with lean meat diets. We wish to point out a decided difference in liver regeneration as compared with serum protein regeneration. Kerr, Hurwitz and Whipple¹¹ found that serum proteins, after a considerable depletion, were regenerated best on a rich protein diet, for example, meat. It is possible that the differences noted may be explained by inherent differences in the proteins which are being formed with unusual speed in the body. Certain foods may contain ingredients especially suited to construct a certain type of body protein but unsuited for another type of body protein. It is significant that the abundant mixed diet gives an optimum regeneration in such instances.

The difference in regenerative power between the parenchymatous organs and ordinary skeletal muscle here noted may be only apparent. Pups were regenerated on liver and kidney, while adults were fed the muscle; there is a possible difference due to age. On the other hand, liver may be better than muscle, because chemically more nearly the

15. Rohde, A.: *J. Biol. Chem.*, 1919. To be published.

equivalent of the tissue which is regenerated. We have shown (Paper I of this series) that the parenchymatous organs are more protective against chloroform injury than skeletal muscle when fed during the days preceding anesthesia.

Thyroid may accelerate synthesis when given in small amounts with foods, as Jänney suggested, but in the experiment here presented it was given in large quantities with no food, and we observed the well known action of increased metabolism, with a continued high nitrogen output, and no increase in liver repair.

The lack of repair on a fat diet is certainly quite striking when compared to the results with carbohydrates, proteins and mixtures of the two. Fat under these experimental conditions has a very minor rôle in tissue building, and apparently is limited to its dynamic function as a fuel in protein sparing.

SUMMARY

A diet of bread and skim milk gives the optimum repair following a unit chloroform liver necrosis. A similar reaction is to be expected with any mixed diet rich in carbohydrate. A liver necrosis involving the central one-half of every lobule (50 per cent. of all liver parenchyma) will usually be completely repaired in from seven to nine days.

A diet of cooked skeletal muscle is not as favorable for rapid liver repair as the rich carbohydrate diet.

A diet of cooked liver or kidney is more favorable for rapid liver repair than a meat diet. This diet of parenchymatous organ tissue approximates the rich carbohydrate diet in efficiency of liver repair.

Beef extract given alone does not favor liver repair, which indicates that meat extractives are not particularly concerned in the reaction of liver repair.

Thyroid powder given in large doses with no food does not stimulate liver repair, but does accelerate tissue katabolism and increase nitrogen elimination. This accelerated katabolism may even impede the liver repair which is to be expected in starvation.

Brain feeding is favorable to liver regeneration and repair. This diet approximates lean meat feeding in its favorable influence on liver repair. In this respect the brain diet stands in marked contrast to the fat diets.

Fat diets (vegetable oils, butter, lard, beef fat, etc.) do not aid in liver regeneration. The same repair is to be observed during fasting control periods. The fat diet can spare the proteins of the animal at the source, but *cannot act in conservation of protein material* by taking an active part in reconstruction of new protein substance.