CIRRHOSIS OF THE LIVER IN "DONOR" DOGS FED A HIGH FAT DIET AND SUBJECTED TO REPEATED BLEEDINGS*

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PLATES 21 AND 22

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The observations reported in this paper suggest that the combination of relatively high fat diet and repeated bleedings results in cirrhosis of the liver, whereas neither diet nor repeated bleeding alone produces such changes in the liver. If confirmed and extended these results might eventually be added to the growing body of evidence tending to incriminate a disturbance of fat metabolism as one of the pathogenic factors in cirrhosis of the liver (1–3).

Methods

The 13 dogs which form the basis for this report were bled repeatedly for plasmapheresis and plasma injection experiments that have been reported elsewhere (4–7). They were large, healthy, adult, mongrel dogs obtained from routine sources. They were kept in the "isolation room", and dipped in creosote, dewormed, and observed for distemper or other infection for 2 weeks and then placed in the "main dog room" in individual cages (on shavings changed 3 times per week). Water was available at all times. They were fed the regular "kennel ration" which has varied from time to time but which has been fed on a uniform basis to all dogs receiving this ration in our kennels.

These variations in "kennel ration" are believed to be significant in the findings described below. The routine source for "kennel ration" for dogs is, and has been for many years, selected table scrap from the University dining halls. At times (vacation and holiday periods) this is insufficient and supplements of Purina dog chow are used. During the period under discussion (from approximately February, 1941, to September, 1942) this routine source was cut off, and beef bones with much adherent fat was the chief source for "kennel ration." The bones were delivered once each week and were kept in a refrigerator (38–40° F.) until fed. During this period the blood plasma of these "donor" dogs was frequently recorded as "lipemic" and dogs that were sacrificed during this period frequently had large masses of fat in the stomach even though the necropsy was performed 18 to 20 hours after the last feeding.

The amount of bleeding varied from time to time and from dog to dog but averaged about 2 times per month for a period that varied from 1 to 3 years. The amount of each bleeding was 200 to 250 ml., or roughly 15 per cent of the total blood volume (of a 20 kilo dog). At this rate a dog would be bled about 10 times its total blood volume during a period of 3 years. The rate of bleeding varied considerably from time to time for any given dog or group of dogs. At times they were bled as often as once per week, then there would be rest periods of 2 to 3 months without bleeding. In general, when the hematocrit reading fell below 30 per cent bleeding was discontinued until after a "rest period."

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The period between cessation of bleeding and postmortem examination has also varied from 0 to 29 months. Most of the dogs were sacrificed by intravenous injections of mercuric chloride (2.0 to 2.5 mg./kg.) or uranyl nitrate (5.0 mg./kg.). All but one of the dogs died after a period of 3 to 14 days following the injection of the heavy metal. The changes in the liver are obviously much older than this period; and, since other dogs injected with the same amount of mercuric chloride or uranyl nitrate have not shown fatty or cirrhotic changes in the liver, I feel confident that this terminal procedure had nothing to do with the changes in the livers of these dogs described below.

EXPERIMENTAL OBSERVATIONS

The data pertinent to sex, age, body weight, period used as a "donor," method of death, and degree of cirrhosis are summarized in Table I.

Of the 13 dogs used repeatedly as donors for 1 to 3 years and fed a relatively high fat diet for 1 year or longer, 3 showed advanced cirrhosis, 7 slight to moderate cirrhosis, and 2 marked fatty change but no definite increase in portal connective tissue. One of the dogs showing advanced cirrhosis (40–71) was in the kennel only 4 months and was bled only 3 times. This dog might properly be excluded from the group and be reclassified as "spontaneous" cirrhosis in the dog. This dog had heart-worm (*Dirofilaria immitis*, a single female worm) infection of the pulmonary artery and conus of the right ventricle of the heart. It was the only one of the group with this infection and it was the only one that had ascites, splenic fibrosis, and evidences of the establishment of collateral circulation (dilatation of the posterior peritoneal veins). In 13 years of experimental work involving the examination of large numbers of dogs, I have seen only one other case of "spontaneous" cirrhosis of the liver in the dog; and, if the control data referred to by Graef, Negrin, and Page (2) are representative, this experience of encountering a single instance of spontaneous cirrhosis in the dog is unusual.

The hepatic changes appear to be identical with those described by Chaikoff and his associates (1) and by Graef, Negrin, and Page (2). In the cases of advanced cirrhosis (grade III) the liver was diffusely nodular (Fig. 1). The nodular pattern, however, was not uniform; areas of nodular hyperplasia were scattered between areas of atrophic fibrotic change. In a few sections the process resembled more closely that seen in subacute yellow atrophy with regeneration, but the over-all picture—especially the diffuseness of the change and the absence of any large scars representing collapse fibrosis of large areas of destroyed parenchyma—more closely resembled Laennec's cirrhosis. Large droplet fatty change in the remaining liver cells and bile duct proliferation and lymphocytic infiltration in the scarred portal areas were marked in all three livers in this group (Figs. 2 to 4). In two of these, small bile plugs were noted within intralobular canaliculi in some of the lobules. None of the dogs developed obvious signs of jaundice or other disturbance of liver function.

The livers with second degree change (grade II) varied considerably. Two

of the 7 livers in this group showed slight fibrotic changes which in one (dog 39–42) were confined to the subcapsular tissue of one lobe and in the other (dog 40–72) to two of the lobes on the left side of the liver. Both of these livers showed moderate large droplet fatty change elsewhere in the liver. One liver in this group (dog 39–36) was the seat of central necrosis involving $\frac{1}{3}$

TABLE I

Summary of Dogs

Dog No.	Sex	Age*	Body weight	Period used as "donor"	Interval before death‡	Manner of death§	Degree of change in liver‖
		yrs.	*kg.*	*mos.*	*mos.*		
39-41	M	4	22.0	22	1	U—5.0 i.v.; 14 days	I
40-76	M	3	22.0	12	0	Sacrificed	I
39-36	M	5	23.0	30	5	Hg—2.5 i.v.; 3 days	II
39-37	M	6	22.0	30	5	Hg—2.5 i.v.; 4 days	II
39-42	M	5	18.0	33	6	U—5.0 i.v.; 8 days	II
40-66	F	4	20.1	20	4	Hg—2.0 i.v.; sacrificed 10 days later	II
40-72	F	4	24.8	21	3	Hg—2.0 i.v.; 10 days	II
41-86	M	2	18.4	10	4	Hg—2.5 i.v.; 8 days	II
40-74	M	7	20.5	15	32	Hg—2.0 i.v.; survived 29 mos.; sacrificed	II
39-35	M	6	26.0	31	5	Hg—2.5 i.v.; 3 days	III
39-38	M	6	18.6	33	7	U—5.0 i.v.; 9 days	III
40-71	F	5	16.0	3	1	Died	III
39-39	M	6	19.6	30	5	Hg—2.5 i.v.; 4 days	0

* Age at time of death.

‡ Interval between cessation of bleeding and death.

§ U—5.0 i.v.; 14 days = death 14 days after intravenous injection of 5.0 mg./kg. uranyl nitrate.

Hg—2.5 i.v.; 3 days = death 3 days after intravenous injection of 2.5 mg./kg. mercuric chloride.

‖ I = fatty change without cirrhosis; II = slight to moderate cirrhosis; III = advanced cirrhosis with regeneration.

to $\frac{1}{2}$ of every lobule. There was considerable hemorrhage and many polymorphonuclear leukocytes in the necrotic tissue about the efferent veins. This liver, in addition, had slight diffuse increase in portal connective tissue. Interpretation of the central necrosis in this liver is difficult but the cirrhotic changes in the portal areas obviously antedated the administration of mercuric chloride 3 days before death. The other four livers in this group showed definite increase in periportal connective tissue with slight to moderate bile

duct proliferation and lymphocytic infiltration—also moderate large droplet fatty change in the hepatic cells (Figs. 5 and 6).

The two livers with grade I change (dogs 39–41 and 40–76) had only moderate to marked large droplet fatty change in the hepatic cells but no definite increase in connective tissue or other change in the portal areas.

There were no known circumstances which might explain the lack of lesions in the liver of dog 39–39.

Two of the dogs (39–35 and 39–42) each had two "splenomata" (localized subcapsular areas of atypical splenic tissue not sharply separated from the surrounding normal splenic tissue but forming gross, spherical, usually pale gray nodules that measured up to 15 mm. in diameter). This change has been observed in other dogs. The number of instances of this condition in these 13 dogs as compared with "controls" does not allow any statement as to whether or not these nodules in the spleen were in any way related to the changes in the liver.

Control Data

Control data are needed for both parts of the experimental procedure-(1) relatively high fat diet, and (2) repeated bleeding. Only the first of these factors deserves serious consideration, for repeated bleeding of dogs over extended periods has been carried out in numerous laboratories during the past 50 years without any reports of hepatic cirrhosis. Direct control data on "relatively high fat diet" are limited to 6 dogs used for long term experiments (that did not involve repeated bleedings) which consumed the regular "kennel ration" during the entire period under discussion. Four of these dogs have been necropsied; none had cirrhosis or marked fatty change in the liver. A much larger body of indirect evidence on "high fat diet" could be selected from the literature, but so far as I am aware the only report of cirrhosis of the liver in the *normal* dog from high fat feeding is that by Chaikoff, Eichorn, Connor, and Entenman (1), and these workers had to resort to forced-feeding which was usually associated with vomiting. The 13 dogs of this report voluntarily ate the beef bones with much adherent fat and maintained body weight and apparently good condition (without vomiting) despite the repeated large bleedings.

DISCUSSION

The experimental data suggest that repeated bleeding in dogs fed a relatively high fat diet results in cirrhosis of the liver, whereas neither diet nor repeated bleeding alone produces such changes in the liver. The simplest explanation for this combined effect is that the repeated bleedings led to more marked lipemia (8) and this augmented the fatty change in the liver due to the high fat diet. Two other possibilities suggest themselves: (1) anoxia, and (2)

removal of something other than hemoglobin (methyl groups?, choline?, vitamins?, lipases?). Any of these factors might add to the "strain" of relatively high fat diet with cirrhosis of the liver the end result. Further conjecture is unwarranted until these results are extended in planned experiments with accurate records of diet and bleedings, with adequate controls, and with chemical analyses of blood and liver lipoids.

SUMMARY

1. In 13 dogs used repeatedly as donors for plasmapheresis and plasma injection experiments and fed a kennel diet that for a period of over a year consisted chiefly of bones with much adherent fat, cirrhosis of the liver occurred in 10. Marked fatty change without definite fibrosis occurred in 2 of the dogs.

2. In control dogs fed the same kennel diet during the same period but not subjected to repeated bleedings, no instance of cirrhosis was observed.

3. In previous plasmapheresis and plasma injection experiments "donor dogs" subjected to similar bleedings over comparable periods but maintained on a different kennel diet did not develop cirrhosis.

4. This series of events suggests that in dogs maintained on a relatively high fat diet repeated bleedings predispose them to cirrhosis of the liver. No data are available to decide the more fundamental question: Do the repeated bleedings remove something (other than hemoglobin) necessary for continued integrity of the liver, or is the cirrhosis the result of relative anoxia or increased lipemia occasioned by the repeated bleedings or perhaps of both combined?

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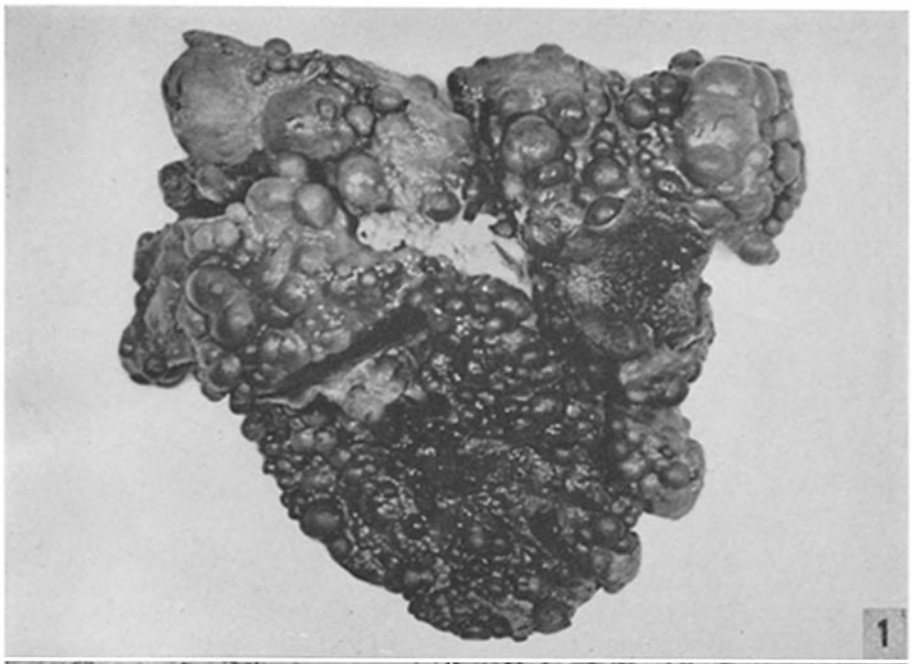
EXPLANATION OF PLATES

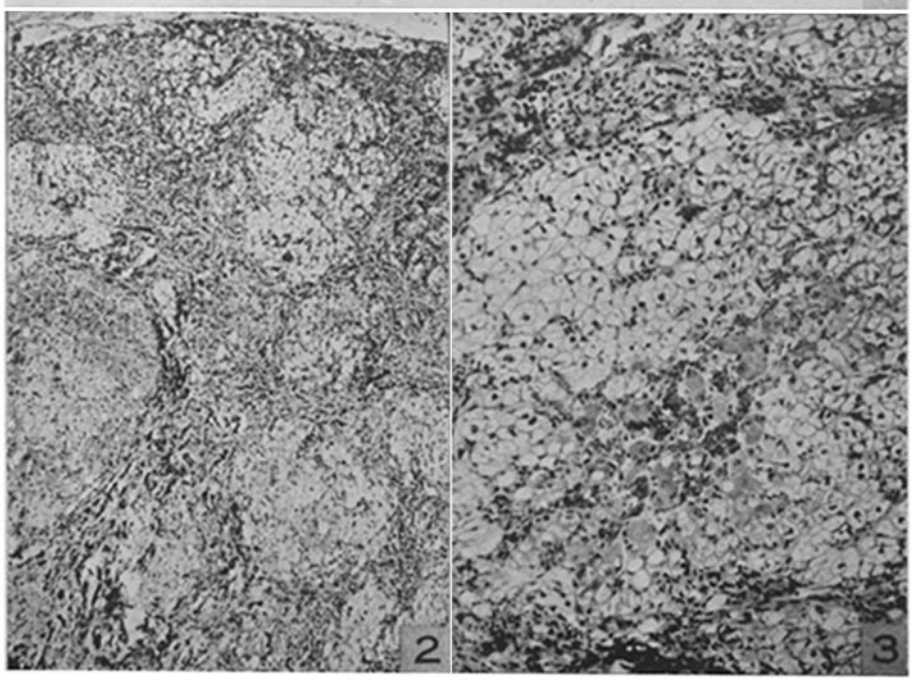
PLATE 21

FIG. 1. Dog 39-38. Advanced cirrhosis of liver with nodular regeneration. Posterior surface. The opened gall bladder is seen near the right center. Half natural size.

FIG. 2. Dog 39-38. Cirrhosis of liver with marked fatty change, disruption of normal architecture, bile duct proliferation, and lymphocytic infiltration. Hematoxylin and eosin. ×50.

FIG. 3. Dog 39-38. Medium power view showing curious pattern of fatty change and some better preserved liver cells about an efferent vein. Fibrosis, bile duct proliferation, and lymphocytic infiltration in portal areas at top and bottom. Hematoxylin and eosin. ×135.





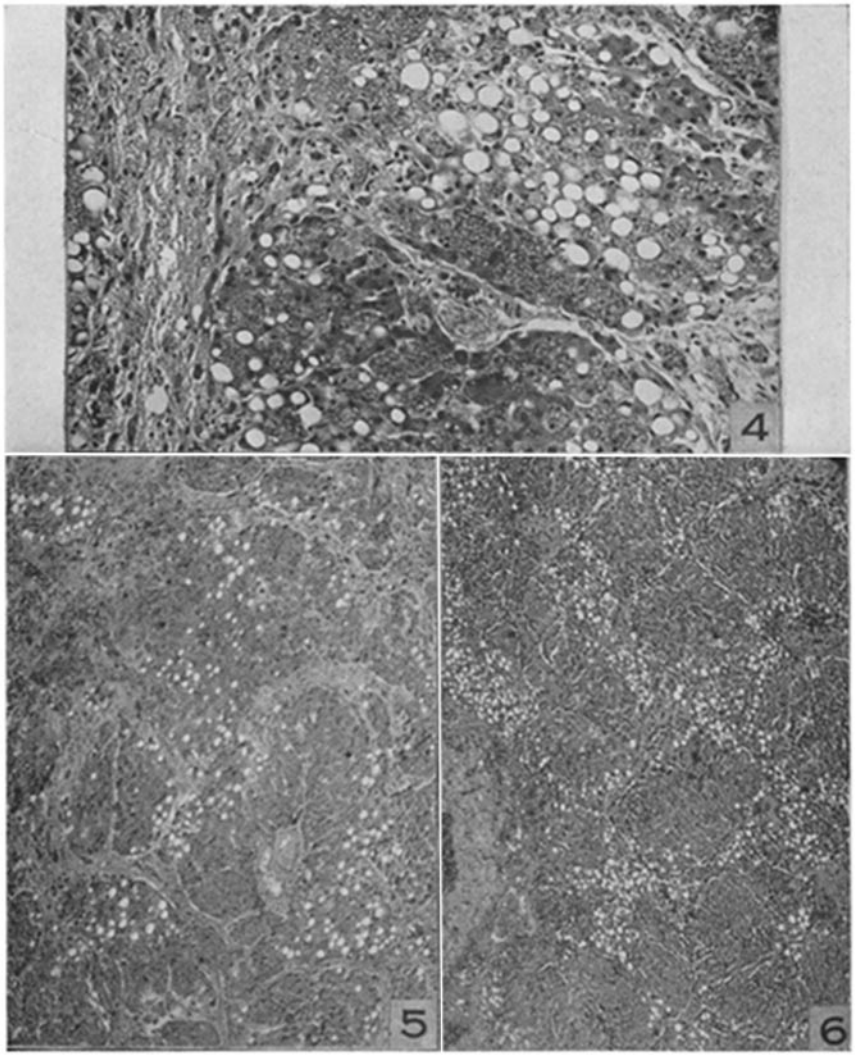
(Holman: Dietary cirrhosis in "donor" dogs)

PLATE 22

FIG. 4. Dog 39-35. Fatty cirrhosis of liver with atrophy of remaining liver cells and marked congestion of sinusoids. Hematoxylin and eosin. ×150.

FIG. 5. Dog 40-72. Large droplet fatty change and moderate cirrhosis. Hematoxylin and eosin. ×45.

FIG. 6. Dog 40-66. Large droplet fatty change and slight cirrhosis. Hematoxylin and eosin. ×45.



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