STUDIES ON THE TOTAL BILE.

II. THE RELATION OF CARBOHYDRATES TO THE OUTPUT OF BILE PIGMENT.

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For two generations the belief has been general that the pigment of the bile is derived wholly from the pigment of destroyed blood; and of late years the quantitative relationship supposedly existing between the two has been utilized as a basis for calculations on the rate of normal blood destruction.[1] Recently, though, Hooper and Whipple[2] have published experiments which have convinced them that the bilirubin is normally formed in considerable part from the carbohydrates of the food. If such be the case, quantitative studies of the bilirubin output and of urobilin in the stools of patients with blood diseases lose largely in significance, as does also much of the accepted reasoning upon the course of normal blood destruction.

The findings of Hooper and Whipple are clear-cut. But they were obtained through quantitations on a rather small fraction of the 24 hour output of bile, and these authors assumed that the fraction was representative of the secretion in general. Is this assumption warranted? Pavy has described the condition of the liver after a meal rich in carbohydrates.[3] It becomes swollen with glycogen, large, pale, and friable, and the capillary spaces are so encroached upon by the parenchyma as to suggest the existence of an actual vascular

[1] Zoja, L., *Folia hæmatol.*, 1910, x, 232. Goodman, E. H., *Beitr. chem. Physiol. u. Path.*, 1907, ix, 91. Abderhalden, E., Lehrbuch der physiologischen Chemie, Berlin and Vienna, 4th edition, 1920, i, 746.

[2] Whipple, G. H., and Hooper, C. W., *Am. J. Physiol.*, 1916, xl, 349. Whipple, G. H., *Arch. Int. Med.*, 1922, xxix, 711; *Physiol. Rev.*, 1922, ii, 440.

[3] Pavy, F. W., The physiology of the carbohydrates; their application as food and relation to diabetes, London, 1894.

compression.[4] May it not be that the increased bilirubin output observed by Hooper and Whipple is a temporary result of these marked changes, produced perhaps by pressure or replacement, and later compensated for by a lessened output? A method recently devised for total bile collection over long periods of time has afforded us opportunity to test the point.

Hooper and Whipple employed dogs with open bile fistulas made according to the classical method of Schwann; and they collected the secretion during 6 or 8 hours of each 24, the specimen of the first 2 hours serving in some experiments as control to that of the later ones. Between whiles the bile was allowed to dribble away from the fistula opening and the dogs could lick it up at will. For some days before each test with carbohydrates a diet of cooked lean meat and liver was fed. The carbohydrate ration,—bread and milk with a small bone,—was then abruptly substituted, or else dextrose was given by stomach tube, or intravenously. A remarkable increase in the output of bilirubin occurred within the next few hours. As the authors say, "On a strict meat diet the bile pigment curve is at its lowest level, but with a sharp transition to a diet rich in carbohydrates, a rise of 50 to 100 per cent in bile pigment output may be noted,"[5] whence the conclusion that "the bile pigments can be modified at will by diet factors."[6]

The most interesting of the protocols are those dealing with the effects of food, since they are indicative of what may perhaps be expected under normal conditions. The direct injection of a dextrose solution into the circulation brings with it a number of extraneous possibilities which must be considered in any interpretation of the results. For these reasons, though we have made both feeding and injection experiments, special emphasis will be laid on the effects of food. The giving of dextrose solutions in large quantity by mouth we have seldom practised (Text-fig. 2, Dog 18; Text-fig. 4, Dog 17), since it frequently induces vomiting.

In our experiments the diets used, the strength and amounts of the dextrose solutions, and the methods in general, have been identical with those of Hooper and Whipple. The dextrose was in part Merck's "highest purity," in part the C. P. product of the Corn Products Refining Company. The water in which it was dissolved had been twice distilled. During a preliminary period the dogs re-

[4] Afanassiew, M., *Arch. ges. Physiol.*, 1883, xxx, 385.

[5] Whipple, G. H., and Hooper, C. W., *Am. J. Physiol.*, 1916–17, xlii, 256.

[6] Wisner, F. P., and Whipple, G. H., *Am. J. Physiol.*, 1922, lx, 119.

ceived cooked lean meat and liver (bullock's or sheep's) in the proportion of five to one. The authors just mentioned gave 200 gm. of liver, but as they do not record the amount of the mixture eaten each day this figure helps but little. After a few days on such diet the appetite of many of our animals flagged and the ultimate contrast in the effects of the diets resolved itself into one between fasting and carbohydrate administration. In certain instances all food was designedly withdrawn for a day or more (Text-figs. 1 and 2) prior to the giving of carbohydrates. The failure of appetite on the meat diet may have been largely due to the circumstance that the dogs were confined in metabolism cages, not exercised daily in a yard as was Hooper and Whipple's practise. Active exercise causes a considerable increase in the bilirubin output as measured in the 24 hour specimen,[7] thus introducing a factor of error that is better avoided. Despite the lack of exercise the animals remained in excellent condition. When the carbohydrate diet was given,—bread moistened with a little milk, and a small bone, as described by the authors just mentioned,—it was practically always taken greedily and in large amount. No better opportunity could have been desired for a demonstration of the influence of carbohydrates. The character and amount of the ingested food find record in the charts (Text-figs. 1 to 6) which serve the purpose of protocols.

The dogs yielded sterile bile to the collecting bag, or one infected in pure culture with an organism harmless both to the animal, as the eventual autopsy proved, and to the bile pigment, as shown by incubation tests with specimens of the same bile and of sterile biles inoculated from it. The 24 hour specimen was always so slightly infected as to appear water-clear. Its pigment content was determined by Hooper and Whipple's modification of the Salkowski reaction, the method used by these authors. A mixture of copper sulfate and potassium bichromate served as the color standard.[7] Two determinations were made on each bile specimen, as in our previous observations.[8]

The urine was followed in every instance for bilirubin (Gmelin reaction, the Salkowski quantitative method). In one animal, sufficient of the pigment sometimes appeared for quantitation (Dog 19, Text-figs. 1 and 2), as is not infrequently the case in apparently healthy animals; but in the others the urine was negative practically throughout. One of the criteria employed in the selection of the dogs was freedom from such "physiological" bilirubinuria. The hemoglobin percentage of the blood was followed by the Newcomer method. Not infrequently it underwent gradual changes during the period of observation, and the changes were usually accompanied by similar fluctuations in the bilirubin yield.[8] Superimposed upon these fluctuations were the day to day changes in the bilirubin, which are relatively slight under ordinary circumstances.

In many of the animals injected with dextrose solution (Text-figs. 4 to 6) a 6 hour collection of bile, corresponding with that of Hooper and Whipple, was

[7] Rous, P., and McMaster, P. D., *J. Exp. Med.*, 1921, xxxiv, 47.

[8] McMaster, P. D., Broun, G. O., and Rous, P., *J. Exp. Med.*, 1923, xxxvii, 395.

made each day, followed by an 18 hour collection. As the charts show, the output of pigment during the shorter period furnishes but a poor index to that of the entire 24 hours. This latter is, as just mentioned, almost constant from day to day under ordinary circumstances. For these reasons 24 hour specimens have been preferred for most of the work.

The charts (Text-figs. 1 to 6) suffice to record the results. It will be seen from them that the ingestion of carbohydrate food in large quantity after a diet of cooked lean meat mixed with liver is wholly devoid of effect on the bilirubin output in the 24 hour bile specimen. It may be objected that our animals, unlike those of Hooper and Whipple, were for some reason incapable of responding properly to the administration of carbohydrates; but they differed from those of the observers mentioned only in that all of the bile was studied instead of a small fraction, that the animals had no open wound, that some of them were mildly anemic, and that the potentially confusing influences of pathogenic infection of the biliary tract, of yard exercise, and of the licking up of bile, were avoided. In certain cases in which a 6 hour specimen was taken separately, a transient hastening in the rate of secretion of bilirubin occurred, giving the effect of a rise in output of the sort recorded by Hooper and Whipple (Text-figs. 4 to 6),—conclusive evidence that the results of our experiments may properly be compared with those of the observers mentioned.

In all save one instance (Text-fig. 6, second injection), in which the dextrose injection was followed by a rise in the bilirubin content of the 6 hour specimen, there was a compensatory drop in the output during the succeeding 18 hours, and the total for the combined periods merely amounted to that of control days. This instance is exceptional in other ways. At the time of its occurrence, the animal was in poor condition, as result of chronic intestinal obstruction, markedly anemic, and showed sudden great fluctuations in the hemoglobin percentage of the blood, and as great ones in the bilirubin output. The red cells of some dogs are so fragile[9] that it would not be surprising to find them injured by intravenous injections of any non-colloid solution.

Comment.

The data here presented would seem to prove that the rise in bilirubin output which follows the administration of carbohydrates is not due to an increase in the amount of the substance manufactured

[9] Rous, P., and Turner, J. R., *J. Exp. Med.*, 1916, xxiii, 219.

by the body, but merely to a temporary hastening in its evacuation. Whether this hastening is consequent on the deposition of glycogen in the liver, an explanation which is suggested by the condition of the organ, has not been determined. Hooper and Whipple make a statement in connection with their 6 hour observations which may bear on the point. They state that: "The diet rich in carbohydrates may give a very high initial curve (perhaps double normal), which is apt to fall during succeeding weeks, but always remains somewhat above the mean curve." This is to say that the changes were much more marked when glycogen was first supplied in quantity to the liver than later when the organ contained the substance in considerable amount from day to day. Furthermore, "there is a tendency for the high bile pigment curve of a carbohydrate diet to approach the mean curve of a mixed diet. Also there is the same tendency for the low bile pigment curve of a meat diet to approach the mean curve of a mixed diet."[2]

Dextrose injections do not cause any consistent alteration in the rate of bile flow, which furthermore is almost regularly less on a carbohydrate diet than on one of meat. The increase in the pigment output can scarcely be laid then to a mechanical flushing out of the liver.

The present experiments have been the necessary first step toward a study of the quantitative relationship between blood destruction and the bilirubin output. It is obviously essential to know in this connection whether bile pigment can be derived from the food. In addition to carbohydrate food, as described, we have fed several diets consisting predominantly of protein, or of mixtures of protein and carbohydrate, all having the character in common that they are poor in fats. None has influenced the 24 hour output of bilirubin; and in this particular our work corroborates that of Hooper and Whipple with 6 hour specimens. Since such is the case no detailed records need be given.

CONCLUSION.

Carbohydrate feeding or injection produces often a temporary increase in the rate at which bilirubin is put forth in the bile, but none in the amount of the pigment secreted from day to day. There would appear to be no ground for the supposition that bilirubin is normally derived in part from the carbohydrates of the food.

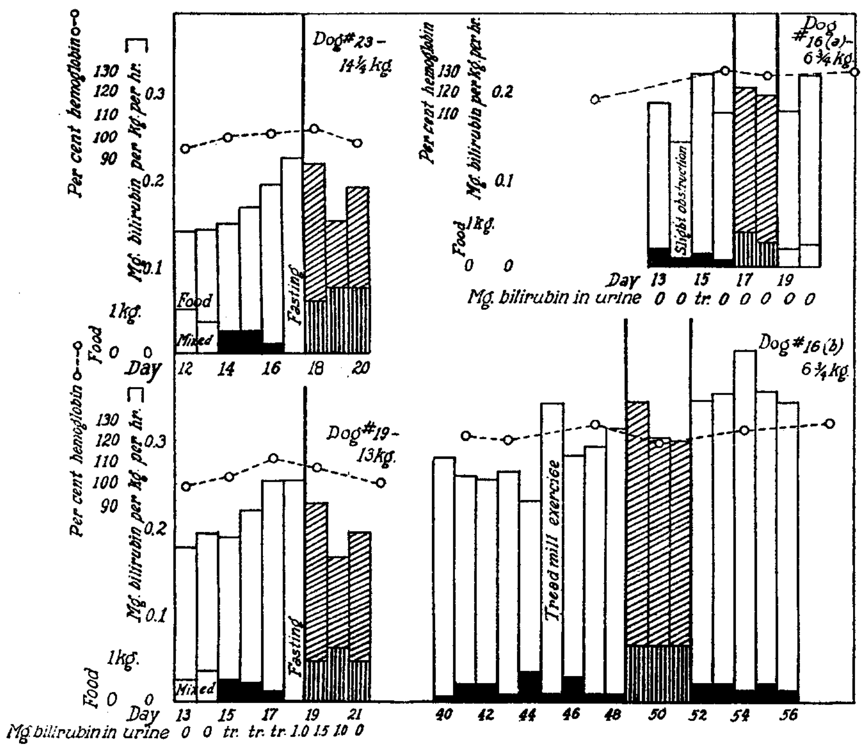
Explanation of Charts.

The bilirubin yield in the bile from day to day, the amount of food taken, and the hemoglobin percentage of the blood are recorded. A black column for the food indicates that it consisted of meat and liver, one vertically lined that bread, milk, and bones were given. For purposes of contrast a heavy vertical line has in some instances been placed to indicate the point at which carbohydrate feeding was begun; and slanting lines have everywhere been drawn across the bilirubin columns for the days upon which carbohydrate was given. In several instances in which 6 and 18 hour collections were made the total bilirubin yield for the combined periods has been indicated by means of a dotted horizontal line. In Text-figs. 4 to 6 the rate of bile flow is recorded. Notes on bilirubinuria are appended for every experiment in which a positive Gmelin reaction was obtained.

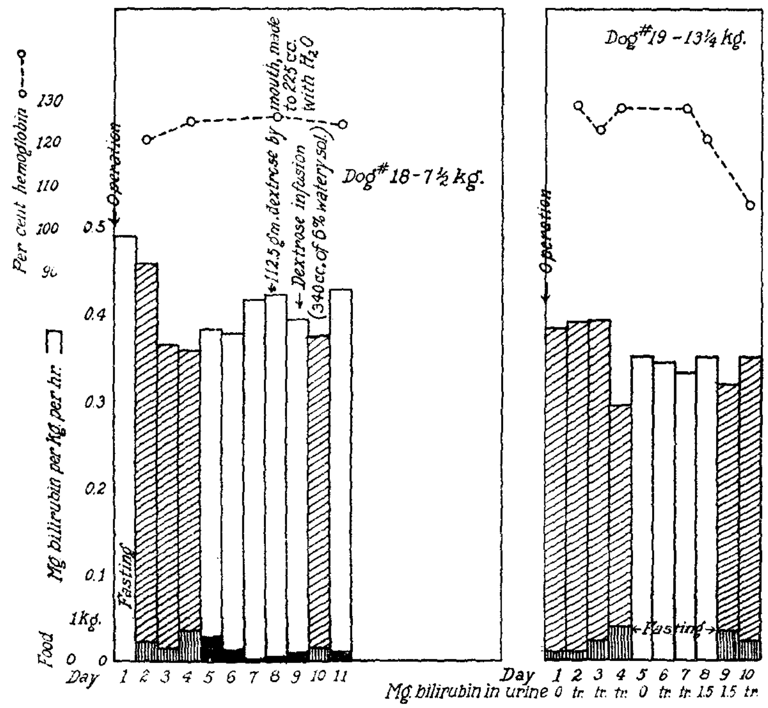
Legend for Text-Fig. 6.

This record should be considered apart from the others owing to the fact that the animal was in good condition only during the early days. It gradually lost weight and strength, because of a chronic obstruction of the small bowel by adhesions, as the autopsy showed. There were loss of appetite, anemia, large fluctuations in the hemoglobin percentage with corresponding ones in the bilirubin output, and, toward the close of the observations, diarrhea, a great drop in hemoglobin, and profound lethargy.

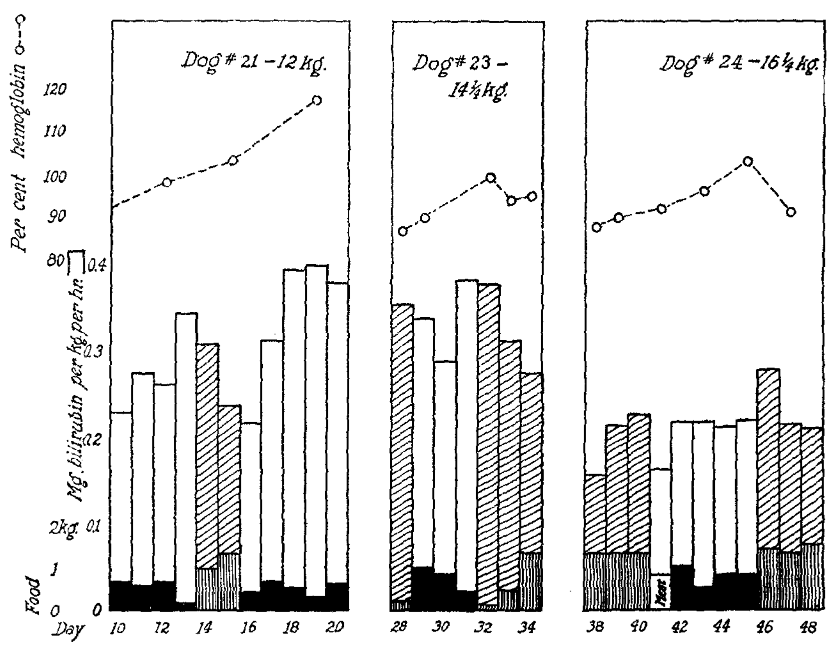
When the first dextrose injection and carbohydrate feeding were carried out the condition of the dog was good. It will be seen that they were without effect on the 24 hour output of bilirubin, though increasing the 6 hour output. The pronounced temporary rise in both outputs after the second dextrose injection occurred at a time when the signs of intestinal obstruction were marked and the condition of the dog was very abnormal. The record has been included for the sake of completeness.


Dog #23 - 14¼ kg.
Per cent hemoglobin
Mg. bilirubin per kg. per hr.
Food
Mixed
Fasting
Day
Dog #16 (a) - 6¾ kg.
Slight obstruction
Mg. bilirubin in urine 0 0 tr. 0 0 0 0 0
Dog #16 (b) 6¾ kg.
Dog #19 - 13 kg.
Treadmill exercise
Mg. bilirubin in urine 0 0 tr. tr. tr. 1.0 1.5 1.0 0

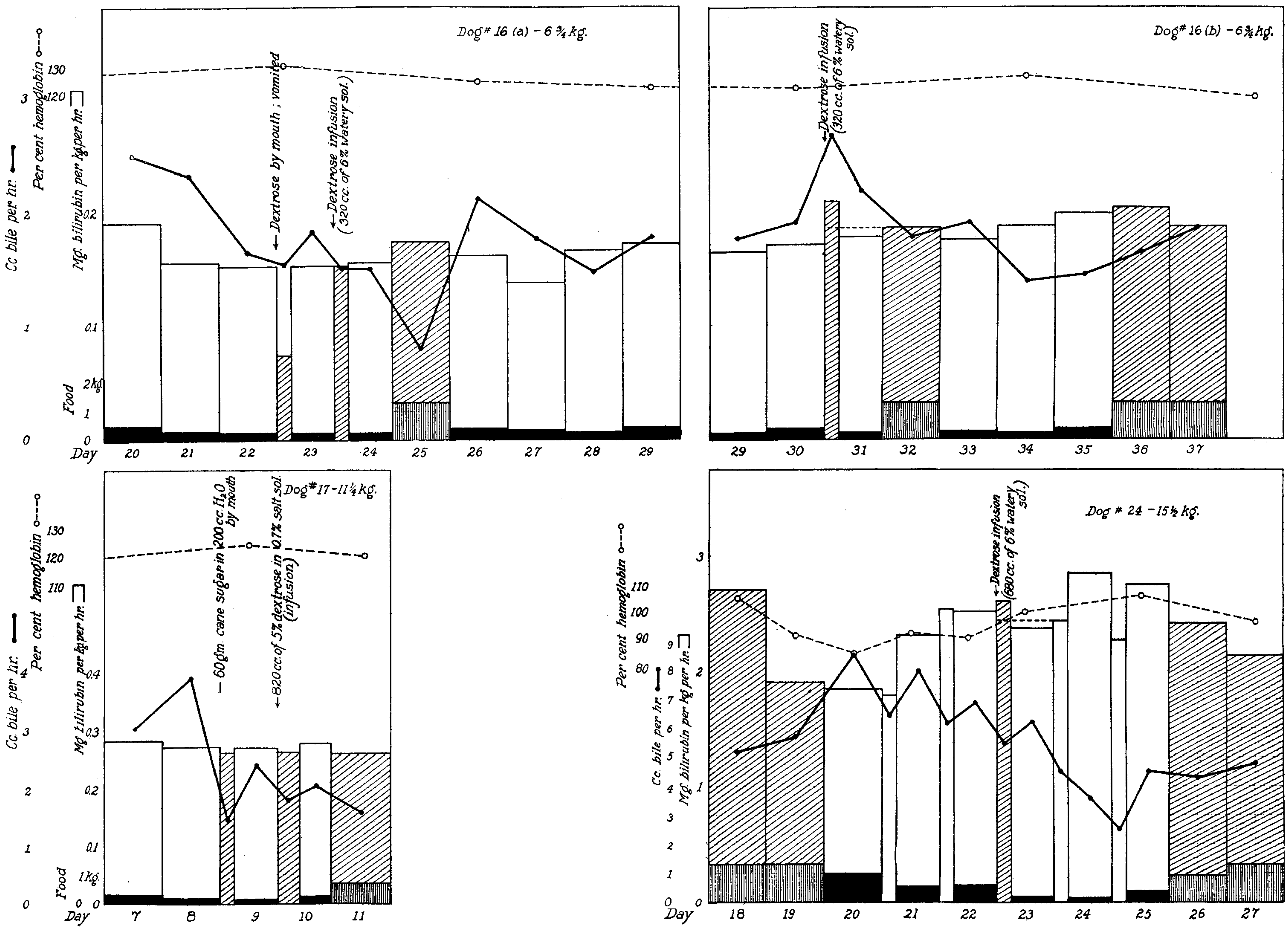
TEXT-FIG. 1.



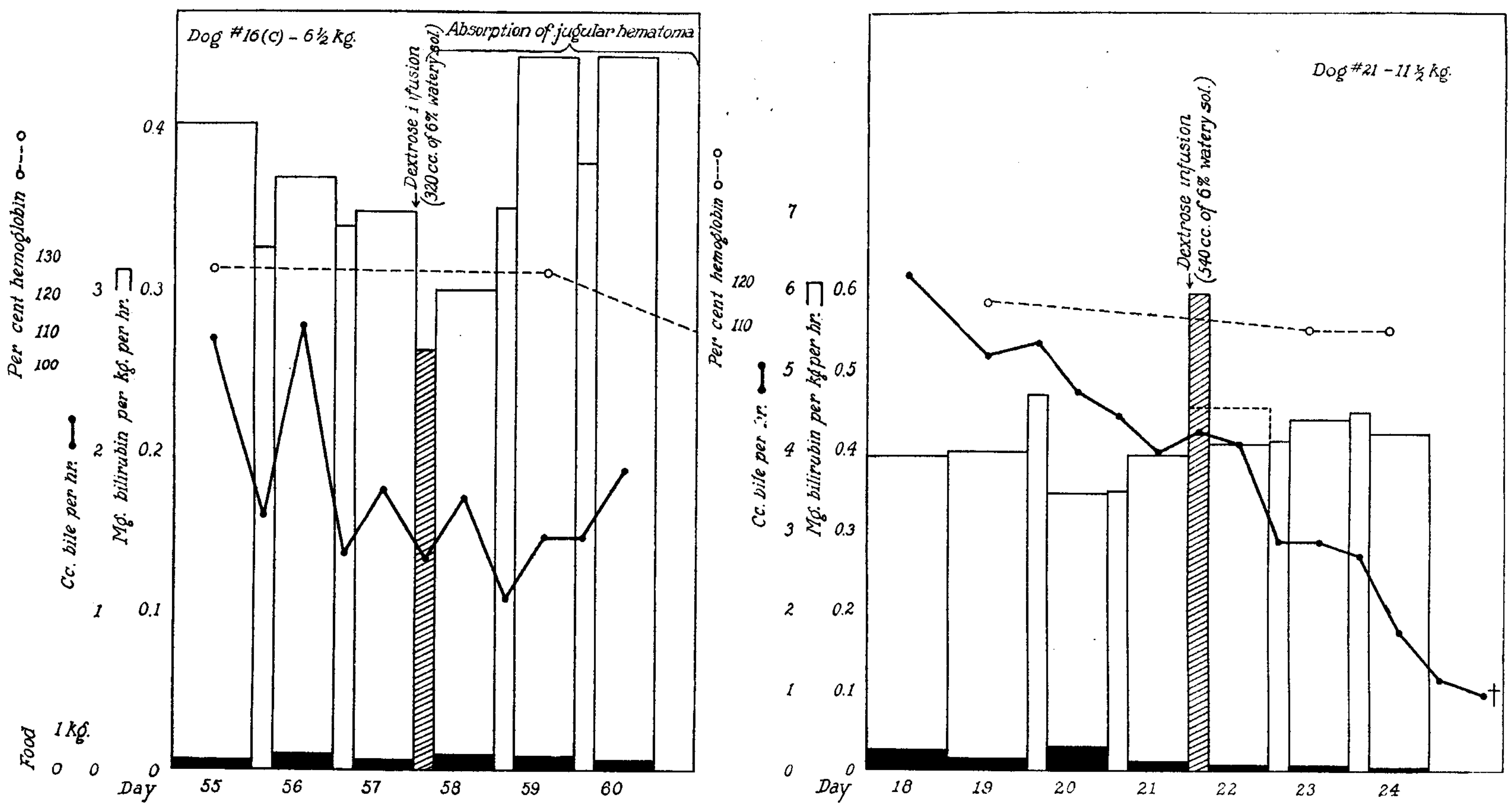
TEXT-FIG. 2.



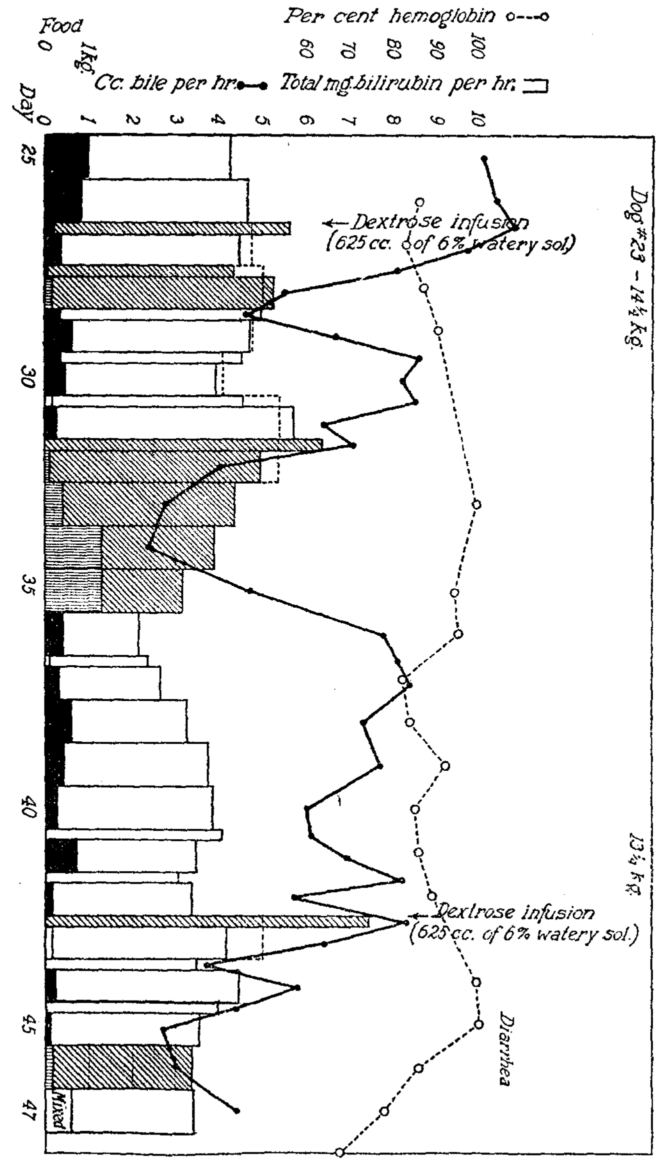
TEXT-FIG. 3.


Dog# 16 (a) - 6 ¾ kg.
Dextrose by mouth ; vomited
Dextrose infusion (320 cc. of 6% watery sol.)
Dog# 16 (b) - 6¾ kg.
Dextrose infusion (320 cc. of 6% watery sol.)
Dog# 17 - 11¼ kg.
60 gm. cane sugar in 200 cc. H2O by mouth
820 cc. of 5% dextrose in 0.7% salt sol. (infusion)
Dog # 24 - 15½ kg.
Dextrose infusion (680 cc. of 6% watery sol.)
Cc. bile per hr.
Per cent hemoglobin
Mg. bilirubin per kg per hr.
Food
Day

TEXT-FIG. 4.


Per cent hemoglobin
130
120
110
100
Cc. bile per hr.
Mg. bilirubin per kg. per hr.
Food
1 kg.
0
Day
55
56
57
58
59
60
Dog #16(c) - 6½ kg.
Dextrose infusion (320 cc. of 6% watery sol.)
Absorption of jugular hematoma
Per cent hemoglobin
120
110
Cc. bile per hr.
Mg. bilirubin per kg per hr.
Day
18
19
20
21
22
23
24
Dog #21 - 11½ kg.
Dextrose infusion (540 cc. of 6% watery sol.)

TEXT-FIG. 5.



Food
0
1 kg.
Per cent hemoglobin
60 70 80 90 100
Cc. bile per hr.
Total mg. bilirubin per hr.
Day
0 1 2 3 4 5 6 7 8 9 10
25
30
35
40
45
47
Mixed
Dog #23 - 14¼ kg.
←Dextrose infusion
(625 cc. of 6% watery sol.)
13¼ kg.
Dextrose infusion
(625 cc. of 6% watery sol.)
Diarrhea

TEXT-FIG. 6.