STUDIES ON HYPOCHROMIC ANEMIA IN DOGS. II. THE EVALUATION OF PREDIGESTED BEEF, IRON AND LIVER EXTRACT ON FORMATION OF HEMOGLOBIN AFTER GASTRECTOMY¹

By STACY R. METTIER, FREDERICK KELLOGG² AND KATHERINE PURVIANCE

(From the Division of Medicine, University of California Medical School, San Francisco)

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In the dog there seems to be a close relationship between gastric digestion and experimentally induced hypochromic anemia. The authors (1, 2) have reported that, following the depletion of hemoglobin-forming reserves by frequent bleedings, the hypochromia of red blood cells thus produced persists if the animals' stomachs are removed by surgical procedures. It was shown that, prior to the production of an artificial achylia gastrica, the average daily output of hemoglobin in the animals on a standard bread ration (Whipple) was 0.93 gram; and the output of hemoglobin when the diet was supplemented with beef was 1.95 grams. Following gastrectomy, the output of hemoglobin in the animals on these diets was reduced to 0.14 gram and 0.08 gram, respectively. It is apparent, therefore, that the gastrectomized dogs are unable to obtain from beef the substances essential to the production of a normal amount of circulating hemoglobin.

In a previous series of observations (3) on a group of female patients with achlorhydria and hypochromic anemia, it was shown that the daily administration of a diet rich in food-iron resulted in formation of sufficient hemoglobin only when the meal fed had been previously digested *in vitro* with hydrochloric acid and pepsin, or when large doses of iron were administered to the patients.

The present study was undertaken to ascertain the effects of feeding predigested beef, iron, and injections of liver extract on the production of hemoglobin in the same series of gastrectomized dogs.

METHODS

Three healthy, adult gastrectomized dogs were placed on the standard bread diet of Whipple and Robschey-Robbins (4). This ration is poor in

factors favorable to the production of hemoglobin, the average output in the normal dog being about 1 to 3 grams of hemoglobin a week over the maintenance factor (4).

Specimens of blood were removed from the jugular vein of each animal twice weekly for the various examinations listed below, the amount of blood withdrawn totaling approximately 40 cc. for each period of two weeks. A quantitative estimation of hemoglobin was accurately determined. Determinations of blood volume and hemoglobin concentration, and estimation of erythrocytes, reticulocytes and leukocytes were made as routine procedures. The technique used in performing these various examinations has been described in a previous paper (2).

RESULTS

1. The effect of a meal of predigested beef on hemoglobin production.

In two gastrectomized dogs, the hemoglobin level had been approximately 3 and 6 grams respectively per 100 cc. of blood for one month prior to the experimental periods. During this period there had been an average daily production of hemoglobin of 0.17 and 0.22 gram. These figures are comparable to those reported by Mullenix, Dragstedt and Bradley (5), who found a greatly reduced daily yield of hemoglobin in gastrectomized dogs while on a stock diet.

The meal used in this experiment consisted of lean beef scraped from its fibrous tissue, to which was added 4 grams of commercial pepsin and a sufficient amount of strong hydrochloric acid to adjust the hydrogen ion concentration to pH_3 . The mixture was then allowed to digest in the incubator at 37° C. for approximately 6 hours. When digestion was complete, the material was adjusted to pH_5 in order to reduce the irritant action of the acid on the intestinal mucosa.

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² Research Fellow of the American College of Physicians.

FIG. 1. THE EFFECT OF PREDIGESTED BEEF, OF IRON AND OF LIVER EXTRACT ON THE PRODUCTION OF HEMOGLOBIN AND RED BLOOD CELLS IN A GASTRECTOMIZED DOG.

It is to be noted in Figure 1 that one animal was given predigested beef during two separate periods of study; and, as is shown in Figure 2, the other dog received the meal during one period only. It soon became apparent that the meal was not a palatable one; even when mixed with the standard bread ration the animals refused to eat more than one-third of their allotted portions. Feeding of the meal in small divided portions through a rubber tube passed into the upper intestinal tract was then attempted, but this procedure was invariably followed by regurgitation of most of the mixture. For another period of feeding, the beef was digested with gastric juice removed from the stomach of normal dogs but this method met with no greater success. From the results reported in Figures 1 and 2 and Table I, it may be seen that hemoglobin production showed no increase, while the animals were

TABLE I
Effect of standard bread (S), of predigested beef, of iron and ammonium citrate and of liver extract on production of hemoglobin in gastrectomized dogs

Dog number	Number of days	Hemoglobin output	Diet and type of therapy
473	20	0.20	Bread (S)
	20	0.24	750 grams predigested beef
	20	1.73	Bread (S) + 1 gram of iron and ammonium citrate daily
	24	0.60	Liver extract, 5 cc. 3 times a week *
394	20	0.04	Bread (S)
	20	2.77	Bread (S) + 1 gram of iron and ammonium citrate daily
	20	0.04	Bread (S) + 5 cc. liver extract daily
393	20	0.10	Bread (S)
	20	0.11	250 grams of predigested beef
	20	1.85	Bread (S) + 1 gram of iron and ammonium citrate daily
	20	0.19	Bread (S) + 5 cc. liver extract daily

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FIG. 2. SHOWS A MARKEDLY INCREASED OUTPUT OF HEMOGLOBIN ON IRON MEDICATION BUT FAILURE TO STIMULATE FORMATION OF BLOOD ON A PREDIGESTED MEAL.

on this regime, over their output during the feeding of the standard bread ration alone. Presumably these results were due largely to the failure of the animals to retain the entire meal.

2. The effect of iron and ammonium citrate on the production of hemoglobin.

As a supplement to the standard bread ration, a capsule containing one gram of iron and ammonium citrate (U.S.P.) was given daily to the same three gastrectomized dogs. Prior to medication, the hemoglobin output of each animal had been less than 0.25 gram daily, whereas during the following 21-day period it was 1.85, 1.73 and 1.75 grams respectively. In the third dog (Figure 3), during a later period of iron administration, there was a greatly increased output of hemoglobin up to 2.77 grams daily. Accordingly, the average daily output for all the dogs was 2.03

grams of hemoglobin. These results are slightly in excess of those obtained by others (5) in gastrectomized dogs. On the other hand, they are only about two-thirds of the daily hemoglobin output found by Robschey-Robbins and Whipple (6) after the administration of iron to healthy dogs in which an hypochromic anemia had been induced.

Figures 2 and 3 show the hematopoietic reaction of the gastrectomized animals to iron as indicated by the reticulocyte response. In the figures it is to be noted that, after 4 days on iron therapy, the reticulocytes rose from a level of approximately 1 per cent to a peak of 3.5 per cent. This maximum, although small, must be considered to represent definite response, when contrasted with the nearly stationary level of 1 per cent which had been observed previously in these animals over a period of weeks. Furthermore, after this rise the reticulocytes dropped to their former level. In view of the microcytic nature of the anemia, with a relatively high red blood cell count, a minimal reticulocyte response is to be expected. Coincidentally with this response, there was an increase in the total number of circulating red blood cells.

3. The effect of liver extract (specific for Addisonian anemia) on the production of hemoglobin.

The same three gastrectomized dogs were maintained in an anemic state by bleeding while on the standard bread ration. Each animal was given 5 cc. of liver extract (Lilly's) parenterally three times a week. Figures 1, 2 and 3 show that the hemoglobin output was not increased. Furthermore, there was a lack of response of the reticulocytes. In each animal, however, there was noted a slight but gradual rise in the total number of erythrocytes similar to the results obtained by Ivy and Mullenix and their associates (7, 5).

DISCUSSION

In undertaking the first experiment recorded here, an attempt was made to obtain by experiments with laboratory animals further information based on previous observations concerning the relationship between gastric digestion and anemia. It was thought that, since an experimentally induced anemia persists in dogs with an artificially produced achylia gastrica, hemoglobin

FIG. 3. SHOWS A MARKEDLY INCREASED OUTPUT OF HEMOGLOBIN ON IRON MEDICATION BUT NEGATIVE RESULTS WITH PREDIGESTED BEEF.

Note the gradual rise of erythrocytes during the injections of liver extract.

output could be augmented by feeding a predigested meal. Accordingly, such an experiment was undertaken; but the dogs were reluctant to eat this mixture voluntarily and did not entirely retain it when it was administered through a tube passed into the upper intestines. Our observations were considerably limited by this handicap, and the experiment is therefore inconclusive in its results.

It is clear from the results recorded in the figures that the animals responded favorably to the large doses of iron. The metal used apparently met the demand for replacement of the iron shortage in the body induced by previous bleeding and the artificially produced achylia gastrica. This is in striking contrast to the failure of the gastrectomized dog to obtain from beef sufficient dietary iron to replace his hemoglobin stores as previously reported (1, 2). The experiment

lends further support to the hypothesis that under conditions of depletion of iron in the body, a state of anemia will persist in the presence of an achlorhydria, but may be favorably influenced by iron medication.

The data obtained from the hematocrit determinations indicate that the type of anemia encountered in gastrectomized dogs is decidedly microcytic and hypochromic. This substantiates the report of Mullenix and his associates. In addition, the failure of liver extract to induce a reticulocyte response or materially to increase the hemoglobin output speaks against the tendency of gastrectomized dogs to develop pernicious anemia.

SUMMARY AND CONCLUSIONS

1. The effect of feeding predigested beef, of inorganic iron and injections of liver extract on the production of hemoglobin and red blood cells was

observed in three dogs with an artificially produced achylia gastrica. Prior to this study the dogs had been rendered anemic by repeated bleedings, and the hemoglobin output maintained at a low level on a standard bread ration; subsequently, gastrectomy had been performed.

2. Hemoglobin output was not increased while the dogs were given predigested beef, presumably due to a failure on the part of the animals to retain the meal.

3. It was shown that the average daily output of hemoglobin was 2.03 grams, while the dogs were given iron and ammonium citrate; whereas before medication, the daily output had been less than 0.25 gram.

4. There was little or no effect on the hemoglobin or red blood cell formation following injections of liver extract.

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