

# STUDIES ON HYPOCHROMIC ANEMIA IN DOGS. I. THE EVALUATION OF A STANDARD BREAD DIET AND OF A MEAT DIET ON FORMATION OF HEMOGLOBIN BEFORE AND AFTER GASTRECTOMY<sup>1</sup>

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(Received for publication January 25, 1936)

The almost constant finding of achlorhydria or hypochlorhydria in patients with idiopathic hypochromic anemia is now well known. Recent studies (1) of patients with this disease indicate that this abnormality may bear a definite relationship to the genesis of the anemia. It was shown that, in the treatment of patients with achlorhydria and hypochromic anemia, by the daily administration of a diet rich in food-iron, satisfactory formation of hemoglobin occurred only when the meal fed had been previously digested *in vitro* with hydrochloric acid and pepsin. This experiment seems of greater significance when it is emphasized that, in some of the patients, the anemia recurred after the predigested meals were discontinued. From these studies it was concluded that in chronic idiopathic hypochromic anemia, gastric dysfunction leads to a failure in the utilization of organic (dietary) iron, and that the disease is due to the resulting deficiency of iron.

Patients with hypochromic anemia comprise a group composed almost exclusively of females many of whom have subsisted on diets deficient in iron-containing foods and whose bodily stores of iron may be influenced by prolonged profuse menses. It seemed apparent, therefore, that these factors might alter the concept of achlorhydria as the sole etiologic agent. Accordingly, it was thought desirable to undertake further studies (2) using a laboratory animal in which these various factors could be more suitably controlled.

Hypochromic anemia has been reported to occur in man following gastrectomy (3, 4, 5). Ivy, Morgan and Farrell (6) have reported the occasional occurrence of a spontaneous anemia in gastrectomized dogs, and have also noted an increased tendency toward anemia in gastrectomized dogs

during pregnancy. These authors concluded that the removal of the stomach reduced the factor of safety in the dog, and that the additional strain of pregnancy was sufficient to produce an anemia. Gutzeit (7), Aron and Bauer (8), and Maison and Ivy (9), in their studies on the rat and dog, found that a similar anemia occurred following gastrectomy. Mullenix, Dragstedt and Bradley (10) reported that their dogs after gastrectomy showed a reduced capacity to form hemoglobin. These authors, however, failed actually to compare production of hemoglobin before and after operation. Other investigators (6, 11) reported that no anemia occurred in their laboratory animals following gastrectomy alone. The discrepancy between these reports suggests that not all dogs under these experimental conditions tend to develop a hypochromic anemia. The iron reserve of the animal and the demands for increased production of hemoglobin are factors which might influence the development of such a state.

Hypochromic anemia has been induced in the dog by Whipple and Robscheit-Robbins (12, 13) following bleedings repeated at frequent intervals. This anemia is an iron-poor anemia, and resembles the anemia in man associated with achlorhydria, gastro-intestinal disturbances, inadequate diet and chronic blood-loss.

The production of an artificial achylia gastrica in the dog by gastrectomy and of an hypochromic anemia by repeated bleeding should, therefore, under controlled conditions, offer a satisfactory means of determining the relationship between diet, digestion and hemoglobin production. An investigation, accordingly, was undertaken to determine the output of hemoglobin in dogs before and after gastrectomy.

<sup>1</sup> Supported by a grant from the Christine Breon Fund for Medical Research.

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## METHODS

Four healthy, adult, mongrel dogs were placed on the standard bread diet of Whipple and Robscheit-Robbins

(12). This bread consists mainly of cereals, supplemented by salmon, tomatoes, yeast and cod-liver oil. Whipple states that this bread is a complete diet for an adult dog, and will maintain the animal in health for long periods of time. Furthermore, this ration is poor in factors favorable for the production of hemoglobin, the average output being close to 1 to 3 grams of hemoglobin per week, over and above the maintenance factor. An analysis of our bread showed an average of 2.6 mgm. of iron per 100 grams of dried bread.

The dogs were bled from the jugular vein sufficiently and at such intervals as to maintain the hemoglobin at a level from between 6 and 7 grams per 100 cc. of blood. A quantitative estimation of the hemoglobin removed was accurately made. The amounts removed represented the maximal production capacity of the bone marrow during the various experimental periods. By bleeding, an anemia was maintained until the production of hemoglobin became stabilized, for a period of from 3 to 8 months, depending on the individual animal. Presumably, at the end of this time the reserve stores of hemoglobin were exhausted.

Examinations of the blood were made by one person, using unvarying technique, throughout the experiments. Specimens of blood were removed at approximately the same hour twice weekly. Blood volume and determinations of hemoglobin, erythrocyte, reticulocyte and leukocyte counts were made as a routine procedure. Blood

volume was estimated using the dye, brilliant vital red, according to the method of Keith, Rowntree and Geraghty (14), as modified by Hooper, Smith, Belt and Whipple (15). The volume of packed cells was determined by the hematocrit method of Wintrobe (16). The erythrocytes were counted in the usual manner, using diluting pipettes and counting chambers certified for accuracy by the U. S. Bureau of Standards. The hemoglobin was determined by the Sahli method, with tubes calibrated so that 100 per cent equaled 14 grams of hemoglobin per 100 cc. of blood.

Studies of the gastric secretion for the purpose of ascertaining the presence of hydrochloric acid were made before gastrectomy. Also, specimens of upper intestinal content were removed after the dogs had completely recovered from the surgical procedure. In addition to the oral administration of 20 cc. of 7 per cent alcohol given to stimulate gastric secretion, each animal received an intramuscular injection of 1 cc. of 1:1,000 dilution of histamine.

All dogs were given a vermifuge prior to the period of investigation, and stools were examined at monthly intervals to determine the presence of parasites and ova.

The technique of gastrectomy<sup>3</sup> performed upon these

<sup>3</sup> The authors wish to thank Drs. F. S. Foote and M. L. Montgomery, of the Division of Surgery, for performing these operations.

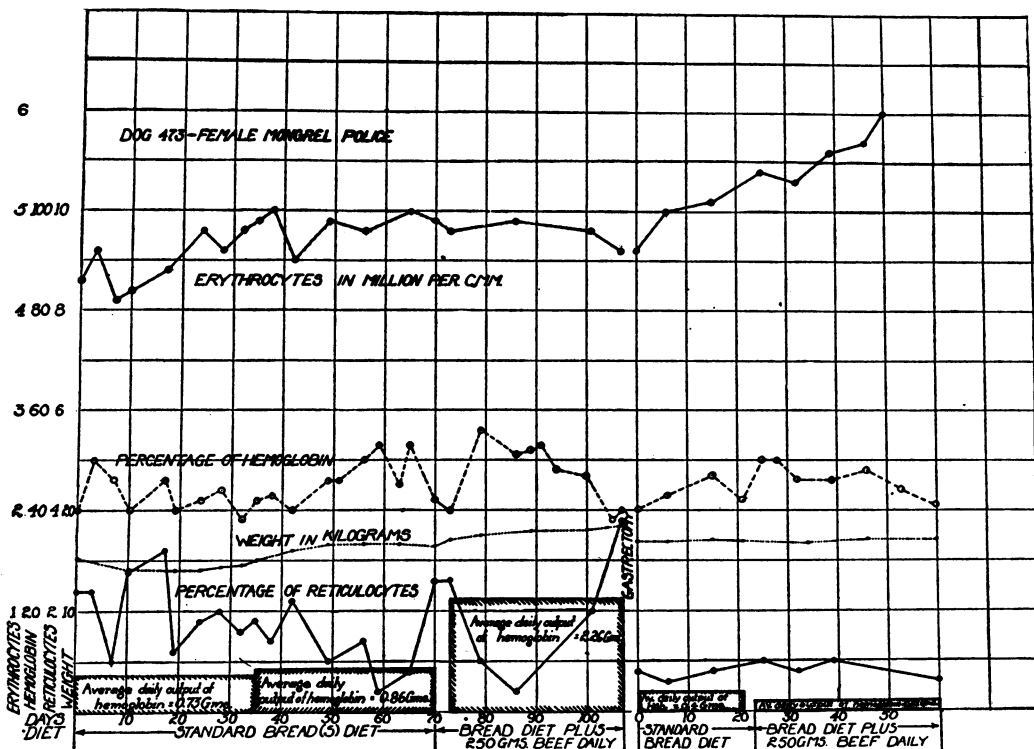


FIG. 1. EFFECT OF STANDARD BREAD "S" RATION AND OF BEEF ON PRODUCTION OF HEMOGLOBIN BEFORE AND AFTER GASTRECTOMY IN THE DOG.

Note the increased number of erythrocytes after operation.

animals was similar to that of Ivy et al. (6) and of Mann and Graham (11) with minor modification. A one-stage operation was performed. The duodenum was severed from the pylorus, and the duodenal stump was closed. The esophagus was freed from surrounding structures, and pulled down into the peritoneal cavity by traction on the stomach. The side of the duodenum just opposite and below the entrance of the common bile duct was sewed transversely to the esophagus with a running suture of silk. Incisions were made on either side of the suture line, and the cut edges of the viscera were sewed with a second inside line of silk. This was continued as a Connell suture about the outside of the stoma. The stomach was transected a little at a time, keeping just ahead of the suture. This allowed the outside cut edge of the esophagus to be held down in the peritoneal cavity by the traction on the stomach.

OBSERVATIONS

Analysis of the gastric secretion following the oral administration of 20 cc. of 7 per cent alcohol showed the presence of free hydrochloric acid in each of the dogs prior to gastrectomy. After

recovery from the operative procedure, the upper intestinal content showed an absence of free hydrochloric acid even after the subcutaneous administration of histamine.

For the purpose of comparison, we include here the hematocrit determinations of Mayerson (17) for the normal values in dogs; volume of packed red blood cells, 38.6; mean corpuscular volume, 59.3 cubic microns; mean corpuscular hemoglobin, 20.0 micromicrograms; and mean corpuscular concentration of hemoglobin, 34.3 per cent. It is to be noted in Table I that at the outset of these studies, after the preliminary period of bleeding, the volume of packed red cells, the mean corpuscular hemoglobin and the mean corpuscular concentration of hemoglobin were considerably reduced below normal. Furthermore, Figures 1, 2, 3 and 4 show at the beginning of the experiments an average concentration of hemoglobin of 6 to 7 grams per 100 cc. of blood.

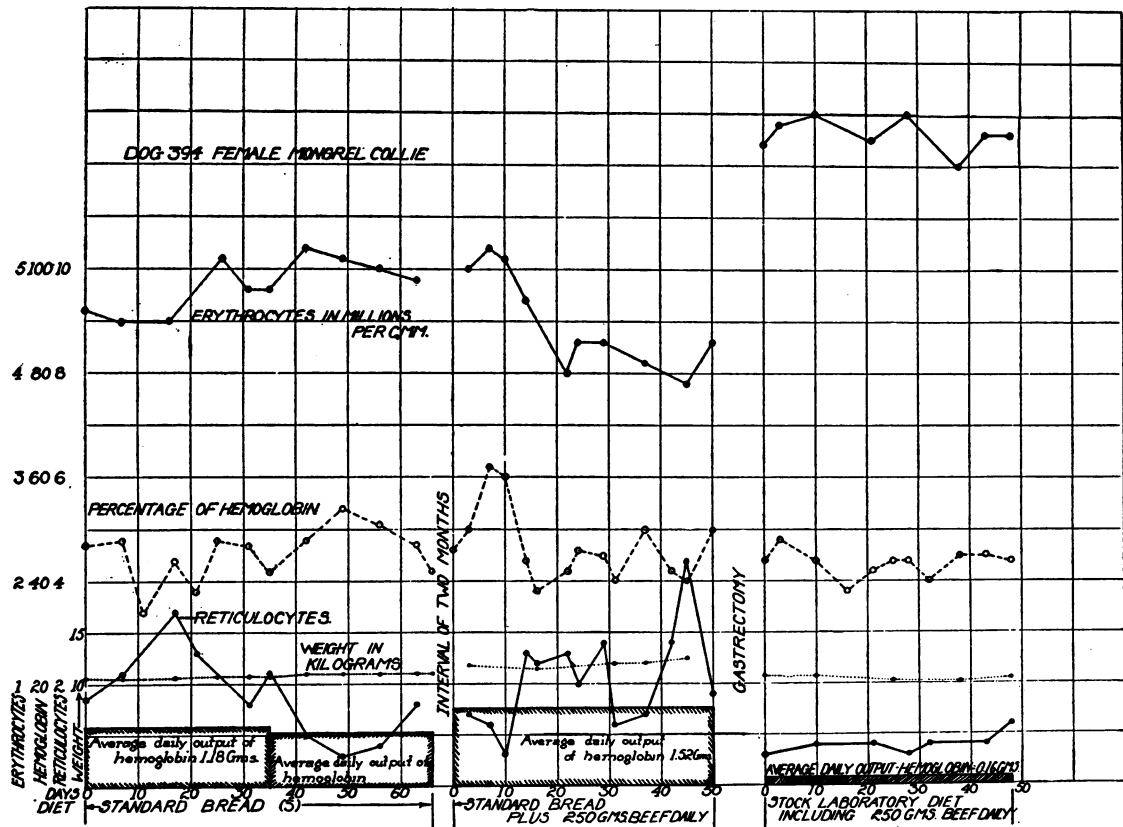


FIG. 2. COMPARISON OF HEMOGLOBIN OUTPUT BEFORE AND AFTER GASTRECTOMY.

Note the rise in erythrocytes following operation.

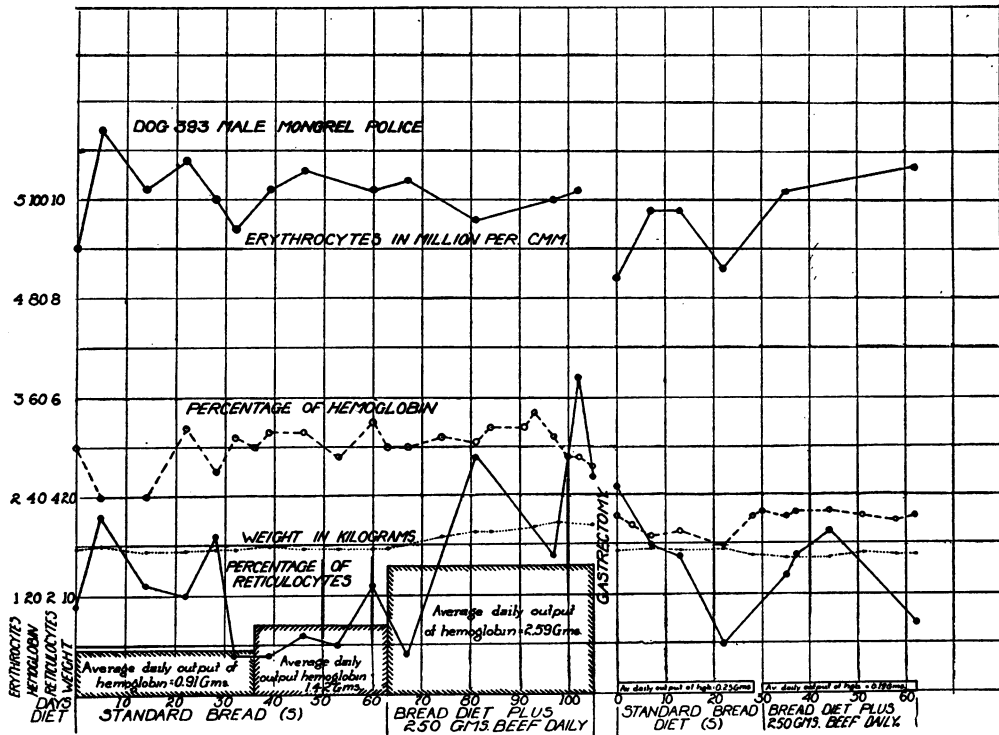


FIG. 3. SHOWS A MARKEDLY INCREASED OUTPUT OF HEMOGLOBIN ON A BEEF RATION BEFORE GASTRECTOMY, BUT A LESSENERED ABILITY TO SYNTHESIZE HEMOGLOBIN FOLLOWING OPERATION.

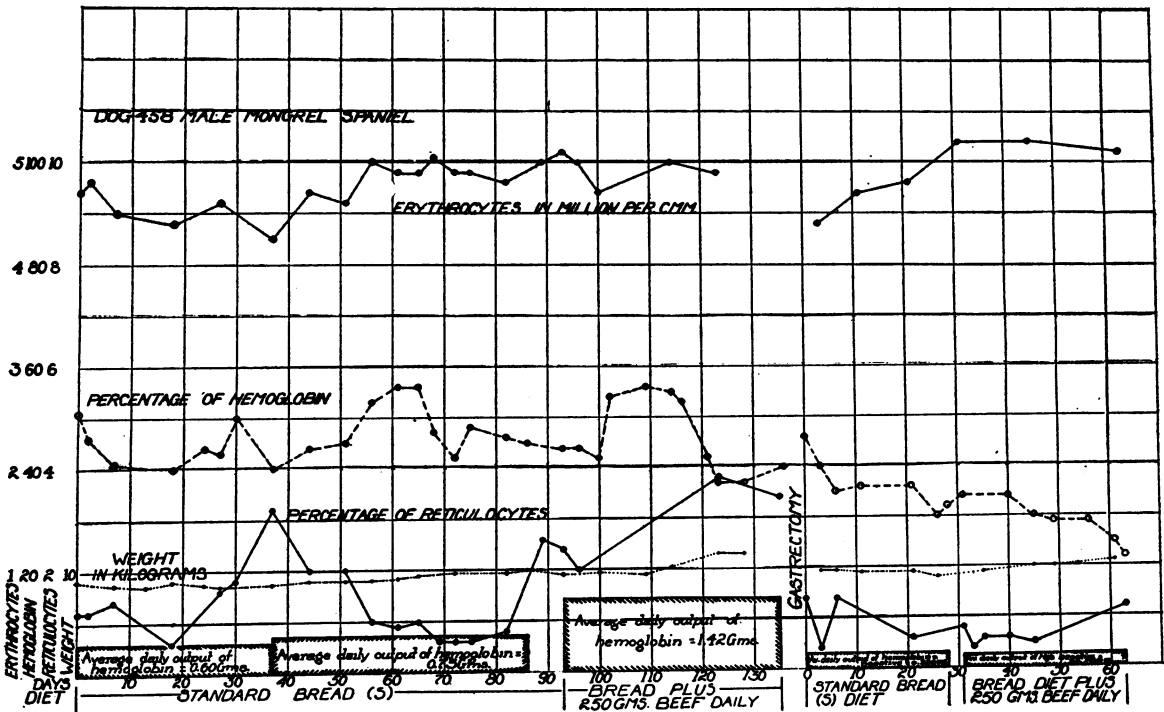


FIG. 4. SHOWS THE LESSENERED ABILITY OF THE DOG TO FORM HEMOGLOBIN FOLLOWING THE SURGICAL REMOVAL OF HIS STOMACH.

It is to be emphasized that the erythrocytes remained at a relatively high level. Accordingly, the average color index was low, and the blood of the dogs was thus in a state of hypochromic anemia induced by the frequent bleedings.

TABLE I

The figures represent the quantities and volumetric aspect of the red blood cell under basal conditions of production of hemoglobin in the dog after bleeding. Each figure represents an average of several determinations on each of the four animals studied. It is to be noted that the hypochromic anemia of blood-loss became additionally microcytic after gastrectomy.

Diet	Dog number	Volume of packed red blood cells	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular concentration of hemoglobin	
			cu. $\mu$	micrograms	per cent	
Before gastrectomy						
Bread "S": 500 grams	393	29.1	58	14.5	25.0	
	458	27.6	56	12.4	22.5	
	473	29.5	58	12.0	20.0	
	394	27.1	59	13.0	22.5	
	Average	28.3	58	13.0	22.8	
Bread "S": 500 grams	393	31.9	63	15.8	25.0	
	458	29.7	57	14.0	25.0	
	473	26.2	51	14.9	29.0	
	394	29.9	59	13.0	22.5	
	Average	29.4	58	14.4	25.5	
Beef: 250 grams	473	26.2	51	14.9	29.0	
	394	29.9	59	13.0	22.5	
	Average	29.4	58	14.4	25.5	
	After gastrectomy					
	Bread "S": 500 grams	393	27.1	57	11.3	19.0
458		26.5	52	12.1	23.0	
473		27.6	53	12.1	23.0	
Average		27.1	54	11.8	22.0	
Bread "S": 500 grams		393	24.9	44	9.2	21.5
	458	21.9	40	7.7	19.2	
	473	26.7	47	11.9	25.6	
	394*	20.3	41	7.9	19.5	
	Average	23.5	43	9.2	21.5	

\* Stock laboratory diet including 250 grams of beef daily.

Following gastrectomy, there was little change in the mean corpuscular concentration of hemoglobin, but progressive decreases in the mean corpuscular hemoglobin occurred, as shown in Table I. Thus the anemia, which before gastrectomy was hypochromic in type, following removal of the stomach became microcytic as well.

### The effect of the standard diet and of beef on the regeneration of hemoglobin before gastrectomy

Since the production of hemoglobin was in a state of minimal output after bleeding the dogs over a long period of time, it was thought that a more accurate comparison of the influence of dietary factors could be made if each phase of the experiments recorded below were conducted over periods of from one to two months or longer. Accordingly, it is to be noted in Table II that the individual animals over a period of at least two months produced a daily average of 1.12, 0.68, 0.80 and 1.11 grams of hemoglobin respectively on the standard bread diet. The average daily output for the four dogs was 0.93 gram.

TABLE II

The effect of standard bread "S" (Whipple) and of beef on the daily production of hemoglobin in dogs before and after gastrectomy. These dogs were in a state of hypochromic anemia of blood-loss, and in a condition of basal hemoglobin production.

Diet	Dog 393		Dog 458		Dog 473		Dog 394		Average daily output of hemoglobin
	Days of observation	Daily output of hemoglobin	Days of observation	Daily output of hemoglobin	Days of observation	Daily output of hemoglobin	Days of observation	Daily output of hemoglobin	
		grams		grams		grams		grams	grams
Before gastrectomy									
Bread "S": 500 grams	63	1.12	86	0.68	70	0.80	66	1.11	0.93
Bread "S": 250 grams	42	2.59	42	1.42	34	2.26	50	1.52	1.95
Beef: 250 grams									
After gastrectomy									
Bread "S": 500 grams	28	0.23	28	-0.32	21	0.40			0.14
Bread "S": 250 grams	30	0.19	32	-0.26	37	0.21	48*	0.16	0.08
Beef: 250 grams									

\* Stock laboratory diet including 250 grams of beef daily.

Table II shows the hematopoietic reaction of the anemic dogs to a diet consisting of 250 grams of the standard bread diet, and 250 grams of lean beef. The daily output of hemoglobin on this meat diet was 2.59 grams, 1.42 grams, 2.26 grams, and 1.52 grams respectively, the average for all dogs being 1.95 grams. Thus the addition of

beef to the diet was followed by increases in production of hemoglobin of 130, 110, 180 and 40 per cent respectively. There was considerable individual variation both in the amount of hemoglobin produced on the basal diet and in the increases in production of hemoglobin following the addition of beef. This individual variation showed the importance of controlled preoperative observations in order to interpret accurately similar studies after gastrectomy. This reaction is comparable to that observed following the use of one of the less potent iron preparations (18), namely, ferric chloride (25 mgm. iron as metal), with a maximal weekly output of 14 grams of hemoglobin.

Our study indicates that the daily output of hemoglobin in dogs with an hypochromic anemia induced by bleeding is approximately doubled when the animal is on a meat diet, as contrasted with the standard bread ration. This is graphically illustrated in Figures 1, 2, 3 and 4. In Table I very little change may be noted in the volume of packed cells or mean corpuscular volume. However, as might be expected, slight rise of the mean corpuscular concentration of hemoglobin occurred.

*The effect of the standard diet and of beef on production of hemoglobin after gastrectomy*

The dogs, following the surgical removal of their stomachs, were in excellent health, and their weights tended to remain constant although at somewhat lower levels than prior to operation. Only one dog (394) refused to eat the standard bread diet, and it was found necessary to place the animal on the stock laboratory food which included 250 grams of beef fed daily.

No actual diarrhea occurred, but the stools of all animals tended to be loose or semi-solid. This is in accord with similar observations made by Ivy, Morgan and Farrell (6). The output of hemoglobin when the animals were on the bread diet fell to a low level during this phase of the experiment, as shown in Table II and Figures 1, 2, 3 and 4. The daily average was 0.14 gram, as compared to 0.93 gram before operation. The addition of meat to the diet did not bring about an increase in the production of hemoglobin. There was to be noted, on the contrary, a slight

decrease to a daily average of 0.08 gram. This decrease was slight enough, perhaps, to be within the limits of experimental error, but it indicates that dietary factors in beef favorable for formation of hemoglobin could not be utilized following gastrectomy as they were prior to operation.

*Spontaneous anemia*

One animal (458) developed a spontaneous anemia after gastrectomy, and showed a negative output of hemoglobin on both the bread and meat diets. In contrast to his kennel-mates, he lost weight from 10.6 to 9.0 kgm., and the level of hemoglobin fell to 3.5 grams per 100 cc. of blood. This dog died three months after operation. At autopsy no cause for death could be ascertained other than anemia. No evidence of infection or neoplasm was to be found. The duodeno-esophageal anastomosis was in good condition. The stomach tissue had been completely removed, and there was no dilatation of the duodenum. By microscopic examination, the various tissues were found to be normal, except the bone marrow. A specimen of marrow from the femur showed a marked diminution of erythropoietic elements. Much of the marrow-space was occupied by fat cells. In some of the intercellular spaces a few clumps of normoblasts and leukopoietic cells were present. Usually a marked hyperplasia of erythropoietic tissue is to be found in the marrow of dogs with anemia after bleeding (18).

DISCUSSION

By the experimental evidence presented, it was shown that the feeding of beef to dogs having an hypochromic anemia induced by bleeding, resulted in a marked rise in hemoglobin output. This is in agreement with the results reported by Whipple and Robscheit-Robbins (13). When, however, the stomachs of these dogs were removed and anastomoses made between the esophagus and duodenum, the regenerative power of hemoglobin was apparently greatly reduced. Before gastrectomy, the output of hemoglobin averaged 1.95 grams daily, while the dogs were on the beef diet; whereas after the operation there was an average output of only 0.08 gram. It seems probable, therefore, that the gastrectomized dogs are unable

to obtain from beef substances essential to the synthesis of hemoglobin.

Some investigators (6, 7) maintain that gastrectomy performed on the dog is not alone sufficient to induce anemia. They state that following the surgical removal of the stomach, only an occasional animal will develop a spontaneous anemia. An explanation for this may lie in the fact that dogs possess an extraordinarily large store of hematopoietic substances. It was our experience that the weekly withdrawal of from 100 to 200 cc. of blood containing from 10 to 20 grams of hemoglobin over a period of from three to eight months, was necessary before the reserves were depleted—a total of approximately 400 grams of hemoglobin. These figures varied somewhat with the individual animal. The amount of hemoglobin appears all the more significant when compared to the total hemoglobin in circulation. Such a dog as treated above, at a weight of 16 kgm., may have a circulatory blood volume of 1200 cc. If his hemoglobin level is 50 per cent, then  $12.00 \times 0.42$  equals 70.8 grams of hemoglobin in circulation. Our normal dogs on a beef diet may be expected to produce this amount of hemoglobin in five weeks.

It is difficult to understand all the chemical and mechanical factors that lead to the development of an anemia due to a deficiency of iron. However, from these experiments it would appear that, once an anemia is induced and the iron reserves depleted, this state will persist when normal gastric secretion is lacking. Clinically, this suggests that any process depleting iron stores, such as deficient diet, prolonged and profuse menses, and frequent pregnancy, in a patient with achlorhydria may lead to an hypochromic anemia, owing to the failure to get from food and to utilize properly hemoglobin-building materials.

#### CONCLUSIONS

1. The effect of feeding a standard bread ration with salmon (Whipple) on the production of hemoglobin, as compared to a diet of beef, was observed in four dogs with an hypochromic anemia induced by repeated bleeding. Subsequently gastrectomy was performed on all four animals, and again studies in the production of hemoglobin were conducted under similar dietary régimes.

2. It is shown that, prior to operation, there was an average daily output of 0.93 gram of hemoglobin on the standard bread ration, and 1.95 grams of hemoglobin after the diet was supplemented with beef.

3. Following gastrectomy, the output of hemoglobin was reduced to 0.14 gram and 0.08 gram respectively.

4. Prior to operation an hypochromic anemia was established by frequent bleeding. This became additionally microcytic following gastrectomy.

5. It is suggested that when the bodily iron reserves are depleted, a state of hypochromic anemia will persist in the absence of a normal gastric secretion, even though an iron-rich diet is ingested.

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