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DIET AND ITS EFFECT UPON BLOOD FORMATION[1]

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THE red corpuscles of the blood and the coloring matter or hemoglobin which they contain are extremely labile substances subject to sudden fluctuations and capable of rapid repair or renewal.

Investigations carried on for many years in our laboratory have definitely demonstrated that the amount of blood regeneration in the animal body can be modified at will by diet factors. This control can be readily demonstrated week by week.

A severe secondary anemia in a dog, produced by withdrawal of blood, offers an excellent means of testing the efficacy of stimuli for blood production. It is absolutely essential that a uniform anemia level be maintained as a constant from week to week. Such a long sustained severe anemia insures a maximal stimulus to red cell and hemoglobin formation. The dog is an experimental animal par excellence for this type of investigation. It is an omnivorous animal and on suitable diets may be maintained in health for many years in spite of an extreme anemia. Our experimental colony contains dogs which have been severely anemic for a period of from six to eight years. All experimental procedures may be executed without the need of resorting to anesthetics—a loss of much time in the handling of animals. Blood can be easily withdrawn from the veins, and the circulating volume of blood is large enough so that it is not disturbed by the necessary frequent sampling.

METHODS

These studies concern an experimental anemia produced in dogs by the frequent withdrawal of blood. The animals employed are born and raised in our own kennels and are of well-known stock whose various reactions are familiar to us. The breeds used for this investigation are represented by a white bull terrier mongrel and a Dalmatian coach hound. They are vigorous and hardy animals of gentle disposition and, in addition, have excellent appetites. This is an important qualification for the continuance of these experiments with a variety of diets of different palatability to the dog, for a period of many years. Being

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cage-raised they are thoroughly accustomed to this place of residence, and to the simple technical procedures of withdrawing samples of blood. Our animals are kept under optimum environmental conditions; the cages are sufficiently large to permit exercise; the dog rooms are well-ventilated and are kept at uniform temperatures by thermostatic control; not more than 12 dogs are kept in any one room and every animal is kept in its own cage. Enclosed court yards to permit out door exercise adjoin each dog room. Extreme care is taken to keep these animals healthy; they are carefully isolated so as to prevent intercurrent infections. As soon as puppies have attained sufficient growth for effective immunization they are vaccinated against distemper, a disease very prevalent in kennels where large numbers of dogs are housed. This disease has an unusually high mortality, particularly among growing animals.

When the dogs have reached adult age they are included in the anemia colony. Their blood picture is then studied, this investigation including determination of the circulating volume of blood, counts of the blood corpuscles and careful estimation of the amount of hemoglobin or coloring matter contained within the red blood cells. Normal red cell counts of our kennel-raised animals range within 6 to 8 million corpuscles per cubic millimeter of blood, with hemoglobin values of 120 to 150 per cent or the equivalent of from 17 to 21 grams per 100 cc. of blood. The dogs are then rendered anemic by frequent bleedings spaced at one or two day intervals. Care must be used to prevent shock caused by too rapid blood removal. Once the desired anemia level of approximately one-third normal or a hemoglobin of 40 to 50 per cent has been attained, further bleedings are graded as to frequency and amounts in order to maintain this level as near a constant as possible. Blood studies are made weekly in addition to the necessary sampling following each hemorrhage. Fluctuations in the plasma or liquid portion of the blood must be taken into consideration in estimating the amount of bleeding necessary.

The hemoglobin contained in the red blood corpuscles serves as an excellent medium of measurement for blood production and its estimation is a simple and very satisfactory procedure. This blood pigment is determined quantitatively in grams in all blood withdrawn. The figure obtained indicates the capacity of that animal to build hemoglobin on a given diet intake, since the anemia level is maintained as a constant as far as possible. Dogs normally have unusually large reserves of potential blood-building stones which enable them to regenerate large amounts of hemoglobin and red cells during the early anemic state. We also know that these dogs, on any favorable diet, will store this reserve to be drawn upon later when the subsequent diet intake is unfavorable for blood regeneration. It is therefore absolutely essential that these large reserves be exhausted before attempting to evaluate the effect of any stimulus to red cell and hemoglobin production. This procedure may take several weeks and even months before one may feel assured of a uniform base level from which to start feeding experiments. Dogs are fed once each day. Actual food consumption is determined daily and any portion of the diet left in the food pans is carefully reweighed. The animals

TABLE 1

Bread (S) = Salmon bread

INGREDIENTS	QUANTITY OF INGREDIENTS	PROTEIN	FAT	CARBOHYDRATE
	grams	*grams*	*grams*	*grams*
Wheat flour	12,000	1,240	125	8,480
Potato starch	6,000			5,400
Bran	2,000	300	86	1,080
Sugar	3,000			3,000
Cod liver oil	1,000		1,000	
Canned tomatoes	2,000	24	4	80
Canned salmon	2,500	545	302	
Yeast, compressed	455	55	2	96
*Salt mixture	150			
Water	7,500			
Total		2,164	1,419	18,136

Protein, 10.0 per cent
Fat, 6.5 per cent
Carbohydrate, 83.4 per cent
Caloric value, 4.8 per gram as fed

* McCollum and Simmonds' salt mixture with ferric citrate omitted.

also are weighed daily so as to insure ample caloric requirements for proper weight maintenance.

The food ration consists of a basal bread mixture plus the unknown diet factor which is to be tested. Sufficient water is added to give a hash of suitable texture for thorough mixing. The various food ingredients are therefore so thoroughly mixed that the dogs cannot pick out any particular morsel which may be more to their taste. A test period for the unknown diet factor consists of a two-week feeding period. This is preceded and followed by a control period of two weeks' feeding of the bread ration alone. In cases where diets favorable for blood formation have been fed, the after bread period may have to be prolonged since reserves may have been stored and will have to be depleted before the desired uniform anemia level is again maintained.

The basic food ration is of the greatest importance for long continued anemia experiments. This food must be palatable and yet permit of minimal hemoglobin and red cell production, since it must serve as a control for the unknown or test-diet factor. The basal ration meeting necessary requirements consists of a bread mixture, the formula of which is given in table 1. This bread represents a complete diet upon which dogs may be maintained in an excellent nutritional state for months or even years. It permits, however, only a slight hemoglobin production approximately 1 to 3 grams per week above that unknown amount utilized for general wear and tear.

DIET FACTORS

A maximum response of blood formation follows liver feeding and a minimum after cereals and bread stuffs. Between these two extremes we observe all types of reactions. To facilitate a review of the results of many experiments, tables 2 and 3 have been prepared. They demonstrate the effect of a variety of diet factors on the production of red cells and hemoglobin in the anemic dog under our experimental conditions.

Dairy products. The addition of large quantities of milk to the bread ration has no significant influence upon blood formation. Whole milk, even when fortified with 100 cc. of cream, will cause only a slight increase in hemoglobin production above the control levels. Supplementing the basal ration with butter results in a hemoglobin production of 10 to 20 grams following a two-week testing period. Adding "American cheese" to the bread diet results in figures for hemoglobin output similar to those obtained by butter feeding. One might suggest that the favorable reaction to cheese may be largely due to the butter fat content.

Whole milk is decidedly inadequate as a diet factor for the production of red cells and hemoglobin. Dairy products are known to contain many invaluable food factors which furnish general nutritional requirements, but their content of substances which aid the body to build red blood cells and hemoglobin are conspicuously meagre. This is an important fact to remember when advocating diets rich in dairy products, particularly in the case of young children where milk is likely to be the main constituent of the diet.

Vegetables. These foodstuffs are credited with much greater potency than they really deserve. Green vegetables are constantly lauded as stimulating blood formation. Their content of iron is particularly emphasized. Our experiments with a liberal intake of various greens furnish no confirmatory evidence of unusual potency. The leafy vegetables such as spinach, chard, beet greens and lettuce may be considered only as moderately favorable foods for blood-building. The response to vegetable material varies considerably in different dogs at different times. One animal, for example, may react more favorably to lettuce at a given time, while on another occasion he may form slightly more hemoglobin on asparagus. Spinach and beet greens seem somewhat more potent than lettuce, although the differences are not striking. Asparagus,

TABLE 2

Hemoglobin production influenced by diet

DOG	DIET IN GRAMS PER DAY	HEMOGLOBIN PRODUCTION PER 2-WEEK PERIOD
		grams
24-25	Bread 450	3
19-104	Bread 350	3
24-42	Milk 450, bread 450	3
19-104	Milk 450, bread 350	3
20-104	Cream 100, bread 350	12
24-45	Cream 100, bread 500	10
24-26	Butter 100, bread 350	10
24-46	Butter 75, bread 450	11
24-49	Cheese 100, bread 450	20
20-1	Beef muscle 200, bread 400	25
24-25	Pig muscle 300, bread 300	20
21-67	Heart muscle 200, bread 300	28
21-67	Codfish 100, bread 300	9
24-45	Fresh fish 250, bread 300	10
20-1	Spinach 250, bread 400	14
20-104	Beet greens 200, bread 300	20

carrots, beets, celery and Brussel sprouts are relatively inert. Minerals, particularly iron, may be more concerned with hemoglobin production than the chlorophyll or plant pigment present in the leafy vegetables. One may state that, on the whole, the presence of large amounts of these various vegetables in the diet modifies but slightly hemoglobin and red cell construction.

Fruits. Experiments with fruits have furnished us with data of extraordinary interest. Some fruits demonstrate considerable potency, while others are quite inert. For example, a considerable quantity of raspberries added to the bread mixture gives completely negative results, whereas the feeding of apricots or peaches stimulates hemoglobin production to the extent of 40 to 45 grams following a daily intake of 200 grams of these fruits for a two-week period. These figures compare favorably with the results obtained with some meat products. Peaches and apricots have been tested in the form of fresh, canned or dried fruit and all have been found to be of equal potency.

Prunes are only slightly less favorable for hemoglobin production, giving values of 35 grams following a two-week feeding period.

Apples in the fresh or dried state show considerable variation in their effect, but rank about equal with raisins and grapes. These fruits give

TABLE 3

Hemoglobin production influenced by diet

DOG	DIET IN GRAMS PER DAY	HEMOGLOBIN PRODUCTION PER 2-WEEK PERIOD
		grams
19-104	Beef liver 400	100
20-104	Beef liver 300, bread 300	50
24-49	Chicken liver 200, bread 250	75
24-25	Fish liver 150, bread 350	0
21-23	Beef kidney 250, bread 250	90
24-45	Pig kidney 200, bread 350	70
24-49	Chicken gizzard 300, bread 200	85
24-46	Bone marrow powder 30, bread 600	25
19-104	Beef spleen 200, bread 250	25
24-49	Brains 300, bread 300	25
24-25	Pig pancreas 250, bread 350	30
24-22	Apricots 200, bread 300	40
21-67	Peaches 200, bread 300	40
24-49	Raisins 300, bread 350	25
24-46	Apples 250, bread 350	35
24-49	Prunes 250, bread 350	35
24-49	Raspberries 250, bread 350	0

values of 20 to 30 grams of hemoglobin above the control levels. One might speculate as to which substances present in the potent fruits might be responsible for the favorable reaction obtained.

Numerous experiments with the feeding of ash, obtained from apricots and peaches, have demonstrated that this inorganic material retains most of the potency of the whole fruit and can exert the same favorable influences upon red cell and hemoglobin production as the fresh fruit when incorporated into the diet of standard anemic dogs.

Minerals. It is very evident from these experiments that minerals play a very definite rôle in the production of red cells and hemoglobin in this type of anemia. A variety of salts based on analyses of the ash of apricots, peaches, liver and kidney have been tested, but the results obtained were somewhat disappointing, in that the majority were proved to be practically inert. Among the minerals which demonstrate only the very slightest or no influence on blood production when tested singly, are manganese, zinc, aluminum, antimony, sodium iodide, potassium and calcium phosphates. All of these salts are found normally in the body and some of them are known to take part in certain metabolic reactions.

The most important mineral concerned in blood formation is iron, since it is contained in the hemoglobin structure. There is no reasonable opposing argument with regard to the positive effect of iron in severe, long continued secondary anemia due to hemorrhage in dogs; there does exist, however, many differences of opinion as to its value in other types of anemia. Iron, for example, may be effective in hastening recovery from loss of blood, and yet be totally inert in an anemia resulting from dietary deficiencies. Considerable controversy also exists as to the particular type of iron which is to be employed in the treatment of anemia. Exhaustion of iron reserves, and therefore a real need for this material, is probably the most important factor concerned in the utilization of iron for hemoglobin formation. Our experiments indicate that the addition of 0.2 grams of an iron salt daily to the bread diet permits a hemoglobin production of 40 to 50 grams following a two-week feeding period.

Copper has come into the limelight within the past few years since the publication of experiments carried on by a group of investigators in Wisconsin (5) who demonstrated the value of copper in a nutritional anemia in rats. Since then copper has occasionally been recommended as a necessary supplement, particularly to milk. It is not amiss to sound a note of warning, however, since copper may be harmful, even in moderate doses. Our experiments have demonstrated that copper belongs to the group of salts which can cause gastrointestinal disturbances in our animals and may therefore be toxic. Copper added to the basal bread ration has not called forth any extensive production of new red cells and hemoglobin in our hemorrhage anemia. One may consider the value of copper as only very moderate in its effect on blood formation under these experimental conditions.

Liver. The most potent substance for the sustained production of hemoglobin and red cells is represented by liver. This extremely favorable reaction is invariable in our dog experiments no matter how long the anemia has existed, or how unfavorable the preceding diet periods may have been. The same response is obtained whether the animal has been anemic for 6 years or for 1 year. For example, if one has removed one-half of the total circulating blood volume of a dog, in several bleedings, and then gives this animal a diet containing 200 grams of cooked liver daily, this entire blood loss plus an excess will be regenerated within a two-week period. Such a dietary permits a hemoglobin production amounting to 100 grams following a two-weeks' feeding of liver. Livers from calves, cows, pigs, sheep and lambs have been tested and have been found to be of very similar potency. Special preparation of the liver material seems to have little influence, there being little or no difference in hemoglobin production whether the liver is fed raw or cooked in any simple fashion.

An extract prepared from liver by the Eli Lilly Company of Indianapolis for the treatment of secondary anemia has a potency of approximately 65 to 76 per cent of that of whole liver (8). One must not, however, confuse this extract for the treatment of secondary anemia with that utilized in pernicious anemia, which is also manufactured by Eli Lilly Company. Each of these liver extracts is a distinctly individual substance to be used in the treatment of one of the two distinctly different types of anemia.

It is of interest to note that chicken livers have been found to be equally as effective as mammalian liver. Fish livers, however, obtained from a large variety of fresh as well as salt water fish are totally inert as blood builders. Only slightly less favorable than liver are chicken gizzards. Calf, beef and pig kidneys are also substances nearly as favorable for hemoglobin and red cell formation as liver, a response of 80 to 90 grams of hemoglobin being obtained.

Many experiments have been carried on with the feeding of beef, veal or pig muscle. Results show considerable variations, appearing more favorable in some cases than in others. The actual figures observed correspond somewhat to those obtained with the feeding of apricots or peaches, a production of 45 to 55 grams of hemoglobin above the control levels following a two-week feeding period.

These reactions obtained in animal experiments by dietary control may give suggestions as to the method of procedure in the treatment of human anemias, advocated by Whipple (4). Subsequent observations must be careful and long-continued in order to establish similarities or differences in the clinical reaction as compared with the experimental. We believe that it is no longer a question of debate that secondary anemias due to blood loss can best be treated by means of diet therapy. Minot and Murphy have successfully applied this same method of treatment in pernicious anemia with excellent results.

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