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ANEMIAS, EXPERIMENTAL AND CLINICAL*

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MR. PRESIDENT, Ladies, and Gentlemen: I wish to thank the president for his pleasant words of introduction, and I need not assure you that it is a great pleasure for me to come back to this State of California, where I have, perhaps, more intimate and close friends than in any other part of this country.

As your president has said, the work was begun here in California with Doctor Robbins and Doctor Hooper, and has been carried on since my removal to Rochester, by Doctor Robbins and other associates there. I wish to mention particularly Doctor Oehlbeck and Doctor Daft, who have been concerned with some of the more recent developments.

WHY THE DOG WAS USED FOR THESE STUDIES

In speaking before a group of practicing physicians, a laboratory investigator is a little at a loss to be sure what may be of particular interest, and I would like to make this talk of interest to you, obviously, and I have tried to pick out things that seem to me to have a bearing on medicine, although I would remind you again that I am a pathologist and have had no direct contact with patients. All my work has been done in the laboratory, with the dog as a constant companion. I may say that in some ways I feel the dog is perhaps a better patient than some that I hear about.

THE STANDARD ANEMIA DOG

This standard anemia dog I shall have to refer to today is something we have gradually worked out. The dog is raised and bred in the laboratory, a standard strain. He is kept under uniform conditions until he attains his growth at about the end of a year. He is vaccinated against distemper. He is taken care of in the most meticulous fashion so that we may control all possible variables. Then we begin to standardize him, and he is reduced to one-third the normal hemoglobin level for the dog and is kept there for the rest of his life, some of them having been under observation for ten or eleven years continuously anemic.

Those dogs react in a constant and uniform fashion to standard diet intakes, good, bad, and indifferent; and, as you know, liver stands at the top of the list. In fact, a dog will react uniformly the first year, the fifth year, and the tenth year, and we have many instances of that character of material that has been reported from time to time.

BONE MARROW VOLUME

I would like to mention bone marrow volume. I find from inquiry that students and people who do not concern themselves particularly with marrow have a rather vague idea as to what marrow volume really is. Anywhere from 10 cubic centimeters up is about the usual impression as to marrow volume. The highest figure I have ever heard was that the volume might be equivalent to that of the spleen.

Because we had to know something about the marrow volume in these experimental animals, the necessary work was done. It is difficult and time-consuming, but it can be done with some accuracy if you have the patience.

In the type of dog we use, the bone marrow volume amounts to about one-half to two-thirds the volume of the liver. The normal spleen is about one-tenth the volume of the liver, so you can readily see that the bone marrow volume in these animals is from five to seven or eight times the size of the spleen. If the bone marrow were only in a nice, isolated lump like the spleen, it would have a very much more respectable position in our knowledge of pathology; but it is all scattered about, and is in a difficult position. Methods are difficult to study it, and for that reason we do not know as much about the bone marrow as we might. But that is the way it is in the dog, a half to two-thirds the volume of the liver, quite a respectable size, and from occasional determinations in humans it appears the same thing is true of the human being, or at least very much the same. It has also been done for rats and monkeys, and the figures are very similar there, too, so I think we can look upon that figure I have given you as a fair estimate.

"MARROW EXHAUSTION"

You have read a lot about marrow exhaustion—at least I have—and it has been said that repeated small hemorrhages would bring about marrow

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exhaustion. Maybe it will, but we have never had the good fortune to see anything of that sort, and we do not believe it occurs in the normal animal and, therefore, in the normal human there is probably the same rule.

A dog, anemic ten years continuously, being bled from one to five times during each week of his life, you might say had been subjected to repeated small hemorrhages for quite a period, a period that in the human would represent fifty or sixty years of life. That brings about no marrow exhaustion, so I am rather skeptical about marrow exhaustion in general.

In the adult, dog or human, there is a very nice adaptation of bone-marrow spaces. In the adult, not all the bone marrow area is filled with active red marrow. A good deal of it, as you know, is filled with fat. If there is a call for increased activity of the bone marrow, the fat is replaced by red marrow cells. After that call has been set aside and they return to normal, fat cells will replace this red marrow, a very beautiful adaptation. I think sometimes the surgeons wish that something like that might happen in the skull when they are dealing with structures there.

When this adaptation occurs in these dogs, you might say, "Well, now, if a dog is anemic continuously for all these years, naturally he would fill up all the standing room; every bit of marrow space ought to be filled with red marrow." We expected to find that. In a few dogs we did find it, but when we began to examine all of the animals carefully we found that rule did not work at all. In other words, there is another controlling factor that controls the bone marrow spread that results from anemia. There is a spread, and it may take up all of the marrow space in the skeleton of that individual, the dog or the human. Again, a litter mate having anemia of the same duration and the same character, and every factor we can control of a similar nature, instead of showing 100 per cent replacement of marrow spaces by red marrow, will show perhaps only 60 per cent; sometimes only 50. So it is obvious that some other factor besides standing-room determines how wide the marrow spread may be. We have gradually come to the conviction that the supply of intermediates of prehemoglobin material made available by that particular animal determines the extent of the marrow that will develop to carry this load through this period of anemia. Probably some marrow cells are much more efficient in turning out red cells than others, just like some dogs have muscles that are much more powerful, where they are more active in running or in any other energy effort.

What about bone marrow exhaustion? It is obvious to you as practicing physicians that sometimes bone marrow does not react properly to substances that ought to form plenty of hemoglobin. Why should that marrow be disturbed in that way, inefficient or partially paralyzed? We got interested in this in an indirect fashion, and it pointed toward infection, and of course infection obviously disturbs arrangements in internal metabolism, and probably has a good deal to do with some of these disturbances of the functioning capacity of the bone marrow.

We thought we could approach it in another way, so we began to take dogs that had been carefully standardized and subjected them to sterile abscess formation, which is a very simple procedure and will be recovered from promptly. The sterile abscess heals up very shortly after it has been opened, or has broken. We got evidence there that was pretty definite, but not very striking, that the sterile abscess production would cause depression of the normal output of hemoglobin of that standardized dog.

AN ACCIDENTALLY PERFECT EXPERIMENT

But the experiment that was a perfect experiment, as sometimes happens in the laboratory, was pure accident. I will tell you about it because it seems to me it is quite interesting in this connection. It was a dog that was one of our very best animals and had been used for metabolism study. We knew to a gram the amount of hemoglobin we could expect to have produced by that animal under given conditions. It had been used in a metabolism experiment, and shortly thereafter this standard reaction of the animal was observed to be falling off. The animal, clinically, was apparently well, although there was some increase in the white count. It ate its food, maintained its weight, and for about six months showed a steadily-falling curve of hemoglobin production, until it came to a point where on a standard liver diet intake, instead of putting out fifty grams a week, as it had always done previously, it did not put out any. It just maintained itself, and on the basal ration there was a fall in hemoglobin without any bleeding at all.

We became much concerned and called in some celebrated consultants and they could not make much out of it, but we thought we had better do an exploratory. There was a little vaginal discharge. An exploratory was done and a greatly enlarged uterus was found. This was removed and the animal made an uninterrupted recovery. Within a week it was right back at the original level of hemoglobin production at which we had carried that animal for six years continuously anemic.

On examination of this enlarged uterus there was found a chronic or subacute endometritis, with great masses of plasma cells, mononuclear cells filling up the submucosa. The mucosa was greatly folded, swollen. In other words, we had an inflammation that had been present since the period of the metabolism test. That called for catheterization. The person doing the catherization was inexperienced and had introduced the catheter into the uterus several times in searching for the urethra. In the female dog that is a very easy thing to do.

We thought we would repeat this, this being a perfect experiment. We do not see many. We would like to see one other. We have tried very hard, but so far we have not been able to repeat. In other words, it was a pure accident, a perfect experiment, to my way of thinking, showing that long-continued, low-grade infection, not severe

enough to cause loss of appetite, may so disturb the mechanism of forming hemoglobin and red cells as to reduce it practically to the level where it can only fulfill maintenance requirements on the best type of diet for producing hemoglobin.

RESERVE STORE MATERIALS

It is important to know that in dogs, as well as in humans, there is considerable reserve store materials, and we mean by reserve store materials probably not hemoglobin or red cells, but reserve materials stored in the body out of which the animal in an emergency may make a lot of new red cells and hemoglobin. I will show you a chart that will illustrate very well what I mean by this reserve store.

PRESENTATION OF CHARTS AND SLIDES

(*Chart is thrown upon the screen.*) This chart shows the experiments on eight dogs which were placed on a diet of liver and bled immediately after weaning. When they had attained maturity, at about ten or eleven months of age, they were then bled carefully to remove all the necessary blood cells and hemoglobin to bring them down to one-third the normal level, which is our standard base line for the anemia dog. If there was no reserve present, the necessary amount to be bled should be roughly two-thirds of this (236). As a matter of fact, they are bled down to this level (66), and to do that you have to remove more hemoglobin than was present on the average in these animals before the anemia was begun. So that the sum of the hemoglobin remaining in circulation, plus that removed, minus what was present, obviously represents the reserve store, or 115 grams. One hundred and fifteen grams of hemoglobin is quite a large order. It could not be stored in the liver as protein, because there is scarcely that amount in this sized animal if all the liver protein was to be made into hemoglobin. Obviously, then, some of it must be stored somewhere else, possibly in the muscles. It can probably be taken from the circulating plasma proteins, but at any rate it is a reserve that is very convenient and is used in emergencies. In studies from anemia experiments, this reserve confuses deductions which may be made as to therapy.

If the animal, instead of being raised on a diet rich in liver after weaning, is raised on the standard bread, that reserve store will amount to about thirty grams. On meat, instead of the standard bread, the reserve will be about seventy grams; but it is an item that must be taken into consideration, and is of interest to people who may be treating anemias that result suddenly for one cause or another.

I want to speak briefly about iron therapy.

(*Slide is thrown upon the screen.*) The next slide will give you two points that are of interest. That is to say, forty milligrams of iron given every day for two weeks will result in about fifty-three grams of hemoglobin output during that period. If you make it ten times more, you just about double, not quite, the output. Either that iron cannot be used, or else it is not taken in. There might be considerable debate on that point, but possibly the next slide will throw some light on that.

(*Slide.*) I do not know that you can all see this slide, but the gist is that during these two weeks iron is given intravenously, so at least we know it got into the circulation, and the total amount as iron was 700 milligrams. If that should be changed quantitatively to hemoglobin, it would amount to 210 grams of hemoglobin. That is the equivalent of 700 milligrams of iron.

During this period the animal was able to put out, on a basal ration, fifteen grams a week for eighteen weeks or 270 grams. The total actually removed from this dog during this period of eighteen weeks was 503 grams. In other words, that iron was quantitatively recovered, and quite accurately for this type of experiment.

We have repeated this with various amounts of iron given intravenously over different periods of time in these carefully standardized dogs, and we have convinced ourselves that iron given intravenously in an animal that needs iron, such as an animal that is continuously anemic, that animal in health will return quantitatively that iron as hemoglobin on the basis of ten milligrams of iron to three grams of hemoglobin—a chemical basis. It does not make any difference what kind of iron you give a dog or inject in a dog, as far as we can determine.

What about the iron that is given by mouth? This evidence would indicate to us that in these animals the reaction shows the amount of iron that is absorbed. It is obviously true that as you increase the amount of iron given, you do not increase in the same measure the amount of iron absorbed.

The factors that influence the absorption of iron are extremely interesting. Doctor Schmidt touched on that point relative to the absorption of the important vitamins, showing that bile salts are concerned. We believe bile also is concerned with the absorption of iron, but just to what extent and what portion of bile, whether bile salts alone or not, we are not prepared to say.

That concludes what I have to say about experimental anemia, and I thought it would be interesting to you to relate briefly something about a very peculiar type of human anemia in which Doctor Bradford and I have been interested for several years.

I will tell you why this anemia is interesting. It used to be called von Jaksch's anemia, but that does not mean anything. It was carefully and accurately described by Cooley, and is sometimes called Cooley's anemia, or erythroblastic anemia. It occurs in people dwelling around the Mediterranean, and we have used the term "Mediterranean anemia," or thalasemia. That is a nice little term that is derived from the same source. It is a peculiar anemia. There is peculiar pigmentation of the viscera, very like that seen in hemochromatosis, which interested Doctor Sprunt many years ago. It has peculiar findings in the bone marrow that suggest scurvy. It is racial; it is familial. I think it is one of the most interesting clinical puzzles I have ever had the opportunity

to study. There must be a good many cases in this community, because in every community where Italians, Greeks, and Spaniards live this type of anemia is to be found.

It develops in early childhood; usually is fatal within five or six years; sometimes not. The cases that recover are going to be extraordinarily interesting to study. There is reason to believe that it may be due to some missing factor which possibly may be found and, therefore, replaced with a curative effect. For that reason I assure you it is an interesting type of anemia, and I should like to illustrate it briefly by a series of slides.

(*Slide.*) The children have a peculiar expression which some people say is Mongoloid, but the clinicians are able to recognize these cases with great accuracy. They are very apt to have a large spleen after the disease is well established.

(*Slide.*) One of the interesting things is the peculiar radial striation in x-rays of the skull. That comes out very beautifully here. I do not know that you can see it at a distance, but the x-ray pictures of the bones are quite characteristic.

(*Slide.*) The long bones do not show well here. They show peculiar mottlings, and when you study the bone marrow and the histological appearance of this, you see there has been a great deal of disturbance of the bony structure within the long bone. Some of this disturbance resembles a picture in scurvy.

(*Slide.*) Here are some museum specimens showing the peculiar striation in the coronal section through the calvaria.

Here is a section through the rib showing the red marrow, the great thinning of the shaft and the fact that there is some marrow underneath the periosteum.

(*Slide.*) The pigmentary deposit has interested me very much and, as I said, it resembles in its distribution the pigmentary arrangement in hemochromatosis. Here is the pancreas with an island, and this black material represents iron-stained material in great amounts in the epithelium of the acini. It occurs very little, if at all, in the islands, but seems to occur in the neighborhood of the islands. This pancreas may attain such a degree of pigmentation as to have a true rusty color in gross, and it is just loaded with iron. It may contain enormous amounts of iron by analysis.

(*Slide.*) This shows a high magnification picture of the pancreas, and these masses stained black are iron deposits within the epithelium of the acini. Occasionally phagocytes in the stroma will contain iron material released from destroyed and degenerated acini that have been loaded up with the iron.

(*Slide.*) This interesting deposit is here in the stomach. This is the muscular wall of the stomach, and here are the glands deep down in the mucosa, and here is the surface of the mucosa, and down deep in the glands you will see a heavy deposit of iron-staining or iron-containing pigment within the epithelium.

(*Slide.*) The liver may be so heavily pigmented as to have a color like that of maple sugar, just as in hemochromatosis. It may have as much iron per 100 grams, by analysis, as a case of hemochromatosis. You see here, these cells are all loaded with black granules, the iron reacting pigment. In the cases that have developed and lasted for many years, you may see a good deal of pigment in the stroma, and an increase of pigment with an early type of cirrhosis, just as you have in hemochromatosis.

(*Slide.*) This shows a spleen with malpighian bodies here and peculiar foamy-looking cells that resemble those of Gaucher's disease. However, the picture is in no way like that in Gaucher's disease, except for these occasional islands of foam cells, which do contain a considerable amount of cholesterol. The spleen may subsequently become very fibrous and hard, enormously enlarged, and may weigh more than the liver. It may be removed, and apparently causes little disturbance one way or the other. There may be erythroblastic crises after splenectomy.

(*Slide.*) This shows the bone marrow, which presents a variety of appearance. There may be nests of lymphocytes in places. There may be areas of bone marrow that look like simple hyperplasia, and there may be nests in the bone marrow that show these same foam cells. This is not a characteristic, but an unusual field. There is tremendous hyperplasia of the red cell elements, and a great many phagocytes are present.

The bony trabeculae are disturbed, as I said. The shafts are decreased in strength, and there may be deposits of bone marrow cells beneath the periosteum, particularly of the ribs.

(*Slide.*) This chart just shows that this child was followed from birth in a hospital, and about the fifth month they began to notice things were wrong with the patient; that it had anemia, and before long it was recognized as a characteristic case of thalasemia. This is the Mediterranean anemia. This was followed, and every conceivable thing done to attempt to modify that anemia, without success. All the gland therapy that could be suggested was tried, because the parents were very eager to have everything tested, as there was another child in the same family. In spite of everything the child died, and some of the material you have seen came from this particular case.

If we could once find the causes underlying this type of anemia, I think we would have a very much better understanding of pigment metabolism, in the first place; of marrow reaction in anemia, in the second; and a host of other problems relating to the complicated story of internal metabolism. So the person who solves this anemia will really have done something. The chances of studying this anemia, I am sure, are open to all of you, because there must be cases here in this State, and I imagine many of them already recognized, but there are really chances for very interesting study that bear on the whole question of pigment metabolism, the formation, production and destruction of pigment in the body about which we know so very little.

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