THE ERYTHROCYTE NUMBER AND HEMOGLOBIN CONTENT OF THE BLOOD OF THE THIRTEEN-LINED GROUND SQUIRREL, CITELLUS TRIDECEMLINEATUS TRIDECEMLINEATUS (MITCHILL), AS INFLUENCED BY HIBERNATION AND SPLENECTOMY

by

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INTRODUCTION

Hibernation is a phenomenon which occurs in various groups of animals. It is usually considered a resting condition in which the animal exists in a state of complete or partial torpidity. When warm blooded (homothermic) animals hibernate, they temporarily resemble cold blooded (poikilothermic) animals.

Internal physiological conditions, especially as influenced by the endocrine glands, as well as the external environmental conditions including lowered temperature, reduced food supply, confined air, and moisture have been believed to have an influence on hibernation. As yet it has not been definitely established what the complete cause or causes of hibernation are.

During the process of hibernation many very marked physiological changes occur within the animal. The general body metabolism is greatly reduced. The heat production is sufficient only to keep the body temperature slightly above that of the surroundings. Respiration rate, heart rate, irritability and the rate of other physiological activities are all much lowered. The rates of tissue destruction and repair are much reduced. When such pronounced changes take

place in the general metabolism, it is to be expected that a considerable modification would occur in the blood. Experiments and observations have shown that in animals during hibernation the circulation of the blood is very sluggish, perhaps being absent in the peripheral vessels. The effects of injected poisons are very much slower to appear during torpidity. The glycogen content of the blood is much reduced during hibernation. Various workers have studied the erythrocyte number and hemoglobin content of the blood of animals in hibernation and have found indications of changes in both.

The spleen is known to be an organ closely associated with the circulatory system. The relationship is such that the spleen might readily influence changes in the blood during hibernation. It is well established that it has certain effects on the blood of active animals.

PURPOSE OF THE WORK

The purpose of this research was to study further the changes in the erythrocyte number and the hemoglobin content of blood as affected by hibernation, especially as they occur in the thirteen-lined ground squirrel. Also an attempt was made to determine to what extent the spleen influences these blood changes and what relationship exists between

that organ and hibernation.

REVIEW OF LITERATURE

The first study to be made upon the changes in the blood cell counts accompanying hibernation was by Vierordt in 1854. He reported finding in the marmot, according to Rasmussen (9) and Dubois (5), 5,800,000 red corpuscles per cmm. at the beginning of hibernation, while at the end of the winter sleep he found only 2,300,000 red cells.

Valentin (10) reported in 1881 that the corpuscles were diminished in size as well as in number.

Quincke (8), in 1882, similarly found in the marmot only 3,500,000 red corpuscles at the end of hibernation, or about 70 per cent of the number found during the summer. The hemoglobin content was determined to be 8.8 per cent or only 64 per cent of the normal amount of this pigment. The blood used for these determinations was taken directly from the pulsating heart.

Quincke thought that he saw abundant evidence of the destruction, during hibernation, of erythrocytes in the spleen, red bone marrow and liver. For example, in leucocytes found in the capillaries of the liver, granules were present which were blackened by ammonium sulphide. His conclusion was that iron from broken down red cells was accum-

ulated in these organs to be later used in regeneration of new corpuscles.

Carlier, as reported by Rasmussen (9), later also observed these iron containing pigment granules in the bile capillaries of the hibernating hedgehog. In the liver and in the large veins coming from the liver he found large, rounded or oval, mononucleated cells which often contained iron pigments in their cytoplasm. Contrary to Quincke, he believed that this pigment was simply accumulated there because of the sluggish state of the excretory function of that organ, rather than a storage of the material for future use. Later the pigment was probably excreted as usual, since he found that the liver did not appear to be a blood forming organ after the animal awoke from hibernation.

Dubois (5) made erythrocyte counts in several marmots at various times from November to May. His counts showed a slight increase in the number of red corpuscles in the large vessels of both the venous and arterial circulation in the early part of hibernation. Toward the end of the dormant period there was a decrease from over 5,000,000 to less than 3,000,000 corpuscles per cmm. while the animal was still torpid and to about 2,000,000 when the animal awakened. These low figures were checked upon by hemoglobin determinations, which showed only 8.5 per cent, while the animal was

torpid with a body temperature of 15° C. It was even lower when the animal was awake and had a rectal temperature of 35° C. The figures for the hemoglobin determination and cell counts check well since there was about double the hemoglobin content at the beginning of hibernation.

Polimanti (7) enumerated the erythrocytes and determined the hemoglobin content of four marmots during hibernation and again when they were awake. His figures were in general agreement with those of previous workers except that the decrease at the end of the dormant period was not nearly as great as reported by them.

Rasmussen (9) in 1916 studied, in the woodchuck, the blood changes accompanying hibernation. He observed little difference in the number of red corpuscles and amount of hemoglobin in the arterial blood of active animals and in hibernating animals, the latter erythrocyte average being but five per cent higher. In three semi-torpid animals, however, he noted an increase of about 1,000,000 red cells per cmm. or 14 per cent over those in the torpid state. The hemoglobin was also considerably increased. After the animals awakened from hibernation, but before they had food or water, the hemoglobin content and cell count were greater than during lethargy. The erythrocyte number had increased

by six per cent. However, after the animals had commenced to eat and drink there was a decrease, amounting to about 20 per cent in the number of corpuscles and amount of hemoglobin.

Dubois and Polimanti both attributed the observed increase in erythrocyte number in the early part of hibernation to an increase in the specific gravity of the blood due to a loss of water. That the blood does lose water during hibernation has been shown by Aeby, as reported by Rasmussen, who found a loss of 4.37 per cent; Dubois, a loss of 4.11 per cent; and Polimanti, a loss of about 4.6 per cent. Rasmussen found little change in the specific gravity of the blood of the woodchuck, but at the same time found in general only small changes in the erythrocyte count and hemoglobin amount. The small changes he did find in the specific gravity led him to conclude that the observed variations in the erythrocyte number and hemoglobin amount were dependent to a large extent upon the concentration of the blood and redistribution of the corpuscles.

Mann and Drips (6) made observations, in 1917, on the spleens of hibernating ground squirrels. They found that within 12 hours after an animal became torpid, the spleen became markedly congested with blood both grossly and microscopically. The congestion increased slightly for a few

days, the condition then being maintained for about 40 days. After this time the congestion decreased until at 70 days the spleen again appeared normal.

It has been quite definitely established that one of the functions of the spleen is to act as a reservoir for red corpuscles. Barcroft, Meakins et al. (3), in a study upon themselves, found that the hemoglobin content as well as the blood volume rose as the surrounding temperature increased. They postulated, then, that the body contains a hidden store of hemoglobin. Only one place in the body, the erythrocyte-laden splenic pulp, could contain sufficient hemoglobin which might be added to the circulating blood to raise its content appreciably.

. Barcroft and . Barcroft (1) found that if carbon monoxide were administered slowly to animals in the air breathed, there was a lag of as much as 30 minutes between the time the carbon monoxide hemoglobin of the circulating blood and that of the spleen reached the same concentration. When equilibrium was reached and pure air supplied, the spleen pulp retained carbon monoxide hemoglobin longer than the circulating blood.

Barcroft, Harris et al. (2) studied the spleen in living cats. They implanted metal sutures into the edge of the spleen and later made X-ray photographs of the spleen under

different circumstances. By preparing models of the spleen from the photographs they were able to compute the size and weight of the organ. Their experiments showed that the spleen is capable of responding to situations making greater demands on the hemoglobin content of the blood. It does this by contracting, thus forcing erythrocytes out of its pulp into the systemic circulation. It was shown that upon bleeding, the spleen in cats may contract to such an extent that if the material squeezed out were red corpuscles, they would correspond to about one-third of the total number in circulation. Moderate exercise could cause the spleen to squeeze out material that, if corpuscles, would correspond to the blood equivalent of about one-fourth the total blood volume.

Barcroft, Murray and Sands (4) showed that splenectomized animals succumbed much sooner to carbon monoxide than did normal animals. The fatal percentage of carbon monoxide hemoglobin was the same for both groups of animals. They also showed that splenectomized animals were killed no sooner than the normals by hydrocyanic acid, a gas which does not poison by making demands on the hemoglobin in circulation.

MATERIALS AND METHODS

For this research, thirteen-lined ground squirrels, Citellus tridecemlineatus tridecemlineatus (Mitchill) were used. A very few of the subspecies pallidus were also included. Those of the subspecies tridecemlineatus were obtained from Wellington in southern Kansas and those of the subspecies pallidus were obtained from Grinnell and Scott City, both in central western Kansas. These ground squirrels seldom reproduce in the laboratory.

The animals were kept in mesh wire cages measuring 24x18x8 inches. Pieces of burlap were placed in the cages for nest material. From one to six animals were kept in each cage. The room temperature was not permitted to drop below 20° C. The animals were maintained on a diet of water, meal and green food. The meal was composed of: corn meal, 38 per cent; ground wheat, 38 per cent; dried milk, 12 per cent; alfalfa meal, 5 per cent; bone meal, 5 per cent; cod liver oil, 1 per cent; and salt, 1 per cent. The green stuff consisted of fresh alfalfa or grass in season and sprouted oats throughout the winter.

Hibernation was artificially induced by placing the animals in a refrigerator where temperatures of from 0° to 10° C. were maintained. Each animal was kept in a metal

bottomed wire cage measuring 10x6x6 inches in which a handful of shavings had been placed. A fresh carrot was provided which served as both food and water while the animal remained awake. Daily observations were made on the animals in the refrigerator; the temperature and the state of each animal, awake or torpid, were recorded. An animal was recorded torpid only after a few shavings, placedon its back the previous day, remained undisturbed at the time of observation.

When it was desired to make an erythrocyte count and hemoglobin determination, a sample of approximately 0.25 cc. of blood was drawn from the heart of the animal. Withdrawal was accomplished through a hypodermic needle, into a small glass syringe. The syringe had been greased with petroleum jelly. The needle was inserted through the skin and abdominal body wall just behind the sternum and between the medial margins of the sternal ribs. The needle was pushed forward through the diaphragm into the thoracic cavity, keeping it a little beneath the ribs and directed slightly to the left of the median line of the animal, until the heart was reached. Occasionally several needle thrusts were necessary before the heart was located. A partial vacuum was produced in the syringe by withdrawing the piston a little, after the point of the needle had been inserted

through the skin. When the heart was punctured, blood would flow rapidly into the syringe. Drawing blood from the heart usually caused no injury. If the animal was not torpid when blood was required, it was lightly anesthetized with ether.

After obtaining blood, the pipette of the hemoglobinometer was immediately filled. The remainder of the drawn blood was quickly transferred to a small 3x5/16 inch test tube containing a few milligrams of anhydrous, powdered, sodium citrate to prevent coagulation. The blood and sodium citrate were rapidly mixed and later used in making the erythrocyte count. The hemoglobin determination was then made.

For this purpose a Dare hemoglobinometer was used. This instrument is essentially a standardized glass plate of varying depth of color which is matched with the color of a sample of undiluted whole blood held in a thin film between two glass plates. In most cases seven readings were made on a sample and the average taken. The readings were made in grams of hemoglobin per 100 cc. of blood.

The erythrocyte count was made by using a Levy hemocytometer with Neubauer ruling. Dilutions of the blood were made with Hayem's solution. The cells in 80 squares were counted in the earlier studies but the number was later reduced to 40 squares.

Splenectomy was a simple surgical operation. The animals at first were anesthetized with ether but Nembutal was soon found to be more satisfactory. Surgical anesthesia was produced within five or 10 minutes by injecting, intraperitoneally, 0.07 cc. of a Nembutal solution (one grain per cc. of solution). A solution prepared by dissolving germicidal discs of potassio-mercuric iodide (Parke Davis and Co.) was the antiseptic used during the operation.

The hair of the left lateral abdominal body surface was removed by clipping and shaving. An incision about five-eighths inches long was made through the skin of the dorso-lateral surface slightly posterior to the margin of the ribs. A somewhat smaller opening was made through the body wall. The spleen was exposed through the incision and the blood vessels to it were ligated with surgeon's silk. The spleen was then freed from the mesentery. The incision in the body wall was closed with a suture of silk and the edges of the skin incision were held in proximity with wound clips.

The spleens removed were fixed in Bouin's solution and preserved in 70 per cent alcohol. Spleens were removed from refrigerated animals while they were torpid, killing them afterward; or if they were not torpid, they were killed quickly and the spleens removed immediately. These organs were later weighed.

RESULTS

A total of 63 different animals were used for the determinations. The erythrocyte numbers and hemoglobin determinations were arranged in various groups according to the conditions under which the animals were studied.

Group I: Normal active ground squirrels.

A. Normal male ground squirrels.

B. Normal female ground squirrels.

Group II: Normal refrigerated ground squirrels.

A. Animals kept in refrigerator 14 days or less and torpid 10 days or less.

B. Animals kept in refrigerator more than 14 days and torpid more than 10 days.

Group III: Active splenectomized ground squirrels.

Group IV: Refrigerated splenectomized ground squirrels.

Determinations of the normal erythrocyte number and hemoglobin content were usually made on each animal before it was used in some other group. Thus, many animals served as their own controls.

Group I

Fifty-nine erythrocyte counts were made on 56 normal active animals. The average of these counts was found to be 8,230,000 cells per cmm. of blood. The individual counts

showed considerable consistency as 80 per cent of them fell within the limits of 7,720,000 and 9,120,000. The counts for the male and female animals were also averaged separately. The difference found was not considered to be of any significance. The average of 21 counts on males was 8,220,000 cells per cmm.; that of 30 counts on females was 8,240,000 cells per cmm.

Fifty-five hemoglobin determinations were made on 52 animals. The average was determined as 16.22 gm. per 100 cc. of blood. The hemoglobin contents were quite consistent as were the erythrocyte numbers. Eighty per cent of the hemoglobin determinations ranged between 14.00 gm. and 19.00 gm. per 100 cc. of blood.

The male animals had a somewhat higher hemoglobin content than the females, there being a difference of 1.12 gm. per 100 cc. of blood. Their respective averages were 16.95 gm. and 15.83 gm. per 100 cc.

Since the difference between male and female ground squirrels in erythrocyte number was insignificant and that in hemoglobin content was small, sex differences were not further considered in other groups.

Group II

Thirty cell counts and hemoglobin determinations were made on normal animals which were kept in the refrigerator

for from eight to 100 days. The average erythrocyte count of all animals of this group was 7,640,000 per cmm. of blood. This figure is 590,000 less than the average for normal unrefrigerated animals.

The average hemoglobin content was also reduced, it being 13.85 gm. per 100 cc. of blood. This is 1.37 gm. less than the average for normal male and female animals.

This group of animals was also divided into two parts: Those animals which had been kept in the refrigerator 14 days or less and which had also been torpid 10 days or less, and those animals which had been kept in the refrigerator more than 14 days and which had also been torpid more than 10 days. The division was made in this way for convenience in making use of a natural grouping as well as for the equality in numbers of animals, there being 15 animals in each division.

The erythrocyte numbers of these divisions did not show any significant difference. Those animals refrigerated and torpid for shorter periods had an average of 7,560,000 corpuscles per cmm. while those refrigerated and torpid for the longer periods had an average of 7,730,000 per cmm.

The average hemoglobin content of these divisions was, however, 1.77 gm. per 100 cc. of blood, lower in the animals kept in the cold for the longer periods. The figures were

14.23 gm. for those cooled the shorter lengths of time and 13.46 gm. for those refrigerated longer.

From the observations, there apparently are reductions in the number of red corpuscles and quantity of hemoglobin in the blood of ground squirrels subjected to temperatures of 0° to 10° C. for periods of days. These reductions in erythrocyte number seem to take place soon after the animals are placed in the refrigerator but do not increase with the passage of time as later determinations did not show further decrease. The hemoglobin content, however, continues to diminish as they remain in the cold for longer periods. These reductions may be the result of the hibernation which was artificially produced by the cooling, or are perhaps merely the effect of the cold environment. However, three animals which had not become torpid after 13 days in the refrigerator, had erythrocyte counts and hemoglobin contents which averaged higher than the average for normal active animals. This would seem to indicate that the cold environment was not the cause of the reductions noted. Of course, the number of determinations on animals in the cold but not hibernating was not sufficient to be the basis for any definite conclusions. No study was made upon animals which had been hibernating for long periods such as would be the condition near the end of natural hibernation.

Group III

Thirty determinations on active splenectomized ground squirrels were made. The average erythrocyte number of these animals was 7,330,000 cells per cmm. of blood and that of the hemoglobin content was 14.37 gm. per 100 cc. of blood. The red cell number was somewhat lower than that of refrigerated normal animals, the difference being 310,000 cells. It also averages 900,000 red cells less than that of normal, active ground squirrels. The hemoglobin content, however, is only 0.85 gm. per 100 cc. of blood less than the quantity found in normal, active animals.

The lowered cell count and hemoglobin quantity cannot be attributed to the immediate effect of the surgical procedure as no determinations were made on animals sooner than 13 days after the operation. Usually longer periods elapsed.

The figures for two splenectomized animals were omitted from this group because their exceptional lowness seemed to indicate disorders other than merely splenectomy. The determinations were made on one 15 days and on the other 46 days after splenectomy.

Group IV

Fifteen determinations were made on splenectomized animals which were refrigerated from 11 to 54 days. They

were also torpid varying from not at all to 26 days. The average erythrocyte number was 6,420,000 cells per cmm. of blood and the average hemoglobin content was 11.61 gm. per 100 cc. of blood.

These averages are considerably lower than those of any other group. The erythrocyte number is lower than that of the unrefrigerated splenectomized animals by 910,000 and lower than in normal active animals by 1,710,000. The hemoglobin content is 4.61 gm. per 100 cc. of blood lower than that of normal active animals and 2.24 gm. less than in refrigerated normal animals.

The reason for these large reductions is not known. It would be interesting to know if the spleen reverts to its embryonic hematopoietic function when the animal is subjected to a cold environment which induces hibernation.

No difference in the hibernation of normal and splenectomized animals was observed. The operated animals, however, did seem to be less resistant to the effects of refrigeration when they were not in a good physical condition for hibernation. This was shown by the frequent deaths of these animals in the refrigerator.

Spleen Weights

The spleens of 21 animals which had been removed by operation were weighed and their weights compared with the

weights of spleens taken from seven animals refrigerated from six to 98 days and torpid from not at all to 69 days. The average weight of spleens from normal animals was 0.325 grams. That of the refrigerated animals was 0.150 grams. The weights within each group were very consistent. In only one case did the weight of the spleen of a normal animal fall within the range of the refrigerated animals. These observations appear to be contrary to observations by Mann and Drips who reported finding the spleens of hibernating ground squirrels enlarged and congested. No explanations for the differences between the two groups of animals used nor between the present research and the earlier work were found.

SUMMARY

1. Normal thirteen-lined ground squirrels were found to have an average erythrocyte number of 8,200,000 per cmm. and a hemoglobin content of 16.2 gm. per 100 cc. of blood.

2. The normal ranges of the erythrocyte numbers and hemoglobin content are fairly wide. However, 80 per cent of the observed erythrocyte numbers were between 7,720,000 and 9,120,000 per cmm. of blood. Eighty per cent of the observed hemoglobin quantities were between 14.0 and 19.0 gm. per 100 cc. of blood.

3. No significant difference between the erythrocyte averages of male and female ground squirrels was found. The hemoglobin content of male ground squirrels averaged about one gram per 100 cc. higher than did that of the females.

4. There was a decrease, on the average, of about one-half million erythrocytes per cmm. of blood when animals were placed in the refrigerator for periods of from eight to 100 days. This diminishing appeared early in the refrigerated animals and did not become more marked after longer periods. There was a decrease, on the average, of 2.4 gm. of hemoglobin per 100 cc. of blood in the refrigerated animals. This decrease was progressive.

5. The average erythrocyte number of splenectomized animals was slightly lower than that of normal refrigerated animals. The average hemoglobin content, however, was only a little lower than that of normal active animals.

6. The erythrocyte number and hemoglobin content of the blood of refrigerated splenectomized animals were considerably lower than those of refrigerated normal animals. The average figures for this group of animals were 6,400,000 erythrocytes per cmm. and 11.6 gm. per 100 cc. of blood.

7. The average weight of spleens from refrigerated animals was found to be less than one-half the average weight of the spleens from normal animals.

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