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Research Article

THE EFFECT OF LIVER AND COMMERCIAL LIVER EXTRACT ON THE BODY WEIGHT, RED BLOOD CELLS, AND RETICULOCYTES OF NORMAL RATS

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The remarkable changes occurring in patients suffering from Addisonian pernicious anemia who have received adequate liver therapy are now well known. Among these changes the rise of the percentage of reticulocytes in the peripheral blood is most striking, and attention has also been repeatedly drawn to the gain in weight associated with improvement in the blood picture (1) (2).

In pigeons treated with liver preparations of known potency in pernicious anemia and given intravenously, there was an increase of immature red blood cells in the peripheral blood (3). On the other hand, normal pigeons or pigeons starved until a nutritional anemia had been produced, when fed an exclusive diet of broiled liver, showed an initial gain of body weight. The normal pigeon, however, on the liver diet became gradually anemic, while the pigeons with the nutritional anemia produced by starvation showed a partial regeneration of the peripheral blood at first, then a fall of both red blood cells and hemoglobin, resulting in a severe anemia. This occurred in spite of rapid gain of weight. An exclusive meat diet fed under similar conditions seemed to be much more adequate for blood formation in pigeons (4). This has been verified by Barlow (5) with regard to meat and only partially for liver because he experienced difficulty in getting pigeons to eat the liver. In our experience the birds will eat broiled liver unless subjected to too long starvation for that particular animal.

The effects on the blood obtained in pigeons with liver fed as an exclusive diet and liver preparations fed or injected intravenously, have been interpreted as being dependent upon the megaloblastic bone marrow found in birds, a type of bone marrow found in pernicious anemia and other anemias of man amenable to liver therapy. To test this hypothesis a series of experiments have been made on rats, an animal that has been used extensively for nutritional studies, and is therefore well standardized. The rat also has a hyperplastic, active bone marrow, but it is predominantly erythroblastic and normoblastic, i.e., similar in type to

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the normal bone marrow of man. The experiment was devised to give a possible answer to the following question:

1. Are the changes observed in pernicious anemia and in grain fed pigeons when liver or liver preparations effective in pernicious anemia are administered, dependent upon the megaloblastic character of the bone marrow?

EXPERIMENTAL METHODS

Twelve healthy male rats of the same age and approximately the same weight were divided into four groups. Daily weights were recorded and reticulocyte counts made every other day, using Cunningham's (6) modification of Hawes' (7) method. Red cell counts were made once a week in a Neubauer chamber, using standard pipettes. Hemoglobin determinations were made at the same time using Haldane's method. During a short control period they were fed on a synthetic standard rat diet [2] supplemented by the salt mixture recommended by Osborne and Mendel (8). Group I was then given raw minced liver which was intimately mixed with the standard food so as to represent 25 per cent of the total amount fed. This amount of liver was given for 15 days and then increased to 50 per cent of the total intake for a further period of 15 days. Finally whole liver without any other food was given for a similar period. Food was always supplied in excess.

Group II was given the standard diet only throughout the whole period of the experiment, thus serving as a control group.

Group III was fed daily for 15 days, 0.5 gram of liver extract number 343 (N. N. R.) derived from 14 grams of whole liver in addition to the ordinary standard food. This was later increased to one gram of liver extract number 343 (N. N. R.), daily for 15 days.

Group IV was given the waste product obtained in the preparation of a fraction potent for pernicious anemia which may have contained extremely small quantities of the effective principle.

EXPERIMENTAL OBSERVATIONS

1. *Red blood cells.* No appreciable alteration was found in the red blood cell counts of any of the animals throughout the period of investigation. Variations were within normal limits, the highest figure being 9,897,000 red blood cells per c. mm., and the lowest 7,720,000. In no case was there a variation greater than 1,000,000 in any one animal. The same was true of the hemoglobin determinations.

2. *Reticulocyte counts.* There was no significant alteration in the reticulocyte count in any of the animals. The greatest variation was in one rat of the control group which fluctuated between 0.3 and 6.9 per cent, but the latter count was exceptional. The average figure for the whole series was 1.54 per cent.

[2] Casein, 20 per cent, pure Arrowroot starch, 56 per cent, fresh creamery butter, 15 per cent, dried yeast, 5 per cent, and salt mixture, 4 per cent.

3. *Weight gain.* The average gain in weight of the three rats receiving the standard diet which contained 25 per cent of whole liver for 15 days was 15.6 grams, compared with 13.3 grams in the control group. When the liver was increased to 50 per cent of the diet the gain in weight was 12 grams, as compared with 8.3 grams in a similar period in the control animals. The total gain in each group during the 30-day period was an average of 28.66 grams for each rat fed whole liver, and 21.66 grams in the control group. When fed whole liver alone for an 18-day period there was a loss of weight averaging 12 grams.

In Group III fed liver extract, number 343 (N. N. R.), there was an average gain in weight of 15.2 grams in the first 15 days when a dose of 0.5 gram daily was given, and of 7.83 grams in the second period of 15 days when 1.0 gram was fed in addition to the standard diet, giving a total average gain of 22.66 grams in 30 days.

Group IV fed the waste product of an effective liver preparation made an average gain of only 5.33 grams in 15 days. This substance was extremely distasteful to the animals, and while taking it they never appeared in good condition. In a 3-week period after cessation of treatment two rats made an average gain of 28 grams, while the third, which had some lung infection as the result of inhaling some of the liver preparation while an attempt was being made to forcibly feed it, made no gain.

It would appear from the above observations that the addition of either whole liver or liver extract to the diet of healthy adult rats for a short period has no effect on either the weight (except perhaps in the rats fed whole liver), the reticulocytes, or the total number of red blood cells. It is recognized that the treatment was maintained for a short period only, but results are obtained both in patients with pernicious anemia and in pigeons during a similar short period, and the results are therefore comparable.

The gain in weight in the animals receiving liver was not apparently significantly greater than in the control group, especially when dealing with such a small series of animals.

DISCUSSION AND CONCLUSIONS

From the above experiments it is seen that when healthy adult rats were fed a synthetic diet supplemented with raw liver or liver extract number 343 (N. N. R.) no change was noted in red blood cells, hemoglobin or reticulocytes. No increase in weight, considered to be significant, was observed in liver fed rats as contrasted with the controls.

It is of interest to note that Vedder (9) and others did not obtain any effect on the blood picture of rats with anemia caused by Bartonella muris-rattis when large doses of liver extract were administered. Rats with posthemorrhagic anemia with either whole liver or liver extract added to their diet did not regenerate red blood cells and hemoglobin

faster than the controls (10). Adlersberg and Gottsegen (11) produced a temporary anemia in healthy dogs and rabbits on an ordinary diet by feeding large doses of commercial liver preparations effective in pernicious anemia (hepatrat and hepatropson). On the other hand, Whitehead and Barlow (12) report a rapid recovery in rats suffering from rice disease when meat or liver was substituted for the rice. The curative effect was better with lean beef than liver. Red blood cells and hemoglobin were completely recovered with lean beef or liver two or three weeks after the body weight had been regained.

It is evident that feeding of liver or commercial liver extract to healthy adult rats on an artificial standard diet, produced none of the effects on the peripheral blood and the weight, as has been observed in pernicious anemia of man and in grain fed pigeons. This is suggestive, and tends to support the hypothesis that a megaloblastic bone marrow is essential to produce the effects obtained by the administration of liver or liver preparations effective in pernicious anemia.

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