EFFECT OF VITAMIN B₁₂ ON THE BODY COMPOSITION OF RATS*

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Of the numerous reports on vitamin B₁₂, few deal with its metabolic rôle. The results of Bosshardt (1), Chow (2), McCollum (3), and Rupp (4) and their associates suggest that vitamin B₁₂ may be involved in the utilization of carbohydrates and its transformation to fat rather than in protein metabolism. In this communication we wish to present some data which serve as additional evidence to substantiate this hypothesis. They deal with the effect of vitamin B₁₂ on the body composition of rats.

EXPERIMENTAL

Vitamin B₁₂-Deficient Rats—Weanling rats of both sexes and adult male rats were used for this study. The weanling rats were born of mothers which were maintained on a soy bean meal diet¹ during the periods of pregnancy and were 25 to 30 days old at the start of the experiment. The average body weight of these animals was 35 ± 5 gm. for both sexes. The adult male rats were born and raised by vitamin B₁₂-deficient mothers and were maintained on the soy bean diet for 4 to 5 months, at which time their body weights ranged between 170 and 220 gm.

Composition of Diets—The two diets used in our experiment were designed to contain minimum amounts of vitamin B₁₂. Diet A consisted of 60 per cent of a commercial preparation of soy bean meal² and 40 per cent sucrose; Diet B of 68 per cent soy bean meal, 4 per cent cottonseed oil, 24 per cent sucrose, and 4 per cent Salts 4 (5). The usual vitamin supplements (6) were added to both diets.

Determination of Composition of Carcass and Organs—Ten weanling rats were distributed at random into two groups of five animals each and

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¹ The soy bean meal diet consists of soy bean meal 62 per cent, corn oil 4.5 per cent, Salts 4 per cent, and sucrose 29.5 per cent plus fat-soluble and water-soluble vitamin supplements.

² Sobee was kindly supplied to us by Mead Johnson and Company. It contains 32 per cent protein, 19.2 per cent fat, 37 per cent carbohydrate, and 8 per cent minerals (ash).

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were fed Diet A ad libitum. The rats on Diet A received 25 m\(\mu\)gm. of crystalline vitamin B\(_{12}\) subcutaneously daily, except Sundays. Food consumption and body weight gains were measured. After a 5 week period the animals were anesthetized with sodium Evipal and most of the blood was withdrawn by heart puncture. Liver and muscle samples were immediately removed for determination of glycogen according to the procedure of Good, Kramer, and Somogyi (7). After removal of blood and the abdominal viscera, the carcass was frozen with dry ice and ground in a meat grinder. The ground tissues were first extracted with hot 95 per cent alcohol, which removed water, part of the fat, and some nitrogenous compounds. To complete the fat extraction the dry tissue residue was refluxed for 72 hours in a Soxhlet apparatus with chloroform. Each of the alcoholic and chloroform extracts was evaporated to dryness at low temperature and extracted several times with ether. The lipide in the ethereal solutions was combined and dried to constant weight after evaporation of the solvent. Protein content (N \(\times \) 6.25) of the carcass was determined from micro-Kjeldahl analyses of a weighed portion of the entire fat-free dry residue. The two ether-insoluble fractions as well as the precipitate which appeared in the ethereal extracts after standing in a cold room overnight were combined with the extracted residue. This mixture was dried to constant weight at 110\(^\circ\). The difference between the wet weight of the carcass and the sum of the weights of body fat and total dry residue was taken as the water content.

In addition to the weanling rats, ten adult male rats were distributed randomly into two groups and were fed Diet B. One group served as controls and the other received subcutaneously 0.5 \(\gamma\) of vitamin B\(_{12}\) three times per week for 12 weeks, at which time both groups of animals were sacrificed for the chemical analyses mentioned above.

Results

Effect of Vitamin B\(_{12}\) on Carcass and Liver Composition of Deficient Animals—Body weight and carcass composition are reported in Table I. As would be expected, greater weight gains were observed with the weanling rats receiving vitamin B\(_{12}\), being 112 gm. for the injected animals and 23 gm. for controls. After the removal of the abdominal organs and blood, the average carcass weight was 117 and 45 gm. respectively. Chemical analyses on the carcass of both groups of animals gave essentially the same percentage of body protein, but a marked difference in the fat content, 16.6 per cent for the vitamin B\(_{12}\)-injected animals and 4.3 per cent for the control group; \(P < 0.001\). Our data show a slightly higher content of muscle glycogen for the vitamin B\(_{12}\)-injected group of young rats, but this difference was not statistically significant.
Similarly, the adult rats which received vitamin B\textsubscript{12} by injection gained 51 gm. more in body weight than did the control animals during a 12 week period. Analysis of the carcass composition of both groups of animals showed no significant difference in percentage of body protein or muscle protein, but significant differences in body fat as well as in body water, with $P$ values less than 0.01 in both cases.

Data on the composition of livers of both young and adult rats in the injected and control groups are presented in Table II. Significant differences in the composition of the livers of either weanling or adult rats were not found. The livers of the deficient rats were relatively heavier.

**DISCUSSION**

The so called animal protein factor is generally believed to be closely associated with vitamin B\textsubscript{12} or identical with it. The name implies that
this vitamin might play a rôle in protein utilization. In harmony with such a concept one may cite the work of Catron et al. (8) and Cunha et al. (9) who demonstrated that vitamin B₁₂ increased the growth rate and, at the same time, the feed efficiency. However, the greater rate of increase in body weight was invariably accompanied by a marked increase in food intake. It is therefore of interest to ascertain the effect of vitamin B₁₂ deficiency and its repletion on the carcass composition of rats. Such information may provide some evidence on the metabolic rôle of vitamin B₁₂. Our data demonstrate that the deficient animals had an abnormally low content of carcass fat which could be increased by the administration of vitamin B₁₂. No change in protein content was observed during the deficiency or after vitamin B₁₂ administration. If the utilization of proteins were improved as the result of administration of vitamin B₁₂, one might expect an increase in nitrogen retention. This phenomenon was not observed after injection of vitamin B₁₂ into our deficient rats. Thus, under our experimental conditions the administration of vitamin B₁₂ did not in any of the ways measured here alter nitrogen retention. Hence, it is probable that the apparent increase in the feed efficiency is related to the better utilization of sources of calories (carbohydrates or fats) rather than proteins. These results support our concept that vitamin B₁₂ definitely plays a rôle in restoring the abnormally low fat content of the carcass of the deficient animals to normalcy.

The body water content of vitamin B₁₂-deficient animals, both young and adult, was approximately 10 per cent higher than in their litter mates receiving vitamin B₁₂. This difference, though statistically significant, might be a reflection of a decrease in carcass lipide without other change. However, it is of interest to note the difference in the ratio of protein to water in the carcass: 1:3.8 and 1:4.4 for the young and adult deficient animals and 1:3.4 and 1:3.6 for the young and adult controls, respectively. In this connection, we have frequently observed slight edema on the paws of vitamin B₁₂-deficient rats, especially after they have been maintained on experimental diets for long periods of time (3 to 4 months). These findings are in harmony with those of Meulengracht et al. (10) who reported that water retention was a characteristic symptom of pernicious anemia in relapse, owing probably to kidney insufficiency as shown by water excretion tests. Thus, the specific gravity of urine of their patients was usually high and the blood urea was higher than normal in the majority of cases. The results suggest that vitamin B₁₂ may also have a rôle in maintaining kidney function. Thus, deficiency of vitamin B₁₂ can cause kidney insufficiency. This hypothesis, though lacking in definite experimental proof, receives some support in the observation of Zucker and Zucker (11), which was later confirmed by Hartman et al. (12) and by
Schultze (13), that high blood urea levels and renal hypertrophy frequently occur among deficient animals. The observations of Chow et al. (14) that the kidneys of rats contained much of the radioactivity of Co\textsuperscript{60}-tagged vitamin B\textsubscript{12} following subcutaneous or oral administration may be of significance in this connection. The fact that vitamin B\textsubscript{12}-deficient rats had higher liver weights per 100 gm. of body weight may be explainable on the basis that such animals were still able to form or maintain their liver tissue near the normal level, while their ability to synthesize body tissues had been impaired. This hypothesis is plausible from the experimental evidence of Rosenthal et al. (15) that, after operative removal of 70 per cent of the liver from rats, both regeneration of liver protein and mitotic activity of the liver cells were not altered by preoperative depletion of protein or postoperative fasting. Thus, the liver seems to have preferential accessibility to the nutritive pool of the body when intake is insufficient to meet all demands.

**SUMMARY**

The administration of vitamin B\textsubscript{12} to the weanling young of female rats deficient in this vitamin enhanced the growth rate and increased the food intake. Analyses of the carcass of rats demonstrated that, on a percentage basis, animals with vitamin B\textsubscript{12} deficiency have low fat, high water, and normal protein contents. The two abnormalities could be corrected by injection of vitamin B\textsubscript{12}. Livers of both young and adult rats in the control and injected groups showed no significant differences in percentage composition of glycogen, nitrogen, or fat. However, in the deficient animals the body weight was smaller relative to the liver weight in comparison with the injected controls. These results are taken to indicate that this vitamin plays a rôile in carbohydrate or fat metabolism rather than in protein metabolism.

**BIBLIOGRAPHY**

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