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Absorption and Tissue Concentrations of Vitamin B_{12} in Obese Rats. (25787)

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Bosshardt *et al.*(1) noted that growth of weanling mice on a high fat diet was markedly improved by administration of crystalline Vit. B_{12}. However, McCollum *et al.*(2) did not demonstrate any significant difference in growth of rats when Vit. B_{12} was added to diets containing different levels of fat in diet regardless of administration of Vit. B_{12}. Fox *et al.*(3) observed that the requirement for Vit. B_{12} by chicks was increased when the diet contained 22% fat, but that methionine alone could replace the increased requirement for this vitamin. They also showed that the higher Vit. B_{12} requirement was due neither to lowered absorption of dietary Vit. B_{12} nor to its subsequent storage in the liver. The involvement of Vit. B_{12} in conversion of pantothenic acid to coenzyme A has been suggested by Yacowitz *et al.*(4). In spite of these reports and the evidence presented by Ling and Chow(5) for the role of Vit. B_{12} in lipid metabolism in rats and, more recently, by Yesair *et al.*(6) in chicks, the metabolic relationship between Vit. B_{12} and lipids has not yet been established. Therefore, it is of interest to study the metabolism of this vitamin in obesity, an abnormal state of fat metabolism. The present communication presents a study of absorption and tissue concentrations of Vit. B_{12} in rats that became obese as a result of consuming a high fat diet.

Methods. Rats of the Osborne-Mendel strain were made obese by feeding *ad libitum* a diet containing 60% of fat by weight(7). They reached 1 kg of body weight in 10 to 14 months. Control rats were fed a 3% fat diet with the same composition as that of the 60% fat diet except that 57% sucrose replaced the Crisco; the control animals in earlier studies received Purina. The Vit. B_{12} concentrations in plasma and livers were determined using Skeggs' media and *Lactobacillus leichmannii* 4797 as the test organism(8). Cobalt-60 Vit. B_{12} with a specific activity of 1 $\mu c/\mu g$ was used for the absorption studies. Fifty $m\mu g$ of Co^{60}-B_{12} in 1 ml aqueous solution was administered orally through a plastic stomach tube to rats which had been fasted overnight. The feces were collected during the next 4 days, homogenized, and radioactivity was measured with a gamma-scintillation counter (9). The difference between the administered dose and amount of radioactivity recovered in the feces was considered as that which was absorbed.

Results. Vit. B_{12} levels in the plasma of obese rats were found to be higher than those in lean animals (Table I) and the differences are statistically significant. This difference is not explainable on the basis of food intake or dietary level of Vit. B_{12}, because both the 3% fat diet for control animals and the high fat diet for obese animals contained the same amount of Vit. B_{12} per gram

TABLE I. Vit. B_{12} Level of Plasma of Obese and Lean Rats.*

Group (% fat)†	Body wt (g)	Plasma B_{12} (mμg/ml)	B_{12} in diet (μg/g)
Control (3)	607	.840 ±.163‡	.200
Obese (60)	1173	1.780 ±.321	.200
Purina (10)	500	.537 ±.104	.036
Obese (60)§	895	1.260 ±.144	.200

* Five rats/group.
† % fat by wt in diet.
‡ Stand. error of mean.
§ Rats were on Purina diet for 10 mo, then placed on high fat diet for 6 mo.

(Table I). Furthermore, the obese rats consumed an average of 16 g of food per day compared to 20 g for the lean rats, therefore, intake of Vit. B_{12} was smaller. The slightly higher plasma Vit. B_{12} levels in the control group on the purified 3% fat diet as compared with those on the Purina laboratory chow may be due to the difference in Vit. B_{12} concentration in the diet. The difference in total plasma Vit. B_{12} would be even greater, since the obese rats have a greater plasma volume than the lean rat(10).

The results of Vit. B_{12} analyses in the livers of the obese rats are shown in Table II. Concentrations of Vit. B_{12} in the livers of obese animals were not significantly greater than those of the control animals. However, since the livers of obese animals were larger in size, total Vit. B_{12} content in the liver was greater in the obese than in the lean.

In view of these findings, it seemed worthwhile to investigate the intestinal absorption of Vit. B_{12} in such animals. The results of 2 experiments are presented in Table III. Radioactivity excreted in the feces following the oral dose of $Co^{60}B_{12}$ was smaller in obese rats than in lean rats and the difference is statistically significant. Thus, obese rats absorbed more of the administered Vit. B_{12}. Comparison of fecal excretion of radioactivity during the period of 4 to 7 days in the 2 groups (Study A) shows that excretion of radioactivity in this period was very small and was practically the same for the 2 groups of rats. Fecal excretion during this period may reflect the biliary and intestinal excretion of Vit. B_{12} which had already been absorbed (11).

To test whether composition of the diet rather than obese state of the animal brought about the difference in absorption rate of Vit. B_{12}, other experiments were carried out to study the effect of the fat content in the diet on absorption of Vit. B_{12}. Adult male rats were divided into 2 groups and fed the diets used in the previous experiments, containing fat at the level of 3 and 60%, respectively, for a period of 2 weeks. The rats were then tested for absorption of Co^{60}-B_{12}. The results (Table IV) indicate that there was no demonstrable difference in absorption rate between the 2 groups. Thus, it is apparent that the high fat content in the diet *per se* was not responsible for the increased absorption rate found in the obese rats.

Discussion. The published reports(1,3,4, 5,6) suggest that Vit. B_{12} may play a role in lipid metabolism, although the evidence is still insufficient. The data presented herein clearly demonstrate that the rats made obese on a high fat diet containing Vit. B_{12} had greater amounts of Vit. B_{12} in the liver and higher levels of plasma Vit. B_{12} than the lean controls receiving a low fat diet although both diets contained the same amount of dietary Vit. B_{12}. Since Vit. B_{12} was determined in fasting plasma, the elevated plasma level was not a post-absorptive phenomenon, but might be metabolically characteristic in obesity. Interpretation and significance of these observations can be only speculation at the moment. The elevated Vit. B_{12} levels may not be a reflection of elevated plasma lipid levels. Total

TABLE II. Storage of Vit. B_{12} in Livers of Obese and Lean Rats.*

Group (% fat)†	Body wt (g)	Liver wt (g)	Vit. B_{12} in liver mμg/g	Vit. B_{12} in liver μg/liver
Obese (60)	1155 ± 7.5‡	25.8 ± 2.34	288 ± 44.0	7.31
" (60)§	896 ± 77.9	17.4 ± .78	234 ± 15.2	4.03
Purina (10)	515 ± 26.3	13.4 ± 1.04	248 ± 10.0	3.35

* Five rats/group. † % fat by wt in diet. ‡ Stand. error of mean.
§ Rats were on Purina diet for 10 mo, then placed on high fat diet for 6 mo.

TABLE III. Fecal Excretion of Radioactivity Following Oral Administration of 50 mμg Co^{60}-B_{12} in Obese and Lean Rats.

	Diet	Body wt (g)	Vit. B_{12} excretion in feces (mμg)		
			Total 4 days	4 to 7 days	Absorbed (mμg)*
A	3% fat (6)†	643 ± 44.7	36.0 ± 2.06	1.2	14.0
	60% " (6)	1173 ± 72.4	24.9 ± 2.92	1.1	25.1
B	3% " (8)	562 ± 23.0	30.5 ± 1.33		19.5
	60% " (8)	995 ± 45.5	25.8 ± 1.34		24.2

* Calculated: 50 mμg − amt excreted in 4 days = amt absorbed. † No. of rats.

blood lipids in obese rats have been found to be only slightly higher than in lean rats under these conditions(12).

The increased plasma concentrations of Vit. B_{12} in obesity are probably due in part to increased absorption as demonstrated in this study, although it is possible that other factors such as decreased rate of utilization and of excretion of Vit. B_{12} are also involved. The increase in absorption in obese rats is not related to body weight of the animals. Earlier work showed that normal animals weighing between 150 and 500 g absorbed practically the same amount of Vit. B_{12} when 50 mμg was administered(13). The data in Tables III and IV also show that animals on the 3% fat diet absorbed the same amount of Vit. B_{12} even though there was a 3-fold difference in body weight of these animals. The increase in absorption does not seem to be directly related to high fat content in the diet, since the last mentioned experiment (Table IV) failed to demonstrate any immediate effect of difference in fat concentration in the diet on absorption of Vit. B_{12}. Rate of passage of Vit. B_{12} through the gastrointestinal tract has been shown to be one of the factors which determines absorption rate of this vitamin(14). The peristaltic movement of the gastrointestinal tract of the obese rats may be sluggish as are most of the physical activities in this abnormal nutritional state, thereby exposing Vit. B_{12} to the absorbing site of the intestinal mucosa longer than in normal lean rats. Although the mechanism for the increased rate of absorption is not clear, it is possible that the observed increase in Vit. B_{12} levels and absorption in obesity is a reflection of some metabolic interrelationship between this vitamin and fat.

TABLE IV. Fecal Excretion of Radioactivity Following Oral Administration of 50 mμg Co^{60}-B_{12} to Rats on High and Low Fat Diets.

Diet	Body wt (g)	Fecal excretion (mμg)	Amt absorbed* (mμg)
3% fat (8)†	240 ± 7.8	32.4	17.6 ± 1.25
60% " (8)	232 ± 8.9	34.9	15.1 ± 1.76

* See Table III. † No. of rats.

Summary. Rats made obese by feeding high fat diet had more Vit. B_{12} in the liver and higher plasma Vit. B_{12} levels than control lean rats receiving same concentration of Vit. B_{12} in low fat diet. The obese rats absorbed more of orally administered radioactive Vit. B_{12} than the controls. The increased absorption in obese rats, however, was not directly due to high percentage of fat in the diet and would therefore be accounted for by obesity. The possible involvement of this vitamin in lipid metabolism as based on these findings has been discussed.

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Clearance of Proteins from Blood of Normal and 6-Mercaptopurine Treated Rabbits.* (25788)

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Intravenously administered protein antigens disappear from the circulation of experimental animals in 3 distinct phases(1): (1) An initial equilibration of the antigen between intra- and extra-vascular compartments takes place during the first 24-48 hours after its injection. During this phase about two-thirds of the injected antigen leaves the intravascular compartments and is distributed in the extra-vascular space (equilibration phase). (2) A logarithmic decline in circulating antigen then occurs. This phase of antigen disappearance is apparently due to catabolism of the injected protein. Circulating antibody has not been detected during this interval, which lasts a variable period of time, depending on nature of the antigen and state of sensitization of the recipient animal (exponential phase). (3) A rapid disappearance of circulating antigen begins next. This so-called immune elimination phase is due to removal of antigen-antibody complexes from the circulation, presumably by the reticulo-endothelial system(2). The exponential phase of antigen elimination has usually been ascribed to a non-immunological mechanism, but some investigators have presented data which suggest that clearance of antigen in the rabbit during this time may be due to formation of antibody, perhaps of a non-circulating type (3). This paper presents evidence that the logarithmic phase of antigen disappearance is substantially the same both in normal rabbits and in rabbits made incapable of responding to antigen by 6-mercaptopurine treatment. This means of suppressing immunity was chosen since it has been demonstrated previously that 6-mercaptopurine can abolish primary immune response of the rabbit to purified antigens(4).

Materials and methods. White, New Zealand strain rabbits weighing between 2 and 3 kg were housed in an air-conditioned animal room and fed water and Purina Rabbit Chow *ad lib.* The drinking water contained NaI, 10 mg/100 ml. Radioiodinated human serum albumin was obtained from Abbott Laboratories (RISA). Bovine gamma globulin (BGG) (Pentex, Fraction II, Lot No. 0609), rabbit gamma globulin (RGG) (Pentex, Fraction II, Lot No. 6903) and rabbit serum albumin (RSA) (Pentex, Fraction V, Lot No. 7503) were iodinated according to the method of Weigle and Dixon(5). Injections, bleedings and measurements of radioactivity were carried out as described previously(4). The T½ of the second phase of antigen disappearance was determined by graphic analysis. Fifteen animals were treated with 6-mercaptopurine according to previously described methods (4).

Results. The results are shown in Table I and Fig. 1 and 2. For each molecular species of protein the half-life of the exponential phase was shorter for heterologous material than for homologous protein. Thus, during this phase, half-life of RGG was 4.6 days, while that of BGG was 2.0 days; similarly, half-life of RSA was 5.4 days, while that of HSA was 3.4 days. Those animals in which antibody formation was inhibited by 6-mer-

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