the ethnic group of the subjects being reported on.

Summary. Serum protein analyses of 3 ethnic groups (American Indian, Negro and Caucasian) revealed the following differences: 1. Indian and Negro subjects have a lower percentage of albumin and a higher percentage of beta and gamma globulins than do Caucasians. 2. Indian subjects have a higher percentage of alpha 1 globulin and a lower percentage of alpha 2 globulin than do Caucasians and Negroes.

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Utilization of Vitamin B_{12} by Rats With Pantothenic Acid Deficiency. (27774)

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The interrelationship between the metabolic roles of Vit. B_{12} and pantothenic acid has been recognized. Livers of chicks deficient in Vit. B_{12}(1) have high pantothenic acid contents. Vit. B_{12}(2) can spare the amount of pantothenic acid needed to restore the growth rate of chicks with pantothenic acid deficiency and vice versa. The same investigators found higher Vit. B_{12} contents in the liver of pantothenic acid deficient chicks and postulated that the lower Vit. B_{12} levels in pantothenic acid treated chicks are the result of rapid exhaustion of Vit. B_{12} from the stores due to growth requirements. In our laboratory, however, it was found that the newborn and young growing rats actually have lower liver Vit. B_{12} stores than the adult. It appears that growth with its accompanying requirement for Vit. B_{12} cannot account for such a difference in liver Vit. B_{12} levels. Therefore, some other mechanism in metabolism or utilization of this vitamin in pantothenic acid deficient rats must be explored. A study, therefore, was undertaken to investigate in rats the effect of Vit. B_{12} on pantothenic acid deficiency, and Vit. B_{12} levels in liver and plasma as well as the pattern of absorption and excretion of Vit. B_{12} in pantothenic acid deficient rats. The results of such a study are now presented.

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Methods. Preparation of pantothenic acid deficient rats: Pregnant rats were removed

TABLE I. Composition of Pantothenic Acid Deficient Diet.

Ingredients	%
Alcohol extracted casein*	24.0
Sucrose	64.0
Salt mixture IV†	4.0
Crisco‡	8.0
To each kg of ration the following vitamins were added:	
Thiamine	2.0 mg
Pyridoxine HCl	2.0 "
Riboflavin	5.0 "
Para-aminobenzoic acid	5.0 "
Nicotinic acid	10.0 "
Inositol	200.0 "
Choline chloride	500.0 "
Vitamin A (from percomorph oil, Mead Johnson)	12,510 I.U.
Vitamin D (from percomorph oil, Mead Johnson)	1,772 "
Vitamin K (menadione)	2.1 mg
Vitamin E (alpha tocopherol)	23.0 "

Fat-soluble vitamins were dissolved in Mazola oil in quantities to yield the above amounts in 2 cc of solution. Water-soluble vitamins were dissolved in 50% ethanol in amounts to yield the desired dosage in 20 cc solution.

* Vitamin test casein, General Biochemicals, Inc.
† Hegsted, Mills, Elvehjem, Hart, *J. Biol. Chem.*, 1941, v138, 459.
‡ Hydrogenated cottonseed oil, Swift and Co.

from the breeding stock cages and put into individual cages 3 or 4 days before casting. They continued on the stock diet until delivery. Several hours afterwards the mothers were fed the pantothenic acid deficient diet (Table I). To prevent the young from falling through, the meshed screened bottoms were removed from the cages. The mothers were provided with bedding material (wood shavings) until the young were 5-6 days old. At this time the screen bottoms were returned.

To reduce the mortality of mothers and young, it was necessary to add 2 mg of calcium pantothenate to each 100 g of the mother's ration. When the young were 28 days old, they were separated from their mothers. The weanlings were kept on the deficient diet and weighed weekly. At the end of approximately 4 additional weeks, their weights remained stationary and the animals were ready for testing.

To produce *adult* rats with pantothenic acid deficiency, they were fed pantothenic acid deficient diets for 7-14 weeks until the gross deficiency manifested itself. The control animals were fed the same diet and received Ca pantothenate by subcutaneous injection (100 μg three times/week). Determination of Vit. B_{12} activity in liver and plasma: Test animals were sacrificed by cardiac bleeding under ether anesthesia. Plasma was obtained from the heparinized blood by centrifugation for Vit. B_{12} determination. The liver was removed immediately and approximately 1 g of it was dissected from the left lobe, weighed and homogenized with cold distilled water in a cold room to yield a 5% homogenate. To 2 ml of the homogenate were added 50 μg NaCN, and the mixture was diluted with acetate buffer to give a final concentration of 0.03 M of the buffer. The diluted homogenate was then autoclaved at 120°C for 20 minutes. Upon cooling, the suspension was centrifuged and the clear supernatant fluid was neutralized with a half volume of 0.15 M Na_2HPO_4. The following amounts of neutralized liver extract were added to 10 ml of Skegg's media: 0.025, 0.05, 0.1 and 0.3 ml. The growth of *Lactobacillus leichmannii* #4797 was measured turbidimetrically after 40 hours of incubation. Plasma Vit. B_{12} determination: Plasma was added to 5 ml medium in amounts of 0.025, 0.05 and 0.075 ml, and the growth of the microorganisms was measured titrimetrically after 70 hours of incubation. Measurement of Vit. B_{12} absorption: Co^{60} labeled Vit. B_{12}‡ with a specific activity of 800 μc/mg was used. One ml of aqueous solution of $Co^{60}B_{12}$ containing 50 mγ in one study and 30 mγ in another study, was fed to rats by stomach tube, and the animals were transferred to individual metabolism cages for collection of fecal and urine specimens for 5 days in 2 periods of 2 and 3 days, respectively. The difference between oral dose and fecal excretion was taken as our measure of gastrointestinal absorption. Five days after administration of radioactive Vit. B_{12}, the animals were sacrificed. Liver, kidneys and the gastrointestinal tract were removed for radiometric measurement in a gamma scintillation counter with a flat crystal of thallium activated sodium iodide installed

‡ Merck's preparation.

TABLE II. Liver Vit. B_{12} Contents of Pantothenic Acid Deficient Rats.

Group	Diet	Treatment	Avg wt	Liver Vit. B_{12} concentrations (mμg/g)
A (10)	PaA def.	None	41.3	.188 $\pm$.020
B (7)	"	PaA-Ca inj.*	59.7	.112 $\pm$.012

Animals were fed PaA deficient diets for 21-23 days from time of weaning.
Numbers in parentheses denote No. of rats used in each group.
* 100 μg $\times$ 3/wk.

in a well-type sample holder. Measurement of excretion and retention of Vit. B_{12}: Twenty μg $Co^{60}B_{12}$ were injected subcutaneously to rats; the radioactivity in urine and feces as well as in various organs was measured.

Results and discussion. Pantothenic acid deficiency and Vit. B_{12} levels of the liver and plasma: *Experiment 1*: Three litters of pantothenic acid deficient weanling rats were divided randomly into 2 groups, (A) and (B). Both groups were offered the pantothenic acid deficient diet for 21-23 days but the control group (B) received Ca pantothenate by injection. Those data (Table II) indicate that the pantothenic acid deficient rats had significantly higher Vit. B_{12} contents in liver ($p<0.05$) than the treated control. *Experiment 2*: Thirty-day-old male rats were divided initially into 2 groups (A and B) and all animals were fed the pantothenic acid deficient diet. However, Group A (control), but not Group B, received Ca pantothenic acid by injection. At the end of 7 weeks, definite signs of deficiency developed in Group B. All animals in Group A and only one-half of the animals in Group B were sacrificed for determination of Vit. B_{12} levels of liver and plasma. The remaining half in Group B were given by injection Ca pantothenic acid 100 μg 3 times/week, for 2 weeks (Group C) and was then sacrificed for the same determinations. The results are given in Table III, and indicate that pantothenic acid deficient rats (Group B) have higher liver Vit. B_{12} contents than those receiving Ca pantothenic acid (Group A). Group C, which received Ca pantothenic acid after the manifestation of deficiency showed an average liver Vit. B_{12} level falling between Groups A and B. However, an examination of the data on plasma Vit. B_{12} levels of the 3 groups demonstrated no difference between Groups A and B. Group C had a higher Vit. B_{12} level than the other 2 groups ($p<0.05$). *Experiment 3*: The effect of Vit. B_{12} on pantothenic acid requirement was investigated by giving 2 different doses of Vit. B_{12} together with 2 different suboptimal doses of pantothenic acid. Survival rates of the animals were measured. Eight litters of pantothenic acid deficient weanling rats were divided randomly into 5 groups on the basis of body weight and litters, and fed the pantothenic acid deficient ration for 8 weeks. Group 1, receiving a physiological saline solution, served as control; Groups 2 and 3 received 2 μg Ca pantothenate mixed with 1 mμg or 100 mμg Vit. B_{12}/injection, respectively, and Groups 4 and 5 received 20 μg Ca pantothenate mixed with 1 mμg or 100 mμg Vit. B_{12}. Each injected dose consisted of 0.2 ml aqueous solution and three injections were given per rat. The number of surviving rats is given together with the liver Vit. B_{12} contents in Table IV.

All animals in Group 5 receiving 20 μg Ca pantothenate and 100 mμg Vit. B_{12} survived during the 8 weeks experimental period, whereas at least half of the animals from the other groups died. The pantothenic acid deficiency symptoms were also less distinct in Group 5. The difference between Groups 4 and 5 is statistically significant and is impor-

TABLE III. Effect of PaA-Ca Injection on Liver Vit. B_{12} Concentrations of PaA Deficient Rats.

Group	Treatment	Avg wt	Liver Vit. B_{12} (μg/g)	Plasma Vit. B_{12} (mμg/cc)
A (5)	PaA-Ca for 7 wk	278	.053 $\pm$ .010	.94 $\pm$ .05
B (6)	None	156	.152 $\pm$ .030	.96 $\pm$ .05
C (5)	Deficient diet 7 wk; PaA-Ca 2 wk	164	.093 $\pm$ .028	1.18 $\pm$ .01

Numbers in parentheses denote No. of rats used.

TABLE IV. Effect of Various Combinations of PaA and Vit. B_{12} on Mortality of PaA Deficient Rats.

Group	Dose of inj*	No. of survival (% mortality) At start	2 wk	4 wk	6 wk	8 wk	Liver Vit. B_{12} (μg/g)
1	—	8	7 (12.5)	3 (63)	3 (63)	2 (75)	.082 ± .008
2	PaA-Ca, 2 μg B_{12}, 1 mμg	8	8	4 (50)	3 (63)	3 (63)	.197 ± .132
3	PaA-Ca, 2 μg B_{12}, 100 mμg	8	8	6 (25)	5 (37)	3 (63)	.270 ± .040
4	PaA-Ca, 20 μg B_{12}, 1 mμg	9	7 (22)	7 (22)	4 (56)	4 (56)	.046 ± .009
5	PaA-Ca, 20 μg B_{12}, 100 mμg	8	8	8 (0)	8 (0)	8 (0)	.146 ± .016

* 3 times/wk.

tant in that 20 μg Ca pantothenate with 1 mμg Vit. B_{12} was not enough to keep all the animals alive, but the addition of 100 mμg Vit. B_{12} brought up the survival rate to 100%. At 2 μg level of Ca pantothenate supplementation of a large dose of Vit. B_{12} did not have any effect on survival rate. This dose of Ca pantothenate is apparently below the suboptimal dose.

The liver Vit. B_{12} contents were also measured at the end of 8 weeks. The mean value of liver Vit. B_{12} contents of Group 4 was lower than that of Group 1 (statistically significant), which is the same finding as in Exp. 1 and 2. In the groups in which 100 mμg Vit. B_{12} was injected 3 times a week, a similar trend was also observed; namely, Group 3 had higher liver Vit. B_{12} contents than Group 5. The difference in treatment among these groups was only the amounts of Ca pantothenate administered. Ca pantothenate, therefore, tends to lower the accumulation of injected Vit. B_{12} in liver. Vit. B_{12} transport from tissue to blood: This transport mechanism seems to be impaired in pantothenic acid deficiency. It has been generally accepted that plasma Vit. B_{12} levels reflect tissue Vit. B_{12} in animals and humans. There has not been demonstrated any abnormal equilibration of Vit. B_{12} between the tissue and plasma, although such an abnormality was suspected to exist in some of the metabolic conditions.

It has also been clearly demonstrated from the third experiment that if rats are kept on a diet containing suboptimal amounts of pantothenic acid, administration of a large dose of Vit. B_{12} has a sparing effect and increases survival rates. This finding seems contradictory in that pantothenic acid deficient rats have high microbiological activity of Vit. B_{12} in the liver, yet require more Vit. B_{12}. However, if utilization of liver Vit. B_{12} is interfered with, or the liver Vit. B_{12} is not metabolically available, administration of a large quantity of Vit. B_{12} could produce a beneficial effect from the injected Vit. B_{12}. In other words, one postulate is that pantothenic acid deficiency, the utilization of liver and plasma Vit. B_{12} is obstructed and this interference is manifested in abnormal distribution of vitamins in liver and plasma Vit. B_{12}. Such derangement of Vit. B_{12} equilibrium in the body makes it hazardous to interpret the state of Vit. B_{12} sufficiency on the plasma Vit. B_{12} levels alone.

From the studies on absorption and retention of injected radioactive Vit. B_{12}, it was shown that the deficient rats have normal capacities of absorption (Table V) regardless of the oral doses of Vit. B_{12} used. When Vit. B_{12} was given to rats by injection, the livers of the deficient rats retained more radioactivity than those of the treated ones on the unit weight basis. No difference in retention by kidneys or digestive tracts or in fecal excretion was observed between groups (Table VI). This fact can explain the accumulation which results in higher concentrations of Vit. B_{12} in the liver. This postulate is compatible with the finding that administration of pantothenic acid to deficient rats raised plasma

TABLE V. Absorption and Organ Distribution of Orally Administered $Co^{60}B_{12}$.

Exp.	Oral dose (mμg)	Group	Avg wt	Absorp.*	Recovery of Co^{60} (as mμg Vit. B_{12}) Liver Total	Liver Per 10 g	Kidney
A	50	Deficient (5)	262	31.9 ± .7	4.48 ± .29	5.98 ± .61	4.46 ± .16
		Treated (4)	309	29.7 ± 1.7	3.66 ± .12	3.85 ± .16	3.48 ± .40
B	30	Deficient (6)	156	11.7 ± 1.3	1.50 ± .17	2.78 ± .40	1.94 ± .11
		Treated (5)	278	13.1 ± 1.0	1.70 ± .31	1.55 ± .30	1.83 ± .21

* Calculated by subtracting fecal recovery of radioactivity from oral dose.

Treated group received 100 μg 3 times/wk of PaA-Ca. Numbers in parentheses denote No. of rats used.

TABLE VI. Excretion and Retention of Injected $Co^{60}B_{12}$.

Exp.	Group	Avg wt	Excretion of Co^{60} (mμg B_{12}) Urine	Feces	Total in 5 days	Recovery of Co^{60} in organs (mμg B_{12}) Liver Total	Liver mμg/10 g	Kidney	Dig. tract
A	Deficient (6)	266	2.17	1.66	3.83 ±.30	2.80 ±.14	3.03 ±.24	3.06	2.83
	Treated (4)	344	2.24	1.89	4.13 ±.39	2.38 ±.14	2.30 ±.19	2.91	3.00
B	Deficient (3)	248	1.75	1.77	3.52 ±.07	2.88 ±.31	3.78 ±.27	2.34	2.36
	Treated (3)	210	2.00	2.02	4.40 ±.10	2.59 ±.10	2.85 ±.19	2.12	2.12

Treated group received 100 μg PaA-Ca 3 times/wk. Numbers in parentheses denote No. of rats used.

Vit. B_{12} levels and lowered liver Vit. B_{12} concentrations as demonstrated in Exp. 2.

The proposed mechanism does not contradict any interrelationship in the intermediary metabolism between Vit. B_{12} and pantothenic acid. There will be more than one mechanism which results in elevated liver Vit. B_{12} levels in pantothenic acid deficiency. Retarded growth and less consumption of tissue Vit. B_{12} may partly account for the altered metabolism, although this does not seem to be an important mechanism. Vit. B_{12} may be related also to pantothenic acid in the intermediary metabolism. Since one of the many metabolic roles of Vit. B_{12} is to reduce –S–S– to –SH state(3), and SH is the active part of the CoA molecule, it is likely that Vit. B_{12} is concerned with the metabolic processes where CoA or pantothenic acid as part of CoA is involved. In fact, in the absence of Vit. B_{12}, CoA failed to be reduced to –SH form, thereby inhibiting the normal pathway of fat metabolism. Other supporting evidence comes from the work of Grasbeck(4), who demonstrated lowered acetylation of sulfonamide in Vit. B_{12} deficient rats. The impaired utilization of Vit. B_{12} in pantothenic acid deficiency demonstrated in our study confirms the close interrelationship between these two vitamins.

Summary. The metabolic pattern and the sparing effect of Vit. B_{12} were studied in pantothenic acid deficient rats with the following results: 1) The liver of pantothenic acid deficient rats had higher Vit. B_{12} content than the control. The plasma Vit. B_{12} level, however, was not elevated in the deficient rats. 2) Administration of a large dose of Vit. B_{12} to rats receiving a suboptimal dose of pantothenic acid had a sparing effect on survival rate. 3) Absorption and excretion of radioactive Vit. B_{12} were not impaired in pantothenic acid deficiency. 4) Radioactive Vit. B_{12} introduced either by intestinal absorption or by injection was retained more in the liver in pantothenic acid deficient rats than in the pantothenic acid supplemented control.

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