

provement on ACTH(1). It is also of interest that some patients receiving ACTH have been observed to develop urticaria as a manifestation of hypersensitivity to the ACTH preparation itself(16).

Summary. (1) Positive Prausnitz-Küstner reactions were consistently demonstrated in volunteers passively sensitized with sera from 2 hypersensitive donors treated with cortisone. (2) Positive Prausnitz-Küstner reac-

tions were similarly demonstrated in *cortisone treated* volunteers passively sensitized with sera of the cortisone treated donors. (3) There was no significant difference in the Prausnitz-Küstner reactions of those volunteers tested before and during treatment with cortisone. (4) It may be concluded that under the conditions of the experiments described, cortisone does not influence passively induced skin hypersensitivity in man.

16. Conn, J. W., Discussion of reference 2, p. 488.

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Diet of Mother and Vitamin B₁₂ Content of Tissues of Infant Rats. (18457)

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The vit. B₁₂ content of various organs and tissues of the rat was determined by a rat growth assay method and the kidney contained the largest amount(1). The vit. B₁₂ content of the liver, spleen, heart and kidney of 28-day-old rats from mothers receiving the basal diet and the basal diet supplemented with vit. B₁₂ concentrates from different sources is reported.

Experimental methods. The composition of the basal diet of the mothers is given in Table I. The females in Group 1 received the basal diet. Those in Group 2 received a supplement of 2.0 g of Merck's APF concentrate No. 3 per kilo of diet. Those in Group 3 received 1.0% of Lederle APF concentrate and those in Group 4 received 3.0% of liver "L." The mothers received the experimental diets from 28 days of age until they had an opportunity to produce at least 4 litters. The young in a few litters from each group were killed at 28 days and the tissues of 2 to 4 rats in each litter were combined into one sample. There were 1 to 3 samples of tissues per litter, depending upon the number of young in the litter. For ex-

TABLE I. Composition of the Basal Diet.

Basal mixture	%
Cerelose	58
Casein	30
Woodpulp	3
Mineral mixture(2)	5
Wesson oil	4
Vit. supplement per 100 g basal mixture	
Vit. A	3000 I.U.*
Vit. D	425 I.U.
	mg
Menadione	2.5
Alpha tocopherol	2.5
Thiamine hydrochloride	1
Riboflavin	1
Pyridoxine hydrochloride	1
Ca pantothenate	4
Niacin	5
Choline chloride	100
Biotin	.02
Inositol	10
Para amino benzoic acid	50

* Vit. A and D were supplied by Mead Johnson's oleum percomorphum.

ample, if there were 10 young in a litter the tissues from 4, 3 and 3 rats respectively were combined to give 3 different samples. Vit. B₁₂ was liberated from the tissues by the procedure described by Couch *et al.*(3) and

1. Lewis, U. J., Register, U. D., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 509.

2. Richardson, L. R., and Hogan, A. G., *J. Nutrition*, 1946, v32, 459.

3. Couch, J. R., and Olcese, O., *J. Nutrition*, 1950, v42, 337.

TABLE II. Effect of Vit. B₁₂ in Diet of Mother on Vit. B₁₂ Content of Tissues of Young.

Group No.	Source of vit. B ₁₂	No. of rats	No. of samples	Avg μg B ₁₂ per 100 g tissue			
				Liver	Spleen	Heart	Kidney
1	None	14	4	6.1 (4.1-7.3)*	7.7 (7.0-8.3)	1.7 (1.4-2.3)	11.3 (8.8-13.8)
2	Merck APF No. 3 2 g/kg (55 μg B ₁₂)	49	17	8.2 (4.0-13.9)	11.4 (1.7-19.5)	4.2 (1.5-7.0)	10.6 (6.2-17.9)
3	Lederle APF concentrate 10 g/kg (6 μg B ₁₂)	13	5	8.4 (5.2-10.9)	11.7 (10.1-15.5)	2.9 (2.2-3.9)	13.5 (10.5-19.3)
4	Liver "L" 30 g/kg (37 μg B ₁₂)	18	6	12.9 (7.5-16.8)	12.0 (10.1-14.6)	3.4 (1.4-5.5)	10.8 (7.3-15.1)

* Numbers in parentheses equal range.

TABLE III. Amount of Vit. B₁₂ in Different Samples of Tissue from the Same Litter.

Litter No.	No. of tissues in sample	μg of B ₁₂ per 100 g tissue							
		Liver		Spleen		Heart		Kidney	
		Single	Avg	Single	Avg	Single	Avg	Single	Avg
1	2	4.0		1.7		5.4		11.2	
		6.2	5.1	13.8	7.8	4.5	5.0	7.2	9.2
2	4	13.9		14.2		3.6		17.9	
		12.2	13.1	14.8	14.5	7.0	5.3	10.2	14.1
3	3	4.9		9.5				8.8	
		7.4	6.2	10.7	10.1	2.3	2.3	9.1	9.0
4	4	4.1				1.5		12.4	
		5.9	5.0	8.3	8.3	2.3	1.9	10.1	11.3
5	4	16.8		12.0		5.5			
	3	15.7		10.1		3.0			
	3	16.5	16.3	14.6	12.2	3.5	4.0	15.1	15.1
6	3	10.9		15.5					
	3	9.3		11.9		2.2		19.3	
	2	10.0	10.1	10.1	12.5	3.9	3.1	10.5	14.9

was determined in the extract by the method described by Skeggs *et al.* (4). Thiomalic acid was added to the medium as a growth factor for *L. leichmannii* 4797 (ATCC) and as a reducing agent to protect vit. B₁₂ during autoclaving. Crystalline vit. B₁₂ was used as the standard and all the assays were autoclaved for only 5 minutes prior to inoculation. Acid production was used to measure growth response.

Results and discussion. The range and the average amounts of vit. B₁₂ per g of tissue are given in Table II. The amount in the liver ranged from an average of 6.1 μg B₁₂ per 100 g for those receiving the basal diet to

12.9 μg for those receiving liver "L." The livers from the groups receiving Merck's and Lederle APF concentrates contained an average of 8.2 and 8.4 μg B₁₂ per 100 g respectively.

The spleens from the basal group contained an average of 7.6 μg of vit. B₁₂ per 100 g and when the mother received a vit. B₁₂ concentrate it was increased to a range of 11.4 to 12.0 μg B₁₂ per 100 g. The hearts from the basal groups contained 1.7 μg of vit. B₁₂ per 100 g and those from the groups receiving a source of vit. B₁₂ ranged from 2.9 to 4.2 μg B₁₂ per 100 g. The vit. B₁₂ content of the kidney ranged from an average of 10.6 to 13.5 μg B₁₂ per 100 g and the difference between groups was not important.

4. Skeggs, H. R., Huff, J. W., Wright, L. D., and Bosshardt, D. K., *J. Biol. Chem.*, 1948, v176, 1459.

In a comparison of the amount of vit. B₁₂ in different tissues, the kidney contained more than the liver with the exception of the group receiving liver "L," which is in agreement with the work of Lewis, Register, and Elvehjem(1). When the diet contained liver "L" the liver contained slightly more than the kidney. The spleens contained approximately the same amount as the kidney with the exception of those in the basal groups. The spleens of this group contained slightly more than half that in the kidney. The amounts in the heart were relatively small compared with that in the other tissues.

The amounts of vit. B₁₂ in different samples of tissue from the same litter are given in Table III. There it may be seen that the values were in the same range, with the ex-

ception of those for the spleen in litter 1, for all the samples within a litter.

Summary. The amount of vit. B₁₂ in the liver, spleen and heart of 28-day-old rats was approximately doubled when a source of vit. B₁₂ was added to the diet of the mothers. The diet of the mother had no important effect on the vit. B₁₂ content of the kidney. In general the kidney and spleen contained slightly more vit. B₁₂ than liver. The heart contained a relatively small amount of vit. B₁₂ in comparison to other tissues.

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Infra Red Spectroscopy of Tissues. Effect of Insulin Shock.* (18458)

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Infra spectra of organic compounds are with a few exceptions, highly characteristic and specific. For this reason study of infra red spectra of tissues has been considered as an adjunct method of tissue analysis(1). In our attempt at evaluating the potential use of infra red spectroscopy for tissue studies 2 characteristics of this physical method became apparent. 1. The locations of absorption bands of molecules are so extremely sensitive even to slight changes of molecular structure that it is necessary to resort to empiricism and to draw conclusions about the structures by correlation between bands which occur regularly with recurring structural features throughout a series of material examined. 2. The infra red energy emitted by all commercial instruments is so small that loss of

energy as may occur in examination of very small structures may make qualitative observations inaccurate and quantitative studies futile. The present paper is a report on our method for infra red spectroscopy of tissues, on qualitative spectroscopy of various tissues especially neural tissue, and on quantitative infra red spectroscopy in brain tissue of control rabbits and rabbits in insulin shock. For this study we have selected to investigate tissue masses rather than smaller units. The selection of brain areas, *viz*: diencephalon, hippocampus, cerebellum, and tectum was largely governed by the clinical manifestations of disordered neural functions displayed during insulin shock.

Preparation of tissue sections. The organs of the animals were rapidly frozen with dry ice immediately after the animal was killed, in almost all instances, by injection of air into the heart. For spectroscopy, frozen sections of 50 μ and, for some purposes, 25 μ thickness prepared by the technic of Adam-

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1. Blout, E. R., and Mellors, R. C., *Science*, 1949, v110, 137.