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Nutritional Effects on the Development and Atrophy of the Thymus.

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In inanition (Jonson)[1] and in Vitamin B complex deficiency (Cramer, Drew and Mottram)[2] low thymus weights are found. Griffith and Wade[3] have reported a graded response of thymus weights when increasing supplements of choline are added to "choline deficient" diets. Christensen and Griffith[4] suggest that "choline probably represents the factor required for maintenance of the thymus."

The thymus data recorded below taken from a larger series of experiments dealing with various phases of the behavior of rats on single deficiencies of B factors (Berg and Zucker)[5] throw further light on thymus growth and thymus atrophy.

Results. The basal diet contained 26.7% of casein. The salt mixture was essentially that of Wesson. The fat soluble vitamins were given as a solution of cod liver oil carotene and wheat germ oil in cotton seed oil, 1 mg of niacin and 10 mg of choline hydrochloride were given daily to each rat. The B factor variables are given in Table 1, columns 1-4. Series A represents controls. In each of the experiments of Series B one of the 4 factors under investigation was completely omitted while Series C represents partial deficiencies. The next 3 columns (5, 6, and 7) give respectively initial and final mean body weights and the mean observed thymus weight. In columns 8 and 9 are found the thymus weights as found in stock diet animals corresponding in body weight to the initial and final mean body weights of the experimental groups. These values were derived from a long series of observations covering the experimentally useful age and body weight range (Stoerk)[6]. They are used as a base line for judging thymus growth during the 4 weeks of experiment (column 9) and the deficit in thymus weight (column 10) shown by the experimental animals as compared with normal animals of similar body weight. The data of column 8 and 9 are quite comparable to those used by Christensen and Griffith for like purposes. Column 12 designates by plus and minus signs the degree of atrophic changes seen in sections.

No attempt is made to put an exacting interpretation on the numerical values but the following indications seem reasonable.

In experiment A1 (positive control) the body weights and thymuses grow as in stock diet animals. From 4 weeks to 8 weeks of age body weight increases from 62 to 150 g and the thymus weight increases by 146 mg. A2 may be considered a negative control in which all four B factors under investigation were omitted from the diet. There is a body weight loss of 11 g and the thymus weight is 248 mg less than that found for normal body weights corresponding to the initial body weight.

With total deficiency of riboflavin and of pantothenic acid (B_1 and B_2) body growth is fair and there is no marked loss of thymus tissue (although the mean values for thymus increase are negative) but the observed thymus weight falls distinctly below that of normal animals corresponding to the final body weights.

With the total thiamin or pyridoxin deficiency (B_3 and B_4) there is also no net loss

1 Jonson, A., *Arch. f. mikr. Anat.*, 1909, **73**, 390.

2 Cramer, W., Drew, A. H., and Mottram, J. C., *Lancet*, 1921, **201**, 1202.

3 Griffith, W. H., and Wade, N. J., *J. B. C.*, 1939, **131**, 567.

4 Christensen, K., and Griffith, W. H., *Endocr.*, 1942, **30**, 575.

5 Berg, B. N., and Zucker, T. F., Abstracts 107th A.C.S. Meeting, Cleveland, Ohio, April, 1944.

6 Stoerk, Herbert C., *Endocr.*, 1944, **34**, 329, and data to be published.

in body weight but the thymuses decidedly fall below the weight at the beginning of the experiments. The deficit below the thymus weights expected for final body weights is not much larger because there was little body growth.

While in all the experiments under B_1 there was a net gain in body weight, the growth was not necessarily continuous. Effects on the thymus weight might therefore be looked upon as inanition phenomena rather than specific effects of the particular vitamin deficiencies. In series C partial deficiencies were produced by giving 1/20 to 1/50 of the approximate requirement of the particular factor under investigation. In these experiments net growth was somewhat better and was in every animal continuous, *i.e.* no weight losses occurred during the 4 weeks of experiment.

In the case of partial deficiency of riboflavin and pantothenic acid there was increase in thymus weight to about half the extent of the thymus increase in optimally growing rats. Still the thymus did not reach weights normal for the final body weights achieved. With partial thiamin deficiency the thymus weight did not deviate much (—26 mg) from that corresponding to the initial body weight. In marked contrast to this the animals with partial pyridoxin deficiency lost an estimated 153 mg of their initial thymus weight while there was better body growth than in the corresponding thiamin deficient rats.

Summary. 1. In otherwise adequate diets complete or partial deficiency of riboflavin, pantothenic acid, thiamin or pyridoxin depresses the thymus weight of female albino rats below that of normally grown controls of corresponding body weight. 2. With complete deficiency of any one of the four factors mentioned, there is mild to marked atrophy of the thymus which may however be referable to losses in body weight. 3. On a diet partially deficient in thiamin which produces small but continuous body weight increases, thymus growth may be arrested for the four weeks duration of the experiment while under similar conditions partial pyridoxin deficiency produces marked atrophy.

The observations all refer to an age range before "age involution" sets in. The atrophic

TABLE I.

		1	2	3	4	5	6	7	8	9	10	11	12	
		B supplements γ/day				Mean body wt, g		Mean observed thymus wt, mg	Mg thymus corresponding to body wt		Thymus inc. over initial	Deficit below expected final thymus wt	Histol. signs of atrophy	No. of animals
		Panto	Ribo	Thia	Pyri	Initial	Final		Initial	Final				
A	1	200	80	40	50	62	150	536	290	536	+146	0	—	12
	2	0	0	0	0	58	47	12	260	240	—248	—228	+++	10
B	1	0	80	40	50	68	105	301	320	420	— 19	—119	—	3
	2	200	0	40	50	63	103	256	300	410	— 44	—154	—	7
	3	200	80	0	50	67	85	128	310	360	—182	—232	++ to +++	6
	4	200	80	40	0	66	88	77	310	370	—233	—293	++ to +++	4
C	1	5	80	40	50	60	114	361	280	440	+ 81	— 79	—	8
	2	200	2	40	50	61	105	354	290	420	++ 64	— 66	—	11
	3	200	80	2	50	59	94	234	260	390	— 26	—156	—	7
	4	200	80	40	1	62	103	127	290	410	—153	—273	+ to ++	7

changes would be classed under the term "accidental involution." They occurred in rats each of which received 10 mg of choline hydrochloride per day.

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Effect of the Endotoxin of *Shigella paradysenteriae* on Pregnancy in Rabbits.

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We have previously reported that the endotoxins of certain gram-negative bacteria, when injected into mice at middle and late pregnancy, induce decidua-placental hemorrhage which often leads to abortion or resorption of the embryos.[1] This hemorrhage induction was considered to be analogous to that induced in implanted mouse tumors by the endotoxins of essentially all gram-negative bacteria. The present study was undertaken to ascertain the effect of such endotoxins on pregnancy in rabbits.

Methods. The acetone precipitate of the residue of dialyzed whole cultures of *Shigella paradysenteriæ* (Flexner) was chosen as containing a representative O antigen of Boivin-type, the principal endotoxin of most gram-negative bacteria. Methods of culture, preparation and standardization are described elsewhere.[2]

The experimental procedure consisted of the intraperitoneal injection into rabbits at various stages of pregnancy of an arbitrarily selected sublethal dose of the Flexner extract. This dose (16 mg) of relatively unpurified material was equivalent to 2 LD_{50} for 20 g white mice or about one-fifth of that lethal to rabbits of 3-4 kg. This high sensitivity of rabbits to the endotoxin of gram-negative bacteria has been noted by other investigators.[3]

The female Rockland rabbits used in this study were multiply mated, a procedure assuring 80-100% true pregnancies. Rabbits so mated received intraperitoneal injections of the endotoxin-containing extract at one of the following stages of pregnancy: (1) 48 hours after mating, *i.e.*, after ovulation and fertilization, but before uterine implantation, (2) 6-7 days after mating, *i.e.*, during the implantation period, (3) 11-12 days after mating, *i.e.*, post-implantation and during development of placental structures, (4) 15-16 days and (5) 19-21 days after mating, *i.e.*, during active growth of embryos and placental tissues, and (6) 25-26 days after mating, *i.e.*, approaching term. Following injection, the animals were observed daily for vaginal bleeding or for evidence of abortion. They were either allowed to proceed to term, or were sacrificed 5 to 10 days after injection, when post-mortem examination was made of the reproductive organs and embryonic tissues. If the embryos and associated membranes appeared to be normal in such sacrificed animals, it was assumed that pregnancy had not been impaired or interrupted.

Results. Of 6 animals injected 48 hours after mating, 5 failed to show further signs of pregnancy. The sixth delivered a normal litter. Of the animals injected at the 6-7-day stage, only one out of 7 underwent interruption of pregnancy. At the 11-12-day stage, 2 out of 4 animals had litters. In the 4 animals at the 15-16-day stage and in the 7 animals at the 19-21-day stage there was uniform interruption of pregnancy although 2 animals of the latter group died within 4 days after injection. At the late stage of 25-26 days 2 out of 9 rabbits delivered normal litters. Among the remaining 7, 3 aborted and 4 died, possibly

[1] Zahl, Paul A., and Bjerknes, C., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 329.

[2] Zahl, Paul A., Hutner, S. H., and Cooper, F. S., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 48; 1943, **54**, 187; Zahl, Paul A., and Hutner, S. H., *Am. J. Hyg.*, 1944, **39**, 189.

[3] Grasset, E., Zoutendyke, A., and Schaafsma, A., *Brit. J. Exp. Path.*, 1935, **16**, 454; Boivin, A., and Mesrobeanu, L., *C. R. Soc. Biol.*, 1935, **118**, 614.