

linear function. We attribute this to an obscure factor(s) which in some manner probably interferes with the activity of Ac-globulin. Our data suggest that this factor may be fibrinogen concentration. In a previous publication(7) and in quantitative data to be published, the importance of fibrinogen on Ac-globulin activity has been stressed. In constructing the reference curves from the normal control plasmas, it was observed that the maximum deviation of areas as well as the variation of slopes became progressively less as the accurate volume range was approached. Moreover, the maximum deviation of areas and slopes was decidedly less with defibrinated than with undefibrinated plasma. When 1 to 5 x 10⁻² ml of human plasma were used, the slopes of the lines derived from undefibrinated plasma were consistently less than those from defibrinated plasma. As the volume of plasma was increased to the accurate range, the slopes of the undefibrinated and of the defibrinated plasma approximated each other. Thus, the observations would indicate that fibrinogen alters the Ac-globulin activity and therefore the slopes of the reference curves, and that Ac-globulin assays can be done more ac-

curately on defibrinated plasma.

Variables, such as temperature, reactivity of the biologic reagents etc. which affect other assay methods in blood coagulation, likewise exert their influence on the assay of Ac-globulin activity. The influence of these variables is manifested as a shift in intercept of the reference control curves from day to day. These variables do not alter significantly the slope of the reference curves. The existence of such variables, however, does demand that the intercept be established each day by the assay of Ac-globulin activity on normal control plasma.

In the assay of Ac-globulin activity, the prothrombin concentration is adjusted so that 2 units rather than 1 unit are present in the clotting tube. As a result, a smaller volume of plasma is required for the estimation of Ac-globulin activity, and the effect of anti-thrombin on the conversion rate and thrombin yield is correspondingly decreased.

Summary. A method for the quantitative estimation of Ac-globulin in plasma has been described. The method is based upon the use of three variables—conversion rate, thrombin yield, and onset of thrombin formation. With the use of all three variables combined, a greater degree of accuracy can be obtained.

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Effects of B Vitamins and Liver on Growth of Immature Rats Maintained at Low Temperatures.* (18153)

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Available data indicate that requirements for a number of nutrients are markedly in-

creased under conditions of low environmental temperature. An increased requirement for thiamine(1,2), riboflavin(3), pyridoxine(4),

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ascorbic acid(5), and vitamin A(6) has been demonstrated in animals maintained for prolonged periods of time at a low environmental temperature. Available data indicate that requirements may be increased for other nutrients as well. Smith *et al.*(7) observed that desiccated whole liver counteracted in part the growth retardation of immature rats maintained at low environmental temperatures. The protective factor was distinct from any of the known B vitamins (with the possible exception of vitamin B₁₂ which had not been isolated at that time). In the present communication data are presented on the comparative effects of various liver fractions and the known B vitamins on the growth and testicular weight of immature rats maintained under conditions of low environmental temperature.

Procedure and results. Five experimental rations were employed in the present experiment: diets A,B,C,D and E. Diets A and B were purified rations containing the B complex factors in synthetic form only. Diets C,D and E were similar in composition but contained desiccated whole liver, water-insoluble liver residue and water-soluble liver concentrate 1-20 in addition to the synthetic vitamins. One hundred and twenty male rats of the Long-Evans strain were selected at 25 to 28 days of age and an average weight of 59.5 g for the present experiment. Animals were kept in metal cages with raised screen bottoms to prevent access to feces and were fed *ad libitum* the diets listed in Table I. Experiments were conducted (1) with animals kept continuously in a large walk-in refrigerator at a temperature of $2 \pm 1.5^\circ\text{C}$ and (2) under standard laboratory conditions at an average temperature of approximately $23 \pm 2^\circ\text{C}$. The dietary groups consisted of 16 rats each in the cold room series and 8 animals per group in the room temperature series. Animals were autopsied after 45 days of feeding, and testes, ventricular and adrenal weights determined.

Findings are summarized in Table II. Gain

TABLE I. Composition of Experimental Diet.

Dietary component	Diet A	Diet B	Diet C	Diet D	Diet E
Sucrose	60	60	50	50	57.5
Casein*	25	25	25	25	25
Cottonseed oil (Wesson)	10	10	10	10	10
Salt mixture†	5	5	5	5	5
Whole dried liver powder‡			10		
Extracted liver residue§				10	
Liver conc. powder 1-20					2.5

To each kg of diets A, B, C, D, and E were added the following synthetic vitamins: Thiamine hydrochloride, 40 mg; riboflavin, 40 mg; pyridoxine hydrochloride, 40 mg; nicotinic acid, 60 mg; calcium pantothenate, 80 mg; 2-methyl-naphthoquinone, 10 mg; and choline chloride, 2 g. In addition to the above each kg of diet B was supplemented with the following synthetic vitamins: Thiamine hydrochloride, 20 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; nicotinic acid, 60 mg; calcium pantothenate, 60 mg; p-aminobenzoic acid, 400 mg; inositol, 800 mg; folic acid, 10 mg; biotin, 4 mg; and vit. B₁₂, 30 mcg. Each rat also received once weekly 4.5 mg of alpha-tocopherol acetate and a vit. A-D concentrate containing 150 U.S.P. units of vit. A and 15 U.S.P. units of vit. D.*

* Vitamin test casein, General Biochemicals, Chagrin Falls, O.

† Hubbel, Mendel and Wakeman salt mixture, General Biochemicals, Chagrin Falls, O.

‡ Whole dried liver powder, Armour and Co., Chicago, Ill.

§ Extracted liver residue, Wilson Laboratories, Chicago, Ill. This material consists of the coagulated, water-insoluble material remaining after the removal of the extractable water-soluble substances.

|| Liver concentrate powder 1-20, Wilson Laboratories, Chicago, Ill. This material consists of the water-extractable material of raw liver.

¶ Nopco fish oil concentrate, assaying 800,000 U.S.P. units of vit. A and 80,000 U.S.P. units of vit. D per g.

in body weight was significantly reduced in all rats under cold room conditions. Animals fed the added B vitamins (diet B) and those receiving the liver fractions (diets C, D and E) gained significantly more weight, however, than those fed diet A. Similar findings were obtained in respect to gonadal weight. The testes of rats fed diet A under cold room conditions were significantly smaller than those of animals fed diets B, C and D and to a lesser extent diet E. Under room temperature conditions, however, the gain in body weight of rats fed diet A did not differ significantly from that of animals fed diets B

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TABLE II. Summary of Experimental Data.
16 rats per group in cold room series; 8 animals
per group in room temperature series.

Dietary group	Initial body wt, g	Gain in body wt over 45 day period,* g	Testes wt,* g
Cold room series			
A	61.4	90.1 ± 4.8 (14)	1.08 ± .14
B	60.7	120.6 ± 6.5 (14)	2.10 ± .15
C	60.1	115.8 ± 4.6 (16)	2.10 ± .12
D	60.4	118.8 ± 4.7 (15)	1.98 ± .12
E	60.9	116.9 ± 5.5 (15)	1.68 ± .07
Room temp. series			
A	56.9	177.7 ± 12.3 (8)	2.30 ± .16
B	57.1	186.1 ± 11.9 (8)	2.77 ± .10
C	55.9	236.5 ± 9.6 (8)	3.02 ± .09
D	57.6	221.7 ± 12.6 (8)	3.01 ± .11
E	56.7	184.7 ± 7.6 (8)	2.52 ± .20

The values in parentheses indicate the number of animals which survived and on which averages are based.

* Including standard error of the mean calcu-

lated as follows: $\sqrt{\frac{\sum d^2}{n}} / \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

and E, although the growth increment was increased in rats fed whole liver (diet C) or extracted liver residue (diet D). The gonads were well developed in all rats in the room temperature series. The weight of the testes in rats fed diet A was smaller than that of animals fed diets B, C, D and E; differences between the various groups, however, were not as marked as in the cold room series. Ventricular and adrenal weights when expressed in terms of mg per 100 g body weight were significantly increased in all rats in the low temperature series, averaging 440.2 mg and 18.3 mg respectively for animals under cold room conditions in contrast to 312.0 mg and 11.4 mg for rats maintained at room temperature. Differences between the various dietary groups, however, were not statistically significant.

Discussion. Available data indicate that

in addition to the major nutrients, there are substances present in our diet which may be required in increased amounts during conditions of stress. Such factors are apparently dispensable under normal conditions or their requirements are so small they may readily be met by amounts present in the diet or through the synthetic activity of the intestinal flora or the animals' own tissues. Body requirements for such nutrients may be increased by such factors as physical exertion, increased caloric intake, fever, thyroid-feeding and other conditions resulting in an increased metabolic rate. Such "stress factors" raise body requirements beyond the usual or average range, accentuate deficiencies and hasten the onset of symptoms on diets that would otherwise be adequate in the absence of the particular stress factor employed (8). Present findings indicate that prolonged exposure to cold is such a stress factor.

Under room temperature conditions diet A is an adequate diet for growth and gonadal development in the rat. Unpublished work from this laboratory indicates that male rats raised to maturity on this ration sire apparently normal young and female rats exhibit regular estrus cycles and give birth to living young although the lactation performance is poor. Present findings indicate that under room temperature conditions supplements of the known B vitamins (diet B) did not augment the gain in body weight of immature male rats over that obtained on diet A although gonadal weight was somewhat larger on the supplemented ration. In the cold room series, however, both body and gonadal weight were significantly greater on diet B. These findings indicate that prolonged exposure to cold increased requirements for one or more nutrients in diet B which were either absent or present to a smaller extent in diet A. Since these diets differed only in their B vitamin content, it would appear that the protective effect of diet B was due to its B vitamin content. Whole liver (diet C), liver residue (diet D), and liver concentrate 1-20 (diet E) also promoted growth and gonadal develop-

ment of immature male rats under cold room conditions. The protective effect of these supplements, however, was no greater than that provided by the synthetic B vitamin supplements. Since previous findings(7) indicate that supplements of the known B vitamins (excepting vit. B₁₂) were without significant effect on the growth of immature rats fed a ration similar to diet A it would appear that the vit. B₁₂ content of diet B was responsible at least in part for its protective effect under conditions of low environmental temperature.[†]

Summary. A marked retardation in body and gonadal weight was observed in immature male rats fed a purified ration under conditions of low environmental temperature. Supplements of desiccated whole liver, water-insoluble liver residue or water-soluble liver

extract resulted in a marked increase in both body and gonadal weight. A supplement of the known B vitamins was similarly effective. It is suggested that the protective effects of the various supplements were due, at least in part, to their vitamin B₁₂ content.

[†] According to data supplied by the manufacturers Whole Dried Liver Powder contained approximately 1.1 mcg vit. B₁₂-like activity per g, Extracted Liver Residue from 0.2 to 0.4 mcg/g and Liver Concentrate Powder 1-20 from 3.0 to 5.0 mcg/g. Since the whole liver and liver residue were fed at a 10% level in the diet and the liver concentrate at a 2.5% level, the vit. B₁₂ content of diets C,D and E were approximately 110 mcg per kg of diet, 20 to 40 mcg per kg of diet and 75 to 125 mcg per kg of diet respectively.

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Toxicity and Effect of ω -Methylpantothenic Acid on Blood-Induced Infections of *Plasmodium Lophurae* in the Chick.* (18154)

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The inhibitory activity of certain analogs of pantothenic acid toward a variety of organisms has attracted attention during recent years. Several analogs, notably derivatives of pantoyltauramide and phenylpantothenone, have been found active for some strains of plasmodia(1,2). The relatively high bacteriostatic effect of ω -methylpantothenic acid

[N-(α , γ -dihydroxy- β , β -dimethylvaleryl)- β -alanine](3) prompted the present investigation on the antiparasitodal potentialities of this compound. The toxicity of this compound for the test animal, the chick, was extended to include its effect on rats, mice and on *Tetrahymena geleii* H.

Toxicity studies. Chicks. Preliminary experiments on a total of 20 chicks, 1-2 weeks old and weighing 53-85 g, showed no toxicity of single doses of sodium ω -methylpantothenate given orally in concentrations as high as 8.4 g/kg body weight, intravenously in concentrations up to 0.4 g/kg and intraperitoneally in concentrations up to 1.0 g/kg. The analog (80% pure) was administered as a neutral 30% solution. Ten birds injected intraperitoneally with higher single doses (3.2-4.5 g/kg) exhibited torpor, loss of appe-

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