

from one patient immediately before injection of the nitrogen mustard, 5 minutes after injection but before tourniquets were released, 2 minutes after tourniquets were released, and 3 hours after treatment was completed. No significant change occurred. The titers, in the order in which the specimens were taken, were 0.0058 ml, 0.0061 ml, 0.0060 ml, and 0.0062 ml per 50% hemolysis unit.

Discussion. This study indicates that in the neoplastic diseases studied there is no consistent deviation of complement activity from normal, although greater variability may occur than in normals. In a series of patients with various infectious diseases, Ecker *et al.*(6) had similar findings. These workers, however, found frequent high complement titers in streptococcus and pneumococcus infections, and frequent low titers in meningococcus meningitis, which differences they felt to be significant. Dulaney(5) has reported frequent low complement titers in malaria. Ecker *et al.*(6) reported a possible relationship of complement to severity of illness and serum protein concentration, but no relation to fever or leukocytosis. In the present work serum proteins were not studied, but there was no apparent relation between complement titer and extensiveness of disease, fever, de-

gree of debility, leukocyte count, or transfusions.

The results of the present *in vitro* experiments with nitrogen mustard and human complement are in general agreement with the results reported with guinea pig complement by Watkins and Wormald(1). The absence of any change in complement titer following clinical use of nitrogen mustard is what would be expected from a consideration of the *in vitro* studies, since the concentrations of mustard attained in patients are far below those which destroyed complement *in vitro*.

Conclusions. The mean serum complement titers of patients with various neoplastic diseases did not differ significantly from that for normal adults. The range of complement values was greater among cancer patients than among normals, but the erratic titers did not appear to be related to clinical status. No depression of human complement titer was observed following the clinical use of nitrogen mustard and x-ray in maximal dosage. Human complement is inactivated by high concentrations of nitrogen mustard *in vitro*, but not by concentrations in the range which is achieved in clinical usage.

Received January 16, 1951. P.S.E.B.M., 1951, v76.

Influence of Vitamin B₁₂ and Liver Extract on Nitrogen Balance of Normal and Hyperthyroid Rats.* (18514)

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The retardation of growth observed upon feeding thyroid hormone can be prevented by simultaneous administration of liver preparations in growing mice(1), chicks(2), rats(3-7), and rabbits(8). Similar effects have been obtained in rats with vitamin B₁₂

(8,9), and with various liver preparations high in antipernicious anemia activity(8). Other

* This work was supported by a research grant from the National Institute of Health. Public Health Service.

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B vitamins which prevent weight loss in the hyperthyroid mature rat(10) are ineffective in this respect in the immature hyperthyroid rat (3).

Prevention of weight loss and continued growth of the hyperthyroid animal by administration of liver preparations or vitamin B₁₂ has been studied in animals fed *ad libitum*. It is believed that increased food intake is in part responsible for the beneficial results of treatment. However, it has been suggested that increased utilization of food may play a role in the prevention of weight loss and/or continued growth of hyperthyroid animals treated with these preparations(4). It was therefore considered of interest to study the influence of liver preparations and of vit. B₁₂ on the nitrogen balance of normal and hyperthyroid rats on a constant food intake.

Material and methods. Male rats, descendants of the Wistar strain (Albino Farms, Red Bank, N. J.) weighing between 190 and 250 g, were used. All animals were tube fed the "mixed diet" described by Ingle *et al.* (11)^{||} twice daily. The animals were gradually adapted to tube feeding, and were then fed a constant amount during the control and the experimental period, the amount selected for each animal being sufficient to main-

tain weight, or to permit a slight weight gain during the control period.

The animals were kept in a constant temperature room in wire screened, individual metabolism cages. Urine specimens were collected at 24 hour intervals in collecting bottles containing 1 g of citric acid, and were analyzed daily by a micro-Kjeldahl method. The urinary N-excretion and the weight of each animal was recorded daily for a control period of 9 days. This was followed by an experimental period of equal length, during which various preparations were injected subcutaneously. Groups 1 and 2 represent normal rats, injected during the treatment period with vit B₁₂ and liver extract respectively. Group 3 was made hyperthyroid by daily injection of 150 γ d-l thyroxine. Groups 4 and 5 were made hyperthyroid in the same way as group 3, but received vit B₁₂ and liver extract respectively. All injections were given at the same time each day.

Results. Results are recorded in Table I, and in Fig. 1. Exp. 1 and 2 show that neither vit B₁₂ nor liver extract influenced the weight or nitrogen excretion of the adult rat on constant food intake.

The results of Exp. 3, 4 and 5 demonstrate that the protein catabolic effect of thyroxine was not abolished by simultaneous treatment with either vit B₁₂ or liver extract; in spite of this treatment the urinary N-excretion increased and the animals lost weight. In Fig. 1 the percentage weight loss and nitrogen loss in rats injected with thyroxine alone (Exp. 3) is compared with that of the thyroxine-treated animals receiving vit B₁₂ or liver extract (Exp. 4 and 5). The thyroxine-induced nitrogen loss was significantly decreased in the animals treated with vit B₁₂. The N-sparing effect of liver extract, with the amounts employed, was slight, and statistically not significant. The weight loss, however, did not differ significantly in the 3 groups.

Discussion. Vit B₁₂ and liver extract failed to induce weight gain or nitrogen retention in the adult rat fed constant amounts of an adequate diet. This indicates that these "growth factors," given in excess, do not exert

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^{||} Diet composition:

Constituent	g
Dried yeast	100
Salt mixture (Osborne & Mendel)	40
Wheat germ oil	10
Cod liver oil	10
Cellu flour	120
Mazola oil	175
Whole milk powder	600

This diet contains approximately 16% protein.

TABLE I. Effect of Vit. B₁₂ and of Liver Extract on N Excretion of Normal and Thyroxin Treated Rats Force Fed Constant Rations. Nitrogen excretion/24 hr.

Exp.	Treatment	No.	Control period*	Treatment period*	Diff.	"p" of diff.	Wt change g/9 days
1	Vit. B ₁₂ , 3 γ twice daily	1	221	231	+10	<.3	+ 0.4
		2	231	215	-16	<.2	+ 2.0
		3	196	201	+ 5	<.4	+ 1.5
		4	232	225	- 7	<.5	+ 0.5
		5	238	234	- 4	<.5	+ 1.5
		6	254	242	-12	<.2	+ 3.0
		7	211	202	- 9	<.3	+ 5.5
2	Liver extr., 0.4 U (0.2 ml)	1	195	197	+ 2	<.9	+ 4
		2	196	204	+ 8	<.1	+ 6
		3	200	193	- 7	<.9	+11
		4	185	189	+ 4	<.6	+ 7
		5	197	196	- 1	<.9	+ 4
3	Thyroxin, 150 γ daily	1	174	246	+72	<.01	-23
		2	174	241	+67	<.01	-30
		3	166	227	+61	<.01	-17
		4	193	304	+111	<.01	-54
		5	158	233	+75	<.01	-30
		6	166	218	+52	<.01	-28
		7	140	217	+77	<.01	-23
4	Thyroxin, 150 γ daily Vit. B ₁₂ , 3 γ twice daily	1	227	283	+56	<.01	-23
		2	254	296	+42	<.01	-35
		3	234	311	+77	<.01	-37
		4	239	259	+20	<.05	-21
		5	229	285	+56	<.01	-33
		6	234	273	+39	<.01	-29
		7	230	278	+48	<.01	-30
		8	220	268	+48	<.01	-24
5	Thyroxin, 150 γ daily Liver extr., 0.4 U (0.2 ml) daily	1	167	216	+49	<.01	-18
		2	183	260	+77	<.01	-28
		3	207	250	+43	<.01	-23
		4	174	263	+89	<.01	-15

* Mean of 9 days.

an anabolic effect. This is in agreement with observations on growing animals(12) and on humans(13), which suggest that liver factor and vit B₁₂ accelerate growth mainly by increasing appetite and food intake.

Thyroxine-induced weight loss is not prevented by either vit B₁₂ or liver extract in adult rats fed a constant ration. This finding is in agreement with observations on growing animals that the prevention of weight loss, or the continued growth of the thyroid-treated growing animal is due largely to increased food intake(4) and not to neutralization of, or interference with the calorogenic and other metabolic effects of excess thyroid hormone (4). The importance of the caloric intake is further suggested by the observation that high fat diets also protect growing rats against

thyroid-induced weight loss(14).

The fact that the nitrogen loss induced by thyroxine, while not abolished, was significantly decreased by vit B₁₂ in the doses employed, suggests that this factor has an action on intermediate metabolism in addition to the influence of food intake. Since, in the force fed rat, on constant intake, the thyroxine-induced weight loss is unchanged, but the nitrogen loss is decreased by treatment with vit B₁₂, it must be concluded that through the action of this factor protein is spared at the expense of other nutrients. This could conceivably be related to the "improved efficiency of food utilization" suggested by Ershoff as a second mechanism in addition to the increased food intake in the *ad libitum* fed animal(4).

Summary. Vit B₁₂ or liver extract ad-

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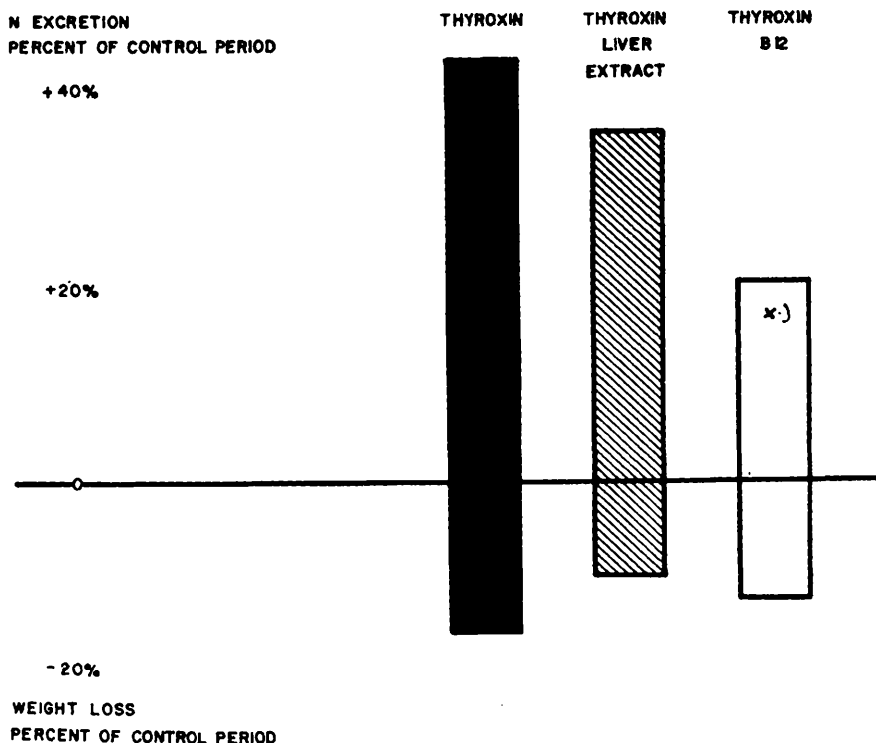


FIG. 1.

N excretion and weight loss. Upper bars indicate increase of N excretion in percent of control period. Lower bars indicate weight loss in percent of weight during control period. x Difference against column one significant $p < 0.01$.

ministered to force-fed rats on constant food intake induced neither weight gain nor N retention. When vit B₁₂ was administered to force-fed rats on constant food intake, made hyperthyroid by injection of thyroxine, the weight loss was identical with that of hyperthyroid animals not receiving vit B₁₂. The N loss resulting from the catabolic action of thyroxine, however, was significantly

smaller in thyroxine-treated rats receiving B₁₂ than in the thyroxine-treated controls. Liver extract had a similar action, but the values obtained were statistically not significant. These results suggest that in hyperthyroid rats vit B₁₂ spares protein at the expense of other body constituents.

Received January 18, 1951. P.S.E.B.M., 1951, v76.

Effect of Aureomycin and Chloramphenicol on Two Recently Isolated Strains of *Listeria monocytogenes*. (18515)

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A survey of the literature revealed no reports on the effects of aureomycin and chloramphenicol on infections with *Listeria monocytogenes*. Two strains of this organism were

recently isolated and found to be virulent for mice. In the present study, the effects of chloramphenicol and aureomycin on these strains were compared *in vitro* and *in vivo*.