ceived 1.5 g a day. There was no change in the peripheral white cell picture, qualitatively or quantitatively, nor did the bone marrow pattern change. This patient expired on the 15th hospital day of thiouracil treatment.

Discussion and Conclusions. Thiouracil was administered to 6 patients with chronic myelogenous leukemia, and to one patient with an acute myeloblastic leukemia. The dosage averaged 1.5 g a day and was well tolerated by every patient. One patient (case 4) received a total of 359.3 g of the drug over a 10-month period. The drug had no effect on the total circulating white count, nor on the differential white cell picture, red cell elements, hemoglobin or platelet values or the bone marrow pattern.

Recently, and after completion of this study, one observer reported that he had tried thiouracil in doses of 3 mg daily in a patient with myelogenous leukemia without appreciable results.[4]

[4] Limarzi, L. R., Kulasavage, R. J., and Pirani, C. L., *J. Hem. (Blood)*, 1946, **1**, 426.

Thiouracil did not influence the metabolic rate of leukemic patients materially when given in a dose of 1.5 g daily. This was true whether it was administered over a period of 2 weeks, 2 months or 10 months. The serum cholesterol rose in only 2 patients (cases 2 and 4), and this rise was only transitory. In one patient (case 3) the serum cholesterol continued to fall, the basal rate steadily rose despite the administration of thiouracil. In one patient reported (case 4), and in 2 others, this drug failed to prolong or modify the remission induced by irradiation. Thiouracil given in the dosage reported in this series had no demonstrable effect on the myeloid dysfunction, basal metabolic rate, the serum cholesterol level or the bone marrow pattern of patients with myelogenous leukemia.

Thiouracil, furthermore, did not modify the clinical course of the disease. It seems certain therefore, that this drug has no value as an adjunct in the treatment of myelogenous leukemia.

15841

Effects of Liver Feeding on Growth and Ovarian Development in the Hyperthyroid Rat.*

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It has been demonstrated that excessive doses of thyroxin or desiccated thyroid cause anestrus and loss of body and ovarian weight in the adult rat and prevent gonadal development in the young animal. The deleterious effects in the adult rat according to Drill *et al.*[1] can be prevented by the administration of increased amounts of thiamine or yeast. Such nutrients are without effect, however, in the young animal.[2] It appears, therefore, either (1) that the young rat is more susceptible to the toxic effects of thyroid insofar as gonadal physiology is concerned or (2) that excessive thyroid feeding may precipitate a deficiency of some nutrient other than the above necessary for gonadal development in the immature rat. In the

* The subject matter of this paper has been undertaken in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the author. They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

[1] Drill, V. A., Overman, R., and Leathem, J. H., *Endocrinology*, 1943, **32**, 327.

[2] Ershoff, B. H., *Endocrinology*, 1945, **37**, 218.

present experiment attempts were made to prevent by dietary means the failure of ovarian development in the immature thyroid-fed rat.

Procedure and Results. Three basal rations were employed in the present experiment: diets A, B and C. Diet A was a purified ration containing the B complex factors in synthetic form only. Diets B and C were similar in composition but contained yeast or desiccated liver in addition to the synthetic vitamins. All 3 rations were supplemented with 0.0 and 0.5% U.S.P. desiccated thyroid. Seventy-eight female rats of the Long-Evans strain were weaned at 21 to 23 days of age and litter mates divided as far as possible among the experimental groups listed in Table I. Animals were kept in metal cages with raised screen bottoms to prevent access to feces, and sufficient food was administered to assure *ad lib* feeding. Subsequent to the 40th day of feeding, vaginal smears were taken daily of all rats. Surviving animals were autopsied on the 60th day of feeding; ovaries were weighed and fixed in 10% formol, and sections prepared and stained with hematoxylin and eosin.

Results are summarized in Table II. In agreement with earlier findings[4] the mortality rate of thyroid-fed rats was greater on the synthetic diet than on rations containing yeast. Diets containing liver were similarly effective in prolonging survival. A significant difference in body and gonadal weight was observed in thyroid-fed rats on the various diets employed. On diets A and B both body and ovarian weight was significantly less in thyroid-fed rats than in animals fed a similar diet with thyroid omitted; and ovaries of the thyroid-fed animals resembled in microscopic appearance those of the immature rat. On diet C, however, no significant differences in body and gonadal weight were observed between animals fed thyroid and those on a thyroid-free ration; and histologically the ovaries of thyroid-fed rats appeared normal in all respects. In the absence of dietary thyroid body and ovarian weights did not differ significantly on any of the diets employed; and histologically ovaries appeared normal in all groups. Data obtained from vaginal smears check with the above findings. On diets A and B thyroid-fed rats exhibited constant leucocytic smears subsequent to the 40th day of feeding in contrast to the regular estrus cycles obtained on diet C. In the absence of dietary thyroid estrus cycles were regular on all diets. Subsequent work indicates that the ovaries of thyroid-fed rats raised to maturity on diet C were normal functionally as well as histologically. When bred to normal males after the 60th day of feeding, such animals while still on the same diet gave birth to apparently normal young.

Available data indicate that the factor in liver responsible for the above effects is not one of the major B vitamins. Doubling the B vitamin content of diet A was without significant effect on the body or gonadal weight of thyroid-fed rats. Individual supplements

TABLE I.
Composition of Experimental Diets.

Dietary component	Diet A	Diet B	Diet C
Yeast*	0.0	10.0	0.0
Liver†	0.0	0.0	10.0
Vitamin Test Casein‡	22.0	22.0	22.0
Salt Mixture§	4.5	4.5	4.5
Sucrose	73.5	63.5	63.5

When thyroid was fed it was included in the above diets at a level of 0.5%, replacing an equal amount of sucrose.‖

To each kg of the above diets were added the following synthetic vitamins: thiamine hydrochloride 72 mg, riboflavin 9 mg, pyridoxine hydrochloride 15 mg, calcium pantothenate 67.2 mg, nicotinic acid 60 mg, 2-methyl-napthaquinone 5 mg, and choline chloride 1.2 g. Each rat also received 3 times weekly the following supplement: cottonseed oil (Wesson) 500 mg, alpha-tocopherol 1 mg, and a vitamin A-D concentrate¶ containing 50 U.S.P. units of vitamin A and 5 U.S.P. units of vitamin D.

* Brewer's Type Yeast No. 200, Anheuser-Busch, Inc., St. Louis, Mo.

† Whole Dried Liver Powder, Armour and Co., Chicago, Ill.

‡ Vitamin Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

§ Sure's Salt Mixture No. 1.[3]

‖ Thyroid Powder, U.S.P., Armour and Co., Chicago, Ill.

¶ Nopco Fish Oil Concentrate, assaying 800,000 U.S.P. units of vitamin A and 80,000 U.S.P. units of vitamin D per gram.

[3] Sure, B., *J. Nutrition*, 1941, **22**, 499.

[4] Ershoff, B. H., and Hershberg, D., *Am. J. Physiol.*, 1945, **145**, 16.

TABLE II.
Effects of Thyroid Feeding on Body and Gonadal Weight.

Dietary group	% thyroid	No. of animals	Avg body wt		Avg ovarian wt* mg
			Initial g	On 60th feeding day* g	
A	.5	20	43.7	116.5 ± 6.7 (4)	23.6 ± 2.2
B	.5	20	42.9	133.5 ± 7.8 (14)	27.8 ± 4.1
C	.5	20	43.3	202.3 ± 8.1 (15)	72.3 ± 7.1
A	.0	6	42.7	198.7 ± 4.1 (6)	53.1 ± 3.3
B	.0	6	43.1	186.6 ± 6.7 (6)	56.8 ± 4.7
C	.0	6	42.7	197.5 ± 4.4 (6)	59.4 ± 5.1

The values in parentheses indicate the number of animals which survived of which this is an average.

* Including standard error of the mean calculated as follows: $\sqrt{\frac{\epsilon d^2}{n}} \;/\; \sqrt{n}$

where "d" is the deviation from the mean and "n" is the number of observations.

of thiamine, riboflavin, pyridoxine, calcium pantothenate, nicotinic acid, inositol, *p*-aminobenzoic acid, biotin or folic acid were similarly ineffective.† Doubling the salt content of diet A or administering an additional 10% casein or 10% wheat germ‡ at the expense of an equal amount of sucrose were also without effect. Only liver of all substances tested gave a positive response in the thyroid-fed rat. Preliminary results indicate that the factor in liver responsible for the above effects is heat stabile.

Discussion. Evidence is accumulating that in addition to the major nutrients, substances are present in our diet which may be required in increased quantities 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. Certain drugs or other "stress factors" may, however, increase requirements for these substances to the extent that deficiencies occur, manifest by retarded growth or tissue pathology, and preventable by the administration in appropriate amounts of the missing nutrient. Present findings indicate that thyroid feeding is such a factor. Although liver did not counteract other manifestations of hyperthyroidism, such as a hypertrophied ventricular wall, reduction in the creatine content of the heart or an increased B.M.R.,[5] it did counteract completely the retardation of growth and inhibition of ovarian development observed on all other rations when thyroid was fed.

Findings indicate that liver contains an or-

† Supplements were added to diet A in the following amounts per kg of diet: thiamine hydrochloride 3.6 g, riboflavin 450 mg, pyridoxine hydrochloride 750 mg, calcium pantothenate 3.36 g, nicotinic acid 3.0 g, inositol 10.0 g, *p*-aminobenzoic acid 10.0 g, biotin 1.0 mg, and folic acid 10.0 mg. We are indebted to Dr. Stanton M. Hardy of the Lederle Laboratories, Pearl River, New York, for the folic acid employed in the present experiment. The remaining B vitamins were obtained from Merck and Co., Rahway, N.J.

Subsequent work indicates that individual supplements of ascorbic acid and the fat-soluble vitamins were also ineffective. These were added to diet A in the following amounts per kg of diet: ascorbic acid 10 g, vitamin A 100,000 U.S.P. units, vitamin D 25,000 U.S.P. units, and 2-methyl-napthaquinone 100 mg. Alpha-tocopherol was administered as a daily supplement at a level of 5 mg per rat.

‡ Defatted Wheat Germ, Vio-bin Corporation, Monticello, Ill.

5 Ershoff, B. H., in press.

ganic factor other than thiamine, riboflavin, pyridoxine, calcium pantothenate, nicotinic acid, inositol, *p*-aminobenzoic acid, biotin or folic acid, a substance not present in significant amounts in wheat germ, casein or yeast, but required for normal growth and ovarian development in the immature thyroid-fed rat.

Summary. The administration of liver completely counteracted the retardation of growth and inhibition of ovarian development observed in immature rats fed toxic amounts of thyroid. Wheat germ, yeast, or increased amounts of salt mixture, casein, thiamine, riboflavin, pyridoxine, calcium pantothenate, nicotinic acid, inositol, *p*-aminobenzoic acid, biotin or folic acid were ineffective in this regard. The suggestion is made that liver contains some factor other than the above required for normal growth and ovarian development in the immature thyroid-fed rat.

15842

Some Pharmacological Characteristics of Bacitracin.*

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Bacitracin, an antibiotic discovered by Meleney and co-workers[1] in the filtrate of surface cultures of the Tracy strain of *B. subtilis*, has become of increasing interest, particularly because of its potential value in the treatment of penicillin-resistant infections. Because of this increased interest, the following preliminary data on the toxicological and pharmacological properties of the antibiotic are presented.

Acute toxicity experiments. Fresh bacitracin† solutions used for oral and intraperitoneal administration contained the dose in 1 cc of water; solutions for intravenous and subcutaneous injections contained the dose in 0.25 to 0.50 cc per 20 g of mouse, or 50 g of rat. All samples gave clear solutions at pH 6 to 7 except No. 187, which dissolved at pH values of 5 to 6. For oral or subcutaneous administration, fine suspensions were administered because of the large doses required. Solutions of earlier samples of bacitracin were brown, but more highly purified later samples, illustrated by No. B-100, were almost colorless.

Experiments were performed with male, white, Rockland Swiss mice weighing 20 to 25 g, and male white rats of the Sherman strain maintained on a stock diet with free access to food and water at all times. Surviving animals were observed for a period of at least 7 days after acute or chronic dosing.

Results of the acute toxicity experiments are shown in Table I. Comparison of the first 4 samples, all administered intraperitoneally, indicates that the toxicity varies independently of the activity. (The fourth sample, No. B-100, is much less toxic than the other 3 and is representative of the batches now being produced). In this connection it may be noted that inactivation of the first sample under mild conditions (pH 7.0 at 37° for 8 days) did not alter the toxicity of the preparation. The LD_{50}, 200 mg per kg, did not differ significantly from the value of 240 mg per kg obtained with the active sample. Neither the scattering of the deaths, nor the toxic signs, nor the pathological lesions in mice differed appreciably

* The work described in this paper was done under a contract between the Office of the Surgeon General and Columbia University. Administration of the contract was directed by Dr. Frank L. Meleney.

1 Johnson, B. A., Anker, H., and Meleney, F. L., *Science*, 1945, **102**, 376.

† We are indebted to Dr. John T. Goorley of the Ben Venue Laboratories for the bacitracin.