to make a quantitative comparison with Rose's work. In general we have found that at the levels fed, the essential amino acids will give rise to almost as much growth as will an equivalent weight of casein.

We found no evidence of toxicity in spite of using racemic mixtures of six of the amino acids. Without knowing the source of the amino acids used by Albanese and Irby we cannot account for the widely different results obtained. Our findings for rats appear

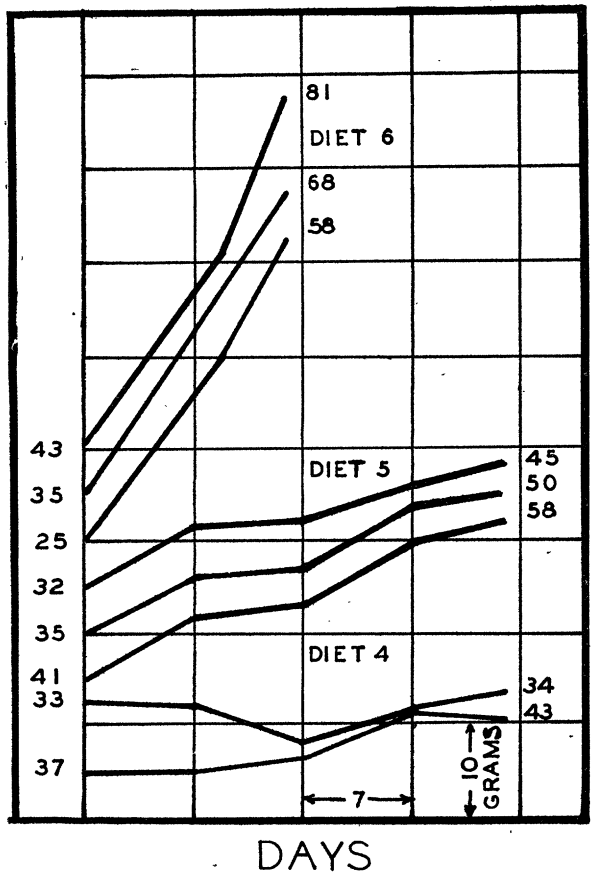

FIG. 2. Growth of rats on diets containing the following as the sole source of nitrogen: Diet 4, 10 essential amino acids at 5.8 per cent. level plus 9 per cent. glycine; Diet 5, 18 per cent. casein—paired feeding with diet 4; Diet 6, 18 per cent. casein—*ad libitum*.

compatible with those obtained in experiments on other animals. For example, Bauer and Berg[5] found slow growth when mice were fed the ten essential amino acids at a 15.6 per cent. level. Furthermore, Madden *et al.*,[6] using dogs, found that plasma protein production after feeding or injecting the essential amino acids was as good as when most proteins were fed in the diet. Moreover, they found no apparent clinical disturbance, even from the rapid injection of the amino acids intravenously.

[5] C. D. Bauer and C. P. Berg, *Jour. Nutr.*, 26: 51, 1943.
[6] S. C. Madden, J. R. Carter, A. A. Kattus, Jr., L. L. Miller and G. H. Whipple, *Jour. Exp. Med.*, 77: 277, 1943.

SUMMARY

Growth was obtained in rats on synthetic diets in which the ten essential amino acids were the sole source of amino acid nitrogen. The growth rate was dependent upon the quantity of amino acids fed and appeared to compare favorably with that obtained when a similar quantity of nitrogen was fed in the form of casein. No increase in growth was observed when nitrogen in addition to that given in the form of the essential amino acids was supplied as glycine. Our experiments would not support the contention that the unnatural forms of the amino acids are toxic.

We wish to acknowledge and thank Phyllis Robison for technical assistance, and Dr. D. Mark Hegsted for helpful suggestions.

V. EVERETT KINSEY
W. MORTON GRANT
HOWE LABORATORY OF OPHTHALMOLOGY,
HARVARD UNIVERSITY MEDICAL SCHOOL

METABOLIC EFFECTS OF THIOURACIL IN GRAVES' DISEASE[1, 2]

EARLY in 1943, Astwood[3] introduced a new type of therapy for Graves' disease. This was based on the goitrogenic effects of sulfonamides and certain derivatives of thiourea as demonstrated in animals by studies of the MacKenzies and McCollum[4, 5] and of Astwood and co-workers.[6] A systematic investigation of such compounds[7] showed thiouracil to be the most potent and least toxic and led to its selection for clinical trial by Astwood[3] and by Williams and Bissell[8] and others.[9] The mode of action of these drugs appears to be an interference with the enzymatic synthesis of thyroid hormone. The exact point of block is still not known, but it is definitely established that the thyroid gland is rendered incapable of utilizing iodine for this process. The resultant thyroid insufficiency leads, via anterior pituitary stimulation, to an ineffectual hyperplasia of the thyroid acinar cells. The peripheral action of administered thyroid hormone, however, remains unimpaired.

[1] From the Russell Sage Institute of Pathology in affiliation with the New York Hospital and the Department of Medicine, Cornell University Medical College.
[2] With the technical assistance of Vincent A. Toscani.
[3] Astwood, *Jour. Am. Med. Asn.*, 122: 78, 1943.
[4] MacKenzie, MacKenzie and McCollum, SCIENCE, 94: 518, 1941.
[5] MacKenzie and MacKenzie, *Endocrinol.*, 32: 185, 1943.
[6] Astwood, Sullivan, Bissell and Tyslowitz, *ibid.*, 32: 210, 1943.
[7] Astwood, *Jour. Pharmacol. and Exp. Therap.*, 78: 79, 1943.
[8] Williams and Bissell, SCIENCE, 98: 156, 1943; *New Eng. Jour. Med.*, 229: 3, July, 1943.
[9] Himsworth, *Lancet*, 245: 483, 1943.

Clinical reports to date[1, 6] on the use of thiouracil (and to a lesser extent, thiourea[7]) in Graves' disease have shown that this drug has a similar action in man; there is a decrease in the basal metabolic rate and in the protein-bound iodine of plasma, an increase in serum cholesterol and body weight and a disappearance of the symptoms of Graves' disease. No improvement in exophthalmos has been noted; and the effect on the size of the gland has been variable. Toxic reactions have been few, the most notable being a non-fatal agranulocytosis.

It would reinforce the value of thiouracil for the treatment of Graves' disease were the beneficial effects noted above also accompanied by the correction of other metabolic derangements characteristic of this condition, such as is achieved by successful thyroidectomy or iodine remission. With this in mind, a series of patients with Graves' disease are being subjected to detailed metabolic study in the metabolism ward of the Department of Medicine at the New York Hospital. They are placed on constant creatin-creatinine free diets and observations made of the influence of thiouracil on the disturbances in creatin, creatinine, calcium, phosphorus and nitrogen metabolism, as well as on several other indices of thyroid activity. In this preliminary report, the data on one such case are presented in detail in graphic form.

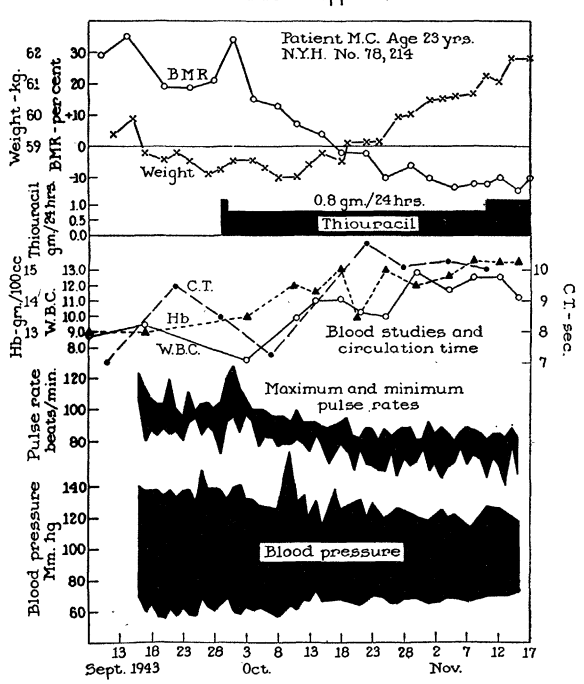

FIG. 1

The patient was a 23-year-old white girl with typical signs and symptoms of Graves' disease of moderate severity and of two years' duration, uncomplicated except for moderate exophthalmos. The gland was moderately enlarged. Balance studies were carried on for two weeks prior to, and for fifty days during the administration of thiouracil. The constant creatin-creatinine free diet provided 2,400 calories, 76 gm protein, 107 gm fat, 283 gm carbohydrate, 1.19 gm calcium and 1.41 gm phosphorus. There was a temporary symptomatic improvement on bed rest alone during the control period, followed by an acute exacerbation of symptoms which was progressive until the fourth day of treatment with thiouracil. From that time on, improvement was rapid and striking in almost all respects. The symptomatic relief was accompanied by an improvement in all the metabolic functions studied, the details of which are given in Figs. 1, 2 and 3.

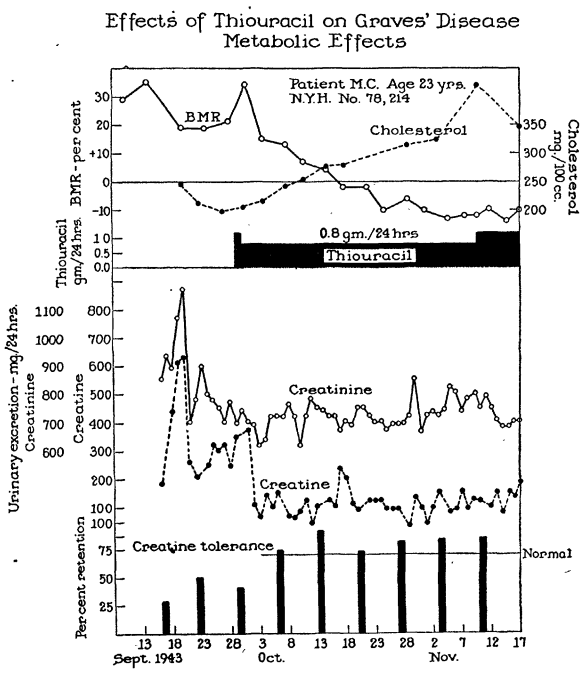

FIG. 2

The creatinuria was sharply reduced from control levels; however, it still persisted at somewhat higher than the average normal levels. The creatin tolerance following the administration of 1.32 gm of creatin rose rapidly to, and remained within, the normal range. An increase in creatinine excretion was not observed but was not anticipated over this short period. Sugar tolerance tests were normal before treatment and remained so. Nitrogen, phosphorus and calcium balances became progressively more positive. The im-

provement in calcium balance occurred chiefly from a reduction in calcium excretion in the stool. In another patient of this series, however, the chief reduction in calcium excretion after thiouracil took place in the urine, calcium content of which fell from the control level, 575 mgs, to the neighborhood of 214 mgs per 24 hours. No changes were noted in the size of the gland or degree of exophthalmos. The purpose of the increased dosage of thiouracil during the last week was to ascertain the degree of thyroid insufficiency which could be achieved; although the basal metabolic rate still remained at −10 per cent. to −15 per cent., serum cholesterol rose to myxedematous levels of 350–415 mgs per cent. during this period.

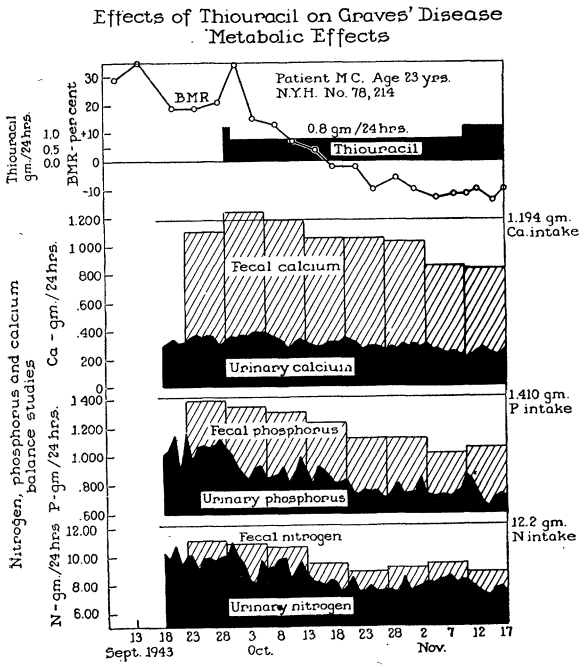

FIG. 3

No toxic manifestations were encountered in this patient. In another patient of the series, mild jaundice with an icteric index of 23 developed after 20 days of thiouracil (0.8 gm daily). There was no demonstrable evidence of hemolysis or hepatic damage at the time. Subsequent gall bladder x-rays and liver function tests were entirely normal. There was a return of the icteric index to normal within 10 days of stopping the drug. Two other patients of a series of 12 treated with thiouracil developed urticarial eruptions which disappeared on discontinuing the drug and reappeared in one of the two patients when treatment was reinstituted a week later. The possibility of toxic hepatitis appears to warrant routine icteric indices during at least the initial stages of treatment.

In conclusion, the effects of thiouracil on the disturbances of calcium, phosphorus, nitrogen and creatin metabolism occurring in Graves' disease are comparable to the beneficial results following successful subtotal thyroidectomy or iodine remission. These findings indicate the physiological nature of the remission produced by this new chemotherapeutic agent.

MARGARET H. SLOAN
EPHRAIM SHORR

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A DIFFERENTIAL TRIPLE STAIN FOR DEMONSTRATING AND STUDYING NON-ACID-FAST FORMS OF THE TUBERCLE BACILLUS IN SPUTUM, TISSUE AND BODY FLUIDS[1]

IN 1932, non-acid-fast forms of the tubercle bacillus[2] were studied by means of several improved counterstain technics. One was devised which gave the most striking results of all for differentiating the acid-fast and non-acid-fast rods and granules.[3] It consisted in staining first by the usual Ziehl-Neelsen method, decolorizing with acid alcohol and then adding to each slide, flooded wth Loeffler's methylene blue counterstain, 6 to 8 drops of an experimentally determined optimum strength of NaOH (0.05 per cent. for avian strains and 4 per cent. (normal strength) for human strains). Whereas this counterstain method was excellent for pure cultures of tubercle bacilli, it was unsuitable for use with sputum, tissues or body fluids, since the background took on and held an intensely blue color which obscured the contrast; thus any other species of bacteria present would also appear blue.

An attempt was made, therefore, to find some means of bleaching out the methylene blue from the background without removing the blue color from the non-acid-fast forms of the tubercle bacilli. Tests were made on pure cultures of tubercle bacilli and of tubercle bacilli mixed with a number of other organisms, including staphylococci, streptococci and *C. diphtheriae*. Controls were made by staining pure cultures of these non-acid-fast species alone. An excellent bleaching agent was found. It is sodium hydrosulfite, a substance used as a discharge or "stripping" agent in the textile industry. This substance in

[1] This work was supported by a grant from the Rosenwald Family Association, and was carried on mainly in the laboratories of Dr. Morton C. Kahn, Department of Public Health and Preventive Medicine, Cornell University Medical College.

[2] M. C. Kahn, *Am. Rev. Tuber.*, 20: 2, 150, 1929; E. G. Alexander, *Proc. Soc. Exp. Biol. and Med.*, 21: 1104, 1934; M. B. Lurie, *Jour. Exp. Med.*, 69: 576, 1939.

[3] E. G. Alexander, SCIENCE, 75: 197, 1932.