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.

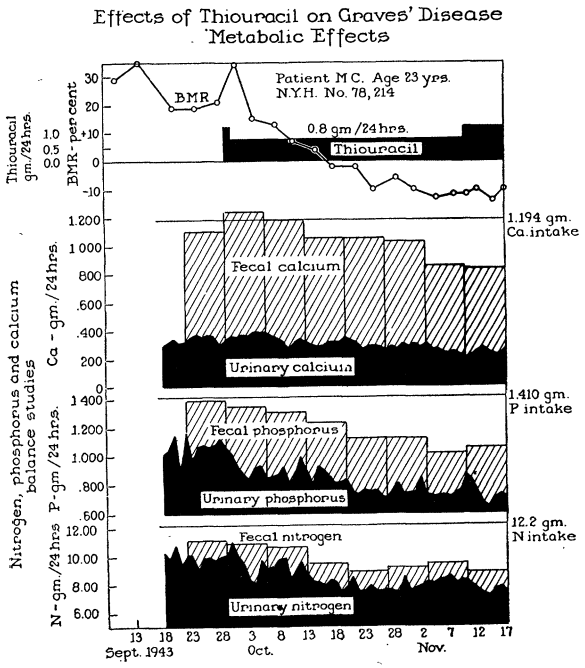

FIG. 3

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.

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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.

a strength of approximately 0.25 per cent. (or a small pinch in about 50 ml of tap water *freshly* prepared *just* before using, selectively bleached the blue color from the background and from all the organisms tested excepting the tubercle bacilli, without affecting the red color of the carbol-fuchsin stained rods or the blue color of the methylene-blue stained non-acid-fast forms of *M. tuberculosis.*

Subsequently, known pure cultures of tubercle bacilli were mixed with non-tuberculous sputums and were stained by the new technic. The results were striking. Acid-fast organisms were red, non-acid-fast forms were blue, partially acid-fast forms mulberry color, while other organisms, tissue cells and mucus formed an effective light green background with the third stain used; this consists of equal volumes of aqueous solutions of 1 per cent. acid green[4] and 1 per cent. acid yellow.[4] Every slide stained by the triple staining method was controlled by a duplicate smear stained by the ordinary Ziehl-Neelsen technic. Thereafter, hundreds of slides of tuberculosis sputum, tissue and body fluids—particularly chest fluids[5]—were stained by the triple stain technic with Ziehl-Neelsen controls, with satisfactory results.

This triple method of staining should be a useful supplement to the usual Ziehl-Neelsen technic since it reveals a number of interesting non-acid-fast forms which ordinarily escape observation.[6] One of these forms is zoogleal, consisting of one or more granules embedded or enmeshed in amorphous material and is not stained by the usual Loeffler's methylene blue or dilute methylene blue counterstains. This form, stained and unstained, has been the subject of intensive study and is being described in detail elsewhere. Ubiquitous saprophytic diphtheroids obtained from a variety of non-tuberculous materials such as normal guinea-pig serum or heart's blood, tap water, or hay, apparently are also able to enter a zoogleal state similar to this newly demonstrated zoogleal state of *M. tuberculosis,* and are distinguished from *M. tuberculosis* by the relative ease and speed with which such forms develop into rods on culture, the non-acid-fast character of all their rod forms, and, of course, by their complete lack of pathogenicity for the guinea pig.

TECHNIC OF THE TRIPLE STAIN FOR TUBERCLE BACILLI

(1) Prepare smears which are not too thick, fix carefully with heat, and stain as usual three minutes with carbol-fuchsin.[7] Decolorize for one to three minutes with acid alcohol (3 per cent. HCl) and wash thoroughly in running tap water.

(2) Flood the slides with a well-ripened Loeffler's methylene blue. Then add with a dropper 6 to 8 drops of normal NaOH with a capillary pipette. Distribute the alkali by tipping the slides gently; let stand for not more than one minute; wash. The NaOH must be freshly prepared about once a month for good results.

(3) Flood the slides one at a time, with sodium hydrosulfite solution (freshly prepared just before using by adding a small "pinch" of hydrosulfite to about 50 ml of tap water in a beaker or flask). Decolorization of the deep blue smear will speedily take place (except for red acid-fast and the non-acid-fast tubercle bacilli, which on microscopic examination appear blue). Wash off quickly in running tap water and immediately flood the slide with the green stain (an aqueous solution of equal volumes of 1 per cent. acid green and 1 per cent. acid yellow). Wash off the green in a few seconds, and blot dry at once. When stained preparations are thick, parts will appear blue rather than green, thus preventing clear differentiation in those areas. On the other hand, if the sodium hydrosulfite solution is too strong, the background and species of bacteria other than the tubercle bacillus will appear grayish.

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[4] National Acid Green L Extra, C.I. No. 666 and National Quinoline Yellow C.I. No. 801 were found suitable.

[5] Most of the material was obtained from the laboratory of Tuberculosis Service at Bellevue Hospital through the kindness of Dr. J. Burns Amberson, director, and Miss Edna Stein, bacteriologist.

[6] E. Alexander-Jackson, *Am. Rev. Tuber.*, 33: 6, 789, 1936.

[7] To protect smears against precipitated particles of carbol-fuchsin stain, it is advisable to lay a strip of clean filter paper across each fixed smear prior to adding the dye.

BOOKS RECEIVED

BARTON, WM. H., JR. *World Wide Planisphere for Finding and Identifying Navigation Stars and Constellations from all Latitudes, North or South throughout the Year.* Addison-Wesley Press, Inc. $2.50.

BARTON, WM. H., JR., and CHARLES O. ROTH, JR. *Basic Problems in Celestial Navigation.* Illustrated. Pp. 56. Addison-Wesley Press, Inc. $1.00.

CHAPIN, WILLIAM H. *Exercises in Second Year Chemistry.* Fourth edition. Revised by WERNER E. BROMUND and L. E. STEINER. Illustrated. Pp. vii + 216. John Wiley and Sons, Inc.

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HAUSMANN, ERICH and EDGAR P. SLACK. *Physics.* U. S. Naval Academy edition. Illustrated. Pp. vii + 857. D. Van Nostrand Company, Inc. $5.50.

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The Technique of Motion Picture Production. A Symposium of Papers presented at the 51st Semi-Annual Convention of the Society of Motion Picture Engineers, Hollywood, California. Illustrated. Pp. viii + 150. Interscience Publishers, Inc. $3.50.