Gaddum and Hetherington attribute this difference in dosage to the possibility that their animals were less sensitive than those of Mørch. From Table II it may be seen that no valid conclusions can be drawn as to the comparative activity of different thyroid preparations since different mice vary markedly in their response to the same dose of a given thyroid preparation, even though administered in an identical manner to animals kept under similar conditions. For example, a given dose of gland No. 27 produced changes in CO_2 output ranging from —0.3 to +17.2, while preparation marked N. J. produced in one case +21.3% and in another case +1.2%.

Our experience with the method of Mørch is disappointing. Possibly observations sufficiently great in number to afford a comparison of the means rather than of individual values might yield intelligible results. The method is long and tedious. Its reliability, even with a large series, is doubtful.

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Assay of Thyroid by Chemical Estimation of the Thyroxine Content.

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To investigate the variation in the thyroxine content of commercial thyroid preparations, thyroid tablets from 15 representative pharmaceutical firms were obtained in the open market and analyzed for total iodine, inorganic iodine and thyroxine. Harington and Randall[1] made a similar survey, and found in tablets "each equivalent to 5 grains of fresh thyroid gland", a variation of about 600% in the thyroxine content, as estimated by the total iodine in the acid-insoluble fraction. However, recent work[2, 3, 4] suggests that abso-

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[1] Harington, C. R., and Randall, S. S., *Quart. J. Pharm. and Pharmacol.*, 1929, **2**, 501.

[2] Kendall, E. C., and Simonsen, D. G., *J. Biol. Chem.*, 1928, **80**, 357.

[3] Leland, J. P., and Foster, G. L., *J. Biol. Chem.*, 1932, **95**, 165.

[4] Gutman, A. B., Benedict, E. M., Baxter, B., and Palmer, W. W., *J. Biol. Chem.*, in press.

lute thyroxine values based on this method may be too high since after 4 hours' hydrolysis with N/1 NaOH the acid-insoluble fraction appears to consist of a heterogeneous mixture of free thyroxine and incompletely hydrolyzed polypeptides containing diiodotyrosine. It was therefore thought desirable to follow a procedure which we had found satisfactory for the iodine partition of pathological thyroid glands.[4] This is based on the Leland and Foster method for the estimation of thyroxine,[5] a modified Kendall method for the determination of total iodine[5] and aqueous extraction of the desiccated gland for estimation of inorganic iodine. All determinations were done in duplicate, the mean discrepancy between duplicates in the thyroxine determinations being 2.7%.

Fifteen different products were found to have the following thyroxine iodine contents, in terms of mg. per one grain tablet. .0099, .0124, .0187, .0226, .0257, .0260, .0270, .0280, .0291, .0305, .0312, .0323, .0325, .0329, .0355. The difference between the minimum and maximum values observed is .0256 mg., a variation of 259% with respect to the minimum figure.

The variations in the physiological activity of commercial thyroid preparations as indicated by biological assay[6, 7, 8] and in the thyroxine content as suggested by the data of Harington and Randall and ourselves, seem to be inconsistent with present therapeutic standards. Adherence to U.S.P. requirements markedly reduces the variation in thyroxine content. However, of the 15 different preparations examined, only 7 contained "not less than 0.17 and not more than 0.23%iodine in thyroid combination" if iodine in thyroid combination is defined as total iodine; while only 6 complied with the requirements, if it is defined as organic iodine. The thyroxine iodine content in mg. per grain tablet in the first group varied from 0.0187 to 0.0312, or 67%, while the thyroxine iodine content of the second group varied from 0.0260 to 0.0329, or only 26.5%. But while the variation in thyroxine content thus appears to be adequately controlled by keeping the organic iodine content within U.S.P. limits, the possible variation is much greater than that actually observed in our series. A preparation with an organic iodine content of 0.17% may have a thyroxine iodine percent of organic iodine of 18%, and another preparation with an organic iodine content of 0.23% may have a thyroxine iodine percent of organic iodine of 35%. Both preparations would satisfy the U.S.P. requirements, but the varia-

[5] Foster, G. L., and Gutman, A. B., *J. Biol. Chem.*, 1930, **87**, 289.

[6] Munch, J. C., *Bioassays*, 1931, 665.

[7] Hunt, R., *Arch. Int. Med.*, 1925, **35**, 671.

[8] Lipschitz, W., and Girndt, O., *Arch. f. exp. Path. u. Phar.*, 1931, **159**, 259.

tion in the thyroxine content of the 2 preparations would be almost 175%. The possibility of adulteration must also be considered. Addition of a cheap, water-insoluble, relatively stable substance such as iodized casein would be very difficult to detect by chemical means if determination of organic iodine or of the acid-insoluble iodine fraction is used for assay.

Standardization of thyroid by direct determination of the thyroxine content after the method of Leland and Foster appears to be the most satisfactory solution of the problem. The determinations can easily be made in cooperating commercial laboratories, an important consideration for any practical method. By maintaining the thyroxine content of thyroid tablets within specified limits, one of the most important of the variables encountered in thyroid medication may be eliminated.

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Retardation of Tuberculous Infection in Guinea Pigs Vaccinated with Killed Tubercle Bacilli as Shown by Cultural Method.

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The effect of vaccination with killed tubercle bacilli upon the resistance of animals to tuberculous infection has been studied extensively. It has been repeatedly shown that guinea pigs can be sensitzed by this means to the intracutaneous injection of old tuberculin (Römer,[1] Bessau,[2] Zinsser, Ward and Jennings,[3] Petroff[4]). Its influence upon the course of subsequent infection, however, has been studied with less conclusive results under widely varying experimental conditions. Römer,[1] Zinsser, Ward and Jennings,[3] Petroff,[4] and L. Lange[5] conclude that such treatment gives some protection; Dold,[6] and Seligmann and von Gutfeld,[7] that it gives none. B. Lange and his associates[8] believe that inoculation with heat-

[1] Römer, P. H., *Beitr. klin. Tub.*, 1909, **12**, 185.

[2] Bessau, G., *Berl. klin. Woch.*, 1916, 801.

[3] Zinsser, H., Ward, H., and Jennings, F. B., *J. Immun.*, 1925, **10**, 719.

[4] Petroff, S. A., and Stewart, *J. Immun.*, 1926, **12**, 97. Petroff, S. A., Branch, A., and Jennings, F. B., *Ibid.*, 1929, **16**, 233.

[5] Lange, L., *Zentralbl. Bakt.*, 1921, **85**, 26.

[6] Dold, H., *Klin. Woch.*, 1925, 1763.

[7] Seligman, E., and von Gutfeld, F., *Deitsche med. Woch.*, 1925, 1064.

[8] Lange, B., and Freund, R., *Zeitsch. Hyg.*, 1926, **105**, 571. Lange, B., and Jochimsen, E., *Ibid.*, 1927, **107**, 426. Lange, B., Jochimsen, E., and Magat, J., *Ibid.*, 1927, **107**, 645.