Relationship between the 3,3′,5-Triiodo-L-thyronine Content of Thyroid as determined by a Thin-layer Chromatographic Method and the Biological Potency assayed by a Rat Anti-thiouracil Goitre Method

In a recent paper[1], we described a method which could be used for identifying thyroxine in a feeding-stuffs additive; its separation from the other components present in the hydrolysed iodinated protein was effected on paper and on starch-bound cellulose powder thin-layer plates.

If the procedures referred to therein are applied to hydrolysed thyroid, both paper and thin-layer chromatograms are capable of effectively resolving a substance having an R_F value corresponding to thyroxine as a well-defined spot. The spot with R_F value corresponding to triiodothyronine is, however, poorly defined on paper chromatograms, but the thin-layer chromatogram shows a distinct spot which can be assessed visually against triiodothyronine standards.

Since it is known that triiodothyronine (T_3) makes a far greater contribution than thyroxine (T_4) to the activity of thyroid when assayed by the rat anti-thiouracil goitre method, it was thought that the triiodothyronine content of hydrolysed thyroid, as determined by visual assessment of thin-layer chromatograms, should provide some indication of the potency. When two samples of thyroid were examined by this means, the quantity of material in the triiodothyronine position present in one sample was distinctively different from the quantity in the other. The first sample was reported to have one-third of the biological activity of the second when subjected to the rat anti-thiouracil goitre test and we estimated that the spots on the thin-layer chromatograms corresponded to 0·025 and 0·06 per cent of triiodothyronine respectively. A third sample, said to be 'biologically unsatisfactory' (the source and type of bio-assay are unknown), showed only a very faint spot, equivalent to about 0·005 per cent of triiodothyronine.

The conditions necessary to obtain spots well separated from other materials and in good alignment with triiodothyronine standards have been worked out in detail. The hydrolysis and extraction of active materials into *n*-butanol are based on the procedure described by Devlin and Stevenson[2]. Thin-layer plates prepared by the method previously described[1] should be used, and should run at a temperature greater than 20° C and preferably near to 26° C in the *t*-amyl alcohol and ammonia solvent system described by Barker[3], special precautions being taken to ensure that the atmosphere is saturated with ammonia. The FFCA reagent of Gmelin and Virtanen[4] is the preferred spray reagent for spot location. The chromatogram is undisturbed by the water-washing necessary to remove excess reagent after spraying. The procedure is improved when a 'blank' enzyme hydrolysate is superimposed on the triiodothyronine standards, so that the hydrolysed sample and standards run in the same environment, as shown in Fig. 1.

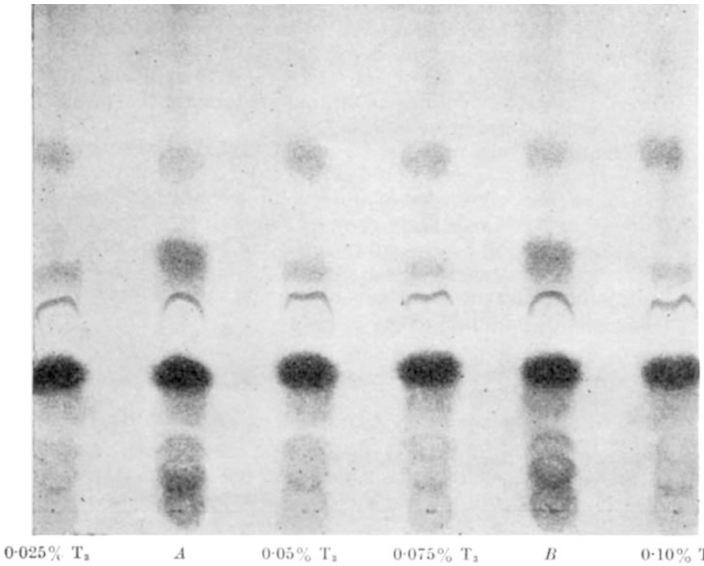

Fig. 1. Thin-layer chromatogram showing 6 μl. of each of two samples of enzyme-hydrolysed thyroid, *A* and *B*, alongside increasing quantities of triiodothyronine (T_3). Six μl. of a 'blank' enzyme hydrolysate have been spotted over each spot of triiodothyronine

This work was undertaken as a result of our membership of Panel 7 of the Joint Committee of the Pharmaceutical Society and the Society for Analytical Chemistry on Methods of Assay of Crude Drugs. The samples of thyroid were selected from those used for work by Panel 7 and bio-assays were supplied by Mr. K. L. Smith of Boots Pure Drug Co., Ltd., Nottingham. Collaborative tests on the method based on these principles is at present being carried out by members of Panel 7 to confirm that the apparent correlation with biological activity can be substantiated on a wider range of samples.

R. L. Clements
Stella J. Patterson

Laboratory of the Government Chemist,
Cornwall House,
Stamford Street,
London, S.E.1.

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BIOCHEMISTRY

Effect of Magnesium Deficiency on Plasma Alkaline Phosphatase Activity

Magnesium activates many enzymes *in vitro*, but very few disturbances in enzymatic activity have been demonstrated during magnesium deficiency *in vivo*[1]. The alkaline phosphatases from various mammalian tissues are among the enzymes known to be activated by magnesium in isolated systems. This communication reports investigations of the effect of magnesium deficiency on plasma alkaline phosphatase activity in the rat.

Two groups of female Wistar albino rats of initial weight 100 g were fed with magnesium-deficient and control diets respectively for 15 days. All animals received an amount of food equal to that consumed by the deficient rats; this gave an initial food intake of 10 g reducing to 8 g/rat/day. Distilled water was provided *ad lib.* throughout the experiment. The magnesium-deficient and control diets were prepared as described previously[2]; their magnesium contents were 0·2 mg/100 g (deficient) and 78 mg/100 g (control). The rats were anaesthetized with ether and killed by bleeding from the heart. Plasma was separated from heparinized blood within 0·5–1 h.

The plasma from each magnesium-deficient rat was divided into two and the magnesium concentration in one portion was raised to normal by adding to it 0·83 per cent of its volume of a magnesium chloride solution containing 165 mg/100 ml. of magnesium. The alkaline phosphatase activity was measured in all samples of plasma, as described by King and Wootton[3], after standing overnight at 4° C. The measurements were repeated after storage for a further six days to allow ample time for the added magnesium