

105–130 c.c. liquid, which is not very appreciably more than the total plasma volume. The latter amounts, in the case of a rabbit weighing 2.3 kgm., to about 90 c.c.

All data given in this note relate to those phosphate molecules which are soluble in petrol ether. We found great differences in the turnover of the different types of phosphatides and this induced us to carry out similar experiments as described above with each of the different types.

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<sup>1</sup> Artom *et alia*, *Archiv. internat. de Physiol.*, **45**, 32 (1937); **47**, 245 (1938); Hahn and Hevesy, *Skandin. Archiv. Physiol.*, **77**, 148 (1937); *Kgl. Dansk. Vid. Selsk. Medd.*, **2**, 14 (1938). Hevesy and Aten, *Kgl. Dansk. Vid. Selsk. Medd.*, **14**, 5 (1939). Chaikoff *et alia*, *J. Biol. Chem.*, **122**, 169 (1937); **128**, 493 (1939). Dols *et alia*, *NATURE*, **77**, 141 (1938).

### Preparation of Thyroxine from Casein treated with Iodine

In a recent paper by Ludwig and Mutzenbecher<sup>1</sup> experiments are described in which treatment of casein with iodine under carefully defined conditions is stated to yield a physiologically active product from which thyroxine can be isolated.

In view of the existing knowledge of the chemistry of thyroxine, these results are so surprising that it appeared to us to be desirable to repeat the experiments. We have therefore carried out three preparations under the conditions laid down by the German workers and we have confirmed the findings of the latter in every respect.

The casein employed in our experiments was a commercial preparation (B.D.H. acetic-precipitated, soluble). Three separate lots of 100 gm. of this material gave, under Ludwig and Mutzenbecher's conditions of iodination, products containing 7.7, 7.7 and 8.7 per cent of iodine respectively; a physiological test on one of these products indicated an activity (as measured by the effect on the basal metabolic rate of the rat) of the order of 1/300 of that of thyroxine.

Hydrolysis of the iodinated casein preparations with barium hydroxide as described by the German workers led to iodine-rich fractions, containing 37–45 per cent iodine, solutions of which in 0.1 N sodium carbonate solution slowly deposited small amounts of a crystalline sodium salt; the latter could be converted in the usual manner into a compound indistinguishable in chemical properties from thyroxine and showing the full physiological activity of the latter; the chemical identification was completed by the catalytic deiodination of the product isolated to give a characteristic preparation of thyronine. The yields of crystalline thyroxine which we have isolated have been of the order of 100 mgm. from 100 gm. of iodinated casein.

There seems, therefore, to be no doubt as to the correctness of the results obtained by Ludwig and Mutzenbecher, and it becomes a matter of great interest to consider the possible mechanisms by which the formation of thyroxine in these experiments may be brought about.

The first point which seems to us to be significant is that the conditions employed by Ludwig and Mutzenbecher for the iodination of the casein, and which according to them must be closely observed, are not such as to bring about maximum substitution

of iodine in the casein molecule but are rather such as to favour the oxidizing action of iodine. Now the hypothesis has been previously advanced by one of us that the biological synthesis of thyroxine may occur from tyrosine through the stage of diiodo-tyrosine, two molecules of which may undergo oxidative coupling with elimination of one side-chain; it has been supposed that an enzymic mechanism may exist in the thyroid gland by means of which this reaction could be brought about. In the experiments of Ludwig and Mutzenbecher the participation of oxidizing enzymes which might be present in the casein preparation could not be completely excluded owing to the conditions of the iodination (maintenance for about four hours at 37° and pH 8.5); we have found, however, that initial boiling of the casein in aqueous suspension does not affect the result, so that enzymic action cannot be involved.

There appear, therefore, to be two possibilities remaining: (1) iodine itself, under the conditions employed, may be able to effect the oxidative coupling of two molecules of diiodotyrosine which is postulated as a stage in the biogenesis of thyroxine; (2) casein may contain preformed thyronine as one of its constituent amino-acids and this may be iodinated to thyroxine.

The first of these explanations is favoured by Ludwig and Mutzenbecher; the difficulties in the way of its acceptance are (a) the inherent unlikelihood that iodine should bring about such a drastic oxidation and (b) the still greater improbability that a reaction of the type required should occur between two tyrosine residues within the protein molecule. On the other hand, although the presence of a small amount of thyronine in casein (and indeed in other tyrosine-containing proteins) might well have escaped detection hitherto, all attempts which have been made in the past to convert thyronine into thyroxine have failed.

We are at present engaged in experiments which it is hoped may throw further light on the chemical basis of the very interesting phenomena which the work of Ludwig and Mutzenbecher has revealed.

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<sup>1</sup> *Hoppe-Seyl. Z.*, **258**, 195 (1939).

### The Cromer Forest Bed Implements

RESEARCHES I have carried out recently, by means of grants from the Royal Society, relating to the artefacts found in the basal layer of the Cromer Forest Bed, have shown that some six periods of flaking are recognizable on these specimens. I conclude, therefore, that the deposit containing them is composed, in part, of the wreckage of a land surface, or surfaces, on which the makers of the flint implements lived prior to the laying down of the Forest Bed. The various groups of artefacts from the base of this deposit differ not only in their patination and condition, but also, it seems, in their forms and flaking. One of the assemblages of specimens referable, apparently, to the earlier part of the Cromer Forest Bed, is that composed of the well-known ochreous-yellow implements, and flakes found upon the foreshore site at Cromer.