

LETTERS TO THE EDITORS

The Editors do not hold themselves responsible for opinions expressed by their correspondents. No notice is taken of anonymous communications.

Presence of Triiodothyronine in Fowl

THE observation of an unknown iodinated compound as a regular constituent of plasma and thyroid tissue of mice and rats¹⁻³ led to the discovery of 3-5:3'-triiodo-L-thyronine⁴⁻⁷. It has been established that triiodothyronine possesses all the thyroid hormone qualities of thyroxine when tested in a large number of species⁸ and is more potent than thyroxine when tested by a variety of methods *in vivo* in mammals and amphibians⁹. Thus triiodothyronine has come to be recognized as being of physiological importance in laboratory mammals and man. However, little is known of the distribution of triiodothyronine in other classes of vertebrates, beyond the fact that it has been found in reptiles¹⁰. It seemed of interest to ascertain whether birds have the capacity to synthesize this compound.

Light Sussex cockerels, 100 days old, were given 250 μ c. of iodine-131 by the subcutaneous route. 24 hr. later they were killed while under light ether anaesthesia, and the thyroid glands removed, minced and placed in ammonium-chloride ammonia:urea buffer (pH 8.6). Trypsin (1 mgm.) was added, and the glands were allowed to incubate for 24 hr. at 35° C. with a further addition of trypsin after 12 hr. After acidification with hydrochloric acid, the hydrolysate (pH 2.6) was extracted three times with butanol and the combined butanol extracts reduced to dryness under diminished pressure. The residue was taken up in methanol:ammonia (3:1) and applied to Whatman No. 1 filter paper for ascending chromatography in *n*-butanol-acetic acid-water (78:10:12 v/v) or *n*-butanol-dioxane (80:20 v/v) saturated with 2 *N* ammonium hydroxide systems. Thyroxine, triiodothyronine, diiodotyrosine, monoiodotyrosine and iodide were added as carriers. The positions of the carriers were visualized in ultra-violet light, and the triiodothyronine and/or thyroxine areas of the paper cut out, eluted with methanol-ammonia and re-chromatographed in a single dimension in *n*-butanol-dioxane-ammonia or in two dimensions in both solvent systems together with the appropriate carriers. The radioactivity of the paper was localized by radioautography and/or scanning with an automatic recording β -counter. The carriers were localized with diazotized sulphanilic acid and palladium chloride.

The results of two such experiments are shown in Fig. 1, and in both cases radioactivity was noted in the area of triiodothyronine. Other experiments,

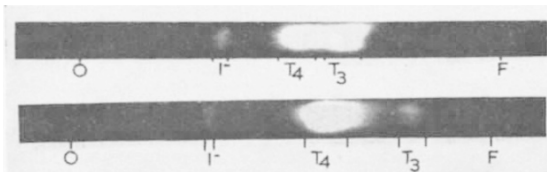


Fig. 1. Autoradio chromatograms of hydrolysed, butanol-extracted bird thyroid glands rechromatographed in butanol-dioxane-ammonia system. Bottom: elution of T_4 - T_3 spot from a butanol-acetic acid-water chromatogram. Top: elution of T_4 spot from a butanol-dioxane-ammonia chromatogram. O, origin; I^- iodide; T_4 , thyroxine; T_3 , 3:5:3'-triiodothyronine

carried out on both males and females, from a cross between Light Sussex and Rhode Island Reds, in some cases following stimulation of the thyroid gland with thyroid-stimulating hormone, have confirmed these observations, but in no case was the percentage of radioactivity ascribed to triiodothyronine higher than 1 per cent of the total radioactivity extractable with butanol from the gland, or more than 5 per cent of the thyroxine radioactivity.

It may be concluded from the present experiments that the thyroid gland of the bird possesses the capacity to synthesize triiodothyronine, and that triiodothyronine presumably has a role in thyroid physiology in this class of vertebrates. This finding may take on added interest in the light of two curious findings, that birds have a low plasma protein-bound iodine value¹¹ and that triiodothyronine is no more potent than thyroxine in various biological tests done in birds^{12,13}.

CLAIRE J. SHELLABARGER*
ROSALIND PITT-RIVERS

National Institute for Medical Research,
The Ridgeway,
Mill Hill,
London, N.W.7.
Jan. 16.

* Special Fellow, National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland.

¹ Gross, J., and Leblond, C. P., *Proc. Soc. Exp. Biol., N.Y.*, **76**, 686 (1951).

² Gross, J., Leblond, C. P., Franklin, A. E., and Quastel, J. H., *Science*, **111**, 605 (1950).

³ Gross, J., and Leblond, C. P., *Endocrinol.*, **48**, 714 (1951).

⁴ Gross, J., and Pitt-Rivers, R., *Lancet*, **i**, 439 (1952).

⁵ Gross, J., and Pitt-Rivers, R., *Biochem. J.*, **53**, 645 (1953).

⁶ Roche, J., Lissitzky, S., and Michel, R., *C.R. Acad. Sci., Paris*, **234**, 997 (1953).

⁷ Roche, J., Lissitzky, S., and Michel, R., *Biochim. Biophys. Acta*, **11**, 220 (1953).

⁸ Barker, S. B., "Ann. Rev. Physiol.", **17**, 417 (1955).

⁹ Selenkow, H. A., and Asper, jun., S. P., *Physiol. Rev.*, **35**, 426 (1955).

¹⁰ Shellabarger, C. J., Gorbman, A., Schatzlein, F. C., and McGill, D., *Endocrinol.*, **59**, 331 (1956).

¹¹ Mellen, W. J., and Hardy, jun., L. B., *Endocrinol.*, **60**, 547 (1957).

¹² Shellabarger, C. J., *Poultry Sci.*, **34**, 1437 (1955).

¹³ Newcomer, W. S., *Amer. J. Physiol.*, **190**, 413 (1957).

Enzymes of the Tricarboxylic Acid Cycle and Cytochrome Oxidase in the Fat Body of the Desert Locust

VARIOUS workers have shown the presence of some or all of the enzymes of the tricarboxylic acid cycle in insect muscle sarcosomes (for example, in *Musca domestica*¹, *Phormia regina*², *Apis mellifera*³) or in particulate fractions prepared from whole insects (for example, from *Aedes aegypti*⁴); but other specific insect tissues have received little attention. The insect fat body is often one of the most conspicuous organs and is known to carry out many reactions involving nitrogen metabolism⁵; but almost nothing has been recorded of other oxidative capabilities. As the biochemical function of the fat body is probably not primarily concerned with energy production (unlike muscle sarcosomes), it appeared of interest to examine this tissue.

The desert locust, *Schistocerca gregaria* Forsk., was used as a source of the fat body tissue which was carefully dissected out and gently homogenized in a Potter-Elvehjem blender. Fifth instar nymphs were used for most of the work. The presence of a number of enzymes associated with the tricarboxylic acid cycle was demonstrated in this tissue for the first