On the Action of Peroxidase on 3,5-Diiodotyrosine

JAN-GUSTAF LJUNGGREN

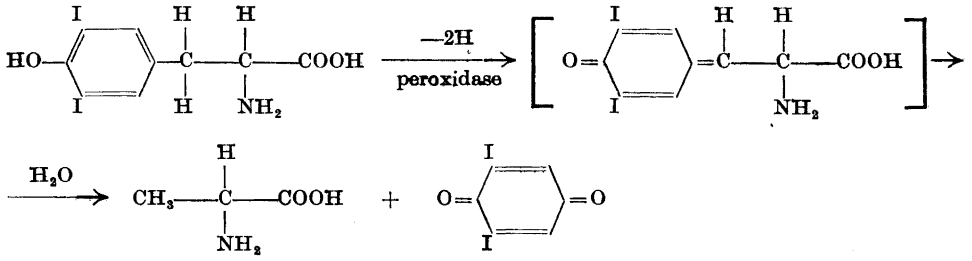
Medicinska Nobelinstitutet, Biokemiska avdelningen, Stockholm, Sweden

It has been suggested [1] that thyroxine is formed from 3,5-diiodotyrosine through the action of peroxidase but no attempts to verify this theory experimentally seem to have been made.

When 3,5-diiodotyrosine, hydrogen peroxide and peroxidase were incubated no formation of thyroxine could in fact be detected. However, another reaction product was observed which might be a possible intermediate in the conversion of 3,5-diiodotyrosine into thyroxine.

3,5-diiodotyrosine was incubated as described below with crystallized verdoperoxidase and hydrogen peroxide and a sample of the solution was studied by paper chromatography. The chromatograms were developed with butanol:acetic acid:water (4:1:1) and the spots visualised with ninhydrin. Alanine was thus identified as one of the reaction products.

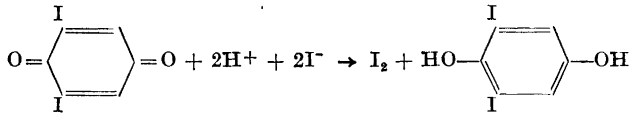
A possible reaction giving alanine as a reaction product would be the following:



The other hypothetical reaction product, 2,6-diiodobenzoquinone, was identified in the reaction mixture by extraction with petroleum ether and crystallization from the same solvent. The melting point of the product was 177°C, as reported for 2,6-diiodobenzoquinone [2], and the mixed melting point with an authentic sample of the quinone prepared according to Ref.[2] was unchanged.

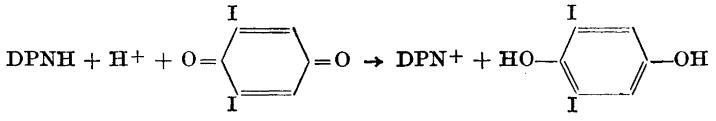
The identity of this reaction product was also confirmed by incubating the latter with potassium iodide in acid solution. Free iodine and a compound identified by paper chromatography as 2,6-diiodohydroquinone were found.

Thus the reaction was the following:



The 2,6-diiodobenzoquinone might be a precursor to thyroxine. It is, however, evident that it must first be reduced to 2,6-diiodohydroquinone.

Wosilait and Nason [3] have previously found that some benzoquinones, *e.g.* 2,6-dichlorobenzoquinone, were easily reduced by DPNH to hydroquinones non enzymatically. We have found that 2,6-diiodobenzoquinone also reacts rapidly with DPNH. The oxidation of DPNH to DPN+ by 2,6-diiodobenzoquinone was followed in a Beckman DU spectrophotometer at 340 mμ



When 2,6-diiodobenzoquinone was incubated with DPNH, 2,6-diiodohydroquinone could be isolated by extraction with ethyl ether, the ether evaporated and the product crystallized from water. The melting point was as reported for 2,6-diiodohydroquinone [4] (144°C) and the mixed melting point between the isolated substance and an authentic sample of 2,6 diiodohydroquinone was the same. They also had the same R_F value on paper chromatogram.

The 2,6-diiodohydroquinone was quantitatively determined both by paper chromatography and a colorimetric method [5]. The latter is based upon the reduction of ferric ions to ferrous ions by a hydroquinone followed by a colorimetric determination of the ferrous ions with *o*-phenantroline. Here DPN^+ does not interfere but the solution must be free from DPNH in order to prevent a new formation of hydroquinone from the benzoquinone formed when ferric ions are reduced to ferrous ions.

The yield of 2,6-diiodohydroquinone was found to be 20 % of theory when 20 ml 3,5-diiodotyrosine (10^{-3} M) in 0.05 M phosphate buffer, pH = 7.2, temperature = 20°C, was incubated with 0.5 ml crystallized verdoperoxidase (3.2 mg VPO/ml) and 0.5 ml H_2O_2 (10^{-2} M). The hydrogen peroxide was added slowly by means of a motor driven micrometer syringe; the 0.5 ml volume requiring 10 h. The hydrogen peroxide had to be added slowly in order to prevent a destruction of the verdoperoxidase, as observed by Agner. When all the hydrogen peroxide had been consumed 1.85 ml DPNH (1 mg DPNH/ml) was added and its oxidation followed spectrophotometrically at 340 mμ.

We have verified that peroxidase is present in the thyroid gland and probably in higher amounts than previously reported [6].

Thus, 2,6-diiodobenzoquinone and 2,6-diiodohydroquinone might be intermediates in the conversion of 3,5-diiodotyrosine into thyroxine. The formation of thyroxine by the condensation of one molecule 3,5-diiodotyrosine and one molecule 2,6-diiodohydroquinone, is now being investigated. Attempts are also being made to purify peroxidase from the thyroid gland. The results of these studies will be reported in this journal.

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1. Westerfeld, W. W. and Lowe, C. *J. Biol. Chem.* **145** (1942) 463.
2. Kehrmann, F. and Messinger, J. *Ber.* **26** (1893) 2377.
3. Wosilait, W. D. and Nason, A. *J. Biol. Chem.* **206** (1954) 255.
4. Seifert, B. *J. prakt. Chem.* **28** (1883) 437.
5. Stephen, W. J. *Metallurgia* **37** (1948) 333.
6. Glock, G. E. *Nature* **154** (1944) 460.

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Hexadecenoic Acid as a Feature of Beef and Horse Subcutaneous Fat

OLLE DAHL

Scans Centrallaboratorium, Malmö, Sweden

Recently the fatty acid composition of some typical fats from domestic animals was examined by the author [1]. Among these fats a beef subcutaneous fat from hide trimmings showed a remarkably high content of hexadecenoic acid, *viz.* 13.0 mole-%. Subsequently, when studying the relationship between iodine value and content of various fatty acids as well as between the content of the fatty acids themselves in natural fats [2], the said subcutaneous fat behaved differently from the others, evidently dependent on the high content of hexadecenoic acid. Furthermore, in an extensive investigation of the characteristics of more than 700 animal fats [3], the beef subcutaneous fats showed a lower saponification equivalent throughout and, besides, the relations between the characteristics were divergent from those of other fats. (In various investigations (for references, *cf.* Ref.[2]) pig subcutaneous fat (back fat) was proved to contain but moderate amounts of hexadecenoic acid, about 3 mole-%.)

These findings gave rise to a further confirmation of hexadecenoic acid as a feature of beef subcutaneous fat. The most abundant depot of subcutaneous fat is the brisket. The brisket fat from 10 cows was collected, mixed and extracted by means of ethyl ether, saponified and esterified by