

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-diiodohydroquinone⁴ (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 method⁵. 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 H₂O₂ (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 m μ .

We have verified that peroxidase is present in the thyroid gland and probably in higher amounts than previously reported⁶.

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. Kehrman, 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 author¹. 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 fats², 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 fats³, 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

Table 1. Component fatty acids of beef brisket fat and horse wither fat.

Brisket fat: iodine value 56.4; sapon. equiv. 278.1; unsap. matter 0.33 %
 Wither » » » 84.2; » » 279.8; » » 0.42 %

Component fatty acids	Beef brisket fat		Horse wither fat	
	% (weight)	% (mole)	% (weight)	% (mole)
<i>Saturated:</i>				
Myristic	3.2	3.7	4.4	5.1
Palmitic	31.3	32.5	26.0	27.1
Stearic	5.4	5.0	2.1	2.0
<i>Unsaturated:</i>				
Tetradecenoic	1.9	2.2	1.7	2.0
Hexadecenoic	16.9	17.7	14.2	14.9
Oleic	38.9	36.6	35.2	33.3
Octadecadienoic	2.4	2.3	6.4	6.1
Octadecatrienic	—	—	9.6	9.2
C ₂₀ -acids	—	—	0.4	0.3

methyl alcohol. The component fatty acids were determined by fractional distillation of the methyl esters as previously described¹. The cows were killed at the end of March, *i. e.* prior to the pasture-feeding.

With regard to pure horse subcutaneous fat, no data of its composition are available whatsoever. It was decided, therefore, to examine the wither fat, which generally is the most abundant horse subcutaneous fat, in the same way as the brisket fat from cows. For this purpose the wither fat from 4 horses — 3 geldings and 1 mare — was collected. The animals were slaughtered at the same time as the cows. The results are summarized in Table 1.

As is seen from Table 1 the content of hexadecenoic acid is considerable both in brisket and in wither fat, the content of the former being still higher than that previously found in beef hide fat¹.

Dugan *et al.*⁴ investigated the composition of beef brisket fat but disregarded the hexadecenoic acid, the content of which was simply included in that of oleic acid. In a review of the formation of animal fats Shorland⁵ refers to unpublished data on the composition of beef subcutaneous fat, indicating a content of 4.6 mole-% hexadecenoic acid only.

The fairly high content of hexadecenoic acid previously found by the author in

horse fats¹ may partly be due to the presence of 17 % wither fat in the fatty tissue mixture.

Finally it may be mentioned that, among fats of land animals, bone fat occasionally holds significant amounts of hexadecenoic acid. Thus, beef bone fat contains up to 10 or even 11 mole-% of this acid (for references, see Ref.³). In hoof oil and "offal oil" from horse, the latter deriving from bones and trimmings mixed in indefinite proportions, Brooker and Shorland⁶ found no less than 20.5 and 11.5 mole-% hexadecenoic acid, respectively.

1. Dahl, O. *Z. Lebensm.-Untersuch. u. Forsch.* **106** (1957) 81.
2. Dahl, O. *Z. Lebensm.-Untersuch. u. Forsch.* **106** (1957) 349.
3. Dahl, O. *Acta Agr. Scand.* To be published.
4. Dugan, L. R., Maroney, J. E. and Petheram, M. J. *Am. Oil Chemists' Soc.* **29** (1952) 298.
5. *Progress in the Chemistry of Fats and Other Lipids*, Vol. 3; Pergamon Press, London & New York, 1955, p. 276; Shorland, F. B. *Formation of Animal Fats*.
6. Brooker, E. G. and Shorland, F. B. *Biochem. J.* **46** (1950) 80.

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