

Spectroscopic Observations of Reactions between Lactoflavin, the Coulter Compound, 'Cytochrome *b*', and Cytochrome *c*

FROM diphtheria toxin, one of us (M. D. E.) has prepared a solution continuing the "complex porphyrin" of Coulter¹, and also a compound characterized by an α -band located in the same position as that of cytochrome *b* (563 $m\mu$). The preparation will be described elsewhere.

When oxidized lactoflavin was added to this solution, at 30° C. and pH 7.4, the 574 $m\mu$ and 563 $m\mu$ bands disappeared instantly. When lactoflavin was reduced by hydrosulphite, the 574 $m\mu$ and 563 $m\mu$ bands reappeared at once. The 574 $m\mu$ and 563 $m\mu$ compounds must therefore be in the reduced state.

Reduced cytochrome *c* was added next to this solution. A paradoxical phenomenon was observed: the 574 $m\mu$ and 563 $m\mu$ bands again disappeared, as if reduced cytochrome *c* had oxidized the reduced 574 $m\mu$ and 563 $m\mu$ substances. Cytochrome *c* was obtained from a stock solution by acetone precipitation; the stock solution had been prepared in Prof. Keilin's laboratory by Dr. F. Stare.

In a separate experiment, oxidized cytochrome *c* was added to the solution of 574 $m\mu$ and 563 $m\mu$ substances. All the bands remained unchanged, indicating that no reaction had occurred.

Lactoflavin is not necessary for the reaction between reduced cytochrome *c* and the reduced 574 $m\mu$ and 563 $m\mu$ substances. This was shown by adding reduced cytochrome *c* to the 574 $m\mu$ and 563 $m\mu$ compounds. The 574 $m\mu$ and 563 $m\mu$ bands disappeared. This reaction can be partly reversed by the immediate addition of a few drops of 5 per cent potassium ferricyanide solution. Oxidized cytochrome *c* appears, and the 574 $m\mu$ and 563 $m\mu$ bands re-appear in diminished strength. The 574 $m\mu$ and 563 $m\mu$ substances alone do not react with ferricyanide. A solution containing the 574 $m\mu$ compound alone reacted exactly like the mixture of the 574 $m\mu$ and 563 $m\mu$ compounds.

The following hypothesis summarizes the observations and interprets the paradox: when the iron atom of cytochrome *c* is in the divalent state, the 574 $m\mu$ and 563 $m\mu$ compounds can be oxidized by the porphyrin ring of cytochrome *c*; but when the iron is in the trivalent state, the porphyrin ring cannot oxidize the 574 $m\mu$ and 563 $m\mu$ substances.

These reactions between cellular catalysts are interesting in themselves. In addition, a more exhaustive investigation will bring out whether or not they are related to the Pasteur reaction.

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¹ Coulter and Stone, *J. Gen. Physiol.*, **14**, 583 (1930/31).

Oxidation of Cholesterol and Dehydroandrosterone by Means of Osmic Acid

UNSATURATED compounds are known to add, under the action of osmic acid, two hydroxyl groups at the place of their double bond¹, these groups being in *cis*-position with regard to each other. The reaction course is analogous to that produced by KMnO_4 . However, action of the latter on cholesterol² yields but 4-5 per cent of *cis*-cholestan-triol. Thus KMnO_4 is unsuitable for the oxidation of the double bond in

sterol-derived hormones, in particular in dehydroandrosterone.

After we had obtained *trans*-androstan-(17)-one-(3, 5, 6)-triol from dehydroandrosterone oxide³ we desired to obtain also the *cis*-compound for the sake of comparing their physiological activities. The method has been worked out for cholesterol⁴, then transferred to dehydroandrosterone.

Equivalent amounts of anhydrous cholesterol and osmic acid, dissolved in water-free ether, are allowed to react for 2-3 days. The ether is then distilled off and the black residue boiled with an aqueous-alcoholic solution of Na_2SO_3 for two hours. When working with cholesterol only traces of the looked-for product—cholestan-triol—are found in the water-alcohol filtrate; most of it is obtained by extracting the black residue with boiling alcohol. The *cis*-cholestan-triol obtained melted, after washing with ether, at 238.5-239.5°. Mixed with the *trans*-compound it caused depression of the melting point.

On oxidizing dehydroandrosterone with OsO_4 almost the whole of the androstanone-triol passes into the aqueous-alcoholic solution (after boiling with sulphite). Yield of the crude product: 70 per cent. Recrystallized from ethyl-acetate; transparent rectangular prisms, m.p. 243.5-244° (corr.; sinters at 242.5°). Analytical data agree with the formula $\text{C}_{19}\text{H}_{30}\text{O}_4$.

We intend applying the same method also to androstene-diol, androstene-dione and testosterone.

The present work is to be published shortly in detail.

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¹ Details in the recent paper by Criegee, R., *Ann.*, 522, 75 (1936), where the literature is cited.

² Windaus, A., *Ber.*, **40**, 257 (1907).

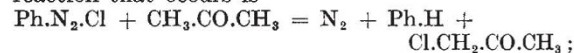
³ Ushakov, M., and Lutenberg, A., "Dehydroandrosterone oxide", in *Report in Journal Obshchei Khimii* and *Bull. Soc. Chim. de France*.

⁴ Criegee (*loc. cit.*) mentions having obtained some *cis*-diols of the sterol group; he does not give any details, nor mention what sterols he worked with.

Decomposition of Benzene-Diazonium Chloride

To test the suggestion¹ that the decomposition of aromatic diazo compounds might be non-ionic in mechanism, I have been investigating the decomposition of benzene-diazonium chloride in the presence of non-aqueous solvents. When suspended in an organic liquid, benzene, diazonium chloride appears to melt at about 50° C. and then immediately a violent decomposition sets in. There is great heat evolution, and, except on the small scale, the reaction tends to become explosively violent. Often hydrogen chloride is evolved, and whenever its formation has been observed, chlorobenzene has been found amongst the reaction products.

From an examination of the decomposition in acetone, it appears possible that the chlorobenzene is produced by a secondary reaction between the decomposing benzene-diazonium chloride and the hydrogen chloride, since when an excess of chalk is added to keep the mixture neutral, the principal reaction that occurs is



for both benzene and chloroacetone were isolated in good yield.