so far as dinitrophenol will cause a drop in the K/Na ratio and ^{42}K uptake. Since the ability of brain mitochondria to phosphorylate is associated with a high potassium-low sodium concentration, a decrease in the intra-mitochondrial potassium could result in decreased phosphorylation (Abood & Gerard, 1954). It is noteworthy that during excitation of the nerve fibre the permeability to sodium is increased, while an equivalent amount of potassium must diffuse out to maintain electrical and osmotic balance (Hodgkin & Katz, 1949).

There is some evidence that GSH is essential for the preservation of the structural integrity of cells (Fegler, 1952), and we have observed that GSH in concentrations of 10^{-3}M will cause agglutination of brain mitochondria (unpublished). If the metabolic effects of electrical currents are in some way related to ion transport, it is possible that the physical alteration of cells or mitochondria induced by currents may be affected by GSH. Brain mitochondria do appear to have a 'membrane' potential and certain metabolic poisons such as dinitrophenol can affect it (Gerard, Abood, Taylor & Falk, 1953). It does not seem improbable, therefore, that mitochondria may be responsive to electrical phenomena. Studies along this general line are now in progress.

SUMMARY

1. The effect of electrical currents on oxidative phosphorylation of rat-brain mitochondria has been studied using a variety of metallic conductors.

2. Maximal inhibition of phosphorylation was obtained with silver-copper and gold electrodes, while with platinum and silver electrodes inhibition was almost absent.

3. When potassium ferricyanide replaced oxygen as electron acceptor in the system, an inhibition of about 45 % in the $\text{P}/2[\text{Fe}(\text{CN})_6]^{3-}$ ratio regardless of the kind of conducting substance used in the construction of the electrode.

4. Glutathione, sodium sulphide, and diethyldithiocarbamate, but not cysteine and many other reducing agents, were able to protect against inhibition of phosphorylation when oxygen was the electron acceptor.

5. A number of thiol-inhibiting agents were tested and were found, unlike electrical stimulation, primarily to depress oxidation, so that the P/O ratio was largely unchanged.

6. Evidence for believing that the effect on phosphorylation is due to the electrical currents and not electrolytic artifacts is discussed.

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REFERENCES

- Abood, L. G. (1954). *Amer. J. Physiol.* **176**, 247.
- Abood, L. G. & Gerard, R. W. (1954). *Amer. Physiol. Soc.*, Madison, Wisconsin.
- Abood, L. G., Gerard, R. W. & Ochs, S. (1952). *Amer. J. Physiol.* **171**, 134.
- Abood, L. G., Gerard, R. W., Tschirgi, R. D. & Banks, J. (1952). *Amer. J. Physiol.* **168**, 728.
- Anson, M. L. (1941). *J. biol. Chem.* **194**, 119.
- Barron, E. S. G. (1951). *Advanc. Enzymol.* **11**, 201.
- Berger, L., Stein, M. W., Colowick, S. P. & Cori, C. F. (1946). *J. gen. Physiol.* **29**, 379.
- Fegler, G. (1952). *Nature, Lond.*, **170**, 624.
- Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
- Folin, O. & Malmros, H. (1929). *J. biol. Chem.* **83**, 115.
- Gerard, R. W., Abood, L. G., Taylor, R. D. & Falk, G. (1953). *19th Int. physiol. Congr. Abstracts*, p. 388.
- Glasstone, S. & Hickling, A. (1939). *Chem. Rev.* **25**, 407.
- Hodgkin, A. L. & Katz, B. (1949). *J. Physiol.* **108**, 37.
- McIlwain, H. (1951). *Biochem. J.* **49**, 382.
- Narayanawami, A. & McIlwain, H. (1954). *Biochem. J.* **57**, 663.
- Rall, T. W. & Lehninger, A. L. (1953). *J. biol. Chem.* **194**, 119.
- Slater, E. C. (1953). *Biochem. J.* **53**, 521.

Serratamic Acid, a Derivative of L-Serine Produced by Organisms of the *Serratia* Group

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During studies aimed at obtaining information on the biosynthetic route by which the pigment prodigiosin (Wrede & Rothaas, 1934) is produced by organisms of the *Serratia* group, attempts to isolate precursors of the pigment led to the discovery of another metabolic product of these organisms which is here given the trivial name of serratamic acid. The organism mainly studied

was isolated in this laboratory from coconut, and like other members of the *Serratia* group was difficult to classify exactly (Breed & Breed, 1926). In culture it resembled *S. indica* (Bergey, 1948). Other highly chromogenic strains, including *S. kiliense* (N.C.T.C. 4619) and *S. marcescens* (Woodbine, Kolasiewicz & Seaman, 1954) were also examined.

On a solid medium containing glycerol and peptone, all the strains used produced abundant growth and pigmentation, together with serratamic acid, after incubation for 48 hr. at 28°. After dilution of an acetone extract of the cells with water and making alkaline, the pigment was removed by extraction into light petroleum. Examination of the aqueous layer led to the isolation of the optically active nitrogenous acidic substance, serratamic acid. It was separated from the acidified aqueous layer by extraction into ether and freed from accompanying traces of neutral substances by transference to sodium bicarbonate solution. On acidification, this solution deposited serratamic acid, which was purified by crystallization from water or ethyl acetate, after decolorization with charcoal. From hot water the acid separates on cooling in an apparently amorphous state, which changes, on standing at room temperature, to long needle-shaped crystals which are waxy to the touch. Aqueous solutions exhibit surface-active properties. Serratamic acid is soluble in alcohols and ketones, sparingly soluble in cold water and ether, and insoluble in hydrocarbons. It dissolves in sodium bicarbonate solution with the evolution of carbon dioxide, is insoluble in dilute hydrochloric acid, but dissolves in the concentrated acid. It gives no colour reaction with ninhydrin.

The analytical figures for C, H and N found in three samples of serratamic acid by different analysts are not exactly accommodated by the formula $C_{13}H_{25}O_5N$, but this formula is consistent with the observed equivalent weight (275) and with the fact that only two products ($C_{10}H_{20}O_3$ and $C_3H_7O_3N$) have been found after hydrolysis. An empirical formula $C_{27}H_{52.54}O_{10}N_2$ is more closely in keeping with the analytical figures, but, in view of the results presented above and the failure to detect the presence of more than one carboxyl group during electrometric titration, this larger formula could not be accepted without further evidence. The titration curve, determined at a concentration of 0.05N in aqueous methanol (50%, v/v), was typical of a weak monocarboxylic acid showing pK 4.3 and strong buffering over the approximate range pH 3.5–5.2. The instability of serratamic acid at the m.p. of camphor has precluded a molecular-weight determination by the Rast method and it is too insoluble in alternative solvents of lower m.p.

Serratamic acid is readily hydrolysed in the presence of either acid or alkali to yield the same nitrogen-free acid, $C_{10}H_{20}O_3$. After hydrolysis with aqueous or methanolic hydrochloric acid there was also obtained a water-soluble hydrochloride giving a positive ninhydrin reaction, from which a pure amino acid was readily isolated. Analytical figures on the hydrochloride and free acid and values for

the optical rotation conformed with the corresponding constants of L-serine.

Because racemization might have occurred during chemical manipulation, the possibility that the amino acid was isoserine ($[\alpha]_D^{20} - 32.6^\circ$ in water, Fischer & Jacobs, 1907a) could not be immediately ruled out. The strongest evidence in favour of its identity with L-serine was obtained by microbiological assay with *Lactobacillus helveticus* and *Leuconostoc mesenteroides*, both organisms having an absolute requirement for serine. When the degradation product was added to serine-deficient media it supported growth of both organisms to the same extent as did authentic serine when incorporated at the same concentration in a similar medium (Stokes & Gunness, 1945).

Confirmatory evidence was obtained from paper chromatography under the conditions employed by Woiwod (1949). Serine, serine hydrochloride, the amino acid from serratamic acid, and the crude hydrochloride of the latter from acid hydrolysates all gave spots with the same R_f value on single-dimensional chromatograms run with butanol-acetic acid. Furthermore, the spots given by the substances derived from serratamic acid could not be further resolved by chromatography in a second dimension with phenol as solvent. The latter observation indicated strongly that L-serine was the sole amino acid resulting from the hydrolysis and, since it was obtained in 80% yield, it was considered to contain all the nitrogen of the serratamic acid molecule.

Any doubt as to the identity with L-serine of the degradation product was removed by qualitative studies of its reaction with periodate under the conditions described by Nicolet & Shinn (1941) and Van Slyke, Hiller & MacFadyen (1941) for the quantitative estimation of serine. After this reaction, ammonia was detected by Nessler's reagent, and formaldehyde was isolated as the dimedone derivative (m.p. and mixed m.p. 189°), in keeping with there being hydroxyl and amino groups on adjacent carbon atoms of the degradation product.

Satisfactory conditions for the crystallization of the second hydrolytic product, an acid, $C_{10}H_{20}O_3$ (isolated as its methyl ester from methanolic hydrogen chloride degradation) could not be found, but it was obtained as a solid, m.p. 43–5°, after purification by distillation in a high vacuum. The formula allotted was supported by analytical figures on the parent acid, its amide, and its *p*-bromophenacyl ester, and also by equivalent- and molecular-weight determinations. The saturated nature of the acid was shown by its stability to cold neutral permanganate in acetone solution and its failure to decolorize bromine in carbon tetrachloride.

This acid is isomeric with the hydroxydecanoic acids, but so little work has been published on these substances that a ready identification of the degradation product with any one of the possible isomers is not easily made. It is interesting to note that a tentative identification as 3-hydroxydecanoic acid has been made in the case of an acid isolated by Jarvis & Johnson (1949) during the hydrolysis under strongly acid conditions of a lipid-like substance, also containing rhamnose, produced by the growth of *Pseudomonas aeruginosa* on a glycerol-peptone medium. There is, incidentally, no evidence of serratamic acid existing in combination with a sugar and the conditions of its isolation are such that a glycosidic linkage is unlikely to be split during the process.

On oxidation with permanganate in acetone solution the C_{10} acid is degraded to an acid $C_8H_{16}O_2$, which has been isolated as a *p*-bromophenacyl ester. The identification of this oxidation product can only tentatively be made as octanoic acid, since cases have been recorded of the esters of isomers of fatty acids showing no m.p. depression on admixture (Wrede & Rothaas, 1934). Hence, until further evidence is available it is not possible to deduce the exact structure of the carbon chain in the C_{10} acid. A decision about the third oxygen atom must be deferred. So far it has been impossible to isolate pure acylated products of the acid by the usual methods. Indirect evidence of the existence of a hydroxyl group is given by the isolation from the methanolic hydrogen chloride treatment of serratamic acid of a chloro ester in addition to the methyl ester of the C_{10} acid. This chloro ester after hydrolysis yields a *p*-bromophenacyl ester identical with that obtained from the directly isolated C_{10} acid.

Since identical nitrogen-free acids are obtained by both alkaline and acid hydrolysis of serratamic acid it appears that the molecule is split in the same position under both conditions. The production of ammonia and failure to isolate serine from the alkaline hydrolysis is in keeping with the known instability of this amino acid at high pH values (Daft & Coghill, 1931). No conclusive evidence has yet been obtained in support of an ester or peptide linkage between the serine moiety and the remainder of the serratamic acid molecule, but the absence of a ninhydrin colour supports the conclusion that there is no free amino group in the substance.

In view of the reported antibiotic activity of strains of *S. marcescens* (Fuller & Horton, 1950; Woodbine *et al.* 1954), each one used in the present study was tested for activity against stock pathogenic strains of *Staphylococcus aureus* and *Escherichia coli*. Tests demonstrated that, though all produced serratamic acid, only the Woodbine strain exhibited antibacterial effects. Neither pure

serratamic acid nor its sodium salt showed any activity. It was thought that serratamic acid, being possibly a peptide, might be related, as a degradation product, to the polypeptide antibiotic marcescin (Fuller & Horton, 1950). However, the lack of activity associated with intact cells of organisms producing the acid precludes this view unless there exists in the inactive organisms an enzyme capable of degrading marcescin.

The ease with which L-serine can be isolated from serratamic acid suggests that the growth of the organisms of the *Serratia* group can be used for the production of the amino acid in quantity. The advantages of such a process are: (a) the biological and chemical techniques are simple and short, (b) no difficult separation from other amino acids is involved and (c) no optical resolution is required as in chemical synthesis.

EXPERIMENTAL

All melting points are uncorrected.

Culture methods. In enamelled trays, measuring $11.5 \times 15.5 \times 2$ in., sterile 5% (w/v) agar-water was poured to form, after setting, a base on which the nutrient materials could be thinly layered, thus effecting an economy in the use of nutrients. To each tray was added 200 ml. of a medium containing (w/v) glycerol, 5%; Difco proteose peptone no. 3, 1%; KH_2PO_4 , 0.2%; $MgSO_4$, 0.6%; and agar 5%. Inocula were prepared by growth of the organism isolated from coconut at 28° on slopes of the same composition. After 18 hr. the organisms were suspended in a similar medium without the agar, approx. 2 ml. of the suspension being added to each tray and spread evenly over the agar surface by brushing with a sterile 2 in. fine-bristle paint brush. Twenty trays, giving a surface area of 3565 sq.in., could be inoculated in this manner by the once-sterilized brush without introducing contamination. The trays were covered by lids of thin aluminium sheeting, and were incubated on edge at 28° for about 40 hr. If the trays were left in a horizontal position, the growth became patchy as a result of condensed moisture falling from the lids.

Extraction of serratamic acid. The thick, evenly pigmented, deep wine-red growth was scraped off the agar with a small sheet of tin plate and added to 200 ml. of acetone agitated by a mechanical stirrer. The organisms rapidly sedimented and the supernatant liquid was then decanted. After sucking the cells dry on a Büchner funnel, they were extracted a second time in the same manner. The combined extracts were diluted with 600 ml. of water, and dilute NaOH was added until the colour of the solution changed to an orange-brown. Four extractions, each with light petroleum (b.p. 40–60°, 50 ml.), followed by one extraction with ether (50 ml.) removed the pigment. After acidifying the aqueous layer to pH 2 with HCl, the serratamic acid was isolated by extraction into ether (10 × 100 ml.). It was then removed from this extract by shaking with several portions sat. aqueous $NaHCO_3$. Finally, the product was precipitated from the solution of its sodium salt by acidification with HCl. It was purified by crystallization from water after decolorization with

activated charcoal. The yield from twenty trays ranged from 1.0 to 1.5 g. Serratamic acid (crystallized from water or ethyl acetate) had m.p. 138° (decomp.); $[\alpha]_D^{20} - 10.2^\circ$ in ethanol (c, 5.0). (Found: C, 57.0, 57.2, 57.3, 57.4; H, 9.0, 8.9, 9.3, 8.9; N, 4.9, 4.5, 4.3%; equiv. by titration, 275. $C_{17}H_{54}O_{10}N_2$ requires C, 57.3; H, 9.4; N, 4.9%; equiv. 283. $C_{13}H_{25}O_5N$ requires C, 56.8; H, 9.1; N, 5.1%; equiv. 275.)

Hydrolysis of serratamic acid. (a) *Aqueous hydrochloric acid.* The substance (2.5 g.) and conc. HCl (50 ml.) were heated together for 30 min. at 100°. The clear solution soon deposited an oil which, after the reaction time, was separated in ether, the mixture having been diluted with water. On evaporation of the aqueous layer under reduced pressure, deliquescent crystals of a hydrochloride remained which gave a positive ninhydrin reaction. The free amino acid was isolated by treatment of an aqueous solution of the salt with an excess of Ag_2CO_3 , filtration from the resulting silver salts, and precipitation of the soluble silver as a sulphide from the filtrate. The silver-free solution was then evaporated under reduced pressure, and the L-serine (0.7 g.) was crystallized from aqueous methanol in stout prisms, m.p. 210–212° (decomp.); $[\alpha]_D^{20} - 6.7^\circ$ in water (c, 7.5); $[\alpha]_D^{20} + 15.1^\circ$ in N-HCl (c, 6.8) (Fischer & Jacobs (1907b) give -6.8° and $+14.9^\circ$ respectively). (Found: C, 34.2; H, 6.8; N, 13.1%. Calc. for $C_3H_7O_3N$: C, 34.3; H, 6.7; N, 13.3%.)

With *p*-toluenesulphonyl chloride and aqueous $NaHCO_3$, the amino acid gave a solution from which *N*-*p*-toluenesulphonyl-L-serine separated in nearly quantitative yield on acidification with mineral acid. It crystallized from water in long needles, m.p. 233° (decomp.). (Found: C, 46.3; H, 4.9; N, 5.7; S, 12.5%. $C_{10}H_{13}O_5NS$ requires C, 46.3; H, 5.0; N, 5.1; S, 12.4%.) This substance did not depress the m.p. of *p*-toluenesulphonyl-DL-serine (McChesney & Swann, 1937).

The oily product (1.5 g.), which was insoluble in hydrochloric acid and was extracted with ether (see above), was separated into neutral and acidic fractions by treatment with a solution of $NaHCO_3$. The oily acidic material (1.0 g.) thus obtained solidified slowly. On treatment of its sodium salt with *p*-bromophenacyl bromide under reflux in aqueous ethanol, it yielded the *p*-bromophenacyl ester, which crystallized in plates or needles, m.p. 105–106°, from petroleum (b.p. 60–80°). (Found: C, 56.2; H, 6.8; Br, 20.6%. $C_{18}H_{25}O_4Br$ requires C, 56.1; H, 6.5; Br, 20.8%.)

(b) *Methanolic hydrochloric acid.* Methanol (50 ml.) was saturated at room temp. with dry HCl gas; serratamic acid (1 g.) was added to it, and the solution was then refluxed for 1 hr., after which the solvent was removed by distillation. Water was added to the cooled mixture, and the oil, which was insoluble in acid, was separated by ether extraction.

By evaporation of the aqueous layer to dryness, a crystalline solid (0.4 g.) was obtained which separated from methanol- $CHCl_3$ in prisms, m.p. 149° (decomp.). (Found: Cl, 24.5%. Calc. for L-serine hydrochloride, $C_3H_5O_3NCl$: Cl, 24.8%.) From this hydrochloride the free amino acid was isolated as described above.

After washing the ether layer with water, it was dried over anhydrous Na_2SO_4 and the solvent was evaporated to leave an oily ester, which was distilled to give fractions: F_1 , b.p. 71–75° at 0.2 mm. Hg (0.4 g.) and F_2 , b.p. 80–100° at 0.2 mm. Hg (0.15 g.). These fractions were redistilled and F_1 yielded a colourless mobile liquid, b.p. 71° at 0.2 mm. Hg containing methoxyl but giving negative

Lassaigne tests for N and Cl. (Found: C, 65.2; H, 10.6%. $C_{11}H_{25}O_3$ requires C, 65.3; H, 10.9%.)

On shaking the pure distillate, F_1 , with ethanolic NH_3 , it gradually gave the amide (60% yield), which crystallized from benzene-ethyl acetate in pearly plates, m.p. 104°. (Found: C, 64.1; H, 11.3%. $C_{10}H_{21}O_2N$ requires C, 64.2; H, 11.2%.)

The ester, F_1 , on treatment with excess 20% (w/v) in KOH methanol at room temp. was rapidly saponified, depositing an insoluble potassium salt from which, after standing for 2 hr., the free acid was isolated in quantitative yield by ether extraction after treatment with mineral acid. Purified by distillation the acid was obtained as a waxy solid, b.p. 125–130° at 0.2 mm. Hg, m.p. 43–45°. (Found: C, 64.0; H, 10.5%; equiv. 195; mol.wt. (Rast) 200. $C_{10}H_{20}O_3$ requires C, 63.9; H, 10.6%; equiv. 188; mol.wt. 188.) The acid yielded a *p*-bromophenacyl ester, m.p. 103–105°, identical with that obtained by aqueous acid hydrolysis of serratamic acid.

F_2 , obtained above, was redistilled and obtained as a colourless oil, b.p. 95° at 0.2 mm. Hg, containing methoxyl and chlorine. Treatment of this substance with dilute aqueous alkali (2N at 100° with occasional vigorous shaking) gave a chlorine-free product from which a *p*-bromophenacyl ester m.p. 103–105°, identical with the corresponding derivatives described above, could be obtained.

(c) *Aqueous alkali.* The acid (0.4 g.) in 20% (w/v) KOH (5 ml.) was heated at 125° for 30 min., during which time a stream of pure N_2 was passed through the mixture, and the effluent gas was scrubbed by dilute HCl. Afterwards the HCl was evaporated and the crystalline residue was treated with a saturated solution of sodium picrate, yielding a derivative which crystallized from water in large yellow needles, m.p. 280–281° (decomp.), and was identified as ammonium picrate. (Found: C, 29.4; H, 2.5; N, 22.4%. Calc. for $C_8H_8N_4O_7$: C, 29.2; H, 2.4; N, 22.7%.)

The alkaline reaction mixture was acidified, the oily acid product was isolated in ether and distilled. The distillate, b.p. 125–130° at 0.2 mm. Hg had m.p. 42–45° (not depressed on admixture with the substance from acid hydrolysis). This was subsequently converted into the *p*-bromophenacyl ester, which crystallized from light petroleum (b.p. 60–80°) in plates (0.2 g.), m.p. 101–103°. (Found: C, 56.1; H, 6.8; Br, 20.5%. $C_{18}H_{25}O_4Br$ requires C, 56.1; H, 6.5; Br, 20.8%.) This ester gave no depression in m.p. when mixed with the corresponding substances obtained from acid hydrolysis of serratamic acid.

Oxidation of the C_{10} acid obtained by hydrolysis. The acid (0.3 g.) was dissolved in a saturated solution of $NaHCO_3$ (1 ml.) and then acetone (25 ml.) added. Powdered $KMnO_4$ (1.0 g.) was added in small portions over a period of 2 hr., the solution being raised to boiling point momentarily after each addition to speed up the slow reaction. Afterwards the mixture was freed from solvent under reduced pressure, the residue suspended in water, cleared by SO_2 and acidified with dilute H_2SO_4 . The products, isolated in ether, were separated by shaking with aqueous $NaHCO_3$. From the alkaline aqueous extract there was obtained, after acidification, an oily fatty-smelling acid which was isolated by extraction into ether. On treatment of its sodium salt with *p*-bromophenacyl bromide under reflux in aqueous ethanol there was obtained *p*-bromophenacyl octanoate which crystallized from light petroleum (b.p. 60–80°) in plates (0.2 g.), m.p. 63–64°. The m.p. was not

depressed on admixture with the authentic derivative of octanoic acid. (Found: C, 56.2; H, 6.1; Br, 23.4%. Calc. for $C_{10}H_{21}O_2Br$: C, 56.3; H, 6.3; Br, 23.5%.)

SUMMARY

1. Serratamic acid, m.p. 138° (decomp.), is produced by several strains of organisms of the *Serratia* group.

2. Serratamic acid is readily hydrolysed by both mineral acids and alkalis. Acid hydrolysis yields L-serine and an acid, $C_{10}H_{20}O_3$, tentatively identified as a hydroxydecanoic acid, which is oxidized to octanoic acid or one of its isomers.

3. The linkage between the amino acid and hydroxy acid appears to be of the peptide type.

4. Serratamic acid does not account for the antibiotic activity of strains of *S. marcescens* producing this acid.

5. The growth of organisms of the *Serratia* group to produce serratamic acid affords a convenient source of L-serine.

REFERENCES

- Bergey, D. H. (1948). *Manual of Determinative Bacteriology*, 6th ed. London: Baillière, Tindall and Cox.
- Breed, R. S. & Breed, M. E. (1926). *J. Bact.* **11**, 76.
- Daft, F. S. & Coghill, R. D. (1931). *J. biol. Chem.* **90**, 341.
- Fischer, E. & Jacobs, W. A. (1907a). *Ber. dtsh. chem. Ges.* **40**, 1058.
- Fischer, E. & Jacobs, W. A. (1907b). *Ber. dtsh. chem. Ges.* **40**, 3718.
- Fuller, A. T. & Horton, J. M. (1950). *J. gen. Microbiol.* **4**, 417.
- Jarvis, F. G. & Johnson, M. J. (1949). *J. Amer. chem. Soc.* **71**, 4124.
- McChesney, E. W. & Swann, W. K. jun. (1937). *J. Amer. chem. Soc.* **59**, 1116.
- Nicolet, B. H. & Shinn, L. A. (1941). *J. biol. Chem.* **139**, 687.
- Stokes, J. L. & Gunness, M. (1945). *J. biol. Chem.* **157**, 65.
- Van Slyke, D., Hiller, A. & MacFadyen, A. (1941). *J. biol. Chem.* **141**, 681.
- Woiwod, A. J. (1949). *J. gen. Microbiol.* **3**, 312.
- Woodbine, M., Kolasiewicz, A. & Seaman, A. (1954). *Nature, Lond.*, **174**, 406.
- Wrede, F. & Rothaas, A. (1934). *Hoppe-Seyl. Z.* **226**, 95.

Paper-electrophoretic Analysis of Protein Extracted at Low Ionic Strength from Fish Skeletal Muscle

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The muscle proteins of fish are considered to be very similar in their general properties to corresponding mammalian proteins. Reay (1935), Reay & Kuchel (1936), Subba Rao (1948) and Dyer, French & Snow (1950) applied the fractionation method of Smith (1934, 1937) in studies of the muscle proteins of several species of marine fish and found protein fractions similar to those present in rabbit muscle. Roth (1947) reported that protein fractions of some freshwater fish resemble in their general properties those in rabbit and frog muscles. Smith's fractionation scheme is, however, out of date and some of the results obtained by the method are incorrect. On the basis of the most reliable values of the above workers, fish muscle seems to differ most characteristically from mammalian muscle only in its very low content of connective tissue and perhaps also in the albumin content.

Using the free electrophoretic method, Hamoir (1949, 1951) has compared carp muscle proteins with those from rabbit muscle and found both similarities and differences between them. The electrophoretic patterns of codling muscle at low ionic strength obtained by Connell (1953a) using the Philpot-Svensson system show the same general characteristics as those of rabbit or frog muscle.

Later Connell (1953b) found considerable differences in minor details of the patterns obtained in free electrophoresis of muscle extracts from several species of fish, but concluded that the components of all the patterns may be resolved into the same mobility groups as those of mammalian muscle extracts described by Jacob (1947).

The present study has been undertaken to determine the electrophoretic properties of fish muscle-protein extracts at low ionic strength by using both paper and free electrophoretic methods. The diagrams for extracts obtained under identical conditions from several species of fish are compared. An extract of pike muscle has been fractionated by ethanol precipitation, and the behaviour of the successive fractions in paper electrophoresis is discussed.

MATERIALS

The following ten species of fish caught on the west coast of Finland were studied in the experiments: Baltic herring (*Clupea harengus membras* (L.)); whitefish (*Coregonus lavaretus* (L.)); roach (*Rutilus rutilus* (L.)); ide (*Idus idus* (L.)); bream (*Abramis brama* (L.)); eel (*Anguilla anguilla* (L.)); pike (*Esox lucius* (L.)); flounder (*Platichthys flesus* (L.)); perch (*Perca fluviatilis* (L.)); pike-perch (*Lucioperca lucioperca* (L.)).