tion constants (see Fig. 2). An unequivocal assignment of the spots to corresponding species is not necessarily possible, e.g., as in the two acid lysinates. The R's subject to temperature fluctuations, appear to correspond, respectively, to the free acid (++o) as 0.30, lysinate (++–) as 0.35, acid lysinates (o+– and +o) as 0.48 and 0.65, and free base (oo–) as 0.74.

The effect is absent in a basic solution, as collidine-water, or in a more economical medium which we have found equally effective for amino acid chromatography, butanol-water-pyridine (1: 1: 0.6). In either solvent the phenomenon does not appear with a polybasic amino acid, such as aspartic acid. It is possible that the use of stronger bases, such as the aliphatic amines, would be more effective with the weakly acidic groups. In phenol-water, arginine also yields long streaks rather than discrete spots, indicating a situation akin to that of lysine. The absence of the effect with histidine may perhaps be correlated with the relative weakness of its basicity.

An explanation of the phenomenon can probably be given along lines similar to those advanced by Westall (*2*) for the separation of inorganic ions in partition chromatography, i.e., basic lysine ions are capable of association with phenol. These associations are essentially new compounds, possessing their own partition coefficients and moving independently without charging the phases. The explanation assumes that in the phenol system the dissociation of phenol is so small that it does not cause drastic acidification of basic ions of lysine. Rough calculation of the volume of water in the phenol > water system bathing the spots shows that it probably does not exceed that used in the original aliquot; there is therefore no appreciable change of the intrinsic pH by dilution.

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Demonstration of a Fatty Acid Oxidase in Frozen Poultry Fat[1]

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Lipoxidases have been shown to exist in plant material (*6*) and in animal tissues (*1, 2*). The Warburg technique has been used to demonstrate the ability of microorganisms to oxidize fats (*3*). The adipose tissue of rats has been shown to be enzymatically active (*3, 5*).

In the course of a study on the causes of rancidity of frozen chicken fat, an enzyme was prepared which oxidizes fatty acids. All birds[2] were killed by sticking, then were drawn, and frozen at 0° F. The adipose tissue was removed by permitting the carcass to thaw enough for skinning. Fat deposits from around the neck, the visceral cavity, and on the body were removed and pooled. Care was taken to include no muscle, skin, or connective tissue. This collected material was kept frozen until used—a period varying from 1 week to 3 months.

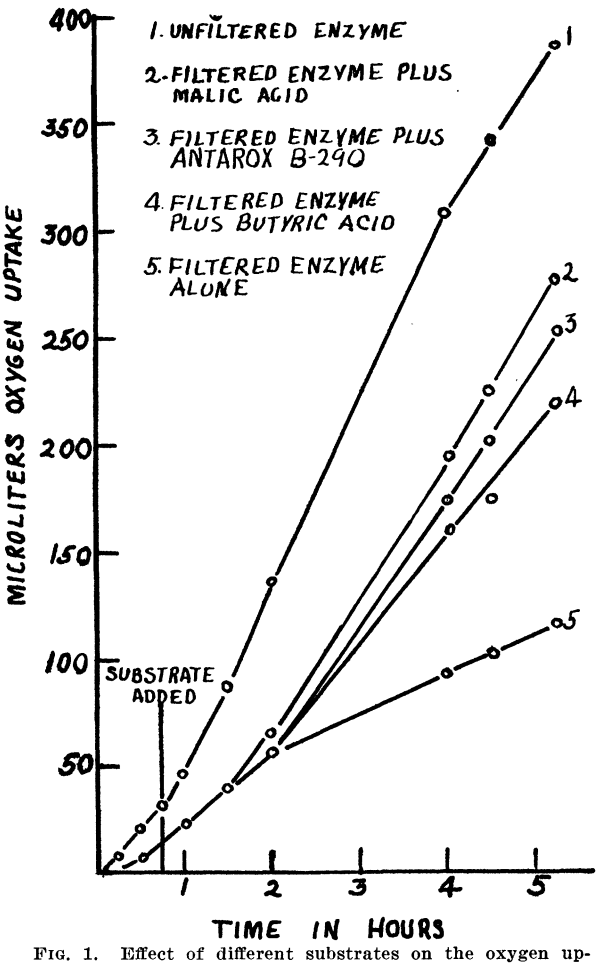

Fig. 1. Effect of different substrates on the oxygen uptake of the fatty acid oxidase.

The enzyme was prepared by expressing the fat away from the tissue portion of the adipose material. The separated tissue was blotted on absorbent paper to remove excess fat, weighed, and homogenized with distilled water in a Potter homogenizer (*4*), 1 g of tissue in 30 ml of water. This mixture was centrifuged, the supernatant collected, and the residue washed twice by centrifuging with distilled water (10 ml of water for 1 g of tissue). Washings were combined with the previously prepared supernatant. Microscopically, the homogenate showed that some cell debris and fat globules were retained.

Using a Warburg Respirometer and an atmosphere of air at 37° C, the homogenate had an induction period of 4–6 hr (average of eleven trials was 5 hr). After the induction period in the presence of phosphate, Mg ion, and adenosine-5-phosphoric acid, there was a steady oxygen uptake, varying from 30 to 100 μl/hr (average of nine trials was 65 μl). Respiration continued for ap-

[1] Published as Scientific Paper No. 848, Agricultural Experiment Station, Institute of Agricultural Sciences, State College of Washington.

[2] We wish to thank Dr. W. J. Stadelman, of the Washington State College Poultry Department, for supplying the birds used in this work.

proximately 24 hr. Attempts to demonstrate the substrate for this respiration were unsuccessful.

The homogenate was combined with 1.2 volumes of 0.01 M phosphate buffer (pH 7.5) and 0.2 volumes of 0.04 M $MgSO_4$, filtered to remove fat globules, and aged at 37° C for 24–29 hr. Very little respiration was shown by 2.4 ml of this preparation with the addition of 0.2 ml of 0.0028 M adenosine-5-phosphoric acid. The addition of glucose as a substrate did not change the respiration. The addition to this mixture of 0.2 mM of oleic acid, linoleic acid, palmitic acid, stearic acid, tripalmitin, tristearin, butyric acid, acetic acid, or 3.3 mg Antarox B-290[3] (a water-soluble castor oil polyethylene glycol ester) approximately doubled the oxygen uptake. Malic and fumaric acids, members of the tricarboxylic acid cycle, also doubled the oxygen uptake. Acids were neutralized with NaOH before using. A portion of the enzyme-phosphate-magnesium ion mixture which had been aged, but not filtered, showed a higher respiration than any of the substrates added to the filtered preparation.

Boiling the aged and filtered homogenate for 30 sec destroyed enzymatic activity. Dialysis of the unfiltered mixture against phosphate buffer and Mg ion during the aging period also decreased activity markedly.

Adipose tissue from five birds was used in this work and it was observed that there were large variations in the activities of preparations from different birds, and fat deposits from different parts of the same bird.

Further work is being carried out on substrates for this preparation and on the path of the oxidation.

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A Simple Jet Type Air Stirrer[1]

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The paddle wheel type of air stirrer has been used in organic laboratories in order to reduce fire hazards. However, this type of air stirrer is noisy, clumsy, and lacks power. The jet type stirrer described in this article is almost noiseless in operation, compact, and has more speed and almost as much power as a variable speed stirrer.[2]

[1] Contribution No. 384, Department of Chemistry. Funds supplied by Office of Naval Research and Bureau of Animal Husbandry under Research and Marketing Act.

[2] Cenco, Central Scientific Apparatus Company, Chicago.

[3] Sample of Antarox B-290 from Antara Products, New York.

The jet type stirrer can be made most conveniently from a 1-in. steel ball and brass or steel jets[3] (Fig. 1). A hole ¼ in. in diam and ¼ in. deep must first be drilled into one side of the steel ball, and a piece of steel ¼ in. in diam and 1¾ in. in length (A) inserted into the hole

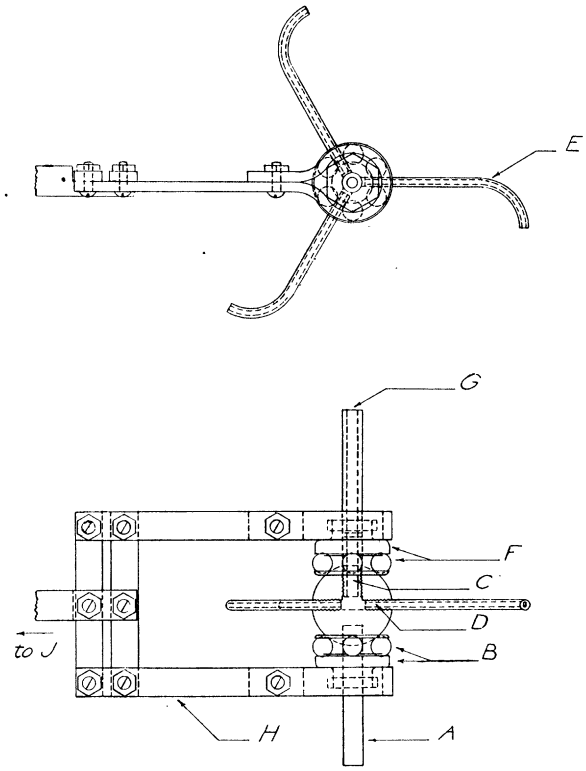

FIG. 1. Diagram showing construction of stirrer (guard around jets of hardware cloth 2 in. in width is not shown).

and brazed to the steel ball. The upper part of this 1¾-in. steel rod serves as a means of holding a ball-bearing race[4] and cone (B) and the lower part serves as a shank to which a stirring rod can be attached with either a piece of heavy rubber tubing or an ordinary steel chuck. A hole ¼ in. in diam and ½ in. deep (C) must then be drilled directly opposite the steel rod, and three holes 3/32 in. in diam (D) at a 120° angle to each other drilled so as to contact the lower half of the hole which had been drilled at (C). The three holes at (D) are then threaded, and threaded brass or steel jets screwed into them. These jets are bent slightly at point (E). The hole at point (C) is also threaded and a ¾-in. threaded steel tube screwed into it. The upper end of the steel tube holds another ball-bearing race and cone and a babbit bearing at (F). Another piece of steel tubing 1½ in. in length is threaded at one end and screwed into the bicycle cone. A piece of heavy rubber tubing is connected to the upper end of this steel tube and serves as an air inlet (G). The apparatus is held rigid by the steel bracket (H) clamped to a ring stand at (J). The speed of the stirrer can be adjusted by regulating the flow of compressed air.

[3] Ford V-8 carburetor jets or ⅛-in. steel capillary tubing.

[4] Front wheel bicycle race and cone.