Under these conditions the rate of growth is quite consistent; the presence of folic acid seems to prevent the acute reversible drops in weight observed in previous experiments (*4*). Of 12 rats studied on this diet, only one rat, number 90, showed a slight drop in weight with food consumption dropping to zero for one day. This rat also showed an early leveling-off of her growth curve on the 15th day of the reported period; the growth of the other rats did not level off until after the 26-day period.

The above experiments indicate that rats do not always lose their ability to utilize homocystine, under the conditions prevailing in these experiments, even though their intestinal folic acid synthesis has been checked by the sulfa drug, since addition of large doses of crystalline folic acid enables some rats to continue growth. The fact that only some rats respond to folic acid indicates that it is not the only factor involved. These animals retain a latent capacity for the utilization of homocystine, which seems to depend for its manifestation on a fairly high level of folic acid and is not destroyed by the sulfa drug. The nature of the variability of the growth response becomes apparent when we realize that other vitamin B factors are probably involved, the most conspicuous one being B_{12}. These factors may be stored in the animals in varying amounts from preexperimental diets and may therefore be depleted in single animals at different periods of the experiment. Work is now under way to elucidate these problems.

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Separation of the Ionic Species of Lysine by Means of Partition Chromatography[1]

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It is a cardinal principle in chromatography that for each compound there is only one position on the chromatogram; this locus is unique in the sense that all of the substance is contained within that position, but not in the sense that entirely different substances may not also occupy that position. As a corollary, it is believed that the appearance of two or more loci of substance indicate the presence of two or more different compounds. It is the purpose here to cite exceptions to this corollary, viz., that the presence of more than one locus of concentration may still be identified with a single substance.

We have found that paper partition chromatography of lysine[3] in a saturated phenol > water system may result in a plurality of spots. If from a relatively large volume of aqueous solution of lysine, small aliquots are taken for partition chromatographs, the number and position of these spots will depend upon the pH of the aqueous solution of the lysine (see Fig. 1). The relative intensities will be in rough accord with the distribution of ionic species for the different pH's as calculated from ioniza-

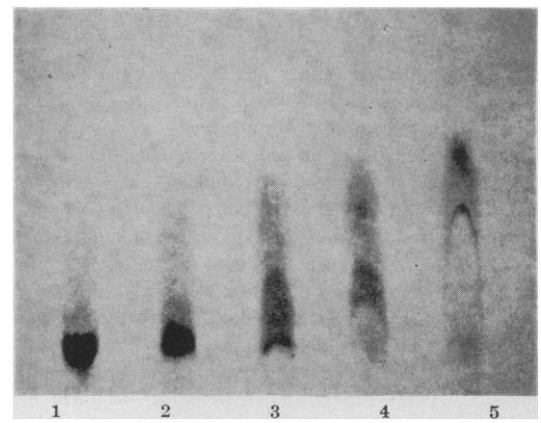

FIG. 1. Ninhydrin-developed spots of lysine aliquoted at various pH's: 1 at 2.20, 2 at 8.45, 3 at 9.50, 4 at 10.62, and 5 at 12.15.

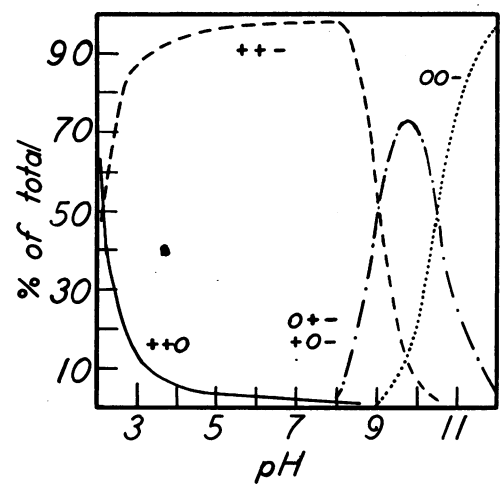

FIG. 2. The distribution of ionic species of lysine at various pH's.

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[2] The author desires to acknowledge the assistance of Mr. Leo Vernon in a portion of this work.

[3] The lysine was purified by two recrystallizations, once as the picrate and again as the dihydrochloride. This procedure was followed for three different sources of material, all of which gave the same results: synthetic DL-lysine (monohydrochloride), naturally-occurring L-lysine (monohydrochloride), and lysine regenerated from the hydrolysis of aminohomopiperidone, and precipitated as the dihydrochloride. Melting points were in agreement with values in the literature (*1*).

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.

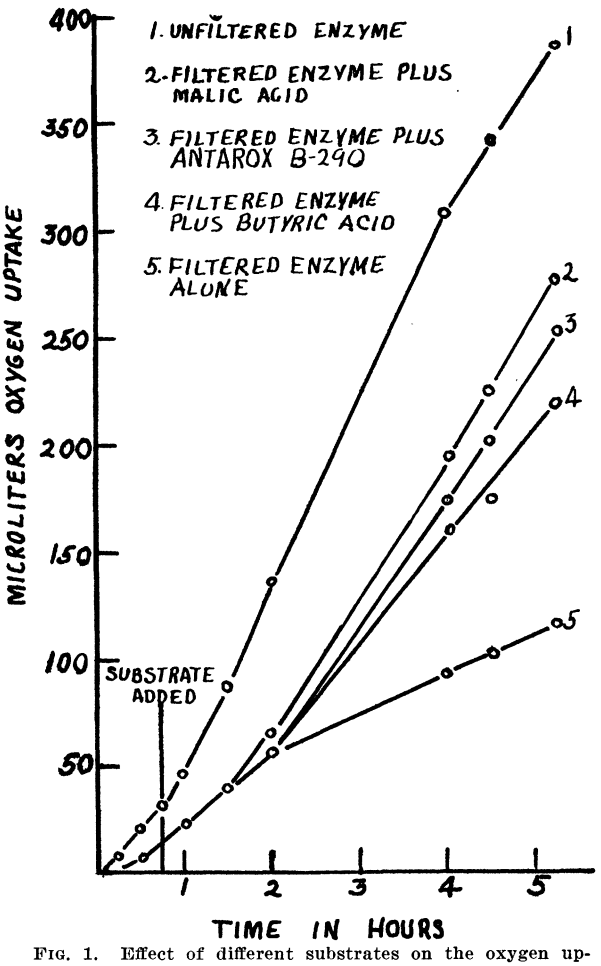

Fig. 1. Effect of different substrates on the oxygen uptake of the fatty acid oxidase.

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-

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