monia, drop by drop, until no more turbidity exists. The solution must be filtered and stored in brown glass bottles. After heating for another 10-min period at 45° C, or until deep brown in color, the increasingly brittle sections are carefully lifted into a Petri dish of distilled water, for a rapid wash.

Reduction is carried out in a 10% solution of formol. If desired, toning in a gold chloride solution (1:500) can be done, with a succeeding wash in distilled water and fixation in "hypo." Dehydration beyond the 97% alcohol stage can be completed with a carbol-creosote-xylol mixture with good results, prior to mounting in balsam.

Chemically clean glassware is essential if precipitate is to be prevented. Reagent chemicals are essential if the delicate nerve terminals are to be impregnated free from overlaying precipitate.

A slow impregnation in the refrigerator in 2% silver nitrate, lasting up to 1 month, has produced some very delicate impregnations with visualization of several synaptic terminals on one nerve cell of the human cerebral cortex.

The mechanism by which the neurofibrillar structures and their terminals take up silver nitrate is not well understood. The reinforcing of this stain by silver carbonate has great value in achieving specific neuronal, as against neuroglial, staining. Gold toning is well suited to the method because it relieves the background of much indistinct material.

It is not contended that all the synaptic endings on a nerve cell body and its processes are made visible by this metallic method, but we believe that even in the cerebral cortex, where the staining of *boutons terminaux* is difficult, a fair proportion can be shown in favorable preparations (Fig. 1a). The postulation of some other method of synapsis in the human brain (*6*) on negative grounds is not supported by our studies on normal tissues. When there has been recent degeneration of nerve pathways in the cerebrum, the increased argyrophilia of the fibers brings into view very large numbers of swollen synapses around individual nerve cells.

The fact that this staining method gives good results on formalin-fixed tissues makes it universally applicable. The use of this staining method for recently lobotomized brains is but one application (Fig. 1b). In addition it offers promise of a simple and fairly consistent method for use in the elucidation of other problems in interneuronal transmission. Refinement of the technique itself, and possibly of methods of tissue fixation, is still needed to bring out its greatest potentialities.

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The Oxidation of Chicken Fat Tissue[1]

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Ellman and McLaren (*1*) reported that a fatty acid oxidase was present in frozen poultry fat. Moore and Nelson (*2*), working with guinea pig mammary gland tissue, stated that this oxidative system (*1*) was probably autoxidation.

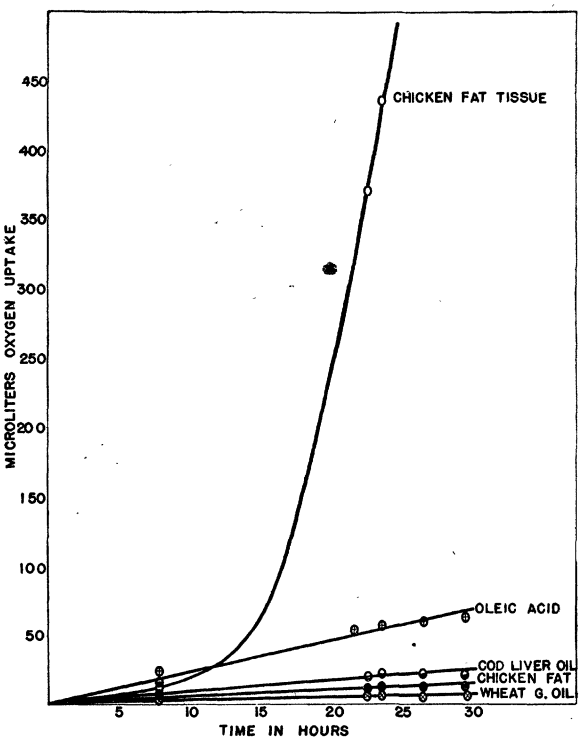

Fig. 1. Comparison by oxygen uptake of chicken fat tissue, an unsaturated fatty acid, and 3 natural fats.

The oxygen-uptake curves for guinea pig mammary gland tissue (*2*) are similar to those for oxygen absorption of unsaturated fats (*3, 4*), as suggested by Moore and Nelson (*2*), although they gave no reference for this comparison. It should be stressed that oxygen absorption curves of fats can be based on methods (*3, 4*), other than the Warburg procedure (*5*).

Comparison by oxygen uptake was made with fresh chicken fat tissue, chicken fat, oleic acid, cod-liver oil, and wheat-germ oil. Chicken fat tissue was isolated from the viscera of a hen, and allowed to set at room temperature for about an hour. Chicken fat

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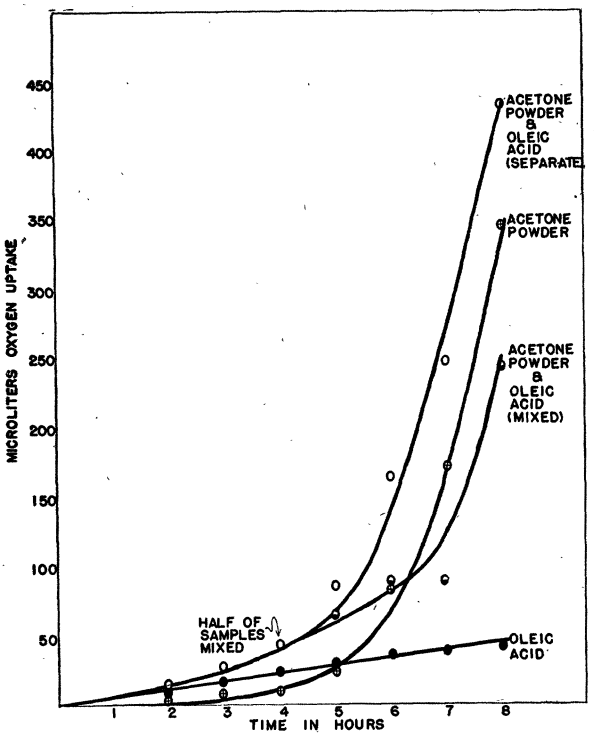

FIG. 2. Qualitative comparison by oxygen uptake of an acetone powder of chicken fat tissue and oleic acid.

as used for these experiments was the fat that melted free of the fat tissue at room temperature. Ten grams of each of the designated 5 substances was homogenized for 1 min with 30 ml of phosphate buffer of pH 7.6 in a Waring Blendor. All samples, except the chicken fat tissue, were boiled under nitrogen for 5 min in order to destroy any enzyme system that may have been present. Samples were then run on the Warburg respirometer, using the direct method, in an atmosphere of air at 37° C.

As can be seen from Fig. 1, the oxygen uptake for the fat tissue was greatly accelerated after 8 hr. Oleic acid, cod-liver oil, wheat-germ oil, and chicken fat all exhibited a progressive linear oxygen uptake. These results could not be accounted for by contamination with microorganisms.

The conclusion drawn from this was that the rapid oxygen uptake of the fat tissue system was dependent on the presence of tissue rather than fat. In order to further verify this conclusion, 45 g of finely chopped fresh chicken fat tissue was homogenized with cold acetone. The precipitate was washed 4 times in cold acetone, dried for 1 hr at 37° C, and resuspended in 270 ml of phosphate buffer of pH 7.6. A qualitative comparison of this suspension with a 2% homogenate of oleic acid was then made.

The acetone powder from the fat tissue took up oxygen very rapidly after an induction period of about 5 hr as is shown in Fig. 2. The primary difference between the oxygen-uptake curve of the chicken fat tissue (Fig. 1) and that of the acetone powder (Fig. 2) is that the fat tissue required a longer induction period before rapid oxidation started. This is possibly due to the presence of fat in the suspension. Additional evidence is that a homogenate of oleic acid lengthened the induction period of a suspension of the acetone powder. This is shown in Fig. 2. Boiling the acetone powder results in loss of activity.

The precise nature of this oxidative system is unknown. The authors do not believe that it can be explained as the autoxidation of a simple fat or fatty acid.

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Enhancement of Lead Excretion in Humans by Disodium Calcium Ethylenediamine Tetraacetate[1, 2]

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Ethylenediamine tetraacetic acid or Versene (EDTA), is a synthetic chelating agent used industrially on a large scale for the control of cations in solution. The compound forms strong, un-ionized, soluble chelate complexes with cations, especially those of the di- and trivalent type. In this way many characteristic effects of metal ions in solution may be controlled. When administered to animals and humans intravenously, the primary acute action of the compound in large doses over short periods was to lower the systemic calcium levels (*1, 2*). At extreme dosage hypocalcemic tetany was observed in animals. In contrast to this effect of EDTA, the administration of the preformed calcium chelate was without effect on calcium homeostasis (*3*). The preformed calcium EDTA was also singularly nontoxic by all routes (*4, 5*). The absence of toxicity of the compound may be attributed to its physiological inertness, since 99% of the material tagged in the methylene position with radioactive carbon could be recovered from the urine after intra-

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