

served until the temperature had reached about 150° K. Then transformation occurred quickly as the colors changed to olive green, at about 160° K to red-brown and at higher temperatures to green, so that above about 150° K, the concentrations of the coexisting species may well have been virtually those at equilibrium. We are unable to decide definitely whether chemical activation energy or the diffusion process limits the rate of the reaction. The hint given by the

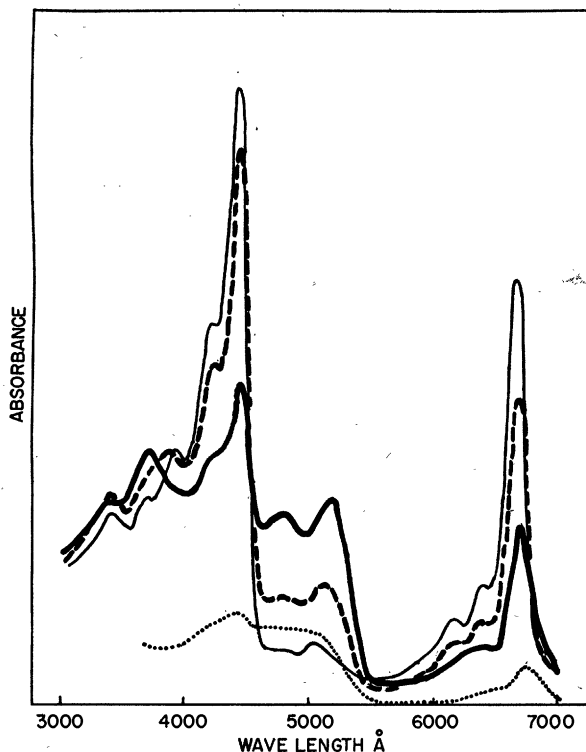


Fig. 2. Chlorophyll *a* in 10% mono-isopropyl amine, 10% isopropyl benzene, and 80% 1:1 propane-propene; — 190° K, ---- 170° K, — 160° K. Curve marked has been derived by Dunicz *et al.* for the intermediate of chlorophyll *a* at 300° K.

concentration dependence of the base would suggest the latter. There seems to be good evidence (2, 3) that the brown intermediate results from an acid-base neutralization which would ordinarily require little chemical activation energy. According to this view, the chlorophyll molecule is conceived as an acid with its hydrogen ion furnished by the labile hydrogen on carbon-10 of the pentanone ring. Activation may then be principally a measure of the lability of the hydrogen. The ion is removed by the base upon neutralization, leaving a negative charge which profoundly affects the resonating circuits of the chlorophyll molecule. In confirmation the fact has been adduced that allomerized chlorophyll does not undergo the Molisch phase test. (In this substance, a methoxy group has supposedly replaced the acidic hydrogen on carbon-10 of the chlorophyll molecule.) We have found, in fact, that a solution of allomerized chlorophyll *a* in iso-

propyl amine does not become brown even at the lowest temperature.

In addition to the transformations we have been discussing, others are taking place which are especially clear at temperatures at which the equilibrium concentrations of the intermediate are small. These seem to have the same characteristics as were found (4) to occur in rapid equilibria between species of chlorophylls in common solvents such as ether.

However, the basicity of the solvent does not appear to be the sole requirement for the formation of the brown intermediate, because with chlorophyll *b* in di-isopropyl amine no such color was observed unless one accepts as indication of its presence a shoulder on the long wavelength slope of the band in the blue region of the spectrum. The color remained substantially unchanged from room temperature to that of liquid nitrogen when hydrocarbon had been added to make the solution fluid at that temperature.

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The Staining of Synaptic Terminals Within the Central Nervous System by Rio-Hortega's Double Impregnation Silver Method¹

George E. Morrison, Jr., and Wm. C. Gibson

Crease Clinic Research Unit and Department of Neurological Research, University of British Columbia, Vancouver, Canada

Since the demonstration by Ramón y Cajal in 1888 (1) of the minute structure of neuronal interconnections in the retina and cerebellum, several workers have described similar synaptic *boutons terminaux* in other parts of the nervous system (2-4). Many such observations have been incidental in nature. Today, however, there is an increasing demand for specific information about the structure of the synapse, and a concern for definitive techniques for its staining. Moreover, it is becoming evident that, for lack of a good method of demonstrating synaptic terminals, neurohistology is lagging behind its more productive counterpart, neurophysiology. In this study Rio-Hortega's double impregnation method (5) for the staining of neurofibrils has been applied to the problem and, with minor modifications, has been found to provide a fairly consistent and simple method for staining the *boutons terminaux* in formalin-fixed material from the human cerebral cortex, cerebellum, and spinal cord. It has been possible thus to establish the

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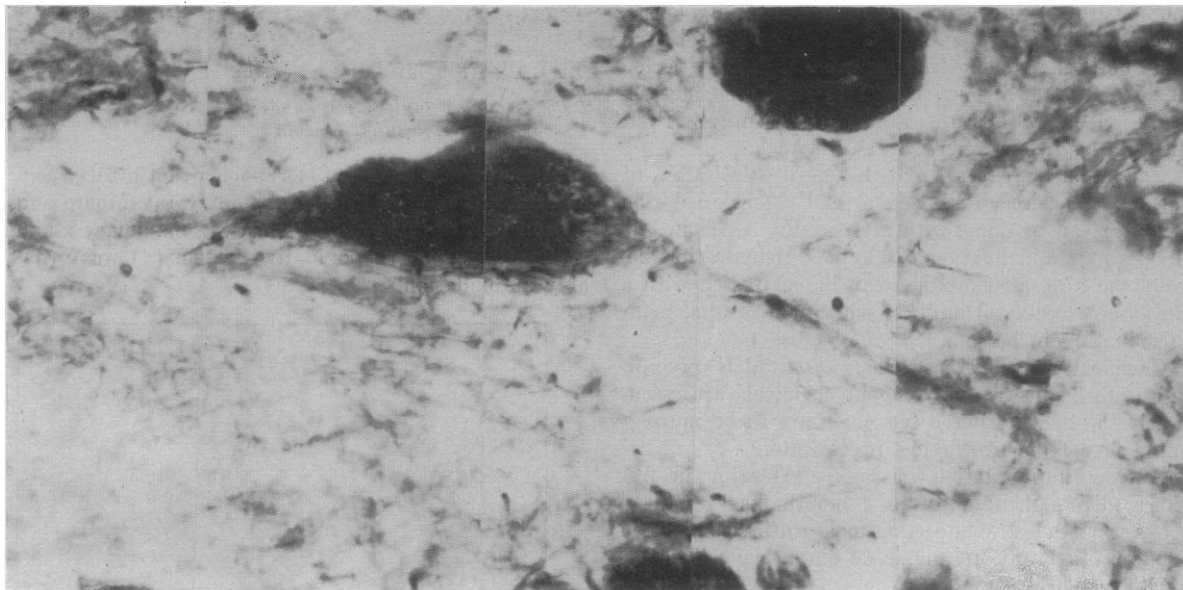


FIG. 1a. Large neurone in the human precentral cortex with a total of 11 ringlike *boutons terminaux* ending upon the cell body or its dendritic processes. $\times 1200$.

ringlike axonal endings as the common denominator of transmission mechanisms throughout the human nervous system.

The annotated method follows: Nervous tissue which has been well fixed in 10% neutral formaldehyde is cut into blocks (5 mm thick by 2 cm square) and sectioned on a freezing microtome at $15\ \mu$. The sections are received into a Petri dish filled with distilled water bearing 10 drops of concentrated ammonia.

By blowing vigorously on the sections it is possible to "wash" them adequately and without damage in this and in two succeeding baths of distilled water. With a small glass "hockey stick" the sections are transferred to a 12-cc Pyrex cup filled with 2% silver

nitrate (C.P.) to which 4 drops of chemically pure pyridine have been added. (Solutions of silver nitrate up to 5% in distilled water may be used, though there is an added risk of fine precipitate being deposited on the sections by the addition of pyridine.)

After gentle heating at 45°C for 10–30 min the sections take on a tobacco color, whereupon they must be washed in a Petri dish of distilled water with further blowing. Sections are then transferred to another chemically clean Pyrex cup holding 4 drops of pyridine and 12 cc of so-called "silver carbonate." This solution is prepared by adding 67 cc of 10% silver nitrate to 267 cc of 5% sodium carbonate (C.P.) and adding distilled water to 1000 cc. The resultant precipitate is dissolved by the adding of concentrated am-

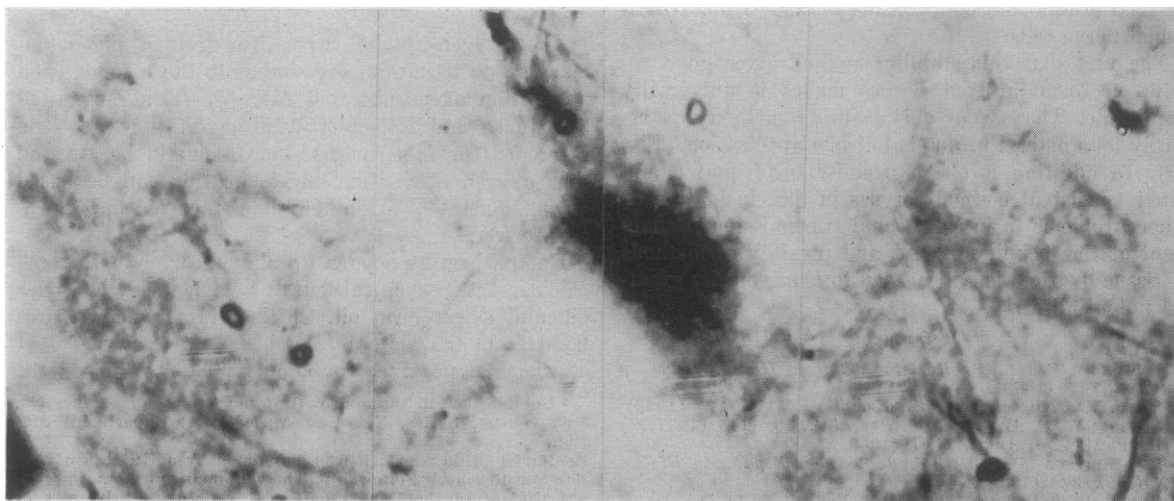


FIG. 1b. Degenerating *boutons terminaux* of the cingulate gyrus in a patient who died 29 hr after frontal lobotomy. $\times 2800$.

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 Tissue¹

H. J. Phillips² and I. L. Williams

Department of Poultry Husbandry,
University of Nebraska, Lincoln

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 autooxidation.

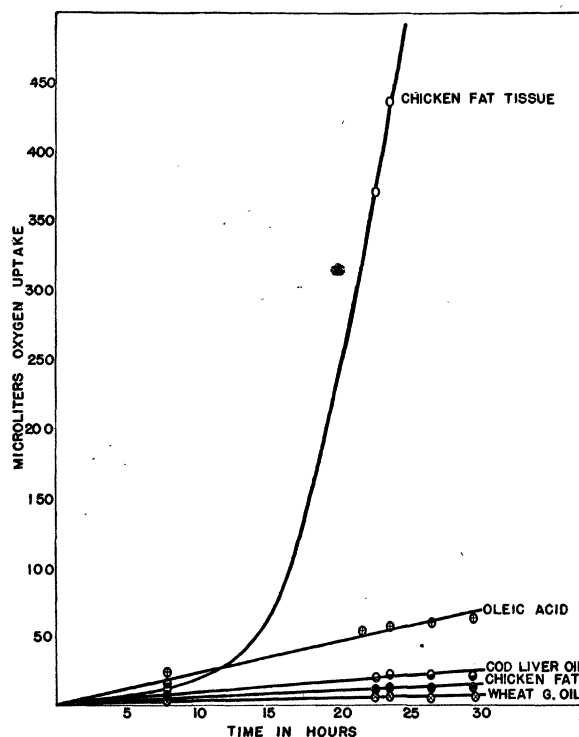


FIG. 1. Comparison by oxygen uptake of chicken fat tissue, an unsaturated fatty acid, and 3 natural fats.

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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