cells after immune hemolysis with guinea pig complement were demonstrated, and it was noted that similar defects were seen in cytolyzed tumor cells (*7*). See also a recent review of steps in immune hemolysis (*8*).

We have estimated that an 88-Å defect, if completely through an 80-Å unit membrane, would have a resistance of 100 megohm in parallel with the remaining cell resistance, with the resistivity of intracellular fluid taken as 75 ohm cm. Hence, one "hole" could produce a 9 percent decrease in resistance in a cell with a 10-megohm input resistance and a 17 percent decrease in a 20-megohm cell. Such initial decreases were seen (frames taken every 1.3 seconds), but the quantity of antibody used and the present uncertainty of the rate at which a "hole" develops prevent resolution of whether the earliest observable changes represent full development of one hole or gradual development of many.

We believe that the electrode recording technique may be of increasing value in the study of sublethal or lethal cell damage, providing an opportunity for temporal resolution of damaging events and possible repair mechanisms in single cells.

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Prostaglandins: Localization in Subcellular Particles of Rat Cerebral Cortex

Abstract. *Homogenates of rat cerebral cortex contain material corresponding to prostaglandins E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$ which are concentrated mainly in the light microsomal and mitochondrial fractions. Only the former fraction exhibits significant ability to synthesize prostaglandins E_1 and $F_{1\alpha}$ from bis-homo-γ-linolenic acid. After subfractionation of the crude mitochondrial fraction, prostaglandin E and F material is found mainly in the cholinergic and noncholinergic nerve endings. We conclude that the nerve endings are a storage site, whereas the light microsomes are the site of synthesis.*

Prostaglandins are hydroxycarboxylic acids, derived by cyclization of ω6 polyunsaturated C_{20} fatty acids; they form a unique class of potent, pharmacologically active compounds (*1*). A mixture of prostaglandins E_2 and $F_{2\alpha}$ (PGE_2 and $PGF_{2\alpha}$) is released from the superfused somatosensory cortex of cats, and increased release is detected following direct, as well as transcallosal and contralateral stimulation of peripheral nerves (*2*). An evoked release of $PGF_{1\alpha}$ from the frog spinal cord is also observed after electrical stimulation of the hind limbs (*3*). In addition, an efflux of material similar to prostaglandins from the cat cerebellum and ventricles has been described, and $PGF_{2\alpha}$ and other prostaglandins have been identified (*4*) in bovine, feline, and fowl brains. The conditions for release of prostaglandins are similar to those governing the release of the transmitter acetylcholine from the cerebral cortex after stimulation of presynaptic nerves (*5*), and thus these substances may be regarded as potential neurohumoral transmitters (*6*). A second criterion for a neurohumor is that application to the postsynaptic membrane should mimic the effects of stimulation of presynaptic nerves. In fact, iontophoretic application of prostaglandins modifies the firing of neurones in the brain stem (*7*). In addition, PGE_1 in chicks has a potent action similar to that of strychnine (*8*).

The successful application, by Whittaker (*9*) and De Robertis (*10*), of the subcellular fractionation technique to brain tissue has made possible the direct testing of a third criterion, namely, that the putative transmitter should be present in the nerve endings. Therefore, prostaglandins were sought in freeze-dried fractions of nerve endings from rat cerebral cortex sent from Argentina by Dr. E. De Robertis. Material similar to prostaglandins was located in the fraction containing light microsomes (*6*). We now report a more detailed study of the prostaglandins contained in fresh subcellular fractions of rat cortices.

Eight to fifteen male rates (Sprague-Dawley, 200 g) were decapitated in each experiment. After removal of the brains, the cortices were homogenized with sucrose (0.32*M*) to a final concentration of 10 percent before differential centrifugation by the procedure of Kataoka and De Robertis (*11*). The *Mic*-20 fraction, which contains the small nerve endings (fraction B) contained little prostaglandin-like material, and therefore only the crude mitochondrial fraction (*Mit*) was further fractionated, either on a sucrose density gradient or by osmotic shock followed by differential centrifugation (*11*). These operations were carried out at + 4°C.

The prostaglandins were extracted from the fractions with ethanol (95 percent) overnight. The supernatant was evaporated, and the residue was dissolved in buffered saline, which was washed three times with an equal volume of light petroleum (boiling point, 30° to 60°C), at *p*H 7.0 and then at *p*H 3.0 to remove other lipids, such as phospholipids, glycerides, cholesterol, esters, and free fatty acids. The prostaglandins were finally extracted into diethyl ether at *p*H 3.0. Recovery of labeled PGE_1 was greater than 90 percent. The prostaglandin E series was separated from the prostaglandin F compounds by thin-layer chromatography on silica gel G in the unequilibrated AI solvent system (benzene, dioxane, acetic acid, 20:20:1 by volume) of Gréen and Samuelsson (*12*), with authentic prostaglandins used as reference compounds. The method used for elution of the silica-gel plates with localization of the prostaglandins by bioassay has been described previously (*2*).

A yield equivalent to 231 ± 25 and 218 ± 36 mμg (eight experiments) of PGE_1 and $PGF_{1\alpha}$, respectively, per gram of active material was localized in

Table 1. Extraction of prostaglandin E and F compounds from subcellular fractions of rat cerebral cortex. Bioassay of the PGE and PGF zones of the AI plate was performed with PGE_1 and $PGF_{1\alpha}$ used as standards. The numbers of experiments are expressed in parentheses. The relative specific concentration is the percentage distribution of prostaglandin as a ratio of the percentage of protein.

Fractions	Contents	Distribution (%)		Protein (%)	Relative specific concentration	
		PGE	PGF		PGE	PGF
Nuc	Nucleus, cell debris, small glial cells	8.2 ± 2.9	3.4 ± 0.6	11.8	0.7	0.29
Mit	Myelin, nerve endings, free mitochondria, lysosomes	52.5 ± 8.9	31.4 ± 7.4	43.2	1.2	0.73
Mic-20	Small nerve endings	6.5 ± 1.0	2.8 ± 2.8	18.7	0.35	0.15
Mic-100	Membrane profiles, endoplasmic reticulum	21.6 ± 7.4	14.7 ± 6.7	9.9	2.2	1.5
Sup	Final supernatant	13.1 ± 2.1 (6)	45.0 ± 17.3 (4)	31.1 (6)	0.42	1.4
A	Myelin	19.2 ± 7.4	31.0 ± 11.4	30.9	0.62	1.0
B	Small nerve endings, isolated synaptic vesicles	14.3 ± 5.1	9.0 ± 3.6	7.8	1.8	1.2
C	Cholinergic nerve endings	22.0 ± 3.8	10.2 ± 3.4	23.6	0.93	0.43
D	Noncholinergic nerve endings	42.0 ± 10.8	27.2 ± 7.5	24.6	1.7	1.1
E	Free mitochondria	6.7 ± 3.0 (5)	28.0 ± 8.5 (5)	13.3 (5)	0.5	2.1
M-1	Nerve-ending ghosts, free mitochondria	43.2 ± 8.9	35.3 ± 4.8	60.6	0.71	0.58
M-2	Synaptic vesicles	25.3 ± 5.6	28.5 ± 6.9	21.1	1.2	1.3
M-3	Final supernatant	31.6 ± 7.9 (4)	36.1 ± 8.4 (4)	17.1 (4)	1.9	2.1

two clearly defined zones of the chromatoplate corresponding to those of the PGE_1 and $PGF_{1\alpha}$ standards. The eluted material was further identified by chromatography on plates impregnated with silver nitrate with the AII solvent system, (ethyl acetate, acetic acid, methanol, 2,2,4-trimethyl pentane, water, 110:30:35:10:100, by volume) which permits separation of individual members of the PGE and PGF series. Most (73 percent) of the material eluted from the PGF zone was found in the $PGF_{1\alpha}$ areas (31 mμg of $PGF_{1\alpha}$ and 19 mμg of $PGF_{2\alpha}$ per gram of fresh tissue, respectively). The material from the PGE zone was also located in two areas, corresponding to 31 mμg of PGE_1 and 46 mμg of PGE_2 per gram; in these experiments the recovery was lower (66 percent). Since losses of 10 to 30 percent usually occur after development of prostaglandin standards in the AI and AII systems (particularly with the more unsaturated prostaglandins) it is likely that most of the prostaglandin-like material detected in the acid diethyl ether extract of the crude homogenate was attributable to prostaglandins; no attempt was made to identify the trienoic prostaglandins.

We examined the ability of rat cerebral cortex to synthesize prostaglandins by incubating portions of crude homogenates, and the primary fractions, for 15 to 30 minutes at 37°C in the presence of the antoxidant hydroxyquinone with 1-C^{14}-bis-homo-γ-linolenic acid, the precursor of the first member of the prostaglandin E and F series and possibly of the A and B series. Under these circumstances more than 90 percent of the precursor was converted into more polar products which, like the prostaglandins, did not partition from an aqueous phase into light petroleum at *p*H 2. Three of these products were identified by thin-layer chromatography as PGE_1, $PGF_{1\alpha}$, and PGA_1; the latter may be an artefact due to dehydration of PGE_1 by the mineral acid used in the extraction procedure (*13*). Of the subcellular fractions, the light microsomes were the most active in synthesizing PGE_1 and $PGF_{1\alpha}$; little or no radioactive prostaglandins were obtained with the other fractions.

Most of the PGE material was located in two distinct peaks associated with the mitochondrial and light microsomal fractions (Table 1). Extraction and bioassay of the subfractions of the crude mitochondria indicated that 66 percent of this PGE material was located in fractions C and D, which contain nerve endings. The prostaglandins contained in nerve endings may be derived from storage sites since biosynthesis of prostaglandins was only significant in the *Mic*-100 fraction. The presence of PGE material in the fraction (B) containing small nerve endings may be due to contaminating synaptic vesicles derived from the noncholinergic nerve endings (D) during centrifugation of the crude mitochondrial fraction on the sucrose density gradient, since the *Mic*-20 fraction, which morphologically is almost identical with subfraction B, contains little prostaglandin (see *11*).

In contrast, the PGF compounds appeared to be more evenly distributed, and more PGF material was present in the nonparticulate supernatant fractions, a reflection perhaps of the greater water solubility of the trihydroxy prostaglandins. This finding would account for the observed preferential efflux of the PGF compounds from tissues consequent to stimulation (*6*).

Evidence for the localization of prostaglandins in the synaptic vesicles (M-2) as opposed to the free mitochondria (M-1) was obtained by treatment of the crude mitochondrial fraction with osmotic shock followed by differential centrifugation. Again, prostaglandins, especially the F series, were present in the supernatant (M-3).

The results of our experiments suggest that the increased amounts of prostaglandins previously detected in superfusates of the cat somatosensory cortex and in perfusates of the frog spinal cord after nerve stimulation, are indeed derived from the central nervous system and, more specifically, that they are derived from the synaptic vesicles in the cholinergic and noncholinergic nerve endings themselves. These experiments have demonstrated that in the rat, the light microsomes are the site of synthesis (*14*) while the nerve endings are storage sites. Whether there is active transport between the two, as there is for acetylcholine and the biogenic amines, remains to be determined.

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Euglena gracilis: A Novel Lipid Energy Reserve and Arachidonic Acid Enrichment during Fasting

Abstract. *In* Euglena gracilis *grown in the dark, wax esters, consisting of a combination of medium-chain fatty acids and alcohols that contain both odd and even numbers of carbon atoms, appear to be a reservoir for metabolic energy. When the organisms are fasted, their pellicular membrane systems become quite rich in long-chain polyenoic acids, mostly of the arachidonic acid family.*

The unicellular (or acellular) flagellate, *Euglena gracilis* (*1*), is a useful model for the study of organismic adaptive processes of the kind that can be induced by shifting environmental conditions. One may derive novel information of general interest about such processes from an inspection of the lipids of euglenas in which adaptive changes are taking place. When grown under continuous illumination, euglenas are chloroplast-bearing and behave as photosynthesizing organisms. As such, they are readily capable of an autotrophic way of life. When supplied with exogenous metabolites (*2*), they thrive in complete darkness as obligate heterophytes. Adaptive changes may be seen in the lipid components of euglenas that are starved, whether through deprivation of exogenous metabolites, in the case of dark-grown organisms, or of light, in the case of light-grown organisms kept in a barren medium. Some of the changes that have been observed in the lipids of starved *Euglena gracilis*, grown in the dark, are given in this report. The composition of the fatty acids of mature euglenas grown in both the presence and absence of light was examined (*3*). The organisms were grown without aeration in a synthetic medium (*2*) at 25°C. The light-grown euglenas were illuminated continuously with fluorescent light, generally at 990 lu/m^2; dark-grown euglenas were shielded from light in a light-proof box. Cultures were harvested as soon as their phase of growth at a logarithmic rate had terminated.

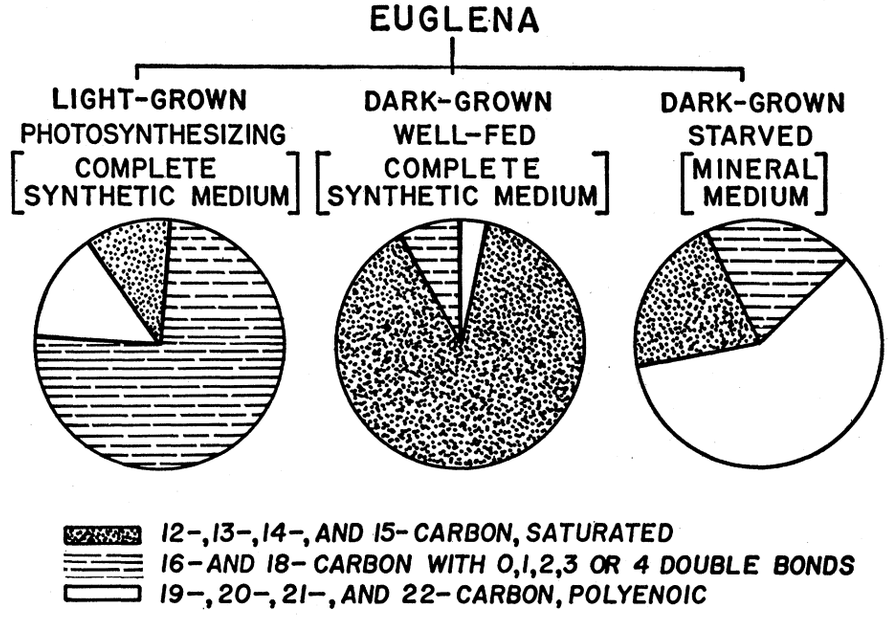

Fig. 1. Distribution of fatty acids by classes in green and etiolated *Euglena gracilis.*

Fatty acids were completely released from lipids of the harvested organisms by lengthy saponification of the whole organisms in methanolic alkali, under nitrogen (*4*) in the dark. The saponified mixture was then rendered strongly acidic with cold HCl, fatty acids were extracted, and methyl esters were made (*5*). The esters were analyzed by a combination of thin-layer and gas-liquid chromatography (*6*).

The fatty acid compositions of light-grown and dark-grown euglenas were quite different quantitatively (Fig. 1). Fatty acids of the light-grown organisms (Fig. 1, left-hand circle) were mainly 16 and 18 carbon atoms in length. The 16-carbon acids had either no double bonds or from one to four double bonds; for the most part, the 18-carbon acids had two or three double bonds. In addition, there were smaller amounts of 20-carbon acids with four or five double bonds, and of acids with shorter chains—12-, 13-, 14-, and 15-carbon acids—with no double bonds.

In contrast, the dark-grown organisms (Fig. 1, center circle) had a preponderance of saturated 12-, 13-, 14-, and 15-carbon acids and a quantity of 20-, 21-, and 22-carbon acids with either four, five, or six double bonds. Acids with 16 or 18 carbon atoms, the major fractions in light-grown organisms, were present, but in much smaller amount.

Attention was drawn to the large amount of 12- to 15-carbon saturated fatty acids in the dark-grown organisms. The occurrence of as large a fraction of shorter-chain fatty acids, especially with odd numbers of carbon atoms, is rare among Protista and higher forms of life. The lipid fraction that contained these acids was analyzed. It migrated on thin-layer plates of silica gel and on columns of silicic acid as a fraction of relatively low polarity (*3*). Consequently, it was readily separable from the other, more po-