ingestion set forth as an acceptable risk for lifetime exposure in the Federal Radiation Council's radiation protection guides for populations at large (*28*).

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2 March 1964

Quantasome: Size and Composition

Abstract. *The quantasome as seen in a two-dimensional crystalline array is 185 Å long, 155 Å wide, and 100 Å thick. The surface of the quantasome appears to contain four or more subunits. The molecular weight, determined from volume and density measurements, is 2×10^6. This is twice the minimum molecular weight calculated from the manganese content and corresponds to a chlorophyll content of 230 chlorophyll molecules per quantasome.*

Both light and dark reactions of photosynthesis in higher plants are localized within the chloroplast (*1*). Electron microscopy and biochemical studies of chloroplast fragments show that the photosynthetic light reactions and associated electron-transport reactions are localized within the chlorophyll-containing lamellae of the plastid, while the carbon cycle or dark reactions are localized in the embedding matrix or stroma. The isolated chloroplast lamellae when illuminated perform electron transport from water to ferredoxin, yielding oxygen gas and reduced ferredoxin. Phosphorylation accompanies electron transport through the lamellae.

The chloroplast lamellar system, seen in cross section by electron microscopy, assumes many configurations. In oxygen-evolving photosynthetic organisms the lamellae exist as double unit membrane systems. The lamella in cross section appears as a flattened vesicle surrounded by a unit membrane. The lamellae are completely separated from one another in blue green (*2*) and red algae (*3*), probably because space is needed between the lamellae for the soluble accessory pigment systems present in these organisms. Stacking of the lamellae commonly occurs in the green algae (*3*) and bryophytes (*4*), and a mixture of stacked lamellae (grana lamellae) and single lamellae (stroma lamellae) is found in higher plants. The intimate morphology of these lamellae undergoes wide variations in response to the environment (*5*). Apparently the conversion of light energy to chemical potential in the lamellae is a consequence of the arrangement of substances within a single lamella and is not related to the detailed lamellar arrangement since all the organisms mentioned are capable of efficient photosynthesis.

Since the light reactions of photosynthesis are localized within the chloroplast lamella, we have been concerned with the morphological, chemical, and enzymological description of the lamella and its components (*6–8*). Morphologically, the chloroplast lamella is a specialized unit membrane. Though the membrane thickness (100 Å) and to some extent its staining characteristics are typical of a unit membrane, its chemical composition is unique (*8*). Most of the lipids, as can be seen from the tabulation below, are, with the exception of the phospholipids, found only in the photosynthetically specialized membrane. This uniqueness will probably also hold for the lamellar proteins when they are fully characterized. In thin section the chloroplast lamella appears smooth when observed by electron microscopy. However, shadowed preparations of isolated lamellae show a repeating structure on the inner surface of the unit membrane. This structure was first observed by Steinmann (*9*) and later described by Frey-Wyssling and Steinmann (*10*). Work in this laboratory has suggested that these repeating structures may be the morphological expression of the physiological photosynthetic unit as formulated by Emerson and Arnold (*11*). For this reason we termed these lamellar units quantasomes.

The quantasome was initially described as an oblate sphere 200 Å in diameter and 100 Å thick (*6*). We have now recognized quantasomes in spinach lamellae as existing in at least three types of packing (Fig. 1). The most crystalline type of packing is shown in Fig. 1*c*. This extended array of quantasomes allows a more accurate determination of quantasome dimensions than was possible previously. The quantasomes in Fig. 1*c* average 185 by 155 Å with a thickness of 100 Å. The crystalline packing of Fig. 1*c* is the least common quantasome-packing arrangement, but the easiest from which to get accurate dimensions. A more

common pattern is the linear array in Fig. 1*b* which is apparently due to partial displacement of quantasomes along the short axis. The most common pattern is the fairly random array in Fig. 1*a*. That all three patterns can exist within one lamella is shown in Fig. 1*d*. The factors controlling the type of packing are unknown. However, we have noted that the crystalline packings are most common in spinach grown under winter conditions in California.

Since we can measure the quantasome volume by electron microscopy, and have determined the effective quantasome buoyant density in the ultracentrifuge as 1.17 (*12*), we can calculate the molecular weight of the quantasome. The molecular weight of a single quantasome from the crystalline array in Fig. 1*c* is 2.0×10^6. This is about two times the minimum molecular weight (960,000) determined by Park and Pon (*7*) on the basis of the manganese content. From these studies on the chemical composition (*7*, *8*) we can calculate that one quantasome contains about 230 chlorophyll molecules if the chlorophylls are assumed to be uniformly distributed. This is surprisingly close to the number of chlorophylls contained in a photosynthetic unit (*13*). The chemical composition of the lamella also supports the supposition that the lamella contains a double lipid layer. The argument follows from the observations that the membrane is 50 percent lipid, 50 percent protein, and 100 Å thick. If one assumes a density of 1.0 for the lipid and 1.4 for the protein, then approximately 65 Å of the membrane thickness must be accounted for by lipid. This lipid thickness is consistent with the existence of a double lipid layer. The distribution of lamellar substances based on a quantasome molecular weight of 1,920,000 is summarized below. This molecular weight is twice the minimum molecular weight and is within the experimental error of the calculated quantasome molecular weight of 2×10^6.

Lipid (composition in moles per quantasome) (*14*)		
230 chlorophylls (*7*, *15*)		206,400
160 chlorophyll *a*	143,000	
70 chlorophyll *b*	63,400	
48 carotenoids (*15*)		27,400
14 β-carotene	7,600	
22 lutein	12,600	
6 violaxanthin	3,600	
6 neoxanthin	3,600	
46 quinone compounds (*15*)		31,800
16 plastoquinone A	12,000	
8 plastoquinone B	9,000	
4 plastoquinone C (*16*)	3,000	
8–10 α-tocopherol	3,800	
4 α-tocopherylquinone	2,000	
4 vitamin K_1	2,000	
116 phospholipids (*17*) (phosphatidylglycerols)		90,800
144 digalactosyldiglyceride (*18*)		134,000
346 monogalactosyldiglyceride (*18*)		268,000
48 sulfolipid (*19*)		41,000
? sterols (*20*)		15,000
unidentified lipids		175,600
Total		990,000
Protein		
9,380 nitrogen atoms as protein		928,000
2 manganese		110
12 iron including two cytochrome		672
6 copper		218
Total		930,000
Total lipid + protein		1,920,000

The 116 phospholipids include 14 molecules of glycerophosphoryl inositol, 52 of glycerophosphoryl glycerol, 6 glycerophosphoryl ethanolamine, 42 glycerophosphoryl choline, and 2 glycerophosphate. The 12 iron atoms in the protein include one as cytochrome b_6, one as cytochrome *f* (*21*), and ten non-heme irons.

Figure 1*c* also shows that the quantasome has substructure. The rows of quantasomes are cut by rows of secondary depressions which in a number of instances yield quantasomes consisting of four or more subunits.

Of extreme interest is the relation between the quantasome of chloroplasts and the elementary particle (*22*) or oxysome (*23*) of mitochondria. Both electron-transport systems are associated with unit membranes, both show electron transport between water and eventually pyridine nucleotide, both are accompanied by phosphorylation, both contain cytochromes of the *b* type and *c* type, and both contain substituted benzoquinones and non-heme iron. The presence of the photochemical-energy conversion apparatus in the quantasome and the direction of electron transport are major differences between the two systems. Nevertheless, one might expect certain morphological similarities. Our efforts at negative staining with electron microscopy have revealed no particle associated with active preparations of chloroplast lamellae which corresponds to the elementary particle. The same procedures did, however, demonstrate the presence of elementary particles in rat liver mitochondria. In chloroplast lamellae, the entire electron transport

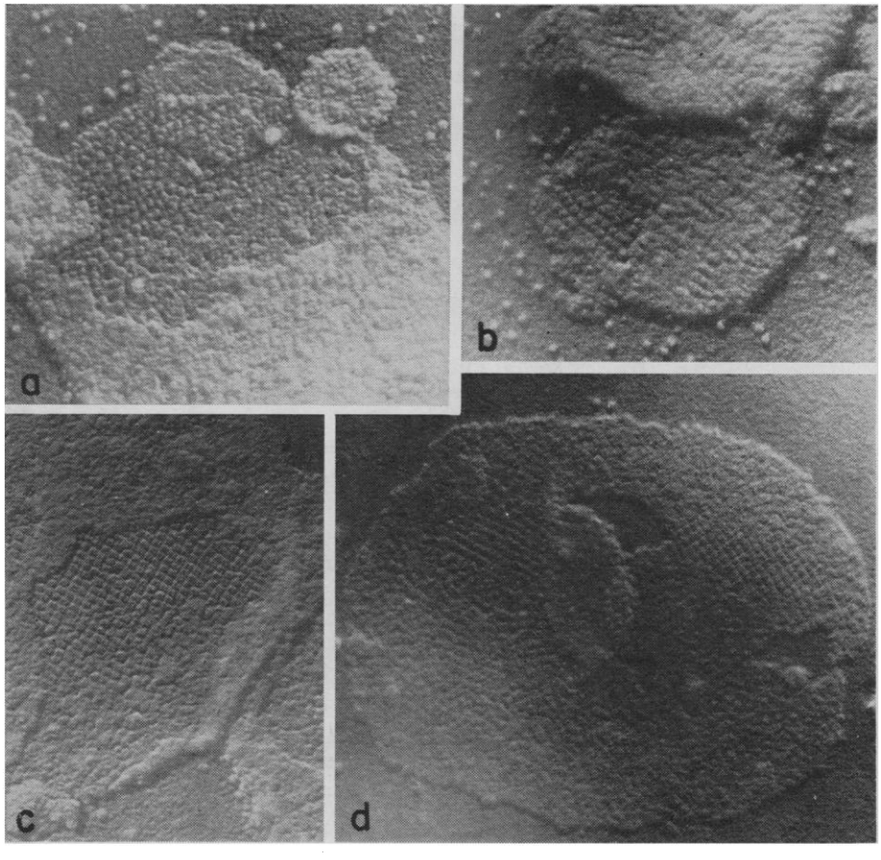

Fig. 1. Chromium-shadowed spinach chloroplast lamellae, all × 71,000. *a*, Random quantasome array; *b*, linear quantasome array; *c*, crystalline quantasome array; *d*, random, linear, and crystalline quantasome arrays within one lamella. These lamellae were prepared according to the methods of Park and Pon (*6*). Such preparations, in the presence of light, are fully active in the Hill reaction and support CO_2 fixation upon the addition of the stroma fraction.

chain leading from water to ferredoxin is located within the membrane. That this may also eventually be true for mitochondrial cristae is indicated by the data of Chance which show that the cytochrome chain is not contained within the elementary particles, but is within the mitochondrial membrane (*24*).

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Synthesis of RNA in vitro Stimulated in Dipteran Salivary Glands by 1,1,3-Tricyano-2-Amino-1-Propene

Abstract. *1,1,3-Tricyano-2-amino-1-propene stimulates by more than one order of magnitude the incorporation in vitro of uridine into the RNA of dipteran salivary glands. The inhibition by actinomycin D is blocked. Higher doses cause a complete inhibition from which a proportion of the glands recover rapidly upon withdrawal of the substance.*

1,1,3-Tricyano-2-amino-1-propene (tricyamp) stimulates net RNA synthesis in rabbit nerve cells in vivo (*1*). Our observations in vitro suggest that there is also a dramatic stimulation in salivary glands. This small molecule, presumably not encountered in normal metabolism, may prove useful for work on other cells as well.

Larval salivary glands of the chironomid *Smittia parthenogenetica* were incubated for 1 or 2 hours at 25°C in standard medium (*2*) containing tricyamp (*3*) at different concentrations. Then they were incubated in the same medium for either 30 minutes with tritiated uridine (40 or 60 μc/ml, 3.0 c/mmole; Radiochemical Centre) or for 45 minutes with tritiated guanosine (50 μc/ml, 0.4 c/mmole). Controls were treated similarly but tricyamp was omitted from the medium. The glands were fixed in trichloroacetic acid, squashed in acetic acid, and then fixed in trichloroacetic acid (*2*). Autoradiographs showed that incorporation of uridine was markedly enhanced in the whole cell after treatment for 1 hour with 100 μg (Figs. 1 and 2). The stimulation is more obvious in chromosomes and specially nucleoli than in cytoplasm, owing to the normally greater incorporation in the nuclear structures. Treatment with 50 μg of tricyamp had no appreciable effect, while treatment with 175 μg inhibited incorporation almost completely, and treatment with 250 μg inhibited incorporation completely. Treatment for 2 hours with 50 μg caused a similar stimulation (see Fig. 2).

Incorporation of guanosine was also markedly enhanced by treatment for 1 hour with 100 μg of tricyamp, though probably less than that of uridine. All label was sensitive to ribonuclease. Pending further characterization, this suggests that the stimulated incorporation is into RNA.

Recovery from the inhibition caused by 250 μg of tricyamp was indicated in about one-quarter of the glands upon withdrawal of the substance and further incubation with tritiated uridine alone. Controls were incubated with tricyamp present or absent throughout the incubations. Incubation for 30 minutes with uridine, immediately after withdrawal of tricyamp or after an intervening 1.5 hours of incubation in standard medium, showed a stimulation of incorporation as in Fig. 2. After 3 hours in standard medium, the incorporation was of the order of controls. Sodium thiosulphate has a detoxicating action on animals poisoned by tricyamp (*4*), but in our experiments the presence of 250 μg of sodium thiosulphate after withdrawal of tricyamp had no effect. These observations are consistent with an initial rapid decrease of the intracellular concentration of tricycamp

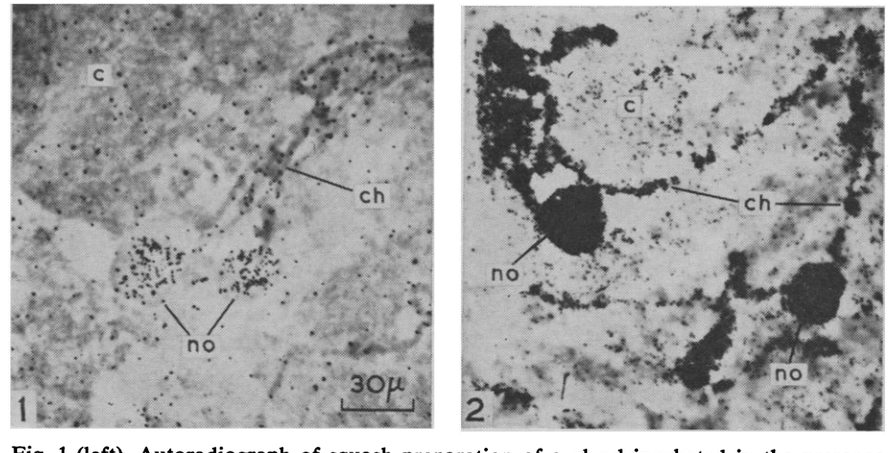

Fig. 1 (left). Autoradiograph of squash preparation of a gland incubated in the presence of ^{3}H-uridine; fixed in trichloroacetic acid and stained with methyl green-pyronin; *no*, nucleolus; *ch*, chromosomes; *c*, cytoplasm. Fig. 2 (right). Same as Fig. 1, except that 100 μg of 1,1,3,-tricyano-2-amino-1-propene (per milliliter) was present before and during incubation.