with 'amorphous' interspaces or irregularly in a fluid-like structure. This concept results from small-angle X-ray diagrams[1].

Electronmicrographs by Park and Pon[2] and Park and Biggins[3] demonstrate that the lipoprotein lamellae consist of particles nearly 200 Å in diameter arranged in a regular way. Park and Pon called these particles 'quantasomes'.

The crystallites already mentioned here are identical with the protein component of the quantasomes. Probably they represent the 'structure protein' of quantasomes only. The proteinaceous mass is predominantly gathered in regions about 27 Å in diameter forming a quadratic lattice within the crystallite[4,5]. The largest period found for this lattice is in :

Isolated aqueous chloroplasts[1]	36 Å
Chloroplasts in living cells[4]	32 Å
Air-dried chloroplasts[4]	38 Å
Lipid-extracted dried chloroplasts	38 Å

In air-dried chloroplasts and lipid-extracted chloroplasts the lattice consists only of 4 lattice points instead of at least 16 lattice points in aqueous chloroplasts.

These results are contradictory in two ways : first, if the crystallite of 16 lattice points disaggregates into 4 sub-units, each one built up of 4 lattice points, a reflexion according to a Bragg-spacing less than 32 Å should occur. Instead we find a reflexion according to a 38 Å spacing. Secondly, on drying the distances in the protein should rather shrink than enlarge.

Small-angle X-ray diffraction patterns obtained from protein-preparations after Weber[7] give an explanation for these contradictions. These patterns show a reflexion according to a Bragg-spacing of 32 Å but also an additional one according to 45 Å. Both spacings are considered to be the (10)- and (11)-distances of a two-dimensional quadratic lattice consisting of 16 lattice points[5].

By this, the results may be interpreted as follows : in Weber's preparations the 'structure protein' of one quantasome is arranged as shown in Fig. 1. Here the 'structure protein' is composed of 4 sub-units each consisting of 4 mass centres. To explain the scattering effect with chloroplasts in living cells (see foregoing) the protein structure of Fig. 1 has to be modified in such a way that the 45 Å reflexion will be suppressed. According to calculations of scattering functions of various models a suppression of the 45 Å reflexion is possible by association of additional particles (perhaps enzymes) either at the outer side (Fig. 2*a*) or in the centre of the sub-units of the structure protein. Furthermore the suppression may also be supported by dislocation of quantasomes (Fig. 2*b*). (In Weber's preparations one or two particles may also be attached to the 'structure protein'. They will have, however, only a negligible influence on the scattering effect.) In dried

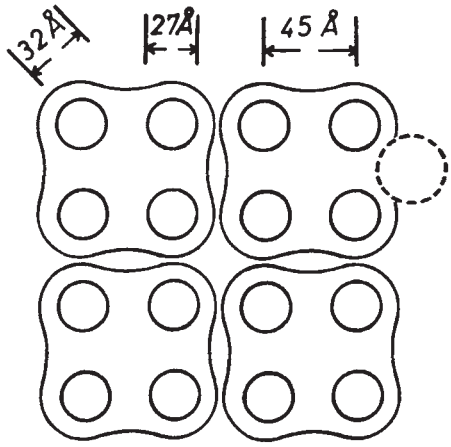

Fig. 1. View of the 'structure protein' of a quantasome with one attached particle (enzyme)

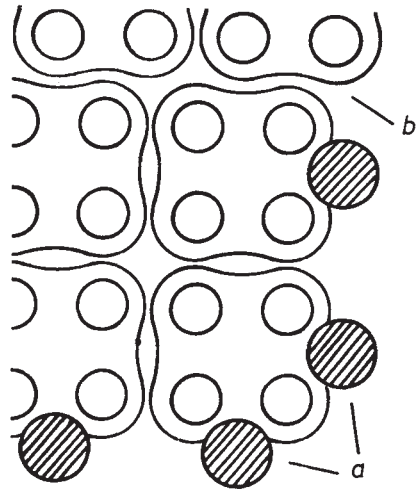

Fig. 2. *a*, Protein particles (enzymes) attached to the outer part of the sub-units of the quantasome; *b*, dislocation of quantasomes

chloroplasts and lipid extracted chloroplasts the protein structure is disordered by a loosening of the sub-units from each other. Under these conditions only the single broad 38 Å reflexion will be caused by pure sub-units or in sub-units with no more than two particles attached to the outer side. With face-centred sub-units, however, no 38 Å reflexion is to be expected at all. Therefore the model of Fig. 2 agrees best with all experimental data. This model explains the suppression of the 45 Å reflexion, the breaking of the lattice in the protein layer after each 16–32 lattice points and the occurrence of one broad 38 Å reflexion with dried or lipid-extracted chloroplasts.

Finally, the suggestion may be noted that the attached particles are enzymes concerned with the primary process of photosynthesis. Therefore it might be possible by further small-angle X-ray investigations to obtain detailed information about the arrangement of the enzyme-chain of the primary process of photosynthesis within the protein layer of chloroplasts.

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W. Kreutz

Max-Volmer-Institut,
für physikalische Chemie,
Technische Universität, Berlin.

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BIOCHEMISTRY

Constituents of *Amanita muscaria*

The fungus *Amanita muscaria* has been the object of intensive investigations for many years, and two of its constituents, muscarine and acetylcholine, are of fundamental importance in pharmacology. The elucidation of the structure of muscarine in the classical researches of Kögl *et al.*[1–3] and Eugster[4] and Waser[5] has overshadowed the existence in the plant of other interesting substances. The fungus has long had a reputation for insecticidal properties in folk-lore as the names 'muscaria', 'fly agaric' and 'fliegenpilz' imply. Ramsbottom[6] describes its use —broken up in milk or sprinkled with sugar—as a fly trap in various parts of Europe.

In the light of recent publications by Takemoto *et al.*[7–11] on the isolation of fly-killing constituents of some Japanese

fungi, we wish to record briefly the occurrence in *A. muscaria* of compounds having interesting effects on insects.

For many years it seems to have been assumed that the insecticidal action of the fungus was due to muscarine, but we were able to show in 1958 (unpublished observations) that pure muscarine was devoid of action when given orally to the ordinary housefly, *Musca domestica*. We thank Prof. Kögl for the sample of pure muscarine which enabled us to carry out the work.

In our investigations into the compounds present in the plant and having action on insects, we evolved a simple testing technique. It was as follows: Pupae of *Musca domestica* were hatched in cylindrical muslin cages at 22°–24°. The insects were fed for 4–5 days after hatching by means of cotton wool moistened with diluted sugar solution. The food was then withdrawn for 17 h and the starved flies counted out into conical flasks. This may be readily done by allowing the insects to pass through a hole in a piece of cardboard separating the cage from the flask. Normally, five flies were counted into each flask and the batches were later transferred to a series of test-tubes 18 cm long by 2·5 cm diameter containing the materials to be tested. The tubes were clamped nearly horizontally and were stoppered loosely with glass wool. Before introducing the flies, the test solution (0·1 ml.) was pipetted into the tube, a few crystals of sugar added as a bait, and the solution taken up on to a tightly rolled ball of 'non-absorbent' cotton wool (0·2 g). This was done in order to avoid external contamination of the flies with the materials. Starved flies readily imbibe from the cotton wool, taking up on the average 10 μg of solution. By using this technique a large number of fractions could be tested simultaneously.

After the flies had imbibed extracts of *A. muscaria* they were usually overcome within 10–15 min. The effects appeared as rapid wing beats followed by apparent loss in the use of the wings. Ability to walk was retained for a while, but this was eventually lost and a state of apparent death then prevailed. This lasted for 50 h or longer, provided the active material was present in concentrations which were not too high. In order to avoid repeated dosing on recovery, the insensible insects were transferred to fresh tubes containing a ball of cotton wool moistened with sugar solution. Recovery in the insects was the reverse of the effect observed on administration of the extracts, power returning first to the legs and later to the wings. This treatment can be repeated at will on the flies without undue harmful effects being observed.

Examination of various parts of the fungus showed that much of the activity was concentrated in a band of yellow material in the cap beneath the red skin, although some slow-acting substance was also present in the stalk.

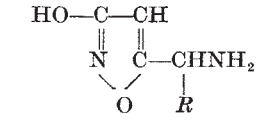
Chromatography of aqueous extracts of the whole plant was carried out on sheets of paper using alcohol/water (5 : 1) as the developing solvent. The active areas were detected by cutting the dried sheets into horizontal strips which were rolled into the form of cylinders and placed in hypodermic syringes (8 cm × 1·5 cm diam.) having the needle exits temporarily blocked. Distilled water was added and the paper macerated in the syringes, the solutions expelled by means of the plunger and the pulp re-extracted again with water in the same way. By this means the active materials were rapidly extracted from the chromatogram strips. The solutions were freeze-dried in small weighed flasks. The dried extracts were re-dissolved in water to a concentration of 25 mg/ml. and tested as described.

By these means three areas of activity were detected on the chromatograms which were designated *Amanita* Factors *A*, *B* and *C*. Electrophoresis of aqueous extracts of the plant on Whatman seed test paper using sodium acetate/acetic acid buffer of *p*H 5 and a potential of 100 V showed after 12 h two areas of activity against *Musca domestica*, one a broad band approximately 5 cm from the origin in the direction of the cathode, and a narrow band which had moved about 0·75 cm from the origin towards the anode.

Similar results to those on paper chromatograms were obtained when larger quantities of material were fractionated through a cylinder of paper in a 'Chromax' column; but the process was very tedious, requiring the collection of hundreds of samples. The use of a column of diethylaminoethyl cellulose showed that Factor *A* passed through the column readily with the alcohol/water (5 : 1) solvent system, the *p*H of the eluate rising to 9 during this stage. Factor *B* was eluted by water, but Factor *C* required 2 N ammonium hydroxide to remove it from the column.

Using 'Deacidite *FF*' (base) resin, Factor *A* was readily eluted at *p*H 6–7, but Factor *B* required a *p*H of 3.

Factor *B* was obtained pure by the method described by Bowden and Drysdale[12] and mass spectrographic and nuclear magnetic resonance data indicated the structure to be I (*R* = H).



This structure was confirmed by the independent work of Takemoto *et al.*[10]. These Japanese workers have isolated the corresponding carboxylic acid, ibotenic acid (I; *R* = COOH) from *Amanita strobiliformis*, *A. pantherina* and *A. muscaria* and it seems likely from the behaviour of our *Amanita* Factor *C* on ion exchange resins that it is ibotenic acid.

We thank Miss M. Davies of the Cooper Research Station for a supply of *Musca domestica* pupae.

K. Bowden
A. C. Drysdale
Smith Kline and French Research Institute,
Welwyn Garden City,
Hertfordshire.

G. A. Mogey
Department of Pharmacology,
University of Leeds.

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'Nothing' Dehydrogenase in the Retina

The separation of isoenzymes by electrophoresis on cellulose acetate paper is now an established technique; the fractions can be located by tetrazolium staining according to the technique described by Barnett[1]. In this laboratory such an approach has been applied to the separation of the isoenzymes of lactic acid dehydrogenase in the retina of the normal and 'retinitis' rat during development[2,3].

A complicating factor in interpreting various isoenzyme patterns, however, concerns the so-called 'nothing' dehydrogenase phenomenon. This is a characteristic pattern obtained by incubating the electrophoretic strip in the absence of substrate. On the basis of the differing pattern obtained in the presence or absence of coenzyme, Barnett