Technical Papers

Observations on the Supposed Intracellular Symbiotic Microorganisms of Aphids

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All aphids, except very old ones, contain numerous large cells in the body cavity (mycetocytes) containing many more or less spherical intracellular particles (Fig. 1). The present consensus regarding these intracellular particles is that they are microorganisms; as given in detail by Steinhaus (*1*) they have been put in the yeast genera *Saccharomyces* and *Aphidomyces,* or have been considered to be bacteriumlike microorganisms. Peklo (*2*) thought they might be *Azotobacter.* If the particles are microorganisms, their universal and massive occurrence in aphids suggests that they play some beneficial role in the economy of the aphid; i.e., they are probably symbiotes. In conformity with Peklo's suggestion that they were azotobacters, Toth (*3*) has claimed that symbiotic microorganisms in aphids fix atmospheric nitrogen. Smith (*4*), however, has repeated some of Toth's experiments with negative results. Smith also fed aphids in an atmosphere containing isotopic nitrogen and was unable to demonstrate nitrogen uptake.

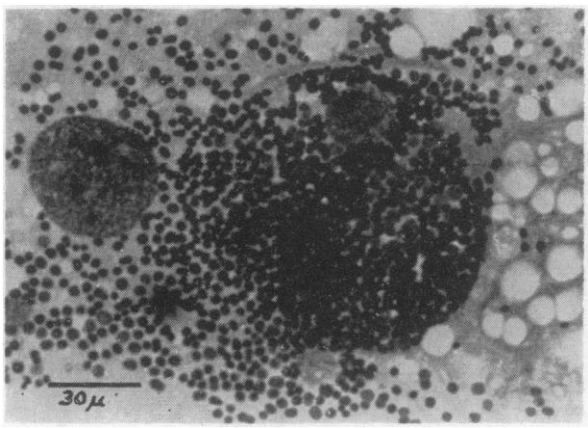

FIG. 1. Ruptured "host" cell with cell particulates; from potato aphid. Stained with Giemsa, without preliminary hydrolysis.

The aphid particles are well suited in some respects for electron microscopy, since they flatten out into thin disks when air-dried. Electron micrograms of particles from the potato aphid (*Macrosiphum solanifolii*), made in the course of the present investigation, revealed electron-absorbing material present inside many of the particles in varying degrees of aggregation (Fig. 2). Owing chiefly to the fact that the particles flatten out so completely, the micrograms resemble those of neither yeasts nor bacteria, at least when these are air-dried, with no special preliminary treatment. When observed with phase contrast microscopy, the particles can be seen to contain small granules evenly dispersed in a relatively dense cortex that prohibits Brownian movement of the granules. Nothing resembling the aggregates visible in the electron micrograms was seen in fresh material with phase contrast, suggesting that they might be artifacts produced by internal dissolution and drying of the particles.

At first sight, the conclusion seems inescapable that the intracellular particles increase in number by a process of division. They often occur in doublets, apparently in all stages of separation, and in slightly elongate forms that could be interpreted as stages preliminary to doublet formation. In preparations stained with Giemsa (without preliminary hydrolysis) there is no gradation in size indefinitely downward; the smallest particles are about one third the size of the largest (which are up to 3 or 4 μ in diam). This would seem to preclude the possibility that the particles arise by growth from submicroscopic precursors. However, if fresh material be examined with phase microscopy, smaller particles can be seen, although they seem to differ from the larger ones in having their shape determined by surface phenomena (i.e., they are perfect spheres) rather than by a more or less rigid cortex. It is conceivable that the smaller particles could develop into the large microbelike particles, attaining their cortex and staining qualities only at a certain stage of growth, and also that two growth centers could be associated early in development, producing a doublet; there is no direct evidence supporting these possibilities, however.

In recent years, it has been possible to demonstrate desoxyribonucleic acid (DNA)-containing particles in bacteria, using a variety of stains following hydroly-

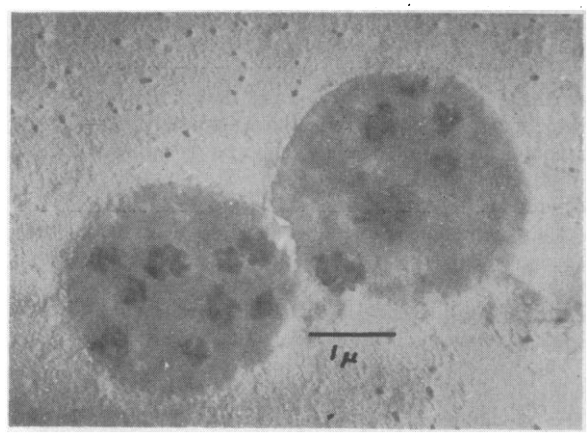

FIG. 2. Electron micrograph of cell particulates from potato aphid. Lightly shadowed with uranium.

[1] A part of this investigation was carried out at the Hopkins Marine Station, Pacific Grove, Calif., under a Faculty Research Fellowship from the University of Michigan.

sis of the cells with 1 *M* HCl at 60° C for 10 min. In the present investigation, attempts to demonstrate DNA in the aphid particles, using similar methods, failed to do so (several species of aphids were used, including the potato aphid and the cabbage aphid, *Brevicoryne brassicae*). Smears of whole aphids, and isolated "host" cells, which were broken open to allow the particles to escape, were used; it is important that the staining reaction of isolated particles be studied, rather than that of blocks of intact cytoplasm containing the particles massed closely together. Nuclei of aphid cells, and microorganisms such as *Saccharomyces* and *Azotobacter,* were subjected to the same treatment as the particles, often on the same slide, and always in the same hydrolyzing and staining jars; in contrast to the particles, the nuclei and microorganisms showed a strong and recognizably similar staining reaction to Giemsa after acid hydrolysis. The intracellular particles do take up Giemsa, especially after long overstaining, but to a very much less degree than either cell nuclei or test microorganisms. With Feulgen no stain was taken up by the particles after acid hydrolysis. These results lead to the conclusion that the aphid particles lack DNA, and that they are not microorganisms, but are cell particulates.

Similar investigations are under way on the supposed intracellular symbiotes of cockroaches.

References

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Determination of Specific Activities of C^{14}-Labeled Organic Compounds with a Water-Soluble Liquid Scintillator

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Recently results have been reported (*1*) on the counting of C^{14}-labeled organic materials by dissolving them in a liquid scintillator—namely, terphenyl-xylene solution. Such a technique for measurement of radioactivity provides essentially ideal geometrical conditions.

Our communication describes measurements thus far obtained employing a liquid scintillator suitable for both organic and water-soluble materials and an experimental arrangement that permits high counting efficiency.

The solution employed was *p*-dioxane saturated with *p*-terphenyl (approximately ½% by weight). Its scintillating properties under external radiation were first reported by Kallmann (*2*), and the possibility of using this combination was also recognized by Raben and Bloembergen. In all our measurements, aliquots of aqueous solutions of C^{14}-labeled compounds were added to the scintillator. All data reported were taken on terphenyl-dioxane solutions containing 5% water. Preliminary results indicated that the extrapolated counting efficiency was not decreased with concentration of water as high as 20%, although there was an appreciable reduction in pulse height.

The solutions were placed in flat-bottom glass vials of 1 in. diameter in contact with the photocathode window of an EMI[1] Type 5311 photomultiplier tube. Good optical coupling was insured by cementing the vial to the face of the photomultiplier tube and cementing aluminum foil to the remainder of the vial with a Dow-Corning grease. Both solution cell and photomultiplier tube were enclosed in a light-tight container. The output pulses from the EMI tube were fed into a conventional linear amplifier with a resolving time of .25 μsec and then into a scaler circuit. All measurements were taken with the entire system at room temperature.

The results obtained thus far are summarized in Table 1. The combustion analyses were performed by

TABLE 1

COUNTING OF SEVERAL WATER SOLUTIONS OF C^{14}-LABELED ORGANIC COMPOUNDS USING A TERPHENYL-DIOXANE SCINTILLATOR

Compound	Expected cps from combustion analysis	Measured cps from scintillation analysis	Relative pulse height
Citric acid	2100	1800	58
Acetylcholine bromide	4900	3700	60
Aniline hydrochloride	2900	2500	55
Sodium acetate	7900	7000	65
Mannonic lactone	440	400	54

the Organic Analytical Group of Tracerlab. The method of Van Slyke and Folch (*3*) was used for the combustion, and the carbon dioxide generated was counted in an ionization chamber with a Lindeman-Ryerson electrometer (*4*). The scintillation measurements were taken in the form of integral bias curves with the photomultiplier tube at a fixed voltage (H.V. = 1300 v). The reported counting rates were obtained by extrapolating the integral bias curves to zero pulse height. In Table 1, the average pulse heights were found by integrating the area under the integral bias curves and dividing by the extrapolated counting rate. Here the average pulse height values are also useful as a cross check on the extrapolated counting efficiency. An external source of Sr^{90} at 25 cm from the scintillators was used as a standard for each solution. Assuming the average energy of the

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