EXPERIMENTS ON THE SILVER NITRATE METHOD FOR THE HISTOLOGICAL DEMONSTRATION OF ASCORBIC ACID (VITAMIN C)

By S. A. BARNETT and R. B. FISHER, Department of Biochemistry, Oxford

(*Received* 18 *January* 1943)

(With Two Text-figures)

A 10 % solution of silver nitrate in 10 % acetic acid has been widely used to show the presence of ascorbic acid in microscopical preparations (Bourne, 1936; Giroud, 1938). When there is ascorbic acid in the cells a black precipitate of metallic silver is obtained. This precipitate is often observed to be on or in the Golgi material, and it has been supposed that where this is the case the ascorbic acid is itself located in the Golgi substance (Giroud, 1938; Bourne, 1935). However, it has been pointed out that this conclusion is not necessarily correct, since the localization of the silver precipitate may be a physico-chemical phenomenon, resulting from the presence of a certain type of interface (Barnett, 1942). Experiments have therefore been carried out *in vitro* to investigate whether silver precipitates tend to accumulate at interfaces in conditions comparable with those in cells.

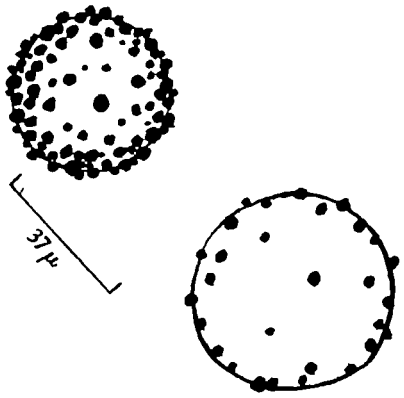

Fig. 1. Drawings of oil drops bearing silver precipitate.

To a 5 % solution of gelatin there were added a few drops of olive oil, plus crystalline ascorbic acid to give a concentration of 200 mg./100 ml. This mixture was passed through a small cream-making machine, in order to emulsify the oil, and allowed to set. Pieces of the gel were left in acid silver nitrate for about 10 min., and were then washed in distilled water; both operations were carried out in brown bottles. Thus the gel was given the same treatment as a piece of tissue in which it is intended to investigate the distribution of ascorbic acid. A small portion of the treated gel was put on a microscope slide and gently compressed under a cover-glass. Examination under the microscope showed that the silver was mainly present as granules dispersed through the gel, but some of the oil droplets had a heavy precipitate attached to their surfaces (Fig. 1): this precipitate was in the form of granules, some rather irregular in shape, and resembled that described elsewhere as occurring on the surface of the Golgi material in certain cells (Barnett & Bourne, 1942) (Fig. 2). In some instances granules also accumulated on the surfaces of what appeared to be dust particles, particularly fragments of thread; this was also observed in preparations made without olive oil.

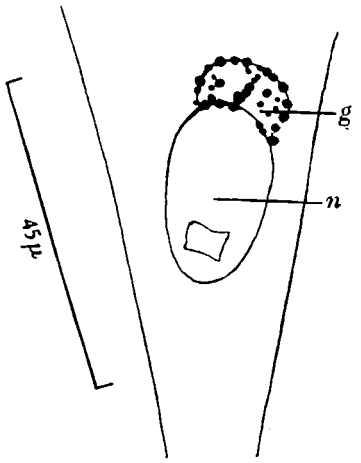

Fig. 2. Part of fibroblast-type cell with Golgi substance (*g*) bearing silver precipitate; *n* = nucleus. Redrawn from Barnett (1942).

In other preparations, instead of olive oil, ground glass or kieselguhr were added to the gelatin solution. On the kieselguhr fragments there was very little precipitate; what there was took the form of isolated granules in the interstices of the diatom skeletons.

the ground-glass fragments there were often coarse deposits.

These experiments show that even in a homogeneous medium such as gelatin solution granular precipitates can occur. The accumulation of some of the granules at the interfaces between foreign bodies and the gelatin solution must be due to processes occurring after the reduction of the silver nitrate. Consequently the form which the precipitates take in these conditions clearly has no bearing on the prior localization of ascorbic acid.

The precipitates observed in cells treated with acid silver nitrate are of three main types: first, spherical granules more or less evenly distributed in the cytoplasm; second, granules adhering to a surface, generally of the Golgi substance (Fig. 2); third, an even precipitate, not visibly granular, evidently on the surface of the Golgi material. In some instances the first type has been observed in cells which contain spherical mitochondria evenly distributed in the cytoplasm, and it has been concluded that the ascorbic acid is located in the mitochondria (Bourne, 1935). However, as we have seen, that the precipitate consists of spherical granules but this need not imply that it is attached to pre-existing structures; and in fact, in some cells a precipitate of spherical granules has been observed where the mitochondria are known to be filamentous (Barnett & Bourne, 1942). There has been no report of the observation of silver precipitates on thread-like mitochondria. It must be concluded that the evidence for the localization of intracellular ascorbic acid in mitochondria is inadequate.

With regard to Golgi material, there is little doubt that the silver precipitates obtained are often located on it: this is shown by both the shape and the position of the deposits. Those of the second type mentioned above, as the figures show, are closely paralleled by those obtained in the experiments with olive oil, but it has not been possible to imitate the third type *in vitro*. It is evidently possible that the tendency for silver precipitates to accumulate at interfaces alone accounts for the localized precipitates observed in cells. The fact that mitochondria, at least when filamentous, do not become covered with precipitated silver, may be attributed to their presenting a different type of interface to that of the Golgi substance.

It is concluded that it is unjustifiable to infer the whereabouts of ascorbic acid within the cell from the site of the silver precipitates obtained by the silver nitrate method.

REFERENCES

BARNETT, S. A. (1942). *J. Exp. Biol.* **19**, 88.

BARNETT, S. A. & BOURNE, G. (1942). *Quart. J. Micr. Sci.* **83**, 259.

BOURNE, G. (1935). *Austr. J. Exp. Biol. Med.* **13**, 239.

BOURNE, G. (1936). *Anat. Rec.* **66**, 369.

GIROUD, G. C. (1938). *L'acide ascorbique dans la cellule et les tissus*. Berlin.