# OBSERVATIONS ON THE DISTRIBUTION OF ASCORBIC ACID (VITAMIN C) IN EXPLANTED CELLS

By S. A. BARNETT[1]

(*Received* 31 *January* 1942)

(With One Text-figure)

A SURVEY was recently made of the distribution of ascorbic acid in the embryonic tissues of the chick (Barnett & Bourne, 1941, 1942). Its results raised a number of problems which can only be solved experimentally. A study was therefore undertaken of ascorbic acid in cultured tissues from chick embryos. Owing to the war this work could not be completed, but it is considered desirable to record the few observations which have been made.

## 1. METHODS AND MATERIAL

Cultures were set up in 'hanging drops' according to the method described by Willmer & Kendal (1932). The medium was composed of equal parts of fowl plasma and Compton-Pannet saline containing 15 % embryo juice. All the cultures examined for ascorbic acid were in their third, or a later, passage: that is, they had been recultured at least twice. Reculturing was carried out at 2-day intervals. The observations were made between 24 and 48 hr. after reculturing, on cells at or near the periphery of the outgrowth. No difference was observed between the 24 and 48 hr. cultures in respect of the ascorbic acid content of the cells.

Ascorbic acid was detected by means of the acid silver nitrate method (see Bourne, 1936). The method is based on the fact that, of all intracellular reducing agents, ascorbic acid is the only one which reduces silver nitrate in acid solution, in the dark, over short periods of time. The evidence for the validity of the method is fully discussed by Barnett & Bourne (1941). The reagent used for combined fixation and impregnation was a 10 % solution of silver nitrate in 10 % acetic acid. The cover-slip bearing a culture to be examined was removed from its cavity slide, dipped in distilled water, and then left in acid silver nitrate for about 10 min. After fixation it was washed thoroughly in distilled water, dehydrated and mounted in a temporary medium. In some instances the preparations were toned with gold chloride before dehydration.

The tissues used were 10-day ventricle (15 cultures), 11-day frontal bone (25 cultures), and 17-day adrenal cortex and medulla (40 and 20 cultures respectively). *L*-ascorbic acid (Roche) was added to the medium used for some of the adrenal cultures: it was weighed in crystalline form and added to the saline in concentrations of 2–8 mg. per 100 ml.

[1] Formerly Christopher Welch Scholar.

## 2. RESULTS

In heart fibroblasts no ascorbic acid was detected.

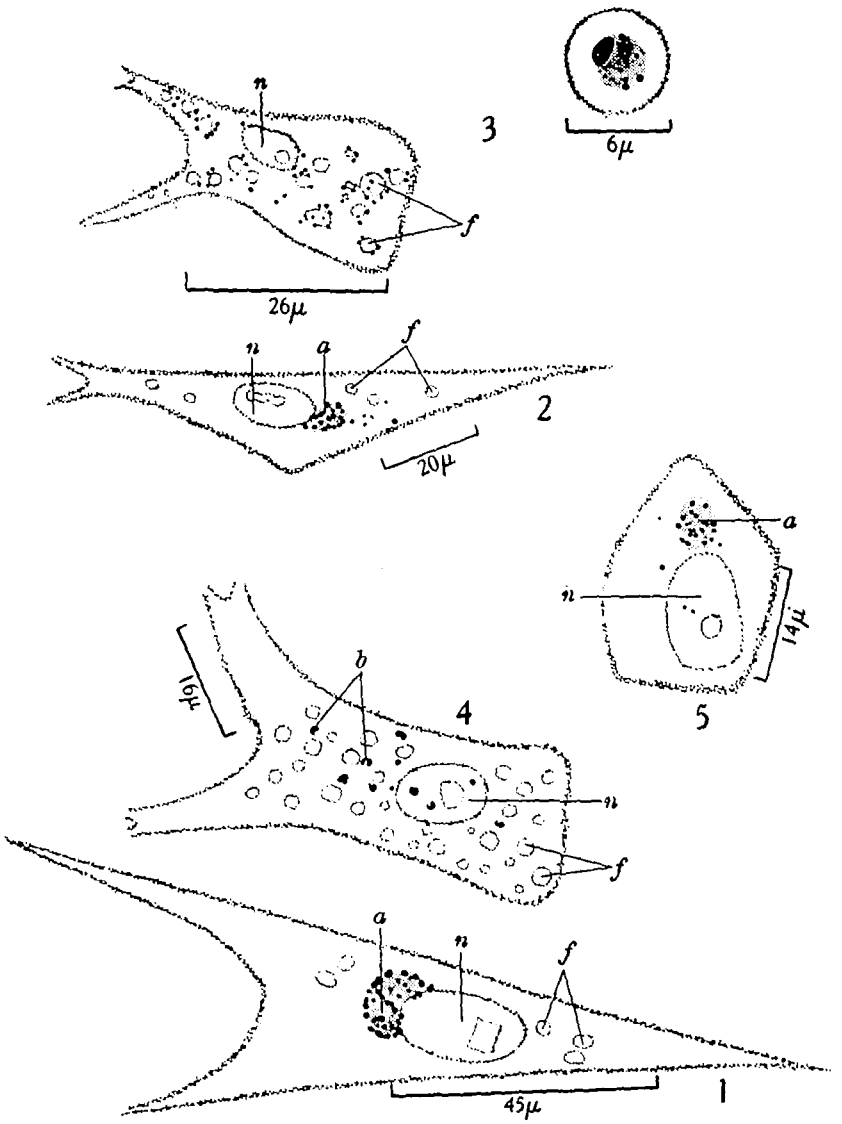
In the bone cultures most of the cells of the outgrowth gave no reaction; this was the case in all those undergoing division. A very small number of cells showed a black precipitate in a localized area of cytoplasm adjoining the nucleus (Fig. 1). There is little doubt that this is the region of the Golgi material (Ludford, 1927, 1935). The appearance in these unstained preparations is of a greyish mass quite distinct from the clear nuclear area, and from the cytoplasm, on or in which are the black granules of precipitated silver. The cells in which this was observed were otherwise normal.

In cultures of adrenal cortex again a majority of the outgrowing cells contained no detectable ascorbic acid. However, some normal 'fibroblasts' were observed with local deposits similar to those of the bone cells which contain ascorbic acid (Fig. 2); again, none of these was dividing. The proportion of these cells was increased, though not very greatly, by the addition of ascorbic acid to the medium. In these cultures there occur a few cells of atypical shape containing much fat in the form of globules: they are evidently degenerate, that is, in the process of dying (Ludford, 1935). They usually contain rather large quantities of ascorbic acid, indicated by a granular precipitate associated with the fat (Fig. 3). The globules themselves, with the silver deposits on them, resemble quite closely the yolk granules described by Barnett & Bourne (1941) as occurring in and below the blastoderm of early chick embryos.

Cultures of adrenal medulla produce mixed outgrowths containing both fibroblast-like cells and epithelium. The former were only observed to contain ascorbic acid when in a degenerate state: the cell drawn in Fig. 4 is an example; the silver deposits are unusual in being not in the form of spherical granules, nor localized, but U-shaped and scattered through the cytoplasm. The impregnation has the appearance of a Golgi network which has broken up; such fragmentation of the Golgi material has been observed in cultured cells by Ludford (1927) and Richardson (1934). In addition to the degenerate cells a few epithelial cells show the presence of ascorbic acid: the silver deposit is in a restricted area of the cytoplasm similar to that observed in the fibroblast types.

## 3. DISCUSSION

In plant seeds the appearance of large quantities of ascorbic acid coincides with the initiation of rapid growth in the tissues concerned (Bonner & Bonner, 1938). There is no reason to think that anything comparable occurs in animals. The tissues used in this study were cultured in a medium optimum for the growth of chick periosteoblasts (Willmer & Jacoby, 1936); very few cells of the outgrowth contained detectable amounts of ascorbic acid, and of these some were isolated and dying: none was dividing. Similarly, in whole chick embryos during the first 4 days of incubation no ascorbic acid can be found except in the extra-embryonic tissues (Barnett & Bourne, 1941). It is evident that histologically demonstrable




The figures are drawings of the cells as seen by means of a 1/12 in. oil immersion objective.

Fig. 1. Normal fibroblast-type cell from 11-day frontal bone, showing a localized impregnation.

Fig. 2. Cell similar to that of Fig. 1, from 17-day adrenal cortex.

Fig. 3. Degenerate cell from adrenal cortex, with granular precipitate associated with fat globules. Only the fat visible in optical section is drawn. The smaller drawing represents a single fat globule, bearing a precipitate, from the same cell.

Fig. 4. Degenerate cell from adrenal medulla, with precipitate evidently associated with fragmented Golgi material.

Fig. 5. Epithelial cell from adrenal medulla, with localized impregnation.

*Lettering*: $a$ = area of precipitation, presumably Golgi material; $b$ = fragmented material bearing precipitate; $f$ = fat granules; $n$ = nucleus.

ascorbic acid need not be present for the occurrence of any of the synthetic processes involved in the multiplication of embryonic chick tissues.

A further question arises in connexion with the site of the silver deposits in the cells. In those illustrated in Figs. 1, 2 and 5 the deposits are evidently associated with the Golgi substance. This association has been frequently observed in the past, and has been assumed to show that ascorbic acid itself is present in the Golgi material (see Bourne, 1935; Giroud, 1938). This is not necessarily the case. Precipitates of this kind often accumulate at the interface between two immiscible liquids, or between a solid and a liquid: the familiar silver mirror is an example. It is possible that a 'mechanical' effect of this kind is responsible for some at least of the localized deposits observed. Bensley has made a similar suggestion in connexion with osmium impregnations of Golgi substance (Owens & Bensley, 1929). If this effect does occur in acid silver nitrate preparations the presence of a precipitate indicates only that ascorbic acid was somewhere in the cell; the exact site of the precipitate must then depend on the existence of a certain type of interface.

## 4. SUMMARY

Heart, bone and adrenal cells from chick embryos were cultured in growth-promoting media in vitro; their ascorbic acid (vitamin C) content was investigated by the acid silver nitrate method. Few cells were found to contain ascorbic acid; those containing most were degenerate; others showed a silver precipitate in the Golgi substance. The results are discussed.

The author is indebted to Prof. E. S. Goodrich and Prof. W. E. Le Gros Clark for facilities provided in the Departments of Zoology and Human Anatomy, Oxford; and to the Christopher Welch Trust for grants for the purchase of apparatus.

## REFERENCES

BARNETT, S. A. & BOURNE, G. (1941). *J. Anat., Lond.*, **75**, 251.
BARNETT, S. A. & BOURNE, G. (1942). *Quart. J. Micr. Sci.* (in the Press).
BONNER, J. & BONNER, D. (1938). *Proc. Nat. Acad. Sci., Wash.*, **24**, 79.
BOURNE, G. (1935). *Austr. J. Exp. Biol. Med. Sci.* **13**, 113.
BOURNE, G. (1936). *Anat. Rec.* **66**, 369.
GIROUD, A. (1938). *L'acide ascorbique dans la cellule et les tissus.* Berlin.
LUDFORD, R. J. (1927). *Proc. Roy. Soc.* B, **101**, 409.
LUDFORD, R. J. (1935). *Protoplasma*, **23**, 180.
OWENS, H. B. & BENSLEY, R. R. (1929). *Amer. J. Anat.* **44**, 79.
RICHARDSON, K. C. (1934). *Arch. Zellforsch.* **16**, 100.
WILLMER, E. N. & JACOBY, F. (1936). *J. Exp. Biol.* **13**, 237.
WILLMER, E. N. & KENDAL, L. P. (1932). *J. Exp. Biol.* **9**, 149.