A Study of the Osmium Techniques for the 'Golgi Apparatus'

By JOHN R. BAKER

(From the Cytological Laboratory, Department of Zoology, University Museum, Oxford)

With one plate (fig. 1)

SUMMARY

The paper is concerned with the action of the osmium techniques for the 'Golgi apparatus' on the intestinal epithelial cell of the mouse. It is shown that, when the fixative has acted, there are many separate lipide spheres in the 'Golgi' region. During postosmication the spaces between these spheres are filled up with a black precipitate. This black precipitate does not represent the distribution of lipide material.

INTRODUCTION

WHEN tissues are treated by the usual osmium or silver techniques for the 'Golgi apparatus', a black or blackish deposit of osmium (probably in the form of hydroxide) or of silver is formed in a particular place (or places) in the cell. According to the view that is usually adopted, this black deposit gives us a representation of the distribution in life of a special lipoidal or lipide-containing substance, the 'Golgi substance'. The unsaturated bonds of the lipide are supposed to have reduced the osmium tetroxide or silver nitrate.

The purpose of the work described in the present paper has been to test this hypothesis of the significance of the black precipitate formed in osmium preparations.

Only one kind of cell has been carefully studied, namely, the absorptive cell of the intestinal epithelium of the mouse. (The Paneth cells of the crypts of Lieberkühn are briefly mentioned on p. 387.) The object has been to ascertain the distribution of lipide material when the cell has been fixed by the standard 'Golgi' fixatives, and to compare this distribution with that of the black precipitate formed during postosmication.

It has been proved, by easily confirmed experiments, that in this particular cell the black substance seen in ordinary 'Golgi' preparations does not represent the distribution of lipide-containing material.

METHODS

The work has been done on adult mice. Since fat-absorption prevents one from getting a clear view of the cytoplasmic inclusions of the cell, mice were usually kept on a diet of bread and water for 24 hours and then with water only for 4 hours, before being killed with chloroform. (I have never found that killing with chloroform affects the distribution of lipides in cells.) Instead, mice were sometimes kept overnight without food (but with water), and killed in the morning. It must be remarked, however, that when food is made con-

[Quarterly Journal of Microscopical Science, Vol. 95, part 3, pp. 383-388, September 1954.]

tinuously available to mice, the stomach and first part of the small intestine are often found to be empty, and good 'Golgi' preparations can then be made.

The part of the intestine chosen for study was between 3 and 8 cm. from the pylorus. Pieces about 1 cm. long were cut out as soon as the mouse was dead, and placed in the fixative. It was found convenient, as a general rule, to slit the piece open longitudinally with fine scissors after the fixative had acted for about 4 minutes. If the slit was made before fixation began, the piece tended to become twisted.

The investigation was carried out in two series of experiments.

In one series the standard 'Golgi' techniques were used. The two techniques chosen for special study were those of Weigl and of Kolatchev.

Weigl himself (1910) did not give very precise instructions. In the present investigation, the pieces of intestine were fixed for 4 hours in Mann's fluid (1894) and then washed overnight in running water. As a general rule, 11 to 14 days' postosmication in 2 per cent. aqueous osmium tetroxide solution at room-temperature (with changes of the fluid) gave the typical 'Golgi' appearance. After being washed, the pieces of tissue were embedded in paraffin, sectioned at 3 μ, and mounted unstained in balsam.

Kolatchev (1916) used various mitochondrial fixatives, washed thoroughly, and then postosmicated. He seems to have favoured Meves's fixative (1908), but also sometimes used Champy's (1911). Those who have followed him have nearly always used either Champy's fluid or Nassonov's (1923) modification of it. I have generally used Champy's fluid and have adhered carefully to Kolatchev's directions. The duration of fixation is 24 hours.

It may be remarked that there is no advantage in using Nassonov's modification of Champy's fluid, though for some unexplained reason nearly everyone who has used the Kolatchev technique in recent years has substituted Nassonov's fixative for Champy's. The latter's fluid consists, as is well known, of a mixture of 3 per cent. potassium dichromate, 1 per cent. chromic acid, and 2 per cent. osmium tetroxide in the proportion of 7:7:4. Nassonov used the proportion of 4:4:2. He does not say why he changed the proportion slightly, nor even that he did so. He simply calls the 4:4:2 mixture 'Champy's fluid'. He recommended the addition of 2 or 3 drops of 0·1 per cent. pyrogallol to 10 c.c. of the mixture. The addition of pyrogallol, a reducer, to about 2,000 times its weight of three strong oxidizers, does not commend itself to reason, and indeed I have found no difference between the action of Champy's fluid and that of Nassonov's modification.

The fixative must be very thoroughly washed out in running water. For the intestinal epithelium, a period of 9 days' postosmication in 2 per cent. osmium tetroxide at 34° C. is about right, though sometimes one will get better results with shorter or longer times. It is best to renew the solution several times during the 9 days. After thorough washing in running water, the tissue is embedded in paraffin, sectioned at 3 μ, and mounted unstained in balsam.

In the second series of experiments, often performed with material from the same mice, the same fixatives were used for the same periods, and the pieces

were then washed thoroughly in running tap-water. They were not post-osmicated, however, but instead were embedded in gelatine and sectioned at 10μ or 8μ on the freezing microtome.

The sections of Mann material were treated with a 1 per cent. solution of iodine in 2 per cent. aqueous potassium iodide solution for a few minutes, to get rid of the mercury precipitate, and then with 5 per cent. sodium thiosulphate solution for about a minute; they were then thoroughly washed in water.

The sections of Mann, Champy, and Nassonov material were coloured with Sudan black according to my usual method (1949). Staining of chromatin with carmalum was generally omitted, however, for it does not work after Champy or Nassonov fixation, and it is not necessary after Mann. A trial of many different mounting media confirmed my belief that Farrants's is as good as any for this purpose. Preparations seem to improve gradually by becoming more transparent over a period of days or weeks.

It sometimes happens that the osmium in the *fixative* makes parts of the cell grey. This could be misleading, for the darkness might be attributed in error to the Sudan black. In cases in which it was thought that error might result from this cause, sections were bleached with 1 per cent. aqueous sodium iodate solution or saturated aqueous potassium persulphate solution before being coloured with Sudan black.

Results

The crucial point of the investigation was the comparison of the results obtained by postosmication with those obtained by the action of Sudan black. If the usual view of the nature of the 'Golgi apparatus' were correct, the form of the apparatus in osmium preparations would be the same as that seen after the use of Sudan black, since the latter agent reveals the sites of lipides.

It will be convenient to describe the Sudan black preparations first. Whether the fixative was that of Mann (fig. 1, A), Champy (fig. 1, C and E), or Nassonov, the appearance given is the same. A large number of spherical or subspherical bodies, coloured with Sudan black, form a group in the cytoplasm near the 'apical' end of the oval nucleus—that is to say, near the end that is directed towards the free border of the cell. I call such lipoidal bodies *lipochondria*. A few of them sometimes occur along the sides of the nucleus, but the majority occur in the group near its apical pole. The extension or non-extension of the lipochondria (and also of the osmium precipitate in 'Golgi' preparations) beside the nucleus appears to depend on the shape of these very elastic cells at the moment of fixation. When the cell is very long and thin, they are generally restricted to a group near the apical end of the nucleus.

The lipochondria are separate from one another. They vary considerably in size in a single cell, but the diameter is commonly between 0·4 μ and 1·4 μ. They are smallest and least strongly coloured towards the base of the villus, and largest and darkest towards its tip. They seem to be homogeneous in these preparations, though when the intestine is fixed in formaldehyde solution and postchromed they often appear to be vacuolated (see Baker, 1949).

If a piece of intestine is fixed in formaldehyde-saline and sectioned on the freezing microtome, and the sections treated with Sudan IV, the lipochondria are not coloured. It is clear, therefore, that they are not simply globules of triglyceride. Their chemical composition is being investigated by Dr. W. G. B. Casselman at Oxford.

In standard 'Golgi' preparations made with the same fixatives, but with postosmication instead of treatment with Sudan black, the appearance is different. There is considerable 'capriciousness' in these techniques, especially Kolatchev's. However hard one may try to reproduce exactly the conditions that gave a successful impregnation, one may fail at the second attempt, only to achieve a good result again on some later occasion. In general, it is more usual to obtain good impregnation at the bases of the villi than at their tips. The cells of the crypts of Lieberkühn are regularly impregnated.

The impregnated region in the absorptive cells of the epithelium of the villi is the same as that in which the lipochondria were seen in the Sudan black preparations: that is to say, it lies in the cytoplasm near the apical pole of the nucleus, and sometimes extends alongside the nucleus.

The structure of the impregnated zone in 'Golgi' preparations is very different from that seen after Sudan black. In any one cell the osmium usually appears in the form of a single black mass of irregular shape (see fig. 1, D, F, and H). Sometimes it contains unimpregnated spheres or 'vacuoles'; some of these are indicated by arrows in the photomicrographs. Similar vacuoles are sometimes seen in Aoyama (silver) preparations (fig. 1, G).

These facts prove that the black material seen in osmium preparations does not represent the lipide that was present in the cell at the end of fixation. On the contrary, it represents either the whole region of the cell in which the lipochondria were situated, or else the parts of the cytoplasm *between and around* the lipochondria. It seems likely that the 'vacuoles', when present, represent the lipochondria, and that the osmium is commonly deposited on their surfaces and between them. Whether the osmium is deposited also *in* the lipochondria, in those cases in which vacuoles are not seen, is uncertain.

It is very enlightening to examine Weigl preparations that have been postosmicated for a shorter period (about 8 days) than that which gives the typical 'Golgi' picture. In such preparations (fig. 1, B), only the lipochondria are impregnated with osmium. One cannot say for certain whether the osmium has been deposited on their surfaces only, or in their substance, but none is deposited in such a way as to fill up the spaces between them. The appearance is very similar to that given by Sudan black, though fewer lipochondria are seen, because the section is much thinner. The facts strongly suggest that further osmication simply fills up the spaces in between.

FIG. 1 (plate). All the photomicrographs represent sections through the intestinal epithelium of the mouse. The free border is uppermost in each case. The letters A, B, C, &c., are given for ease of reference in the text. The arrows point towards 'vacuoles' in classical 'Golgi' preparations.

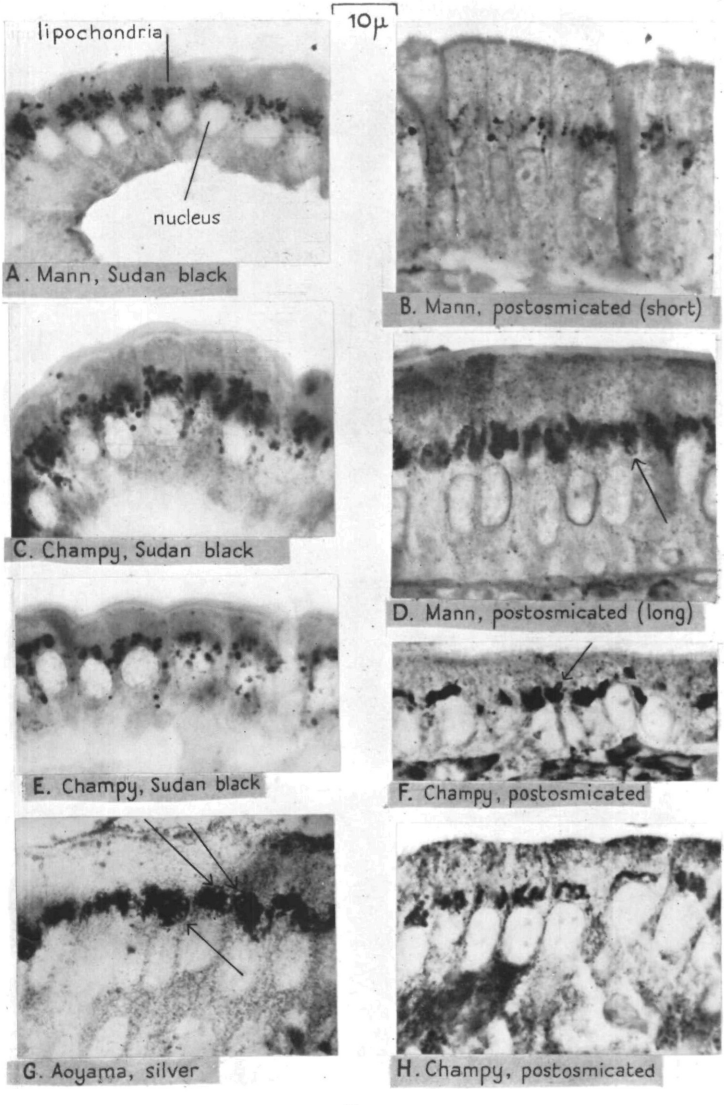

FIG. 1

J. R. BAKER

Thomas (1948) used the Weigl technique in his study of the sympathetic nerve-cell of the mouse. He postosmicated for different periods from 12 hours upwards. He found that when the period of postosmication was short, only the 'spheroids' that exist in the cytoplasm of nerve-cells during life were impregnated. When the period of postosmication was increased, osmium began to be deposited partly on the mitochondria, and partly also at random (that is to say, in such a way as not to represent anything that was present in the living cell). The findings recorded in the present paper confirm Thomas's discovery that at first only the spheroids (lipochondria) are impregnated by Weigl's method.

Mann and Champy fixation followed by the colouring of gelatine sections with Sudan black are useful techniques for showing lipide droplets in a wide variety of different cells. I have tried many different fixatives, simple and mixed, including many new mixtures. I have not found any fixative that is better than Mann's or Champy's for this purpose, though Meves's is also very good.

Discussion

It follows inevitably from the easily repeatable experiments described in the present paper that the greater part of the osmium seen in 'Golgi' preparations of this particular cell does not represent the site of lipide that was present there at the end of fixation. Two possibilities present themselves:

(1) The osmium was deposited on the surfaces of (or perhaps sometimes in the substance of) the lipochondria, and further particles of osmium were added one to another, like silver in a photographic plate during development, until the intervening spaces were filled. If so, the standard 'Golgi' techniques give a very misleading impression of what was present at the end of fixation.

(2) There exists a reducing substance, *not a lipide*, between and around the lipochondria. Of this possibility it can only be said that we have as yet no clear evidence of the existence, far less of the chemical composition, of the hypothetical substance. Still, its existence remains a possibility.

The large spherical granules of the Paneth cells in the crypts are not coloured by Sudan black, nor are the spaces between them. In 'Golgi' preparations a three-dimensional network of osmium extends between the granules. It is obvious that in this case also the osmium does not represent the site of lipide, and the two possibilities just mentioned present themselves.

I have described elsewhere (1949) how the villi may be removed and placed in saline solution for microscopical examination in a fresh condition. Spheroids can be seen in the cytoplasm near the apical pole of the nucleus. It can be argued that these may represent lipide droplets that have separated from lipoprotein complexes as a result of the treatment of the villi, and that they would not have been seen in this form if the cell could have been examined while the villus remained in connexion with its blood supply. Similarly, the lipochondria seen in Sudan black preparations may conceivably represent a result of separation of lipide from lipoprotein, caused by the action of the fixative. This possibility does not alter the inevitable conclusion that must be drawn from the

facts recorded in this paper. The lipochondria are certainly present at the end of 'Golgi' fixation, whether one is going to postosmicate or to colour with Sudan black—and there is no lipide between them.

Prof. A. C. Hardy, F.R.S., has kindly continued to support my studies of the lipide constituents of cells. I acknowledge a great deal of skilful practical assistance from Miss B. M. Jordan. Dr. W. G. B. Casselman has given me the benefit of his criticism of the manuscript of this paper.

REFERENCES

BAKER, J. R., 1949. Quart J. micr. Sci., **90**, 293.
CHAMPY, C., 1911. Arch. d'Anat. micr., **13**, 55.
KOLATCHEV, A., 1916. Arch. russes d'Anat. d'Hist. d'Emb., **1**, 383.
MANN, G., 1894. Zeit. wiss. Mikr., **11**, 479.
MEVES, F., 1908. Arch. mikr. Anat., **72**, 816.
NASSONOV, D., 1923. Ibid., **97**, 136.
THOMAS, O. L., 1948. Quart. J. micr. Sci., **89**, 33.
WEIGL, R., 1910. Bull. internat. Acad. Sci. Cracovie B (no vol. number), 691.