The Structure and Chemical Composition of the Golgi Element.

By


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With Plates 1 and 2, and 7 Text-figures.

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PART I. STRUCTURE.

INTRODUCTION.

The object of this part of the investigation was to find whether there is a single structural plan to which the fully developed Golgi element of diverse cells can be referred, and if so, to reveal it.

It was thought better to concentrate attention on a few kinds of cells, rather than to make a less complete study of many. The cells which have been intensively studied have been chosen on the following grounds. They should belong to animals widely separated in systematic classification, and the animals should be very common, so that anyone who doubts any of the findings might have the least possible difficulty in repeating the observations. The cells should be diverse in function, and possess a well-developed Golgi element. They should be reasonably large, so as to be convenient for study, and some at least of them should be easy to examine alive. Extremely aberrant cells, such as spermatozoa or striated muscle, should be avoided. It was desirable to include the nerve cell of a Vertebrate, on account of its being a classical object for the study of the Golgi element.

The cells chosen to fulfil these requirements were the following:

The primary spermatocytes and early spermatids of the common snail, Helix aspersa. (The secondary spermatocytes are too short-lived to be suitable for intensive study.)

The absorptive cells of the intestinal epithelium of the smooth or common newt, Triturus vulgaris.

The nerve cells of the anterior mesenteric ganglion of the rabbit, Oryctolagus cuniculus. (This ganglion is much easier to dissect out and more convenient in size than the dorsal root ganglia, which would otherwise be equally suitable.)

The spermatocytes, spermatids, and intestinal epithelial cells are convenient for intra vitam studies, the two former because they fall apart loosely when the ovotestis is snipped
up, the latter because the whole epithelium, one cell thick, can be stripped off from the underlying connective tissue, spread flat, and examined under the microscope.

A few other cells were also studied, but only incidentally to the main research.

In this paper the details of technique are kept separate from the general remarks on methods. It is essential that the details should be given, so that anyone who doubts any statement can repeat the work for himself; but some readers will find it a convenience to skip the sections headed 'Details of Technique', or to refer only to certain parts of them.

The usage of the words lipoid, lipide, and lipine in this paper is defined on p. 40.

Methods.

Most investigations of the structure of the Golgi element are open to serious criticism on the ground of the empirical nature of the procedures followed. The structure and chemical composition of what is to be investigated are not known; complicated procedures are followed, involving the use of a succession of chemical reagents whose reactions with the object of investigation are not known; and the appearance resulting from these reactions is carefully described. Emphasis is laid on the necessity for getting 'sharp' images. One is forcibly reminded of the desire of the earlier histologists to get a 'sharp' nuclear network, though it was later found that the network produced was the result of coarse protein precipitation. Students of the Golgi element have tended to work towards a desired sharp image, without questioning whether in fact the structure is sharply defined in life. Tarao (1939), a recent student of the Golgi element, has used these words: 'But we must confess without hesitation that the familiar Golgi apparatus impregnated with care shows too beautiful a figure to be considered as an artifact.' Nevertheless, the sharpness and beauty of the Golgi element in impregnated preparations are irrelevant, unless it can be shown to be sharp and beautiful in the living cell.

The accepted methods have taught us much about the
almost universal occurrence of the Golgi element in the cells of Metazoa, about its position, its size, and its relation to the processes of cellular secretion; but it may be questioned whether unknown reactions on unknown substances can give much certainty on matters of structure or chemical composition. I do not in any way minimize the important part that the standard procedures have played in giving certain kinds of information about the Golgi element, but I believe that the time is ripe for the introduction of entirely new methods of a less empirical kind.

Wherever possible, the Golgi element should be studied in the living cell, so that there may be a standard of comparison for fixed material. Methods for studying the Golgi element in certain living cells are carefully described in this paper (p. 10). Unfortunately, the whole of the structure cannot be revealed in this way, and for studies of chemical composition the cell must inevitably be killed. For these reasons a suitable fixative must be designed. It is here that my first principle of research on the Golgi element comes into play: not to use a fluid that will give 'sharp' figures, but to design a fixative to give life-like appearances, using nothing but reasonable deductions from existing knowledge as guides.

In considering how to fix the Golgi element, one must take into consideration what evidence already exists as to its chemical composition. The evidence is considered on p. 40 of this paper. Here it will suffice to say that there has long been evidence from many sources that substances which are not triglycerides but which are soluble in lipoid-solvents are an important constituent. As will be shown below, my own experiments confirm and extend this evidence. Now ordinary histological fixation is above all protein-fixation. In the present investigation the plan of action was to try above all to preserve and demonstrate lipoids.

Protein fixation usually means protein precipitation. In the precipitation of the proteins of the cytoplasm into a sponge-work, delicate structures such as the Golgi element tend to be distorted or destroyed. It is not only that delicate threads are broken. Vital observations show that there are commonly
vacuoles in the Golgi element, which are seldom well seen after fixation. It is likely that the precipitation of the surrounding proteins breaks down their walls, and their cavities become continuous with the artificial cavities of the sponge-work. For this reason the decision was reached to omit all protein precipitants from the fixative. It will be recollected that the standard fixatives for the Golgi element (Mann’s, Champy’s, Cajal’s, Da Fano’s, and Aoyama’s) all contain protein precipitants (mercuric chloride, chromic acid, uranium nitrate, cobalt nitrate, and cadmium chloride respectively). The range of possibilities is thus limited to fixatives which are not protein precipitants and which do not destroy lipoids. The best fixatives fulfilling these requirements are potassium dichromate (unacidified), osmium tetroxide, and formaldehyde.

It is usual to use mixtures of two or more fixative substances; but when this is done, one usually cannot ascribe the effect to one substance rather than to the other, and one therefore does not know what is happening. Further, the fixatives usually penetrate at different speeds, and different appearances are therefore given at different depths in the piece of tissue fixed; and there is no sure guide to indicate which is the proper level for study. Again, one fixative often reacts chemically with another, or may affect its action in some other way. Even when only a single fixative is used, it is hard enough to interpret the reactions with the various components of the cell: when two or more are used, it is impossible to know what one is doing. Fixative mixtures are useful empirically in routine histological and micro-anatomical work, but do not serve the investigator’s purpose when he wants to know as exactly as possible what he is doing.

For this reason a single fixative substance must be chosen for the further investigation of the Golgi element.

Potassium dichromate preserves lipines, but has the disadvantage of changing the shape of mitochondria, rounding up short rods into spheres and swelling them. Since there is reason to believe that one constituent of the Golgi element resembles mitochondria in composition, potassium dichromate is to be avoided. Osmium tetroxide has the grave disadvantage
of very slow penetration, resulting in different appearances being given at different depths in a piece of tissue. Of all the fixatives whose effects on single cells were studied by Strangeways and Canti (1927), osmium tetroxide and formaldehyde distorted the cell and its component parts the least. Formaldehyde has the advantage of very rapid penetration (Medawar, 1941). It is true that Strangeways and Canti found that it tended to divide thread-like mitochondria into rows of spheres, but these authors used a solution in distilled water, and it is likely that the result would have been different if they had used a suitable saline solution. Zirkle (1933), in a comparative study of the action of aldehydes as fixatives, found none superior to formaldehyde. Although we do not know exactly how this substance reacts with proteins, at least its reaction with amino-acids is known (Lloyd, 1926), so that in using it we are not working completely in the dark. It has the great practical advantage of leaving tissues in a convenient state for the cutting of frozen sections. Within wide limits the concentration at which it is used does not appear to have much effect on the result, and the usual solution of 10 c.c. of ‘formalin’ to 90 c.c. of water or saline is convenient. The formaldehyde is here present at roughly 4 per cent.

More than sixty years ago Berthold (1882), in a paper which has been overlooked in writings on this topic, showed that various fixatives preserve marine Algae in a much more life-like way when dissolved in sea-water instead of distilled water. Carleton (1922) and Young (1935) have shown that the presence of salts at a concentration roughly equivalent in osmotic pressure to that of the body-fluids of the animal under investigation is helpful to good fixation. The presence of the formaldehyde makes the total osmotic pressure far greater than that of the body-fluids (Baker, 1933), but Young has given reasons for thinking that only the osmotic pressure due to the indifferent salts is effective. My own studies suggest that the exact concentration of the indifferent salt is unimportant, but I have had my best results when it is somewhat less than equivalent in osmotic pressure to the body-fluids. For terrestrial Vertebrates, Teleosts, and terrestrial Invertebrates, 0·7 per cent.
sodium chloride is a convenient standard concentration. If anyone doubts its effect, he should cut out the ovotestes from two snails and place one in 4 per cent. formaldehyde dissolved in distilled water and the other in the same fixative dissolved in 0.7 per cent. sodium chloride solution. The ovotestis placed in the former will be seen to swell greatly.

It remains to decide what salt to use for its osmotic effect. It is known that lipines, such as lecithin, can go into colloidal solution in water, and that this tendency is prevented by the presence of divalent cations (Hammarsten and Hedin, 1914). I therefore use calcium chloride instead of sodium chloride, and thus kill two birds with one stone. A 1 per cent. solution of the anhydrous salt has approximately the same osmotic pressure as 0.7 per cent. sodium chloride solution, and this is therefore the chosen concentration.

The fixative is therefore:

**Formal-calcium**

Formalin (formaldehyde of approximately 40 per cent.) 10 c.c.
Calcium chloride, anhydrous, 10 per cent. aqueous . 10 c.c.
Distilled water . . . . . . . . . 80 c.c.

Some calcium carbonate, in the form of precipitated chalk, is kept in the bottle containing the fluid in order to neutralize the formic acid resulting from the gradual oxidation of the formaldehyde. When the fixative is to be used, the bottle is shaken and the cloudy fluid poured into a capsule.

The reaction of formalin with proteins is slow (though penetration is quick), and I therefore fix for three days as a rule. It is well known that lipines very gradually disappear from tissues lying in solutions of formalin, and the following fluid is used as a preservative of lipines whenever prolonged soaking is necessary or convenient:

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1 The word is often spelled formol, but the standard abbreviation for aldehydes is -al (e.g. chloral, furfural). The ending in -ol is best reserved for alcohols and phenols. There is no more reason for writing formol than there would be for writing phenol.
Formal-calcium-cadmium

Formalin . . . . . . . . 10 c.c.
Calcium chloride, anhydrous, 10 per cent. aqueous 10 c.c.
Cadmium chloride, 10 per cent. aqueous . . 10 c.c.
Distilled water . . . . . . . . . . 70 c.c.

This fluid, like formal-calcium, is kept free from formic acid by the presence of precipitated chalk. The tissue may be transferred to it at any time after the formal-calcium has acted for 24 hours. The cadmium chloride has no precipitant effect on proteins after formaldehyde has had time to act, but it forms an insoluble compound with certain lipines and thus prevents their gradual loss. The fluid is convenient not only for the preservation of tissues in bulk but also for other purposes which will be mentioned below. When it is used for the temporary preservation of sections, it should be filtered in order to avoid the adherence of particles of chalk. If fixation in formal-calcium lasts only three days, one may proceed to wash out without using formal-calcium-cadmium.

When fixation is complete, the tissue is washed in running water. The use of solvents for lipoids is involved in embedding in celloidin or paraffin, and the tissue is therefore embedded in gelatine and cut on the freezing microtome. The sections are firmly attached to glass microscopical slides. Full instructions are given on p. 13.

The next consideration is how to colour the lipoids. I have discarded impregnation, except as an ancillary method, because the result is not to be trusted so far as finer structure is concerned. Our knowledge of how the image grows on a photographic plate suffices to show that dependence on impregnation images is illusory unless there is confirmation from a surer source. The process of reduction throws the metal down in an elaborate network whose details are irrelevant. Further, the appearance given depends on arbitrary decisions as to the various procedures, and there is no guide to the selection of one image as true rather than another.

The most dependable information of all, apart from what can be seen in the living cell, comes from the use of a coloured
substance which simply dissolves in the lipoids. Such a substance has no tendency to form connecting bridges between one structure in which it is deposited and another—and that is what particles of silver or osmium hydroxide do in impregnations. The problem is to find a substance which is sufficiently soluble in lipoids other than triglycerides. Sudan III and IV, excellent for triglycerides, are very deficient in this respect. I have chosen sudan black as almost perfect for the purpose, on account of its enormously greater solubility in lipoids other than triglycerides. I use the product of Mr. G. T. Gurr. Sudan black, like sudans III and IV, is not a dye and does not act by adsorption. It dissolves in all lipoids and thus colours them intensely. It was introduced into microscopical technique by Lison (1934, 1935), who used it for colouring the myelin of nerves. I follow Lison in using a saturated solution in 70 per cent. alcohol and counterstaining in Mayer's carmalum. The section is finally mounted in glycerine jelly or laevulose syrup. Lipoids are coloured blue-black.

I call the technique described above the formal-sudan-black technique. For full details, see p. 12. This technique is the basis of my study of the Golgi element in sections, and has played a large part in the histochemical work as well, as will appear in the second part of the paper. The fixation is remarkably life-like.\(^1\) Even delicate cells which are easily crumpled and distorted by ordinary cytological treatment appear in their original form. This is probably due to the preservation of the lipoids of the cytoplasm and of the cell-membrane, which are both preserved and coloured. I would recommend formal-calcium fixation followed by gelatine embedding to all biologists to whom it is important to preserve the shapes of cells accurately, especially embryologists.

The finished slide, coloured by sudan black, will surprise the observer. One is so used to looking at slides which show mainly the proteins (including nucleoproteins) of cells, that a slide

\(^1\) See fig. 7, Pl. 1. Although the form of cells is well preserved, yet there are certain cells which seem inevitably to get much smaller during the making of permanent preparations. The primary spermatocyte of the snail is a good example.
which concentrates attention instead on the lipoid components is quite unusual in appearance. It is common to hear of 'masked' lipoids in cells, so-called because they are not shown by sudan III or IV. Sudan black unmasks them and presents an altogether new view of the cell.

The routine methods for showing the Golgi element were also used for purposes of comparison. In addition, a new impregnation technique was devised in which the use of a precipitant fixative is avoided. This formal-osmium technique is described on p. 16.

**Details of Technique.**

**Vital Methods.**

For the ovotestis of the snail I have found this saline solution satisfactory:

**Sodium and Calcium Chloride Solution.**

| Sodium chloride, 0·7 per cent. aqueous | 100 c.c. |
| Calcium chloride, anhydrous, 10 per cent. aqueous | 0·2 c.c. |

This is the solution in which the ovotestis is teased when the cells are going to be examined without staining, and also as the vehicle of the dye for intra vitam ('supravital') observations.

For some years I have been accustomed to teach zoology students at Oxford to use vital dyes to demonstrate the Golgi element in the primary spermatocyte of the snail, and I give here a method which is reliable.

All instruments and glass-ware should be clean, but sterility is unnecessary. Cut out the ovotestis of a common snail as described on p. 12, place it in a watch-glass, and immediately add a few drops of sodium and calcium chloride solution. For a large ovotestis use 5 drops, and proportionately less for smaller ones. Snip the organ up finely with small scissors. Suck some of the suspension into a pipette, avoiding lumps, and transfer three drops of it to a clean deep watch-glass. Add three drops of the following solution:

| Dahlia or neutral red, 0·1 per cent. aqueous | 1 c.c. |
| Sodium and calcium chloride solution (as above) | 9 c.c. |
COMPOSITION OF THE GOLGI ELEMENT

(The 0-1 per cent. stock solutions of the dyes are in distilled water, as the dyes are not permanently stable in saline solutions.)

Mix the cell-suspension with the dye solution by sucking it into a pipette and pressing it out again. The dye is now at 0-005 per cent. Cover the deep watch-glass and leave the preparation for 20 minutes. The six drops of suspension expose a large surface to the air inside the watch-glass, and the reduction of the dye to a colourless compound by the vital activity of the cells is thus counterbalanced by oxidation by atmospheric oxygen. (This is particularly necessary with dahlia, or again with janus green, as these dyes are more easily reduced than neutral red.)

Put two drops of the suspension on the glass of a compressorium and lower the coverslip in such a way as to include some air with the suspension. Cover the edge of the compressorium with soft paraffin (e.g. vaseline) to prevent concentration of the salts in the suspension by evaporation. Examine under the immersion lens.

It is important to use a compressorium or some other device that will prevent the cover-glass from moving when the immersion lens is focused. It is best to work in a partially darkened room. If not, one should at least sit with one's back to the window. The iris diaphragm should be closed to rather a small aperture until a general picture has been obtained, and then opened as widely as the transparency of the object will allow for critical examination. A few cells will be seen whose nuclei are stained. These have been damaged during the manipulations and are dead. Attention is concentrated wholly on the living cells.

The intestine of the newt may be examined intra vitam by slitting it open longitudinally under 0-7 per cent. sodium chloride solution and placing it flat in the compressorium, epithelium upwards. The muscular and connective tissue layers, however, interfere somewhat with critical illumination, and it is therefore desirable to strip off the epithelium and examine it alone. To do this, pin out a length of intestine under 0-7 per cent. sodium chloride solution and slit it longitudinally with
fine scissors. Now, working from one end, very carefully separate the epithelium from the underlying connective tissue with a scalpel. It is necessary to use a dissecting microscope, and a good deal of practice is required. The operation is much easier if the crested newt, *Triturus palustris*, is used, on account of the larger size. The epithelium may now be examined as it is, or vitally stained.

To stain the epithelium, put it in the following solution contained in a deep watch-glass:

Dahlia or neutral red, 0·1 per cent. aqueous . . . 0·5 c.c.
Sodium chloride, 0·7 per cent. aqueous . . . 9·5 c.c.

Cover the watch-glass, leaving an air-space below the cover. After waiting for 20 minutes, put the epithelium in the compressorium and gently adjust the cover-glass until the folds are just flattened out. It is a great convenience to use a reversible compressorium, because it is very difficult to keep the extremely thin film of epithelium always the same way up during the manipulations, and it is essential to have it with the free-border side upwards during microscopical examination, because the Golgi element lies on that side of the nucleus.

The *Formal-sudan-black Technique*.

I am above all anxious to persuade others to try my standard formal-sudan-black technique on the object for which it was first elaborated, the ovotestis of the common snail. I therefore give precise instructions to supplement those given above.

The ovotestis is at its largest and contains most spermatoocytes and spermatids about July (in England), but these cells are present at all times of year. Kill a fully grown snail by decapitation. (If the head is not protruded, put the snail in tepid water until it crawls out and then leave it on a wet board, when it will soon extend itself.) Next remove the shell with strong forceps. Identify the albumen gland and cut right across the spiral visceral hump immediately above it with scissors. Place the part of the spiral hump above the cut on a clean sheet of paper, identify the ovotestis exposed on the surface of the inner side of the spiral, and snip away all the ‘liver’ which
covers its other sides, until nothing but the milk-white organ remains. Take the utmost care not to grasp the ovotestis in forceps nor to injure it in any other way. Throw it at once into formal-calcium (p. 7), and leave it there for three days. Wash in running water for four hours, and then transfer to a gelatine sol made as follows:

Wet 25 gm. of gelatine with 100 c.c. of a 0.25 per cent. aqueous solution of cresol. After an hour apply warmth until the gelatine dissolves. Strain the warm sol through muslin. (The cresol preserves the gelatine indefinitely.)

The gelatine should be ready in an incubator maintained at 37° C. when the ovotestis comes out of the running water. Leave it in the gelatine sol for 20–24 hours. Then make the gelatine into a gel by cooling (preferably in a refrigerator), and cut out a block containing the tissue with a safety razor blade or sharp scalpel. Harden the block for 20–24 hours in formal-calcium-cadmium (p. 8), and then wash it for 3 or 4 hours in running water. Section the block on the freezing microtome at 15 μ.

Fix the sections to slides by a slight modification of the method of Leach (Carleton and Leach, 1938), as follows. Make up a gelatine solution in exactly the same way as that described above, except that it contains only one-tenth as much gelatine. Place this in the paraffin oven, put clean microscopical glass slides in it and allow them to warm up. Remove them one by one. Dry the back of each slide with a duster, and prop it up against a wall to dry, with the wet side facing but not touching the wall. (The place must of course be free from dust.) When the slide is dry, place it with the gelatine side upwards in a petri dish containing distilled water and the section which is to be attached. Float the section over the slide and raise the latter carefully from the dish. Hold the section in place by a pin while the water runs off. Dry the slide round the section with a duster and prop it up once more in the same way as before to dry again. As soon as it appears dry, expose it for 10 minutes in a closed slide-jar to the vapour of formalin (full strength), to harden the gelatine. Then put it into filtered formal-calcium-cadmium and leave it till you are ready to
subject the section to further treatment. The drying of the section, which is not so complete as to remove every trace of water, causes no change of appearance in the tissue that can be detected by any process of microscopical examination. The sections adhere well even when subsequent treatment is rather rough.

Next proceed to colouring the section with sudan black. It is essential that the solution in 70 per cent alcohol should be completely saturated. In this respect there is a marked contrast from the behaviour of true dyes, which act in almost identically the same way whether they are nearly or quite saturated. The action of the sudan black depends on its partition between the 70 per cent. alcohol and the lipoids in the tissue. If the 70 per cent. alcohol is not completely saturated, the power to colour cell-constituents is much reduced. Saturation occupies several days, with repeated shakings. The solution is filtered on the day of use. (The used solution may be poured back into the stock bottle, which must always contain an excess of the colouring matter.)

Remove the slide from the formal-calcium-cadmium and wash it for 3 minutes in running water. Transfer it to 50 and then 70 per cent. alcohol for a few minutes and then to the saturated solution of sudan black in 70 per cent. alcohol. To avoid the falling of undissolved particles on the section, use a wide staining jar and lean the slide with the section facing downwards. (Precipitates on the surface of the section are usually not altogether avoided, but it is easy to disregard them in a thick (15 μ) section examined with the highest powers of the microscope.) Leave the slide in the sudan-black solution for 7 minutes. Pass it successively through three lots of 50 per cent. alcohol, allowing it to remain 30 seconds in each. Rinse in distilled water. Stain for 2 minutes in Mayer's carmalum (a saturated solution of carminic acid in 5 per cent. potassium alum, preserved by the addition of 0·5 c.c. of formalin to 100 c.c.). The purpose of the carmalum is simply to show up the general structure of the tissue by tingeing the nuclei lightly and the cytoplasm more lightly still, in a colour contrasting with the blue-black of the sudan; but it is often best to omit the carmalum staining
altogether. Wash the slide for 3 minutes in running water to get
rid of the alum, rinse in distilled water, and mount in glycerine
ejelly or syrupy laevulose. Lison used the latter, but sudan
black fades more slowly in the jelly.

Whatever appears blue-black consists of lipoids, for sudan
black is not soluble in other substances contained in cells and is
not adsorbed like a dye. (See Lison, 1936.)

Anyone who has tried this technique will be in a position to
perform the histochemical extraction-experiments without diffi-
culty (p. 54).

With other tissues experiment must determine the best time
to leave the slide in the sudan black. The absorptive intestinal
epithelial cell of the newt is so loaded with lipoids that the
sections must be very thin and the period in sudan black
reduced to 4 minutes. Whenever the blue-black colour is found
to be extracted by 50 per cent. alcohol, the period in it must be
shortened. A quick dip in each of the three jars suffices with
some tissues.

The following method of embedding in gelatine is used when
thin sections (5 μ) are required.

Use the same gelatine sol as in the standard technique, but
put it with the tissue in a deep watch-glass, uncovered, in a
dessicator containing anhydrous calcium chloride, the dessi-
cator itself being already warm inside an incubator maintained
at 37° C. Allow the evaporation of water from the gelatine sol
to proceed for about 30 hours. It is best to stop just before the
sol sets to a gel. The time will depend mainly on the dryness
and amount of the calcium chloride. Then remove the watch-
glass to the refrigerator and treat it in the same way as before.
The gelatine gel is naturally much harder than usual and it is
easier to cut thin sections.

Impregnation Methods.

For the reasons explained on p. 3, less reliance was placed
on these than on vital observations and on the formal-sudan-
black technique. The routine methods used were the following:

Silver methods:
Golgi’s arsenious acid and silver nitrate (1908).
Da Fano's.
Aoyama's.
Osmium methods:
  Weigl's (Mann-Kopsch).
  Kolatchev's (Champy, postosmified).
  Nassonov's modification of Kolatchev's method.

In addition, a new osmium method was devised, to avoid the use of a protein precipitant in the fixative. The technique is as follows (cf. Sjövall, 1906):

Fix for 24 hours in:

| Formalin (40 per cent. formaldehyde) | 10 c.c. |
| Sodium chloride, 10 per cent. aqueous | 7 c.c. |
| Distilled water | 83 c.c. |

Keep some precipitated chalk in the solution.

Wash for 5 minutes in each of two lots of 0.7 per cent. sodium chloride solution. This does not completely remove the formaldehyde, and the part of it that remains in the tissue accelerates the reduction of the osmium tetroxide in the next procedure. Put the tissue in a 1 per cent. solution of osmium tetroxide in 0.7 per cent. sodium chloride solution, contained in a specimen tube. Close firmly with a rubber bung. Place in an incubator maintained at 30° C. The period in the warm osmium tetroxide solution must be determined experimentally, but at the first attempt with a new tissue the periods should be about 24, 36, and 48 hours. (It is convenient to start impregnating in the evening, to remove one tube the next evening, a second the following morning, and the third the evening of the same day.) On removal from the incubator, wash for 6 hours or overnight in running water and then dehydrate through the alcohols. It is convenient to keep the organs which have been impregnated for 24 and 36 hours in 70 per cent. alcohol until they are joined by the one impregnated for 48, and then bring them all through together. Embed by Peterfi's (1921) methyl-benzoate-celloidin method (celloidin and paraffin). Cut sections are 3 or 4 μ. Mount them unstained in balsam. Overimpregnated sections may be brought down to water and differentiated in a saturated aqueous solution of potassium persulphate, but the best results
are obtained by impregnating to the right degree so that no differentiation is required.

The best object on which to learn this technique is the skin epithelium of the mantle region of the common snail. It is hardly possible to fail with this object, and it has the advantage that ordinary paraffin embedding may be used, instead of celloidin-paraffin. Reduction of the osmium tetroxide is very rapid, and impregnation should last only 15 hours.

The advantage of this method, which I call the formal-osmium technique, is that no protein precipitant is used in the fixative, so that there is less liability to distortion than in the standard Golgi methods. The process is very quick, because the formaldehyde retained in the tissue helps the reduction of the osmium tetroxide. The disadvantages are that there is sometimes reduction on cell- and nuclear membranes, and that the tissue has a strong tendency to crack during ordinary paraffin embedding. One of the effects of the osmium tetroxide is to preserve and harden the gel-structure of the protoplasm produced by the formaldehyde, and prevent its being distorted by the action of alcohol. There are thus no meshes, such as exist everywhere after ordinary fixation, into which the paraffin can penetrate. It is for this reason that double embedding by Peterfi’s method is recommended.

Miscellaneous Remarks.

Snails were always used on the day on which they were caught or on the following day. The method of killing is described on p. 12.

Newts were kept without food for about 10–14 days, so as to leave the small intestine empty. (This occasions them no harm, for like many Urodèles, they can be kept indefinitely with only occasional feeding.) Each is put in a separate covered glass bowl with enough water to keep the atmosphere damp, but not enough to cover the whole of the bottom of the bowl. When faeces or bits of moulted skin appear, they are washed out to prevent the newt from eating them. Newts are killed by decapitation.
The rabbit is held up by the back legs and killed by a downward blow on the back of the head.

All the photomicrographs illustrating this paper were taken by the method which I have described elsewhere (Baker, 1942a), in which the use of a focusing-screen is eliminated. I wish to thank Mr. P. A. Trotman for the care and skill which he has devoted to the enlargement of my negatives. The photomicrographs are all untouched.

**Results.**

The Primary Spermatocyte and early Spermatid of the Common Snail, *Helix aspersa*.

Photomicrographs of these cells are shown in figs. 1 to 14 on Pl. 1. Text-fig. 1 shows the position of the Golgi element in the primary spermatocyte. Text-fig. 2 is a diagram showing my opinion of the structure of the Golgi element in this cell.

The reader is referred to Gatenby's paper (1917) for an excellent account of the disposition of the cells in the ovotestis of the snail.

The primary spermatocyte is sometimes spherical and loose in an alveolus of the ovotestis, sometimes drawn out at the point of attachment to another cell. The nucleus is excentric, so that a larger mass of cytoplasm is left on one side, which is the side of attachment in the case of attached cells. The Golgi element is situated in the middle of the larger mass of cytoplasm.

In what follows the word 'primary' will generally be omitted. The secondary spermatocytes are short-lived and there are therefore relatively few of them in the ovotestis at any given time. They were not specially studied in this investigation.

When a spermatocyte is examined under the microscope while still alive, without any staining, rods are seen in the Golgi region. These are the 'lepidosomes' of Parat (1928). They are shown in fig. 1, Pl. 1, which represents a living, unstained cell. A touch on the fine adjustment would bring other lepidosomes into view, but in the unstained cell only the two that are precisely in focus are seen (at I). The lepidosomes do not transmit quite so much light as the ground cytoplasm and therefore...
TEXT-FIG. 1.
Diagram of the primary spermatocyte of the common snail, *Helix aspersa*, to show the position of the Golgi element. *g*, Golgi element; *n*, nucleus.

TEXT-FIG. 2.
Diagram illustrating the author’s opinion of the structure of the Golgi element in the primary spermatocyte of the common snail, *Helix aspersa*, as seen in a thin section. The neutral-red vacuoles are seen as clear spheres. The following conventions are used in Text-figs. 2, 4, 6, and 7. The dense lipoid-containing substance is shown in black. (In the primary spermatocyte of the snail it forms the lepidosomes.) The diffuse lipoid-containing substance is indicated by small black dots. The Golgi-product (not present in the primary spermatocyte of the snail) is indicated by hollow dots.
appear very pale grey. It is probable that there are usually less than fifteen of them in the cell. They are mostly curved and arranged with the concavities of their curvatures directed inwards towards the centre of the Golgi region, but there is not strictness in this arrangement. They vary in length in a single cell, but are commonly between 2 and 5 μ long. They are only about 0.4 μ thick. They stain well with dahlia in the living cell, but are quite untouched by neutral red. The dahlia also stains the mitochondria. Janus green stains the lepidosomes less well than dahlia, but is of course good for the mitochondria.

Fixation somewhat shortens and thickens the lepidosomes, so that they become banana-shaped. They can be seen as very pale grey bodies in unstained sections of formal-calcium material (fig. 5, Pl. 1). They are powerfully coloured by sudan black (fig. 6, Pl. 1) and therefore contain much lipid material. They have reductive powers and are easily impregnated by osmium (figs. 8 and 10, Pl. 1). Fig. 8, Pl. 1, shows that the mitochondria also reduce osmium tetroxide, but much less strongly.

When a living cell is stained with neutral red, an altogether different picture is presented by the Golgi region, which is now seen to contain spherical 'vacuoles'. Neutral red is such a convenient dye for showing the vacuoles of the Golgi region in diverse cells, that it is convenient to call them the 'neutral red vacuoles'. This is done without any suggestion that neutral red never stains anything else in living cells. Scorn has been poured on 'neutral-red cytology', but in fact no such cytology exists. It is merely a question of making appropriate use of a dye which is remarkably non-toxic and particularly convenient for certain purposes. Thanks largely to the work of Koehring (1930), we probably know more about its action than about that of any other vital dye.

The neutral-red vacuoles were seen by Platner in 1885, without the use of a dye. They are shown in his fig. 11 on Pl. XXIII. The cell, which he calls a 'Spermatogenie', is almost certainly a primary spermatocyte.

The neutral-red vacuoles are seen in figs. 3 and 4, Pl. 1. They vary in diameter from about 1½ μ downwards. They are not seen to increase in size when neutral red is used in the way
described on p. 10. There is no proof that neutral red, used in this way, produces artificial appearances. It is difficult to state the number of the vacuoles, because one does not know whether to include the very smallest dots. Nine undisputed neutral-red vacuoles would be about the average, though I have counted as few as six and as many as fifteen. These vacuoles are usually not seen in sections, though they sometimes appear in formal-sudan-black and formal-osmium preparations, as transparent spheres.

It is not possible to state definitely that one lepidosome is attached to one neutral-red vacuole. In a preparation stained with dahlia and neutral red at the same time (a technique that I do not in general recommend), I have seen a lepidosome lying apart from any stained vacuole. The radius of curvature of the largest lepidosomes is much greater than that of the largest vacuoles. In sudan black preparations one sometimes sees a little vacuole attached to the concave side of a lepidosome. A large and a small lepidosome with vacuoles attached are seen in fig. 7, Pl. 1. In dahlia preparations the neutral-red vacuoles can sometimes be seen, unstained, and the lepidosomes sometimes appear to lie on their rims, generally extending about half-way round. They lie on a grand circle, and in the case of the small vacuoles extend nearly (perhaps quite) all the way round. I do not know whether this appearance represents a particular stage in the development of the spermatocyte. The various appearances of the relation between lepidosomes and vacuoles are shown in Text-fig. 2. I have not found support for the view of Monné (1938) that the lepidosomes lie on the rims of lens-shaped vacuoles which themselves contain smaller vacuoles.

Long impregnation with osmium or long colouring with sudan black reveals that the whole Golgi region contains a diffuse osmiophil and sudanophil substance, the 'lipoides diffus périvacuolaires' of Parat. This is shown in fig. 9, Pl. 1, in which the whole Golgi region is black with osmium. It appears in fact to occupy the whole space of the Golgi element except that occupied by the lepidosomes and vacuoles. It is a far less obvious constituent of the Golgi element than in many other kinds of cell.
The structure of the Golgi element of the early spermatid was not studied in such detail as that of the spermatocyte. It is not easy to describe the disposition of the lepidosomes in this cell better than in the words of Platner, written nearly sixty years ago: 'Diese [Stäbchen] an Zahl variirend, meist sind es 4-6, sind von verschiedener Länge, theils grade, theils leicht gebogen und zu einer zusammenhängenden polyedrischen Figur geordnet.' The lepidosomes are confined to the outer limit of the diffuse osmiophil substance, which is better represented than in the spermatocyte (see fig. 12, Pl. 1). The vacuoles are situated in the diffuse osmiophil substance, among which they are not always easy to see.

The Absorptive Cell of the Intestinal Epithelium of the Smooth or Common Newt, Triturus vulgaris.

The intestine of the newt has no villi, but is thrown into a few longitudinal ridges, which are more numerous in its wider, anterior part. (No glands of Brünner being present, the term duodenum is inappropriate.) Towards the bases of the ridges, on the side of the epithelium away from the lumen of the gut, there are clumps of cells which seem to correspond to the glands of Lieberkühn, but have no lumina. They probably serve as reserves for the two types of cells which constitute the single-layer epithelium of the intestine, the absorptive and goblet (mucous) cells. I have studied the cytology of the intestinal epithelium of the newt for years, in connexion with the structure of the free border of the absorptive cells (Baker, 1942b), but have never seen any reason for believing, as Nassonov (1923) did, that the absorptive cells become modified into goblet cells. Two kinds of cells occur in the reserves, in one of which mucin already exists, and it is likely that one kind gives rise to the absorptive and the other to the goblet cells. I have never seen mitosis nor any intermediate stage between an absorptive and a goblet cell in the epithelium. Mitosis occurs frequently in the reserves. These facts are stressed because I do not believe that the Golgi element of the absorptive cell has anything to do with the secretion of mucin.
The absorptive cells produce large globules, whose function is not yet determined. They are shown in fig. 30, Pl. 1. The cells near the troughs between the ridges do not contain large globules, but as one passes up from the trough towards the apex of the ridge, one sees them appear and get progressively larger and larger until finally, near the apex, they often attain a diameter of some 6 $\mu$. At the actual apex of the ridge, the cells appear to undergo decomposition. It is likely that cells are continually moving up from the reserves, developing large globules as they go, until finally they reach the apex of the ridge and decompose. In this study attention was directed mainly to cells about half-way up the ridges.

The end of the absorptive cell next the lumen of the intestine will be called the apical or upper end, the other the basal or lower.

Photomicrographs of the Golgi element of the absorptive cell are shown in figs. 15 to 30, Pls. 1–2. Text-fig. 3 shows the position of the Golgi element in the cell. Text-fig. 4 is a diagram showing my opinion of its structure.

The Golgi element is situated near the nucleus, on the upper side of it. It occasionally happens that the nucleus is as it were thrust through the Golgi element, which then surrounds its upper part (fig. 28, Pl. 2).

When the living epithelium is examined in surface view under the microscope, vacuoles are seen in the region of the cytoplasm above the nucleus. These vacuoles, which are visible without any staining (fig. 15, Pl. 1), are easily coloured by neutral red (figs. 19, 20, and 21, Pl. 1). Their diameter varies from more than 6 $\mu$ downwards. The largest, which were referred to above as 'globules', occur only in the cells near the apices of the ridges. When the epithelium, stained with neutral red, is flattened in the compressorium and examined under the low power of the microscope, the position of the ridges is at once announced by the large red globules, the smaller vacuoles of the sides of the ridges being much less obvious. The smaller vacuoles are spherical, but the larger ones depart somewhat from this shape.

Dahlia has a remarkable effect on the vacuoles. It tends to colour them yellow or red, with a blue surface layer. The red
colour is often remarkably intense, considering the very low concentration at which this dye is used *intra vitam*. On the larger vacuoles the blue superficial part becomes no longer a uniform covering, but occurs in the form of irregular, generally crescentic patches on their surface (fig. 18, Pl. 1). It is these which give the non-spherical effect.

One cannot fail to be surprised on turning from the living cell to that seen in sections, for at first there seems to be little in common between the Golgi regions of the two, apart from the
largest vacuoles. The formal-sudan-black technique has an extraordinary effect. Almost the whole of the cytoplasm is so loaded with what would ordinarily be regarded as 'masked' lipoids, that scarcely anything is to be seen except a blue-black mass. The only parts of the cell which are free from lipoids are (1) the nucleus; (2) the outer part of the free border (the basal granular layer of the border contains them. (Baker,
1942b)); (3) a narrow, sharply delimited strip of cytoplasm immediately below the granular layer; and also, I think, (4) the little processes at the extreme basal end of the cell, by which it makes contact with the connective tissue. When thin sections are used and the time in sudan black reduced, it is seen that there are three regions where the lipoid material is present in greatest amounts. These are (1) the region of the basal group of mitochondria; (2) the region of the upper group of mitochondria; and, separated from the latter by a region less rich in lipoids, (3) the Golgi region, just above the nucleus (fig. 22, Pl. 1). It is not possible to make out the structure of the Golgi region very distinctly, but there appear to be lipoid-free vacuoles and threads of lipid substance running parallel to the long axis of the cell. The superficial part of the large vacuoles, which is coloured blue by dahlia intra vitam, is shown by sudan black to consist of lipoid, while the internal part is not. (Large vacuoles are not seen in fig. 22, Pl. 1.)

The Weigl (Mann-Kopsch) technique strongly impregnates longitudinal threads in the Golgi region, but generally destroys all the vacuoles except the largest. Sometimes, however, the small vacuoles are quite clearly seen (figs. 26 and 27, Pl. 2). Nassonov’s modification of Kolatchev’s technique often shows the small vacuoles clearly (figs. 24 and 25, Pl. 2), and it is strange that this author did not see them himself in this cell, thinking that vacuoles occurred only in mucous cells. This technique shows the diffuse osmiophil substance of the Golgi element, surrounding the vacuoles. It also shows the longitudinal osmiophil threads, not as cylindrical bodies, but as irregular strands running from vacuole to vacuole and spreading over parts of their surfaces. In fixed preparations the vacuoles are often seen to be arranged in longitudinal rows, and some of the strands run between the rows, as though pressed tightly between them. There is no sharp distinction between the dense osmiophil substance of the strands and the diffuse osmiophil substance. This is a marked contrast to the condition in the spermatocyte and spermatid of the snail, in which the dense osmiophil substance of the lepidosomes is sharply marked off from the diffuse substance.
COMPOSITION OF THE GOLGI ELEMENT

The photomicrography of the Golgi element of this cell as seen in osmium preparations is very difficult. The least movement of the fine adjustment to and fro will bring an understanding of the structure which it is impossible to convey by means of a photographic image, and even a whole series of photographs at different foci would show less than a mere glance down the microscope.

Rows of vacuoles with osmiophil substance in between are sometimes seen in formal-osmium preparations (fig. 23, Pl. 2).

I have never succeeded in impregnating the strands of the Golgi element of this cell with silver. M. and M. Parat (1930) record that they only succeeded on a single occasion. I have tried Golgi’s, Da Fano’s, and Aoyama’s techniques and used very young as well as adult newts.

Frozen sections of material fixed in formaldehyde solution occasionally show the Golgi element when stained with paracarmine and picronigrosin. The vacuoles, unstained, are surrounded by a substance which stains blue-black with nigrosin (fig. 31, Pl. 2, representing an absorptive cell of Triturus palustris). Paraffin sections treated with Mallory’s methylene blue or other basic thiazine dyes or with pyronine sometimes show staining of the Golgi region, without revealing its structure.

I have never been able to see the longitudinal threads of the Golgi region intra vitam. M. and M. Parat, however, succeeded in colouring them with Janus green. Unfortunately they give no particulars of their technique. I have tried this dye, but without success in this respect.

The conflicting evidence of intra vitam and other studies can only be reconciled by the following hypothesis, which is illustrated in Text-fig. 4. The Golgi element is concerned in making a product, the large vacuole, which is seen without difficulty by various techniques. It is not shown in figs. 22–8, Pl. 1–2, because in these cells it has not yet appeared. The vacuoles are very small when they arise in the Golgi region. When they have grown beyond a certain size they usually break away from the Golgi region, passing upwards towards the free border, but not reaching it. In doing so they carry away with
them on their surfaces part of the lipoid component of the 
Golgi element, which at this stage stains blue with dahlia. 
The internal part of the vacuole stains red with neutral red at 
all stages (though the very small vacuoles have a yellowish 
appearance, which is probably a dichromatic effect). The lipoid 
part of the Golgi element consists of a diffuse substance in 
which the vacuoles lie and of longitudinally arranged strands of 
dense lipoid material which pass from vacuole to vacuole, 
spreading over their surfaces. There is no sharp line of distinc-
tion between the diffuse and dense lipoid substances.

The function of the fully formed vacuoles, which I regard as 
the Golgi product, is not known. They were described in 
Salamandra maculosa by Steinhaus in 1888. This author 
derived them from the nucleus, but no subsequent worker seems 
to have agreed with this. The vacuoles seem to reach their 
greatest size in the newt, in which animal they have been 
described by several authors. Nicolas (1891) called them 
'grains' and 'boules' according to size. He recognized that the 
boules were formed of two substances, differing in their reactions 
to dyes. He regarded the boules as 'le substratum sur lequel se 
déposent les substances (spécialement les graisses en solution) 
qui pénètrent par imbibition dans les cellules épithéliales'. 
Champy (1911) distinguished a series of bodies of various sizes, 
which he called 'grains colorables comme les chondriocontes', 
'petits grains colorables à l'alizarine', 'boules colorables au 
rouge neutre', 'boules et grains imprégnés par ma méthode à 
l'osmium', 'vacuoles', and 'plasmopyrènes'. Only the 'plasmo-
pyrènes' correspond with what I have called the largest vacuoles. 
Champy was doubtful of the origin of these, but he regarded 
the rest as a series of stages in the transformation of mito-
chondria. M. and M. Parat (1930) called the largest vacuoles 
'boules lipoidiques', and derived them from the vacuoles of 
the Golgi region, with which interpretation I concur. Koehring 
(1930), in her general study of the neutral-red reaction without 
special reference to the newt, concluded that this dye is an 
indicator of the presence of proteolytic enzymes.

To find whether the material of the largest vacuoles is an 
external secretion which is passed through the free border into
the lumen of the intestine, a newt was injected with pilocarpine. This drug was chosen because it is well known to stimulate the extrusion of external secretions from cells. 0.2 c.c. of a 1 per cent. solution of pilocarpine nitrate in 0.75 per cent. sodium chloride solution was injected subcutaneously into a newt which had been kept without food for a fortnight. The injection was repeated two hours later, and the newt killed the same day. The intestine was fixed in formaldehyde solution and sectioned. The absorptive cells were not noticeably different from those of the control, which had received no injection of pilocarpine. Large vacuoles occurred in the cells of both. (The goblet cells of the intestine of the newt which had received the pilocarpine had poured out great quantities of mucus.) This experiment suggests that the substance of the large vacuoles fulfils its function, whatever that may be, within the cell itself.

The remarkable phenomenon of Chlopin's (1927) 'Krinom' was confirmed on this cell. 0.2 c.c. of 0.25 per cent. neutral red dissolved in 0.75 per cent. sodium chloride solution was injected into the peritoneal cavity of a newt, from which food had been withheld as usual. The injection was repeated the next day. The following day the newt was killed and the intestine fixed in Helly's fluid. The larger neutral-red vacuoles (not only the very large ones) were now found to stain with basic thiazine dyes and also with Ehrlich's haematoxylin (fig. 29, Pl. 2), which does not happen in a newt which has not been injected with neutral red. Why this dye should not only collect in vacuoles, but also produce inside them a substance which is readily stained by routine histological techniques, is quite obscure.

It may incidentally be remarked that the goblet or mucous cell of the intestine of the newt, at the height of mucus-production, possesses a Golgi element closely resembling that of the absorptive cell but considerably larger. The Golgi element in its most active form is shown in fig. 32, Pl. 2, which represents a Kolatchev preparation. The vacuoles, some of them lying in rows, are very clearly seen, as well as the threads of dense osmiophil substance and the diffuse osmiophil substance. The threads and the diffuse substance are shown by the formal-
sudan-black technique to contain lipoids. Like the corresponding objects in the absorptive cell, they are not sharply marked off from one another.

As Nassonov (1923) showed, the spheres of mucus originate in the vacuoles of the Golgi element of this cell. They subsequently leave this region and pass upwards through the cytoplasm to become crowded together in the goblet.

The Nerve Cell of the Anterior Mesenteric Ganglion of the Rabbit, Oryctolagus cuniculus.

Photomicrographs of this cell are shown in figs. 33 to 41 on Pl. 2. Text-fig. 5 shows the position of the Golgi element in the cell. Text-fig. 6 is a diagram showing my opinion of the structure of part of it.

The nerve cell is much the most puzzling of the three chosen for special study, because formal-sudan-black preparations give a picture which cannot at once be reconciled with the classical Golgi 'net'. The latter is shown in typical form in the Da Fano preparation, fig. 41, Pl. 2. It must be emphasized, however, that silver preparations often show different appearances in cells at different distances from the surface of the piece of tissue, unless the latter is very small. It is not unusual for the Golgi element to appear in the form of separate bits, which are often in the form of rings (see fig. 42, Pl. 2).

The formal-osmium technique is somewhat capricious with nerve cells, but the characteristic figure which it gives is vacuolar (fig. 38, Pl. 2). Sometimes rows of vacuoles are connected by osmiophil strands (fig. 39, Pl. 2), or groups of vacuoles are bunched together and provided with an investment of osmiophil substance (fig. 37, Pl. 2). Careful study of the typical Da Fano preparation (fig. 41, Pl. 2) will show an area in which the structure is exactly the same: a group of vacuoles is held together and surrounded by an argentophil substance.

The formal-sudan-black technique shows that the whole of the cytoplasm is divided into an inner sudanophil (lipoid-containing) part, and an outer part from which lipoids appear to be absent. This is shown particularly clearly in fig. 33, Pl. 2, in which the symbol lim marks the limit of the region of
Diagram of the nerve cell of the anterior mesenteric ganglion of the rabbit, *Oryctolagus cuniculus*, to show the position of the Golgi element. *lim*, limit of region of diffuse lipoid-containing substance; *n*, nucleus; *vB*, neutral-red vacuole surrounded by dense lipoid-containing substance.

Diagram illustrating the author's opinion of the structure of the Golgi element in the nerve cell of the anterior mesenteric ganglion of the rabbit, *Oryctolagus cuniculus*, as seen in a thin section. For an explanation of the conventions used, see legend to Text-fig. 2, p. 19.
diffuse lipoid, while $cm$ is the cell membrane. The intervening area is untouched by sudan black. The nucleus, being in another plane, is not shown in this photograph. The lipoid-containing part of the cytoplasm lies against the nucleus, often appearing to surround it on all sides. Within this region of diffuse lipoid, strongly sudanophil bodies are sometimes seen. The appearance is by no means always given, and I have found no means of reducing the capriciousness of the technique when applied to this object. The strongly sudanophil bodies are sometimes seen as rings, sometimes as crescents, and sometimes as 'granules'. They are shown in figs. 33 to 37, Pl. 2. In fig. 35, Pl. 2, they appear mostly as rings, while fig. 34, Pl. 2, shows rings and a crescent, the latter being particularly clear, because it lies above the completely non-sudanophil nucleus. Since the rings are all approximately circular, there is no possibility that the structure is actually ring-shaped. Real rings distributed at random in space would appear as circles, ovals of various shapes, and rods. Such rings do occur in the Golgi element of the skin of the snail. Here, in the nerve cell of the rabbit, they do not. The apparent rings are optical sections of the thick skins of spherical vacuoles. The vacuoles, which are not themselves sudanophil, have intensely sudanophil skins, which are not uniformly thick all over. When the departure from uniform thickness is particularly marked, the appearance is of a crescent. When the skin is particularly thick in relation to the size of the vacuole, or when the centre of the vacuole is not focused, the appearance is 'granular'. The real structure can be made out by careful focusing.

Fig. 37, Pl. 2, is particularly interesting. The vacuoles are here seen to be crowded into groups, with an intensely sudanophil substance in between and all round. The appearance here is the same as that shown in parts of the formal-osmium and Da Fano preparations (figs. 40 and 41, Pl. 2, respectively). Text-fig. 6 is a diagram showing my opinion of the structure of the Golgi element in this nerve cell. It consists of a region of diffuse lipoid, partly or quite surrounding the nucleus, and containing within it non-lipoid vacuoles. Some of these are quite separate. They have an investment of dense lipoid-
containing substance, which is not uniform in thickness and appears sometimes not to cover them completely. I have thought it right to show in the diagram groups of vacuoles held together by dense lipoid-containing substance. I do not know whether this condition occurs in life, but it seems significant that it is sometimes shown by the formal-sudan, formal-osmium, and Da Fano techniques.

The view that the Golgi element of the nerve cell is vacuolar is consistent with the vital observations of Covell and Scott (1928). These authors could find nothing in the living nerve cell resembling the classical Golgi net. They found only vacuoles which stained with neutral red. These vacuoles had a tendency to be arranged in rows, a tendency which was greatly accentuated by fixation. The vacuoles reduced both osmium tetroxide and silver nitrate. Figs. 11 and 14 of these authors suggest strongly that it is the surface of the vacuole that is the reducer.

The classical Golgi network of the nerve cells of Vertebrates is presumably produced as follows. The vacuoles arrange themselves in rows or groups under the influence of the fixative. The dense lipoid substance which invests them runs together to form strings where the vacuoles were in rows, or a meshwork when they were in groups. The osmium tetroxide or silver nitrate is then reduced on the dense lipoid substance. The diffuse lipoid substance is not shown unless impregnation is carried so far as to obscure the classical image. The dense lipoid investment of large, separate vacuoles is sometimes impregnated in the form of large, separate rings. These are genuinely ring-shaped structures, but they are artifacts and do not indicate that the dense lipoid substance was present in the form of a ring during life. It was then a complete, though irregular, investment of the vacuole.

**Conclusion and Discussion.**

The Golgi elements of the three cells carefully studied have a good deal in common, despite their apparent differences. In conjunction with what is known of the Golgi element in other cells, they provide the basis for a general theory of the
fully developed Golgi element. A diagram consistent with this theory is shown in Text-fig. 7. According to this theory, the fully developed Golgi element consists of four parts:

(1) The 'neutral-red vacuoles'.

(2) The dense lipoid-containing substance.

(3) The diffuse lipoid-containing substance.

(4) The Golgi-product.

The neutral-red vacuoles are spherical and vary in size in the same cell. They do not contain sufficient protein to produce the appearance of a 'granule' in a routine histological preparation, and they are usually destroyed by precipitant fixatives. Some authors have objected to the word vacuole, but it is
useful for its antithesis to 'granule'. The substance of the vacuoles is fluid, presumably watery, and the colour given by neutral red shows that it is acid. The vacuoles have a very great power of segregating high concentrations of certain dyes, particularly neutral red, from dilute solutions during the life of the cell. The synthetic product of the Golgi element appears within the neutral-red vacuoles.

The dense lipoid-containing substance is intensely sudanophil and osmiophil and generally argentophil. It is usually situated in close relation to the neutral-red vacuoles. It appears in different forms in different kinds of cells and sometimes in the same cell. The different forms, some of which are probably artificial, are the following:

1. A complete investment to two or more vacuoles.
2. A partial investment to two or more vacuoles. The substance then appears as irregular strands running from vacuole to vacuole and spreading out over parts of their surfaces.
3. A complete investment to separate vacuoles.
4. A partial investment or cap to separate vacuoles, giving the appearance of a crescent in optical section.
5. A crescent-shaped rod on the surface of a vacuole.
6. A ring passing right round a vacuole.
7. A curved rod with a vacuole attached near its centre.
8. A rod, often curved, not closely related to a vacuole.

All these different forms are shown in Text-fig. 7, with corresponding numbers.

It is possible but not proved that the whole surface of the vacuole is always covered by at least a thin layer of the dense lipoid-containing substance.

The diffuse lipoid-containing substance fills the whole of the region occupied by the Golgi element, except the parts occupied by the other constituents. The substance is osmiophil as well as sudanophil, but in a much less degree than the dense lipoid-containing substance. The diffuse lipoid-containing substance is often absent from the cells of Invertebrates and sometimes from those of Vertebrates. The Golgi element then consists of vacuoles invested with dense lipoid-containing substance, the
invested vacuoles being either separate or joined into small groups by the investment. A Golgi element of this kind has been particularly clearly described by Worley (1943), who studied living cells in the embryos and veligers of Molluscs.

The Golgi product arises in the neutral-red vacuoles. When fully formed, it leaves the Golgi zone, with part of the dense lipoid-containing substance at first adhering to its surface. It is sometimes not formed until the cell has undergone division. For instance, the vacuoles of primary spermatocytes form no product until they have become the vacuoles of the spermatid, when they produce the acrosome of the spermatozoon. The Golgi product may either remain within the cell (e.g. acrosome, Golgi yolk, nematocyst) or be cast out (extra-cellular enzymes, fat of sebaceous and oil-glands, mucus, bile, &c.). The chemical composition of the Golgi product is extremely diverse in different cells. Sometimes no visible Golgi product appears within the neutral-red vacuoles (e.g. nerve cells).

Although the method of functioning of the Golgi element is not the subject of this paper, it may be pointed out that just as the cytoplasm maintains a special chemical composition by the selective action of a lipoid-containing membrane, so also the neutral-red vacuoles, in which the synthetic product of the Golgi element arises, are surrounded by a lipoid-containing substance. It is, however, much thicker than the ordinary cell-membrane. (See p. 64.)

The dense and diffuse lipoid-containing substances together form the ‘Externum’ of Hirschler and of Hirsch (1939). I do not understand why the latter, in his lucid and extremely valuable book on the Golgi element, does not separate the Externum into its parts. No student of the male germ-cells of the snail could fail to do so, and the distinction is often clear enough. It must be admitted, however, that in many Vertebrate cells, other than nerve cells, the distinction is not sharp. As we have seen, there is not a sharp distinction in the Golgi element of the absorptive intestinal epithelial cell of the newt.

Some authors have felt very strongly that the word ‘Golgi’ should apply only to the osmiophil or argentophil part of the
object, because Golgi used a precipitant fixative and did not see the vacuoles. As we have seen, the net-like appearance of the classical Golgi 'apparatus' of the nerve cell is probably an artifact. The Golgi net of other cells is often an artifact produced in another way. The neutral-red vacuoles are shattered by the precipitant fixative and the lipoid-containing substances are left as a meshwork, which is impregnated by osmium hydroxide or silver. In the living cell the vacuoles are an essential constituent of the fully developed Golgi element. In many Vertebrate cells, the Golgi element would be almost structureless without them. No one would describe a mass of putty containing rubber balls as a 'network', or insist that the name applied to the whole mass should refer to the putty only. If the name of Golgi be applied to the lipoid-containing substances only, a new name must be invented to cover the whole object, which would surely be undesirable. It is not worth while to argue at length over words, but I suggest that the convenience of calling the whole object the Golgi element outweighs any disadvantage.

In cells which are not functioning actively, and particularly in reserve cells set aside to replace functioning cells, vacuoles sometimes either do not occur or else are so small that their presence cannot be demonstrated. The Golgi element then seems to consist of a mass of lipoid-containing substance, which Hirsch calls the 'Praesubstanz.' There is not yet a differentiation into dense and diffuse substances, and the product is naturally absent in such cases. The Golgi element in such cases is small. Good examples of this condition are provided by the spermatagonia of the snail and by some of the cells of the 'reserves' of the intestinal epithelium of the newt.

When the Golgi element of a cell which has been resting awakes to activity, neutral-red vacuoles appear and swell among the lipoid-containing substance, which differentiates into a diffuse part and into denser structures generally closely associated with the vacuoles. The whole object grows. Finally, the product is synthesized within the vacuoles and moves out of the Golgi region. This evolution, as we have seen, is not always completed without the intervening occurrence of one or
more cell-divisions. The behaviour of the Golgi element at cell-division is not considered in this paper.

The investigation described in this paper in one way supports and in another contradicts the view of the Golgi element put forward by the French cytologist, M. Parat, in his *magnum opus*, 'Contribution à l'étude morphologique et physiologique du cytoplasme' (1928), and upheld in several lesser works. Although, of course, Parat was not the first to study neutral-red vacuoles, yet he did more than any other single person to show how they are associated with the zone of Golgi. This was an important step forward. Parat, however, went farther. He was inclined to regard the classical 'Golgi apparatus' as wholly an artifact, caused by the deposition of silver or osmium hydroxide on the surface of vacuoles, that deposition representing nothing pre-existent in the living cell (except when sometimes it showed the vacuoles as such). It is true that silver nitrate and osmium tetroxide can be reduced on surfaces of various kinds, and the resulting black particles can therefore suggest the presence of non-existent objects apparently lying near those surfaces. This criticism, however, can scarcely be levelled against a dye, and it is quite impossible to level it against a substance which acts solely by solution in pre-existent substances. That is the great merit of sudan black for studies on structure: it is scarcely possible that this colouring agent could produce an artifact (although of course it could reveal one produced by a fixative). The formal-sudan-black technique removes all possibility that the osmiophil or argentophil substance is simply non-existent in the Golgi element.

Parat always allowed the existence of osmiophil (argentophil) substances in the male germ-cells of the snail, and he used the word lepidosomes for the structures formed of this substance. He also allowed that what he called the 'chondriome actif' or 'chondriome périvacuolaire' was sometimes a part of the Golgi element in other cells (though he avoided the expression Golgi element). The lepidosomes and 'chondriome actif' are both included under what I have called the dense lipoid-containing substance. Parat did well to point out the general resemblance in substance between the mitochondria and the 'chondriome
actif'; but the name was unfortunate in view of the absence of proof (or even likelihood) that the structures named are derived from mitochondria. Parat was well acquainted with the 'lipoides diffus périvacuolaires', and it seems probable that he would have been in general agreement with the theory of the structure of the Golgi element presented in this paper. It is a pleasure to acknowledge my indebtedness to the work of this great cytologist, whose early death was such a misfortune to the science. His views were often attacked with vigour by those who had not taken the trouble to understand them.

PART 2. CHEMICAL COMPOSITION.

INTRODUCTION.

The object of the work described in this second part of the paper has been to find the chemical composition of the lipoid-containing or osmiophil part of the Golgi element. The composition of the vacuoles has not been studied. Attention has been concentrated chiefly on the dense lipoid-containing substance, because it gives stronger histochemical reactions than the diffuse.

Nearly the whole of the chemical work has been done on the dense lipoid-containing substance constituting the lepidosomes of the spermatocytes and young spermatids of the common snail, Helix aspersa. These cells were chosen for special study because the lepidosomes are visible during life, without the use of any dye or other cytological reagent. In investigating the chemical composition of these bodies one is relieved of any doubt that one may be studying the composition of an artifact. The lepidosomes are regarded by some authorities as banana- or thread-shaped objects, and by others as rims round spherical or lens-shaped vacuoles (see p. 18), but their existence is not disputed by anyone.

In order to possess reliable evidence as to the chemical composition of the lipoid-containing substance of the Golgi element, it would be desirable to isolate it as mitochondria have been isolated by Bensley and Hoerr (1934), by Claude (1941) and by Chargaff (1942), and subject it to direct chemical analysis.
Since no one has succeeded in isolating it, the guidance of three lines of evidence is required:

(1) The substance should give a positive reaction to a reliable histochemical test.

(2) It should resemble in solubility the substance or group of substances suggested by the histochemical test.

(3) The substance or group of substances suggested by the histochemical test should react similarly to the lipoid-containing substance when subjected to the standard techniques for the Golgi element.

Evidence of these three kinds is presented in this paper.

It is necessary to state the exact meanings attached to certain words used in this paper, for unfortunately the nomenclature of fatty and fat-like substances is confused. Some authors use the word lipoid to mean all substances soluble in ordinary fat-solvents, others use it to mean conjugated fats (phosphatides, &c.), and others again to mean those substances which resemble fats in solubility but do not contain a fatty acid radicle. The last-named is the meaning which the inventor of the word, Kletzinski, attached to it, and some of the best modern authorities adhere to this usage. There is a great convenience, however, in having a single word to include all substances which occur in plants and animals and are extracted by fat-solvents. Overton (1901) re-introduced the word lipoid with this meaning, and he has been followed by many biochemists including the writers of well-known text-books (Hammarsten and Hedin, for example); and no other word has been so frequently used for the same purpose. This is the usage which I follow in this paper, on account of its practical convenience. Thus used, the word lipoid has a physical but not a chemical meaning. I use lipide to cover all substances containing a fatty acid radicle, and lipine for those lipides which contain either nitrogen or phosphorus or both these elements. The words lecithin, cephalin, and sphingomyelin are used as group-names for the substances classified under these heads.

Critique of Previous Studies.

As Owens and Bensley (1929) particularly have insisted, the
mere capacity to reduce osmium tetroxide under certain conditions gives no reliable histochemical information; and the resistance of the reduced substance to extraction by turpentine does not by itself provide evidence on which histochemical identification can be based. The fact that the Golgi element can be seen in paraffin sections made by routine methods proves nothing about chemical composition. That which resists extraction during embedding and after-treatment may be merely the silver or osmium hydroxide. The action of turpentine on osmicated lipoids has not been studied from the chemical standpoint. It is not known whether it acts by dissolving lipoid, or only by oxidizing osmium hydroxide to tetroxide. Silver nitrate, again, can be reduced by the most varied agencies. The standard methods for the Golgi element are not histochemical tests, though any substance that is thought likely on strictly histochemical grounds to exist in the Golgi element should be subjected to the standard Golgi techniques by way of comparison, since a negative result would suggest an error in identification.

The suggestion that the Golgi element contains or consists of lipoid did not arise from strictly histochemical studies. It was simply a conclusion drawn from the fact that fixatives containing lipoid-solvents do not preserve the Golgi element. Sudan III and IV do not usually colour it when applied in the usual way to formaldehyde-fixed frozen sections. Ma and Chang (1928) succeeded in colouring the Golgi element of living cells by relying on the solubility of sudan III in a saturated solution of neutral red. It is probable that the sudan III helped in the colouring, but it is unfortunate that its colour is not more different from that of neutral red. Boyle (1937) showed that the chromophobe part of the Golgi element in the neurones of Helix can be coloured by sudan IV in frozen sections of material treated by Aoyama's technique.

Nusbaum stated so far back as 1913 that the main component of the Golgi element is a lecithin-like compound, but he gave no evidence for this conclusion beyond the fact that fixatives that do not preserve lecithin do not preserve the Golgi element. A number of cytologists (Karpova, 1925; Weiner, 1926; Parat,
1928; Subramaniam, 1939) have based conclusions about the chemical composition of the Golgi element on the results of Ciaccio’s test. Ciaccio himself (1910) demonstrated what is almost certainly the Golgi element in mammalian spermatids by the use of this method. His test, however, has been proved unreliable by the detailed investigation of Kaufmann and Lehmann (1928), who showed conclusively that a positive result is given by various unsaturated lipoids, and that it is not specific for any particular group or groups of such substances; though a positive reaction indicates the presence of a lipoid in the wide sense. As Lison (1936) says of Ciaccio’s test, ‘rien n’est moins certain qu’elle soit spécifique’ for the particular groups of lipoids mentioned by Ciaccio.

Other workers have used the Smith-Dietrich test, and their studies are much more worthy of critical consideration. It will be recalled that the test is carried out as follows. The tissue is fixed with formaldehyde and frozen sections prepared. These are treated with hot potassium dichromate solution, to oxidize the lipines and mordant them. They are then stained with warm Kultschitzky’s haematoxylin, and finally differentiated ‘overnight’ with Weigert’s alkaline ferricyanide solution, the purpose of which is to remove any haematoxylin not firmly held by the mordant. In their extremely detailed studies, Kaufmann and Lehmann (1926 a and b) showed that sphingo-myelin (alone or mixed) and lecithin mixed with other lipoids gave a positive (black) reaction with this test. Lecithin alone usually did not. Galactolipines gave a grey-black reaction. Thus the lipines tested gave on the whole positive results, while a very large number of other substances that were tested all gave negative results. There is accordingly justification for saying that a positive result with the Smith-Dietrich test indicates the presence of a lipine. It must be mentioned, however, that Kaufmann and Lehmann did not test cephalin.

The Smith-Dietrich test was applied to the Golgi element by Karpova (1925) and by Parat (1926). Parat used a special modification of the test, which he described in a separate communication (1926). It is important to note that Kaufmann and Lehmann’s elaborate studies were devoted to the standard
Smith-Dietrich test, not to Parat's modification of it, which involves the use of Helly's fluid as fixative instead of formaldehyde alone. There is no proof that Parat's modification is specific for lipines. He himself did not so regard it, but considered that it should be controlled by Ciaccio's test.

Karpova (1925) announced that the osmiophil part of the Golgi element in the male germ cells of *Helix pomatia* gives a positive reaction with the Smith-Dietrich test, and this has been the most concrete evidence available that the Golgi element may contain lipines. Unfortunately she did not state how long she differentiated in Weigert's ferricyanide solution. In the standard Smith-Dietrich test the period of differentiation is 'über Nacht' (Dietrich, 1910): this is confirmed by Lison (1936). This period may be taken to be 15 hours. Now if one applies the Smith-Dietrich test to the ovotestis of *Helix aspersa*, differentiating for 15 hours, the Golgi element of the male germ-cells gives no trace of a positive reaction. This fact can be proved by anyone who will try it, and it is very unlikely that *Helix pomatia* differs in this respect. When the section has been stained by Kultschitsky's haematoxylin, every part of every cell is black; and if one were to make a personal decision as to when to stop differentiating, one could decide that any chosen part gave a positive reaction.

Tanaka (1932) obtained a blue coloration of the Golgi element with Nile blue in living tissue-culture cells. A blue reaction with this dye provides no histochemical information (Lison, 1936). Tarao (1939) finds that the Golgi element of the liver cells of the mouse, after the action of digestive enzymes, is coloured 'blue with red tone' by Nile blue. This is suggestive, though blue with red tone can hardly be regarded as a positive reaction with Nile blue. A positive (red) reaction would have suggested the presence of an unsaturated triglyceride (see Lison, 1936).

Walker and Allen (1927) mixed lecithin and cephalin with proteins, smeared the mixtures on glass slides, and treated them by the Weigl (Mann-Kopsch) technique. On microscopical examination they found appearances resembling the Golgi element. Instead of drawing the conclusion that the
latter may be formed of lipine, they suggested that there is in fact no such thing as the Golgi element: that it is an artifact produced by the fixative, which separates the proteins from the water of the cytoplasm, allowing the lipines—previously homogeneously dispersed—to become spread over the protein-water interface. This conclusion was effectively disputed by Ludford (1928) on several grounds. In particular, the Golgi element does not appear at random anywhere in the cytoplasm, but has a characteristic position in each kind of cell. It is shown by widely different techniques to occupy these characteristic positions.

Tennent, Gardiner, and Smith (1931) extracted the lipoids from the ovaries of the sea-urchin, *Echinometra*, and sought to find which of them resembled the Golgi element in reaction to the standard Golgi techniques. The work was very carefully done and of great interest, but it is unfortunate that they chose cells so loaded with lipoids that the Golgi element itself does not seem to have been recognized with certainty. They concluded that as free fatty acids give a black reaction with osmium tetroxide and the black material is resistant to extraction with turpentine, the Golgi element consists of fatty acid. They regarded it as an evanescent cell-inclusion, only present when fats are being synthesized or broken down. If the Golgi element in fact consisted of free fatty acids, one would expect to be able to extract them from all kinds of tissues in considerable amounts, which is not so. Free fatty acids scarcely occur in the tissues of most animals, except in the intestine and liver (Lovern, 1942). A study of Table 17 in Tennent, Gardiner, and Smith's paper shows that cephalin gives with osmium tetroxide a 'black and grey' material which still appears black and grey after soaking in turpentine, so that this property is not confined to free fatty acids.

Monné (1939) found that the Golgi element of the spermatocytes and spermatids of two species of snails (*Helix*) is scarcely or not at all doubly refractive. Certain experimental procedures, however, particularly treatment with chrysoidin, produce birefringence in the Golgi element.

The suggestion that the Golgi element contains protein does
not rest on strictly histochemical evidence. It is perhaps unlikely that any part of the cell is totally lacking in proteins, but histochemical tests for them have not been shown to give positive reactions in the Golgi element. Nath (1925) recorded that the Golgi element of the spermatocytes of Lithobius is not completely destroyed even by Gilson's or Bouin's fluid, which suggests (without proving) the presence of proteins in this particular case. Holmgren long ago used Weigert's resorcin-fuchsin to show his 'Trophospongium', after treatment of the tissues with a fixative that does not preserve lipoids. In 1904 Bergen confirmed that the 'Netzapparat' of certain cells can be stained by resorcin-fuchsin ('Fuchselin'), and also in some cases by iron haematoxylin. Weigl (1910) confirmed the resorcin-fuchsin effect. Among other dyes, aniline blue (Kirkman, 1937) and nigrosin (see first part of this paper) have been shown to stain the Golgi element in certain cells. It is certainly interesting that the dyes which are sometimes effective for this purpose are those which are used to demonstrate protein fibres, namely resorcin-fuchsin (for elastin) and aniline blue and nigrosin (for collagen). These dyes, however, especially the last two, are not specific and cannot give reliable histochemical information.

Bourne (1934 and 1935) and later workers have brought forward strong evidence that ascorbic acid is associated with the Golgi element in certain cells, but it is not suggested that the osmiophil substance is ascorbic acid.

Horning (1933) has called it a 'fact' that the Golgi element is a protein substance compounded with a lipin'. He quotes two papers, by Harvey (1925) and Cowdry (1918), as his authorities for this statement. Neither of these papers contains evidence for the opinion that the Golgi element is a protein substance compounded with a lipine.

This review of existing knowledge shows that there has been no reliable information about the chemical composition of the Golgi element, beyond the fact that it contains a lipoid (in the widest sense of that word).
HISTOCHEMICAL COLOUR-TESTS.

The Smith-Dietrich Test.

This test for lipines has shown itself to be more useful than any other colour-test in throwing light upon the chemical composition of the Golgi element. It may be recalled that the principle of the test is as follows. The tissue is fixed in formaldehyde solution (formal-calcium in my experiments) and then treated with hot 5 per cent. potassium dichromate solution. Smith (1909) used temperatures varying from 37° to 65° C., while Dietrich (1910) used 37° to 40° C. Kaufmann and Lehmann (1926) showed that the temperature should be 60° C., for if it be less, negative results are often given. The lipines are oxidized by the potassium dichromate and form a complex with the chromium. Frozen sections are made and stained with Kultschitzky’s haematoxylin, no mordant being added. (To avoid waiting for six months for the haematoxylin to ripen, I add 0·2 per cent. of sodium iodate, and thus have a solution ready for immediate use.) The haematoxylin unites with the chromium held by the lipines to form a resistant black lake. The slides are soaked in Weigert’s differentiator (potassium ferriyanide with borax), which oxidizes away all the haematoxylin except that held in combination with chrome. Differentiation proceeds ‘overnight’. Whatever is still black is considered to consist of or contain lipine.

The exact technique used in this investigation was as follows. Fix the tissue in formal-calcium, embed in gelatine, section on the freezing microtome at 10 or 15 μ, and attach the sections to slides in the way described on p. 13. Place the slide in a slide-jar containing 5 per cent. potassium dichromate solution. Put the jar in a paraffin oven maintained at 60° C. (not the usual 57° C.). (Do not put the slide straight into a hot solution, or the section may come loose. It is best to stand the slide-jar on a wooden block, so that the solution will warm slowly.) Leave for 48 hours. Raise the slide occasionally during the first few hours to free air bubbles. Cool. Wash for 10 minutes in several changes of distilled water. Place the slide in Kultschitzky’s haematoxylin (ripened by the addition of 0·2 per
cent. sodium iodate), already standing in a slide-jar in an incubator at 37° C. Leave it for 5 hours. Wash in distilled water. (It is generally convenient to leave the slide overnight in distilled water at this stage, to avoid night-work. If so, one may leave it in the refrigerator.) Differentiate for 15 hours in Weigert’s borax and potassium ferricyanide. Wash in running water for 5 minutes. Mount in glycerine jelly. Lipines, black.

Under special circumstances the period of differentiation may be reduced to 8 hours (see p. 52).

Lecithin and sphingomyelin have already been subjected to the Smith-Dietrich test, by Kaufmann and Lehmann (1926 a and b), but cephalin has not and it was therefore necessary to test it. It was prepared from the brains of sheep by Weil’s method (1930), and emulsified at 37° C. in the proportion of 0.2 gm. of lipine to 1 c.c. of distilled water. (In some of the experiments blood-plasma was substituted for water, so as to surround the cephalin particles with protein.)

Elder pith was boiled in absolute alcohol to free it from lipoids and cut into cubes of side about 3 mm. The pith cubes were pierced with a needle to allow the emulsions to enter. They were soaked in the emulsions for 4 hours at 37° C., the pressure being repeatedly reduced by a vacuum hand-pump to help to draw the emulsions into the pith-cells.

The pith cubes containing the emulsions were then fixed for 24 hours in formal-calcium (see p. 7). The calcium salt prevents the lipine from being lost by colloid solution before the formaldehyde has exerted its effect. The cubes were treated exactly as though they were pieces of tissue, and sections were subjected to the Smith-Dietrich test. The period of differentiation was 15 hours.

Cephalin gave a positive (black) reaction to this test. Sphingomyelin (also prepared by Weil’s method) was tested at the same time, and the positive reaction obtained confirms

1 The blood-plasma of the rabbit was usually used in the experiments described in this paper. That of the cock was sometimes used instead.

2 The cephalin prepared by this method was probably very pure, while the sphingomyelin may have been contaminated to some extent with galactolipines.
Kaufmann and Lehmann's finding. Lecithin alone, as these authors found, does not respond satisfactorily to the test. I think the cause is that treatment with formaldehyde does not prevent it from disintegrating when the sections are placed in water. When other lipoids (themselves negative to the test) are present as well, lecithin gives a positive reaction, as Kaufmann and Lehmann showed.

Cephalin and sphingomyelin, then, respond positively to the Smith-Dietrich test, and so does lecithin when other lipoids are also present. Galactolipines give a grey-black reaction. Since Kaufmann and Lehmann tried a very large number of lipoids other than lipines, pure substances as well as mixtures, and got negative results with all of them, a positive result with the Smith-Dietrich test is strong evidence of the presence of a lipine. Since galactolipines do not occur in significant amounts outside nervous tissue, a positive result with the Smith-Dietrich test in non-nervous tissue may be taken as indicating the presence of lecithin, cephalin, or sphingomyelin, or of a mixture of these substances.

Details of Histochemical Technique.

Except where the contrary is stated, the histochemical colour-tests were all carried out on ovotestes fixed in formal-calcium, embedded in gelatine, and sectioned on the freezing microtome; and the sections were attached to slides in the way described on p. 13. They were carried out as follows.

Sudan Black for Lipoids.—This technique has already been described in detail (p. 12).

Sudan IV for Lipoids.—This colouring agent is very much weaker in its action on lepidosomes than sudan black. It was only used as a confirmation of the result given by sudan black. A special technique was devised to change its partition between the vehicle and the lepidosomes in favour of the latter. This was done as follows. Saturate 70 per cent. alcohol thoroughly with sudan IV at $37^\circ$ C. (Leave the bottle in the incubator for some days with excess of the colouring matter, shaking occasionally.) Put two clock-glasses in the incubator, and allow them to warm up. Warm the slide by dipping it in some 50 per cent.
alcohol already in the incubator, and then put it face down in one of the clock-glasses. Add sufficient of the solution of sudan IV to reach the section, and cover with the other glass. Leave for 5 minutes. Now transfer the clock-glasses and their contents to a refrigerator maintained at about 0° C. and leave for about 2 hours. The solubility of the colour in the alcohol is reduced, and it seems to be forced into the lepidosomes. Dip quickly in two lots of 50 per cent. alcohol at about 0° C. and then put in distilled water at the same temperature. Mount in laevulose.

Nile Blue.—Make a saturated solution of Nile blue (sulphate) in distilled water. To 100 c.c. add 2.5 c.c. of strong sulphuric acid. Boil for 2 minutes. Cool. Leave the slide in this solution for 8 minutes. Differentiate for 30 seconds in 1 per cent. acetic acid. Wash thoroughly in distilled water. Mount in glycerine jelly. A red reaction indicates the presence of olein. For a critique of the Nile blue reaction, see Lison (1936). (One must avoid being misled by a metachromatic staining of mucin.)

Windaus's Method for Free Cholesterol (modified for histological purposes).—Leave the slide for 4 hours in a \( \frac{1}{2} \) per cent. solution of digitonine in 50 per cent. alcohol. Rinse in 50 per cent. alcohol. Mount in glycerine jelly. Examine between crossed nicols. Cholesterol-digitonine appears as birefringent needles and rosettes.

Schultze's Modification of Liebermann's Method for Cholesterol and its Esters.—Place the slide in a petri dish of distilled water and expose to the sky on a flat roof for 9 days in sunny weather (to oxidize the cholesterol). Dry thoroughly with filter paper. Add two drops of a mixture of equal volumes of acetic and sulphuric acids (both water-free). Examine in the reagent. Cholesterol and its esters, blue to purple, changing to green.

Aldehyde Test for Tryptophane.—Experiment has shown me that a modification of Raspail's century-old reaction (1828) gives a stronger colour in cytological work than when formaldehyde or vanillin is used instead of sucrose. My technique is as follows: Dissolve 18 gm. of sucrose in 10 c.c. of
distilled water. Dry round the section carefully with a duster. Add 2 drops of the sucrose solution. Add one drop of 10 per cent. mercuric sulphate solution. Leave for 10 minutes. Rinse momentarily in distilled water. Dry rapidly round the section again. Add 1 drop of concentrated sulphuric acid. Cover. Tryptophane only gives a purple appearance when in massive form. Small objects appear yellowish brown or brown. (See Dippel, 1882.) This is presumably a dichromatic effect. The aldehyde concerned in this test is probably hydroxymethyl-furfural, produced by the action of the sulphuric acid on the sugar.

**Millon’s Test for Tyrosine.**—Leave the slide in the reagent (Bensley and Gersch’s formula (1938)) for a few hours at 57° C., and examine without replacement of the fluid.

**Xanthoproteic Test.**—Dry round the section with a duster. Cover it with concentrated nitric acid for a few minutes. Rinse off and dry again. Add a few drops of concentrated solution of ammonia. Cover. Tyrosine, tryptophane, and phenylalanine, yellow to orange.

**Sakaguchi’s Test for Arginine.**—The test was adapted to cytological work as follows: Flood the slide with 2 c.c. of 1 per cent. sodium hydroxide solution. Add 2 drops of a 1 per cent. solution of α-naphthol in strong alcohol (e.g. 95 per cent.). Do not drop the alcohol directly over the section. Add 1 drop of 10 per cent. sodium hypochlorite. Rock the slide to mix the fluids. Leave until colour develops. Pour off most of the fluid. Cover. Arginine, pink.

**Joyet-Lavergne’s Test for Glutathione.**—Cover the section with 10 per cent. potassium cyanide solution (to have all the glutathione in the reduced form). Rinse in distilled water. Add 1 drop of freshly-made 5 per cent. sodium nitro-ferricyanide solution and then a drop of a concentrated aqueous solution of ammonia. Cover. Glutathione and related substances, red or red-violet. The same test was also applied to part of an ovotestis freshly snipped up on a glass microscopical slide, the rinsing in water being omitted to avoid washing away the cells.

**Bourne’s Test for Ascorbic Acid** (see Barnet and
Bourne, 1941).—Fix for 30-45 minutes in a 10 per cent. solution of silver nitrate in 10 per cent. acetic acid. Wash thoroughly in distilled water. Dehydrate through the alcohols. (Carry out all these processes with the tissue in a bottle made of brown glass, to prevent reduction of the silver salt by light.) Embed in paraffin. Cut sections at 5 μ and mount them in balsam. Ascorbic acid, black.

The Response of the Golgi Element to Histochemical Colour-tests.

That the Golgi element of diverse cells contains lipoid is shown by the fact that it takes up sudan black, as was recorded in the first part of this paper. This reaction is regarded by Lison (1936) as proof of the presence of lipoid. The rest of the histochemical work was done on the lepidosomes of the spermatoocytes and young spermatids of Helix aspersa.

The strong reaction of the lepidosomes with sudan black is illustrated in Fig. 6, Pl. 1. They are not coloured by sudan IV when it is used in the ordinary way, but they are weakly (though definitely) coloured when it is used as described on p. 48. The failure of sudan IV to colour the lepidosomes when used in the ordinary way suggests the absence of triglycerides. Their absence is confirmed by the lack of any red tinge with Nile blue, for triglycerides generally include some unsaturated fatty acid radicles in animal cells.

The Smith-Dietrich test, as was stressed above, is of exceptional importance to the student of the Golgi element. The one trouble about it is that differentiation must proceed for the unstated period described as ‘overnight’ (Dietrich, 1910). This may be taken to mean about 15 or 16 hours. Now the test is commonly applied to the myelin of nerves, which is generally very thick in comparison with the thickness of a lepidosome of the Golgi element. An ordinary large Mammalian nerve, held at right angles to the line of vision, presents in the middle a thickness of about 10 μ of myelin (5 μ on each side of the axon). A lepidosome in the primary spermatoocyte of the snail, however, is only about 0.4 μ thick. The discrepancy in size has two effects. In the first place, the differentiator attacks the lepidosome at
once from all sides, while large parts of the myelin sheath of
a nerve are protected from immediate attack by the thickness
of the substance. Secondly, 25 lepidosomes would have to be
placed one behind another to give the thickness of the myelin
in a single large Mammalian nerve, and if they were each only
a pale grey the effect would then be blackness; but each single
lepidosome, viewed separately, would have to be regarded as
giving a negative result.

If differentiation be allowed to proceed for 15 or 16 hours, the
lepidosomes for these reasons give a negative reaction to the
Smith-Dietrich test. Nothing in the germ-cells gives a positive
reaction except the relatively thick tails of the spermatozoa.
I have therefore arbitrarily chosen 8 hours as the period of
differentiation, and it seems very probable that any minute
object which appears black or dark grey at the end of this time
consists of or contains lipine. It must be stated, however, that
proof cannot be regarded as complete until some one has under-
taken an investigation similar to that of Kaufmann and Leh-
mann, using 8 hours as the period of differentiation. Unfortu-
nately these authors did not say what period they used, and it
must be supposed that it was 'overnight'.

When the period of differentiation is restricted to 8 hours,
the Smith-Dietrich test sometimes gives a strongly positive
reaction for the lepidosomes of the spermatid and a less strong
positive reaction for those of the spermatocytes. It also
gives a positive reaction for the diffuse lipoid substance of the
Golgi element of the spermatid, and sometimes also for that of
the spermatocyte, but the reaction is much less intense in the
region of the diffuse lipoid than in the lepidosomes. The Smith-
Dietrich reaction is illustrated in figs. 11, 13, and 14, Pl. 1. These
figures provide strong evidence that the lepidosomes, and
probably the diffuse lipoid-containing substance also, contain
lipine. This evidence should be viewed in the light of the
critique of the Smith-Dietrich test given on p. 46.

It is interesting to notice that during spermatogenesis the
mitochondria, like the Golgi element, show a progressive
increase in the intensity of their response to the Smith-Dietrich
test. They give a negative reaction in the spermatocyte,
positive in the early spermatid, and a progressively more and more intense reaction as the spermatid develops, until finally they mass together to produce the sheath of the tail of the spermatozoon, which remains black even after Weigert's differentiator has acted for 16 hours.

Both Windaus's method for cholesterol and Schultze's for that substance and its esters give negative results. The lepidosomes also have no yellow or red colour which would suggest the presence of a carotene or allied pigment. As we have seen, they appear a very pale grey. This is perhaps slightly intensified by the addition of concentrated sulphuric acid to the formal-fixed section, but there is no black reaction suggestive of carotene.

The cytoplasm of the spermatocytes and young spermatids of the snail was coloured by the aldehyde test for tryptophane, by Millon's test, and by the xanthoproteic test, though the colour was shifted away from purple, red, and orange towards yellow (presumably a dichromatic effect). The lepidosomes were in no case more strongly coloured than the surrounding cytoplasm. They were clearly seen after the application of the aldehyde and xanthoproteic tests, but only glimpsed after Millon's. The conclusion is the negative one that the lepidosomes do not contain an appreciably higher proportion of tryptophane, tyrosine, or phenylalanine than the cytoplasm.

Sakaguchi's test for arginine was strongly positive for the nuclei of the spermatozoa, faintly for other nuclei, and negative for the cytoplasm and lepidosomes.

Joyet-Lavergne's test for glutathione was negative, both in sections and on fresh material. The freshly snipped ovotestis gave a macroscopically positive reaction, indicating the presence of glutathione or a related substance in the organ, but microscopical examination failed to localize it in any part of the spermatocytes or spermatids.

Bourne's test for ascorbic acid was negative for the lepidosomes. It produced black particles in the acrosomes of the spermatozoa, and also gave a positive reaction for what appeared to be the osmiophil part of the Golgi element of the oocytes. (It was also positive in the Golgi element of the
mucous cells of the intestine of the newt. The black particles appeared to indicate the position of the vacuoles. It was negative in the Golgi element of the absorptive cells.)

The only conclusion that can be drawn from the histochemical colour tests is that there is strong evidence, not in itself amounting to proof, that the lepidosomes of the male germ-cells of *Helix aspersa* contain or consist of lecithin and/or cephalin and/or sphingomyelin. The next section of this paper is concerned with the light thrown on this conclusion by tests of solubility.

**Solubility Tests.**

**General Remarks.**

It would be easy to study the chemical composition of the Golgi element if solvents could be applied directly, but lipoid-solvents exert such a damaging effect on fresh cells that the results of their action on the osmiophil substance cannot be followed. One can only say that if lipoid-solvents are applied to fresh cells, the Golgi element cannot subsequently be distinguished in the great majority of cases. This suggests that it contains lipoid, but carries us no farther.

In order to make more exact solubility tests, it is necessary to preserve the cell first. I have chosen to use formaldehyde (formal-calcium) for this purpose, because it leaves the lepidosomes of the male germ-cells of *Helix* in a state in which they can readily be coloured by sudan black. Various solvents can be applied, and subsequent trials with the colouring agent will show whether the lipoid contained in the lepidosomes has been wholly or partly dissolved. What is being studied, therefore, is the solubility of the lipoid of the lepidosomes in various solvents after treatment with formaldehyde. Concrete information can be obtained on this subject.

Solubility tests of this kind can be applied not only to cells, but also to lipines contained in pith. Solubility tests of lipines are described below (p. 57). The purpose of these studies has been to find whether the tests of solubility confirm or invalidate
the conclusion, based on the result of the Smith-Dietrich test, that the lepidosomes contain lipine.

**Solubility Tests on Lepidosomes.**

When the ovotestis of the snail has been fixed in formal-calcium, it is washed and brought into the solvent whose effect is to be tried. When the solvent has acted at a stated temperature for a stated time, the ovotestis is washed and embedded in gelatine. The block is hardened as usual and frozen sections cut. (For particulars of these processes, see p. 13.) The sections, 15 μ thick, are attached to slides, coloured by the standard sudan black method, and mounted in glycerine jelly or laevulose. The appearance at once tells whether the solvent has or has not left any lipid in the lepidosomes. The sudan black is used here simply as an indicator of the presence or absence of lipoids. Conclusions about the chemical composition of the lepidosomes may be drawn from the effects of various solvents on the subsequent reaction with sudan black.

The solvents used are named in the Table on p. 57. 57°C was chosen arbitrarily for some of the extractions simply because this is the temperature of the paraffin oven.

Acidified alcohol-ether is this:

- Absolute alcohol . . . . 100 c.c.
- Ether . . . . . 150 c.c.
- Concentrated hydrochloric acid . . 4·5 c.c.

The idea for this solvent was suggested by the work of Anderson, Reeves, and Stodola (1937). These authors found in tubercle bacilli complexes composed of lipid material and carbohydrates. A large part of the carbohydrate was split off by the use of hot acidified alcohol-ether, leaving the lipid free to dissolve. This solvent was therefore used to throw light on the question whether the lipine of the Golgi element is combined with carbohydrate.

'Xylene-acetic' is a mixture of xylene and glacial acetic acid in the proportion of 30 : 70 by volume. The trial of this solvent was suggested to me by Mr. P. B. Medawar. It was evolved empirically by him and Mr. W. Holmes for the purpose of
clearing away myelin from formal-fixed nerves, and thus per-
mitting silver impregnation of the axon (Holmes, 1942).

The standard way of dehydrating ovotestes of snails for sub-
sequent extraction with solvents was to leave them for about
half an hour in alcohols of each of the following strengths: 30 per
cent., 50 per cent., 70 per cent., strong industrial spirit (ca. 95 per
cent.), and then half an hour in each of three lots of absolute
alcohol. After extraction by the solvent the ovotestes were
soaked for about half an hour in absolute alcohol and then
carried through the other strengths in reverse order. Three of
the solvents required no previous dehydration (sodium oleate
solution, normal hydrochloric acid, and boiling distilled water).

Further details about the extraction experiments are shown
in the Table on p. 57.

The results of the extraction experiments were little short of
astonishing, for the lepidosomes showed enormous powers of
resistance to extraction. Cold absolute alcohol followed by cold
ether does not extract at all, nor does boiling distilled water.
Normal hydrochloric acid at 57° C. extracts slightly. Boiling
absolute alcohol followed by boiling ether extracts to some
extent from the lepidosomes of the spermatocytes but not from
those of the spermatids. Acidified alcohol-ether also has some
solvent effect. Only two of the methods of treatment have
strong extractive powers for the lipoid material of the lepido-
osomes. These are sodium oleate followed by alcohol and ether,
and xylene-acetic.

So great is the resistance to extraction, that ovotestes may
even be embedded in paraffin wax according to routine histo-
logical procedure, yet the sections will still show the lepidosomes
when treated with sudan black. The preparations are not so
good, however, as frozen ones, owing to partial extraction of
the lipoid.

The lipoid (sudanophil) substance of the mitochondria is
much more easily extracted than that of the lepidosomes. Of
all the solvents tried after formal-calcium fixation, they only
resist cold alcohol followed by cold ether. Most of the other
solvents seem to disperse the sudanophil substance of the mito-
chondria all through the cytoplasm, which thus becomes pale
Table showing Particulars of the Extraction Experiments.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Reaction of lepidosomes with sudan black after action of solvent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>III</td>
</tr>
<tr>
<td>Boiling distilled water (½ hour)</td>
<td>III</td>
</tr>
<tr>
<td>Ether (overnight)</td>
<td>III</td>
</tr>
<tr>
<td>Normal hydrochloric acid at 57° C. (4 hours)</td>
<td>II+</td>
</tr>
<tr>
<td>Boiling abs. alcohol (½ hour), then boiling ether (½ hour)</td>
<td>III in spermatids</td>
</tr>
<tr>
<td>Acidified alcohol-ether at 50° C. (5 hours)</td>
<td>II</td>
</tr>
<tr>
<td>Sat. Na oleate (overnight), then ether (overnight)</td>
<td>I in spermatids</td>
</tr>
<tr>
<td>Xylene-acetic at 57° C. (overnight)</td>
<td>0* in spermatocytes</td>
</tr>
<tr>
<td></td>
<td>* Traces of a positive reaction in a few cells.</td>
</tr>
</tbody>
</table>

Solubility Tests on Lipines.

The lipines studied were egg-lecithin, cephalin, and sphingomyelin. The two latter substances were extracted from the brains of sheep by Weil's method (1930). The lipines were emulsified and incorporated in pith cubes, as has been described above (p. 47), and the cubes were fixed for 24 hours in formal-calcium. They were then washed, embedded in gelatine and sectioned on the freezing microtome. The sections were stuck on glass slides with gelatine. The slides were then placed in various solvents (after appropriate dehydration where necessary). The sections were next coloured with sudan black as has already been described for the lepidosomes in the first part of this paper.

As has already been mentioned, the lecithin sections had some tendency to disintegrate when taken from the microtome into distilled water, and were therefore not so satisfactory as the
cephalin and sphingomyelin sections. The control (unextracted) cephalin sections were coloured particularly strongly by sudan black.

The experiments at once revealed two facts about the effect of formaldehyde on lipines:

1. It affects their solubilities, rendering them less easily extracted by solvents.

2. It eradicates the differences in solubility of the various lipines, so that they are no longer distinguishable from one another in this way (apart from the tendency of lecithin, after treatment with formaldehyde, to retain a liability to disintegrate in water; see also the footnotes on this page and the next).

Weil (1929) concluded that the preservation of tissues by formaldehyde does not fix the lipines. He found that the amount of cephalin and sphingomyelin in the central nervous system is gradually lessened on prolonged soaking in formaldehyde solution. However, he did not test the change of solubility in lipid-solvents. Further, some of the lipines may have been lost in emulsion before the formaldehyde had had time to act, for no calcium salt was included in the preservative. Again, formic acid was presumably present as well as formaldehyde, since neutralization was not mentioned. When using formaldehyde as a fixative for lipines it is desirable to have a soluble calcium salt present to prevent emulsification, and calcium carbonate also present in powdered form to prevent acidification. This is achieved by the use of formal-calcium, which was the standard fixative in all my experiments.

It follows from what was said above that all three lipines can be considered together. Their reactions to solvents are as follows, under the conditions of the experiment. The cell-walls of the pith probably make solution slower than it would otherwise be.

The following solvents do not extract or scarcely extract formaldehyde-fixed lipines:

- Ether (24 hours).\(^1\)
- Absolute alcohol (24 hours).
- Acetone (24 hours).

\(^1\) There is a very slight effect on cephalin.
Boiling absolute alcohol (1⁄2 hour) followed by boiling ether (1⁄2 hour).
Paraffin wax at 60° C. (1 hour), preceded and followed by cold xylene.
The following solvent extracts formaldehyde-fixed lipines to some extent:
Ligroin (24 hours).\(^1\)
The following solvents extract formaldehyde-fixed lipines completely or nearly completely:
Pyridine at 60° C. (24 hours).
Xylene-acetic at 60° C. (24 hours).
Saturated aqueous sodium oleate solution (24 hours), followed by boiling absolute alcohol (1⁄2 hour) and boiling ether (1⁄2 hour).
Ditto, with sodium ricinoleate substituted for sodium oleate.

A Comparison of Lepidosomes with Lipines.
In response to these tests there is a remarkably close resemblance between the lipines on the one hand and the lepidosomes of the male germ-cells of Helix aspersa on the other. As was shown above, the formaldehyde-fixed lepidosomes are not extracted by ether, nor by xylene and melted paraffin wax, so that they retain part of their lipid content during paraffin embedding. They are not extracted by boiling alcohol followed by boiling ether. They are extracted, on the contrary, by saturated sodium oleate followed by alcohol and ether, and also by hot xylene-acetic. In every one of these respects the lepidosomes behave in the same way as the lipines.\(^2\)

The Response of Lipines to the Standard Techniques for the Golgi Element.
It has been shown above that a histochemical test gives strong evidence of the existence of lipine in the lepidosomes and that this conclusion is supported by the behaviour towards

\(^1\) Sphingomyelin is not extracted.
\(^2\) The effect of acetone, ligroin, and pyridine on the lepidosomes has not been studied.
solvents after formaldehyde fixation. It remains to find whether lipines behave like the Golgi element when subjected to the standard techniques for showing this cytoplasmic inclusion.

Egg-lecithin, brain-cephalin, and brain-sphingomyelin were incorporated in pith cubes in the way described above (p. 47). The cubes were then subjected to the Weigl (Mann-Kopsch) technique, exactly as though they were pieces of tissue. Post-osmification lasted for four days at 30° C. Paraffin sections were cut and mounted in balsam.

The appearance under the low power of the microscope varied according to the thickness of the section from grey to black. In thin sections minute particles could be seen separately, each appearing black or dark grey when focused separately under the highest powers of the microscope. Sphingomyelin also gave a grey to black reaction, according to the thickness of the sections, but separate particles could not be seen.

It is regarded as characteristic of the Golgi element that it remains dark in Weigl sections when they are soaked in turpentine for 15 minutes, while other lipoid bodies are completely decolorized. Some of the sections were therefore soaked for 15 minutes in turpentine before being mounted. These sections revealed a sharp difference between lecithin and cephalin on the one hand, and sphingomyelin on the other. The osmified lecithin and cephalin were only slightly extracted, the particles remaining grey to black. The osmified sphingomyelin, on the contrary, simply disappeared. Only a few black dots could be seen under the microscope lying here and there against the walls of some of the pith cells, and there is no certainty that these dots represented the sphingomyelin.

Lecithin and cephalin, then, both resemble the osmiophil substance of the Golgi element, while sphingomyelin does not. For this reason further experiments were confined to lecithin and cephalin.

Egg-lecithin emulsion incorporated in pith was subjected to Kolatchev's technique as follows. Fixation in Champy's fluid lasted overnight. Washing-out in running water was very thorough. (It was found that if the washing-out at this stage was not thorough, the impregnation did not succeed.) Post-
osmification lasted for 5–6 days at 35° C., 2 per cent. osmium tetroxide solution being used. The material was then washed, embedded in paraffin, and sectioned. The sections were mounted in balsam. The lecithin was black in its deeper parts, even in thin (5 μ) sections. The lecithin near the surface of the pith was far less deeply impregnated than the deeper parts. This agrees with the fact that the Golgi element often does not reduce osmium tetroxide near the surface of a piece of tissue, as Kolatchev (1916) remarked in the paper in which he introduced his method.1

Pith cubes containing emulsions of egg-lecithin and of brain-cephalin were subjected to Aoyama’s technique. They were fixed overnight, the period in the fixative and also in the impregnating fluid being lengthened in conformity with Aoyama’s instructions for invertebrate material. Impregnation with 1½ per cent. silver nitrate solution lasted for 15–24 hours and reduction for 5. Paraffin sections were mounted in balsam, without toning.

The experiments with lecithin gave remarkable results, for the procedure has a powerful disintegrating action on lecithin-emulsion. I think that the disintegration occurs during reduction. Scarcely any lecithin remains at the outside of the pith cube, and it only occurs here and there in the central pith-cells; though those cells that contain it are often nearly full. The silver colours the emulsion in all shades between brownish-yellow and black, just as it colours the Golgi element in snail ovotestes. The deposition of silver is particularly dense round minute spaces (? air bubbles) in the emulsion.

In order to try to hold the lecithin in position, experiments were made with lecithin emulsified in blood-plasma. Here again disintegration occurred all round the edges of the pith cube. The lecithin remained only in some of the pith-cells, where silver was intensely deposited in it, so that separate silver particles could not be seen.

Blood-plasma without lecithin was tried as a control. Here no dissolution occurred, all the outer pith-cells remaining full. The plasma reduced the silver with the production of minute

1 Cephalin emulsions in water were not tried by Kolatchev’s method.
yellow to black particles, mostly visible separately and here and there arranged in lines showing the Liesegang effect. The appearance was quite different from that of the massively impregnated lecithin emulsions.

Disintegration can be studied macroscopically by treating small lumps of lecithin by Aoyama's method. At the stage when the reducer is poured out of the capsule, each lump collapses on the bottom and frays at its edges. When water is added and the whole gently stirred, the lumps disintegrate into ropy and irregular masses, while emulsification takes place and the water becomes yellowish-brown.

The effect of Aoyama's technique makes one suspect that the Golgi nets often seen in finished silver preparations may be artifacts produced by the flow and anastomosis of the lipine.

Cephalin does not tend to disintegrate like lecithin. The pith-cells contain plenty of emulsion and the latter reduces silver nitrate, though not so massively as lecithin-emulsion. (The experiments were done with cephalin emulsified in blood-plasma.)

Both lecithin and cephalin apply themselves to the cellulose of the pith, making the cell-walls reduce silver nitrate strongly. This occurs even in pith-cells from which the rest of the lecithin has disappeared. It is not observed in controls containing blood-plasma without added lipine.

These experiments show that lecithin and cephalin resemble the osmiophil and argentophil substance of the Golgi element in their reactions to the standard Golgi techniques.

**DISCUSSION.**

Everything recorded in this paper is consistent with the view that the osmiophil substance of the Golgi element in the spermatocytes and young spermatids of the common snail, *Helix aspersa*, contains or consists of lecithin or cephalin or both. The three lines of evidence mentioned in the introduction to this second part of the paper all point in the same direction:

First, the lepidosomes react in the same way as lipines to the
Smith-Dietrich test, if allowance is made for their minute size by shortening the period of differentiation to 8 hours.

Secondly, they show the same resistance to extraction by solvents as lipines, after formaldehyde fixation in both cases.

Thirdly, lecithin and cephalin react positively to the standard tests for the Golgi element, and the black reaction-product of the Weigl (Mann-Kopsch) technique is not easily extracted by turpentine.

The fact that the Golgi element does not as a rule resist routine histological procedures shows that it usually contains little or no protein. When lecithin is associated with the protein vitellin to form yolk, the resulting substance is one of the most indestructible with which the histologist has to deal, contrasting strongly with the delicate osmiophil substance of the Golgi element. It would not be necessary to stress the radical difference between yolk on the one hand and the osmiophil Golgi substance on the other, were it not that text-books sometimes contain statements that would lead one to infer close similarity in chemical composition. The fact is that the one contains much and the other little or even perhaps no protein. (The yolk of hens' eggs of course contains lecithin and cephalin and in this respect resembles the osmiophil substance; and since the time of the pioneer studies of Gatenby and Woodger (1920) we have known that fatty yolk sometimes arises in relation with the Golgi element.)

The action of soaps suggests that the lipoid-containing substance of the Golgi element may be a complex like the lipoid-protein complexes of Macheboeuf (1929a and b; see also Macheboeuf and Tayeau, 1938). Tests for proteins show, however, that the lepidosomes do not contain appreciably more protein than the cytoplasm in general, and there is no proof that they contain any at all.

The great resistance of the lepidosomes to extraction by lipoid-solvents after formaldehyde fixation excludes the possibility of their being formed of triglycerides.

It is likely that much of the lipoid matter, other than triglycerides, extractable from fresh tissues by lipoid-solvents comes from the Golgi element and mitochondria. Only a
relatively small part of it may in many cases be derived from the so-called 'structural lipoid' which has been regarded as spread homogeneously throughout the cytoplasm. Quite a significant proportion of the dry matter of an animal must be contained in its Golgi element and mitochondria.

There is much evidence that in secreting cells the product of secretion arises within the neutral red vacuoles which are commonly associated with the lipoid-containing substance in the fully developed form of the Golgi element. It may be suggested that the lipine acts as a selective membrane allowing the synthesis of substances which could not be formed in the cytoplasm outside. This would be comparable to the action of the lipoid of the cell-membrane in allowing synthesis in the cytoplasm of substances that are not formed outside the cell.

ACKNOWLEDGEMENTS.

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SUMMARY.

Structure.

1. Reasons are given for believing that the methods used to produce the classical Golgi network cannot be relied upon to give an accurate picture of the structure of the Golgi element during life.

2. In this investigation reliance has been placed chiefly on vital observations and on the results of the formal-sudan-black technique. Sudan black is a colouring agent with an intense affinity for lipoids, whether 'masked' or not.

3. The Golgi element was studied in the following cells:

   (1) the primary spermatocyte and early spermatid of the common snail, Helix aspersa;

   (2) the absorptive cell of the intestinal epithelium of the smooth or common newt, Triturus vulgaris;

1 The words lipoid, lipine, &c., as used in this paper, are defined on p. 40.
(3) the nerve cell of the anterior mesenteric ganglion of the rabbit, Oryctolagus cuniculus.

4. In its fully developed condition, the Golgi element of diverse cells consists of four parts:

(1) the 'neutral-red vacuoles';
(2) the dense lipoid-containing substance, generally in close relation to the vacuoles in the form of strands, 'lepidosomes', caps, crescents, rings, or complete investments;
(3) the diffuse lipoid-containing substance, which fills all the space in the Golgi element not occupied by the other constituents;
(4) the Golgi-product, which arises in the vacuoles and is the result of the synthesis achieved by the Golgi element.

**Chemical Composition.**

1. A critique of the literature reveals that there has been no reliable evidence of the chemical composition of the osmiophil substance of the Golgi element, apart from the fact that it contains lipoid (in the widest sense).

2. The lepidosomes (Golgi batonettes) of the spermatocytes and young spermatids of the common snail, Helix aspersa, were chosen for the study of chemical composition, because they are visible without staining in living cells and there is therefore no possibility that one may be investigating an artifact.

3. The results of the Smith-Dietrich test strongly suggest that the lepidosomes contain lecithin, cephalin, or sphingomyelin.

4. After formaldehyde fixation these three lipines all closely resemble one another and also the lepidosomes in their resistance to extraction by various solvents.

5. Emulsions of lecithin and of cephalin, incorporated into the cells of pith, respond to the standard techniques for showing the Golgi element in the same way as the lepidosomes. Weigl (Mann-Kopsch) preparations of these lipines are not decolorized by soaking for 15 minutes in turpentine. (Sphingomyelin, though blackened by the Weigl technique, differs strongly from...
the osmiophil substance of the Golgi element in that the black material is rapidly extracted by turpentine.)

6. It is concluded that the lepidosomes of the spermatocytes and spermatids of Helix aspersa consist of or contain lecithin or cephalin or both.

DESCRIPTION OF PLATES 1 AND 2.

A scale representing 10 μ is placed at the top of each plate. The following symbols are used:

- **cm,** cell membrane; **fb,** free border; **g,** Golgi element; **gn,** Golgi net (artificial appearance); **gp,** Golgi product; **gr,** Golgi ring (artificial appearance); **k,** Krinom of Chlopin; **l,** lepidosome; **lm,** limit of region of diffuse lipid-containing substance; **m,** mitochondria; **n,** nucleus; **nm,** nuclear membrane; **vA,** neutral-red vacuole; **vB,** neutral-red vacuole surrounded by dense lipid-containing substance; **vC,** crescent of dense lipid-containing substance on a neutral-red vacuole; **vD,** several neutral-red vacuoles crowded together and invested by dense lipid-containing substance.

**PLATE 1.**

The Primary Spermatocyte of the Common Snail, Helix aspersa.

Fig. 1.—Unstained living cell. Two lepidosomes are seen at 12 o'clock. (Photomicrograph ref. 441.)

Fig. 2.—Living cell, stained with dahlia. Four lepidosomes are seen, one of them bent into a U. (Ref. 436.)

Fig. 3.—Living cell, stained with neutral red. Four neutral-red vacuoles are in focus. (Ref. 431.)

Fig. 4.—The same cell as Fig. 3, at a deeper focus. Four different neutral-red vacuoles are in focus. (Ref. 430.)

Fig. 5.—A cell fixed in formal-calcium, unstained. Two lepidosomes are clearly seen and others glimpsed. (Ref. 413.)

Fig. 6.—A formal-sudan-black preparation. The lepidosomes are intensely coloured by the sudan black. (Ref. 418.)

Fig. 7.—A formal-sudan-black preparation. Two of the lepidosomes (large and small) have each a vacuole attached. (Ref. 417.)

Fig. 8.—A formal-osmium preparation. The lepidosomes have reduced the osmium strongly, the mitochondria slightly. (Ref. 384.)

Fig. 9.—A formal-osmium preparation, showing the whole Golgi region impregnated with osmium hydroxide. (Ref. 364.)

Fig. 10.—A Kolatchev preparation. The lepidosomes are strongly impregnated. (Ref. 428.)

Fig. 11.—A Smith-Dietrich preparation. The lepidosomes give the
reaction for lipines strongly, the diffuse lipid-containing substance less strongly.

The Early Spermatid of the Common Snail, Helix aspersa.

Fig. 12.—Living cell, stained with dahlia. The lepidosomes are arranged round the edge of the Golgi region. The mitochondria are also stained. (Ref. 438.)

Fig. 13.—A Smith-Dietrich preparation. Three lepidosomes are seen, giving a strong positive reaction for lipines. (Ref. 422.)

Fig. 14.—Another Smith-Dietrich preparation. The whole Golgi element appears black in each cell on account of the strong positive reaction for lipines shown by the lipid-containing substance (dense and diffuse). G, Golgi element. (Ref. 420.)

The Absorptive Cell of the Intestinal Epithelium of the Smooth or Common Newt, Triturus vulgaris.

Figs. 15–21 are surface views of the epithelium, which was spread out as described in the text. Figs. 22–30 represent parts of transverse sections through the intestine.

Fig. 15.—Living cells, unstained, showing the vacuoles of the region above the nucleus. (Ref. 332.)

Fig. 16.—Living cell, stained with dahlia. (Ref. 328.)

Fig. 17.—Living cells, stained with dahlia. (Ref. 322.)

Fig. 18.—Living cells, stained with dahlia. One of the larger vacuoles has a very obvious mass of dense lipid-containing substance on its surface, intensely stained (blue) with dahlia. (Ref. 327.)

Fig. 19.—Living cells, stained with neutral red. This figure shows the characteristic size of the smaller neutral-red vacuoles. (Ref. 347.)

Fig. 20.—Living cells, stained with neutral red, showing small and also larger neutral-red vacuoles. (Ref. 366.)

Fig. 21.—Living cells, stained with neutral red, showing one of the vacuoles of the largest size (‘plasmopyrène’ of Champy), as well as other vacuoles of various sizes. (Ref. 352.)

Fig. 22.—A formal-sudan-black preparation. Part of the Golgi element shows a strongly positive reaction for lipoids. (Ref. 449.)

Plate 2.

Fig. 23.—A formal-osmium preparation, showing the vacuolar structure of the Golgi element. (Ref. 355.)

Figs. 24 and 25.—Nassonov preparations, showing the vacuolar structure of the Golgi element. (Refs. 289 and 296.)

Figs. 26 and 27.—Weigl (Mann-Kopsch) preparations, showing the vacuolar structure of the Golgi element. (Refs. 306 and 305.)

Fig. 28.—A Weigl (Mann-Kopsch) preparation, showing the nucleus
thrust through the Golgi element, which now surrounds its upper part. (Ref. 298.)

Fig. 29.—A Chlopin preparation, showing the 'Krinom' in the form of spheres stained with Ehrlich's haematoxylin. (Ref. 303.)

Fig. 30.—A cell fixed in 1 per cent. osmium tetroxide solution, unstained. The cell contains vacuoles of the largest size as well as smaller ones. They appear black owing to the reduction of the osmium tetroxide on their outer parts. (Ref. 389.)

**The Absorptive Cell of the Intestinal Epithelium of the Crested Newt, Triturus palustris.**

Fig. 31.—A cell showing the staining of part of the Golgi element with nigrosin. Vacuoles are clearly seen, unstained by the dye. (Ref. 390.)

**The Mucous Cell of the Intestinal Epithelium of the Smooth or Common Newt, Triturus vulgaris.**

Fig. 32.—A Kolatchev preparation, showing vacuoles, strands of dense lipoid-containing substance, and diffuse lipoid-containing substance. (Ref. 302.)

**The Nerve Cell of the Anterior Mesenteric Ganglion of the Rabbit, Oryctolagus cuniculus.**

Fig. 33.—A formal-sudan-black preparation, showing the limit of the region of diffuse lipoid-containing substance. The edge of the cell is marked cm. The dense lipoid-containing substance is seen distributed throughout the diffuse region. (Ref. 404.)

Fig. 34.—Another formal-sudan-black preparation. A crescent of dense lipoid-containing substance is particularly clearly seen because it lies over the lipoid-free nucleus. A number of vacuoles surrounded with dense lipoid-containing substance are seen. (Ref. 415.)

Figs. 35 and 36.—Other cells from the same preparation as fig. 33 (formal-sudan-black). Vacuoles covered with dense lipoid-containing substance are seen. (Refs. 410 and 406.)

Fig. 37.—A formal-osmium preparation, in which the dense lipoid-containing substance is seen to form an investment to several vacuoles. This should be carefully compared with parts of the right-hand sides of figs. 40 (formal-osmium) and 41 (Da Fano). (Ref. 408.)

Fig. 38.—A formal-osmium preparation, showing vacuoles with osmiophil rims. (Ref. 378.)

Fig. 39.—A formal-osmium preparation, showing strands of osmiophil substance linking vacuoles together. (Ref. 381.)

Fig. 40.—A formal-osmium preparation, showing the osmiophil substance forming an investment to several vacuoles. (Ref. 377.)

Fig. 41.—A Da Fano preparation, showing the classical Golgi network.
On the right-hand side an area is seen in which the argentophil substance forms an investment to several vacuoles. (Ref. 426.)

The Nerve cell of the Dorsal Root Ganglion of the Cavy, Cavia cavia.

Fig. 42.—A Da Fano preparation, showing the argentophil substance in separate pieces, some of them ring-like. (Ref. 427.)

REFERENCES.

Baker, J. R., 1933.—'Cytological technique.' Methuen, London.
Bergen, F. von, 1904.—'Arch. f. mikr. Anat.', 64.
—— 1935.—Ibid., 13.
—— 1928.—Ibid., 40.
Champy, C., 1911.—'Arch. d'anat. micr.', 13.
Ciaccio, C., 1910.—'Arch. f. Zellforsch.', 5.
Dippel, L., 1882.—'Das Mikroskop und seine Anwendung.' Vieweg, Braunschweig.
Horning, E. S., 1933.—*Erg. Enzymforsch.*, 2.
— 1928.—Ibid., 270.
Ludford, R. J., 1928.—*Nature*, 121.
— 1929 b.—Ibid.
Monné, L., 1938.—*‘Protoplasma’,* 30.
— 1939.—Ibid., 32.
Overton, E., 1901.—Quoted by MacLean and MacLean, 1927.
Steinhaus, J., 1888.—*Arch. de Physiol. norm. et path.*, 2.
Subramaniam, M. K., 1939.—Quoted by Hirsch, 1939.
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Weigl, R., 1910.—Quoted by Bowen, 1928.
—— 1930.—'Pflügers Arch.', 223.
Weiner, P., 1926.—'Arch. russes d'anat., d'hist. et d'emb.', 5.
Zirkle, C., 1933.—'Protoplasma', 20.