THE WANDERING CELLS OF THE ALIMENTARY CANAL. By W. B. HARDY, M.A., *Fellow of Gonville and Caius College, Cambridge,* and F. F. WESBROOK, M.D., *John Lucas Walker Student and Professor of Bacteriology in the University of Minneapolis, U.S.A.* Plate V.

(*Physiological Laboratory, University of Cambridge.*)

For the purposes of this research the following animals have been examined:—the frog and newt representing Amphibia, the common grass snake representing Reptilia, and among Mammals, dogs, cats, and ferrets as carnivorous types, and guinea-pigs, rabbits, oxen and sheep as herbivorous types. In addition to these the rat was used as an animal of omnivorous habit, and the hedgehog as a type of a group, the Insectivora, which in its anatomical characters lies in many ways midway between the carnivora and the other deciduate mammalia. Lastly as young animals puppies and kittens were used, and fœtal calves were also examined.

The regions examined were the stomach, chiefly the pyloric end, small and large intestines and rectum. Attention was chiefly directed to the small intestine.

PART I.

Methods employed. It is absolutely necessary to fix the pieces of gut intended for examination within at most a minute or so after the cessation of the blood flow. Therefore immediately the animals were killed, usually by beheading, short pieces of the gut were removed, cut open, and plunged in the fixing fluid.

The reagents employed for fixation were absolute alcohol and a saturated solution of corrosive sublimate in 0·6 $^0/_0$ salt solution. These were employed boiling, at the temperature of the room (16 to 20° C.) and at 0° C. Absolute alcohol is the only reagent which preserves all the varieties of cells. In the case of this reagent it is the rule that fixation at high temperatures is best for basophile matter, while fixation at medium to low temperatures is best for oxyphile matter. Corrosive sublimate is useful in checking the results obtained with absolute alcohol in respect to the number of oxyphile cells.

Gold chloride and osmic acid vapour were employed occasionally.

In all cases the histological analysis of tissues was made with a variety of stains. Those employed were Ehrlich's neutral mixture, and the Ehrlich-Biondi mixture; the acid stains, eosine, methyl eosine, orange G, acid fuchsin in presence of acetic acid, sodium sulphindigotate, and hæmatoxylin. The acid stains were used in aqueous, glycerine, and alcoholic solutions after the manner described by Kanthack and Hardy[1]. The basic stains employed were methylene blue, thionin, and dahlia in saturated alcoholic solutions, and the intensity of the staining power

[1] This *Journal*, XVII. 81. 1894.

was controlled by the percentage of alcohol present. A solution in 85% alcohol is perhaps most generally useful, but by using various strengths of alcohol accurate comparative determination of the affinity of particular substances for the dye may be made.

The basophile cells of the gut are always exceedingly sensitive to the presence of even minute traces of water, therefore in basic staining the percentage of alcohol should not fall below 80. It also follows from this fact that rapid dehydration with absolute alcohol is essential to the preservation of these bodies.

It is necessary to say a few words concerning the effect of temperature on staining. We distinguish two processes in the colouring of a tissue with a dye, one a physical process in which the dye is precipitated probably by surface tension acting in the minute capillary spaces, and another in which a chemical union between the dye and some particular substance occurs. The physical action leads to the diffuse colouration of the tissue, and this precipitated colour can generally be removed with ease by washing with some solvent of the dye. Further it is obvious that the general colouration of the tissue by this physical process will be diminished by increasing the solvent power on the dye of the medium in which the dye is applied. Thus, in the case of methylene blue, if a saturated alcoholic solution of this dye be heated it becomes an unsaturated solution since methylene blue is more soluble in hot alcohol than in cold alcohol. Therefore a hot alcoholic solution of methylene blue produces less physical (diffuse) colouration, but on the other hand the chemical processes are intensified and the true dyeing action is more marked. On the other hand with cold solutions the physical process is accentuated and the chemical process diminished or, it may be, arrested.

Turning to the facts of the case it was found that during the cold weather of last winter the temperature of the solutions at times fell to a point at which the chemical processes—namely the dyeing of the basophile granules (dog) with methylene blue—took place with such exceeding slowness that in eight hours only a faint trace of colour was visible here and there. This was because the critical temperature for the union of the granule substance of the basophile cells of the dog and methylene blue is about 13° C. At this temperature however the tissue generally colours deeply.

With a boiling solution of the dye very different results are given—the basophile granules colour very intensely while general colouration is almost absent—if the hot solution is rapidly blotted off the section.

This brings us to the practical issue that basic staining with methylene blue is in most cases best effected by boiling solutions of the dye.

Imbedding was always performed by means of chloroform.

The tissue was passed very gradually from absolute alcohol into chloroform, which was then saturated at a moderate temperature with paraffin, and the chloroform driven off by moderate heat. After the sections were cut they were fixed to coverslips and the paraffin removed by xylol or chloroform.

MORPHOLOGY AND HISTOLOGY.

In the bodies of all the Metazoa so far as we know there exists a tissue composed of isolated units each of which is a cell endowed with the power of amœboid movement to a greater or less extent. These cells have for their home the fluids of the body and, in the lowest animal types, the cells are all of one kind while in the higher forms they have become differentiated into three kinds; the oxyphile cell bearing discrete granules which stain with acid dyes, the basophile cell bearing discrete granules which stain with basic dyes, and the hyaline cell which does not form discrete granules. These wandering cells are derived from the mesoblast, and to them as a whole the name "sporadic mesoblast" has been given.

So far as is known all the members of the Craniata possess these three types of wandering cells, but in the higher forms—namely in those animals which constitute the Mammalia—the three types are no longer uniform in character throughout the body as they are in the lower Craniata but differ in certain structural details. In these animals the sporadic mesoblast is further differentiated into portions, each composed of three kinds of cells, oxyphile, basophile, and hyaline, and each related to a special part of the body.

In an earlier paper[1] two of these groups were recognised, namely a hæmal group related to the blood system, and a cœlomic group related to the great cœlomic spaces, and, a closely related structure, the peripheral lymph system. This present paper has for its object a consideration of the minute structure and function of those wandering cells which lie in the interspaces of the mucous coat of the gut, and of the relation of these cells to those of the cœlomic and hæmal groups.

[1] Kanthack and Hardy, *loc. cit.*

To these cells of the gut the term "splanchnic" cells might conveniently be given.

Oxyphile cells. The oxyphile cells of the gut in shape are round or irregular and each bears a round or horse-shoe nucleus, resembling in this particular the coarsely granular oxyphile cell[1]. The cell substance is crowded with discrete granules which stain with acid dyes, the size of the granules and the intensity of their affinity for acid dyes varying in different animals.

Thus in Amphibia (frog and newt) and Reptilia (grass snake) the splanchnic oxyphile cells agree in every particular, in the size of the cell, in the nuclear characters, and in the size, highly refringent nature, and staining reactions of the granules with those found generally in the blood, lymph, and connective tissues of the body (cf. Fig. 1 *a* with Fig. 1 *b*). They are cells of about 10 μ in diameter with an eccentrically placed nucleus which is in most cases elongated, and bent round to form a more or less complete horse-shoe.

In the Mammalia the splanchnic oxyphile cells differ from those found elsewhere in the size and shape of the cell and in the size and shape of the granules. The nucleus, as in the case of the cold-blooded animals, is mostly horse-shoe in shape though a varying and sometimes large percentage of the cells present in any section show round nuclei.

In type these cells most closely approach the oxyphile cell of the cœlomic group, that is the coarsely granular oxyphile cell, and in the ox, sheep, and rabbit the difference is solely one of size. The same typical crowding of granules, characterised by a high refractive index and an intense affinity for acid dyes is observed in both, but the splanchnic cells and granules are somewhat smaller (Fig. 2). Thus in the rabbit the splanchnic oxyphile cell averages 7 μ in diameter while the cœlomic oxyphile cell averages 10 μ.

In the rat too these splanchnic oxyphile cells closely resemble those of the cœlomic system except in the matter of size but here the difference is greater than in the previously mentioned forms, the splanchnic oxyphile cell averaging 6 to 6·5 μ while the cœlomic oxyphile cell averages 10 μ (Fig. 3). The splanchnic oxyphile cells of the hedgehog resemble those of the rat in type.

In the carnivora, ferret, cat and dog, the splanchnic oxyphile cells depart very widely from the types found elsewhere (Fig. 4). The granules are small and have a much diminished affinity for acid dyes,

[1] Kanthack and Hardy, *loc. cit.* p. 86.

in point of fact the granules agree in size and reaction to dyes much more with the finely granular oxyphile cell of the hæmal system than with the coarsely granular oxyphile cell of the extra vascular fluids. On the other hand the cell has a rounded or curved nucleus and never shows the characteristic irregular nucleus of the hæmal cell. The granules are smallest and the cell is most extreme in type in the dog and cat, while in the ferret the granules have a very appreciable size and lustre, so that the structural features more closely resemble those found in the splanchnic oxyphile cells of the rat and hedgehog.

It will be noticed that we have pointed to the arched or horse-shoe nucleus of the splanchnic oxyphile cell as an important characteristic, and we have done this because we regard the rounded nucleus of the hyaline cell, the curved elongated nucleus of the cœlomic oxyphile cell, and the irregularly branched nucleus of the hæmal oxyphile cell as being true morphological features, and not as has been suggested, accidental appearances dependent upon the process of fixation.

It has been supposed[1] that the shape of the nucleus in fixed cells is a measure of the activity of the cell. The statement which has been made is twofold, (1) that in the position of rest of these cells both cell body and nucleus are spherical, and (2) that the extent to which the nucleus departs from the spherical shape in preparations is a measure of the ante-mortem activity of movement of the cell. Thus in histological preparations we find the coarsely granular cell for the most part with a more or less curved nucleus, and the finely granular oxyphile cell with a very irregular and lobed nucleus, and, according to the above hypothesis, both curved and lobed nuclei are departures from the shape of the nucleus in the living cell when at rest due to the fact that, in the process of fixation of a mobile cell, the cell substance contracts and is fixed so much more rapidly than the nuclear substance that the latter remains more or less extended in an irregular shape.

This hypothesis appears to us to be opposed to the facts of the case, and to be based on evidence which, strictly speaking, does not touch the question at issue.

In the first place so far as the histological facts are concerned it is inconceivable how, if the shape of the nucleus chiefly depended on such chance factors, it should be so remarkably constant a feature of the different kinds of cells, and so typical in certain animals that the coarsely granular oxyphile cell of the rat can be identified as belonging to that animal eight times out of ten by its nucleus alone.

[1] Sherrington. *Proc. Roy. Soc.* Dec. 14. 1893.

In the second place the statement that the nucleus of the living cell when at rest is always spherical in shape is apparently based entirely on the observation that when any of these cells are allowed to die slowly the nucleus is found to be spherical[1].

If we assume that the morphological structure of a cell is the same when it naturally and slowly reaches the condition of non-living matter by the gradual ebb of its store of energy, in other words, when it has reached what may be called the absolute zero of life, as when it is in the condition of rest of the living cell, or the conventional zero of life, then the truth of the hypothesis naturally follows.

But this is a great assumption to make, for even in the condition of rest, that is when rate of change of energy is minimal, the living cell, in virtue of the potentialities involved in the statement that it is "living," constitutes a dynamical field marked by molecular stresses induced by balanced forces; and, until direct evidence to the contrary is forthcoming, we may well believe that those forces maintain the nucleus in a shape which it would not possess if it yielded to those external agencies such as surface tension which, as in the case of an oil globule suspended in water, would otherwise reduce it to the spherical shape.

Further, though we cannot see the nucleus in the healthy living cell, and therefore are not able to appeal to direct observation to settle this question, yet in the case of those cells which bear refringent granules, the disposition of the latter in immobile individuals may help us to form a pretty shrewd guess as to its position and shape by noting the shape of the space unoccupied by granules. In most cases this space is clearly curved in the case of the coarsely granular oxyphile cells (see for instance the photograph reproduced in Fig. 1 of Prof. Sherrington's paper), and equally clearly a flattened spheroid is outlined in coarsely granular basophile cells.

Direct observation may be brought to bear by observing the staining of spherical cells in a hanging drop faintly tinged with some dye. If for instance a trace of methylene blue be used the cells die and stain so slowly as to afford ample time for observation and the making of sketches. A quiescent cell was brought into the field of the microscope *before* the addition of methylene blue. Figures 15 *a*, *b* and *c* represent stages in the slow appearance and staining of the nuclei. The drop was from "inflamed" lymph of a frog and the cell happened to be one of the apparently truly multinuclear oxyphile cells sometimes found in that condition.

[1] Sherrington, *loc. cit.* p. 187.

In discussing this question it is important to bear in mind that the nucleus may play a part in the movements of the cell purely as a mechanical factor. The junction of the nuclear substance and cell substance forms an internal surface which must have a certain directive effect on energy liberated within the cell. In this way the difference in the character of the movements shown by the various wandering cells may be in part referable to differences in the form of the nuclei.

Basophile cells form a very remarkable feature in the mucous coat of the gut. They are especially striking in Carnivora both as regards their number (as many as three to five hundred are present in a single villus of a dog) and the orderly arrangement they exhibit. In Herbivora on the other hand these cells are both less numerous and less ordered.

In all the animals examined the splanchnic basophile cells agree in one feature of peculiar interest, namely the exceeding instability of the granule substance in presence of even small quantities of water. After death water induces a complete disruption of the granules of so marked a nature that it involves the whole cell body, swelling it up to a bladder-like form or even destroying it so completely that no traces can be recognised by subsequent treatment. In the latter case, breaks in the continuity of the tissue appear where the basophile cells previously lay.

It follows from this liability to change in the presence of water that these cells rapidly disintegrate after death. They can only be preserved intact by very rapidly dehydrating small pieces of the gut with absolute alcohol. Corrosive sublimate in aqueous solution fails to preserve them, though sometimes a few imperfect specimens persist.

The instability of the granule substance of these cells is so great that even after being acted on by absolute alcohol for as long as eight and a half months, exposure to water for a few seconds[1] suffices to destroy or alter them in such a way that subsequent treatment with stains completely fails to show their presence. Alcohol, even as high as 50 °/₀, only serves to slightly retard the destructive action of water[2]. Therefore staining must be carried on in solutions of dyes with not less than 70 °/₀ of alcohol present. Owing probably to the instability of these cells they have, so far as we know, been overlooked by previous observers.

[1] By immersing a section fixed on to a coverslip in distilled water.

[2] On damp, muggy days the moisture in the atmosphere or in the blotting-paper used to blot off excess stain sometimes suffices to destroy the granules.

Though nothing is known of the chemical nature of these granules yet there is reason for believing that they are not simply proteid in nature. Thus even after prolonged exposure to absolute alcohol they are unstable in presence of cold water, and of boiling water; and are even destroyed by a saturated aqueous solution of corrosive sublimate. The destructive action of the saturated solution of corrosive sublimate was determined as follows in the case of the splanchnic basophile cells of the dog, ox, and rat. Two successive sections from a ribbon were mounted on coverslips, freed from paraffin by xylol, and brought into absolute alcohol. One section was then plunged for a few seconds into a saturated solution of corrosive sublimate and, after blotting off excess, was then placed in absolute alcohol containing free iodine. The other section was placed directly into the iodine solution, and both were allowed to remain there for some hours. The iodine was then completely removed by prolonged washing with 95 % spirit and the sections stained with methylene blue. The section which had been exposed to the corrosive sublimate solution showed no trace of basophile cells. Control experiments were made to determine whether, for instance, the abrupt transference from alcohol to a watery solution was responsible for the change by, among other methods, first drying the sections before immersion in the watery solution, but, so far as we could determine, the sole cause of the change was the presence of water.

Slight differences in the degree of instability of the granules of the splanchnic basophile cells were found in different animals; those of the rat for instance being a little more resistant than those of the rabbit. The difference is not great but, however slight, it is interesting when we remember the peculiarly great stability, in the face of reagents and treatment, of the granules of the coarsely granular (cœlomic) cell of the rat, and the equally marked instability of those of the rabbit[1].

The differences between the splanchnic and cœlomic basophile cells of the rat are exceedingly striking both as regards the size and stability of the granules and the size of the cells[2].

It should be noted that the splanchnic basophile cells occur only in the mucous coat of the gut, and a section treated with water will still show coarsely granular basophile cells of the ordinary type in the submucous and muscular coats.

The splanchnic basophile cells themselves vary much in shape, and

[1] Kanthack and Hardy, *loc. cit.*

[2] Compare fig. 5 *a* with fig. 5 *b* reproduced from Kanthack and Hardy, *loc. cit.*—both drawings to the same scale.

in sections of the gut they may appear elongated and flattened or rounded and lobed. They are usually more or less flattened in a plane parallel to the internal surface of the gut and are irregular in shape (Fig. 10). The cell substance is commonly so crowded with granules as to obscure the nucleus. When that structure can be seen it is ovoid or spherical.

In Amphibia and Reptilia, these cells resemble those found elsewhere both in the size of the cells and of the granules. In Mammalia the splanchnic basophile cells always differ from the coarsely granular (cœlomic) basophile cell in being smaller and possessing usually very much smaller granules. In the rat for instance the difference between the large basophile spherules of the cœlomic basophile cells and the fine dust-like granules of the splanchnic basophile cells is especially striking (Figs. 5 and 6).

The arrangement of these cells in the villi of Carnivora is very remarkable. It can be most readily studied by mounting entire villi which have been fixed in absolute alcohol and stained with methylene blue in 80 °/₀ alcohol, in Canada balsam. The whole villus then appears closely studded with basophile cells and, in optical sections, one sees that they form a defined layer lying immediately under the epithelium (Figs. 12 and 13). Each cell is flattened in the plane of the surface of the villus and is of an irregular shape. Allowing for contraction in preservation it is clear that during life they form a practically continuous layer. We thus find this feature, characteristic of the villi, and of the villi only, namely that a layer of flattened granular cells underlies the epithelium, and to this layer from its histological characters the name "basophile layer of the villi" might fitly be given.

In addition to the basophile cells of this (basophile) layer, others, but only a few, lie scattered in the parenchyma of the villus, and as we proceed downwards into the crypt region, these scattered specimens become more and more numerous so that a considerable number lie between the crypts and in the basal region. In other words the basophile cells of the crypts and basal region do not show such regularity of arrangement, as marks their disposition in the villi.

The cells vary in shape. Those of the basophile layer may be rounded so that they fail to touch one another, or they may be so irregular in shape as to be almost branched and to overlap. The overlapping may be much more considerable than appears on first sight, since it is impossible to determine how far the unstaining cell substance may extend beyond the granule-bearing region, and we are therefore unable to fix the extreme limit of the cells.

Judging from our entire experience of these cells we are inclined to believe that, though they usually have a fixed habit, they perform movements of extension and retraction which result in large alterations of the shape of the cells. Thus the layer may be described as composed of mobile cells, which are laden with basophile granules, and which, in virtue of their mobility, form a fenestrated membrane immediately underlying the epithelium which has the power of altering the size of its perforations. As we shall see later the splanchnic basophile cells under extreme conditions move from place to place.

We have failed to demonstrate the existence of such a well-defined basophile layer in mammals other than Carnivora. In the ox however a similar but very much less perfect structure is seen in some of the villi. Basophile cells are abundant in the mucous coat of the intestine of this animal but they mainly lie dispersed irregularly throughout the parenchyma. The sheep agrees with the ox in possessing a large number of splanchnic basophile cells, but we did not succeed in demonstrating a definite and continuous basophile layer.

In the hedgehog splanchnic basophile cells are numerous but, in the two examples examined by us, they appeared to be specially unstable. In these animals the bulk of the cells lie in the parenchyma, though many occur immediately beneath the epithelium. Those which occupy the latter position are too far removed from one another to allow us to speak of them as a definite basophile layer. The rat resembles the hedgehog except that the cells are relatively less numerous. Lastly in the rabbit the cells are still less numerous in the small intestine, though they are relatively abundant in the cæcum and vermiform appendix.

In the frog, newt, and snake basophile cells, resembling in size and appearance those found elsewhere, are found in the gut in fair numbers; they are scattered, sometimes in groups.

Putting these facts together we see that in the Carnivora, especially the dog and ferret, the basophile cells found in the villi are arranged beneath the epithelium in such a way as to form a definite basophile layer; whereas in the non-carnivorous forms they lie scattered throughout the parenchyma. When we compare these non-carnivorous animals one with another we find in some a greater, in others a less proportion of the scattered basophile cells lying immediately under the epithelium, and in this we probably have an expression of the manner in which the arrangement in the Carnivora has been arrived at, namely by the segregation of the basophile cells into a particular region in immediate relation to the epithelium.

The *Hyaline cells* found in the mucous coat of the gut are rounded or angular in shape and possess a rounded nucleus. They vary considerably in size, being from 5 to 10 μ in diameter, and the nuclei show considerable differences in the amount of basophile matter present.

As Heidenhain[1] pointed out the nuclei sometimes stain a deep opaque colour with nuclear stains, or they may show an open network with or without nucleoli (Fig. 14).

The dark staining nuclei lie in small cells with scanty cell protoplasm, the open, lighter staining nuclei are larger and occur in larger cells.

Two extreme conditions of the splanchnic hyaline cells may be recognised, in the first condition the cells present appear to be all of one order, resembling one another in size (about 7 μ) and in the fact that the nuclei all show the open network and do not stain intensely (Fig. 14 *b*). Here and there however are a very few of the smaller cells with deeply stained nuclei. In the second condition cells are present in all the varying stages from small young cells with darkly staining nuclei, so small and with such scanty cell substance as to deserve the term "lymphocyte," to hyaline cells larger than common and reaching to a diameter of 10 μ (Fig. 14). Under these conditions the percentage of small cells with dark nuclei varies markedly in different regions of the gut and is maximal in the neighbourhood of solitary follicles or Peyer's patches. To this point we shall return later. The larger cells in the second condition sometimes manifest phagocytosis and when swollen with ingesta they may be larger than 10 μ in diameter (Fig. 16). We believe that these hypertrophied hyaline cells are identical with the large phagocytes which Metchnikoff includes under the term "macrophage."

In Mammals the hyaline cells usually form the larger proportion of the cells present in the mucous coat. Various countings give them from 40 to 70 %. In frogs they are sometimes less numerous than the oxyphile cells.

Splanchnic wandering cells of the fœtus. In the course of this work we were fortunate enough to secure two fœtuses from slaughtered cows sufficiently fresh to enable us to examine the condition of the wandering

[1] *Pflüger's Archiv* 43, Suppl. 1888.

cells of the gut at this period. In both cases the organs were fully formed. Sections of small intestine showed that the villi though prominent structures were very imperfect. The adenoid tissue of the mucous coat was dense and very richly supplied with cells in the basal region. Here obviously rapid growth was taking place. Nearer to the lumen of the gut however, that is to say in the region of the crypts, it was looser and in the villi it was represented only by a network with such large interspaces, occupied by so few loose cells as to give one at first sight the impression that the villi were hollow structures consisting of a wall of epithelium on a basement membrane which enclosed a large central space.

The wandering cells present comprised both oxyphile and basophile individuals, while non-granular free cells were abundant. The oxyphile cells were quite typical but very infrequent (an average of not more than one to an entire transverse section). On the other hand, and this was the remarkable feature of the intestine, basophile cells in all respects resembling the basophile cells of the adult were present in relatively considerable numbers, even in the villi, and were fully charged with granules.

Thus in the fœtal intestine basophile cells are already present and functional.

The cells of the parenchyma of the intestinal mucous coat have been described at some length by Heidenhain[1]. He distinguishes "wandering" cells, "sessile" cells, and "phagocytes," of which the sessile cells represent merely the resting condition of the wandering cells. Unfortunately neither the description in the text nor the figured representation (Taf. III. Fig. 17) have enabled us to identify these "sessile" cells. With regard to the phagocytes we are in agreement with Professor Heidenhain. He regards them as hypertrophied leucocytes, and the Fig. 22 in which he illustrates the stages in their development clearly shows that in his opinion they are derived from the non-granular or in other words the hyaline cell.

By staining with Ehrlich-Biondi's fluid Heidenhain distinguished (1) a small cell with dark staining nucleus and scanty cell substance. This is the lymphocyte or young hyaline cell. (2) A larger cell with clear cell substance and round nucleus. This is the hyaline cell. (3) A cell with round or bent nucleus; the cell substance charged with granules staining purple. This clearly is the oxyphile

[1] *Pflüger's Arch.* 43, Suppl. 1888.

cell. Lastly Heidenhain distinguishes cells, not always present, with dark intensely staining nuclei and cell substance colouring intensely and evenly purple. These he regards as dying or dead cells—for cells which have been ingested by phagocytes show similar staining reactions. Hoyer[1], a pupil of Heidenhain, verified this conclusion by showing that if pieces of lymphatic gland were kept at the temperature of the body for some hours after removal from the animal they contained a largely increased number of cells which stained in this way.

Thus Heidenhain distinguished the hyaline and oxyphile cells but overlooked the basophile cells.

PART II. ACTIVITIES OF THE SPLANCHNIC WANDERING CELLS.

When we turn from the relatively secure ground of the structural characters of the splanchnic wandering cells and attempt to trace out the nature of their activities we are met with a variety of phenomena which, though fairly simple in themselves, yield curiously contradictory results when an attempt is made to correlate them with obvious processes taking place in the alimentary canal.

What we have to say on this point and the conclusions we offer we regard as being tentative. The reader will readily gather from what follows how contradictory the results may appear on the surface, and how large a series of experiments would have to be conducted to secure anything approaching a complete history of the relation of these splanchnic wandering cells to the processes going on in the alimentary canal.

Broadly speaking the cells undergo changes (1) in total and relative number, (2) in the size and composition of their granules, and (3) in their distribution, as influenced by their own proper movements, in the mucous coat.

The immediate stimulus which leads to these changes is of a chemical nature and consists of alterations in the chemical composition of the fluid, the lymph, that is to say, which occupies the interstices of the mucous coat.

The chemical composition of this fluid is dependent (1) upon the blood stream in the capillaries of the mucous coat, and (2) upon the absorptive and secretory activity of the lining epithelium.

The nature of the diet affects these factors in two ways. In the

[1] *Arch. f. mikr. Anat.* xxxiv. 208. 1889.

first place the chemical character of the substances absorbed from the gut will be modified by changes in the composition of the diet. But in addition to this obvious relation we must bear in mind the fact that the chemical nature of the end products of digestion—*e.g.* leucin, tyrosin, ptomaine—vary in accordance with alterations in the *quantity* of food given even though the quality of the food remain constant.

Taking our experiences as a whole we find that the splanchnic wandering cells are affected by (1) the presence of food in the gut, (2) the absence of food from the gut, and (3) the extent to which the gut is invaded by micro-organisms. The most difficult case to deal with experimentally is the absence of food from the gut, for starvation so frequently leads, especially in herbivorous animals, to a striking increase in the number of micro-organisms present.

A further consideration to be borne in mind is that starvation, when uncomplicated by secondary phenomena, has a twofold effect. Initially it leads to a hypertrophy of such structures as suffer loss of substance during digestion, *e.g.* the loading of the alveolar cells of the pancreas during starvation; while secondly if pushed far enough it will cause a diminution of these same structures as well as of the other structures of the body. An instance of the first action is furnished by the splanchnic oxyphile cells, these in certain pure cases of starvation have been found to increase in numbers and in the loading of individual cells with granules (p. 517).

We will as far as possible deal with the different cells in order in considering the changes which they show under various conditions.

Oxyphile cells. The splanchnic oxyphile cells may occur throughout the entire depth of the mucous coat of Mammals from the epithelium, where they lie imbedded between the cells, to the peculiar 'basal region' which is placed between the bases of the crypts or glands and the muscularis mucosæ; or they may be restricted entirely to this basal region.

So far as these cells are concerned we agree with Heidenhain in regarding the basal region as their place of origin and from this level, in response to certain changes in the constitution of the contents of the lumen of the gut, they move up toward the inner surface.

It is impossible to make a similar statement with regard to either the hyaline or the basophile cells, for these latter never lie wholly in, nor can they be said to have any special relation to, the basal layer of the mucous coat, though both forms occur there together with the splanchnic oxyphile cells.

A comparison of all the mammals examined by us leads us to the conclusion that the moving upwards of the oxyphile cells from the basal layer towards the free surface of the gut is at first associated with an increase in their numbers. If therefore we are right in regarding the basal layer as the place of origin of the oxyphile cells, and in viewing the movement thence towards the lumen as an indication of activity on their part, we may say that, at the onset of a period of activity, the cells migrate towards the lumen of the gut and increase in numbers, and some of them pass into the epithelium and through it into the lumen of the gut (Fig. 18).

The appearances seen towards the close of a period of activity would naturally vary according to whether the rate of production of these cells, whatever the machinery for that might be, was or was not maintained, and whether it was less, equal to, or in excess of the rate of destruction of the cells in the epithelium and in the lumen.

If we trace the oxyphile cells into the epithelium we find that they thrust themselves between the bases of the cells and move between the cells towards the lumen. Some of the cells suffer degenerative changes while still within the epithelium (Fig. 19). The granules disappear and the nucleus alters. The latter loses all trace of a network and passes into a condition in which it stains evenly. It may also break up into two or three masses. These degenerated nuclei frequently stain a remarkably intense and brilliant green with Ehrlich-Biondi's fluid. Sometimes the nucleus suffers these extreme changes long before the granules have completely disappeared. The appearances presented force one to the conclusion that the oxyphile cells perish in the epithelium and indeed they may shrink there, as though dissolved, and leave cyst-like spaces.

However all the oxyphile cells which enter the epithelium do not perish there, some, an unknown but possibly at times a very large percentage, traverse the epithelium and gain the lumen; others again may re-enter the parenchyma. Degeneration of the cells may occur at any level and in the same section one may find more or less degenerated cells together with individuals which so far as one can see are absolutely intact, in situations ranging from the base to the inner, free surface of the epithelium.

As might be expected free cells in the lumen of the gut are only found in cases of starvation, the presence of digestive juices would no doubt almost instantly destroy them. On the other hand the oxyphile cells are found in the epithelium of animals in full digestion and, in the case of mammals, intact cells have been seen in lowest portions of the lumen of the crypts of Lieberkühn. It appears to us to be clear that immigration of the oxyphile cells into the epithelium and thence into the lumen is a process of constant occurrence, at times so slight as to be barely detectable, at other times so excessive that the epithelium appears to be riddled with these bodies.

The most obvious structural change which the oxyphile cells manifest within the epithelium or in the lumen of the gut is a diminution in the number of the oxyphile granules even to the total disappearance of these structures. This appears in figure 18, where a small portion of a crypt of Lieberkühn is shown containing oxyphile cells some of which are completely charged with granules, others have only a few, while one has lost its granules. If we limit ourselves strictly to the evidence furnished by stained sections it is impossible to decide whether this change in the oxyphile cells is or is not necessarily followed by death and complete disintegration.

We have never witnessed any signs of the possession of phagocytic powers by the splanchnic oxyphile cells—in this negative feature they agree with the coarsely granular oxyphile cells.

The oxyphile cells may lie evenly dispersed in the mucous coat, or they may be collected into foci. In the frog the cells very commonly lie in groups, and over each group the epithelium is sometimes astonishingly crowded with immigrating cells. If we ask why immigration should be so marked over certain areas of the epithelium and practically wanting in intermediate regions, absolutely no answer is forthcoming. The cells of the epithelium in a region of migration are naturally displaced to a certain extent by intrusive cells, but the hyaline border may be normal and shows no signs of rupture other than the scanty presence of rounded perforations leading into cyst-like spaces immediately below it from which the oxyphile cells have crept. The epithelium cells themselves, so far as appearances go, exactly resemble those found in regions not infiltrated with intrusive cells.

Parasites in the epithelium appear to have little effect on the oxyphile cells ; thus in one frog, and the case is interesting as showing the different behaviour of the hyaline and oxyphile cells, the epithelium of the small intestine was largely occupied by a parasite which occurred with a single nucleus, a fragmented nucleus, or as a heap of spores. Oxyphile cells were practically absent from the gut, but hyaline cells were present in number in the epithelium where they ingested the parasites and, in some cases, obviously digested them.

Mammals resemble the lower forms in the fact that the oxyphile cells do not enter the epithelium indifferently at any point, but a curious and apparently permanent specialisation has taken place, namely that the oxyphile cells migrate especially by way of the crypt epithelium: and further when migration of the oxyphile cells is not very marked, the lower one goes down the crypts the more marked is it until a maximum is reached absolutely at the extreme lowest point of the gland. On the other hand when migration of the oxyphile cells is marked they may be found in the epithelium quite at the apices of the villi. In point of fact the apparent preference of the cells for the crypt epithelium may be very largely due to the fact that it is so much nearer than the epithelium of the villi to the basal layer from which, as we have already stated, the oxyphile cells appear to start, but we do not regard this as the sole cause since oxyphile cells may be present in the villi though absent from the villous epithelium, while at the same time they are present in the epithelium of the crypts. Further, as we have already pointed out, the rare migration of basophile cells follows precisely similar lines. We have never yet seen a basophile cell in the epithelium of a villus.

This limitation of immigrated oxyphile cells to the crypt epithelium is, so far as our experience goes, much more marked in the case of the dog, cat, and ferret than in other mammals. It is doubtful whether oxyphile cells find their way into the epithelium of the villi of dogs except under very extreme conditions. Paneth has described the existence of granule-bearing cells at the base of the crypts of Lieberkuhn, and we were careful to convince ourselves that these had not been mistaken for intrusive oxyphile cells[1].

It will be wise here to attempt to give some idea of the number, both absolute and relative, of the oxyphile cells. Comparison is made between sections of similar thickness about 8μ thick.

In frogs apparently normal and taken at random from the tank oxyphile cells may be present to the number of three to four hundred in the mucous coat of a transverse section of the small intestine; or only 50 to 60 in an entire transverse section. When abundant the cells lie in groups and at each focus from 35 to 64 can be counted in a single field of a $\frac{1}{12}$th.

From 20 to 60 or an even higher percentage of the oxyphile cells may lie either in the epithelium or so closely attached to the base of

[1] See note at the end of this paper. Also Figs. 21 and 22.

the cells that they remain adherent to the epithelium when that structure is torn away from the subjacent connective tissue.

The above figures are from sections through the upper portion of the small intestine. It is not the purpose of this paper to consider accurately the distribution of the cells throughout the length of the gut, but we may note that this was determined in the case of four frogs, and they agreed in showing a continuous and very marked decrease in the number of oxyphile cells as one passed from the duodenum either towards the rectum or towards the lower end of the œsophagus.

It is difficult to compare the relative number of the three kinds of cells in the frog's intestine. The oxyphile cells are readily seen, the hyaline cells are even more readily overlooked, while the basophile cells are only preserved with difficulty. Careful countings seem to show that the oxyphile cells frequently may form as much as 50 °/₀ of the total number of wandering cells present. In the mammals examined the oxyphile cells were found to form from 10 to 50 per cent. of the total number of wandering cells present in the mucous coat.

We will now proceed to the connection of the movements and number of the oxyphile cells with processes taking place in the gut.

The most striking fact we have met so far is that these cells are, if one may use the expression, finally used up either within the limits of the endodermic epithelium or even in the lumen of the gut itself. The movement of the cells from the basal layer, through the parenchyma to the epithelium, we rank as a phenomenon falling under the heading of chemiotaxis.

It is certain that the oxyphile cells perform some work either as such, or in virtue of the products of their disintegration, when in the epithelium and also when in the lumen of the gut, but we have no evidence to offer as to the nature of this work. It is also equally certain that they are 'used up' in the performance of certain functions while still within the parenchyma—if proof be needed of this it is found in the fact that ingestion of effete oxyphile cells on the part of the larger hyaline cells occurs within the limits of the parenchyma. Here again evidence sufficient to determine the part the oxyphile cells play is at present lacking.

What our experience seems to show is that the movement of the oxyphile cells into the epithelium is increased (1) by the presence of an unwonted number of micro-organisms in the gut, (2) by the onset of starvation, (3) by the onset of a period of digestion when it follows a short period of starvation. The difficulty in arriving at certain conclusions has already been dealt with. As we have pointed out during a period of starvation micro-organisms usually increase in the gut and one cannot then determine how far the changes observed are due to the absence of food and how far to the micro-organisms. But this is only an example of one side of the difficulty for it is equally difficult to obtain a measure of the activity of the cells. It is obvious that when the activity of the cells results in migration and destruction the total number present is of no use to us. It merely represents the balance for the time being between the rate of production and the rate of destruction.

This may be illustrated by reference to the hyaline cells. In starving rabbits these cells were found in excessive numbers in the epithelium of the small intestine, but the total number present in the parenchyma was very obviously below the normal. In other words here for the time being expenditure was far in excess of income.

An estimate of the number of the cells in the epithelium might be regarded as an accurate criterion but this again is useless unless we assume that the rate of progress through or from, and the rate of destruction in, the epithelium do not vary. To take an instance. In rabbits and rats deprived of food and prevented from consuming their own fæces, in two days the stomach was occupied by incredible masses of inwandered cells, yet on examination the living epithelium was found to contain a ridiculously inadequate number of intrusive cells. In point of fact it was difficult to be certain that they were more numerous than in the normal animal, although there is always a paucity of intrusive cells in the lining epithelium of the stomach. In view of these difficulties we have taken the percentage of the total number of cells present in the entire mucous coat which is formed by those lying within the epithelium as the best guide to the migratory activity, and, in the case of the oxyphile cells of mammals, we have been guided in our estimate also by the position of the cells in the mucous coat, and by the extent to which they are or are not limited to the basal layer.

The action of micro-organisms when present in the gut on the oxyphile cells was, it seemed to us, prettily illustrated by experiments on frogs. Diluted cultures (broth) of anthrax bacilli and cholera vibrios were injected into the intestines of six pithed frogs. The intestines of three of the animals were removed and fixed after 5½ hours, and from the remaining three, 19 hours after the operation. In one case a length of intestine was removed from a recently killed frog, and after having some cholera culture injected into its lumen, it was hung up in a moist chamber. In this and in all the experiments the result was the same, namely a gradual diminution of the oxyphile cells present, with at the same time a continued increase of the proportion of the total number of these cells present in the epithelium, until the number there formed 100 % of the whole; and finally, a total disappearance of oxyphile cells from the gut.

In attempting to determine the relation of the oxyphile cells to periods of feeding and of hunger we were met with the difficulty that, in spite of the utmost care directed to keeping the animals clean and to prevent them eating their own fæces, the micro-organisms increased in the small intestine and penetrated even as high as the duodenum. We believe that our failures in this respect were largely due to the fact that we used the rabbit and the rat for the purpose, our experience going to show that in animals of purely carnivorous habit the small intestine remains free from any obvious number of micro-organisms during at any rate short periods of starvation.

Whatever may be the true effect of starvation on the oxyphile cells there can be no question but that when it is complicated by the presence of micro-organisms in the gut, a large percentage are found in the epithelium. In digesting mammals and frogs the oxyphile cells are more numerous in the duodenum and decrease in number as we proceed down the small intestine. In starving animals whose intestines have become invaded by micro-organisms, this condition is usually altered, the oxyphile cells are more numerous at the lower end of the small intestine or in the large intestine, than in the duodenum, and this is in keeping with the fact that the invasion by micro-organisms takes place from below upwards.

Those cases of starvation in which the gut remained free from detritus agreed in the fact that the oxyphile cells increased in numbers to a great extent. They were abundant in the basal layer and extended thence right to the tips of the villi. On the other hand a comparison with the condition of the cells in other animals fed on the same diet, and under the same conditions, but killed when in full digestion, revealed the fact that the migration of the oxyphile cells was very much less in the starving than in the fed animals. In other words the accumulation of oxyphile cells in the case of starving animals appears to be due to a diminution of the drain on the cells which occurs in feeding animals, especially on a flesh diet, and in starving animals when increase occurs in the micro-organisms present.

The conditions which we have met with often seem curiously contradictory, but taking them together this seems to be the most satisfactory interpretation, and it is further borne out by the fact that when accumulations of wandering cells appear in the gut as a result of starvation they are exclusively of the hyaline type.

In his experiments on feeding and starving animals Heidenhain[1] found that the oxyphile granules were larger in the former; and he illustrates the two conditions of the cells in a starving and a full-fed dog on Plate IV., Figs. 27 and 28. Our experience goes to show that these relations do not always hold and indeed in very clear and pure cases of starvation, that is free from any excess of micro-organisms in the gut, the reverse condition may occur, the granules being larger in the starving animal. This was noticed for instance in the experiment further detailed on page 517; and in connection with this and like experiments we have been led to think that the partial hypertrophy of the oxyphile cells and granules was due to a diminished drain. It is a phenomenon of hunger, as it modifies this tissue in a thoroughly well-nourished animal.

The results of an attempt to determine experimentally the effects of a flesh diet are given on page 516. The most marked oxyphile granulation met with by us among dogs, when the granules were large and the cells very numerous, occurred in a portion of small intestine from a fat bitch. The mucous coat however was injured by the presence of worms, and migration was particularly marked, the number of oxyphile cells in the crypts of Lieberkühn being very striking. Fig. 18 is drawn from this animal.

Hyaline cells. The hyaline cells, unlike the oxyphile cells, are distributed evenly throughout the parenchyma, and are always present, though in variable numbers, in the epithelium. We have already pointed out that the hyaline cells vary largely in character. The cells differ in size, and the nucleus may be either very full of basophile material so that they stain as dark bodies, or they may be relatively free from basophile matter showing only the open nuclear network.

The most obvious phenomena displayed by the splanchnic hyaline cells are migration into and through the epithelium and phagocytosis. The latter may be manifested both in the parenchyma, in the epithelium, and, under favourable conditions, in the lumen of the gut. They also display changes, too obscure to be dealt with at present, in

[1] *loc. cit.*

the staining reaction of their nuclei and cell substance. Practically the discoverable activities of the hyaline cells are limited to migration and phagocytosis, and the variations in the nuclei ('dark' or 'open') and in the size of the cells are as Heidenhain pointed out correlated with the general activity of the cells as displayed in these two ways.

Heidenhain notices that when migration is unusually marked (that is as determined by the number of cells present in the epithelium), a large proportion of hyaline cells with small dark nuclei are found. As a matter of fact this is only a part of the change, for whereas when migration is slight the cells are all of about the same size and all have open nuclei, when migration is marked variations on both sides of this mean position occur, small cells with 'dark' nuclei being present and so with all intermediate conditions to cells considerably larger than common with nuclei containing only a very loose network. In other words, not only is the tissue occupied by numbers of the youngest cells but growth of individual cells progresses under the particular stimulus, whatever it may be, which gives rise to the condition of greater activity, to a point beyond that reached in the relatively inactive state.

From Heidenhain's description and figures one gathers that only the smaller hyaline cells were found in the epithelium. As a matter of fact this is not so, the cells in the epithelium, probably as a result of the staining of the tissue about them, appear much smaller than they really are—actual measurement shows that the smallest cell does not occur in the epithelium but that both intermediate and larger ones migrate, the intermediate ones being apparently more active in this respect than the larger ones.

Hyaline cells migrate chiefly into the epithelium of the villi. In carnivora the distinction between hyaline and oxyphile cells in respect to their place of migration is sometimes exceedingly sharp, the former being confined to the villous, the latter to the crypt epithelium. Hyaline cells, as a result of their ingestive activity, may contain intrusive bodies (parasites), micro-organisms, other wandering cells, *e.g.* oxyphile cells[1], and amorphous *débris* probably derived from one or other of the above.

So far as can be seen there is no necessary connection between the migratory and phagocytic activities of the hyaline cells. Thus migration may be excessive and yet one may be unable to detect any trace of

[1] Figure 9, compare also Hardy and Lim Boon Keng, This *Journal*, xv. 361. 1894.

phagocytosis, and, on the other hand, the number of cells within the epithelium may be by no means excessive when instances of ingestive activity occur both within the epithelium and in the parenchyma.

In point of fact the ingestion of solid particles is an act infrequent in its occurrence and of minor importance among the processes carried out by these cells. It has been shown[1] that the hyaline cells of the lymph of the frog will remove from the lymph plasma foreign substance dissolved there. The particular substance employed in the experiment was methylene blue and it was found precipitated as solid masses within vacuoles in the cell substance.

Similarly, by feeding with peptonate of iron, we have been able to show that the splanchnic hyaline cells remove from the interstitial lymph of the villi substances which are in solution there.

Absorption of iron by splanchnic wandering cells. Macallum[2] found that when the "peptonate" of iron is given to animals (guinea-pigs) the metal may be detected not only in the epithelium of the villi but also in wandering cells which crowd to the tips of the villi and there load themselves. It seemed to be probable that the hyaline cells and these only were the iron carriers. We accordingly repeated the experiments, using Denayer's peptonate of iron. Guinea-pigs and rats were fed with this for one to four days, and then killed. The intestines were preserved in absolute alcohol and sections were tested for iron both with ammonium sulphide and with hydrochloric acid and potassium ferrocyanide, while other sections were stained according to the methods already described. It was found that clean staining of the oxyphile granules was possible after the application of the ferrocyanide test to sections. Very beautiful preparations can be made in this way. After the application of the iron test, the section should be washed fairly quickly in distilled water and then very thoroughly in several changes of re-distilled spirit (95%). They are then lightly stained with hæmatoxylin, washed in tap water, stained with very dilute aqueous eosine, dehydrated, cleared in cedar wood oil, and mounted in balsam. Bismark brown is perhaps a more effective nuclear stain than hæmatoxylin. In these various ways we demonstrated the presence of iron in large numbers of wandering cells in the villi and in the spleen. These iron-holding cells were in all cases hyaline cells (Figs. 16 and 17).

[1] Hardy and Lim Boon Keng, *loc. cit.*

[2] This *Journal*, XVI. 268. 1894.

It seems to us that in this we have a clear case in which the hyaline cells perform important work directed to the removal of dissolved substances from the fluid in which they are bathed. This result is important since it at once widens our conception of the activities which may be ascribed to these structures—they are not only as phagocytes concerned in the removal of solid particles from the fluids of the body, but we may also conceive them as actively and directly controlling the chemical constitution of those fluids by removing dissolved constituents.

The presence of iron in quantities above the normal sometimes induces a marked immigration of oxyphile cells into the epithelium of the deeper portion of the crypts. The oxyphile cells not only pass into the epithelium but continue through and are found in the lumen. This fact is the more remarkable and inexplicable when we remember that there is no evidence that the iron salt is absorbed by the lining epithelium of the crypts.

Basophile cell. In many respects this is the most interesting cell in the splanchnic series. Unlike the other forms the basophile cell does not lie free in the interspaces of the mucous coat but is attached to the supporting framework of the adenoid tissue. We were for a long time under the impression that these splanchnic basophile cells were not in the strictest sense wandering cells, we supposed that their movements were limited to changes of shape of the cell body and did not carry the cell from place to place. This idea however must be given up since, in two cases—the first of a starving rat in which the lower part of the small intestine contained very large numbers of bacilli, the second of rats fed for 2½ months on fresh flesh from the butcher,—undoubted basophile cells were found thrust between the cells of the endodermic epithelium.

These two cases stand alone and we must regard the migration of the basophile cells as being an event of very rare occurrence. The fixed nature of these cells is shown by the fact that, though the numbers present do vary in different animals, even in different individuals of the same species, yet we have never met with any increase or decrease in number sufficient to warrant us in thinking that they commonly vary very greatly.

The striking changes which the basophile cells exhibit are limited to changes in their granules. The cells are markedly granular in well-nourished animals and become less granular during starvation though the latter change is not readily brought about and it is difficult to produce extreme exhaustion of the granules (Fig. 8)[1].

If however the condition of starvation be complicated with increase in the micro-organisms of the gut, or in any case where micro-organisms are abundant, the basophile cells are much distended with granules which are often much more stable than those found normally (compare Fig. 7 with Fig. 5).

The hypertrophy of basophile cells which commonly occurs when abnormal chemical substances are present, *e.g.* during inflammation, is an old observation of Korybutt-Daskiewicz and Ehrlich, and in a paper dealing with the wandering cells of the frog, Kanthack and Hardy suggest that the basophile cells remove certain substances, probably either of the nature of foreign substances, or the more extreme products of the general metabolism, which may be present in solution in the lymph or blood plasma. The facts which we have stated concerning the splanchnic basophile cells lend support to this view, that is so far as it confers on the basophile cells important chemical activities. The position of the remarkable basophile layer of carnivora is such that the fluid elaborated by the activity of the endodermic epithelium from the contents of the gut must flow past it and be exposed to the action of the basophile cells before it reaches the blood vessels and lymphatics. Similarly the hypertrophy of the granulation when micro-organisms are very abundant in the gut points in the same direction.

As we have said we have never met with any very striking hypertrophy of the splanchnic basophile tissue as a whole but Dr Sherrington has described cells, apparently basophile in nature, in the intestines of cholera patients as being present in numbers apparently far above any observed by us, and Mr Hankin writes to one of us that at the time Dr Sherrington demonstrated his preparations the disposition and appearance of the cells strongly impressed him with the fact that they were present in order to absorb the microbic poison streaming in from the lumen of the gut.

The further question of the exact nature of the action of these cells on the chemical substances brought to them by the cells of the epithelium is one we do not propose to discuss at any length here. The knowledge we possess of the chemical processes taking place in the

[1] Small though they sometimes are these granules show signs of being complex in substance. Strictly speaking the change above mentioned is limited to that portion of the granule which fixes the basic dye.

walls of the gut points to their being mainly synthetic in character, as an instance we have the condensation of peptone to the level of more complex proteids; and we will leave the question of the part played by the basophile cells with the statement that evidence has been accumulating for some time past showing that the basophile granule is a centre of synthetic activity.

The presence of basophile cells in a fœtus at a period of life when the gut is not occupied in the digestion of food, does not necessarily conflict with the view that, at a later time, they take part in the manipulation of the absorbed products of digestion. In the broadening of our physiological ideas we have been led of late to a much less narrow view of function and to a clearer recognition of the interdependence of the chemical processes which are carried out in the various organs. Each part of the body as we now know is a blood gland and in the gut for instance the blood and lymph is exposed to chemical action probably chiefly synthetic in character, by which products of metabolism in other tissues suffer change. Such processes will go on even before digestive activity is manifested and in the splanchnic basophile cells of the fœtus we may see a part of the mechanism involved.

The differences between carnivorous and other forms in respect to the arrangement and number of the basophile cells and in the character of the oxyphile granulation led us to attempt to determine whether these differences had any special relation to a flesh diet. Rats were chosen for the experiment mainly because we had examined a greater number of these animals than of any other species and could therefore better judge what might be called the normal condition. Four tame rats were isolated, in a large wire cage kept scrupulously clean, and fed for 2½ months on fresh butcher's meat freed from fat and obvious masses of connective tissue. One rat died after about a fortnight, the others remained very healthy and active to the end of the experiment. After 2½ months one rat was starved for 3½ days and then the three were killed by beheading, and portions of intestine were fixed in absolute alcohol cold and boiling, and corrosive sublimate both cold and boiling. Apart from the special differences between the starving and fed examples which will be dealt with later there were obvious changes in the condition of the splanchnic wandering cells, all in the direction of an approximation to the carnivorous type. The oxyphile cells did not depart from those found in rats generally either in size, or numbers, or in the size of the granules. On the other hand they differed widely from normal rats and approached the carnivorous type in two respects, (1) the granules were scarcely preserved by absolute alcohol, and (2) they did not stain very readily. The basophile cells were present in more than normal numbers especially in the mucous coat of the stomach, they were however scattered in the parenchyma and there was absolutely no formation of a definite basophile layer. But though there was no formation of a basophile layer an unparalleled state of affairs was found, for basophile cells in considerable numbers were in the epithelium, thrust between the cells and either spherical, irregular, or elongated in shape. These intrusive basophile cells were remarkably abundant in the walls of the upper portion of the pyloric glands, in the small intestine they occurred in the walls of the crypts.

It was very clear that the basophile granule had altered considerably, especially in its chemical composition. The granules were smaller than in the splanchnic basophile cells of normal rats, indeed one might describe them as being powdery. The change in the composition of the granule was striking. The most satisfactory preservative of splanchnic basophile granules is boiling absolute alcohol, but these rats were quite unique in our experience since boiling absolute, completely destroyed all trace of the basophile granules though they were preserved by absolute alcohol at 19° C. The difference in chemical composition was further shown by the fact that union between the granule substance and methylene blue could only be brought about by high temperature, and in a solution saturated with the dye at the boiling point. On the other hand once the combination was effected the stain was intense and very characteristic, the granules showing a bright rose colour.

So far as this initial experiment goes, it points to the conclusion that the peculiar condition of the oxyphile and especially of the basophile cells in the gut of carnivora is a direct result, an acquired feature, impressed on them by the nature of their diet. This is rendered almost certain so far as the basophile cells are concerned by the fact that in three-days-old puppies, though basophile cells were present in considerable numbers, we were unable to demonstrate the presence of a complete basophile layer.

The differences in this experiment between the starving and full-fed digesting rats were that the oxyphile cells were more numerous and particularly full of granules, while the basophile cells possessed an exceedingly scanty granulation in the starving animal. On the other hand there was little or no migration of the oxyphile cells in the starving animal, the increased number of the cells therefore appeared to be due to a diminished drain on this tissue. It should be remembered in this connection that the animal was exceedingly well nourished up to the onset of the short period of starvation.

PART III. ORIGIN OF THE SPLANCHNIC WANDERING CELLS.

Oxyphile cells. It is clear from what we have already said that the splanchnic oxyphile cells of mammalia betray in their structural features a close affinity to the cœlomic oxyphile cells. In Herbivora, Rodents and Insectivora the differences between the two are very small and it is not until we come to the Carnivora that any marked divergence appears. This suggests that the splanchnic oxyphile cells are closely connected with, or are a specialised portion of, the cœlomic cells, and we accordingly find in the frog that a focus of proliferation of oxyphile cells may supply both the small intestine and the peritoneal cavity (Fig. 20).

The walls of the body spaces—pleural, pericardial and peritoneal—of all animals contain areas, usually related to lymphatic capillaries and to blood vessels, which are crowded with wandering cells. In some areas the wandering cells are oxyphile, other areas again, notably about the diaphragm, contain only vast numbers of basophile cells. These areas are probably foci for the proliferation of particular kinds of wandering cells, and if this be the case they are comparable in this respect to the nodules of lymphoid tissue in spleen, or lymphatic gland. We have met with such foci in rabbits, guinea-pigs, rats, and frogs—in all animals in short in which they have been looked for—and Klein noticed proliferating areas in his study of the cœlomic spaces as portions of the lymphatic system[1]. In the frog a focus of oxyphile cells exists in the connective tissue which accompanies the hepato-pancreatic duct, and in sections taken through the small intestine at the point where the hepatic duct opens into it one sees that this focus of oxyphile cells extends into the walls of the gut, and continues a short distance upwards and downwards, gradually thinning out until it merges into the scattered oxyphile cells of the gut walls (Fig. 20). In other words the splanchnic and cœlomic oxyphile cells in this region clearly have a common origin.

Hyaline cells. There is no special level in the mucous coat from which the hyaline cells may be said to originate.

[1] Klein, *Anatomy of the Lymphatic System*, I. 1873.

In the solitary follicles and Peyer's patches however we find within the reticulum hyaline cells of various sizes, the larger number being of the smallest kind, and in cases of inflammation of the gut (enteritis) we have found the mucous coat near these structures flooded with an amazing number of hyaline cells. The lymphatic glands of the small intestine thus form an obvious source of hyaline cells. On the other hand the distribution of these structures is very irregular and large tracts of the intestine are free from them. Further, when, in the condition mentioned above, the lymph glands are flooding the mucous coat with hyaline cells only a part, and that possibly a small part, remain there, the rest passing into the lacteal vessels in such numbers that they appear in sections distended with packed masses of hyaline cells.

These cells which are so drained off are mainly small cells with dark nuclei—in other words what we recognise as the young form of the cell.

General Conclusions.

Although this enquiry has led to only very partial and tentative results so far as the elucidation of the function of the three forms of wandering cells present in the gut is concerned, yet it has established certain solid facts of structure, notably in determining the presence and peculiar arrangement of the splanchnic basophile cells, and in establishing the solidarity which exists between the characters of the wandering cells of the gut and those found in the cœlomic spaces, the peripheral lymph system, and the blood system, in respect to the presence there of the three great types, oxyphile, basophile, and hyaline cells.

It has been claimed for the wandering cells that they form a distinct tissue in the body which has undergone a peculiar development converting it from a system of free cells all possessed of similar characters to one characterised at first by being composed of three kinds of cells[1], while later in its history it becomes specialised in different portions of the body, in the blood, the great extravascular spaces, and, as we now see, in the walls of gut. In each of these places the three kinds of cells are present, though the cells of any one place differ from those found elsewhere sufficiently to enable us to recognise them as being distinct. The magnitude of these structural differences in the case of the gut of carnivora is brought home to us by the fact that when

[1] Hardy, This *Journal*, Vol. XIII. Kanthack and Hardy, *Trans. Roy. Society*, 1893.

Heidenhain submitted his preparations of the intestine to Ehrlich the latter was unable to rank the cells there present (those which contained granules staining with acid dyes, *i.e.* the oxyphile cells) with those which he had classified from other parts of the body.

These views receive fresh support from this work not only from the facts of structure set forth in the preceding pages but also from such scanty light as has been thrown on the activity of the splanchnic cells, for we have seen throughout how distinct are the activities displayed by the oxyphile, the hyaline and the basophile cell. The oxyphile cells for instance may be very numerous without, so far as one can see, any immediate reference to the number of hyaline cells; the oxyphile cells too chiefly wander into the crypt epithelium, while the hyaline cells chiefly wander into that of the villi. Again in the case of the absorption of iron we saw that the hyaline cells alone charged themselves with the iron compounds.

The absorption of iron by the hyaline cells to which reference is made has, as has been pointed out, an interesting significance, since it widens our conceptions of the activities of these structures. We now know them to ingest solid particles and also to absorb into vacuoles in their cell substance and precipitate there matters previously in solution in the fluids which bathe them.

Finally, if we turn to the specialisation of the sporadic mesoblast in different parts of the body the structures found in the gut suggest certain pregnant reflections.

The gut of the mammalia with the exception of the œsophagus and extreme portion of the rectum contains, in its mucous coat, a sheath of lymphoid tissue crowded with wandering cells. Placed here and there in this sheath are foci of proliferation specially related to the lymph stream and apparently peculiarly the seat of origin of hyaline cells. These are the solitary follicles and Peyer's patches. No such development of lymphoid tissue is found in the gut either of amphibia or of reptilia, nor are wandering cells so constantly present in large numbers. How then does this large development of lymphoid tissue and of wandering cells in the wall of the gut in mammalia fit with what we know of the development of the lymphatic system in vertebrates?

We have already alluded to the fact that there appears to be a specialisation of the wandering cells in different regions of the body. A comparison of the condition of the lymphatic system and lymphoid tissue of the body as a whole in different vertebrates presents this fact in a new light.

In the less-specialised lymphatic system of amphibia and reptilia the peripheral part consists largely of irregular spaces rather than of defined vessels, and lymphoid tissue is not distributed along their course in a manner comparable to the lymphoid masses which lie as lymphatic glands on the course of the lymph vessels of mammalia. In the gut, as we have seen, the amount of lymphoid tissue is exceedingly small, and the contrast in this respect between a section of the small intestine of a frog or snake and that of a mammal is very great, and the spleen of a frog does not contain masses of lymphoid tissue such as form the malpighian bodies of the spleen of mammalia.

In these groups of animals in place of the widespread development of lymphoid tissue in lymphatic glands, in the gut wall and the solitary follicles and Peyer's patches of the gut &c., there is a concentration of this tissue in one organ, namely the thymus gland.

In the mammalia a very different condition is found. The lymphatic vessels now ramify as specialised tubes to the most remote parts of tissues, and the lymphoid tissue instead of being gathered into one mass is scattered about the body in masses having special relations to the lymph stream from definite areas of the body.

The significance of this arrangement is obvious when we consider the effect of localised inflammatory lesions. If an infection of some part of the body take place, such for instance as the leg, the effects of the lesion may be traced along the lymphatic vessels of the thigh as far as the lymphatic glands of the groin, but there it abruptly ends. The afferent vessels of those glands bring lymph in quantities above the normal, laden, probably with noxious substances in solution in the plasma, certainly with dead and dying wandering cells and even with microbes. With this disorganised lymph the glands deal, eliminating its poisons and destroying its effete corpuscles, and so long as the glands are capable of coping with the difficulty so long will the lesion remain localised[1].

In the processes which go on during inflammation we probably see merely a gross exaggeration of events occurring under normal conditions. Each group of glands in the body is related to a definite group of tissues in that it receives the lymph flow from those tissues: and we must suppose that the numberless events to which the body is exposed affect the delicate balance of the chemical process, so that from time to time metabolites appear which so far depart from the common

[1] Cf. Hoyer, *Arch. f. mikr. Anat.* xxxiv. 208. 1889.

either in quantity or quality as to deserve the title "abnormal." The effect of excessive exercise may be cited as a case in point.

On such bodies, if we may trust the phenomena of disease, lymphatic glands act so as to preserve the mean composition of the lymph[1]. In the spleen again we have tissue possessing broadly the same histological characters related to the blood stream as lymphatic glands are related to the lymph stream. The processes which we know to occur in the spleen resemble those which are so obvious in inflamed lymphatic glands. In the spleen effete solid matter, such as red and white corpuscles, and bacteria are eliminated from the blood and there too the plasma suffers chemical changes.

An instance of the action of the spleen on the blood plasma is furnished by animals fed with peptonate of iron—in these the spleen will be found to contain an incredible amount of arrested iron held in part by wandering cells entangled in the reticulum, and apparently in part by the cells of the reticulum itself. Unless we make the rash assumption that the iron is carried from the intestine wholly by wandering cells—an assumption which scarcely agrees with the fact of the rapid absorption of iron by the stomach and the paucity of leucocytes there—then some of this very large amount found overloading the spleen must have been removed from the blood plasma.

If we turn now to that portion of the lymphoid tissue and wandering cells which forms the special subject of this paper we see that the great development of this tissue in the gut of mammalia is only a part of the developmental process which has perfected the peripheral vessels of the lymphatic system and placed on their course masses of lymphoid tissue each having a special functional relation to the tissues drained by its afferent lymph vessels.

And as these masses of lymphoid tissue modify and control the histological structure and chemical composition of that peculiar overflow of the tissues and vascular system called lymph, so the lymphoid tissue of the gut with its contained wandering cells modifies the composition of that special lymph which owes its composition chiefly to the activity of the endodermic epithelium.

Note on changes in the cells at the base of the crypts of Lieberkühn in feeding and hungry animals. The presence of large granules in the cells at the extreme base of the crypts of Lieberkühn was first noticed

[1] Hofmeister (*Arch. für exp. Path. u. Pharmak.* XIX. 1885) starting from a consideration of the fate of peptone in the body arrives at a similar view of the utility of the chemical processes carried out in lymphatic glands.

by Paneth[1]. The possession of these bodies seems to be a peculiar character of these basal cells, marking them off from the rest of the epithelium lining the crypts.

In the course of our work on the wandering cells our attention was called to changes in the extent of the granularity of Paneth's cells and a comparison of various preparations showed that those from well-fed animals agreed in possessing a scanty granulation (Fig. 22), while those from hungry animals agreed in possessing numerous and large granules (Fig. 21). This change was observed in rats, and it would appear to show that the granules, like those of the salivary glands, pancreas, and other digestive glands, suffer loss during digestion.

PLATE V.

Figures 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 14, 16, 17, 18, 19, 21, and 22 are drawn with camera lucida and Ocular 4, Zeiss Objective $\frac{1}{12}$th, Leitz. T. L. 170. Same scale as in plate Kanthack and Hardy *loc. cit.*

Figures 10, 12, 13 are camera lucida drawings with Ocular 4, Objective D, Zeiss.

Figure 20 is camera lucida drawing, Ocular 2, Objective A.

Figure 15 *a*, *b* and *c* are sketches from Ocular 4, Objective $\frac{1}{12}$th.

Figures 1, 2, 3 and 4 show oxyphile cells from mucous coat side by side with oxyphile cells from peritoneal cavity, or blood.

Fig. 1. Frog. (*a*) Oxyphile cell from mucous coat of intestine.
(*b*) Oxyphile cell from peritoneal cavity.

Fig. 2. Rabbit. (*a*) Oxyphile cell from small intestine, (*b*) coarsely-granular oxyphile cell from omentum.

Fig. 3. Rat. (*a*) Oxyphile cell from small intestine, (*b*) coarsely-granular oxyphile cell from peritoneal fluid.

Fig. 4. Dog. (*a*) and (*b*) Oxyphile cells from small intestine, (*c*) coarsely-granular oxyphile cell from blood.

Fig. 5. Rat. Basophile cells from mucous coat of small intestine.

Fig. 6. Rat. Basophile cell from peritoneal fluid.

Fig. 7. Rat. Basophile cell from small intestine with micro-organisms very abundant in lumen. Animal starved but ate its own fæces.

Fig. 8. Rat. Basophile cell from small intestine, 3½ days' starvation, gut healthy.

Fig. 9. Ferret. Basophile cell from group shown in Fig. 10. Animal digesting.

[1] *Arch. f. mikr. Anat.* xxxi. 112. 1888.

Fig. 10. Ferret. Tangential section through apex of villus.

Fig. 11. Dog. Almost completely disrupted basophile cell. Small intestine. Compare with this *Journal*, Vol. XVII. Plate 11, Fig. 13.

Fig. 12. Dog. Optical section, longitudinal through villus of dog showing layer of basophile cells at base of epithelium. Eosine and methylene blue.

Fig. 13. Dog. Optical section, transverse through villus of dog showing the basophile layer.

Fig. 14. Dog. Hyaline cells, various sizes.

Fig. 15. (*a*) Living oxyphile cell in hanging drop of inflamed lymph. Frog. Trace of methylene blue added. Successive stages in staining of nucleus shown in (*b*) and (*c*).

Fig. 16. Guinea-pig. Hyaline cell holding an ingested oxyphile cell, and absorbed iron as droplets in its cell substance. Animal fed with Denayer's peptonate of iron. Absolute alcohol. Potassium ferrocyanide and hydrochloric acid. Methyl-eosine and methylene blue. Before the application of the iron test the droplets appear bright yellow.

Fig. 17. Guinea-pig. Hyaline cell holding iron.

Fig. 18. Dog. Portion of crypt of Lieberkühn about $\frac{3}{4}$ down showing wandering of oxyphile cells into lumen. Corrosive sublimate, methyl-eosine, and methylene blue.

Fig. 19. Dog. Portion of epithelium of crypt of Lieberkühn showing degenerated oxyphile cells. Corrosive sublimate, methyl-eosine and methylene blue.

Fig. 20. Frog. Near junction of hepato-pancreatic duct with intestine. (*a*) focus of oxyphile cells which continues into gut wall and into peritoneal membrane, (*b*) pancreas.

Fig. 21. Rat. Cells at base of crypt of Lieberkühn in starving animal ($3\frac{1}{2}$ days), (*a*) oxyphile cell.

Fig. 22. Rat. The same cells in full-fed animal.

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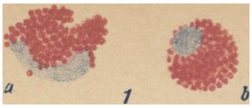

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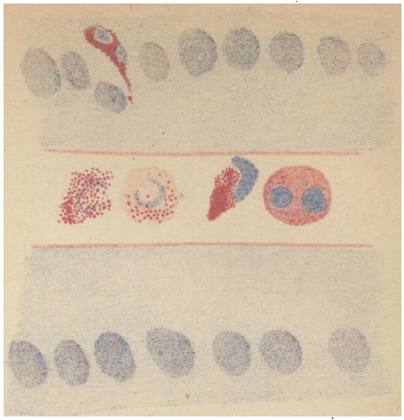

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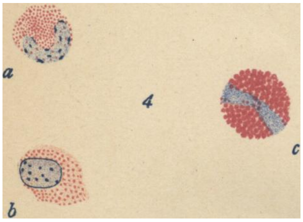

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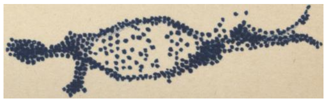

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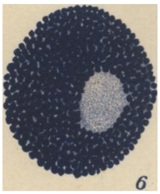

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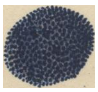

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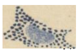

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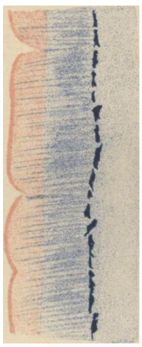

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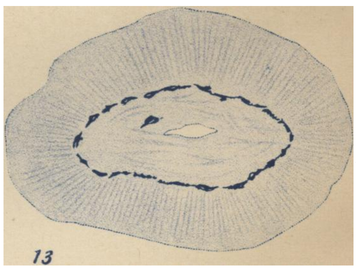

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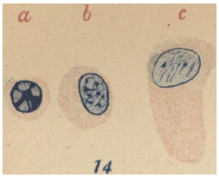

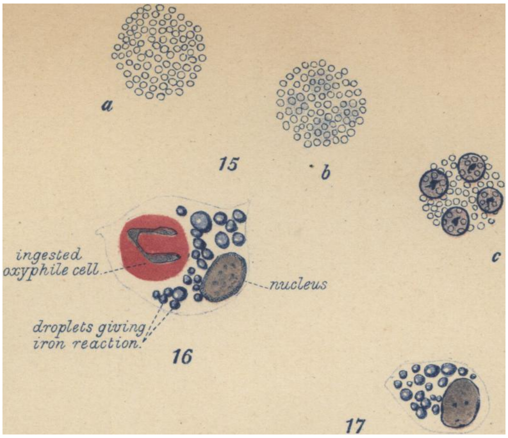

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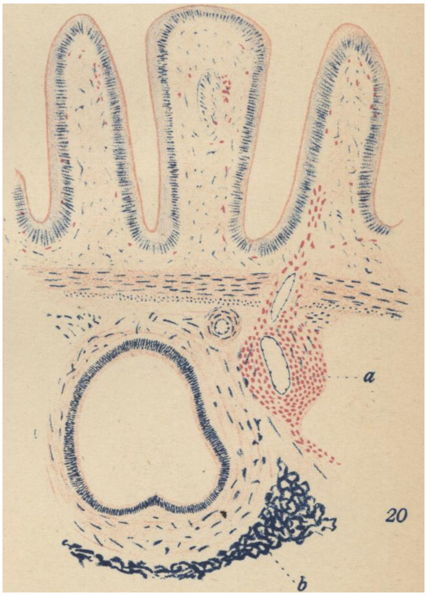

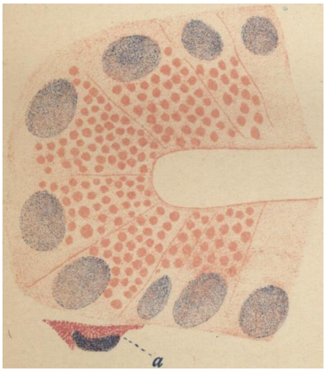

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