Section of Pathology

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Cellular Associations in Normal and Abnormal Lymphocytes

(Illustrated by a Film)

PRESIDENT'S ADDRESS

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COLLECTORS of Churchilliana may like to be reminded of the story of the pathologist who performed a blood count on him. On reviewing his troops, as the Prime Minister described the analysis of the differential count, he commended the polymorphs and monocytes for their meritorious service. "But what are these lymphocytes and eosinophils doing?" The pathologist admitted that he did not know—"but we find them very useful in the laboratory." "Sir", was the stern reply, "The human frame was built before the laboratory. Run away and find out what they are for." So far as I know, however, these orders have not yet been fulfilled, so difficult is it to extend a biological horizon.

It is indeed astonishing that no essential function has been ascribed to the lymphocyte. According to Osgood (1954) the human body contains 1,300 grams of lymphocytes outside the blood, lymph nodes, and bone-marrow; and Yoffey (1956) estimated that their weight in the bone marrow was 70 grams. Osgood again (1954) estimated that there were 3 grams in the circulating blood, and 100 grams in lymphatic tissue. The weight of the human liver is 1,600 grams, not very much more than the total weight of the lymphocytes. Yet Trowell (1958) could say at the end of his survey of the literature "it must be regretfully concluded that the office of this Cinderella cell is obscure".

I propose to discuss whether any suggestions derive from the study of the living lymphocyte collected from normal and pathological conditions. I shall do so in the main by presenting my material as a cinematographic record of some seven years' study, most of which has not previously been presented. This has been carried out as a combined operation with many colleagues, without whose help it would not have been possible.

At the very beginning I must emphasize that it is always a mistake to try to isolate the function of any cell in the body. Cells do not operate in a vacuum. We readily accept the interlaced pattern of hormones, reciprocally influencing endocrine organs; but all the organs of the body and all their cells are integrated. Perhaps indeed there is a simple mechanism whereby the chemical composition of all cells is kept basically uniform. In tissue culture cells may often be observed to sequestrate portions of their cytoplasm, and many, perhaps most cells, constantly take up from their environmental fluid small droplets by the mechanism known as "pinocytosis". In this way the special components of one cell may be, in however small a concentration, incorporated into the substance of others. In the case of many tissues "cell-lines" may be established and readily subcultured *in vitro*. We have isolated such strains from many types of pathological thyroids including carcinoma, from malignant melanoma, Hodgkin's disease, seminoma of testis, and fœtal human liver. We were late-comers in this field; but in common with all others we find that such easily cultivated cells quickly deviate from their early form to a primitive cell indistinguishable from the HeLa cell of Gey. Moreover in the case of the thyroid these cell lines cease to be able to synthesize hormones *in vitro*. They differ from the freshly isolated thyroid in many other ways; for example, in their reaction to certain viruses, in the rotation of the nucleus, and in the manner in which one cell is related to another (Pulvertaft *et al.*, 1959). It seems that at least in some cases normality can only be maintained when specialized cells are in constant relationship with others.

It is on the interrelationship of cells that I wish to focus attention. I shall consider the lymphocyte as a symbiont, if biologists will permit me to misuse a word not altogether appropriate in such a complex system as a living animal. From the enormous literature relating to this cell I shall extract at first two established facts only: first, that lymphcoytes are not uniformly distributed in the tissues, but characteristically are aggregated in foci; secondly, that they are motile. Motility in cells must imply a special function; we are in no doubt

what that function is in the case of spermatozoa, polymorphs and monocytes. When motility ceases, we must assume that the objective has been attained, as journeys end in lovers meeting. The cliché "round cell infiltration" establishes the fact that the wanderings of the lymphocyte are not invariably aimless, but that under certain conditions they clearly reach their destination and cease to move.

Parts of the film show that they move towards certain cells, and remain attached for long periods; if they leave they return again; their cytoplasm licks, as it were, the cytoplasm of other cells; in the cases shown these are malignant cells (Fig. 1). No other motile cell does this;

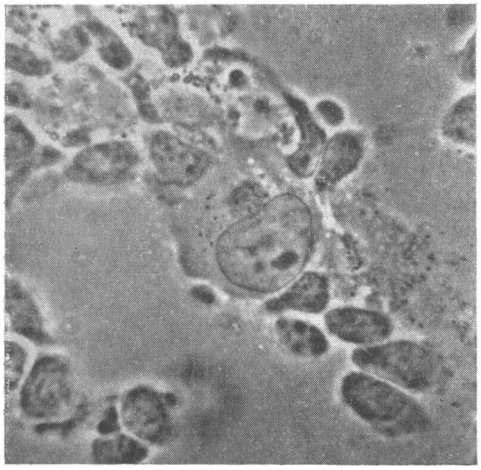

FIG. 1.—Lymph node, Hodgkin's disease. Serum agar. Adhesion of lymphocytes to Dorothy Reed cell. ×850.

Figs. 1–5 are all phase contrast photographs of living cells at 37° C.

for example the polymorph, the eosinophil and the monocyte never attach themselves to other cells in this way. It is true that we cannot deviate the lymphocyte from its apparently purposeless wandering by any chemical bait or by small particulate objects as we can so readily do in the case of the monocyte and polymorph. No one has ever established the existence of chemiotaxis in the case of the lymphocyte. When you see the lymphocyte moving in a time lapse or accelerated film you may be reminded of Graves' poem on the white butterfly:

> "The butterfly, the cabbage white,
> (His honest idiocy of flight)
> Will never now, it is too late
> Master the art of flying straight."

On the other hand, on occasions when the lymphocyte approaches close to certain other cells its movements are directed towards them. Dare we introduce a new word for this and call it "cytotropism"?

We first noted this kind of association in a "blunderbuss" examination of pathological material coming to the laboratory. Phase contrast microscopy opens our eyes to cellular detail which stained smears or sections exclude. We do not appreciate the fact that, with the exception of nucleated and adult red cells, practically every cell in the body is motile. We found that any motile cell tends to adhere to megakaryocytes, but that the lymphocyte differs in that once attached to megakaryocytes it never leaves them, whereas other wandering cells eventually escape. Obviously the megakaryocyte, like the platelet, is very sticky (Pulvertaft and Humble, 1956). It is, as it were, a cellular flypaper. The next feature we noted was that lymphocytes and only lymphocytes became attracted to areas of mitosis. Megaloblasts can be stimulated to divide by folic acid *in vitro* and when they enter into mitosis the lymphocytes are concentrated in their area. Most strikingly they tend to cross the dividing cells still united by a thread of cytoplasm; this gives a ludicrous appearance of "cutting the umbilical cord". Finally, whereas polymorphs, eosinophils and monocytes show no tendency to adhere to malignant cells, in many cases lymphocytes quite definitely do.

When freely suspended in fluid the lymphocyte adheres poorly to glass, indeed some people say that it does not adhere at all. It certainly does not spread out in the same way as a polymorph, and it is readily removed by washing the glass surface. It becomes elongated and detail in nucleus and in cytoplasm cannot be resolved. But when it is placed on a serum-agar surface (Pulvertaft *et al.*, 1956) and compressed under a cover-slip in a special chamber it behaves quite differently. In this case the depth of fluid is of the order of 50μ and it is repelled by the agar and attracted to the glass. It now becomes flattened out and moves characteristically, and its mitochondria can be clearly seen. However, it has been suggested that the adhesive phenomenon to which I have drawn attention may be an artifact, since large cells may produce a sulcus in the agar, and the lymphocyte may move around in the groove so produced. The greater part of the film was not taken with this type of chamber but in a different type in which agar is not used, but in which a small slide cell is made by sticking a ring made of plastic to a cover-slip and a slide. In these cells the lymphocytes move towards other cells and remain attached to them in exactly the same manner. We observed these associations as soon as we examined pleural exudates, malignant tissue, bone-marrow, and lymph nodes, for the

lymphocyte is ubiquitous, and most of our material came from cases of malignant disease. The most striking fact observed, however, was that occasionally the lymphocyte was found inside the cytoplasm of other cells, and that it circulated there for long periods. We gave the term "emperiopolesis" signifying "inside, round-about wandering" to this phenomenon (Humble *et al.*, 1956) (Fig. 2). When we first noted it we were unaware that we had long been anticipated. Indeed the process has been recorded cinematographically by Koller and Waymouth (1953). Trowell (1958) has collected many references.

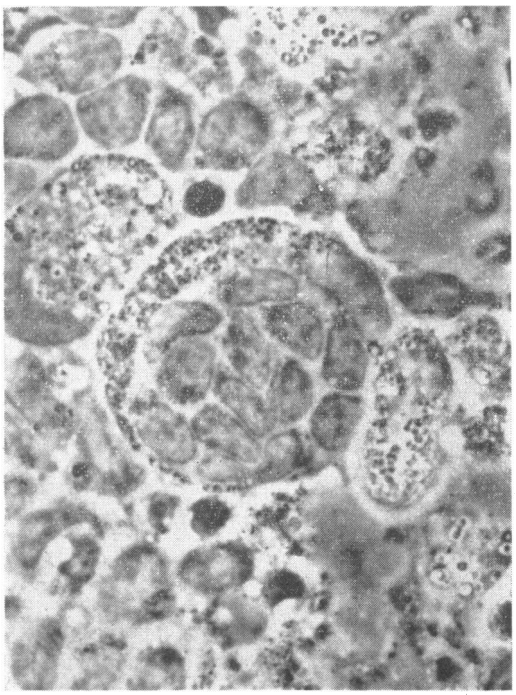

FIG. 2.—Intracellular lymphocytes in culture of human lymphatic leukæmic bone-marrow. Serum agar. ×885.

Some of these observations were made on stained preparations and these I think must be taken with caution, since it is hard enough to be certain of one's facts when living cells are examined. However, Fischer and Dolschansky (1929) observed the phenomenon in cultures of chick spleen. I take particular pleasure in recalling Dolschansky's work since, although my first introduction to tissue culture was in 1919 from Strangeways, it was Dolschansky who in Jerusalem taught me the discipline. Trowell (1949) saw it in mixed cultures of rat thymus and chick fibroblasts, and Darcy (1952) noted it on thyroid (homografts) in rabbits. These last two observations are of particular interest to me for my film deals with mouse thymus and chick fibroblasts as well as with human thyroids and lymphocytes taken from many sources. When we began our studies we were unaware of this earlier work; indeed, one of the interests of biological research is that all who undertake it find themselves irresistibly carried into the same channels, themselves often very bizarre.

Recently Emma Shelton and Mary Rice (1958) have isolated a strain of murine leukæmia in which the leukæmic cells are characteristically within histiocytes in the ascitic fluid which this form of murine leukæmia evokes.

So far as human cases are involved, the phenomenon is best seen both in the bone marrow and in the lymph nodes in cases of Hodgkin's disease and in lymphosarcoma. We have never seen it in normal bone-marrow or lymph nodes. We have examined many normal cases, the nodes being collected from gastrectomy cases suffering from peptic ulcer, and the bone marrow being selected from cases suffering from malignant disease not affecting the bone-marrow. It is customary to examine the bone-marrow before extensive radiotherapy is undertaken.

I therefore take it as established that the lymphocyte has been observed by many competent workers within other cells, and that in certain human diseases it can readily be so demonstrated in appropriate preparations when examined immediately after being set up. That is to say, it is not an artifact of culture.

I turn now to a different but closely allied matter, the duration of life of the lymphoctye *in vitro*. When explanted without other cells and in the conditions of these experiments that life is very short. I have been able to work through the courtesy of Dr. Hewitt with the strain of murine leukæmia isolated by him (1958). At 37° C. the life of these cells is measurable in hours or even in minutes. The cells very rarely indeed flatten themselves or move. A high proportion of them are in mitosis when first examined, but division never takes place. In twenty-four hours the cells have for practical purposes all autolysed.

Although there are a few examples of successful culture *in vitro* of murine leukæmia nearly everyone who has worked with the condition has failed. It would take far too long for me to relate all the variations of culture medium and technique which I have used in an attempt to prolong the life of these cells. I have even collected the cells and set up cultures in complete darkness. Embryo extracts, however concentrated, and whether from mouse or chick, do not extend the murine leukæmic cells' life. Horse, human or mouse serum, mouse ascitic fluid, yeast extracts,

lactalbumin hydrolysate, tryptic digests of serum, culture in pure oxygen, air, and pure nitrogen with the addition of 5% CO_2 all fail. Whenever I have read of a method recommended for culturing murine leukæmic cells I have used it, but always without success. Indeed, one of the very few, and certainly the first successful culture of murine leukæmic cells was that of De Bruyn (1949) where they grew only in the presence of mesenchymal cells; "a kind of symbiosis".

Lymphocytes collected from human reticuloses have *in vitro* and when by themselves as short a life as murine leukæmic cells, although human leukæmic cells are not so short-lived. If we pulp a lymph node in Hodgkin's disease or sometimes in lymphosarcoma the lymphocytes and other cells autolyse at 37° C. almost immediately. The process may be delayed by using soya bean trypsin inhibitor, but this is of limited value.

Lymphocytes from normal human lymph nodes and from many other sources, however, usually survive much longer. As a rule they are fully motile for twenty-four hours, and often survive for four days by themselves. There is continuous autolysis, and at the end of a week the survivors become very large, non-motile, and quite unrecognizable. There is much controversy as to the final destination and morphology of this cell and I shall not further discuss this question here. The significant fact is that the vast majority autolyse within four days. The vulnerability of the lymphocyte is exemplified by its reaction to radiation, since relative lymphopenia is an early indication of radiation damage. This can be demonstrated *in vitro* (Humble *et al.*, 1954). Since I shall develop the argument that lymphocytes and malignancy are closely associated, it is tempting to suggest that some of the beneficial results of radiotherapy may be associated with destruction of lymphocytes.

The picture is very different when other tissues are present. One experiment which I carried out particularly impressed me. I took a very early whole mouse embryo about 2 mm. long and incorporated it in chick fibrin with a portion of murine leukæmic thymus. In a day or two these fragments fused, and the embryo heart continued to beat for six weeks. There was a moderate outgrowth of fibroblasts, but the cells of this chimæra for the most part did not migrate into the fibrin clot. The leukæmic cells could be clearly identified in large numbers and over the course of weeks infiltrated into the embryo tissue and could be recognized around the heart. It appeared that the leukæmic cells slowly increased in number. I cannot tell how long they would have survived had I contented myself with replacing the fluid medium. Unfortunately at the end of six weeks I added fresh chick plasma in order, as I thought, to help the leukæmic cells to migrate. This they did, as well as fibroblasts from the chick embryo. The heart ceased to beat and the migrating leukæmic cells quickly lysed when they had wandered away from the embryo and into the fibrin clot.

In this case, therefore, I had observed a strikingly long survival of the leukæmic cells when other tissue was present. I had noticed the same phenomenon many times in human tissues *in vitro*. For example, malignant melanoma is very readily cultured *in vitro*, and the tissue explanted often contains lymphocytes. I have noted survival of human lymphocytes for six weeks under these circumstances. Human thyroid gland is also quite exceptionally easy to maintain alive *in vitro* (Pulvertaft *et al.*, 1959) and often contains lymphocytes. These are found alive after many weeks.

These observations stimulated an attempt to discover how murine and human lymphocytes would behave when presented with monolayers of other cells. The preparation of monolayers is to-day a routine technique among virologists. They are easily prepared by trypsin dispersal (Dulbecco and Vogt, 1954). Not all tissues are equally suitable but mouse and chick embryo or chick fibroblasts are very suitable and, fortunately, human thyroid. I say fortunately because human thyroid tissue is readily available from the operating theatre. Moreover, it survives for many weeks and does not multiply *in vitro* until what is known as transformation takes place. Embryo tissue unfortunately multiplies *in vitro* within a few days of setting up experiments, and this overcrowding of culture chambers sets a limit to the duration of experiments.

The results were quite convincing. When the

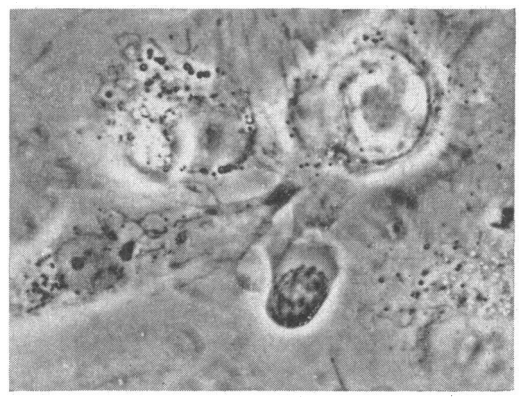

FIG. 3.—Human chronic lymphatic leukæmic cell from blood, with human thyroid. Lymphocyte between thyroid cells and cover-slip. Ring culture in fluid. ×850.

lymphocytes at first sedimented on to the cell sheets they were evenly distributed, but within a few hours as a rule aggregated into clumps. The individual cells remained spherical, but moved around each other. At the end of eighteen hours the picture was quite different.

I may take as an example two cases of cells from human lymphosarcoma which came for examination within a short time of each other. The cell sheets on this occasion were chick embryo. The lymphosarcomatous cells were in eighteen hours concentrated entirely around the areas where there were embryo cells. The sheet was discontinuous, and where there were no embryo cells, there were no lymphosarcoma cells. Most of the lymphosarcoma cells were clearly within the embryo cells. Later these experiments were repeated using human thyroid sheets and cells from a buffy coat from chronic lymphatic leukæmic blood and from the bone-marrow in late lymphosarcoma. In this case the lymphocytes moved freely between the cover-slips and the cell sheets, that is to say the lymphocytes were originally in the free fluid above the thyroid cells, and in eighteen hours were all between them and the cover-slips (Fig. 3).

A large number of tests were then carried out using murine leukæmic cells of Hewitt's strain. As a rule the lymphocytes were obtained from the thymus and in the original experiments sheets of chick or mouse embryonic cells were used as a source. The leukæmic cells were clearly seen to have penetrated the embryo cells, and they divided within those cells on many occasions. Individual leukæmic cells were also seen to autolyse within the embryo cells (Figs. 4 and 5). Survival of the leukæmic cells was noted for many weeks, although the experiments were invariably terminated by crowding of the culture chambers through cellular multiplication, and on no occasion was subculture successful.

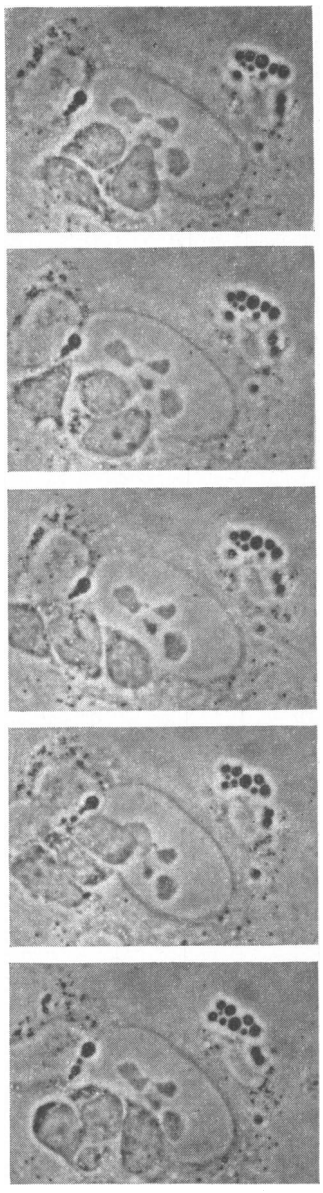

FIG. 4.—Murine leukæmic cells with chick embryo. Penetration of embryo cells. Ring culture in fluid. ×780.

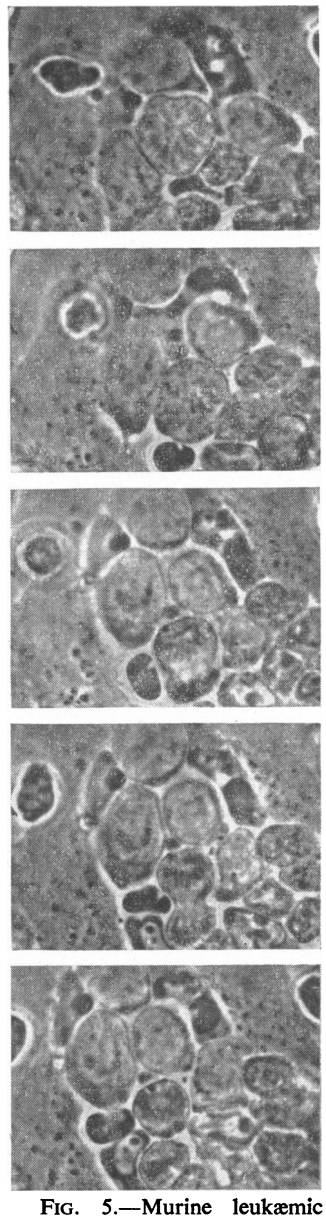

FIG. 5.—Murine leukæmic cells with chick embryo. Multiplication and lysis within embryo cells. Ring culture in fluid. ×780.

Similar results were found with human thyroid sheets, although penetration of thyroid cells was not observed. The movement was apparently always between cover-slips and thyroid cells, but the long survival was equally clear. The experiments here were terminated by the death of the thyroid cells in about a month.

I should make it clear that only a very small proportion of the murine leukæmic cells actually survived. Several can sometimes be seen in one field but the majority autolyse and only those which establish themselves under the thyroid cells survive.

This may be a suitable place to define the criteria for judging that lymphocytes are truly intracellular. I do not think that it is possible to be certain about this in stained preparations. In living preparations unequivocal evidence is provided only when the lymphocytes are seen to move over a nucleus and under a cytoplasmic granule of the investing cell, and where the limits of the investing cell are never transgressed. This I have frequently seen; the granule, the lymphocyte and the nucleus of the investing cell are in three superimposed optical planes and for this reason the appearance is less decisive in cinematographic record than it is to the observer of the tissue culture. Less conclusive, but to my mind quite satisfactory evidence, which is often seen in my film, is available when lymphocytes circulate always within the perimeter of one investing cell, displacing its cytoplasmic granules and distorting its perimeter.

The more frequently observed phenomenon is the penetration of leukæmic cells and lymphocytes through cell sheets and between those sheets and the glass cover-slip. It is difficult to understand in what medium they are now moving as the cell sheets are quite firmly adherent to the glass, and often require trypsinization for complete removal; in many cases they cannot be washed off with culture medium alone. The leukæmic cells and lymphocytes can be seen to squeeze between cells in a remarkable and almost indescribable manner. When cell sheets are touched with a glass rod they often retract, but the presence of the lymphocytes never disturbs them.

Hamster mesentery flattened over agar also makes a good cell sheet and the lymphocyte moves between the cover slip and the mesentery in exactly the same way.

When fully extended on glass lymphocytes and leukæmic cells are quite unrecognizable to an observer familiar only with stained preparations. They cover surprisingly large areas, and the nuclei are markedly patterned. The leading edge of the cytoplasm characteristically bulges, now in one direction and now in another, and the prenuclear cytoplasm is always free of organoids. These trail behind, usually in a pointed tail. Lymphocytes appear to attract each other when inside cytoplasm and under cell sheets where, even when present in small numbers, they constantly converge towards each other. Since by nature of the experiment the field is always illuminated, phototaxis is not ruled out, or even a small local rise of temperature in spite of a heat filter which is always used.

The lymphocytes in Hodgkin's disease and acute leukæmia may show very large granules which by phase contrast are very dark; these do not stain in Leishman films. Transitional forms of lymphocytes and plasma cells are often seen. The plasma cells in myelomatosis move sluggishly and they are unique in my experience because the nucleus is trailed behind. Again in lymphosarcoma, although these cells in general resemble lymphocytes, on occasions an undulating "organdie" edge of cytoplasm may be seen which resembles the movement of monocytes.

I do not wish to confuse my narrative by undue reference to other experiments but it is perhaps permissible to add that certain mouse ascites tumours such as S37 behave in my hands like the leukæmic cells in that they cannot be cultured alone but survive well on cell sheets, and that I have an indication that certain human malignant material, such as carcinoma of the breast, may behave in the same way.

The results show that the lymphocyte is attracted to other cells and cannot long survive without them, may enter into their cytoplasm, may there divide and may there autolyse. And I ask, how does this behaviour fit in with or throw light on familiar facts in the natural history of leukæmia and malignant disease?

I begin with the familiar association between malignant metastases and lymphoid tissue. This of course is often held to be an attempt by the reticulo-endothelial system to remove foreign cells; as we all know it is an unsuccessful attempt. In my hands when malignant cells are collected from metastases in lymph nodes they survive very well, while a primary growth in the breast survives very poorly. Malignant cells in serous exudates where lymphocytes are always present survive reasonably well.

Again we often find in sections a close association between malignant disease and round cell infiltration. One type of carcinoma, the lymphoepithelioma of Régaud, is characteristically rich in lymphocytes. I have seen two cases of chronic lymphatic leukæmia in squamous cell carcinoma on top of leukæmic deposits in skin (Pulvertaft, 1936).

However, undoubtedly the work of Gross and his successors on mouse leukæmia is in this context the most stimulating. Gross (1951) found that malignant tumours could be induced in the progeny of mice inoculated with centrifugates of leukæmic tissue. In his hands leukæmia as well as malignant disease was conclusively produced with these cell-free filtrates. This work has had much confirmation and I would like to refer in particular to the very recent work of Salaman (1959) who finds that newborn mice inoculated with centrifugates of leukæmic blood themselves after short latent periods develop more than one

type of malignant disease, but not leukæmia. There is a large body of confirmatory evidence on this most significant discovery and, although several workers have failed, it seems clear that this failure is more likely to be due to variation in technical method and in strains of mice than to any other cause. It is clear as Salaman emphasizes in his review that, where donor and recipient mice differ antigenically, induced leukæmias and tumours have been of recipient cell type and not of donor.

In Salaman's own series 40 tumours appeared in 15 out of 23 inoculated mice which survived three weeks or more—the first tumour after eleven weeks. The tumours included carcinoma apparently of salivary glands and of mammary tissue in males as well as females. Metastases were found in the lungs. There were also four sarcomas. It is even suggested by certain workers, such as Hays *et al.* (1957) and Latarget *et al.* (1958) that extracted nucleic acid alone may be effective. There are further suggestions that the lymphocytes may enter other cells and be re-utilized for the manufacture of new lymphocytes in the work of Hamilton (1954, 1956): his experiments indicate that some lymphocytes survived eighty-five days, and that others either had a half-life of three hundred days or were used by stem cells for the production of fresh lymphocytes.

To sum up, normal lymphocytes when alone have a short life; supported by other cells, a long one. "Malignant" lymphocytes have frequently a still shorter life in tissue culture; but here again when in symbiosis their life term is greatly extended. There is a close analogy also between their behaviour and the life of certain parasites such as protozoa and viruses.

Lymphocytes, whether normal or malignant, apply themselves closely to other cells, and are in some cases attracted by malignant cells; the cytoplasm of lymphocytes appears at time to be "licking" that of the malignant cell.

"Malignant" lymphocytes are often found inside histiocytes when first collected from cases of reticulosis. They move inside them, not in vacuoles, but freely in the cytoplasm. "Malignant" lymphocytes readily invade tissue cultures of chick and mouse fibroblasts, and are found freely motile inside their cytoplasm in considerable numbers. In murine leukæmia they divide and autolyse within embryonic cells.

There is a distinction between the behaviour of lymphocytes with thyroid sheets and embryonic sheets. I have never observed penetration of thyroid cells, but only movement between them and the cover-slip, while in the embryonic cells there is obvious cellular penetration.

A large series of lymph nodes and bone-marrow preparations has been examined in which there was no diagnosis of malignant reticulosis; in no case was there evidence of histiocytes with included lymphocytes when freshly examined. On the other hand, all lymphocytes, normal and abnormal, penetrate between sheets of cells and cover-slip.

It may be suggested that there is some special property of embryonic cells and of thyroid cells by virtue of hormone production, which encourages longevity of lymphocytes. I have no observations which might support or contradict such a suggestion.

A profoundly significant fact is that neither I nor any other observer has recorded any cell except the lymphocyte as living and moving within histiocytes. Polymorphs, eosinophils, monocytes and many "blast" cells were present in a number of my preparations, but only lymphocytes were found intracellularly. The moment of penetration was never seen; but from examination of film sequences I am convinced that histiocytes on occasion become enormously extended in thread-like form; the long process becomes curved around a group of lymphocytes, and later the ends of the cytoplasmic thread become joined up; eventually the vacuole thus formed becomes filled in by the cytoplasm of the histiocyte. I believe that the histiocyte is in part at least the aggressor.

We must ask ourselves in what way these observations may be related to the development and propagation of the malignant process. Here we enter the realm of hypothesis, and I do so without apology, for observations and experiments are useless unless they point the way to new conceptions. Research is like "blind man's buff": we touch our target for a moment in the darkness but can neither hold nor name it; only at long last do we grasp it firmly and claim with confidence to recognize it without a doubt. For most of the time we must be content with half-knowledge and uncertain guesses. At any one time "the truth" is merely the closest approximation to reality which we can attain in the light of our present knowledge.

One of the functions of the lymphocyte may be to bring to selected areas concentrations of enzymes and of nucleic acid. The lymphocyte has a cycle; it is derived from stem-cells in lymph nodes and in the reticulo-endothelial system generally, and returns to that system for re-utilization of its specialized chemical components.

The lymphocyte may, like every other cell, become malignant; it is no part of our argument to say how or why. Once malignant its nucleic acid is carcinogenic, and by incorporation into another cell imposes malignancy on that cell.

In the case of lymphatic leukæmia or lymphosarcoma penetration of a stem cell induces the

production of malignant descendants. But we may envisage a state of affairs in which a stem cell may be "invaded" at a stage in which some degree of differentiation had already taken place. In such a case, the adult cells derived from this "invaded" cell will be of various types and we find, as in Hodgkin's disease, a pleomorphic malignant picture with "malignant" polymorphs, eosinophils, monocytes and lymphocytes.

In murine leukæmia the extracts of the malignant lymphocytes display the widespread carcinogenic potency of the "malignant" nucleic acid; not only malignant lymphocytes, but also mammary, salivary gland and connective tissue malignancy are induced. Successful experiments have so far only been performed on newborn mice; and carcinogenic power of lymphocytes in adult animals is not suggested, so far as I know, by any recorded work.

If, however, we examine human malignant tissue in paraffin sections determined to incriminate this cell we can find it guilty at least of association. Anyone who cares to can examine the point at which a Grade I carcinoma of rectum invades normal epithelium and here he will always find large accumulations of lymphocytes. At this point too, he will never find a transition between the mucin-forming normal columnar epithelial cell, with a small basal nucleus, and the malignant cell, with its cytoplasm practically full of nucleus. What is happening? Is the normal tissue just pushed aside by the extending and multiplying malignant tissue? Or is there any possibility that "normal" cells, at this point, are being transformed into malignant cells? If so, by what mechanism? Could it be by invasion by lymphocytes?

Trowell (1958) has collected many references to the existence of lymphocytes within epithelial cells, e.g. Kelsall (1946), Andrew and Burns (1947), Andrew and Andrew (1949). I have no difficulty whatever in identifying lymphocytes apparently within normal epithelial cells at the junctional point of normal and malignant rectal epithelium.

It is regrettably only too easy to support any hypothesis, however preposterous, with selected evidence. A thousand observations contradict the notion of continuous transformation of cells by a continuously acting agent of any kind. Only perhaps in a Presidential Address, where convention frowns upon discussion and contradiction, could such a suggestion be made: and then only with the admission that it is taking an unfair advantage of a long-suffering audience. All I can really claim, with confidence, to have shown is the dependence of all lymphocytes on other cells for survival, and their power of survival inside other cells when "malignant". What light this fact throws on the nature of malignant disease must be for others to determine. It may be that the lymphocyte holds within it the keys of life and of death.

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