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The Cytology of the Contagious (Venereal) Tumour of the Dog.

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Introduction.

No attempt has hitherto been made to elucidate the finer details of the structure of the so-called contagious venereal tumour of dogs. This is the more surprising when one considers what controversy has surrounded the nature and classification of this neoplasm; how much interest attaches to this unique example of a malignant tumour whose constant mode of origin is extrinsic to the body of the host, viz., by a natural grafting of the neoplastic cells from one animal to another; and the importance of studying in closest detail a malignant disease which produces an immunity in those patients that do not succumb to it.

The literature dealing with the nature of this tumour has previously been reviewed (Jackson, 1936). It may here briefly be repeated that while earlier ideas of the non-neoplastic nature of the disease no longer merit consideration and while a considerable body of investigators have been non-committal about the pathological classification of the tumour, opinion has centred mainly on its lymphocytomatous nature (lymphosarcoma; "round-celled sarcoma")—e.g., Sticker (1904), Folger (1917), and (with reservations) Feldman (1932); or alternatively it was suggested (Jackson, 1936), that the cells were originally of neurectodermal origin and the tumour therefore a neuroblastoma (arising originally from precursors of autonomic neurones).

The former opinion was based on what will be shown in this communication to be the inadequate technique of ordinary histological sections (usually paraffin-embedded, or frozen and dehydrated) subjected to general tissue stains; fixatives and stains suitable for cytological studies were not used, nor were any methods employed other than sections. The latter theory arose not so much from morphological considerations as from the discovery that certain rare *spontaneous*(1) canine tumours which have the structure of the contagious tumours arise, not in lymphoid tissue, but in the situation of the primordia of sympathetic ganglia, namely, the heart-base ganglia. In the seven years which have elapsed since this theory was advanced, it has not been criticised. During this period only one further communication on the subject has been made: Kaalund-Jørgensen and Thomsen (1937) conclude

(1) That is, tumours not contracted through contagious transfer of malignant cells and thus of intrinsic origin, viz., composed of the body cells of the subject itself.

that the cells are stellate in shape (reticulum cells), of mesenchymal origin, and the tumour, therefore, a sarcoma. They reject the neuroblastoma theory, without, however, examining the arguments on which that theory is based.

But the present communication is concerned less with theoretical arguments about the nature of the tumour than with the detailed study of its cytology, an aspect which in the past has been neglected by all investigators, not least by those who have been most confident in their opinions of the nature of the tumour cells.

Material and Methods.

The chief source of material has been (artificial) subcutaneous grafts, into young dogs, from naturally occurring tumours, while the latter have also been used to supplement the investigation. Methods used have included examination of the living or unfixed cells, moist- and dry-fixed smears, frozen sections, and gelatin-, paraffin-, celloidin-, and colloidin-paraffin double-embedded tissue. A wide variety of fixatives have been tried, including formol, formol-saline; Zenker, Helly, Regaud; Helly-osmic, Altmann, Champy, Flemming, Bensley (A.O.B.), Hermann, Benoit; Bouin, Bouin-Allen, alcohol, picric-acetic-formol-alcohol; Mann's osmic-sublimate, da Fano, Aoyama; and Ciaccio's acetic bichromate. All phases of the technique, e.g., fixation times and temperatures, washing times, dehydration methods, clearing media, and infiltration methods, have been widely and systematically varied. Stains employed in addition to haemalum-eosin and van Gieson have included Sudan III and Scharlach R (together with modifications to be mentioned later), Nile blue sulphate, Fischler's copper-acetate haematoxylin, Smith-Dietrich; Iron haematoxylin, Altmann and modifications, Benda, Champy-Kull, Hollande's chloro-carmine; Iodine and Best's carmine; Mallory and modifications; Silver impregnations for Golgi(²), neurofibrils, and reticulum; Osmic impregnations for lipides and Golgi.

(²) With reference to silver impregnation of the Golgi apparatus of tumour cells, one feels that an over-optimistic impression is conveyed by da Fano's (1921) remark that the optimum fixation time, when once determined for a given strain (of transplantable neoplasms), is constant. His recommendation—an excellent one—is to take many small blocks and transfer to silver every hour from the 2nd or 3rd to the 8th or 10th; when usually it will be found that the 7th or 8th hour is the most favourable. But one suspects that the size of the pieces may be a crucial factor, and since this is difficult to control precisely, in practice an element of chance plays a greater part in successful impregnation than he is inclined to admit. However this may be, successful impregnations of one and the same tumour (viz. the contagious venereal tumour) result from such widely differing fixation times (as well as silvering times) that it appears meaningless to speak of an optimum time unless block-size were accurately stipulated and controlled. Thus from a single biopsy specimen I obtained good preparations after fixation times *varying* from 3½ to 8 hours (followed by silvering times varying from 12½ to 45 hours). The time of washing between fixation and silvering, as also between silvering and reduction, is also difficult to control accurately, being so short, and may play an important part in success. These varying factors of block-size and washing times are quite probably responsible for the fact that one block may turn out excellently while duplicates, carried through in the same containers and, so far as is humanly possible, given identical treatment throughout the technique, may prove complete failures. At one time I attempted to institute accurate control of the block-size factor, in this as well as in other fixation technique, by the use of a twin-bladed knife designed to cut slices of tissue of constant thickness, and even by punching out from such slices discs of constant diameter by means of a canula or cork-borer. I was unaware that Ellermann had previously devised a double-bladed knife, but not surprised to read subsequently that Fibiger (1928) had found its use irksome in practice.

From the thousands of preparations—good and bad—that have been made, it may be said at once that in obtaining adequate pictures, corresponding with the structure of the living cells, considerable difficulties are presented; and that in general the ordinary methods, especially after paraffin-embedding, are largely doomed to failure in this critical respect. Osmic-containing fixatives are a *conditio sine qua non* for the accurate preparation of the cells in embedded sections, and in my experience it is only with Helly-osmic that a fixation of such quality is imparted that the deleterious effects of ordinary paraffin-embedding are resisted. Formol and sublimate-bichromate and formol-bichromate combinations especially are not suitable(3) for this tissue, at least if ordinary embedding technique is to be employed. For the cell globules to be described later, best results are obtained from a specially devised Acetic-Carbol-Sudan stain previously described by me (Jackson, 1944); for the mitochondria by double celloidin-paraffin embedded sections of tissues fixed in Helly-osmic and stained by a modified Altmann-Bensley-Cowdry method in which aniline-acid fuchsin is preferably replaced by carbol-acid fuchsin.

The difficulties and distortions experienced when this tissue is subjected to the usual routine techniques fully explain the current erroneous conceptions of the structure of the tumour cells. It will be necessary to return to some important aspects of the technique in relevant context later.

It should be emphasized that the preparations on which the following observations were based were made from vigorously growing grafts or from parts of tumours not showing evidence of regression or degeneration, as judged from their high mitotic indices and also from the circumstance that in every case sub-inoculation of the material described or illustrated was successful. This is pointed out especially lest it should be thought that the lipide globules to be described were associated with degenerative changes in the tumour cells. Moreover, in all the material used, anaplastic changes, previously described by me in naturally occurring tumours, were minimal. Marked variation in cell size and shape, nuclear size and shape, and multipolar mitoses in numbers worth mentioning were absent. I am now inclined to the opinion that such changes are by no means essential or typical features of the contagious venereal tumour, but very probably accompany regressive changes and are indicative of reduced activity or conditions of difficult growth for the cells.

Explanatory Remarks on the Histology.

The contagious venereal tumour is composed of a highly vascular collagenous and fibroblastic stroma which supports solid groups or alveoli of the neoplastic cells, between which reticulum fibrils are absent. The parenchyma is regularly infiltrated by small lymphocytes and macrophages, and plasma cells may also be seen. Neutrophil leucocytes occur in variable numbers but are chiefly associated with areas of secondary change, e.g. following ulceration or degeneration. Eosinophils and mast cells may also occur sparsely. The stroma is more heavily infiltrated with lymphocytes and macrophages.

(3) The "suitability" of a fixative must be judged on the extent to which it enables a given tissue to withstand the deleterious effects of the subsequent steps to which it is exposed, and is therefore a relative term. I have on occasions obtained good results with Helly-fixed contagious venereal tumour tissue, embedded in paraffin; but only after the dioxane technique.

Description of the Tumour Cells.

1. *The Living (Unfixed and Unstained) Elements.*

(i) The cells when teased in serum or saline become rounded, measuring up to *ca.* 17μ in diameter. The ovoid nucleus is sharply outlined by the nuclear membrane and reveals no structure except the prominent single or sometimes double nucleolus. The cytoplasm contains few to very many, but virtually constant, highly refractile spherical globules or droplets varying from *ca.* 0·4μ to 2·6μ in diameter. They have a predilection for the peripheral part of the cytoplasm. From ruptured cells they float out into the medium, retaining their shape and size. They are sharply outlined bodies having a pale sea-green colour in transmitted light. A typical appearance is their occurrence in chains, several globules, often of very unequal size, each in contact with the other. This adhesion may be maintained when they float free from the cells (see Fig. 1). No other formed bodies, e.g., mitochondria, were seen with this technique: using a 1·5 mm. (N.A. 1·3) oil-immersion objective with immersed condenser of N.A. 1·4, the cytoplasm, apart from the globules, appeared quite homogeneous under the bright-field microscope.

(ii) Similar preparations under dark-field illumination reveal, in addition to the above features, the presence of numerous fine granules or rodlets (mitochondria?) in the cytoplasm. They are closely packed, so that apart from the highly illuminated globules, a striking black network of the non-refractile intergranular cytoplasm is the chief visual impression received.

(iii) With polarised light the globules are isotropic and no doubly-refractile material was seen.

2. *Supravital Preparations.*

In supravitally stained suspension preparations (neutral red-Janus green film) the globules take on no colour. After long periods of observation the granules may take on a faint and doubtful tinge from the Janus green. The cells remain for days apparently unaltered and this faint staining may well be an agonal phenomenon. Typical and rapid supravital staining was not obtained with this technique, although comparable control emulsions of other cells, including malignant lymphocytes, showed readily the mitochondrial staining.

When the cells are suspended in dilute Nile blue sulphate-saline, the granules stain much more readily and deeply (Fig. 1). In such preparations the globules appeared of a more golden tinge.

3. *Fixed Material: Sections and Smears.*

(a) The Lipide Globules.

The globules (as tested in formalin-preserved tissue) are insoluble in strong acids and alkalis and are soluble in ether, chloroform, acetone, and cold absolute alcohol. It is, therefore, only in frozen or gelatin embedded (non-dehydrated) sections that they are fully retained in their original condition. Embedded sections may be partially successful in this respect, but only if heavily pre- or post-osmicated. This osmication has to be carried to a degree where the tissue becomes very refractory to staining and one has to resort to prolonged bleaching before the application of aniline dyes. In

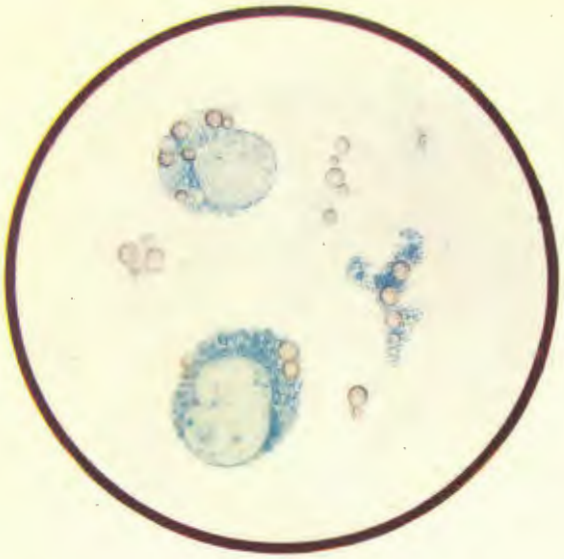


Fig. 1.—Unfixed tumour cells suspended in Nile blue sulphate-saline, showing granules and refractile globules. Note also these constituents set free from ruptured cells.

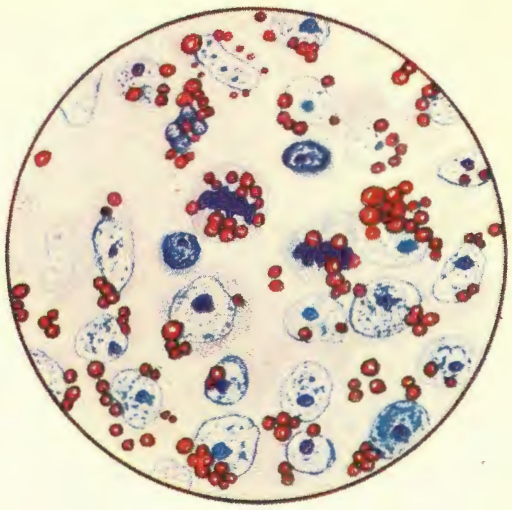


Fig. 2.—Acetic-carbol-sudan staining of the "sudanophobe" (!) lipides. Note, in addition to the resting tumour cells, two cells (centre) in metaphase of mitosis, one infiltrating neutrophil and two fibroblasts of the stroma, all containing lipides, and two infiltrating lymphocytes free from lipides. (Counterstained with Delafield's haematoxylin.) X 1,000. (25116.)

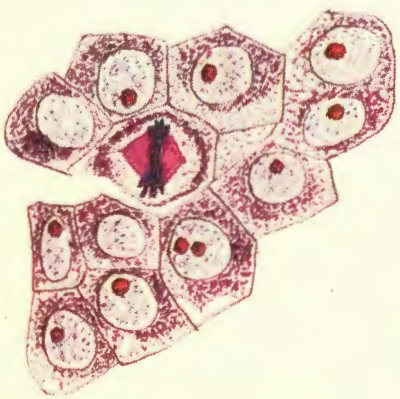


Fig. 3.—Granules (mitochondria), cell membranes, and general features of the tumour cells. Helly-osmic fixation, celloidin-paraffin embedding, and carbol acid fuchsin-toluidine blue staining. Cell in metaphase of mitosis shows achromatic spindle, centrosomes, clear area round spindle, and mitochondrial ring. In some of the resting cells, vacuoles from which lipides have been dissolved may be seen in the peripheral cytoplasm. (Leitz oil-immersion objective n.a. 1·3, with compensating ocular 7 X and immersed condenser of n.a. 1·4.) X 1350. (26865 D3 C8.)

Fig. 4.—Golgi apparatus of the tumour cells. Compare attenuated apparatus of infiltrating lymphocytes. (Da Fano, toned, and counter-stained acetic-neutral red.) X 1130. (23724 AH3.)

most paraffin-embedded material—however carefully cleared, e.g., by the drop method—there is a pronounced tendency for the central or perinuclear part of the cytoplasm to retract from the peripheral part of the cell, which, being composed largely of the globules, has been dissolved. The appearance seen is that of a nucleus surrounded by a zone of granule-rich cytoplasm retracted a considerable distance from the cell-membrane.

This is indeed the essential explanation of the erroneous conception which has been built up regarding the structure of the contagious venereal tumour cells and which has led to the idea that they are round cells, viz., lymphocytes. This shrinkage following dissolution of the globules can largely be avoided by double embedding in celloidin and paraffin; also by Helly-osmic fixation followed by simple paraffin embedding, although from such preparations the globules themselves are still dissolved out.

In unstained, undehydrated frozen sections of formalin-fixed material, the globules are preserved and easily visible owing to their refractility and greenish colour. But they are refractory to the ordinary staining methods. Further, both smears and sections fixed in formalin gave consistently negative results with dyes of the Sudan series, using the techniques usually employed for the demonstration of neutral fats [70 per cent. alcoholic solutions, alkaline-alcoholic solutions according to Herxheimer, 70 per cent. alcohol-acetone solutions; using either Sudan III or Scharlach R of a variety of brands, or Sudan IV according to Kay and Whitehead (1935)].

At this stage of the work, the only methods I had of staining the globules involved the use of osmic acid. It was anomalous that when the osmicated globules were bleached (H_2O_2, H_2SO_3, Cl_2), they could then be stained with Sudan dyes, employing the latter according to ordinary methods. One has not seen this phenomenon referred to in the literature on the staining of " sudanophobe " lipides; it is worth mentioning in case it is desired to stain lipides in embedded section with Sudan dyes. The globules were also positive with Bell's (modification of Ciaccio's) method; but the cells shrink greatly and the globules, running together, appear much exaggerated in size. With Fischler's copper-acetate haematoxylin (" for fatty acids ") and the Smith-Dietrich method (" for lipoids ") negative results were obtained. In frozen sections of formol-fixed material the globules also fail to stain with Nile blue sulphate, even with polymerised solutions which control sections, containing both neutral fat and fatty acid, showed to be reliable. With neutral red, as applied for lipide staining, entirely negative results are obtained, even when heat is used to a degree where the mitochondria start to take up this dye.

It was not until a late stage of this work that the non-stainability of the globules with Sudan dyes (after formalin fixation) was fully overcome. The technique which proved successful has been published separately (Jackson, 1944), but was so intimately associated with the investigation of the contagious venereal tumour that it may be of interest briefly to repeat how it was evolved:—

It will be remembered that Romeis (1927) published a technique of staining with 40% alcoholic solutions of Sudan, whereby he claimed to avoid the solvent effects of the usual 70% alcoholic solutions. This method gave on the whole disappointing results in the hands of Froboese and Spröhnle (1928), who concluded that whatever virtue resided in the solutions of Romeis lay not in their superior preservative effects on fats, but was due to an association between their highly colloidal nature and their actual staining power. The work of Kaufmann and Lehmann (1929) has been interpreted as supporting Romeis in this controversy.

Although Romeis (1929) then published improved and indirect methods of preparing and using the 40% alcoholic solutions, which he found to be greatly superior to the original "direct" method, none of these methods has come into general use. Apparently the brand of Sudan used is an important factor for success, but this was not disclosed by Romeis; moreover, rather elaborate precautions were stipulated in the actual technique of staining, which in themselves suggested that the methods were capricious.

For the purpose of staining the lipide globules of the contagious veneral tumour, both methods of Romeis were tried out extensively. Using the 1927 or "direct" method, I found that most samples of the dye available to me gave quite unsatisfactory results. On occasions, good results were obtained with the well-known Batch 55572 of Sudan IV (British Drug Houses) used according to Romeis; but the method was capricious and precipitates were troublesome.

Ultimately a striking improvement in respect of reliability, constancy, and completeness of staining these refractory lipides was obtained by the use of highly colloidal solutions of the Sudan dyes, prepared by the addition of carbolic acid to the stock solution. The method of preparing the stain (Acetic-Carbol-Sudan) is described in my publication referred to above.

With the acetic-carbol-sudan method the globules stain brilliantly orange-scarlet (Fig. 2). There is no question that it succeeds on account of the staining powers of the solution, not (as the findings of Kaufmann and Lehmann, on lipides in general, might suggest) because of the preservative effect of the somewhat reduced alcoholic content (60 per cent.). Sections immersed in 70 per cent. alcohol for 20 minutes (i.e., the usual time of staining with 70 per cent. Sudan) still contain numerous globules. Smears actually stained in 70 per cent. alcoholic Sudan and found to be completely negative for lipides in the tumour cells can still be stained with acetic-carbol-sudan. Lastly the globules can be stained with carbol-sudan solutions of 70 per cent. alcohol content, although this concentration is not the optimum one.

In addition to the globules in the tumour cells, this method reveals also considerable numbers of fine fat globules in the infiltrating neutrophils, when these are present; and the macrophages which are found among the tumour cells and in large numbers in the stroma are seen to be densely packed with coarse fat globules, which are, however, also stainable (although as a rule weakly) with the ordinary Sudan III or IV methods. Even the fibroblasts of the stromal septa contain fat globules in rows running from the poles of the nuclei out into the cytoplasmic processes. The ubiquitous infiltrating small lymphocytes are by contrast entirely negative (Fig. 2).

Both in smears and in sections the globules have some tendency to fuse with one another, producing elongated or oval structures. In material which had been preserved for some considerable time in 10 per cent. formalin, unstained sections showed that some of the tumour cells contained elongated crystals, which are also stainable with acetic-carbol-sudan and which are anisotropic. As such crystals were not seen in the living or freshly fixed cells, it is quite probable that they represent a change undergone by the globules when they lie a long time in formalin solutions. Also in such old formalin material a small proportion of droplets may start to show a weakly positive reaction (blue or bluish violet) with Nile blue sulphate.

(*b*) *The Granules* (*Mitochondria?*)

These may be stained by Altmann, Benda, Heidenhain, or Bensley-Cowdry methods after a variety of fixatives, but often one does not succeed.

They tend to stain more weakly than the mitochondria or other cells (infiltrating lymphocytes) in the same preparation. The best staining occurred in Helly-osmic fixed tissue, double-embedded, cut at 2μ, and treated as follows:—

> After removal of paraffin with xylol, absolute alcohol and ether (ãã) to remove celloidin; absolute alcohol; descend to aq. dest; bleach according to Lustgarten-Pal (0·25 per cent potassium permanganate, then 1 per cent oxalic acid and 1 per cent. potassium sulphite ãã for say 4 minutes each; but time to be ascertained by trial). Stain according to the Bensley-Cowdry acid fuchsin-methyl green method, except that instead of aniline-acid fuchsin, carbol-acid fuchsin is used.(4) Much more acid fuchsin will dissolve in 5 per cent, aqueous carbolic acid than in the time-honoured aniline water, and the staining is correspondingly more intense. Light green, toluidine blue, etc. may sometimes advantageously replace methyl green as the counter-stain.

The results obtained with this technique are illustrated by Fig. 3.

It is important to immerse the condenser when studying the granules, since sections in which they are fully preserved and stained are considerably opaque and also many of them are exceedingly minute and may otherwise be missed. Even with the careful technique as suggested above, I was not satisfied that these preparations always give an absolutely faithful picture of the granules. By comparison with the unfixed cells or with such silver or osmic Golgi preparations in which the granules are impregnated,(5) they often appear fewer and coarser than they are in the natural state. Further, it is a matter of considerable difficulty simultaneously to secure optimum differentiation of the tumour cells and the elements of the stroma, owing to the danger of over-differentiating the former consequent on the comparatively weak affinity of their granules for the fuchsin. But the carbol-fuchsin suggested helps considerably in this respect.

During division of the cell, the granules arrange themselves in the form of a thick ring around the achromatic spindle and they seem constantly to be coarser and more intensely fuchsinophilic than in the resting cells (Fig. 3). Between the mitochondrial ring and the most peripheral fibres of the spindle there often appears a granule-free zone. This may be a shrinkage artefact, but I incline to the opinion that it is a real area of agranular and chromophobe cytoplasm, which will again be referred to later.

Owing to the common occurrence of the cytoplasmic retraction referred to earlier, one should not fall into the error of concluding from inadequately prepared material that the granules are exclusively juxtanuclear in distribution.

(*c*) *The Golgi Apparatus.*

Successful de Fano and Mann-Kopsch preparations give identical pictures of the Golgi apparatus, which is revealed as a compact robust wreath, about one-third to one-half the size of the nucleus, and composed of dictyosomes which, tending to be arranged vertically to the circumference

(4) Dissolve 40 gm. (!) acid fuchsin by warming in 100 c.c. of 5 per cent. aqueous carbolic acid, cool, and filter. This concentrated solution may be diluted before use if differentiation proves difficult on account of overstaining.

(5) Da Fano regards this as a *desirable* feature of Golgi preparations of tumours, saying that cobalt nitrate-silver technique, when good, nearly always shows up mitochondria as well. Yet the more modern tendency is to strive to make the technique as specific as possible for the Golgi apparatus: surely it was this very unspecificity of imperfect technique which gave grounds for at least some of the objections against the validity of Golgi preparations in general.

of the wreath, give it a spiked or burred appearance (Fig. 4). Often the apparatus is seen to cap one pole of the nucleus, but this is quite inconstant and in many cells it lies adjacent to one side of the nucleus. In the metaphase of mitosis the migrated dictyosomes can be seen re-grouping at each pole of the spindle.

(*d*) *The Centrosome and Achromatic Spindle.*

In preparations in which the mitochondria are unpreserved or, if preserved, are differentiated until decolourised or weakly stained, one can demonstrate a centrosome in many of the cells. It appears as a single granule or a pair of slightly elongated granules or rodlets (diplosome) surrounded by a spherical area of chromophobe cytoplasm (attraction sphere). The centrosomes were best seen in moist-fixed Helly smears stained with iron-haematoxylin (Fig. 6). It is best not to carry the differentiation of such smears to a point where the nucleolus becomes sharply defined from the nucleoplasm, by which time many of the centrioles will have become decolourised. In smears, in many of those cells in which at first glance a centrosome appears to be absent, it will be found to be lying on the nuclear membrane, if the latter is examined in profile or in surface view. In sections, the centrioles, especially of dividing cells can be seen in the mitochondrial preparations (Fig. 3). Those of resting cells may under favourable conditions be distinguished from mitochondria by their coarser size and the clear area (attraction sphere) surrounding them [Fig. 5 (*b*) and (*d*)].

The achromatic spindle as well as the centrosomes showed up in the Helly-osmic fixed double-embedded sections when stained as for mitochondria (Fig. 3). During metaphase (equatorial plate), the spindle axis is strikingly short and the angle subtended at the spindle pole (Mitosenwinkel) is a very wide one. In these respects there is a great contrast with the much longer and narrower spindles of lymphoid cells as pictured and measured by Ellermann (1923). The marginal chromosomes protrude but slightly beyond the equator of the spindle. A fibrillar structure of the spindle was often seen, more especially during anaphase but also during metaphase(6). However, astral rays (as distinct from spindle fibres) were not revealed by the technique employed. In late anaphase what is known as the *Spindelrestkörper* (fusorial bundle—Cajal) was sometimes well seen. It has a striking hour-glass shape. The phenomenon, as is known, is due to a crosswise contraction and lengthwise expansion of the spindle and is considered to be instrumental in completing the migration of the chromatids by *pushing* them to the poles.

(*e*) *The Cell Membrane.*

This is best shown up in double-embedded sections of material fixed in osmic-bichromate or Helly-osmic. Here the polygonal shape of the cells, each surrounded by a sharp fine line, is well revealed (Fig. 3). In such

(6) Contrary to what might generally be thought, the appearance of a fibrillar structure of the spindle, so familiar from text-book illustrations, is presumably indicative of a *less* perfect technique than when the spindle presents a homogeneous appearance. That spindle fibres are an artefact is suggested by observation of living cells and confirmed by microdissection (Chambers, 1924). But reflecting an actual reality in the living cell, viz. the convergent linear pattern in which the more highly hydrated portions of the spindle substance are arranged, this dehydration effect may therefore be described as a *characteristic* artefact, as defined by Darlington (1932).

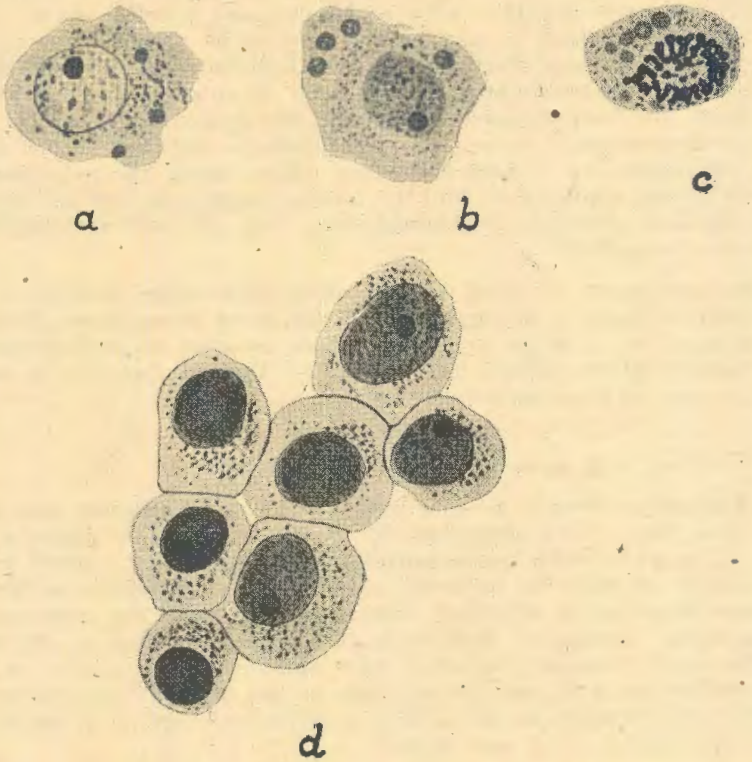


Fig. 5.—Cells from a moist-fixed Champy smear, stained with iron haematoxylin: (*a*) granules and weakly osmiophilic globules; (*b*) contains in addition a probable diplosome surrounded by a clear area (attraction sphere?); (*c*) granules and globules in a dividing cell (metaphase); (*d*) coherent group of cells showing cell membranes and granules, but with globules either unstained or dissolved out during dehydration (note clear peripheral zones of cytoplasm). In the uppermost cell a canalicular structure ("negative Golgi image"?) is seen at one side of the nucleus, enclosing a heavier granule (centriole?). A similar canaliculus is seen in one of the lower cells. X1350.

Fig. 6.—Centrosomes in cells of a smear preparation. Diplosome surrounded by unstained attraction sphere in resting cell. Polar view of equatorial plate of dividing cell, with centriole in focus. (Helly moist-fixed smear, iron haematoxylin.) X 1130. (25116C1).

preparations the cells are seen beyond doubt to be arranged as an epithelium, i.e., closely cemented together without leaving any possible room for intercellular reticulum, which in any case is seen to be absent when impregnations or stains for such fibrils are used (Bielchowsky and modifications, Mallory). The membrane may also be seen in smears—in such groups of cells as have not been dissociated during the spreading (Fig. 5). It is excellently demonstrated in preparations made by teasing (formalin-) fixed material and mounting in Lugol's iodine. Here again the polygonal shape of the cells is well appreciated, and the nuclear membrane and cell membrane stand out as dark greenish lines contrasting with the yellowish nucleoplasm or cytoplasm respectively.

Unless one bears in mind the technical difficulties (solubility of the globules with collapse of the cytoplasm away from the cell membrane) previously referred to, it seems incredible, when looking at good preparations, that so many authors should have described the cells as poorly outlined, rounded or even as forming a syncytium.

(f) The Nucleus; with Remarks on Cell Division.

The nuclear structure, as seen with the usual techniques, and also the nucleoli have been fully described by me in a previous communication. However, it is only with osmic-containing fixatives that, apart from the nuclear membrane and the nucleoli, an absolutely homogeneous picture of the nuclear structure is obtained, corresponding with the appearances in the living cells. Altmann's fluid is in this respect the best of the fixatives used. The more usually employed solutions reveal the robust chromatin network contrasting with the nuclear sap, as has always been pictured; but which, like such details in other kinds of cells, may well be a fixation artefact, however constantly it may appear.

In the prophase of mitosis, there is resolution of the chromosomes within an intact nuclear membrane, and at this stage the nucleolus still persists. The chromosomes tend to arrange themselves so that one limb stands perpendicularly to the nuclear membrane. This appearance is characteristic and should not be mistaken for a regressive change, viz., marginal hyperchromatosis, with which it might be confused, the more especially since, in mitosis, one expects fairly early disappearance of the nucleolus, and as has been stated this does not yet occur(7). At this stage the Golgi apparatus also is intact.

The lipide globules persist during mitosis, and have a tendency to be drawn from their peripheral position in the resting cell into the spindle area (Fig. 2).

As has been mentioned, in later phases of mitosis there is in many dividing cells a striking, pale, ring-like area immediately surrounding the achromatic spindle, due to absence of mitochondria from the cytoplasm of this zone. In favourable circumstances " negative Golgi fragments " can be seen here and there in the zone, which possibly represents the *path* of migration of the dictyosomes, and apparently is not a fixation artefact.

(7) It is of course well known that the nucleolus may persist even to metaphase, at least in invertebrate cells, but the appearance is not usually encountered in animal pathology. It appears that the nucleolus of these tumour cells disappears during early anaphase, in which it is sometimes still to be seen.

Another striking feature about cell division is the great frequency of simultaneous mitosis in pairs of contiguous cells (see, e.g., Fig. 2). Usually, but not invariably, the two spindle axes are at right angles to the line of apposition of the cell membranes. These details suggest that the phenomenon of mitotic pairs of cells does not depend on rapidly successive division of two isogenous elements, since in that case one would on general principles expect that the axes would be parallel with the common cell membrane. Therefore it seems more likely that the effective stimulus to division tends to operate on two neighbouring cells at the same time, these cells not necessarily being daughter cells of a common parent cell.

During early metaphase, coinciding with the disappearance of the nuclear membrane, there constantly appear in the cytoplasm a number of rather coarse basophilic bodies, which migrate to the extreme periphery of the cell and persist throughout metaphase and anaphase. I am inclined to believe that they are of nuclear origin. They were seen in frozen sections stained with very well ripened Delafield's haematoxylin, and require further study.

Discussion.

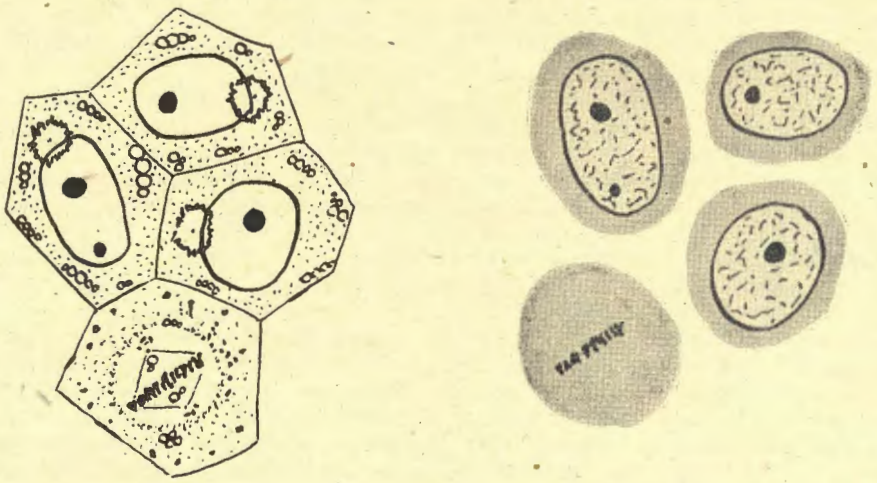
The descriptions and illustrations hitherto available are seen to be quite inadequate (compare Figs. 7 A and B). They show a fairly characteristic nuclear structure (which is of diagnostic importance although very possibly an artefact), a nucleolus, and no cytoplasmic structure (Fig. 7B). Faint traces of the cell membranes have sometimes been pictured, but in general the conception has been that of poorly outlined, rounded cells, widely separated from their neighbours, or, according to Kaalund-Jørgensen and Thomsen, appearing to be in syncytial continuity with one another (stellate cells). The first interpretation has favoured the idea that the cells are of the large lymphocyte type, the second that they may be reticulum cells. Such ideas tend to persist, even though it has been pointed out that the absence of a reticular fibrillar intercellular matrix is, according to general experience, inconsistent; and moreover there is absolutely no evidence of a maturation of the cells (in the direction: reticulum cell→large lymphocyte→medium lymphocyte→small lymphocyte) from which they might derive support, or which indeed one might well demand as proof.

It has been pointed out (above) that this picture depends entirely on artefacts—exclusive reliance on dehydrated material, or failure to use a technique which avoids that shrinkage of the cytoplasm away from the cell membrane which follows dissolution of the lipide globules, the presence of which has not been suspected by the proponents of the sarcoma theory. By comparing well-preserved material with routinely-prepared sections and with the illustrations of previous authors, it is readily seen that the appearance of round cells depends upon complete separation of the cytoplasm from the cell membrane, while the picture of stellate cells results from a less complete separation in which strands of interglobular cytoplasm still maintain a connection with the membrane.

The nature of the globules at first presented a problem. "Vacuoles" in embedded sections were previously described by me (Jackson, 1936) and it was at that time stated: "Unexpectedly the fatty nature of the vacuoles could not be established by frozen sections".(8) Yet the refractility,

(8) In another case the presence of Sudanophil fat was mentioned, but this was considered to occur in areas of degeneration, as indeed it probably did.

droplet form, and refractory behaviour to ordinary stains strongly suggested a fatty substance, and much labour was wasted because of my erronous conclusion, based on the customary methods, that this substance was " sudanophobe ". The chemical implications of the affinity for Sudan only under certain conditions of preparation of the latter(9) cannot be fully discussed in the present state of our knowledge of the microchemistry of lipides, and only chemical analytical methods would appear to be capable of throwing further light on this problem. This work is well worth doing, since it is not unreasonable to ponder whether their very high content of lipides, differing in details of their microchemical reactions from the known fatty substances, may not in some way be associated with the peculiar properties (e.g. extraordinary ease of homoplastic transplantation, powers of survival outside the body, and immunity production) of the cells of the contagious venereal tumour.



A. B.

FIG. 7.—A: Composite diagram of the structure of the tumour cells according to author's conception. Polygonal shape of cells, cell membranes well defined and in apposition (epithelial habitus), lipide globules in peripheral cytoplasm, granules (mitochondria), Golgi apparatus, centrosome, nuclear membrane, homogeneous nucleoplasm, nucleoli. In dividing cell (in addition) achromatic spindle (towards and into which the globules have been drawn), centrioles, re-grouping dictyosomes, clear zone around spindle and outside this a ring-like condensation of mitochondria; outermost, the scattered basophilic bodies which appear constantly during mitosis and which apparently are of nuclear origin. B: The same cells as they would appear with routine technique, illustrating the limitations of the latter and representing current conceptions of the structure of the cells.

While it should be noted that some authorities take a conservative, not to say pessimistic view of the possibility of micro-chemical identification of various of the lipides,(10) and while, therefore, one sets out the ensuing

(9) Namely, in the opinion of Froeboese and Spröhnle (1928) and Jackson (1944), in such a way that they are highly colloidal, instead of molecular.

(10) The crux of the difficulty in practice is that the best observations have been made on the chemically pure lipides rather than on the substances as they occur in nature.

remarks with some reluctance and more for the sake of illustrating the difficulties of the problem than with any desire to draw conclusions; yet according to such criteria[11] as are available, the following substances could be excluded, for the reasons mentioned (often in addition to others):—

Neutral fats should stain with (routine) Sudan methods, stain red with Nile blue sulphate, and be negative with Ciaccio. *Fatty acids* should be positive with neutral red, Fischler, and Smith-Dietrich; if unsaturated (oleic) they should stain deep blue with Nile blue sulphate. *Soaps*, ditto. *Cholesterin esters* would be anisotropic[12], negative with Ciaccio[12], red or reddish with Nile blue, and relatively insoluble. *Cholesterin-fatty acid mixtures* should be positive with Smith-Dietrich, negative with Ciaccio, reddish with Nile blue; and unless proportion of cholesterin very low, would be optically active. *Cholesterin-glycerin ester mixtures* should be negative with Ciaccio and positive with Smith-Dietrich.

PHOSPHATIDES.—*Lecithin*[13] is anisotropic, insoluble in acetone (Maclean, 1918), positive with Smith-Dietrich (Kutchera-Aichbergen, 1925) and with Ciaccio (Ciaccio, 1913, Kasarinoff, 1910). *Cephalin* should be positive with neutral red and with Smith-Dietrich. *Sphingomyelin* is optically active, positive with neutral red, Smith-Dietrich and Fischler, and negative with Ciaccio. "*Cuorin*"—not a chemical compound according to Levene and Rolf (1921)—is insoluble in acetone (Maclean).

CEREBROSIDES.—*Phrenosin* and *Kerasin*: are anisotropic, positive with neutral red, and negative with Ciaccio.

Thus the questions arise: Is the lipide material of the contagious venereal tumour an unknown compound; or (much more feasibly) a lipidal mixture whose microchemical reactions have not hitherto been worked out? Alternatively, are the tests, by which the known substances and the familiar mixtures appear to be excluded, completely reliable? The phenomena previously mentioned as having been casually observed in old formalin-preserved material may perhaps give a hint as to the probabilities, but it is scarcely necessary to say that, in the present state of our knowledge, such investigation does not lie within the province of histopathology.

Regarding the bearing of the globules on the identification of the cells, it may be mentioned that globules of this kind have not been encountered by me in any noteworthy or comparable numbers in malignant lymphoblasts

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[11] These are given (except where other authors have been cited) by Herxheimer (1924) and Leupold (1935), and are based largely on the extensive investigations of Kawamura (1911).

[12] A discrepancy of views may be noted here. One of the esters of cholesterin—cholesterin oleate—according to Kasarinoff (1910) is isotropic and, like cholesterin palmitate, positive with Ciaccio. This opinion is not shared by Kawamura, Herxheimer, Leupold or by Siegmund (1935), who treat these esters as a group and mention no exceptions in the reactions. In any case, there would still be the objection that cholesterin oleate is stated specifically by Herxheimer to stain red (and cholesterin esters in general by Leupold to stain reddish) with Nile blue. One could wish, however, that this difference of opinion between Kasarinoff and Kawamura had been satisfactorily resolved, and it is the more unfortunate that in Kawamura's table (*loc.cit.*, p. 18) occurs an error which further obscures an issue quite possibly relevant to the very problem we are discussing.

[13] The reaction of lecithin with neutral red remains in doubt.

(e.g., the cells of canine lymphoid aleukaemia) examined under similar conditions; and according to Wiseman (1932) non-staining refractile " vacuoles " are absent from lymphoblasts. " Vacuoles " are, however, known to occur in *lymphocytes*: see for example Bloom (1938), who further states that " supravital staining has shown (them) to be of fatty nature ".

The granules or mitochondria of the tumour cells are more numerous, finer, more evenly distributed in the cytoplasm, and less rod-like in form than one might expect if the cells were lymphocytes. Wiseman (*loc. cit.*) states that lymphoblasts have very numerous mitochondria, but mostly in the form of *large* rods. These granules of the tumour cells show some differences of staining from those of lymphocytes and most other body cells. It may be pointed out that this applies also to the normal elements of the nervous system, so that it is not yet certain that granules of nerve cells (which by Held were termed " neurosomes ") and of glia cells (known as gliosomes) are to be wholly identified with the mitochondria of cells in general.

Like the globules and the granules, the Golgi apparatus has not previously been described. It is unfortunate that the Golgi of lymphoblasts, especially the cells of lymphocytomatous tumours, has not been satisfactorily investigated. Cowdry (1921) has pictured the apparatus in erythroblasts and states that in lymphocytes it is similar. But obviously it is to small or medium lymphocytes that reference is made. Maximow (1932) gives an excellent picture of the Golgi in cells from the germ-centre of a lymph-node, but again it would appear that these are medium lymphocytes.([14]) It would be easy to say that the apparatus in the cells of the contagious tumour is much larger than in those cells in which hitherto it has been impregnated and described; but it would be unfair to the protagonists of the lymphosarcoma theory if one compared the tumour cells with any cells of the lymphocytic series except the *large* lymphocytes or lymphoblasts.

Among other findings that may be of importance in identifying the cells of the contagious venereal tumour and especially in distinguishing them from cells of the lymphocytic series are: The shape of the nucleus, usually regularly oval as compared with the lymphoblast nucleus—usually round (Wiseman, *loc. cit.*) or with the lymphocyte nucleus—typically showing an indentation. And lastly, the mitotic angle:—

It has been mentioned that Ellermann (1923) devised a technique of measuring the mitotic angle (or, as it might preferably be called, the spindle angle) of dividing cells. Dealing only with the precursors of the elements of the blood, he found very constant average differences between cells of different types. Later he (1924) actually applied this criterion to distinguish (in man) between myeloblasts and lymphoblasts of acute leukaemias, in which, owing to the failure of the cells to mature, other means of distinction are lacking. These variations in the average spindle angle are of no mean order, e.g., erythroblasts 21°, lymphoblasts 40°, myeloblasts 69°. Ellermann further showed that these findings are constant for the leukaemic counterparts of the normal lymphoid or myeloid cells. His work has been confirmed, at least in so far as myeloid cells are concerned, by Petri (1926), whose own figures agree very closely with Ellermann's.

([14]) As the magnification is stated, one can calculate that these cells are in the neighbourhood of only 12μ diameter.

The following values were obtained for a random sample of measurable spindle angles of the contagious venereal tumour cells in Helly-osmic or Champy fixed, celloidin-paraffin embedded material:—

121	115	141·5	106
115	103·5	138·5	107
121·5	100	112·5	102
126·5	112·5	133	105·5
124	130·5	110·5	132·5
99·5	105·5	121	103
118	114·5	113·5	116
116·5	112·5	106·5	97
100	109	100	116
125·5	100	110	113
109·5	110	107	107·5
118·5	104·5	118·5	104·5
117	102	110	115
137	119	117	107·5
117	105	99	106
122·5	123	118	94
100	122·5	128·5	108·5
107·5	127·5	118·5	123
131·5	129·5	105	115
119·5	115	97·5	

Mean = 114°. Std dev. = 10·6.

Utilizing the *t*-distribution and the standard deviation of the mean angle, viz., 1·2, it will be seen that the range of the " true mean " is given by $114° \pm 3·2°$ at a probability level of $P = ·01$. This means that in hundred-fold repetition of the experiment this statement would be rejected once. It also follows that the mean angle will always remain an obtuse one.

Now Ellermann's data show that it is justified to distinguish myeloblasts from lymphoblasts on the basis of a difference of 29° between their mean spindle angles.([15]) It may be pointed out that the mean spindle angle of the contagious venereal tumour cells differs from that of lymphoblasts by more than twice that amount, viz., 73°, so that in this case distinction should be obvious from mere casual inspection, the more especially because in the case of lymphoblasts the mean angle is always acute, whereas in the case of the contagious tumour cells it is always obtuse. It may further be noticed that Ellermann in fact never observed an obtuse angle in a lymphoblast, while I did not see an acute angle in the tumour cells.

([15]) The so-called " small sample methods " applied above were unknown at the time of Ellermann's work; but although his ranges of variation ($\pm 2\sigma$, $\pm 3\sigma$), based on the so-called " Normal Theory ", are not accurate, a re-test of his data shows that he was fully justified in distinguishing between the cell types which he studied. A direct comparison of the present results with those of Ellermann may be instituted here. Supposing, as Ellermann did, that 16 angles were measured in each case, the ranges (at $P = ·01$) respectively become:—

Ellermann { Erythroblasts: 21° ± 4·7.
Lymphoblasts: 40° ± 8·3.
Myeloblasts: 69° ± 9·1.

C.V.T. Cells: 114° ± 3·5.

The mean spindle angles of those cell types for which data now exist are compared graphically in Fig. 8. It is of some interest to note that the contagious tumour cells differ from the others (*known* mesenchymal derivatives!) in having an obtuse angle. Fig. 9 shows a comparison of the frequency distributions of spindle angles of lymphoblasts (calculated from Ellermann's data) and contagious venereal tumour cells; the frequencies are in both cases expressed as percentages, so that the comparision may be direct. It will be seen that the distributions do not even overlap.

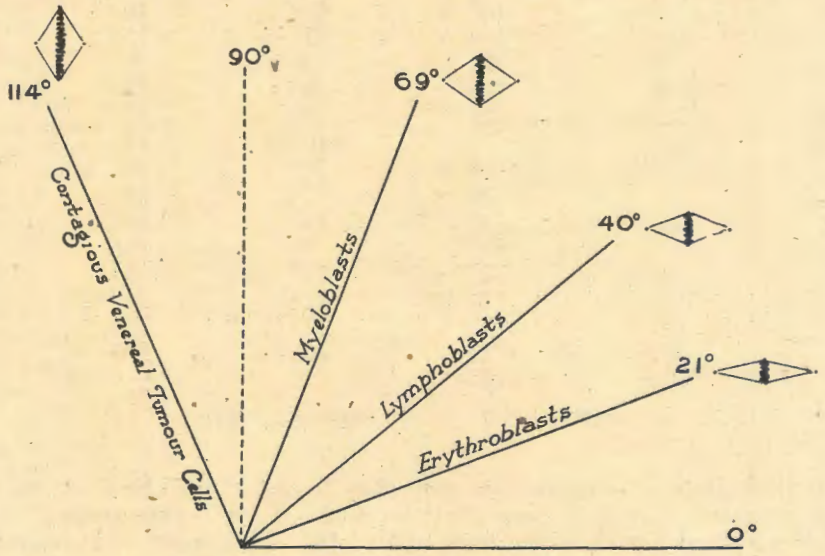

Fig. 8.—Mean spindle angles of cells of various types: Precursors of human blood cells (including lymphoblasts), according to Ellermann's data, compared with the contagious venereal tumour cell.

A weakness of the present discussion is that, in the absence of comparative data on the spindle angles of lymphoblasts, one has had to compare the tumour cells with human lymphoblasts. It must be left for future work to determine whether this is justified, namely whether spindle angles remain constant for given cell types irrespective of species—at least within mammalia.

No data exist which permit a comparison of the spindle angle of the contagious tumour cells with that of known neurectodermal derivatives. Indeed, the usual neuro-histological methods do not demonstrate the achromatic spindle, which has seldom been pictured in cells of neurectodermal derivation. Mallory (1923), a pathologist whose technique was so meticulous that spindles and centrosomes are often quite casually depicted in his text-figures, gives illustrations in which the spindles of glioma cells are to be seen; but unfortunately, although in some cases it is even possible to measure the angles, the number is far too small for purposes of comparison.

Regarding the attempted identification of the contagious venereal tumour cells with cells of the lymphocytic series, until more accurate information becomes available about the cytology of the large lymphocytes, when

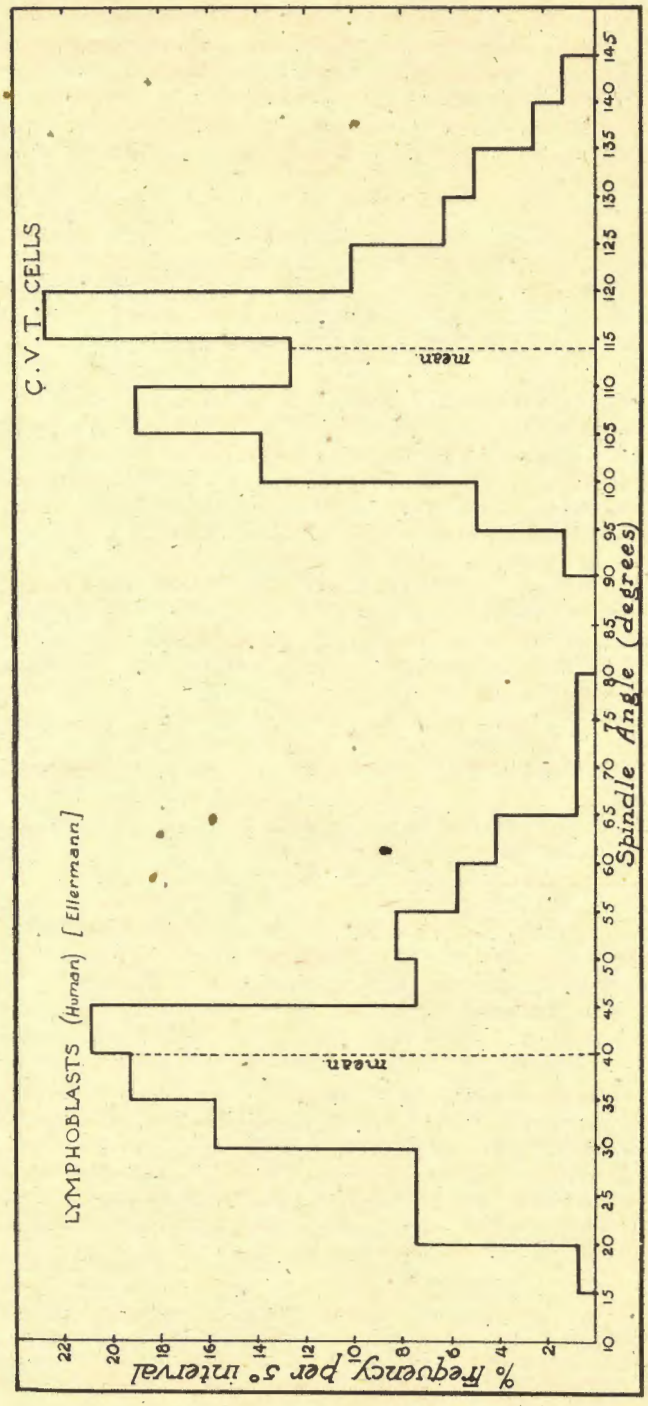

Fig. 9.—Frequency distribution of spindle angles of the contagious venereal tumour cells compared with that of lymphblasts according to Ellermann's data.

the various features discussed may assume a greater significance, it would appear that it is in the highly developed cell membrane, the polygonal shape of the cells, their epithelial arrangement, and the lack of intercellular reticulum that the most certain departures from the morphology of cells of the lymphocytic series—according to current conceptions—are brought to light. But once more the reservation may be made that it is possible that the large lymphocytes, if prepared according to meticulous technique, might hold some surprises for us.

Still less have the cell characters described been worked out for apolar neuroblasts, neurological embryology having concerned itself but little with cytological technique. Nevertheless, it is at this stage fair to say that evidence against the lymphocytomatous nature of the tumour tends to support the alternative theory that it may be a neuroblastoma.

The term " neuroblastoma " has been used by various authors for neoplasms which more modern analysis, correlating embryological with morphological considerations, has shown to comprise several different types. In the widest sense, stating the matter roughly, " neuroblastoma " has included all those malignant tumours of neurectodermal derivation whose cells are not differentiated in the direction of neuroglia proper; in other words, neuroblastoma and glioma are contrasted. The work of Bailey and Cushing (1926) and Bailey (1927) especially has shown what a heterogeneous group this comprises. Wright (1910) used the term " neuroblastoma " for neoplasms, previously confused with lymphosarcomata (round-celled sarcomata), which are known to arise from the precursors of sympathetic nerve-cells and which to-day would presumably be grouped with the tumour known as medulloblastoma. They were characterised by the " pseudorosettes " of Bailey and Cushing, not containing neurofibrils. Bailey and Cushing, who have brought so much order into the confusion surrounding tumours of neuro-ectodermal derivation,([10]) restrict the term neuroblastoma to tumours whose type cell is in fact differentiated as a neuroblast. This is, of course, the only logical course to pursue, but it necessitates wordy explanations when one has to discuss the older literature. A great practical difficulty, however, exists in the recognition of some neuroblastomas (in this restricted sense). Neuroblasts may be apolar, unipolar, bipolar, or multipolar. Now the three types of polar neuroblasts are easily recognised by silver impregnation of their processes, but play little part in the composition of tumours. The apolar neuroblast—probably the most important in the series in so far as its participation in neoplasia is concerned—is difficult to recognise as such. Theoretically, one should, as in the case of its normal counterpart in embryological material, be able to impregnate with silver a fibrillogenous zone of Held: an area of argentophilic cytoplasm at one pole of the cell from which the process would later arise if developmental potentialities were realised. But in practice this has not been achieved. Hence even Bailey and Cushing do not go further than to suspect certain of their tumours of being apolar neuroblastomas, saying " . . . It seems probable that the cells are

([10]) For which, however, they can find no better term than " tumours of the glioma group ". It may be suggested that this group be referred to as the neurectodermatogenous neoplasms. The term, if perhaps a little clumsy, has the merit of conveying exactly what is implied by the desire to place these neoplasms in a single class, viz. their common embryological origin.

apolar neuroblasts ''. I have likewise failed to demonstrate a fibrillogenous zone in the cells of the contagious venereal tumour. In spite of disappointment, efforts to do this should be continued.([17])

Meanwhile, it should be pointed out that the general appearance of these probable apolar neuroblastomas, as illustrated, is indistinguishable from that of the contagious venereal tumour—compare Bailey and Cushing's (1926) Fig. 38 (*b*) with any moderately well fixed section of the contagious venereal tumour stained with a general tissue stain; or with Feldman's (*loc. cit.*) Fig. 172.([18]) In both cases the cell membranes and polygonal shape are well depicted, so far as this is possible with routine technique, and one sees unmistakably the characteristic shrinkage of the cytoplasm away from the membrane, as occurs in fact as a sequel to solution of the lipide globules. Nuclear characters are likewise similar. Even the cytoplasm is in both these publications described as granular. It is not too much to say that the more one's reading and experience of the difficult problem of tumours of neurectodermal origin increases, the more acutely does one suspect where the truth about the supposedly mysterious nature of the contagious venereal tumour lies.

So far as the routine diagnosis of the contagious venereal tumour is concerned, the observations presented show that diagnosis is quicker and surer if fresh material is teased in serum or saline with or without the addition of Nile blue sulphate. Where only fixed material is received, the tissues may similarly be teased, and here the use of Lugol's iodine is an advantage. It is quite remarkable how much can be learned about the tumour cells by such simple methods, given good optical conditions. Most instructive preparations can be made by the simple expedient of mounting a formalin-fixed section in Lugol's iodine and examining under oil, with immersed condenser and a powerful concentrated source of light (e.g., " point-o'-light "). Again the cell membranes are well seen and the polygonal outlines of the cells are undistorted. The cytoplasm appears uniformly stippled with slender rodlets, the granules, which somewhat obscure the globules. (The latter require careful focussing in Lugol mounts, and are better studied in sections mounted in water, in unfixed material, or above all with the acetic-carbol-sudan technique). The nuclear membrane

([17]) It does not seem beyond the bounds of possibility that impregnation of the Golgi apparatus represents success in technique towards this end, while impregnation at a " fibrillogenous zone " constitutes a partial failure, i.e. is the result of a less specific technique. It is indeed surprising that no one has remarked on the similarities between cells impregnated to show the " fibrillogenous zone " and cells which have become over-impregnated in Golgi apparatus technique. The zone of Held is about the size of the Golgi, it is confined to one pole of the cell, it appears in striking fashion to cap the nucleus. If one imagines the archoplasm within the Golgi net to have also become filled up with silver and the dictyosomes to have acquired a fused appearance due to deposition of silver between as well as on them, it is not difficult to see in the " fibrillogenous zone " an overimpregnated zone of the Golgi. This suspicion is further pardonable when one considers (*a*) that the relationship between the Golgi and fibrillogenous zone has never been stated; and (*b*) that at least we do know that the Golgi pole and the fibrillogenous pole of the cell are identical, according to Cajal (1933). His fig. 399 (*loc. cit.* p. 463), shows vividly how the development of the primary process (and probably even of the secondary processes) of the neuroblast is intimately associated with and quite possibly governed by the primitive localisation (and subsequent expansion of) the Golgi.

([18]) For a comparison of the appearances at lower magnification, see Bailey's (1927) plate xxiv fig. 2 (presumed apolar neuroblastoma) and Feldman's figs. 171 and 173 (contagious venereal tumour).

and nucleolus are well seen, and with patient focussing one can make out in many cells a system of unstained canals, apparently corresponding with a Golgi apparatus. Or the sections may be mounted in a drop of such a stain as well-ripened Held's molybdic acid-haematoxylin, and the progressive staining watched under the microscope.

It will be clear from what has been said that by simple methods one actually gains more information about the cytology than from *routinely* prepared sections, from which the diagnosis of contagious venereal tumour is in my experience sometimes overconfidently and erroneously made: In view of the occurrence of mast-cell tumours in the skin of dogs—a disease that is seldom recognised but is undoubtedly far commoner than is suspected—great caution is necessary in excluding this differential diagnosis before identifying a given neoplasm as a contagious venereal tumour, the more especially in view of the striking prognostic differences between the two diseases. Mast-cell granules are soluble in the watery fixatives ordinarily employed, and when dissolved out or poorly preserved and the tissue submitted to routine stains, no trace of them may be seen in the cytoplasm. Under these circumstances the cells of mastocytoma do not reveal any certain diagnostic features: they appear as " undifferentiated " elements and it is very doubtful whether nuclear or other cytoplasmic features—at least as seen with routine stains—would enable one to distinguish them from contagious venereal tumour cells.

A second differential diagnosis, which might be ignored by those with limited experience of cutaneous tumours of dogs, is from endotheliomata of the solid (i.e., deficiently angioplastic) type. Here methods for reticulum fibres and the demonstration of rudimentary capillary lumina must chiefly be relied on. The differential diagnosis from basal-cell epithelioma has been dealt with in my previous communication (*loc. cit.* pp 303-394).

Summary.

1. The contagious venereal tumour of dogs has been examined for the first time by modern cytological technical methods and the cytoplasmic structure revealed.

2. Adequate technique, controlled by observations on the living cells, shows that these tumour cells are exceedingly rich in lipide globules, the presence of which has been overlooked (*a*) because they are dissolved out from paraffin sections and (*b*) because in frozen sections they are refractory to the customary methods for the demonstration of fatty substances. To stain these globules, the use of a specially devised acetic-carbol-sudan method is recommended.

3. Previously employed methods of studying the tumour cells have been misleading—as indeed they were doomed to be—not merely because of failure to demonstrate the lipides, but still more because of the extensive artefacts which result from failure to take precautions against shrinkage of the cells which follows dissolution of this constituent, which occupies so large a part of the cytoplasm. Current views—that the contagious venereal tumour is composed of round cells or of stellate cells (reticulum cells)—depend entirely on the examination of material showing such fixation or rather post-fixation artefacts.

4. The cell membrane is highly developed and the cells are polygonal in shape and closely aggregated together as in an epithelium.

5. The granules (mitochondria?) are distributed throughout the cytoplasm and are less constantly stained by the classical mitochondrial techniques than are the mitochondria of most other cells. Technique suitable for their demonstration is described.

6. The Golgi apparatus is well developed and has the form of a spiked wreath, one-third to one-half the size of the nucleus.

7. The centrosome and the achromatic spindle have been demonstrated and described. Measurements of the spindle angle reveal a mean value of 114°, contrasting with the angle of cells of the lymphoid series as measured by Ellermann.

8. Routine diagnosis of the contagious venereal tumour is more rapidly and certainly accomplished by means of teased preparations than by the *usual* sections. Pitfalls in the differential histopathological diagnosis have been explained, especially from mastocytoma and endothelioma.

9. According to present conceptions of the structure and habitus of large lymphocytes (lymphoblasts) it would be difficult to identify the tumour cells with these elements.

10. Accordingly, further support is provided for the author's alternative theory that the contagious venereal tumour may be an apolar neuroblastoma.

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