ON THE OCCURRENCE OF CRYSTALS IN TUMOURS.[1]

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IN examining sections of tumours which have not passed through alcohol there are often to be seen crystals of a fatty nature,[2] either among the cells or in the connective tissue.

The tissues should be fixed in formalin, and sections cut by the freezing method. They may then be mounted in Farrant's solution, either unstained or after faint staining with a watery solution of Nile-blue. For the investigation of the crystals it is necessary to use polarised light and a warm microscope stage.

The microscope stage.—The stage which I use carries a thermometer reading to 100° C., and is warmed by water obtained from a special constant level water-bath which is heated by four bunsen burners. The temperature of the water-bath is regulated by two gas regulators, which are connected with separate gas supplies, but which both control all the four burners. One regulator is placed in the water-bath and the other in a vessel through which the outflow from the stage passes. Either regulator can thus be used at will and, if each is set for a different temperature, the temperature of the stage can be made to vary between two fixed points by turning on the two gas supplies alternately.

The water from the water-bath passes first through a stopcock by which the rate of flow can be regulated, and then to the stage through a three-way stopcock. The third branch of this stopcock is connected with the cold water tube which supplies the constant level attachment of the water-bath, so that, by turning the stopcock, either hot water or cold can be admitted to the stage.

The outflow from the stage passes to another three-way stopcock and thence, according to the way in which the stopcock is turned, either directly to waste or through a vessel containing the second gas regulator above referred to. Hence, by means of this stopcock, this second regulator can be put out of action if desired.

By means of this apparatus the stage can be heated or cooled rapidly or slowly, the delicate variations being made by altering the adjustments of the regulators and the rapid alterations by means of the three-way stopcock, which admits either hot or cold water to the stage. Any temperature between 15° and 95° C. can be obtained and maintained for any length of time.[3]

The stage can be standardised by observing the melting-points, in the field

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[2] I am not here concerned with other crystals, such as pigment, leucin, tyrosin, etc.

[3] The glass regulating apparatus, which must be made of Jena glass with hollow plugs in the stopcocks, was made for me by Mr. Otto Baumbach, 10 Lime Grove, Manchester, and the water-bath was made by Messrs. Griffin & Sons, Kingsway, London, W.C.

of view of various pure chemicals whose melting-points are known. With my apparatus I find that the stage temperature of 95° C. corresponds to a temperature in the field of view of 79° C., the difference diminishing with diminution of temperature until it vanishes at the temperature of the room.

In taking melting-points of pure chemicals or of the crystals in the tissues a low power should be used, as the high power exerts a considerable cooling effect on the preparation owing to its proximity to the cover-glass. The alteration in temperature must be made extremely slowly, each observation taking sometimes two or three hours. If this be done the melting-point can be determined with great accuracy, a difference of less than half a degree determining liquefaction or solidification.

The Various Kinds of Crystals met with.

If we examine a section of a tumour with polarised light, the nicols being crossed, we find various crystals which appear bright on a dark background, and sometimes showing iridescence. The crystals are of various kinds:—

a. Irregular plates, sometimes showing a distinct rhomboid shape which do not melt at any temperature obtainable with the apparatus described.

b. Long needles or prisms, usually in bunches, which do not melt.

c. Smaller needles, usually in bunches, which melt at varying temperatures into globules which solidify on cooling. When melted they resemble fat globules.

d. Small needles, closely resembling *c*, which melt at varying temperatures into globules which, on cooling, become anisotropic.

e. Fluid crystals, usually in the form of anisotropic globules and cylindrical myelin forms.

It must be remembered that we are not, as a rule, dealing with pure chemicals, but with mixtures. Hence the melting-points are not always sharp, and may vary in crystals which are close together. As a rule, however, the crystals in a given section either show a fairly uniform melting-point or they melt in groups showing two or three distinct melting-points. For example, in one of my specimens, on warming the section slowly, some of the crystals in a particular field of view melted at 40° C. On further raising the temperature some more melted at 48° C., but there were still some crystals which did not melt until the temperature rose to 68° C. On cooling the section these groups retained their differences, each group becoming visible at a temperature corresponding to the melting-point of the group. This observation could be repeated indefinitely, each group maintaining its own melting and anisotropic points. Similar results were obtained from other specimens.

The difference in the melting-points depends chiefly on the varying proportions of the constituents of the crystals, and, to a less extent, on the position of the crystal in the section. I have taken as the melting-point of a group the highest temperature at which any of

the crystals of that group in the centre of the field of view become invisible.

The Nature of the Different Kinds of Crystals.

a and *b*. These are evidently nearly pure cholesterin, *a* being the purer. Both of them, on heating to 70° to 80° C. for some time, give off cylindrical anisotropic myelin forms resembling those given by cholesterin and soap solutions. I find that commercial cholesterin (Merck and Kahlbaum) when heated in Farrant's solution also gives these myelin forms, whereas purified cholesterin requires to be heated to about 125° C. in glycerin before it will show this reaction. Pure cholesterin will, however, give myelin forms when heated on the stage in glycerin containing alcohol. The impurity present in these crystals is probably a fatty acid, an alcohol, or lecithin.

c. These are crystals of ordinary fats, often containing fatty acids, as shown by the staining reaction with Nile-blue, when melted. The melting-point varies, but is usually between 45° and 55° C. In each specimen the melting-point is usually well defined. The solidifying point is also sharp, and is either identical with or a little below the melting-point.

d. These crystals differ from the preceding in that, on cooling after being melted, the globules do not solidify, but assume the crystalline fluid condition, appearing as anisotropic globules. When melted they resemble fat globules closely, but do not stain so readily. In this condition they are invisible with crossed nicols, but on cooling to a definite temperature (anisotropic point) each globule becomes anisotropic, appearing bright with a dark cross on it, dividing it into four equal bright segments. This anisotropic point is usually about two degrees below the melting-point. On heating these globules they become isotropic at a temperature (isotropic point) usually about one degree below (never above) the melting-point of the original crystals.

In some cases the typical globules are not easily seen on cooling, because the entanglement in the tissues prevents the fluid crystals assuming the globular shape. They can be easily demonstrated, however, by a little pressure on the cover-glass which displaces them into the surrounding medium, where they can readily assume the globular form. In size these crystals and the resulting globules vary considerably, some being seen with difficulty with a Zeiss E objective, whilst others form brilliant objects with a low power. In melting-point they also vary, some melting at as low a point as 30° C., while others melt at 70° C., and others again at various intermediate points. When once the section has been heated and cooled so as to produce the globules these remain permanent in the specimen for several days. In all probability they exist during life in the crystalline fluid condition.

I have shown (1908) that there is reason to believe that these

crystals consist of a loose combination of cholesterin with fatty acids or other substances (lecithin, glycerin, etc.). The globules and the small needle-shaped crystals can be reproduced exactly by mixtures of the pure chemicals. The presence in them of cholesterin can be shown by treating the section with 85 per cent. sulphuric acid when the globules assume a brown or purple colour, and often on heating a section stained with Nile-blue the globules stain a blue colour, indicating the presence of fatty acids. In other cases they stain pink.

c. These are identical with the globules and myelin forms given by crystals *a*, *b*, and *d*. They are met with occasionally in formalin fixed specimens, and more frequently in fresh sections which have not been treated with formalin. In one case also I found them in a specimen which had been fixed with potassium dichromate. They usually show a definite isotropic point on heating, but some do not become isotropic at any temperature obtainable.

Thus the crystals *a*, *b*, *d*, *e* are all of the same nature, consisting of mixtures or loose combinations of cholesterin with lecithin, fatty acids, or other substances, the crystals *a* containing the highest proportion of cholesterin.

Occurrence of the Different Types of Crystals.

Carcinomata.—I have found crystals *d* or *e* in almost every carcinoma that I have examined, usually by themselves, but sometimes in association with crystals *a*, *b*, and *c*. The carcinomata have included growths from the lip, tongue, pharynx, breast, stomach, pancreas, and liver. Occasionally I have not been able to find them, but in most cases where they have not been visible in one section a section from another part of the tumour or from a metastatic nodule has shown them distinctly. They are irregularly distributed in the tumour, hence any given section may not show them. Sometimes they are few in number, and at other times they are very conspicuous. They occur often without any evidence of degeneration, and are in or among the apparently healthy cells. Often they occur as minute single intracellular crystals, at other times as groups of crystals in or between the cells. The stroma is free from them, or may contain a few scattered crystals. They do not occur, as a rule, in necrotic areas, but occasionally they may be found in the border zone between the healthy cells and the necrotic mass.

On the other hand, the crystals *a*, *b*, and *c* are found especially in the necrotic areas, and are not found among the proliferating cells. In most cases, however, the necrotic areas are quite free from crystals of any kind.

Sarcomata.—The crystals *d* and *e* are also to be found in sarcomata. I have found them in two cases of sarcoma of the femur and in three cases of melanotic sarcoma. In a chloroma starting from the

periosteum of the femur there were numerous cells crowded with minute anisotropic globules, and some globules were lying free among the cells. A few globules were found in the cells of the growth in the liver and in a lymphatic gland.

Adenomata.—In three specimens of adenoma of the breast examined large numbers of crystals *d* were present. In two of these cases, which were large tumours of the intra-canalicular fibroma type with a large amount of connective tissue, there were large masses of crystals in the connective tissue visible to the naked eye as dead white areas. In the third case the epithelial cells contained minute intracellular crystals, so that the outline of the tubes could be seen quite clearly mapped out by the crystals with the polarised light.

Apart from tumours, crystals have been found in the following conditions:—

Adrenals.—Crystals *d* occur in the adrenals under normal conditions. I refer to these in another communication to this *Journal* (*infra*, p. 11).

Kidneys.—In three cases of acute nephritis there were large numbers of crystals *d*, in two cases being so numerous that they were visible to the unaided eye as dead white streaks. In two of these cases the crystals were in the connective tissue, in the other case they were in the epithelial cells. In one case of compensatory hypertrophy of the kidney there were large numbers of crystals *d* in the epithelial cells. Other cases of fatty kidney showed crystals *c* only.

Liver.—Two livers were examined in which there were large areas of proliferated connective tissue containing masses of crystals which were conspicuous to the naked eye. One of these was from a case of cholangitis; the nature of the other was not clear. The crystals in both cases were the crystals *d* and a few crystals *a*. One case of fatty degeneration of the liver associated with carcinoma of the stomach showed globules *e*. Otherwise fatty livers only showed crystals *c* (fats).

Adipose tissue.—This shows crystals *c* only.

Atheroma.—Several cases of atheroma of the aorta were examined and showed crystals *a*, *b*, *c*, *d*, and *e*. Of these *a* and *b* were the most numerous.

Fat Necrosis.—This showed crystals *c* (fatty acids), together with some crystals which did not melt, probably calcium soaps.

Other conditions.—In a case of dilated lacteal filled with crystals, due to obstruction by new growth, only crystals *c* were found

In a mesenteric cyst, macroscopically resembling a dermoid but containing no dermoid structures, were found crystals *c*, *d*, and *b*. In a true dermoid cyst only crystals *c* were found, and a chemical examination of its contents showed that no cholesterin was present. It contained arachidic and other fatty acids, and an alcohol melting at about 55° C. together with other unsaponifiable matter.

In one case of local thickening of the pia mater, from a case of hydrocephalus, masses of crystals *d* were present.

In a case of chronic mastitis the cysts contained crystals and myelin forms which did not alter at a temperature of 80° C. Similar refractory globules and myelin forms are sometimes found in the dilated ducts of breasts affected with carcinoma. A lactating breast showed some crystals *c* in the contents of the alveoli and ducts, and a few crystals *d* in the epithelial cells.

In two cases of tuberculosis, one of the peritoneum and the other of the lung, minute crystals *d* were found only in the areas where proliferation was proceeding, the caseous areas being quite free from crystals.

In examining sections care must be taken to exclude adventitious crystals, such as form in specimens and sections which have been kept for some time. These are distinguished by their being, as a rule, deposited on the surface of the section, but some of those present among the cells may be accidental. The adventitious crystals, however, are distributed over the section, irrespective of any histological boundaries, whereas the crystals which are not adventitious show a definite relation to the tissues. They are present, for instance, in or among the epithelial cells, and absent from the stroma, or *vice versa*. Also they are to be found in sections cut the day after the specimen is obtained. Adventitious crystals appear to be always of the *a* or *c* type, and I have never found any of the *d* type.

Significance of the Crystals.

As to the significance of these crystals, I am not prepared to give a decided opinion at present, but there are one or two points to be noticed. Crystals *a* and *b* are found only in association with necrotic changes. Crystals *c* also, apart from their occurrence in ordinary fat, are mostly connected with degenerative changes, but they are sometimes to be found between apparently healthy cells, where they may be adventitious.

Crystals *d* and *e* are not necessarily associated with evident degeneration, although they may be found in degenerated areas. They occur especially in areas where the cells appear quite healthy, and where proliferation is taking place. Often they can only be detected with polarised light, the tissue appearing quite healthy by ordinary methods of examination.

The appearance of crystals in a tissue does not necessarily imply that the substances of which they are composed are newly formed, or that they are present in excess. It may only mean that they have changed their state, *e.g.*, from a colloidal to a crystalline condition. Cholesterin is present in every part of the body, and is always in association with lecithin or fatty acids. Apparently these substances help to keep one another mutually in solution in the body fluids, from which they may, under certain conditions, separate in the crystalline form. It has long been known that lecithin forms a fine emulsion or a colloidal solution in water, and it does this because of the cholesterin which it contains. When freed from cholesterin it no longer becomes emulsified with water. Similarly, cholesterin mixed with fatty acids forms a fine emulsion with water, especially if a trace of alkali be present. These emulsions or colloidal solutions are unstable, being easily precipitated by the addition of acids or salts.

I have noticed, occasionally, that the number of anisotropic globules in a section has appeared to increase after it has been heated and

cooled several times, as though substances, invisible before, had been rendered visible by the alternations of temperature.

It would thus appear—(*a*) that cholesterin occurs in the body normally in association with lecithin, fatty acids, etc., as a colloidal solution or a fine emulsion; (*b*) that under certain circumstances these associations assume the crystalline fluid condition as the crystals *e* which may, before or after death, solidify as crystals *d*; (*c*) that crystals *a*, *b*, and *c* result from the dissociation of these loose combinations into their component parts.

It would appear that cholesterin plays an important part in the economy, and that it is not a mere curiosity or waste product; and the presence of the crystals *d* in the proliferating areas of cancers, etc., suggests that cholesterin may, in some way or other, be associated with the regulation of proliferation. Craven Moore (1907) has pointed out that cholesterin, owing to its peculiar physical properties, probably plays an important part in the cell economy (3).

It is interesting to note that gall-stones are two and a half times, as common in patients suffering from carcinoma as in patients of similar ages suffering from other diseases, and this frequency is independent of the site of the primary growth (Colwell, 1905). Also, spermatozoa are particularly rich in cholesterin.

Undoubtedly the crystals *d* appear in degenerated areas, but they are not necessarily a sign of degeneration, since they appear more frequently in and among cells otherwise healthy. Their presence in degenerated areas can be accounted for by precipitation from the state of colloidal solution by the decomposition products which are formed in such areas.

Another point of interest arising out of the presence of crystals and globules in cancer cells is that it is probable that some of the innumerable so-called parasites of cancer consist of these globules. They stain easily by Weigert's method for staining myelin sheaths, but I have not investigated their staining properties further.

Crystals in tissues do not seem to have attracted much attention in pathological literature. Cholesterin is usually said to be recognisable by the typical shape of its crystals. In my experience, however, it is far more frequently present in the form of needles or prisms than as the typical rhomboid plates. Crystals occurring in fat cells are often erroneously called fatty acid crystals, whereas they are usually composed of neutral fats. The crystals *d* do not seem to have been definitely described before. The only description I have found which may possibly refer to these crystals is one by Podwyssotzky (1898), in which he describes crystals and hyaline globules occurring in the stroma of a cancer of the upper jaw. He thinks that the crystals and globules have some connection with Altmann's granules, and that the crystals arise from the globules. In this case, however, the crystals and

globules stained with eosin, which I have not so far found to be the case with crystals *d*.

Anisotropic globules have been described in different conditions by numerous authors, and have usually been ascribed to cholesteryl esters (1).

Note.—Mr. J. Holden Webb, of Melbourne (1901), suggested that cancer was due to the crystallisation of cholesterin from the living cells.[1] He did not detail any evidence on which this hypothesis was founded.

SUMMARY.

1. Crystals consisting of a loose combination of cholesterin with fatty acids, lecithin, or other substances occur in or among the cells of malignant tumours, and in some other conditions.

2. These crystals seem to be associated with cell proliferation rather than with degeneration.

3. The crystals found in degenerated areas are mostly either cholesterin, fatty acids, or fats.

4. It is suggested that cholesterin may be associated in some way or other with the regulation of cell proliferation.

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[1] Webb J. Holden, "Cancer, its Nature and Treatment," *Lancet*, 1901, vol. ii. p. 976.