but, looking about the room, he asked whence the strong smell of violets came.

I inquired as to the revival of long-past impressions, and it seems that occurrences which took place before his present memory existed, had been revived and verified, but still more interesting was his experience in reference to a mathematical formula which he had forgotten. Being hypnotized he dictated it, and though when once more awake he did not remember it, when shown what he had just dictated he recognized it as the lost formula. This, of course, is in a way parallel to the solution of difficult problems during sleep.

I feel that in passing to the subjects of experimental psychology, and more particularly to hypnotism, I am on what might be called dangerous ground. Many of my colleagues, I know, shake their heads and doubt the utility of hypnotism, and the fitness for the subject being seriously considered. But one of the great pieces of advice which Harvey gave was that we were to follow truth without fear as to whither it may lead. I am doing my best to direct the line of advance by means of observation, and it seems more than likely that the two opposing forces as they appear at present—experimental psychology and hypnotism—may, after all, unite in the scientific advance.

And now, gentlemen, it is time for me to bring this my Harveian Oration to a conclusion. No one can be more conscious of its shortcomings than myself, but I have done my best to present to you a brief survey of the great Harvey and his environment, and I have naturally dealt with the subject from the point of view of my own special line of work. Let me take leave of him with a few final words of reverence and appreciation.

Amongst the most prominent characteristics of Harvey was the singleness of purpose of his life. He had formed definite aims, or, rather, he determined to look for himself into things without fear or prejudice. And his power for steady scientific work did not prevent him from entering into practical professional duties of all kinds. He was socially acceptable alike in England and in Italy; but, with all this, he seems to have disregarded the great men of his time. Shakespeare was living, and writing, and acting; Milton was in full evidence by his politics and his poetry; Chancellor Bacon was known, but was not appreciated; and so with many others of the very distinguished men of his time. Science was his mistress; literature, as such, did not appeal to him. Though the exact sciences, like mathematics, formed an interest up to the end of his life, he still followed the anatomical and the physiological rather than the mathematical sciences. Religion did not seem to take a prominent hold of his character, and, though living in the most stormy political period, he seemed to be little moved by the tragic events through which he passed. He was a man of kindly family affection, yet his one object—the pursuit of truth—so dominated him that little else appears upon the horizon.

Let me conclude with his own words: "I therefore whisper in your ear, friendly hearer, and recommend you to weigh carefully in the balance the exact expression of all that I have delivered in this my exercise."

At the annual meeting of the Society of Medical Officers of Health on October 8th, Dr. H. C. Pattin of Norwich was installed in office as president, and delivered an address, entitled, State Standardization of the National Life. The fifty-third annual dinner of the society took place the same evening, and was attended by the Dean of Norwich, who described himself as the commercial traveller of the section of the Poor Law Commissioners which had signed the Minority Report; by Mr. Sidney Webb of the London County Council; by Dr. Newsholme, Principal Medical Officer to the Local Government Board; by Sir R. L. Morant, Secretary of the Board of Education; and by Surgeon-General Evatt, and Sir James Crichton-Browne, Presidents of the Poor Law Officers' Association and the Sanitary Inspectors' Association respectively. In response to the toast of the evening, the president spoke of medical officers of health as analogues of Wesley, in that as preachers of the gospel of hygiene, they took the whole people for their parish. He hoped that before long they would find incorporated with them all officers engaged in public medical aid work. Their general aim should be to standardize the healthiness of the nation, and to aid in the development of a communal conscience to whose bar all social activities could be brought for judgement.

A Report ON CANCER RESEARCH.

AN INVESTIGATION BY "IN VITRO"
METHODS.

BY

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FOR more than twelve months we have conjointly been endeavouring to throw some light on the subject of cancer,* by studying the disease from what appears to be a new standpoint.

In August, 1908,† we found that the blood of cancer patients appeared to contain a substance which, like the artificial excitant of methylene blue and atropine, already described,‡ excites amoeboid movements in leucocytes, and shortly afterwards we described how lymphocytes in the presence of both these excitants extruded what at that time we believed to be flagella.¶ These observations led one of us (C. J. Macalister) to suggest the advisability of instituting a combined clinical and pathological research upon the lines which will be referred to in this paper, especially since the study of cancerous tissues by *in vitro* methods had thrown some light upon the behaviour, and possibly also upon the character, of the cancer cell.

In order to carry on this research efficiently it was manifestly preferable that the whole time of one of us should be devoted to this and kindred subjects; and this became possible owing to the great generosity of Sir William Hartley, who, together with Mrs. George Holt, Mr. and the Misses Allan Paton and a number of their friends, furnished the necessary funds. A committee for administering the fund was formed under the chairmanship of Professor Sherrington, and one of us (H. C. Ross) was invited to become director of the research, which is being carried on in the Hartley Laboratories at the University of Liverpool. The committee and medical staff of the Royal Southern Hospital in Liverpool also associated themselves with the work, and have provided laboratory accommodation for carrying out those portions of it which are more immediately concerned with the clinical study of the disease.

The points which we shall set forth in the course of this report, which is the first to be submitted, are based partly on previously published investigations and upon the suggestions of others, some of which have been very important, but largely upon certain well-known facts concerning cancer with which it has been necessary to reconcile each step of the work.

Among other points we shall refer to the existence of what may be described as an excitator of reproduction for human lymphocytes, and so far as we are aware a chemical agent which, when absorbed by an individual resting human cell, will cause that cell to endeavour to reproduce itself forthwith, has not been previously described.†

Apart altogether, however, from the possibility of a similar substance being associated with cancer (and the facts rather point to such a conclusion), we submit that the finding of a chemical agent by the employment of which one is enabled at will to cause human lymphocytes to reproduce themselves on a microscopic slide is in itself a point of considerable scientific interest, especially as the mitoses induced are not of the somatic but of the reproductive type. We shall explain how these mitoses have thrown light upon the morphological differ-

* The word cancer in this paper refers to carcinoma.

† Loeb has caused artificial development up to a certain point in the ova of starfish by the action of certain solutions. (*Die chemische Entwicklungserregung des tierischen Eies*, Berlin, J. Springer, 1909, and former publications.)

ences between lymphocytes and polymorphonuclear leucocytes;* and, lastly, we shall show that when a cell has responded to the exciting agent, it appears to convert it into a poison which, under the experimental conditions, seems to be a virulent one, since the cell invariably dies, and that the production of this poison may have some bearing upon the phenomena seen in cancer. Other facts have presented themselves in the course of our investigations which we believe to be important to cancer research; and we propose, after endeavouring to interpret or trace the significance of the known cardinal features of the disease, which, although somewhat meagre, are fairly definite and must form an important basis for research, to enumerate our experiments in the order in which they were made, together with the principles upon which they were founded. We wish especially to be assured that our opinions as to the possible explanation of the significance of our fundamental facts are within the realms of reasonable supposition, and, since the principles underlying cancer research are mainly clinical ones, we have thought it appropriate to read this report before a medical society and to publish it in a medical journal.†

Methods.

Before proceeding further it is necessary that we should give some account of the methods of research which have been employed. We deal only with living human cells, and our problems are therefore associated, not with the phenomena seen in dead structures but with those of vitality and stimulation. In this report, therefore, the word "cell" refers to a living cell unless otherwise specified, and we submit that the staining of living cells is an advance on the older fixation methods, which give pictures of dead structures. Perhaps one does not obtain quite such useful observations of the arrangements of the cells by this method, but it enables us well to observe some of the phenomena concerned in life and death—and now, we may add, in reproduction.

The appearance of the living cell thus examined is not merely that of a flattened diagram, for by careful focussing the arrangement of the cellular elements can be seen from above downwards, as well as from side to side. In the case of leucocytes, active movements may be excited while the cells are staining. The rapidity of the staining may be observed, and one can stain cells slowly or rapidly as may be desired, the highest possible immersion objectives being employed in the usual manner.

There is, however, one drawback to *in vitro* staining. The specimens cannot be kept. When a cell dies, or if it is killed by the staining of the nuclear chromatin, its cytoplasm liquefies, and then it becomes achromatic.² If the cell has been stained its stain fades. If it is dead and has not been stained it may now refuse to stain. After death there is no alternative but to discard the specimen, owing to this onset of achromasia which cannot be prevented. All attempts to fix the dead cells have failed owing to technical difficulties. Hence, one has no cabinets of slides to refer to, and therefore it has been necessary to repeat each experiment over and over again to assure ourselves that the result which was first obtained could be repeatedly demonstrated.

This method is simple, and it has already been described in detail in former papers^{1,3,4,5}. Briefly, it consists in the mixing of some stain ‡ with agar jelly containing a little sodium citrate and sodium chloride. The jelly is melted and the whole mixture is boiled, a drop of it then being run on to a slide where it is allowed to set. The cells to be examined are suspended in citrate solution, and a small quantity of the suspension is placed on to a cover-glass which is then inverted and allowed to fall flat on to the film of jelly. The cells spread out between the cover-glass and the jelly, and if the latter has been allowed to set thoroughly on the slide, the arrangement of the cells will always be found to be suitable for examination, and consequently one never finds "bad specimens" by this method. Soon after the cells come to rest on the jelly the

stain will, under suitable conditions, diffuse into them. Of course if the jelly contains other substances as well as stain, these substances will also diffuse into them. The cells continue to live, and we can thus observe the effects of the stain and other substances upon them.

Two things must be remembered in *in vitro* staining. First, that so soon as the nuclear chromatin of a cell stains, death occurs.¹ Any attempt to stain the nucleus causes death; and when death has occurred, the nuclear chromatin stains bright scarlet. After death achromasia ensues and the stain gradually fades as the cell becomes disorganized.

The second point is that the diffusion of substances, including stain, into living cells appears to depend almost entirely upon certain physical laws, and unless these laws are adapted to the conditions required in each specimen, successful results will not be obtained.

The main principles involved in the diffusion of substance into living cells concerned in researches of this nature are as follows: Some classes of cells absorb substances more rapidly than others. Hence we say that different classes of cells have different "coefficients of diffusion," but it must be borne in mind that the rapidity of diffusion also depends upon the concentration of the substance in the liquid surrounding the cell. It will readily be understood from this that the greater the concentration of the substance in the medium in which the cell rests, or the lower the coefficient of diffusion (*cf*) of the cell itself, the more rapidly will the substance be absorbed. There are other factors concerned in this diffusion of substance into cells—for instance, heat and alkalis both accelerate it, and it must be obvious that time is an important factor, because the longer the time which is given for the diffusion to take place, the greater will that diffusion be.

A simple experiment will explain these points: The jelly which has been referred to, and which for convenience we call "coefficient jelly,"⁵ consists of a standardized preparation of agar jelly which is faintly acid and contains certain quantities of sodium citrate and sodium chloride. It is made up in tubes, each of which contains 5 c.cm. To one tube of this jelly we can add a given amount of liquid stain, and to another similar tube we can add a greater quantity of the stain, distilled water is then added to each tube until it contains a total of 10 c.cm. The tubes are then steeped in boiling water until the jelly melts, so that complete mixing is effected. It will be apparent that the first tube will contain a low concentration of stain and the second tube a higher concentration, while both contain equal concentrations of agar and salts. If the cells which are going to be examined are placed upon films made from these tubes and kept at equal low temperatures, say that of the room, the diffusion of the stain into the cells will gradually take place, but more rapidly in the case of the film containing the greater amount of stain. If the slides are incubated at 37° C., for instance, the diffusions will take place more rapidly, and the longer the slide remains in the incubator the more deeply will the cells be stained, always bearing in mind that the jelly which contains the larger concentration will stain the cells before and more deeply than the other. Hence, by playing off these factors—that is, the concentration of the stain, the heat, and the time—against each other we can cause the staining, or prevent the staining, of the cell to any degree we please.

To a tube of coefficient jelly a variety of substances can be added; and since one invariably makes it up or completes it with water to 10 c.cm., by a simple calculation we are able to tell how much of any given ingredient a given tube of jelly contains. We have already indicated that alkalis accelerate diffusion and that acids and neutral salts delay it, and by means of these we can still further modify the rapidity and degree of staining. The cytoplasmic granules of cells stain first and then their nuclei.

We have already mentioned the fact that the absorption of substances into cells depends not only upon the degree of their concentration and the several factors which accelerate it, but also upon the coefficient of diffusion of the particular cell which is being observed. This coefficient may be determined by a procedure for the full details of which we must refer the reader to a paper by one of us, "On the determination of a coefficient by which the rate of the diffusion of stain and other substances into

* In future in this report the word "leucocyte" means the polymorphonuclear leucocyte.

† An epitome of this report was read at the Liverpool Medical Institution, October 14th, 1909.

‡ The word "stain" in this report refers to Unna's polychrome methylene blue (Grubler) which has been found to be the most useful dye to employ.

living cells can be measured," published in vol. 81, B, No. 546, of the *Proceedings of the Royal Society*.

As this is a recent publication, it may, perhaps, be well briefly to reiterate the principles with which it deals. It consists in the measurement of the several factors already alluded to—namely, heat, time, alkali, concentration of stain and salts in certain units. A series of tubes of coefficient jelly are made up to 10 c.cm., as before, but each tube contains a certain known number of units of stain, alkali, and salts. Since salts delay the diffusion, the total number of units of them is subtracted from the total number of units of stain and alkali, and the number which remains is called the "index of diffusion" (fx) of the jelly. To find the coefficient of a given cell it is placed on to, first, a jelly with a low index of diffusion, and then on to higher jellies, until one is found which will just cause the nucleus to stain. Time and temperature are also divided up into suitable units, and the total number of units of these two factors added to the index of diffusion of the jelly gives a grand total number, which is the coefficient of diffusion of the cell under examination. The units have been measured by experiment, and are so arranged that they are all of equal value—that is, the unit of one factor is equal in its effect, as regards the acceleration or delay of diffusion, to a unit of any other factor. The units are, in point of fact, interchangeable; consequently, if a jelly is found which will just stain the nucleus of a cell, its index of diffusion may be plotted thus:

$$fx = (s + a) - (c + n),$$

s being the unit of stain, a the unit of alkali, c the unit of sodium citrate, and n that of sodium chloride.

But the coefficient of diffusion of the cell consists of the index of diffusion of the jelly which will stain the nucleus *plus* the total number of units of time and temperature. Thus:

$$cf = (fx + h + t),$$

where h = the unit of temperature, and t = the unit of time.

Therefore it may also be said that*—

$$cf = (s + a + h + t) - (c + n).$$

The lymphocyte being the important cell in the present research it may be well to use it as an example and indicate its cf by means of an equation. Thus: A drop of fresh blood was placed on a film of jelly which was made from a tube of coefficient jelly to which had been added 0.5 c.cm. of stain, 5 units of alkali, and was completed with 4 c.cm. of water up to 10 c.cm. The coefficient jelly contained its usual content of three units of sodium citrate and one unit of sodium chloride. The slide was kept at 37° for ten minutes (one unit of time) when it was found that the nuclei of the lymphocytes had just stained. What was the coefficient of diffusion of these cells?

$$cf = (5s + 5a + 7h + t) - (3c + n) = 14.$$

This is the principle which has been employed throughout these researches in investigating some of the properties of cells. In practice it is quite easy, although it is difficult to describe shortly. We always employ the standardized coefficient jelly, but it is important to remember that when stain and other substances are added to it it must always be completed up to 10 c.cm., and that it should contain the correct amount of alkali (that is, it should have the correct fx) to cause the stain, or whatever it contains to diffuse into the cell with due regard to the latter's cf . The equation is the guide. Since all the units of the several factors are equal, they are interchangeable with the cf , and thus if any one of them is an unknown quantity, it may be ascertained by bringing the known coefficient to the other side of the equation, and substituting for it the unknown quantity required. It was through acting on these principles that the reproductive excitant to which reference has been made was noticed, and they must, in making experiments of this nature, be rigidly adhered to, for, if the index of diffusion of the jelly is not correct for the coefficient of diffusion of the cell, it may contain a great concentration, say, of the reproductive excitant, but

*The actual amount of each factor contained in one unit will be found in the paper referred to, but we may repeat here that the unit of stain is 0.1 c.cm. of Grubler's solution and that of alkali is 0.1 c.cm. of a 5 per cent. solution of Na_2CO_3 . A unit of time is ten minutes, the unit of temperature is every 5° of temperature from 10° C. upwards.

the cell will refuse to respond to it, no matter how much of that substance be present.

The coefficient of diffusion of healthy human cells appears to be constant, but can be lowered by certain agents. Although the coefficient of diffusion varies in different classes of cells, it appears to remain constant in each class, provided the cells have not lost vitality. This appears to be the case with blood cells and those tissue cells which we have tried. On the other hand, approaching death of a cell lowers its coefficient of diffusion, and certain poisons also have this effect.⁶ The cells of persons suffering from chronic illnesses also show a low coefficient of diffusion, and this we have found to be marked in cases of cancer. As we shall show later, some substances have the property of lowering the coefficient of diffusion without being very poisonous, and it is possible that the low coefficient of diffusion found in cancer may be promoted by some such substance.

We have stated that our research has been based upon certain well-known facts with which it has been necessary to reconcile each step of the work; and we shall now say a few words about each of these facts or axioms, and the theories which have suggested themselves concerning them, before proceeding to explain the experiments arising from their consideration.

Age-Incidence.

The age-incidence of cancer is strikingly important. It is a disease which occurs mainly in persons over 40 years of age, and its prevalence appears to diminish in old age. Bashford and Murray state that "Any explanation of the etiology of cancer must accord with the circumstance that, when considered statistically, cancer is a function of age, and when considered biologically, it is a function of senescence."¹² Among its characteristics we must recognize that during youth the disease is rare, and that when the age of its incidence has been reached its tendency is to attack the robust rather than the infirm. It occurs in persons who appear to have perfect vitality, and in this respect contrasts remarkably with the incidence of infective diseases. On the other hand, a loss of vitality in a person's cells seems to be unfavourable to the onset of cancer, and we find that it does not occur to any great extent among very old people.

We are thus brought face to face with the problem, What is it that occurs in the tissues after the age of 40 which renders them liable to the onset of cancer? The data upon which we have to find an answer to this question are so indefinite that suggestions can only be made vaguely. We must be prepared, however, to appreciate that the onset may be induced by the oversetting of a normal balance. The body is mainly composed of living cells, all of which have been derived from a pair of cells, and they constitute an elaborate combination of living factors. We know that in certain tissues these cells are continually dying and being replaced, so that it is evident that birth and death must be going on incessantly in the body. What happens to the dead cells? They of course liquefy and become disorganized, and their constituents are presumably excreted or converted into other compounds. While this is happening it seems probable that some of the products of the remains of dead cells may be absorbed by their neighbours, for it must be remembered that the diffusion of substance into living cells appears to be a physical process over which they exercise no control. There are doubtless some cells which remain alive for long periods; for instance, it has been estimated (and we are informed that it is practically certain) that some cells of the central nervous system live throughout the life of a man. Many cells, however, only live a very short time, the length of their lives perhaps varying in different parts of the body, so that the remains of dead cells are probably always present in the body fluids. In this connexion, however, we have to keep in mind the physiological curve expressive of the relationship between anabolism and katabolism. There are only three stages of life if it is viewed from this point of view, the first terminating at about the 30th year, when a man reaches his prime, and up to which period cellular birth must preponderate over its death-rate. For some years it may be suggested that anabolism and katabolism remain balanced, and that after the age of 40 quite physiologically, so that nothing occurs to make a man aware of it physically, these conditions

begin to be reversed and more of the products of katabolism—that is, the remains of dead cells—tend to exist in the body fluids than was the case before middle age.

Here we have a fact incidental to the cancer period which suggests the possibility that these products of katabolism may in some way predispose to the onset of malignancy. It cannot possibly be suggested that they are the cause of the disease, for if such were the case everybody over the age of 40 would die of cancer; but assuming that some product of katabolism may possibly favour the onset of the disease, we may enlarge upon the speculation and say that it is a certain morphological (or chemical) element in a dead cell which may be the agent. For the sake of argument it may be derived from either the cytoplasm, the cell wall, the nuclear wall or the linin or it may be the chromatin itself.

Vitality.

We have mentioned incidentally that cancer is a disease of those who appear to be in robust health, and the question of the vitality of the individual or of the cell is germane to our argument. It is obvious that the response of a given cell to a given excitant depends upon two conditions—namely, the rate of absorption of the excitant (a subject which has been discussed) and the vitality of the cell itself. The lower the vitality of a cell the greater will be the amount (or potency) of the excitant required to bring about a certain response, and such a point of lowered vitality may be reached that the cell will not respond, however much excitant may have diffused into it. In old people, when the incidence of cancer appears to be on the wane, it is possible that the cells concerned are of comparatively low vitality, or that the proportion of young cells which are liable to be attacked is smaller than it was in middle life, and that this may in some way account for the fact that cancer is not so common in old age as during senescence. It is possible that many factors are responsible for the onset of malignancy, and that unless they are present in correct combination and hit upon a cell which has sufficient vitality to respond they will be ineffectual.

Irritation.

The next factor which plays an important, if not an essential, part in the development of the disease is irritation. Chronic irritation is always associated with an attempt at repair, and healing means the presence of leucocytes and lymphocytes. These two elements are also usually present in large numbers in cancerous growths. Where there is chronic irritation there must also be cell destruction going on with its attendant products of katabolism, so that irritation will produce locally the product which may predispose to the disease, and, supposing that this substance is also present in the body fluids owing to senescence, it will occur in great excess at the seat of irritation.

Bashford lays much emphasis on the point that cancer is a disease which originates in a circumscribed area. We consider it possible that it may begin in a single cell. Apart from the predisposing influences of mechanical and long-continued pathological irritation, it must be remembered that certain chemical irritants undoubtedly favour the onset of the disease more than others, especially some coal-tar products.

Excitation of Reproduction.

We know very little about the influences which control cell reproduction in the body. It is difficult to imagine that it is automatic in each individual cell, and we do not know how much nervous influences have to do with it, but we shall show presently that the reproduction of human cells can be caused by chemical agency, and it is possible that the normal balance may be maintained by something of this nature. If our suggestion is at all correct, that some product of katabolism favours the onset of cancer, it may do so by in some way accelerating the reproduction of cells. This cannot be due to mere mechanical irritation, which, which, we all know, does not necessarily lead to cancer, or even to tumour.

Death.

There are two more axioms which are worthy of serious consideration in relation to our search for the cause of cancer, and the first of these is that it causes death.

Benign tumours do not lead to death, as a rule, but if the progress of a malignant one is not successfully interrupted by surgery, it almost invariably leads to a fatal termination.

Why should death occur in the one instance and not in the other? It will occur in cancer irrespective of the presence of metastases, or even of the relation of the growth to vital structures, and we speak of death resulting from those vague conditions styled exhaustion and cachexia.

Bashford,¹⁰ in his address before the Medical Congress at Budapest, indicated his belief, so we understand, that in cancer "there is no evidence of toxic products injurious to the hosts," and that "death is not due to poisons." He points out that in experimentation with mice, when the tumours have exceeded certain sizes, the growths live at the expense of their hosts. But surely this is not applicable to men, who, unlike mice, do not develop pendulous cancers of large size. It has been shown in former papers^{8,11} that there appears to be no poison in the peripheral blood of cancer patients which shortens the lives of polymorphonuclear leucocytes which are susceptible to infective toxins, but this does not show that no poison is causing the death of cancer patients. General cell death must be extensive in the later stage of the disease, as evidenced by the wasting and cachexia, and at present it is difficult to avoid the suggestion that some poison promotes dissolution. One may feed a cancer patient *ad libitum*, yet he will die. The problem of the cause of death is a difficult one, on which artificially induced reproduction may throw some light.

Cancer is a Disease which Aggravates Itself.

Our last fact is that cancer is a disease which aggravates itself. We have evidence of this in the circumstance that the disease begins in a circumscribed area, possibly in a single cell in that area, and that if the area is removed the patient may recover.

It is usually supposed that cancer having started, say, in a single cell which divides, the two daughter cells are also cancerous, and that the growth of the cancer takes place in this way. Again, the metastases of cancer are generally supposed to result from embolisms derived in the first place from the circumscribed area in which the growth begins, but if these secondary deposits so-called were always due to embolism, one would expect them to occur at the onset, as well as during the advanced stages of the disease, and we might also ask the question, Why does embolism practically only occur in *malignant* growths?

There is yet another possible way in which the disease may progress, a speculation based on the remarkable fact that a growth appears to confer protection against other growths experimentally planted. This fact has been shown by the Imperial Cancer Research. The protection is presumably due to the formation of antibodies in the body fluids, and these antibodies must be formed against *something* produced by the living growth, for a dead growth, or an extract of a growth, will not confer protection. Hence, it is reasonable to suppose that the living cancer cell may be secreting or otherwise producing a substance which either aggravates the cause of cancer or assists the normal cells to respond to it more readily.* This speculation was borne out by the fact that we found that the excitant for leucocytes which is present in the blood of these patients is found also to a greater extent in the juices of the growth.⁸ Presumably, if a substance is secreted by a cancer cell, it will first be absorbed by its neighbours, and will pass also into the lymph spaces, and so on to the lymphatic channels and glands which have so remarkable a tendency to become infected. The larger the growth becomes the more secretion will be produced, each secondary growth contributing to the ingravescence of the trouble. On this suggestion one may explain why cancer so frequently spreads eccentrically, in contradistinction to any of those growths which spread concentrically, or only by reproduction of the original cells. Lastly, the aggravating substance may assist to cause the death of the patient.

The question may now be asked, "Why is it that when a growth is completely removed it sometimes does not recur, although the cause of the original growth is still

* It should be remembered that the transplantation of a dead growth, or an extract of a growth, will not cause cancer.

present?" Why does not a recurrence invariably take place immediately in the healing and irritated scar? This is also probably a question of antibodies. Presumably, if the growth produces something which favours the aggravation of cancer, antibodies are also produced against that something, and when a growth has been removed these antibodies will remain in excess of the original cause of the disease, and the amount of the antibody in the blood may account for the longer or shorter period which elapses before recurrences take place.

These, then, are our axioms and theories. We are aware that it is dangerous to theorize, but it is impossible to undertake a cancer research without having hypotheses on which to work. Our endeavour has been to keep to theories which are as reasonable as possible and in accord with known facts.

Former Researches.

It has been shown by Galleotti and Hertwig, and Farmer, Moore, and Walker, and others who have been in the field of research, that irregular mitoses frequently occur in cancer cells, and Farmer, Moore, and Walker,¹³ studying the irregular number of chromosomes in cancerous and other cells, described how the former resemble the reproductive rather than the somatic type, frequently showing the meiotic divisions with 16 chromosomes instead of the somatic number of 32. They also pointed out how the longitudinal splitting of the chromosomes is a characteristic of the first (heterotype) meiotic phase, and that cells undergoing these mitoses may show "sinaptic contractions." These observers also suggest that leucocytes may be ingested by cancer cells, and that a combination of the chromatin of the two cells may play a part in the onset of the disease. The intracellular Plimmer's bodies are said (by them) to be identical with the archoplasmic vesicles, which together with archoplasm are said to be characteristic of reproductive cells, and appear in the first meiotic division. Walker¹⁴ also suggests that all leucocytes are of the reproductive type, and have a reduced number of chromosomes. He describes archoplasm in them. Unfortunately he does not distinguish between the different varieties of leucocytes, and as far as we can gather he examined leucocytes from the internal organs for the most part.

Benjamin Moore¹⁵ has shown that the alkalinity of the inorganic constituents of the blood plasma in cancer is increased. He also states that "irritation and inflammation are accompanied by increased alkalinity of the lymph which is a factor in stimulating cell growth." It should be remembered in this connexion that alkalis accelerate the diffusion of substances into cells.¹⁶

H. Becton, in a paper read before the Pathological Section of the British Medical Association at Belfast on July 28th, 1909, remarked upon the absence of Altmann's granules in the cells of malignant tumours, although in innocent growths and usually in normal cells they can be found. This is an important point which we can confirm, and we can also testify that it is true, in spite of the fears entertained, so we understand, by Professor Lorrain Smith, that the appearance or absence of the granules may depend on the fixative used, because we employ no fixatives.

An Artificial Excitant by which Leucocytes can be Stimulated to Extrude Pseudopodia.

In a former paper⁴ one of us recorded a remarkable excitation of amoeboid movement which occurs in leucocytes, even at low temperatures, when they are placed on a jelly film containing polychrome, methylene blue, and atropine.* The stain is the important part of this excitant, as it can be made to be effectual if no atropine is present, but atropine and some other alkaloids accelerate its action. The action of this exciting jelly is very constant provided its index of diffusion is correct for the leucocytes, in which case the cytoplasmic granules of the polymorphonuclear and eosinophile cells will stain while the cells are actually extruding and retracting pseudopodia. In this respect they differ from lymphocytes, which while under the influence of the excitant are the most amoeboid of all the cells, but they will not continue to show movements after all their nuclear granules have stained.

* Since this paper describing this excitant was published, it has been ascertained that Professor Osler many years ago noted that certain alkaloids and curare increased amoeboid movement.

An Excitant for the Leucocytes of Healthy Persons found in the Plasma of Cancer Patients.

In a paper in the *Proceedings of the Royal Society of Medicine*⁶ we pointed out how normal leucocytes, when mixed with the citrated plasmata of a series of cancer patients, appeared to show, under suitable conditions, the same remarkable exaggeration of amoeboid movement as they do when the stain and atropine jelly is used. Control experiments did not demonstrate the same marked movements. Hence we conclude that there is in the blood in cancer some substance which, in effect, resembles the methylene blue and atropine excitant. To distinguish these excitants we call the combination of stain and atropine the *artificial* excitant, and that found in cancer plasma the *pathological* excitant.

The Flagellation of Lymphocytes in the Presence of Both Excitants.

In a subsequent paper⁷ we showed that flagellation of lymphocytes takes place when blood is mixed either with the artificial or with the pathological excitant found in cancer plasma. The flagella, which can be watched during the whole process of extrusion, are filamentous and usually have a dot of chromatin at their ends. They break off in time, and in our description of them we speculated that they might be spermatozoa. They certainly resemble the flagella of malarial parasites, but we have now reason to believe that our speculation was erroneous.

EXPERIMENTS.

Pathological Excitant Concentrated in Juices of Malignant Growths.

We have previously referred to the fact that the pathological excitant seems to exist in a more concentrated form in the juices of malignant growths than in the peripheral circulation.

We have investigated this by expressing the juice, citrating it, and mixing it with a small quantity of fresh blood. In most cases the leucocytes responded readily and extruded long pseudopodia. Furthermore, we have found that if some blood is squeezed from a growth immediately after it has been removed, and spread upon a jelly film containing stain and alkali but no atropine, the leucocytes sometimes show exaggerated movements which normal leucocytes will not do on such a jelly.

These experiments suggest that the excitant is actually being produced in some way by the growth itself. It must be remembered, however, that we have found it in the peripheral plasma of persons who only have had very small growths. In addition to demonstrating the presence of an excitant in the plasma, these experiments have also elicited the fact that cancer juice lowers the coefficient of diffusion of normal leucocytes considerably.

An Investigation of the Nature of the Artificial Excitant.

Since this excitation of amoeboid movement appears to be associated with malignant disease, and since in effect it resembles the action of the artificial excitant, the following experiments were made to ascertain whether this excitation increased phagocytosis. The artificial excitant being so constant in its action was the most suitable one to investigate. A suspension first of typhoid bacilli and then of staphylococci was made in citrate solution. To one portion of each of these the correct proportions of atropine and methylene blue were added, thus producing a liquid form of the artificial excitant.⁹ Some fresh blood was mixed with both portions, and they were kept at 37° C. for fifteen minutes. The number of bacteria ingested by the leucocytes was counted by the usual methods and by *in vitro* staining, many samples being observed, and the averages showed that the excitant certainly did not increase phagocytosis, and in many instances it seemed to delay it.

Secondly, we placed a mixture of leucocytes and bacteria on jelly which excited the leucocytes. It was noticed that the pseudopodia were extruded without regard for the bacteria, and if a pseudopodium happened to impinge against a bacterium, the latter was usually pushed out of its way. This experiment has often been repeated, and in the presence of the excitant we have never seen the time-honoured engulfing of bacteria by a leucocyte.

Mr. Simpson, who at that time was one of the Residents at the Royal Southern Hospital, suggested an ingenious experiment to test whether the extrusion of pseudopodia was due to a chemotactic action. Two jellies, one containing the excitant and the other only sodium citrate and sodium chloride, were boiled and allowed to run together on a slide, and a drop of blood under a cover-glass was placed over the junction of the two jellies. If the excitant was a chemotactic one, it was presumed that the cells would throw out their pseudopodia in the direction of the excitant. But they did not do so, for their excitation depended on the amount of excitant they absorbed, and not upon any chemotactic action, for as often as not they thrust out their pseudopodia in a direction remote from the jelly which contained the stain and atropine. Hence these experiments did not explain the nature of the excitant, but showed that it did not increase phagocytosis.

The Cytology of the Lymphocyte.

The cytology of the lymphocyte concerns us very immediately in connexion with this research. These cells differ from other forms of leucocytes in the facts that their nuclei are single, and that they do not contain Altmann's granules in their cytoplasm. But lymphocytes do contain granules arranged on the walls of their nuclei, and these granules give the chromatin reaction with stain. When a polymorphonuclear leucocyte dies, its cytoplasmic (Altmann's) granules show Brownian movement owing to liquefaction of the cytoplasm. The nuclear granules of the lymphocyte under the same conditions remain immobile. When fixed and stained by the older methods the granules of the latter cell merge into the nucleus, which appears as a homogeneous chromatin-staining mass, or the chromatin is arranged as a spireme. By *in vitro* methods the nucleus consists of a transparent membrane studded with chromatin granules. Within the nucleus there is always one, and sometimes more than one, "ring." These rings also stain, as if they were composed of chromatin, and have been described as nucleoli. In a former paper* we have stated that we have on a few occasions seen these rings lying outside the nuclear wall, and we therefore suggested that they might be centrosomes. Seen normally, however, they are within the nucleus.

The Examination of the Cells of Growths by In Vitro Staining.

The examination of the cells of growths by *in vitro* staining has furnished us with some interesting facts. Small portions of the growths were scraped or teased out in a drop of citrated solution on a cover-glass, which was then inverted on to the film of jelly. The tissues were always examined as soon as possible after removal, and those parts of the growth from the extending edge were examined as well as others from the centre of the mass. Jellies with different indices of diffusion were employed, and at first it was difficult to say which of the cells could be styled cancer cells, but it was soon discovered that in every growth there were a large number of cells which had a coefficient of diffusion of about 14, which is the same as that of a lymphocyte, whereas the same jelly would not usually stain ordinary epithelial cells.* No matter what part of the body the growth was taken from (breast, uterus, stomach, rectum, floor of mouth, and tongue),† these cells were invariably present, and showed many characteristics in common. They might have different shapes, but they were very large in size and nearly all contained a large round nucleus, and within the nucleus were rings similar to those seen in lymphocytes. Each one, however, usually had more than one ring, and sometimes had as many as six. They contained no cytoplasmic granules,‡ but only nuclear granules like lymphocytes. In point of fact they resemble huge lymphocytes. In some growths these cells formed the majority, and we noticed that it was practically in them alone that archoplasmic vesicles and archoplasm were to be seen.

* Some further experimentation is required with reference to the coefficient of the other cells.

† Only specimens proved to be carcinoma by the older methods were employed.

‡ When these experiments were made we were not aware that Becton had already arrived at the conclusion that Altmann's granules are absent in cancer cells.

Archoplasmic Vesicles (Plimmer's Bodies) and Archoplasm, and Their Relations to the Casting out of Chromatin by the Cells.

Plimmer's bodies are said to be identical with archoplasmic vesicles. They consist of rounded, deeply staining bodies lying in the cytoplasm. They stain as if they were composed of chromatin, and often appear to contain short rods or dots of chromatin. We have also seen what appear to be chromosomes lying in pairs free in the cytoplasm. As a specimen is watched, the vesicles become more homogeneous, and then appear like diffusion vacuoles (red spots). Archoplasm is a patch of cytoplasm, which also stains scarlet. We believe that the vesicles are the chromosomes cast out from the nucleus, and which have collected together in the cytoplasm to form an archoplasmic vesicle. The vesicle then appears to liquefy, and the chromatin diffusing through the neighbouring cytoplasm gives the picture of archoplasm. The suggestion that the "cancer bodies" were derived from chromosomes originally came from Borel in 1893, but was disputed by Benda on the ground that chromosomes coexist with the vesicles. We do not think that this is sufficient to disprove that the vesicles and archoplasm arise in the manner above stated, and we wish to uphold Borel's hypothesis. The striking resemblance of the vesicles and archoplasm to chromosomes and diffusing chromatin is well shown by the *in vitro* method. Ultimately it is possible that the chromatin is shed and diffuses out of the cell. If this is the case the cells may be extruding chromatin, and a growth may produce a considerable quantity of it.

A Suggestion by Professor Harvey Gibson.

Professor Harvey Gibson made the important suggestion that it might be useful to experiment with nuclein in cancer, and he founded this idea on the well-known fact that in the sexual generation of the normal alternations of generations of plants the nuclei have only half the number of chromosomes which are present in the nuclei of the asexual generation, and that what is normal in the plant appears to resemble what is pathological in the human being's cancer cells. It is thus suggestive that a cancerous growth might be looked upon as consisting of abnormally induced "gametophytic" or sexual tissue. Professor Gibson, with this in his mind, suggested that it might be possible by some means to induce the nuclei deficient in nuclein to absorb more, and so get back to the normal somatic condition. Farmer and others have shown that it is possible to induce such changes in the tissue of ferns, and for many months one of us (C. J. M.), acting on this knowledge, treated some cancer patients with nuclein, which was made by Professor Reynolds Green, but without proof that it conferred benefit. We, however, determined to experiment with it on individual cells. As a matter of fact, H. Gibson considered that the experiments, which will presently be referred to, rather lead to the belief that there is some irritant at work which causes the cells to throw out their chromosomes in the way that we shall presently explain, and that this would correspond very closely to what takes place abnormally in ferns when the asexual generation with the $2x$ number of chromosomes gives rise to a prothallus, or gametophyte, directly with the x number of chromosomes.

The Effect of Nuclein on Leucocytes.

Dr. Reynolds Green very kindly supplied the nuclein. It is a brown powder, which is only sparingly soluble in water. A saturated solution of it was made in citrate solution, and a volume of it was added to an equal volume of blood. It was found that it lowered the coefficient of diffusion of the leucocytes considerably in a few hours, but did not markedly shorten their lives. The solution was then mixed with stained jelly, and leucocytes were examined on it. After twenty minutes' incubation of the slide at 37° C., some curious deeply staining bodies sometimes appeared in the cytoplasm of the cells, but their appearance was not constant.

The Effect on Leucocytes of a Solution Supposed to Contain Chromatin.

The effect of nuclein in lowering the coefficient of diffusion of leucocytes was striking when it is remembered that the juice from a growth also has this effect;

and, again, that the blood cells of cancer patients usually show a lowered coefficient of diffusion. But nuclein was too insoluble to enable us to experiment with it in any variety of concentration. We have already indicated that cancer cells appear to secrete chromatin, and since, as has been suggested, the aggravation of the disease may be due to some secretion from the growth itself, with a view to investigating the effect of this substance on leucocytes, we laked some blood with water and tried the filtrate, assuming that it contained the chromatin of the ruptured leucocytes. It was citrated and mixed with fresh living leucocytes as before. Again the lowering of the coefficient of diffusion was apparent.

Chromatin of Lymphocytes.

Our next step was to experiment with an extract supposed to contain the chromatin of lymphocytes. We were led to this partly because lymphocytes exist in such large numbers in cancerous growths as well as in irritated tissues, but also owing to the fact that we had observed that the chromatin of cancer cells seems to be arranged in a manner similar to that of lymphocytes, and because it is through the lymphatic channels and glands that secondary infections frequently take place.

The small prevertebral (haemal) glands of lambs supplied the lymphocytes. These glands are composed almost entirely of lymphocytes floating in a viscous fluid. To obtain the chromatin we adopted a principle based on the phenomenon of achromasia. In a former paper² it was suggested that achromasia may be due to the chromatin passing out of the dead cells as they become disorganized. It is accelerated by heat and the presence of salts. We therefore mixed up a large number of broken up haemal glands—which were removed from the lambs immediately they were slaughtered—with a solution containing 3 per cent. sodium citrate and 1 per cent. sodium chloride. The mixture was kept at 60° C. for twenty-four hours. This, of course, killed the cells, and they soon became achromatic. The mixture was then filtered, and the filtrate, a yellowish-brown liquid, was again kept at 60° C. for twelve hours to sterilize it. Some blood was mixed in a capillary tube with an equal volume of this extract and incubated at 37° C. for three hours. A drop of the mixture was then examined on jelly which excites amoeboid movement in leucocytes.⁴ It was found that the coefficient of diffusion of the leucocytes had fallen greatly—a greater fall than we had ever seen before except that produced by the action of morphine.⁶ The nuclei actually stained on this jelly which will never stain the nuclei of normal leucocytes. But it was also noticed that the cells contained round or oval vesicles within their cytoplasmata. These vesicles could be seen before the cells stained; but as the stain diffused in, the vesicles became bright scarlet, and appeared to contain rods and dots like "Plimmer's bodies." Every leucocyte contained one or two of these bodies. As the specimens were watched, the vesicles appeared to liquefy, when they resembled large vacuoles (red spots). At first they were quite discrete, but on keeping the mixture of blood and extract in the incubator for a little time longer the leucocytes developed what appeared to be patches of archoplasm, diffuse scarlet-staining patches, resembling those seen in cancer cells. This experiment has been repeated many times, and the appearance of the vesicles is remarkably constant.

Unfortunately we cannot say that these vesicles are the same phenomena as those seen in cancer and reproductive cells. Farmer, Moore, and Walker give a warning that great care must be exercised in forming an opinion as to these bodies, and we can endorse this view. Leucocytes are supposed to be "reproductive" cells, and to undergo mitotic divisions, and archoplasm has been described as being present in normal leucocytes when examined by the older fixed film methods. We wish to state, however, that we have never seen anything in normal living leucocytes which we could possibly call archoplasm, and we certainly had never before seen anything like the vesicles or scarlet staining patches in the cytoplasm of leucocytes until they had been suspended in the extract. It was possible, however, that what looked like vesicles might really be ingested substances or bacteria. To eliminate this possible fallacy we therefore took every aseptic precaution, and filtered the extract most carefully before we repeated the experiment. Still, the vesicles and supposed archoplasm

appeared. Again, the vesicles might have been diffusion vacuoles (red spots), although we had never seen these structures appear in the same way. The extract, therefore, was rendered neutral, and afterwards actually acid, and retried. Still the vesicles appeared, but no red spots appeared in control experiments which contained no extract. These experiments, however, did not prove that the extract produces archoplasmic vesicles, and we could think of no means of deciding the point. Still it was sufficiently promising to warrant our following the matter up, especially as it was coupled with the great lowering of the coefficient of diffusion of the cells.*

Supposed Chromosomes Lying in the Cytoplasmata of Lymphocytes under the Influence of the Extract.

We mixed up a small quantity of the extract with jelly which contained a little stain (4 units). This jelly had the correct index of diffusion to stain the nuclei of lymphocytes in ten minutes at the room temperature. On one or two occasions what appeared to be chromosomes were seen in the cytoplasm of a lymphocyte as the cell stained. They appeared as short rods, staining scarlet, and were independent of the nucleus, which was, of course, studded with granules. We could not be certain whether these rods were chromosomes.

The Excitation of Reproduction.

This last experiment was now repeated, but with a concentrated extract made of 50 grams of haemal gland chopped up in 50 c.cm. of the citrate solution and prepared as described above. A tube of jelly was made thus: To a tube containing 5 c.cm. of "coefficient jelly" 0.5 c.cm. of stain (5 units) and 0.8 c.cm. of alkali (8 units) were added, together with 3 c.cm. of the extract and 0.7 c.cm. of water. The jelly was boiled and a film prepared on a slide. A drop of fresh citrated blood from the finger was placed on a cover-glass, which was inverted on to the film. The endeavour was to stain the nuclei of the lymphocytes very deeply in ten minutes at 37° C. The jelly therefore contained an extra unit of alkali for the coefficient of diffusion of the cells, which is 14. This is the equation (where x is the 3 c.cm. of chromatin extract, which is alkaline and which contains 3 per cent. sodium citrate and 1 per cent. sodium chloride):

$$cf = (5s + 8a + 1.5x + 7h + t) - (6c + 1.5n) = 15.$$

We may say now, although we found out the point later, that it is better to keep the slide at the temperature of the room for two minutes to allow the cells to come to rest on the jelly, and then to insert it in the incubator for about eight minutes.

The object of making this experiment was to see whether the lymphocytes, at their normal temperatures, would more readily show chromosomes in their cytoplasm. But the result was very different and surprising, for the majority of the lymphocytes in the specimen were found to be undergoing mitosis. Many of the figures were typical of the mitotic phase, the number of chromosomes never exceeded sixteen, and although the figures presented different aspects to the observer, the several phases of the phenomenon could usually be recognized quite easily. This experiment has been repeated many times, and if the jelly is correctly prepared, mitoses can practically always be seen in the lymphocytes. Thus we may say that the jelly contains a true cell reproducing excitant, for it must be remembered that, so far as we can ascertain, mitoses have never hitherto been seen in normal lymphocytes from the peripheral circulation, no matter by what methods they have been examined. There is another reason, however, for believing that the jelly contains a reproductive excitant, which is, that unless it is correctly prepared with an index of diffusion suitable for a cell with a coefficient of diffusion of 14 or 15, no mitosis will appear in lymphocytes however much of the excitant may be present, and the cells remain in their resting stage. Hence we may say that the jelly contains a substance which has an "auxetic" effect on lymphocytes, and the substance may be called an auxetic (*αὐξ*, multiplication or increase, and *εἶκος*, an

* As a further control experiment we tried lymph which was obtained for us by Professor Sherrington, who kindly operated on a dog and tapped the thoracic duct. No vesicles or archoplasm were induced in the leucocytes by its action, nor did it lower their coefficient of diffusion to any great extent.

excitor).^{*} Now these mitotic figures immediately directed our attention to some important points. Looking at a cell which presented its side to the observer, it was seen that it had become oval or hour-glass shaped. The first question asked was, What had become of the granule-studded nucleus and its nucleoli? It became apparent that the phantom nucleus had formed the spindle, the granules had collected together round the equator of this spindle and had divided into sixteen portions, which now appeared as chromosomes. At the poles of the spindle the nucleoli were seen, and these "rings" now appeared as the centrosomes. Cells in all the earlier stages of mitosis have been seen, and this is what occurs: The cell first becomes elongated. Then the granules at the poles open out, leaving a clear space through which the nucleoli emerge. The extrusion of the centrosomes pulls the phantom nucleus into the shape of a spindle. The granules curl outwards and towards the equator, where they collect as a solid belt of chromatin surrounding the waist of the spindle. In a cell which presents one of its poles towards the observer, it will be seen that the solid belt of chromatin divides usually into sixteen portions, each of which becomes semilunar shaped, with its points inwards and in contact with the points of its neighbour. Thus the cell presents the typical rosette. We are not quite certain about the next stage. On one occasion, certainly, the chromosomes appeared to be seen dividing longitudinally, but we should not like to state whether this is the rule.[†] Having divided into two, one portion of a chromosome travels up towards one pole, and the other portion to the other pole. The spindle becomes constricted in the centre, and the cell becomes hour-glass shaped. If the cell only contained one ring (nucleolus centrosome), a dot of chromatin took the place of the other.

We cannot describe what occurs after the separation of the chromosomes, because death overtakes the cell before the division is complete. This is the rule, although we have seen one exception, in which a cell had just divided, and even then the two daughter cells were dead. We cannot succeed in preventing this death, and it was because of it that we have been able to follow the stages of the mitosis, for, when it occurred, the chromosomes stained, and thus the figures were demonstrated. In every case, with the one exception, the cells have died in the act of reproduction, and they remain in the stage which we have described until they become disorganized and achromatic.

The Parts Played Respectively by the Stain and Chromatin Extract in Exciting Reproduction.

The appearance of the nucleoli at the poles of the spindles led to the discovery of a fallacy in the last experiment. We remembered that we had already seen on two or three occasions the "rings" lying outside the nuclear wall in the cytoplasm. This fact was mentioned in a former paper⁷ published nearly a year ago, and at that time we certainly had never used the chromatin extract. The suspicion was therefore aroused that we had already seen the early phases of mitoses although we had been unaware of the fact. In the early days we had been using a jelly which only contained stain and atropine; and, since this mixture is an excitant, we made a fresh jelly which contained concentrated artificial excitant and no chromatin extract. To our surprise we found that at 37° C. this artificial excitant containing stain and atropine is itself an auxetic. We next found that the atropine could be dispensed with if 10 units of stain were employed. We then repeated the experiment by which we first recognized the mitoses, employing only the 5 units of stain but omitting the chromatin extract, when it became evident that this low concentration of stain did not cause reproduction. Hence it is clear that 10 units of stain *per se* is a reproductive excitant; that 7 units of stain *plus* atropine has a similar effect; that 5 units of stain *per se* is not sufficient to cause it, but that 5 units of stain *plus* chromatin extract promotes the mitotic figures in a marked degree.

Our next step was to try a jelly which contained extract but no stain, and, as far as could be seen, no effort at

reproduction occurred, for the cells did not become oval or rosette shaped.

Thus stain contains an auxetic but chromatin accelerates its action markedly, for the combination will cause reproduction even if a very small quantity of stain is employed, which could not of itself cause the mitotic changes.

SUMMARY.

We have here placed on record some facts regarding a chemical substance which causes a remarkable train of events when it is absorbed by human lymphocytes in that it promotes, first, excitation of amoeboid movement, then reproduction, and, lastly, death. These events are closely related to each other, and follow in quick succession under the experimental conditions. More than a year ago the fact was noted that there appears actually to exist in the body fluids of persons suffering from carcinomata a substance which causes the first of these events in the chain—namely, the excitation of amoeboid movements. When it is remembered that the excitation of reproduction and death constitute two of the cardinal facts known about cancer—for the cause of cancer, whatever it may be, must be an excitant of cell reproduction, which ultimately leads to the death of the individual—the inference may be drawn that this first event in cancer may be followed by the succeeding ones, reproduction and death, which are produced by the artificial substance under the microscope. Although this chain of events occurs on the slide in the space of a few minutes, it must be borne in mind that the conditions are experimental in the extreme, and must be most harmful to the vitality of the cells. In the body, however, similar events, supposing such to exist, would probably be spread over several generations of cell-life in their occurrence, and a much smaller quantity of the exciting agents would be required to bring about the same delayed results.

By way of recapitulation of the points which we have set forth in this report, may we once more direct attention to the main facts? A mixture of methylene blue and atropine excites amoeboid movements in leucocytes. So does the blood of a cancer patient. The same mixture will cause lymphocytes to extrude flagella with a particle of chromatin at their ends; so will the plasma of a cancer patient. We have shown that an extrusion of chromatin also appears to be a phenomenon which occurs in cancer cells, and that cancer cells appear to produce "something" which aggravates the disease. It is possible that this "something" is chromatin, and in support of this theory is the fact that an extract which probably contains chromatin or a derivative of it accelerates the action of the artificial auxetic. The deduction is that this chromatin thrown out by the cancer cells may be the element which, by affecting some other unknown substance, may promote a repetition of the changes which cause the three events in the chain to which we have referred. The promoter of the first of these events (excitor of amoeboid movement) has already been described as existing in the blood of cancer patients, and to a greater extent in the juice of a growth. If chromatin, or a derivative of it, is the "something" which aggravates the disease, in order to produce a chain of events similar to that which has been observed in lymphocytes, it will have to aggravate the action of a substance resembling in effect that contained in the aniline dye which causes first reproduction, and then death. It may also be noted that chromatin is a product of katabolism which, as has been suggested, may predispose to the disease in persons over the age of 40.

Such are the hypotheses on which we are working. Experimentally one can induce mitoses in lymphocytes, and, as we have shown, these cells present characteristics resembling in many ways those appertaining to cancer cells. Cancer cells frequently show the first (heterotype) mitotic phase; but whether the mitoses induced in lymphocytes are of the first gametic variety remains to be proved. We are not certain yet whether the lymphocyte is normally of the somatic or of the reproductive type. Still, the chain of events is striking, and worthy, we think, of further investigation, which must now be directed to solve the reason for the premature death of the cell, for it would appear that some virulent poison must be at work within the cell to cause it to die in the height of its reproductive activity. This problem now becomes a cytological one,

^{*} This term was suggested by Professor Harvey Gibson, and is more correct than the word "aphrodisiac," which had been thought of.

[†] An important point. Longitudinal division is said to be characteristic of the first (heterotype) mitotic phase.

which should be elucidated as a preliminary to further experimentation.

The research which we have conducted has shown that the actual phenomena of mitosis in human lymphocytes can now be demonstrated. The remarkable differences between these cells and the other leucocytes are now apparent. Lymphocytes respond to the auxetic and endeavour to reproduce themselves, whereas leucocytes do not. The cytoplasmic (Aitmann's) granules of leucocytes can be stained without harm to the lives of the cells. The nuclear granules of the lymphocytes, on the other hand, are composed of chromatin, and somatic death occurs when they stain. The fact that the nucleoli of lymphocytes form the centrosomes is also interesting, but not unique, from a cytological point of view.

In concluding the present report, we may mention that extracts from organs other than haemal glands are effective in assisting to promote mitotic changes—for instance, suprarenal gland and the udder of a ewe—but thyroid gland and udders of lambs do not appear to contain the necessary accelerator of reproduction, and, furthermore, they do not prevent it. The mitoses seen when chromatin extract is used sometimes show a remarkable "lop-sided" appearance, but we have been unable to satisfy ourselves that this was connected with sinapsis, although suggestive of it. In performing the experiments to produce the mitoses which we have described care and accuracy in carrying out details are essential, for unless the alkalinity of the jelly is correct and the temperature accurate no mitoses will be seen, and the cells will remain at rest. The mitoses occur only at a temperature of 37° C., and in the few moments which immediately precede death and the staining of the chromosomes.

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THE DIAGNOSIS OF MALIGNANT DISEASE BY MEANS OF THE ANTITRYPTIC INDEX.

BY

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By the antitryptic index is meant the power of inhibiting tryptic digestion possessed by any given serum, compared with such inhibitory power possessed by a normal standard serum; or, in other words, the ratio of the powers possessed by different serums for neutralizing tryptic ferments.

Dr. F. L. Golla,¹ in a paper on the clinical value of the antitryptic index in tuberculosis, pointed out that the variations in the antitryptic powers of serums were not only interesting laboratory phenomena but could be applied for clinical and diagnostic purposes. He employed the electrical conductivity method introduced by Victor Henri for the study of ferment action, and it is this method that I have used throughout, employing the technique described by Dr. Golla in his paper.

Mr. E. C. Hort in the same month published a paper² on a chemical method for estimating the antitryptic index, especially in relation to diagnosis of malignant disease. In July, 1909, Mr. Hort read a paper before the British Medical Association at Belfast on the diagnosis of cancer by examination of the blood. Here also he used the chemical method, and found that the antitryptic index was raised in 94 per cent. of cases of malignant disease.

Dr. Golla, Mr. Hort, and myself, working independently, have observed that most processes involving cell destruction, whether bacterial, mechanical, or cytolytic, produce a raising of the antitryptic index. The phenomenon can, therefore, be in no way regarded as specific, but this lack

of specificity, though it narrows the scope of the reaction, still leaves an ample margin where its utility as an aid to diagnosis in malignant disease is clearly shown.

Thus, in the absence of bacterial infection symptoms of obstruction or pressure accompanied by a raised anti-tryptic index would indicate the presence of a malignant rather than a benign growth or aneurysm, and a normal index would contraindicate the presence of malignant disease.

It has been observed that the serum of cases of gastric ulcer give a nearly normal reaction, and a diagnosis between this condition and carcinoma of the stomach can therefore be facilitated by the antitryptic test.

Perhaps it may be useful to give a few more details as to quantities than were mentioned in Dr. Golla's paper. I have used 5 c.cm. of the formalized casein solution, 0.5 c.cm. of serum, and 2.5 c.cm. of a fresh and filtered, roughly 10 per cent. solution of pure active pancreatin. The observations were made after the mixture had been in the thermostat ten minutes and four and a half hours, which I found to give the maximum of variation. All results were corrected for the different electrical capacities of the bottles, and the majority of the estimations were determined in duplicate. Instead of expressing the index as Dr. Golla has by + and - signs, I have used the same notation as that employed for the opsonic index and taken 1 to express normal, so that an index expressed by Dr. Golla's + 0.6 is in my notation 1.6 and one of - 0.6 is 0.4.

My results confirm those obtained by Mr. Hort with the chemical method, but as the chemical method depends on the matching of shades of colour, I think that the electrical method, which registers quite clearly and unmistakably very minute differences in the amount of digestion, will be found to be both the more reliable and easier for those without very much practical chemical experience.

Dr. Golla has lately introduced a new method by which he estimates the antitryptic index by means of the viscosity of the serum; but as the estimation of rate of flow requires accurate measurement of quantity, space, and time, there is ample opportunity for errors in observation in unskilled hands. With the electrical method, however, providing that the bottles are standardized sufficiently often, there seems to be but little opportunity for experimental error.

	Site of Cancer.				Antitryptic Index.
1.	Carcinoma of cervix	...	...	...	1.58
2.	" "	...	...	...	1.4
3.	" "	...	...	...	1.17
4.	" "	...	...	...	1.63
5.	" "	...	...	...	1.63
6.	" "	...	...	...	1.33
7.	" "	...	...	...	1.6
8.	Carcinoma of the uterus	...	...	...	1.22
9.	" "	...	...	...	1.6
10.	Carcinoma of the breast	...	...	...	1.25
11.	" "	...	...	...	1.48
12.	" "	...	...	...	1.45
13.	" "	...	...	...	1.05
14.	" "	...	...	...	1.21
15.	" "	...	...	...	1.72
16.	Carcinoma of the rectum	...	...	...	1.42
17.	" "	...	...	...	1.37
18.	" "	...	...	...	1.63
19.	Carcinoma of the lip	...	...	...	1.06
20.	" "	...	...	...	1.4
21.	Carcinoma of the penis	...	...	...	1.31
22.	Carcinoma of the antrum	...	...	...	0.67
23.	Carcinoma of the larynx...	...	...	...	1.57
24.	Carcinoma of the intestine	...	...	...	1.4
25.	Carcinoma of the sigmoid	...	...	...	1.4
26.	Carcinoma of the stomach	...	...	...	1.69
27.	Carcinoma of the tongue...	...	...	...	1.09
28.	Carcinoma of the bladder	...	...	...	1.37
29.	Carcinoma of the nose	...	...	...	1.68
30.	Carcinoma of the soft palate	...	...	...	1.6

Of the 30 cases of cancer that I have given above, 26 (86 per cent.) show a markedly raised index. Of the remaining 4 cases, 3 (13, 19, 27), = 10 per cent., show practically normal indices, and one (22) shows a sub-normal index. This case was a very advanced one, and died before I had the opportunity of obtaining another specimen. The section showed the tumour to be an epithelioma. Of the 3 cases showing normal indices, No. 13 was an early case ten days after operation; No. 19 was an early case before operation; and No. 27 was awaiting removal of the tongue.