New Research
INTO THE
ORIGIN OF CANCER.

[THE two communications which follow mark an event in the history of medicine. They form a detailed description of a prolonged and intensive research into the origin of malignant new growths, and they may present a solution of the central problem of cancer.]

THE ÆTIOLOGY OF MALIGNANT NEW GROWTHS.

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INTRODUCTION.

IN 1911 Peyton Rous [1] described a sarcoma of the fowl which differs from mammalian tumours in one very important way—namely, that it can be transferred from chicken to chicken by inoculating dead cells, killed by drying or with 50 per cent. glycerol, or by means of a cell-free Berkefeld filtrate. The mammalian tumours, with the possible exception of a lymphosarcoma of dogs,* have so far been transferred only by inoculating living cells. This chicken tumour —No. I. in the series examined by Rous—is a spindle-celled sarcoma which metastasises freely and generally kills the host within 28 days. The malignancy of the tumour has increased with passage. It cannot be transferred to mammals or to any other species of birds than the hen, either by inoculating living cells or filtrates. Its specificity at first was exceptionally strict; successful transplantations of the tumour could be obtained only in blood relations of the bird—a Plymouth Rock—in which the original tumour occurred. The tumour now grows freely in other varieties of fowls, but the Plymouth Rock is still by far the most suitable variety of bird for experimental study.

A second filterable tumour of the hen was described by Rous [2] in 1912 (Tumour VII.). This tumour is an osteochondrosarcoma. The primary growth was benign, but it proved to be transplantable and with repeated passage became malignant. It differs from tumour No. I. not only in its histology, but also in its general behaviour, rate of growth, site of metastatic deposits, &c. It can be transferred with dead cells or with filtrates.

The third of these filterable tumours—No. XVIII.—was described by Rous in 1914.[3] It is a spindle-celled sarcoma with blood sinuses into which growth occurs. The tumour may be propagated with living cells, or with Berkefeld filtrates, but not by means of the dried tumour.[4]

There are, then, three well-described filterable tumours—all sarcomata and all growths of hens—among many thousands of animal tumours which have been transplanted and studied. It is very improbable that every neoplasm which has been described has been tested thoroughly as to its filterability, and it is therefore possible that one or more filterable tumours have been overlooked. But the general statement may be made: First, that no mammalian tumour has been propagated by cell-free filtrates; secondly, that a large number have been tested with entirely negative results; and thirdly, that if filterable mammalian tumours occur they are exceptions to the general rule. Thus the filterable chicken sarcomata differ, apparently, fundamentally from other tumours. This difference has led to expressions of doubt as to their being true new growths. It is unnecessary to discuss this point, as the work to be described will show, but it must nevertheless be pointed out that Rous studied the filterable tumours most carefully and showed clearly that they answer every valid test which has been put forward as a criterion of new growths. Indeed, no set of tumours has been more thoroughly studied from this point of view. In my judgment the classical papers published by Rous between 1911 and 1916 settled this point conclusively.

The problem which remained when Rous concluded his work on these tumours was the determination of the nature of the filterable agent. Rous and his collaborators brought forward strong evidence in favour of its being a filterable virus—a living but extremely small microbe. Thus the agent is destroyed by exposure to a temperature of 55° C. for 15 minutes; by chloroform and toluene; by phenol in concentrations as low as 0·5 per cent., and by other antiseptics; and the tumour may be kept immersed in glycerine and it still retains its infectivity. In spite of this evidence Rous used the term "agent" rather than "virus," since the final proof that the agent is animate its cultivation outside the body was missing.

The work which is to be described in this paper began with the study of chicken sarcoma No. I. Before plunging into details of this work a few words on the parasitic hypothesis of the origin of tumours may not be out of place.

The "parasitic hypothesis" of cancer may be stated briefly as follows: Malignant new growths constitute a specific disease and have as their essential cause a specific parasite. To some advocates the parasite is a microbe—e.g., the *Micrococcus neoformans* of Doyen; to others a protozoon; to others again, it is a new form of organism which is capable of existing as "unorganised plasm," as a filterable virus, in coccal or bacillary forms or as a fungus.

The opponents of the parasitic hypothesis—and they are chiefly those who have devoted themselves to cancer research—have no difficulty in refuting the theory in the form in which it has been presented. In the first place there has been no agreement amongst the advocates as to which of the many organisms brought forward as the "cause" of cancer is the true one, and in the second place it has never been possible to reproduce a tumour with a culture of any one of the organisms. Further, when all the definite knowledge we possess on malignant disease is arranged in due order an impressive case can be made out against the general conception that malignant disease has an extrinsic specific cause. The evidence, however, is largely negative, and is really only destructive of the view that tumours are the pathological consequence of the simple conjunction of host and parasite.

The Rous group of filterable tumours has been an embarrassment to both sides. Those who oppose the parasitic hypothesis have been content either to regard these tumours as entirely exceptional—a view that by implication rejects the concept of new growths as a homogeneous, specific disease and places them as a consequence amongst the inflammations—or to deny that the tumours are true new growths. Those who favour the parasitic hypothesis have inclined to ignore the Rous tumours for different reasons; these tumours make the theory too complicated altogether.

One of the outstanding features of new growths is their specificity. A tumour of the mouse, transplanted in fragments of living cells, will not grow in the rat or vice versa. The species specificity is very strict.

* During the year 1924 I collaborated with the late Dr. B. R. G. Russell in the study of this tumour. The work has been continued in conjunction with Dr. A. M. Begg, and the results will be given in another paper.

It may be argued that this is probably an example of general cellular specificity and is therefore not a particular property of tumour cells. And the argument is probably sound. But cell-free filtrates of the chicken sarcomata are just as strictly specific; they are incapable of producing a tumour in any bird save the hen, and much less in a mammal. Moreover, the filtrate from each tumour reproduces only a tumour of the same structure and general behaviour as that from which it is derived. The specificity, in other words, applies not only to the species but even to tissues. If we accept the conclusion that these tumours are caused by living viruses and argue—justifiably—therefrom that other tumours have similar causes, we are inevitably driven, in the present state of knowledge, to the conclusion that for every species of animal there is at least one group of viruses and for every tissue a particular virus. We are thus led by the logical application of undisputed facts to an apparently absurd position. It does not require a penetrating insight into pathological problems to see that an essential fact is missing and that its discovery may be the key to the general problem of tumours.

This strict specificity, to which is probably related the conspicuous and unique cellular phenomena which are so attractive to pathologists, forms the chief obstacle to all attempts to connect ætiologically the great variety of tumours of so many species of animals with one external agent or class of agent. The complete failure to provide a unifying conception of the disease has led cancer specialists to look upon the malignant transformation of a cell as a physiological reaction—or a perversion of a physiological reaction—to long-continued irritation. In other words, the disease is regarded as a particular problem in cell physiology, and however clearly external agents may be demonstrated as factors in the chain of causation of tumours their influence is considered to be entirely subsidiary to internal cellular capacities, and particularly to those which relate to cell division. In the final analysis of the pathology of tumours all this may turn out to be true, but as relatively little is known in this branch of physiology it is premature to indulge in speculation.

I have no desire in the present preliminary publication to discuss the very numerous theories, some of which are merely fantastic, which have been advanced to explain the origin of tumours. It is sufficient to draw attention to this conflict of opinion on the parasitic hypothesis. The work which I now report, and which has been carried on during the last two years, provides, I think, the reconciliation of the two views.

TECHNICAL NOTES.

1. *Filtration.*—An exaggerated importance has been attached to the filterability of a virus. This is reflected in the common use of the term "filterable virus" as a description of the whole class of very small organisms. The objections to the term come from two sides: first, the relatively gross spirochætes pass through bacterial filters readily, and cannot on any grounds be classed with the very small organisms which are invisible, or which, at least, cannot be resolved with the best microscopes in common use; and, secondly, for various reasons, some undoubted viruses are not filterable under the conditions in which they occur. The virus of vaccinia, for example, as it occurs in calf lymph is not filterable; also, the virus of herpetic encephalitis as it occurs in the brain tissue of rabbits is held back by filter candles. Filterability is determined by a number of factors, one of the most important being the properties of the fluid in which the virus is suspended. This is shown very easily with the Rous chicken sarcoma No. 1. If the disintegrated tumour be insufficiently diluted, or if the diluent employed be distilled water, the filtration is difficult and the filtrate is generally non-infective.

The best method of obtaining an infective filtrate is as follows. Healthy tumour tissue is selected and is minced with sharp scissors; the mince is then rubbed with sterile sand in a Wedgewood mortar in order to break up the cells. This is done very thoroughly. Saline or Ringer's fluid is then added to the mass in the proportion of 100 c.cm. to about 1 g. of tumour. After mixing thoroughly the muddy suspension is filtered under gravity through layers of paper pulp and sand, arranged in a long tube-filter, in order to remove sand and remnants of tissue. The filtrate thus obtained—the "sand filtrate"—is usually a faintly yellow transparent fluid, which passes, under moderate pressure, through a Chamberland L_2 or a Mandler candle with ease. The final "candle filtrate" when inoculated into a chicken in a dose of 1 c.cm. causes the formation of a tumour which is palpable in 14 days and almost always kills the chicken within 28 days. Rous showed that the addition of kieselguhr promotes the development of the new growth. In all the experiments described later a trace of silica has been added to the inocula.

2. *Media.*—In the course of this work a large variety of media has been prepared and tested. The formula of one only, that which has proved most satisfactory, will be given. The basis of the medium is Hartley's broth[5] to which is added 0·2 per cent. KCl. The broth is tubed in 5 c.cm. lots and is sterilised by steaming—never in the autoclave. To each tube of 5 c.cm. is added 1 c.cm. of fresh rabbit serum, after which the tubes are incubated at 37° C. for two days in order to test sterility. When sugars are required the sugar is added to the broth in bulk to give a concentration of 0·5 to 1 per cent.

3. *Aseptic Excision of Tumours.*—Bacteriological technique which involves the use of fresh animal tissue is complicated by the occurrence, especially after prolonged incubation, of contaminations. With chicken tumours the common contaminant is a white staphylococcus; with mouse tumours a Gram-negative bacillus; with rat material a Gram-negative bacillus sometimes occurs and about as often a streptococcus which under anaerobic conditions is very small, but which on solid media, incubated aerobically, assumes a larger size. When contaminations occur attempts to produce a tumour fail; in mice and in rats abscesses often follow the inoculation of material which is slightly infected.

When tubes have been incubated anaerobically it is not always immediately evident that a tube is contaminated; turbidity is not by any means constant. A state of affairs is attained which might be called "concealed contamination," the contamination being revealed only by means of subcultures, aerobic and anaerobic, in broth and on agar.

The following technique has been followed in order to reduce these contaminations to a minimum. The tumour-bearing animal is killed with chloroform or coal-gas and the carcass is then soaked in warm 2 per cent. lysol for five minutes. The subsequent procedures are carried out in a sterile room. The animal is pinned out on a sterile cork slab and the skin reflected so as to expose the tumour, the cutting of the skin and necessary dissection being performed by means of a cautery. After searing the surface, pieces of tumour are excised by means of a dry sterile knife and are dropped into tubes of medium. Unless such strict precautions are taken contaminations occur with disheartening regularity.

EXPERIMENTAL STUDY OF ROUS CHICKEN SARCOMA NO. 1.

It has already been remarked that the injection of 1 c.cm. of a Chamberland L_2 candle filtrate is followed by the formation of a tumour which appears in a fortnight and kills within a month. When the infectivity of such a filtrate is tested with doses of varying size it is shown that a proportionality between size of dose and time of appearance of tumour exists. This is well shown in the following protocol.

Experiment June 20th, 1923.—Chickens were inoculated with L_2 filtrate, all into the pectoral muscle, in quantities indicated in Chart 1. This experiment, modified in various ways, has

CHART 1.

20.6.23. CHICK	INOCULUM	DAY 7TH	14TH	21ST	28TH	35TH
5	1cc L_2 FILTRATE.	—	●	●	†22ND DAY	
6	0·5cc L_2 FILTRATE + 0·5cc SALINE.	—	•	●	●	● † 35TH DAY.
7	0·25cc L_2 FILTRATE +0·75cc SALINE.	—	—	•	•	•
8	0·1cc L_2 FILTRATE + 0·95cc SALINE.	—	—	—	—	—
9	0·01cc L_2 FILTRATE + 0·99cc SALINE.	—	—	—	—	—

In the second column of this chart, Chick 8, for 0·95 read 0·90.

been repeated over and over again and the result is constant with filtrates through close candles. The interpretation of the result is not possible without a count of the number of organisms per c.cm. in the infective fluid. Superficially it appears to support the view that the infective "agent" is a chemical substance, since proportionality, in such narrow limits, between dose and effect is not characteristic of living microbes.

INFECTIVITY OF "PRIMARY CULTURES."

A primary culture—a term of convenience—is obtained by placing a fragment of tumour in a tube of medium. The infectivity of the supernatant fluid is retained for a variable length of time depending upon the medium employed and the method of incubation. Retention of infectivity is favoured by anaerobiosis; the more completely oxygen is removed the longer the fluid remains infective. Similarly, the addition of rabbit serum to the broth helps to preserve infectivity. The findings may be summarised as follows:—

(1) When a piece of tumour—roughly 0·5 g. in weight—is dropped into 5 c.cm. of broth and the tube is incubated aerobically at 37° C., the supernatant fluid may remain infective in doses of 1 c.cm. for as long as two days. If the tube is incubated anaerobically the infectivity remains as long as four days, particularly if, before incubation, the air dissolved in the medium or "entangled" in the piece of tumour be removed by means of a Geryk pump.

(2) When the medium contains rabbit serum—5 c.cm. of KCl broth to which has been added 1 c.cm. of sterile serum—infectivity is retained after three days' incubation aerobically. If the aerobiosis be increased by using an Erlenmeyer flask instead of a test-tube infectivity is lost sooner. If a piece of tumour is placed in serum broth and the culture incubated anaerobically the supernatant fluid remains infective often for as long as seven days.

(3) When glucose, maltose, or lævulose are added to the medium an acid reaction occurs, the pH rising often as high as 4·0 to 4·5. With mannite, sucrose, galactose, lactose, dulcite, and salicin fermentation does not occur. The addition of a fermentable sugar tends to increase the time during which infectivity is retained, provided the acidity produced is not too great.

This summary of results of experiments with primary cultures is illustrated by the protocols below, but it must be noted that the size of the piece of tumour added to the medium is of primary importance. If a large mass of tumour—e.g., 2 g.—be added and the culture be incubated aerobically, then it is often possible to produce a tumour with the clear supernatant fluid after as long as seven days' incubation at 37° C. The general impression derived from a long experience of such cultures is that an "agent" diffuses from the tumour tissue and that it disappears slowly in the presence of rabbit serum and under anaerobic conditions, and more rapidly in the presence of oxygen and absence of serum. The larger the piece of tumour the greater the quantity of "agent" to diffuse, and consequently the longer the infectivity is retained.

Experiment Nov. 6th, 1923.—Chicken 44 was inoculated in the right pectoral muscle with 1 c.cm. of a culture made by adding a fragment of tumour (about 0·5 g.) to 5 c.cm. of glucose broth, the tube being incubated aerobically for

CHART. 2

6.11.23. CHICKEN 44	DAY 7TH	14TH	21ST	28TH	35TH
RIGHT BREAST	—	—	—	—	—
LEFT BREAST	—	—	•	●	● †40TH DAY.

three days at 39° C.; in the left breast with 1 c.cm. of an acrobic culture in rabbit serum glucose broth, incubated three days at 39° C. (Chart 2). Post mortem, there was no tumour in the right breast; in the left a large tumour.

This experiment shows that serum tends to preserve infectivity.

Experiment Dec. 27th, 1923.—Two sterile Erlenmeyer flasks were taken; into each 5 c.cm. of rabbit serum glucose broth were placed. A piece of tumour tissue was now placed in the medium in each flask. One flask was incubated aerobically for three days at 39° C., the flask being covered

CHART 3

27.12.23. CHICKEN 47	DAY 7TH	14TH	21ST	28TH
RIGHT BREAST	—	—	—	—
LEFT BREAST	—	•	●	†22ND DAY.

with a rubber cap to prevent evaporation; the second flask was incubated anaerobically for three days at 39° C. Chicken 47 was then inoculated in the right breast with 1 c.cm. of the aerobic culture and in the left breast with the anaerobic culture (Chart 3). Post mortem, there was no tumour in the right breast; in the left a large tumour.

This experiment shows the advantages of anaerobiosis.

DESTRUCTION OF THE AGENT WITH CHLOROFORM.

Rous [6] showed that carbolic acid, toluene, and chloroform all destroy the agent in a relatively short space of time. I have confirmed all his statements, but here I wish particularly to describe the method of rendering "sand filtrate"—which will produce a tumour in doses of 0·05 c.cm.—innocuous by treatment with chloroform.

Healthy tumour tissue is taken and is disintegrated with sand. The mass is then mixed with Ringer's fluid, the volume of fluid used being 100 c.cm. to about 5 g. of tumour. The mixture is filtered through well-packed paper pulp and sand and the filtrate should be quite transparent. Ten c.cm. of the clear sand filtrate is placed in a boiling tube by means of a pipette. The fluid is delivered neatly to the bottom of the tube without wetting the sides. A few drops of chloroform are then run down the sides of the tube, which is held in a slanting position. In this way, although some of the chloroform sinks to the bottom of the tube to form globules, a portion remains for a time as a thin film on the surface. The tube is then placed in a beaker of water, the temperature of which is 37° C., and is kept in the incubator. After half an hour has elapsed the chloroform globules are taken up in a Pasteur pipette and mixed repeatedly with the bulk of the filtrate. The object of this manœuvre is to facilitate the saturation of the fluid with chloroform. The tube is again incubated in water kept at 37° C., and is left until three hours' incubation has been completed. The chloroform is then removed completely by means of a Geryk pump. "Sand filtrate" which has been treated in the above fashion now fails to infect in doses of 2 c.cm., whereas a control tube treated

similarly, but with saline instead of chloroform, is still infective in doses of 0·2 to 0·25 c.cm.

The following experiment shows the importance of temperature and of complete saturation of the filtrate with chloroform in the destruction of the virus.

Experiment Dec. 12th, 1923.—Chicken 49 was inoculated with 1 c.cm. sand filtrate which had been saturated with chloroform and kept at a temperature of 37° C.; Chicken 50 with 1 c.cm. of the same filtrate which had been saturated with chloroform and kept at room temperature; Chicken 51 with 1 c.cm. of the same filtrate incompletely saturated with chloroform and kept at 37° C. for three hours (Chart 4).

CHART 4

12.12.23. CHICKEN	DAY 7 TH	14 TH	21 ST	28 TH	35 TH
49	—	—	—	—	—
50	—	—	●	●	† 33 RD DAY.
51	—	—	•	●	† 38 TH DAY.

For the rapid destruction of the virus the filtrate must be saturated with chloroform and the temperature be kept in the neighbourhood of 37° C. It is to be noted that it is insufficient merely to place the tube in the incubator and expect the contents to attain the temperature in a short time; the tube must be placed in water at the required temperature.

EXPERIMENTS TO SHOW THAT TWO FACTORS EXIST IN INFECTIVE FLUID.

Experiments have already been described in which it is shown that "primary cultures" in broth and in rabbit serum broth become non-infective in two to seven days, the time depending upon the amount of tumour tissue, the composition of the medium, and the conditions of incubation. From the beginning of this work on the Rous sarcoma I suspected that this loss of infectivity does not depend upon the death of a virus, but upon the disappearance of an accessory chemical factor which governs infection of cells. The following experiment shows that this is true. The accessory factor, a product of tumour cells, is obtained by killing the virus in a "sand filtrate" with chloroform in the manner already described. The suspension of the virus consists of a non-infective primary culture.

Experiment Jan. 14th, 1924.—A culture was made by placing a fragment of healthy tumour tissue in 5 c.cm. of rabbit serum glucose broth, contained in a small Erlenmeyer flask, and incubating aerobically at 37° C. for three days. Clear "sand filtrate" was treated with chloroform in the manner already described and the chloroform removed completely under the pump. Chickens were now inoculated as shown in Chart 5.

CHART 5

14.1.24. CHICK	INOCULUM	DAY 7 TH	14 TH	21 ST	28 TH	35 TH	42 ND
66	1 cc CULTURE.	—	—	—	—	—	—
67	1 cc TREATED FILTRATE.	—	—	—	—	—	—
68	0·5 cc CULTURE +0·5 cc FILTRATE	—	—	●	●	●	† 38 TH DAY.

It is clear from this experiment, one of many, that a tumour extract—the "sand filtrate"—treated with chloroform and thereby rendered innocuous has a property which, when aided by a property of an inert primary culture, can bring about the formation of a neoplasm. The interpretation of these results, which were first obtained in a rather different form in November, 1923, is as follows. The chloroform-treated "sand filtrate" contains a labile chemical substance which in some way, as yet unknown, renders the cells susceptible to infection by the virus contained in the primary culture. The proof that this is an accurate conception of the process will be made clear later.

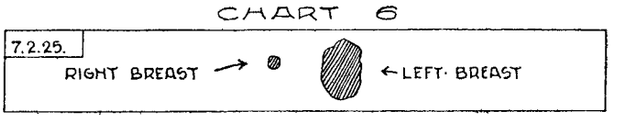
The existence of two factors in primary cultures can be shown by means of the centrifuge.

SPINNING EXPERIMENTS.

The interpretation of the results obtained with infective fluids which are spun at high speed depends in a large measure, but not entirely, upon the fact that a proportionality exists between size of dose and effect. This is illustrated by the following experiment.

A "primary culture" in rabbit serum glucose broth, incubated at 39° C. anaerobically in a McIntosh and Fildes tin for five days, was taken and the clear supernatant fluid pipetted off without disturbing the tumour tissue at the bottom of the tube. The acidity of the fluid was between pH 5·0 and 5·5. The fluid was spun in a conical tube for two hours at a speed of 9000 revolutions per minute. Rather more than 1 c.cm. of the top layer of the spun culture was then taken off with a Pasteur pipette, the greatest care being taken to avoid disturbing the small amount of deposit at the bottom of the tube. Next, 1 c.cm. was taken from the lowest layers, the deposit being included. A chicken was then inoculated in the right breast with 1 c.cm. of the fluid from the top layer, and in the left breast with 1 c.cm. from the lowest layer. The chicken died in 20 days and when examined post mortem the tumours in the breasts were relatively as indicated in the chart here given (Chart 6).

CHART 6



Such an experiment has been taken to indicate a "shift" of the particulate agents, whether purely under centrifugal force or whether as part of a general shift of large colloid protein masses which form in medium containing tissue. In an ordinary infective disease, the clinical signs of which are elevation of temperature, appearance of a rash or vesicles, and so on, a delay in the appearance of signs would be more difficult to interpret. But with a tumour which always occurs locally at the site of injection and which manifests itself as a swelling, the hazards are less.

With candle filtrates and with five-day cultures in media which do not contain fermentable sugars—i.e., when the pH is 7·8 to 7·9—the shift of virus is so small as to be open to doubt. The effect of acidity in aiding spinning is thus very great; all attempts to spin out the virus completely, even with acid cultures, in this way have failed. An additional advantage is obtained by lining the centrifuge tubes with a thin film of nutrient agar. The agar becomes detached during spinning and, when the machine is stopped, it is found at the bottom of the tube. The exact part which agar plays has not been determined, but it was first employed to provide a material upon which it was imagined the virus would stick firmly. This, however, may be passed over for the present; the fact is that by using agar films acid infective fluid can, by a short period of spinning, be rendered inert in doses double and often treble the usual infective dose, as the following experiment indicates.

Experiment Feb. 7th, 1925.—A portion of a five-day culture of a tumour in rabbit serum maltose broth was spun for 35 minutes in a tube lined with agar. The pH of the culture

CHART 7.

7.2.25. CHICKEN 144	DAY 7 TH	14 TH	21 ST	28 TH
RIGHT BREAST	—	—	—	—
LEFT BREAST	—	•	●	● † 32 ND DAY.

had increased from 7·8 to 5·0 during incubation. After spinning, Chicken 144 was inoculated in the right breast with 1 c.cm. of the supernatant fluid; in the left breast with 0·5 c.cm. of the portion of the culture which had not been spun. Chart 7 shows the result. Post mortem, there was no tumour in the right breast.

When the deposit which is obtained by spinning a five-day anaerobic primary culture in rabbit serum glucose broth is washed twice with saline, it is found that the final emulsion of deposit is inert. This might readily be supposed to be because the virus has been washed away. It is not so, however, as can be proved by inoculating a chicken with a mixture of washed deposit and innocuous supernatant fluid, when a tumour forms in the usual time. This is well shown by the following experiment:—

Experiment Sept. 2nd, 1924.—Five-day anaerobic cultures in rabbit serum glucose broth were taken and spun in four conical centrifuge tubes, two of which were lined with agar and two were not. The first spinning was for 40 minutes at 9000 revolutions; the topmost layers were pipetted off from the two agar-lined tubes—in all 4 c.cm. were taken—and put aside. The fluid in the two remaining tubes was pipetted off with care, leaving only the small amount of deposit which had formed. The deposit in each tube was mixed thoroughly with 5 c.cm. of saline and the two tubes again spun at 9000 r.p.m. for 40 minutes. Again the fluid was removed and the deposit mixed with saline and the mixture spun. In this way the deposit was washed twice. It was finally emulsified in 1 c.cm. of Ringer's fluid and the following experiment performed.

Chicken 150 was inoculated with 2 c.cm. of the fluid taken from the agar-lined tubes; Chicken 151 with 0·6 c.cm. of washed deposit plus 0·8 c.cm. Ringer; Chicken 152 with 1 c.cm. of fluid from the agar-lined tubes to which was

CHART 8

29.9.24. CHICK	INOCULUM	DAY 7 TH	14 TH	21 ST	28 TH	59 TH
150	2 cc SUPERNATANT FLUID.	—	—	—	—	—
151	0·6 cc WASHED DEPOSIT.	—	—	—	—	—
152	1 cc SUPERNATANT FLUID + 0·4 cc WASHED DEPOSIT.	—	●	●	† 20 TH DAY.	

added 0·4 c.cm. of washed deposit. Chart 8 shows the result of the experiment.

Chickens 150 and 151 were killed on Oct. 31st, 1925—the fifty-fourth day—and there were no signs of a tumour.

It is not easy to obtain such a perfectly clean result. A more frequent result is indicated in the next experiment.

Experiment Dec. 13th, 1924.—Spinning and washing were carried out as before. Chicken 15 received 1·5 c.cm. of supernatant fluid from agar-lined tubes; Chicken 16 received 0·5 c.cm. of washed deposit plus 0·5 c.cm. Ringer;

CHART 9

13.12.24. CHICK	INOCULUM	DAY 13 TH	18 TH	24 TH
15	1·5 cc SUPERNATANT FLUID.	—	—	○
16	0·5 cc WASHED DEPOSIT + 0·5 cc RINGER'S FLUID.	—	—	—
17	0·75 cc SAND FILTRATE + 0·25 cc WASHED DEPOSIT.	●	●	† 25 TH DAY.

and Chicken 17 received a mixture of 0·75 c.cm. supernatant fluid and 0·25 c.cm. washed deposit. The result was as shown in Chart 9.

Chicken 15 eventually died on Feb. 2nd, 1925, 51 days after being inoculated; Chicken 16 was killed three months later and there was no sign of a tumour.

These experiments have been carried out 15 times; in two instances the cultures proved to be non-infective—in each case there was a "concealed contamination"; in two experiments tumours have developed in the chicken inoculated with washed virus, once after 30 days and once after 28 days. The remainder of the experiments are fairly represented by the protocols given.

When candle filtrates of a tumour are taken, made acid with NaH_2PO_4, and spun in the same way the results obtained are of the same kind, but it is more difficult to render the fluid in the agar-lined tubes non-infective.

Experiment Nov. 11th, 1924.—A tumour filtrate (Mandler candle) was acidified with NaH_2PO_4, the pH being brought to 5·5. The acidified filtrate was then spun, the deposit in the two tubes being washed, as in the protocols already given (Chart 10). Post mortem, it was found that the

CHART 10

11.11.24. CHICK	INOCULUM	DAY 7 TH	11 TH	14 TH	21 ST
184	2 cc SUPERNATANT FLUID.	—	—	●	● † 21 ST DAY.
185	RIGHT BREAST: 0·6 cc WASHED DEPOSIT + 0·7 cc SALINE.	—	—	—	—
	LEFT BREAST: 1 cc SUPERNATANT FLUID + 0·3 cc WASHED VIRUS.	—	●	●	● † 21 ST DAY.

tumour in Chicken 184 had penetrated the peritoneal cavity into which hæmorrhage had occurred, causing death. The tumour in Chicken 185 was confined to the pectoral region and was much larger than that in Chicken 184.

It will be noted that my practice has been to give double doses of supernatant fluid and washed virus when these are inoculated separately. This, of course, is the recognised safe method of control. If, however, the first and third chickens of an experiment be inoculated with the same volume—usually 0·75 c.cm.—of supernatant fluid diluted with Ringer's fluid for the first chicken and washed virus for the third; then the results are similar to those shown in the first protocol (Experiment Sept. 2nd, 1924).

The experiments show that both in primary cultures and in candle filtrates of the tumour there are two factors which are necessary to the production of a tumour; the one is particulate and is therefore probably a virus; the other, being uninfluenced by spinning, is probably a chemical substance. Neither of these factors operating alone will cause the formation of a sarcoma.

The bearing which these findings have upon the pathology of the Rous tumour and, as will be shown, upon tumours generally, is most important. To return to the central difficulty of tumour ætiology, specificity. It seems to me obvious that, since the virus alone is not infective, the specificity of the Rous tumour must be carried by the second—the chemical—factor derived from cells. (That it is not a toxin or other product of culture is evident from the results of spinning candle filtrates of tumours and from the fact—shown later—that subcultures alone are not infective.) It is very probable that the virus of the Rous tumours VII. and VIII. is the same as that of No. 1†; the chemical factor in each case would, I presume, be different. In this way the specificity is again related to the cells, but in the case of the filterable tumours, to a cell derivative which can be separated from the cells. I propose to call this chemical substance the "specific factor."

At this point it is convenient to refer to attempts to stain and see the virus. Most of the stains recommended for the study of small organisms have been employed in this research. Giemsa's fluid in particular has been thoroughly tried. In order to obviate repetition in the later parts of this paper it is as well to state at the outset that all attempts to stain and see the virus, using for the purpose a 2 mm. apochromatic lens with eye-pieces ranging from 6 to 20, have failed. The "granules" which can be stained in films made from a medium containing tissue have been seen but that they are not the virus is shown by the following observation. A film was made from a five-day primary culture of the Rous tumour in rabbit serum glucose broth; a chicken was then inoculated with 1 c.cm. of the culture. Next the culture was spun at 9000 revolutions per minute for

† This cannot now be tested as the tumours VII. and VIII. have been lost. Dr. Rous very courteously sent me dried powder of the tumours, but they proved to be inert.

40 minutes in an agar-lined tube. The upper part of the fluid was removed with a pipette and a film made; 1 c.cm. of the fluid was then injected into a chicken which did not develop a tumour; the chicken inoculated with the unspun fluid died of a large tumour three weeks later. The films were passed through fixative together and then stained together with Giemsa's long method; both showed innumerable pink granules on the border-line of resolution. Such experiences as these have led me to the opinion that such granules are not the virus. The visual discovery of such small organisms is obviously a special problem in optics. Fortunately, my colleague, Mr. J. E. Barnard, F.R.S., has undertaken this work. His report upon it follows this paper.

THE CULTIVATION OF THE VIRUS IN VITRO.

Whilst the results of spinning experiments show that one factor in the tumour-producing filtrate is particulate and is therefore probably a virus, the proof of multiplication in vitro is essential for the final justification of this conclusion. To describe completely the attempts, successful and unsuccessful, to cultivate the virus would expand this paper unreasonably; details will be left to the technical journals. Here it is sufficient to say that the most useful medium employed is that which has been described already. To 6 c.cm. of rabbit serum KCl broth a fragment of chick embryo is added. The age of the embryo used has generally been 12–16 days. The tube is then inoculated with a loopful of primary culture and is now called the first subculture. It is incubated in an anaerobic tin at 35°–36° C. for four days, when the second subculture is made into similar medium containing freshly removed embryo. The maximum growth as judged by the results of experiments on chickens is obtained on the fourth to fifth day of incubation.

The dilution with each subculture is at least a thousand times; in a fifth subculture, therefore, the original inoculum has been diluted 10^{15}, or a thousand billion times. If tumours are produced with subcultures beyond the fifth we may be certain that growth has taken place. The following protocols show results obtained with such subcultures.

Experiment Sept. 18th, 1924.—Chicken 157 was inoculated with 1 c.cm. of sand filtrate which had been treated with chloroform; Chicken 158 with 1 c.cm. of a fifth subculture of

CHART 11

18.9.24 CHICKEN	DAY 8 TH	14 TH	21 ST	42 ND
157	—	—	—	—
158	—	—	—	—
159	—	●	●	† 25 RD DAY.

the tumour virus; Chicken 159 with 0·5 c.cm. of the sand filtrate plus 0·5 c.cm. of the fifth subculture. The results are seen from Chart 11.

Experiment Dec. 4th, 1924.—On Dec. 2nd, 1924, fragments of tumour were placed in tubes of glucose broth and the tubes incubated anaerobically in a McIntosh and Fildes tin for two days at 39° C. On Dec. 4th supernatant fluid was taken from the tubes without disturbing the tumour

CHART 12

4.12.24 CHICK	INOCULUM	DAY 14 TH	21 ST	28 TH	52 ND
206	1 cc SUPERNATANT FLUID.	—	—	—	—
210	0·5 cc SUPERNATANT FLUID + 0·5 cc OF A FIFTH SUBCULTURE OF VIRUS.	—	●	●	† 31 ST DAY.
211	2 cc OF THE SAME FIFTH SUBCULTURE OF VIRUS.	—	—	—	—

tissue. The fluid was spun in agar-lined tubes for an hour and three quarters at 9000 revolutions, and the supernatant fluid pipetted off. Chickens were then inoculated as shown in Chart 12.

Experiment April 27th, 1925.—In this experiment an eighth subculture of virus was used. Chloroformed sand filtrate was prepared as before and chickens inoculated as shown in Chart 13.

CHART 13

27.4.25 CHICK	INOCULUM	DAY 7 TH	14 TH	21 ST	28 TH
168	1 cc SAND FILTRATE.	—	—	—	—
169	1 cc 8 TH SUBCULTURE.	—	—	—	—
170	0·5 cc SAND FILTRATE + 0·5 cc 8 TH SUBCULT.	●	●	●	† 28 TH DAY.

MAMMALIAN TUMOURS.

Four standard tumours of rats and mice have been studied. These are: (1) A spindle-celled sarcoma of the mouse, strain 37/S of the Imperial Cancer Research Fund; (2) the Jensen rat sarcoma; (3) a carcinoma of the mouse, strain 63; and (4) a rat carcinoma, strain 9, both of the Imperial Cancer Research Fund. The mouse tumours 37/S and 63 give 100 per cent. of takes in mice, metastasise, and always kill the host. The rat tumours often retrogress. All four tumours are accepted as genuine neoplasms by competent pathologists in this country and abroad.

The first part of the investigation was a re-examination of the filterability of the sarcomata. In this work, during the early months of 1924, I enjoyed the assistance of my colleague Dr. W. J. Purdy. The mouse tumour 37/S, especially, was thoroughly tested. The method of preparing extracts was similar to that described for the chicken tumour. The candle used was a Chamberland L_1, which permits *B. prodigiosus* to pass when a rich broth culture is filtered; cells, however, are invariably kept back. In different experiments the tumour tissue was ground with sand at freezing-point, at room temperature, and at 37° C. The diluent was varied from time to time; the pH was varied from extreme acidity to extreme alkalinity. But under no conditions were we able to produce a tumour with a cell-free filtrate. It may be taken as certain that the mouse sarcoma 37/S is not a filterable tumour in the sense that the Rous chicken sarcoma is. The same may be said of the Jensen rat sarcoma, though fewer experiments were made with this tumour. The carcinomata were not reinvestigated.

Mouse Sarcoma 37/S.

The survival of the tumour cells in vitro was first tested. This was repetition of unpublished work which I had first carried out in 1918–19 whilst on the staff of the Imperial Cancer Research Fund. The general result of these studies is that the tumour cells die relatively rapidly when incubated at 37° C. in Ringer's fluid or broth. This is illustrated by a typical experiment.

Several tumours were excised aseptically and minced with sharp scissors; 1 c.cm. of the mince was then delivered by means of a syringe into 5 c.cm. of Ringer contained in a test-tube. Another portion of the mince, contained in a sterile capsule, was kept in the cold. The tube of Ringer containing tumour cells was now placed in a beaker of water the temperature of which was 37° C. and kept at this temperature for 18 hours. Two series of 12 mice were now inoculated, one with the incubated tumour cells which had been separated from the Ringer by spinning; the second set with the tumour emulsion which had been kept in a sterile capsule in the cold. The second set of mice developed tumours in the usual way, whilst those injected with incubated cells remained exempt and were eventually killed.

The explanation of the early death of cells is certainly not simple. The addition of serum tends to preserve the viability of cells, as does also an abundant supply of oxygen. Absence of oxygen—for incubation

in Ringer is partial anaerobiosis—plays an important part in the lethal process. When a piece—about a gramme—of healthy tumour is dropped into a tube of rabbit serum KCl broth and the tube is incubated 24 hours in an anaerobic tin and the clear supernatant fluid then injected into mice, about 50 per cent. of the animals develop tumours which appear usually on the fourteenth to eighteenth day and attain a large size—2–3 g.—in four to five weeks. The tumours thus produced have the structure characteristic of 37/S, and behave identically when transplanted in other mice. Are they produced by inoculation of an occasional cell which survives anaerobic incubation? Or are they genuine new growths produced by infecting the host's own cells with a virus derived from the incubated tumour tissue? It is probable from what has already been said that the second explanation of their origin is the correct one. The following experiments prove this.

A number of healthy tumours were removed and placed—about 2 g. per tube—in tubes of rabbit serum KCl broth. The tubes were now put into a McIntosh and Fildes anaerobic jar which was evacuated with a Geryk pump. As the pressure in the jar fell gases bubbled out of the tubes and especially from the surface of the tumour tissue. Pumping was continued till no more gas was given off. Hydrogen was then run into the jar and the coil of platinised asbestos warmed with an electric current in order to achieve complete anaerobiosis. The jar was incubated at 37° C. for three hours and was then again evacuated. This time the medium in the tubes, being warm, boiled under the reduced pressure. In this way the air entangled on the tumour was completely removed. The jar was made anaerobic and then incubated for 24 hours at 37° C. The jar was now opened, the fluid in the tubes pipetted off, spun in the centrifuge, and the topmost layers of fluid taken off and inoculated into mice. The mice had well-developed tumours in 14 days, which were removed in 21 days and the experiment repeated with them. The same result was again obtained. Thus by incubating under perfectly anaerobic conditions the percentage of tumours obtained *may* reach 100. The following is the protocol and chart of the second experiment.

Experiment April 30th, 1925.—About 1 g. of healthy tumour tissue was placed in a tube of rabbit serum KCl broth; incubated 25 hours anaerobically in a jar. On May 1st

CHART 14

30 4 25 MOUSE		DAY 7 TH	14 TH	21 ST
1	BLACK & WHITE ♀	—	●	●
2	YELLOW ♀	—	●	●
3	WHITE ♀	—	●	●
4	CHOC & WHITE ♂	—	—	○

four mice were inoculated, each with 1 c.cm. of supernatant fluid (Chart 14). Lest doubt remain that cells have survived anaerobic incubation and originated tumours, the following experiment was performed.

Experiment May 22nd, 1925.—Cultures were made as before by adding healthy tumour tissue to medium and incubating 24 hours at 35° C. The cultures were now mixed in a boiling tube and one half inoculated into mice (A); the liquid remaining was filtered through a Chamberland L_1 candle. Chart 15 shows the result of the experiment. Primary cultures of 37/S, incubated for three days in this way fail to produce tumours. Proof that this is not due to death of the virus will be given in another paper. Charts 14 and 15 are the best results obtained. When dry necrotic tumours are used the results of inoculation may be quite negative.

It is now certain that a typical mammalian sarcoma (37/S) can be transferred with a cell-free filtrate, under the special conditions described. The Rous chicken tumour is thus, in reality, not exceptional; it differs from the mammalian sarcoma 37/S merely in the fact that its accessory chemical factor is either more abundant or more stable. All the evidence points to the oxygen lability of the chemical factor and it is possible that the explanation of the uniform failure

CHART 15

MOUSE		7 TH DAY	14 TH DAY	21 ST DAY
A. MICE INOCULATED WITH UNFILTERED CULTURE.				
1	YELLOW ♀	—	●	●
2	FAWN ♀	—	●	●
3	DARK FAWN ♀	—	●	●
4	BLACK & WHITE ♀	—	●	●
B. MICE INOCULATED WITH FILTERED CULTURE				
1	BROWN ♀	—	●	●
2	BROWN ♀	—	●	●
3	BLACK ♀	—	●	●
4	BLACK & WHITE ♀	—	●	●
5	BLACK & WHITE ♀	—	●	●
6	BROWN & WHITE ♀	—	●	●

to transfer 37/S with tumour filtrates is due partly to the oxidation of the specific factor in grinding with sand, and partly to the excessive dilution necessary to form a filterable mixture.

Jensen's Rat Sarcoma, Rat Carcinoma 9, and Mouse Carcinoma 63.

It will be observed that proof of the existence of an extrinsic cause of 37/S sarcoma proved to be a lengthy and laborious research. For the remaining three tumours an indirect method has been adopted to obtain the same information. The method consists in substituting the virus of one of these tumours for that of the Rous sarcoma in the manner indicated in the following experiments:—

Experiment April 17th, 1925.—Sand filtrate of Rous chicken sarcoma was prepared from healthy tumour tissue. The filtrate was perfectly clear, faint yellow in colour, and viscid. 10 c.cm. were saturated with chloroform and then incubated in a beaker of water kept at 39° C. The chloroform was completely removed by means of a Geryk pump and the following experiment was made:—

Chicken 251 was inoculated with 1 c.cm. of the filtrate; Chicken 252 with 0·5 c.cm. of the filtrate to which was added 0·5 c.cm. of a culture made by adding a fragment of 63 carcinoma to rabbit serum KCl broth and incubating

CHART 16

17.4.25 CHICKEN	DAY 7 TH	14 TH	21 ST	42 ND	70 TH
251	—	—	—	—	—
252	—	●	●	† 21 ST DAY.	
253	—	—	—	—	—

anaerobically for three days at a temperature of 36° C.; and Chicken 253 was inoculated with 1 c.cm. of the culture of 63 carcinoma. The result is shown in Chart 16.

It is evident from this experiment, which has been repeated many times, that the culture of 63 carcinoma can replace the virus of the Rous tumour. The tumours thus produced cannot be distinguished histologically or biologically from the Rous tumour.

The next protocol is included to show how important is the preparation of the specific factor.

Experiment May 9th, 1925.—Clear sand filtrate was prepared from a Rous tumour and two lots, each of 10 c.cm., were taken. To one a few drops of chloroform, insufficient to saturate the mass, were added and the mixture incubated for three hours; to the second lot of 10 c.cm. rather more than 1 c.cm. of chloroform was added and saturation facilitated by repeated mixing. The second tube was incubated for 5 hours. Chickens were inoculated as follows:—

(*a*) Chicken 284 was injected with 1 c.cm. sand filtrate insufficiently treated with chloroform; Chicken 285 with 0·5 c.cm. to which was added 0·5 c.cm. of a three-day primary culture of carcinoma 63; and finally, Chicken 286 was inoculated with 0·5 c.cm. of filtrate diluted to 1 c.cm. with 0·5 c.cm. saline. The result is indicated on Chart 17.

CHART 17.

9.5.25 CHICK	DAY 7TH	DAY 14TH	DAY 21ST	POST MORTEM
284	—	—	●	KILLED 23RD DAY. SMALL TUMOUR. NO METASTASES.
285	—	●	●	DIED 22ND DAY. LARGE TUMOUR. DEPOSITS IN LUNGS AND LIVER.
286	—	—	—	KILLED 42ND DAY. NO TUMOUR.

(*b*) Experiment with sand filtrate treated with excess of chloroform and incubated five hours. Details are shown on Chart 18.

CHART 18

9.5.25 CHICK	INOCULUM	DAY 7TH	DAY 21ST	DAY 42ND
287	1 cc FILTRATE.	—	—	—
288	0·5 cc FILTRATE + 0·5 cc CULTURE OF MOUSE CARCINOMA 63.	—	—	—
289	0·5 cc FILTRATE + 0·5 cc CULTURE OF RAT CARCINOMA 9.	—	—	—

Rat Carcinoma 9 and Jensen's Rat Sarcoma.

These tumours will be taken together in order to save space. Cultures of them are made in the usual manner by adding a fragment of healthy tumour to a tube of rabbit serum KCl broth. Carcinoma 9 usually becomes very hæmorrhagic and a young tumour must therefore be taken. In the case of the Jensen rat sarcoma retrogression is so common that it is advisable to chart the tumour frequently in order to be certain that it is taken in the growing phase. Such cultures may now be substituted for the Rous cultures, as in the experiments already taken from protocols for tumour 63.

Experiment May 15th, 1925.—10 c.cm. of water-clear sand filtrate of a Rous tumour was taken and treated with chloroform. The following chickens were inoculated: Chicken 291 with 1·5 c.cm. treated sand filtrate (specific factor), Chicken 292 with 0·5 c.cm. filtrate plus 0·5 c.cm. saline, Chicken 293 with 0·5 c.cm. filtrate plus 0·5 c.cm. of a twelfth subculture of the Rous tumour, Chicken 294 with 0·5 c.cm. filtrate plus 0·5 c.cm. of a culture of rat carcinoma 9, and Chicken 295 with 0·5 c.cm. filtrate plus 0·5 c.cm. of a culture of the Jensen rat sarcoma (Chart 19).

Again, the tumours produced with the specific factor of the chicken sarcoma, together with cultures from the rat tumours, do not differ in structure from the ordinary Rous No. 1.

It has now been shown that the non-specific factor can be obtained from three mammalian tumours. Experiments with subcultures of these tumours are not yet complete, but there is no reason to doubt that the element provided is a virus.

HUMAN TUMOURS.

Tests with three tumours only have been completed. One of these, for which I am indebted to Dr. A. G. L. Reade, was a scirrhous carcinoma of the breast. Cultures were made, but proved to be contaminated. Nevertheless, chickens were inoculated with the culture and specific factor but results were negative. The second was a sarcoma of the thigh, pieces of which were kindly given to me by Sir Cuthbert Wallace. The cultures which were made from this tumour were uncontaminated, but experiments on chickens failed

CHART 19

15.5.25 CHICK	INOCULUM	DAY 7TH	DAY 11TH	DAY 14TH	DAY 21ST	P.M
291	1·5 cc SPECIFIC FACTOR.	—	—	—	—	† 35TH DAY. NO TUMOUR.
292	0·5 cc SPECIFIC F. + 0·5 cc SALINE.	—	—	—	—	† 35TH DAY. NO TUMOUR.
293	0·5 cc S.F. + 0·5 cc 12TH SUBCULTURE OF ROUS TUMOUR VIRUS.	—	●	●	●	† 24TH DAY.
294	0·5 cc S.F. + 0·5 cc 3RD DAY CULT. OF RAT TUMOUR 9.	—	●	●	† 18TH DAY	
295	0·5 cc S.F. + 0·5 cc CULTURE OF JENSEN RAT SARCOMA.	—	?	●	† 18TH DAY	

completely. The third was an adeno-carcinoma of the breast; I am indebted to Prof. G. E. Gask for the opportunity to test it. Cultures were made both from the tumour itself and from an axillary gland which was enormously enlarged. There was no contamination in the cultures. The experiment was made on May 23rd, 1925. Chart 20 shows the result.

CHART 20

23.5.25 CHICK	INOCULUM	DAY 7TH	DAY 14TH	DAY 21ST	DAY 28TH
296	1 cc SPECIFIC FACTOR OF CHICKEN SARCOMA.	—	—	—	—
297	0·5 cc S.F. + 0·5 cc CULTURE OF HUMAN TUMOUR IN R.S.KCl BROTH, 4 DAYS AT 36°C.	?●	●	●	†
298	0·5 cc S.F. + 0·5 cc CULTURE OF GLAND IN R.S.KCl BROTH, 4 DAYS AT 36°C.	—	●	●	†

Chick 297 died on the twenty-third day; and Chick 298 on the twenty-eighth day.

The tumours produced were again indistinguishable from the Rous tumour.

One human tumour then behaves like the rat and mouse tumours. The negative result with the human sarcoma may mean that a group of viruses—not interchangeable—exist which cause tumours, but obviously more experiments must be done before an opinion can be formed. The failure with the scirrhous tumour can be ignored owing to the contamination of the culture.

DISCUSSION.

It has now been shown that the Rous tumour No. 1 is caused by a virus which has been cultivated; that the mouse sarcoma 37/S can be transmitted with cell-free filtrate; that the rat tumours 9 and J.R.S. and the mouse carcinoma 63 and a human breast carcinoma all provide a factor which can replace the virus of the Rous tumour in the genesis of a chicken sarcoma.

The common factor of the last four tumours is almost certainly a virus. The production of tumours with remote subcultures remains to be done. Further, it has now been shown that the virus alone is unable to bring about the malignant transformation of a cell. An adjuvant, fortunately provided in abundance by the chicken sarcoma, is necessary; this adjuvant

I have called the specific factor. Superficially, it might be thought that Bail's term "aggressin" would appear satisfactory, but a casual analysis of the facts relating to an aggressin shows that this is not so. As an example of an aggressin the toxin of *B. welchii* will be taken. The toxin is separated from the bacilli by filtration and the minimal lethal dose for mice determined. Bacilli, conveniently grown on solid medium are washed free of toxin, and are then found to be perfectly harmless to mice. If, now, mice are inoculated with a sublethal dose of toxin to which has been added washed *B. welchii*, an infection is set up, more toxin is produced in vivo, and the mouse eventually dies of a spreading gangrene with toxæmia. When the bacilli added to the toxin are *B. welchii* the infection produced is characteristic of *B. welchii* infections; when *Vibrion septique* is added the disease produced is characteristic of *Vibrion septique*;[7] and similarly, toxin-free harmless tetanus spores mixed with sublethal doses of *B. welchii* toxin produce tetanus.[8] Thus, the specificity in these cases depends upon the microbes; the aggressin, acting equally well for all three, produces a microscopic lesion which in some way, not yet fully understood, enables these anaerobic organisms, which alone are harmless saprophytes, to proliferate in the tissues and there to make their characteristic poisons. These facts have been established by experiments similar to, but much easier to perform than, those which have been employed in the work described in this paper. The differences between an aggressin and the specific factor of a tumour are evident.

These researches have led me to look upon cancer —using the term in its widest sense—as a specific disease caused by a virus (or group of viruses). Under experimental conditions the virus alone is ineffective; a second specific factor, obtained from tumour extracts, ruptures the cell defences and enables the virus to infect. Under natural conditions continued "irritation" of tissues sets up a state under which infection can occur. The connexion between the specific factor of a tumour and an irritant remains to be investigated. Some of the relatively unimportant "irritants" are known, such as coal-tar, paraffin oils, &c. The virus probably lives and multiplies in the cell and provokes the cell to continued multiplication.

The methods of experiment now described, and the obvious extensions of them, give the means of classifying the viruses, of investigating the nature of the specific factor, and of testing suspected "irritants," such as preservatives in foods, which may play a rôle in the genesis of a tumour.

I am indebted to the Executive Committee of the Imperial Cancer Research Fund for placing at my disposal their unrivalled series of animal tumours. My thanks are due to my former chief, Dr. J. A. Murray, F.R.S., for his continued critical interest in the work. To my colleague, Dr. W. J. Purdy, I am indebted for assistance in many experiments, for help in the routine inoculation of tumours, and for the preparation of charts; over and above this direct help, I desire to acknowledge the advantages I have derived from his criticisms both of experiments and the interpretations placed upon results. Finally, I wish to state that I owe much to my laboratory servant, Mr. W. Hall, for his willing service.

REFERENCES.

1. Peyton Rous: Jour. Med., 1911, xiii., 397.
2. P. Rous, J. B. Murphy, and W. H. Tytler: Jour. Amer. Med. Assoc., 1912, lix., 1793.
3. P. Rous and J. B. Murphy: Jour. Exp. Med., 1914, xix., 52.
4. P. Rous: Jour. Exp. Med., 1913, xviii., 416–427.
5. P. Hartley: Jour. Path. and Bact., 1922, xxv., 482–483.
6. P. Rous and J. B. Murphy: Studies from the Rockefeller Institute for Med. Res., 1913, xvi., 253.
7. Bullock, W. E., and Cramer, W.: Proc. Roy. Soc., B, 1919, xc.
8. W. J. Tulloch: Proc. Roy. Soc., B, 1919, xc., 145.

THE MICROSCOPICAL EXAMINATION OF FILTERABLE VIRUSES

ASSOCIATED WITH MALIGNANT NEW GROWTHS.

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(With Illustrative Plates.)

Introduction.

THE search for filterable viruses by microscopical methods presents problems of great difficulty, due for the most part to the optical limitations of the microscope. The evidence that such organisms exist is based upon numerous experiments in which infective material has retained its infectivity after passing through a filter that will not pass the smallest known visible organism. There is at present no method in general use by which such filters can be standardised, and it is therefore not certain that filterable viruses can be regarded as organisms of any definite order of size. Other factors than size of the organism influence the result of filtration experiments, not the least being variability of the filters themselves and the viscosity of the filterable material. If filtration did give more exact information as to the size of any given organism it would then perhaps be advisable to adopt some other standard of reference than permeability. Such a standard might be the limits of microscopical resolution, by which all bodies of less than 0·25 μ in their greatest diameter should be regarded as filter-passers, although in that case some other more suitable name might well be found.

It is necessary to consider the main factors limiting both visibility and resolution in the microscope as these limits operate so definitely in any attempts to see or to photograph small bodies. It is also of interest to realise the probable size of filterable viruses in relation to the smallest micro-organism on one hand, and a large molecule on the other. The latter limits are shown diagrammatically in Figs. 1 and 2. The inner circle A in Fig. 1 may be regarded as a hæmoglobin molecule, molecular weight 16,600 approximately. The circles B, C, D, represent the orders of microscopical resolution obtainable if certain ultra-violet radiations are used of wave-lengths 232 μμ, 257 μμ, and 275 μμ respectively; they do not indicate the magnitude of relative wave-lengths. The circle E represents the relative position of the green line in mercury wave-length 546 μμ, with which the visual observations have been made. Between the positions A and B, therefore, no method at present exists by which microscopical resolution can be obtained. (The millimicron μμ = one-thousandth of a micron, 10^{-7} cm.) It is convenient to use the micron for microscopic dimensions and the millimicron for wave-length estimations. To enable a comparison to be made with an organism of known size, reference must be made to Fig. 2, which is on one-tenth the scale of Fig. 1. In this the relative wave-lengths are represented by the small inner circles B, C, D, and E, and the outer circle F represents a body of 1·0 μ in diameter, the average size of a small micrococcus. On reference again to Fig. 1 it will be seen that to obtain the necessary microscopical resolution, a term which will be explained later, and which is proportional to the wave frequency of the light used, it is necessary to use light of wave-length 275 μμ if a body of 0·1 μ in diameter is to be represented, whereas wave-length 257 μμ would resolve down to approximately 0·075 μ, a dimension that is of importance in the present investigation. Fig. 2 gives some idea of the possible sizes of filter-passers. It is certain that they are made up of a considerable number of complex molecules, but no exact limit is assignable in either direction. There are, however, two physical considerations that do suggest a limit of smallness. One is the difficulty there would be for a small body to overcome its