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SOME RECENT WORK IN EXPERIMENTAL CANCER RESEARCH*

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The pioneers of cancer research, especially Borrel, were well aware that the study of transplanted tumours is only the study of cells which are already "cancerized," and can throw no light on the complex changes that occur when a cancer forms. It was clearly understood that the aetiology of cancer is divisible into two parts: the causes which lead up to the occurrence of a tumour and the cause which explains the property of the malignant cell. This distinction is not artificial or merely plausible, invented to explain an apparently irrational multiplicity of facts concerning cancer. It is a deduction from general pathological observations, and is now a self-evident conclusion from recent work with carcinogenic agents. Clinically it has long been known that epithelioma of the skin may develop as a consequence of the action of tar, pitch, lubricating oils, or x rays on the skin; and that the autonomous growth of the malignant cells is independent of the action of the tar or oil or other causative agent, since the metastases, remote from any possible action of the causative agent, behave as malignant cells. Further, a tumour brought into existence by applying a carcinogenic agent, chemical or physical, can be transplanted indefinitely in normal animals of the same species. Thus the agent which is responsible for starting a tumour plays no further part in the progress of the disease once malignancy has occurred. This distinction between causes which act on normal cells and lead to malignant transformation and the intracellular cause which is responsible for malignancy is not fanciful; it is a necessary deduction from universally accepted facts. Ewing has designated these processes as the "causative genesis" and the "formal genesis" of cancer. The simpler terms "remote causes" and "the proximate cause" are perhaps preferable. Included under the term "remote causes" are the very numerous chemical substances, radiant energy, and gross parasites which have been proved capable of initiating new growths. The second part of cancer aetiology, the proximate cause, gives rise to keenest discussion and will be dealt with later. The advances in our knowledge may be conveniently grouped under these respective terms.

Remote Causes of Cancer

It is now common knowledge that cancer can be started at will by the application of one or other of the "carcinogenic agents." This knowledge has robbed cancer of much of its "mystery" and has helped to create a healthy optimistic outlook in research. It has demonstrated that the sort of investigation which has proved so successful for other diseases can lead to great advances in our knowledge of cancer.

Space does not permit of even a brief survey of the carcinogenic agents. They are already numerous and are being added to every year. In this field of research clinical observation has usually anticipated laboratory experiment. Percivall Pott more than 150 years ago observed that chimney-sweeps suffered excessively from scrotal cancer, and attributed this to soot. That x rays may induce epithelioma was proved by the pioneer radiologists, and the causative association of parasites with cancer was observed long ago in bilharzia. Laboratory investigations have amplified and extended these observations. They have led to the preparation of pure chemical substances which can induce cancer formation, and have given rise to fruitful working hypotheses. A whole series of carcinogenic hydrocarbons with a phenanthrene nucleus have been discovered by Professors Kennaway and Cook and their collaborators. One of these compounds, methyl cholanthrene, can be obtained from bile acids by chemical transformations theoretically possible in the body. Moreover, these carcinogenic substances are related chemically to oestrone, to cholesterol, and to other substances which are naturally present in the body. The hypothesis that all cancer-producing substances possess the phenanthrene nucleus is not, however, tenable: Japanese pathologists have been successful in inducing cancer with aniline dyestuffs.

Yoshida has proved that *o*-amino-azo-toluene induces hepatic cancer in rats which are fed with the substance dissolved in olive oil. Kinoshita has shown that dimethyl-azo-benzene—"butter-yellow," so called because according to Kinoshita it is used commercially as a colouring matter in butter—likewise causes cancer of the liver when fed to rats. He found that 176 mg. fed over a period of fifty days was the smallest dose able to give rise to a hepatoma. These substances, administered by mouth, are probably changed by the liver and excreted in an altered form. Thus *o*-amino-azo-toluene is known to be broken up and eliminated as *p*-acetyl-toluylenediamine in the urine. Butter-yellow, fed to rats, is detectable in the liver, and to a less extent in the kidneys and spleen: elimination from the body is not easy, but it is probably broken up and partially excreted as dimethyl-*p*-phenylenediamine.

The induction of cancer by means of oestrone is of exceptional interest because its mode of action has given us a glimpse of what may be described vaguely as an internal carcinogenic environment. The story begins with the study of heredity in cancer, which led to the production by inbreeding of strains of mice in which 70 per cent. or more of the females died of cancer of the breast. Leo Loeb, working with such a strain, showed in 1916 that when very young females were ovariectomized the percentage of cancer fell significantly. His observations were confirmed by Cori and later by W. S. Murray, who

*A summary of a lecture given at the Royal College of Surgeons of England on February 16, 1938.

succeeded also in proving that young males into which ovaries from litter-sisters were grafted may suffer from mammary carcinoma. It was concluded from these experiments that an ovarian hormone is in some way related to the occurrence of cancer of the breast. This conclusion was finally clinched by Lacassagne, who proved that the injection of folliculin in male mice of a cancer strain results invariably in cystic hyperplasia which passes into malignancy. These observations have been confirmed abundantly.

Oestrone, it should be noted, does not cause tumour formation at the site of application; it acts at a distance on an organ, the mamma, with which it is physiologically related. Its mode of action is being closely studied. My colleagues, Drs. Cramer and Horning, have already shown that under its influence complex changes occur in the ductless glands generally. The most conspicuous changes are in the adrenals and the pituitary body. In the adrenals brown degeneration in the zone between medulla and cortex takes place. The pituitary body, after an early period of hyperaemia, becomes greatly enlarged and congested, and frequently adenomas composed of chromophobe cells are found.

Cramer and Horning deduced from their studies of these phenomena that one or more of the hormones of the anterior lobe are capable of neutralizing the effects of oestrone on the pituitary gland. They proved by careful experiment that the thyrotropic hormone has this power. Injected weekly it keeps in check the mammary and pituitary changes produced by prolonged oestrinization, and in female mice having a high incidence of mammary cancer it prevents the spontaneous development of cancer of the breast.

The Proximate Cause of Cancer

It has already been pointed out that a tumour which has been started by applying one or other of many agents, chemical or physical in nature, may be propagable through an unlimited series of normal animals, and that the continued growth and multiplication of the malignant cells is independent of the action of the agents used in starting the tumour. How can we explain the change that has taken place within a cell when malignancy occurs? Verbal explanations are easy. It might be said that the cell has undergone "an irreversible change"; and doubtless a term of this kind, giving as it does an air of scientific validity, might prove satisfactory to chemists or physicists, who are accustomed to working with comparatively simple material under well-regulated conditions. But the explanation carries us no further. Similarly a solution of the problem might be conveyed by suggesting that the malignant cell is a somatic mutant. Such an explanation again is merely verbal. It has no factual basis, it ignores some well-established facts, it is arrived at by the dangerous process of reasoning by analogy, and it offers no prospect of further advances in knowledge. It is unprofitable to spend time in considering such explanations. I shall confine my attention to the view that a virus may be the proximate or intimate cause of malignant growths. The reason for doing this is that there are many tumours in which a virus cause has been established, and this fact gives a feeling of security that we are not living in regions of pure speculation.

Mode of Action of a Tumour Virus

First of all it must be made clear that the mode of action of a tumour virus is different from the action of carcinogenic agents. The virus acts promptly, is specific both to the animal and to the tissue, and can be recovered

again from the tumour which follows inoculation. With a filterable tumour like the Rous sarcoma No. 1 or the Imperial Cancer Research Fund's strain of endothelioma known as Mill Hill No. 2, tumour formation begins in a week or less after inoculation; each virus produces only its own tumour in only one species of animal; and the virus increases in amount with the growth of the tumour. When a tumour is started with the most active of carcinogenic agents—with benzpyrene, for example—the story is quite different. In the first place, the time interval between application of the chemical substance and the appearance of a tumour is, even in the most sensitive animals, a matter of months. In the second place, the kind of tumour produced depends upon the site of application: if the skin is painted a squamous carcinoma results, but if the substance is injected into the subcutaneous tissues a sarcoma follows. There is no specificity of action. And, in the third place, the chemical substance, though present in the primary tumour, is not necessary to the growth of the tumour and is not recoverable from remote generations of transplants. It is puzzling to understand how it comes about that viruses are so often classed with the carcinogenic agents when their mode of action is so manifestly different.

I must now hasten to state that the tumours for which a virus cause has been established are comparatively few in number; or, rather, are almost confined to birds. They consist of tumours of the domestic fowl, a rabbit papilloma which goes on to malignancy, and an adenocarcinoma of the kidney of a certain species of frog. Other tumours may assume a filterable phase, as Parsons has shown for a mouse sarcoma which had been induced by injections of a complex hydrocarbon. The great majority of tumours used in experimental research—tumours of mice, rats, guinea-pigs, dogs, etc.—can be propagated only with living cells. If attention is restricted to these it must be observed that nothing could appear more improbable than the notion that these tumours are caused by an intracellular virus. They give no evidence of any sort that a virus is at work. It is largely because of this that the majority of pathologists have until recently paid scant attention to viruses in cancer. At present, it is fair to remark, most workers in cancer would agree that any coherent explanation of cancer must include an explanation of the filterable tumours.

The division of tumours into two kinds—those which can and those which cannot be transmitted by cell-free extracts—is one of the major puzzles of experimental cancer research. Why some tumours readily yield an infective agent and others subjected to the same treatment should give innocuous extracts is at present inexplicable. But recent work has shown that failure to demonstrate an intracellular causative agent by direct means—that is, by serial transmission of tumours with non-cellular material—must not be interpreted too dogmatically. This conclusion is supported by two pieces of research—one by Foulds, who worked with a dibenzanthracene tumour of a fowl, and the second Peyton Rous's revolutionary work with the Shope papilloma.

Foulds's Work with Non-Filterable Dibenzanthracene Tumours

The tumour studied by Foulds is a fibrosarcoma of the domestic fowl. It was brought into existence by injecting a hen with a solution of dibenzanthracene in lard.* The original purpose of inducing a tumour in this way was to determine whether an artificially induced tumour is trans-

* The dibenzanthracene was generously provided by Professor Kennaway.

missible with cell-free extracts. The object of the work was, in other words, to put to the test of experiment the thesis that dibenzanthracene or similar agents act upon cells by sensitizing them to the action of a virus. It may be stated at once that all attempts to transmit this tumour with cell-free extracts have failed, though it can be propagated fairly easily with living cell grafts. In some of the experiments in which these tests were made it is not even justifiable to use the term "cell-free extract," for it has repeatedly been found that a saline extract which has been spun for a few minutes at 5,000 revolutions is incapable of giving rise to a growth. This artificially induced sarcoma therefore differs in no wise from tumours of mice and rats. It has one great advantage as material for experimental study, since it is possible to test in an indirect way whether the cells, though providing no direct evidence of the presence of a virus, do or do not contain an antigen related to the infective agents of filterable fowl tumours. We cannot make such tests with either spontaneous or artificially induced tumours of rats and mice because we have no filterable rat or mouse tumours to provide virus to test immune sera. The experiments carried out by Foulds were briefly as follows. Rabbits were bled in order to obtain samples of normal serum and were then injected repeatedly with Berkefeld filtrates of the dibenzanthracene tumour. After a time it was found that the sera of the rabbits acquired an antibody which neutralized completely the virus of the Rous sarcoma. This was proved by mixing the serum with active filtrates of the Rous sarcoma, the control being a similar mixture of active filtrate and serum taken before the immunizing injections were made; the mixtures were incubated at 37° C. for an hour and then injected in different sites of four chickens. In the sites where mixtures containing immune serum were placed no tumours developed, whereas in the control sites large rapidly growing Rous sarcomas formed.

It is clear from the experiments, which have been fully described by Foulds (*Amer. J. Cancer*, 1937, **31**, 404), that although a filtrate of the dibenzanthracene tumour is inactive—that is, is unable to provoke tumour formation—it nevertheless contains an antigen which is capable of giving rise to antibodies that readily neutralize the virus of the Rous sarcoma. That this antigen is not some indefinite component of normal chicken tissues was proved in several ways—for example, by attempting and failing to absorb the antibody by means of chicken tissue, and by attempting and failing to produce a similar antibody by injecting rabbits with extracts of normal chicken tissues. There is therefore something closely related to the infective agent of the Rous sarcoma in these inactive filtrates of a dibenzanthracene tumour. Whether it is a virus or part of a virus we do not yet know. For the present it is sufficient to emphasize that these experiments are a warning against too confident and too dogmatic conclusions which may be drawn from negative results of filtration experiments.

A comment on criticisms of this work may be allowed before passing on to a brief account of work on rabbit papillomas. It has been objected that the virus which is present in the dibenzanthracene tumour may be merely carried in the tumour passively, as a passenger is carried in a train—that it is unrelated causatively to the tumour. The foundation of this criticism lies in the well-known fact that viruses can be made to lodge in tumour growths—for example, the virus of vaccinia can be carried by mouse tumours. It is inferred from this fact that it is possible, if not probable, that a dibenzanthracene tumour growing in a chicken attracts virus which may normally exist in chicken tissues and provides suitable conditions for

the existence, and presumably for the growth and multiplication, of the virus. If this were so the production of immune sera by the methods employed would be misleading. This criticism is not relevant to the facts. It is sufficient to point out some obvious differences between the two cases. We prove that vaccinia virus is being carried in a mouse tumour by showing that an extract of the tumour causes vaccinia; we cannot prove that the virus of the Rous sarcoma is present in the dibenzanthracene tumour by causing a Rous tumour with extracts: the extracts are completely harmless. The recognition of the supposed virus in the dibenzanthracene tumour is dependent on its resemblance to a tumour-producing virus. Further, when the dibenzanthracene tumour is artificially contaminated by the virus of the Rous tumour the contamination is recognizable by injecting chickens with a cell-free extract of the tumour, when a Rous sarcoma is produced. This fact, which we owe to Sir Edward Mellanby, pretty well disposes of the theoretical objection under discussion.

Rous's Work on the Shope Papilloma

We can now turn to the work of Peyton Rous on the Shope papilloma. This tumour was discovered by Dr. R. E. Shope on the skin of a cotton-tail rabbit. Shope found that the tumour is transmissible with filtrates and with glycerinated tissue both in cotton-tail and in domestic rabbits. The skin is shaved and then scarified as in vaccinating a person with calf lymph, and a filtrate or extract of glycerinated tissue is rubbed into the scarified area. After two to four weeks small translucent papillomas appear; they grow and eventually form large cauliflower-like masses of tissue. In cotton-tail rabbits the tumour can be transmitted in an endless series in this way, but in domestic rabbits, although the papilloma in them is larger and more fleshy as well as more aggressive in character, it has been found as a rule that transmission beyond the first generation is not possible. Thus the papilloma can be started in domestic rabbits, but thereafter the virus appears to be lost. Shope, however, has succeeded during the last few years in separating a strain of the virus which can, with some difficulty, infect domestic rabbits serially. There are now in most laboratories two strains—the "cotton-tail strain" and the "domestic rabbit strain."

The aggressive character of the papilloma in domestic rabbits, already referred to, is indicated by the frequency with which it becomes malignant. Experience in the laboratories of the Imperial Cancer Research Fund is that almost all Shope papillomas in domestic rabbits become malignant within two years. If the primary papilloma is very large malignancy may supervene within six months. In the cotton-tail rabbit, on the contrary, the papilloma rarely passes into carcinoma; Professor Berry of Rochester University has observed a case and Peyton Rous several out of hundreds of papillomas.

The position has thus been reached that a papilloma of the rabbit, caused by a virus, regularly passes into a state of malignancy. The carcinoma invades tissues locally and neighbouring lymph nodes and internal organs, especially the lung, leading to the death of the host animal. Is the virus the cause of the malignant growth also? If a cell-free filtrate of the growth would start a new malignant growth then the conclusion that the virus is the cause would be incontestable. But this is not so. Filtrates are completely inactive. It appears superficially that as soon as the papilloma becomes an invading true malignant neoplasm the virus disappears, and we are confronted with the same apparently insoluble problem presented by the generality of mammalian cancers.

Again we have to seek indirect evidence. Peyton Rous succeeded in propagating the carcinoma through two generations of rabbits by the process of grafting fragments of living carcinoma cells taken, in order to avoid the possibility of including papilloma cells in the inoculum, from an "infected" lymph node. As the implanted cells grew to form "daughter tumours" it was found that the blood serum of the rabbits acquired an immune body which neutralizes the papilloma virus. The conclusion which must be drawn from these observations is that, although filtration experiments with the carcinoma give negative results, the carcinoma cells do nevertheless carry the papilloma virus. The final proof that the virus acts causatively is more difficult, though it is fair to observe here that if we were now discussing any other subject than cancer the *prima facie* evidence that the virus is acting as the cause of the pathological condition under investigation would be scarcely questioned. At least it may be said that the negative filtration results do not warrant the conclusion that the papilloma virus is absent. It is very probable, indeed almost certain, that conditions exist within the cells of the tumour under which a virus-protoplasm complex is formed and that filterability may depend upon an easy or a particular dissociation of the complex.

Relation Between Carcinogenic Agents and Viruses

Passing now to the relation between the action of carcinogenic agents and viruses, it is necessary to discuss an experiment with coal tar and papilloma virus carried out by Peyton Rous. When coal tar is applied once a week to the skin of a rabbit's ear papillomas form after a few months, the time depending upon the quality of the tar. Some of the papillomas may, after a much longer time—a year or more—become malignant. The experiment carried out by Rous was as follows:

A series of rabbits were painted with coal tar for about three months, when small papilloma began to appear. Some of the rabbits then received intravenously an injection of virus obtained from a papilloma of the cotton-tail rabbit. Painting the ears with tar was continued on all the rabbits, control and experimental alike. After two to three weeks—the incubation period usually observed with the virus in its action on the skin—the tarred areas in those rabbits which had received virus developed true anaplastic rapidly growing squamous carcinomas. The cancers appeared both at the sites of papilloma formation and on tarred areas devoid of papillomas. No cancers appeared on areas that had not been tarred. The control rabbits, which had been treated with tar only, showed no cancer at the time nor later, a long while after the others had died of generalized cancer.

This experiment has been repeated and confirmed, and has excited world-wide interest. Like so much work of Peyton Rous it goes to the heart of the problem. It will doubtless be subjected to criticisms similar to those already discussed in dealing with Foulds's work with non-filterable dibenzanthracene tumours—namely, that it is possible to cause viruses of all sorts to lodge in tumour growths. But these criticisms are invalid; for, as Rous himself has pointed out, when a virus such as Virus III is carried as a contaminant in a transplantable rabbit carcinoma the virus has no effect on the tumour, whereas in his experiment the Shope virus causes quickly a definite specific change in the tar lesion, and the change corresponds with changes which it is known that the virus may induce slowly under normal and presumably less favourable conditions. It is more reasonable to look upon the action of tar as being of a preparatory kind, as affecting the cells in such a way as to render them very susceptible to the action of the virus. If this view is correct—and it certainly is in conformity with the fact that carcinogenic

agents like tar have a fundamentally different mode of action from that of a virus—it permits us to postulate as a working hypothesis that when a tumour is induced in an animal by applying carcinogenic agents the vital change from hyperplasia to malignancy is probably associated with the entry of a virus. This presupposes the widespread existence within the animal body of viruses of low infectivity but capable, when suitably conditioned, of entering cells and setting in motion the train of events described as cancer. Some difficulty is felt in accepting the supposition, but it can be pointed out that where it is possible to make experiments capable of yielding information some supporting evidence has been found. Thus it is now known, following the first observations by Pentimalli, that immune bodies which neutralize the viruses of avian neoplasms appear in the blood in a high percentage of apparently normal fowls more than 18 months old. These immune bodies are presumably formed in response to a virus related to or identical with the viruses found in actual neoplasms.

An outline of cancer work in progress must necessarily be incomplete; each section could be expanded into several lectures. But I hope I have given evidence that progress is being made in many directions and that what has been done already justifies us in our optimism.

VITAMIN C DEFICIENCY IN PEPTIC ULCERATION AND HAEMATEMESIS

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Although frank clinical scurvy rarely follows the modern conservative methods of dietetic treatment of peptic ulceration (Davidson, 1928; Barling, 1935; Wood, 1935; Platt, 1936) it is now being realized that early "pre-scorbutic" or "subclinical" states may occur quite frequently. The experimental evidence put forward by McCarrison (1919, 1931), Magee *et al.* (1929), McConkey (1933), and Smith (1933) shows that ulceration of the stomach and duodenum is very apt to occur in animals in a state of vitamin C deficiency, while the importance of vitamin C in the normal reparative processes has been further emphasized quite recently by Lanman and Ingalls (1937), who have shown that a deficiency of this vitamin is a potent factor in delayed wound healing.

Harris *et al.* (1936) found that nineteen patients with peptic ulcers had distinctly subnormal daily urinary excretions of ascorbic acid, but in that particular inquiry 84 per cent. of all the patients examined showed subnormal daily urinary excretion values. An important contribution was that of Archer and Graham (1936), who showed that six out of nine patients with peptic ulcers, for which they were receiving dietetic treatment, were in the "subscorbutic" state. In the course of our investigations we have had the opportunity of examining the