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Experimental Cancer Research

BY SIR FREDERICK BANTING

T. D'ARCY LEONARD, CHAIRMAN:—Members of the Canadian Club. I have the honor to introduce to you today a speaker who needs no introduction. The name of Banting anywhere in the world is sufficient introduction, for everywhere he is known for the enormous benefits he conferred on diabetics when he discovered insulin. Now his task is research into cancer, and today he will tell us something of the work done and advances that have been made at the Banting Institute.

Sir Frederick is one of our few honorary members, and I think you will agree with me that Ontario is fortunate in having so distinguished a research worker to take up the battle against the disease that is the second biggest killer of mankind. Gentlemen, Sir Frederick Banting.

DR. BANTING:—Mr. President and Gentlemen. The animal body, with the exception of the cuticle, nails and hair, is entirely made up of cells. These cells have different structures and functions in the various organs and tissues of the body. Cancer is an uncontrolled growth of normal cells which serves no useful purpose. Cancer originates as a local disease and may develop in any organ or tissue of the body, which accounts for the large number of different types. A cancer is said to be benign when it remains within a capsule and does not invade surrounding tissue. Benign cancers frequently become malignant. A malignant cancer invades and destroys the surrounding normal tissues and may be carried in the blood stream to organs in distant parts of the body; the lymph vessels may also carry cancer cells to the lymph glands which thus become involved in the malignancy. If a cancer originates in the supporting structure of the body such as muscle, bone, or fibrous tissue,

it is called a sarcoma; if it originates in the skin, in the gastro-intestinal tract or in the glands along the intestinal tract it is called a carcinoma.

Many cancers arise without apparent cause, while others have a definite history of long-continued local irritation of physical or chemical origin. The most unfortunate thing about cancer growth is that it does not of itself cause pain. Pain usually does not occur until the cancer has affected some vital organ and caused a disturbance in its function, or until the cancer has eaten through to a surface and become infected by bacteria.

Cancer is a very old disease, but experimental work on the problem has been carried out for less than fifty years. In 1889 Hanau observed a rat with a lump, which he removed and examined microscopically and found to be a cancer. He injected a fragment of this tumor under the skin of other rats. These fragments grew to be large tumors and these he again transplanted. Hanau believed that he had a means of investigating the cancer problem. He was, however, unable to persuade other people of the importance of his discovery and because of his disappointment he committed suicide. Many attempts were made at that time to produce cancer in animals by injecting them with human cancer, as had been done in the case of tuberculosis, but all attempts failed. Human cancer did not grow even when transplanted into the chimpanzee; as a result the prevailing opinion of that time was that there was no relationship between human and animal cancer.

Very little experimentation with cancer was carried on until 1902, when the Imperial Cancer Research Fund was established in England. This organization consisted of laymen and research workers. By the establishment of this Fund, men were able to devote their lives to cancer research and to plan a continuity of experiments. One of the first things that was done was to send out a call for animals with tumor. People responded by bringing old dogs, cats, mice and rats. The slaughter-houses permitted the examination of carcasses of old animals. Within three years, over 1000 cases of tumor in mice were examined and studied microscopically. This group of workers were thus able to place cancer experimentation on a firm basis by establishing

the fact that cancer is fundamentally the same in animals as in man. This conclusion was reached by a thorough study of animal tumors. They found that cancer was not uncommon in domestic animals that were allowed to live out their natural span of life. Thus the age incidence of cancer was found to be the same in animals as in man. By a detailed study of the spontaneous tumors in mice and other animals they found that the cancer cells in humans and in animals were identical in appearance, in power of invasion into normal cells, in formation of secondary cancer in distant parts of the body and in their response to radium and x-ray. This is the foundation on which all experimental investigation on cancer was built. Of all experimental diseases produced in animals, few, if any, resemble the disease in humans as closely as does cancer.

Having established the similarity of animal and human cancer, Bashford and Murray, and later, Cramer showed that when fragments of mouse tumor were transplanted into another mouse, the cells of the fragment grew and gave rise to a new tumor. This propagation could be carried on from mouse to mouse in an indefinite number of tumor generations. The cells of the mouse into which the transplant was made, took no part in the malignant process. The cancer cell differs from all normal cells in that it can be transplanted and retransplanted from animal to animal of the same species. Transplantation of cancer was found to be possible only within the species from which the cancer was derived. A mouse tumor did not grow in a rabbit nor even in so closely allied a species as the rat. This special specificity explained why all efforts to transmit the human cancer to animals had failed. No one has yet been able to transmit human cancer to an animal.

By 1908 the work of the Imperial Cancer Research Fund had created a world-wide interest in experimental cancer. Rous and a group of associates at the Rockefeller Institute in New York examined a large number of fowl tumors. They made an offer to the wholesale chicken dealers to pay \$5.00 for every live chicken with a tumor. In this way the famous Rous chicken sarcoma No. 1 was discovered. This tumor occurred in a pure-bred Barred Rock chicken and

fragments of the tumor were transplanted into a number of birds of different varieties. Tumors only grow in blood relations of the original chicken. This again illustrates the specificity of the tumor cell. After the tumor had been re-transplanted from bird to bird a number of times, it assumed an established rate of growth and became less specific. Later, it was found that the tumor could be propagated in other ways. Cells of the tumor were ground in a mortar with sand and extracted with saline. This extract was then filtered through a special filter which removed all the cells. This cell-free extract, when injected into the breast muscle of Barred Rock chickens, produced a tumor similar in every way to the original spindle celled carcinoma. It was also found that the Rous tumor cells could be killed by drying, without effecting their power to produce tumors. These findings created a marked distinction between chickens and mammalian tumors, as the latter can only be reproduced by transplantation of living tumor cells. A further distinction can be pointed out, for, as previously stated, when a rat or mouse tumor was transplanted, it was the injected fragment which grew and not the surrounding host cells, but when cell-free extract or dried, powdered cells are injected, the normal cells of the host are rendered malignant by the tumor-producing agent.

This original work of Rous and the later work of Gye of London has established the fact that the Rous tumor in chickens is due to a virus. A virus is an organism which is so small that it cannot be seen under the highest power of a microscope and is able to pass through filters which hold back visible bacteria.

In addition to the original Rous No. 1, which consisted of spindle cells alone, Rous found a second type of chicken tumor which was slower in growth and contained immature cartilage and bone cells. Both these tumors could be transmitted by an extract which was free from living cells and the resulting tumors in each case were identical with the tumor from which the extract was made. The extract, although free from cells, carried with it not only the power to produce tumor, but also the ability to produce a tumor of the same character as the original one.

The question then arose, was the character of the new tumor the result of a different virus in each case, or was it the same virus with an accompanying factor which accounted for the specificity? Gye, working at the National Research Council of London, sought to explain these facts and advanced the hypothesis that all tumors were due to a virus and that the variation in tumors in the same animal and the species specificity of tumors in general, was due to a specific factor attached to the virus. Gye's hypothesis as to the cause of tumor stimulated a tremendous amount of the recent research work.

In 1904, Yamagiwa and Itchikawa, two Japanese workers, produced malignant tumors in rabbits by painting the ears each week for many months with coal tar. This was a great step in cancer research, because it proved that normal cells could be transformed into malignant cells by the application of a low grade irritant over long periods of time. Coal tar was selected by these workers because it had been repeatedly observed that chimney-sweeps so frequently developed cancer of the scrotum. It was found that tumors produced by the application of tar could also be transplanted from one animal to another of the same species by the injection of a small quantity of the living tumor cells.

Some years later, Kennaway, Cook, Hieger, and others, working at the Cancer Free Hospital in London, made a detailed investigation of the particular constituent of tar which would produce cancer. They started with two tons of gas works pitch, which was the residue from the distillation of coal tar. By means of distillation, extraction and crystallation they reduced this volume to about 2 ounces of a very active cancer-producing material. On further purification, this compound was found to be a complex hydrocarbon which had been unknown up to that time, but which was identified as 1:2 benzpyrene. Other related hydrocarbons had been prepared synthetically by Cook, many of which were found to be carcinogenic. The one most frequently used experimentally is 1:2:5:6-dibenzanthracene. The cancer produced in mice by these substances develops very slowly so that it took six to nine months to ascertain whether or not they were carcinogenic. When the chemical

identity of these carcinogenic substances had been established, it was found that they closely resembled that of certain newly isolated sex-hormones.

The next step was to ascertain if these substances had the same physiological action as the hormones. It was found that the cancer-producing substances did induce the estrus cycle in young mice, i.e., they were oestrogenic as well as carcinogenic. It would appear from the most recent work that if certain of the oestrogenic substances are applied in sufficiently large quantities over long periods of time, cancers are produced. They differ from tumors by tar products in that they do not appear at the site of application but in the breast or pituitary gland.

Normal bile also contains a substance from which methylcholanthrene can be isolated. This substance is the most active cancer-producing substance known.

The reason why so much attention was paid to the chemicals which produce cancer was because it was hoped that it would prepare the way for a chemical understanding of the cancer process and it might even be possible to neutralize these cancer-producing substances by other chemicals.

The outstanding feature of tar cancer and the cancers produced by chemical substances derived from tar is that cancer does not result immediately after the application. No visible change takes place in the tissue cells for weeks or months. Benign, warty growths first develop. These warts persist for weeks and months before malignancy develops. The explanation of this phenomena is not yet forthcoming. It would appear, however, that the effect of the tar is to prepare the normal cell for the malignant change.

Cancer may also follow the chronic irritation produced by physical means. Smokers' cancer of the lip was common in the days of the short clay pipes. Carcinoma of the oesophagus was a common disease among the upper class Chinese men who ate hot rice. It was not found in their women because they ate after the men had finished, and the rice was cool. In the early days of X-ray and radium, the workers did not protect themselves and many of them developed skin disease which later became malignant. Although these tumors have not been used experimentally they illustrate the

fact that a wide variety of irritations may prepare the cell for the malignant change.

It would be of great value to know the exact role of heredity in the production of cancer. The impossibility of such an investigation in the human is evident. Observations cannot be extended over a sufficient number of generations, breeding cannot be controlled, accurate records cannot be acquired because of lack of postmortem examinations. All of these difficulties can be overcome in the experimental laboratory.

In 1909, Maude Slye began an investigation of the heredity factor in the production of cancer. She commenced the selective breeding of mice in which a spontaneous tumor had been observed. The normal span of life of the mouse is from 2-3 years. She has now followed the occurrence of cancer in many generations of pedigreed stock and has, by careful postmortem examination of many thousands of mice, shown that the tendency to cancer is a hereditary factor which may be bred in or bred out of a given strain of mice. As the result of the work of Maude Slye and others, we may now obtain a strain of mice 90 per cent. of which will develop spontaneous tumor if they survive to the tumor age. On the other hand, strains of mice can be obtained which will die of old age with an incidence of spontaneous tumor less than 10 per cent. These strains are called tumor and resistant strains.

About ten years ago the Department of Medical Research at the University began work on the cancer problem. Owing to limited facilities it was necessary to begin in a small way, but from time to time, as facilities increased, we have been able to widen the scope of the research and attack the problem from various angles. At the present time we have 12 full-time workers on the cancer problem in the Department.

Our first experiments were done with Rous chicken carcinoma. This tumor was selected because it is one of the most malignant of the experimental tumors and never fails to develop when transplanted. A tiny fragment of this tumor when injected into the breast muscle of a Barred Rock chicken increases in weight as much as 1,000 times in less than a week. The birds usually die of tumor in about three

weeks. If they survive longer, small secondary tumors are frequently found in the heart, lungs, and liver. As previously stated, this tumor can be transmitted by a cell-free extract, in which case the tumors develop more slowly and the tumor-bearing birds survive a longer period of time. Transmission by cell-free filtrate is not as reliable as by transplantation of the living cells.

In order to keep a supply of tumor tissue on hand for experimental work, transplants are made into three Barred Rock chickens once or twice a week. The chickens for all our experimental work have been obtained from the Ontario Agricultural College at Guelph. When an experiment is carried out with the cell-free filtrate it is always necessary to inoculate the original extract into control birds because sometimes the filtrate is inactive or may give rise to very slowly growing tumors.

In 1928, we observed that a large tumor in a bird began to get smaller and in about a month it had entirely disappeared. The bird was again transplanted and a small tumor grew which also disappeared and on subsequent transplantation no tumor was produced. This bird thus developed a spontaneous immunity to Rous sarcoma. This immunity to Rous tumor is very rare. Gye found that only one Rous tumor in 5,000 regressed. Gye had used very young birds in which the tumor develops more rapidly and kills more quickly, which may account for his small number of regressions.

Since 1928, we have been endeavoring to produce this immunity in chickens. Up to that time our facilities for cancer work were very inadequate, but with slight improvement we were able to keep birds under observation for longer periods of time. This made a very great difference to the trend of the work, and between 1928 and 1933 we had seven tumors regress in a total of 1,140 tumor-bearing birds, i.e., one tumor in 164. We have never observed a bird to be immune without having had a tumor which regressed.

Many experiments were carried out with the serum of these immune birds. A cell-free extract of Rous tumor was made. Two portions of 3 cubic centimeters each were meas-

ured into test-tubes. To one portion 1/10 of a cubic centimeter of immune chicken serum was added. Both tubes were incubated at body temperatures for one hour. At the end of this time three birds each received 1 cc. of the untreated extract and another three received 1 cc. of the extract treated with immune serum. Ten days later the three birds which had received the untreated extract had well-defined tumors which developed and killed the birds. The birds receiving the treated extract did not develop tumors. This meant that the serum of the immune birds was able to destroy the tumor-producing virus which was present in the extract.

We then tried the effect of treating birds which had tumor with immune serum. Large amounts of the serum were injected intravenously in tumor-bearing birds, but the tumors continued to grow and the birds died of tumor. The serum had no effect on the growth of the tumor because it did not reach the virus which was within the tumor cell. Finely divided tumor cells were incubated in immune serum at body temperature for many hours. They were then injected into chickens, with the result that tumors were produced. It would appear from this that the wall of the tumor cell prevented immune bodies of the serum from reaching the virus.

There are certain dyes and stains that readily pass into the tumor cell. Hence we tried mixing the immune serum with these dyes, in the hope that the dye would carry the immune serum into the cancer cell. So far results have been unsatisfactory.

Although we have a powerful specific, neutralizing substance in the serum of the bird which is immune to cancer, we have no means by which its destroying effect can be applied to the virus within the living animal.

Dr. Mendel and his group are investigating the cancer problem by means of the Warburg technique. By means of a special apparatus the respiration of normal and tumor cells can be measured with great accuracy. Very thin slices of tissue are suspended in a nutrient solution which contains the same food stuffs as blood. The slices are gently shaken in a vessel in a water bath at body temperature. The

cells survive for many hours and behave in the same way as they do in the animal body, i.e., they breathe, take up food stuffs and burn them. During this time the activity of the cell can be measured by its uptake of oxygen and production of carbon dioxide and lactic acid. Warburg, who received the Nobel prize for his outstanding work, found that cancer cells differ in a very striking way from the normal cells in their utilization of food stuffs. All normal body cells require oxygen in order to live, but the cancer cell can survive and grow for weeks without oxygen. The reason for this is that the cancer cell has a special ability to derive energy for life and growth without oxygen by the fermentation of glucose. In this regard the cancer cell behaves like yeast. This property of the cancer cell is largely responsible for its unregulated growth and many attempts have been largely made to specifically check this process.

Dr. Mendel has been endeavoring to find substances which would specifically correct this abnormality and restore a normal type of respiration to the cancer cell. He was the first to point out that extremely small amounts of a substance called "glyceric aldehyde" checked the fermentation of the cancer cell. Recently he has worked with sodium ferricyanide, which has this same property. After full investigation was carried out with tumor slices, the substances were tested on tumor-bearing animals. In some instances he has been able to prolong the life of a tumor-bearing animal by temporarily checking the growth of the tumor with sodium ferricyanide, but so far he has not been able to bring about the disappearance of a tumor.

As previously stated, the cancer cell has the ability to live with oxygen or without it. It is now fairly well established that the effect of X-ray and radium on a cancer cell is to destroy its power to use oxygen. Dr. Franks sought to augment the effect of radiation by the administration of glyceric aldehyde so that both respiration and fermentation would be checked at one and the same time and as a result the cancer cell would die. In this work, Dr. Franks has had the co-operation of Dr. Richards, of the Cancer Clinic. His procedure is as follows: Mice are transplanted with Balogh

mouse sarcoma, which is the most malignant of all mammalian tumors. About 6 days after the transplantation, when the young tumor is about the size of a small marble, X-ray, in mild dosage which of itself would not kill the tumor, is applied. Four days later, when the X-ray is exerting its maximum effect, the animals are given a light anaesthesia and injected over a three hour period with a solution of glyceric aldehyde. To date the results have been interesting and encouraging, but because of the poor blood supply to the tumor, it would appear that the glyceric aldehyde, which is rapidly destroyed by blood serum, does not reach the tumor cell.

Dr. Franks and his group are attacking the cancer problem from yet another angle. There is a great deal of evidence to show that when an animal has recovered from a tumor, it remains immune just as a patient who has had mumps or measles very rarely has a second attack of the disease. This immunity is due to the fact that the causative agent calls forth a protection on the part of the body. The only substance that we know which elicit this immunity are proteins or substances linked with proteins. Dr. Franks therefore conceived the idea of linking a purified tumor-producing substance, such as 1:2:5:6-dibenzanthracene with a foreign protein and utilizing the combined substance to invoke immunity against the tumor. After three years of effort, with the assistance of Mr. Creech, the chemical linkage was finally effected, and they now have under observation a large number of rabbits, rats, guinea pigs and mice which have been so treated. There is already some evidence that this combined protein tumor agent behaves like a bacterial vaccine. Many months will be required before results will be obtained.

Experimental cancer research requires a large number of animals which must be kept in good health for long periods of time. In order to do this it is essential that the animals be kept clean, be fed scientifically, be kept free from epidemics and not overcrowded.

In view of all the experimental work, we must be cautious in regard to reputed cancer cures which are reported from time to time. These so-called cures could be tested on ex-

perimental animals and if they had any value it would be demonstrated by their effect on animal tumors. In the early part of this century the beneficial value of radium and X-ray, and radium are the only means of proven value in Research Fund. From this work there arose the clinical application of radium and X-ray. In like manner it was found in laboratory animals that if a tumor was completely removed it did not recur and the animal was cured of its tumor. If a small portion of the tumor tissue remained it frequently took on more rapid growth. So far, surgery, X-ray, and radium are the only means of proven value in the treatment of cancer

I have endeavoured to give you some idea of the tremendous effort that is being put forth by various cancer research groups. Cancer of many kinds can be produced at will in the laboratory and are available for experimentation. Scientific facts are being brought forth and related. More progress has been made in cancer research in the last thirty-five years than in the whole history of the world previous to that time. Progress is slow but sure. The solution of the cancer problem will not come by chance, but by further research. What is needed in the treatment of cancer is a specific. When that is discovered it will replace all other treatments.