THE PROPAGATION OF MOUSE TUMOURS BY MEANS OF DRIED TISSUE*

BY

W. E. GYE, M.D., F.R.C.P., F.R.S.

Director of the Imperial Cancer Research Fund

My first duty is to say that anything which is new in this lecture is the joint work of my colleagues, Dr. A. M. Begg, Dr. James Craigie, F.R.S., Miss Ida Mann, F.R.C.S., and myself. I have great pleasure in thanking them for their permission to mention researches for which we are all jointly responsible.

Most of us have heard the remark made that too much and too little is known about cancer. We know so much now about starting cancers by physical and chemical means and so much about the chemistry and biochemistry of cancer that the literature has become confused. But we do not yet know the cause of cancer, and of course we cannot, except in early favourable cases, cure it with certainty; and all this appears to be lamentable. Are we quite certain that we have studied carefully enough the work of the pioneers of cancer research—that we have not missed some clue which was clearly revealed forty or more years ago?

A brief survey of the early work of the Imperial Cancer Research Fund, which is representative of the researches of the earliest workers in experimental cancer research, is useful and rewarding.

The work which proved that the familiar animal tumours of mice, rats, and other small mammals are pathologically and biologically of the same nature as tumours of man may be passed over, though even now there are diehards in the ranks of the profession who apparently consider that no work counts unless done on man. Similarly the statistical work of Bashford, the study of the zoological distribution of cancer by Murray, and the variety of histological forms of the disease may for present purposes be neglected. Let us confine our attention for the moment to a consideration of the prime interests of the pioneers like Jensen, Bashford, Ehrlich, Leo Loeb, Borrel, and the many others who helped to lay the foundations of cancer research.

First of all Bashford clearly asked himself the question, What do I mean by the cause of cancer? He knew, like all the medical world of his time, that soot caused an excess of skin cancer in chimney-sweeps, and that coal-tar was responsible for much skin cancer. Indeed, in the famous memorandum on research which Bashford drew up for the Committee of the Fund one of the items specifically mentioned—this was in 1902—was the proposal to test whether Pott's observations on the carcinogenic properties of soot on man applied also to mice. For various reasons this was not carried out; it was left for Passey to do in 1920. As everybody knows, soot does start cancer in mice.

Bashford in his inquiries into the ethnological distribution drew attention to the occurrence of squamous epitheliomas of the abdominal skin of the natives of

India who wear the Kangri basket for warmth. He arrived at the obvious conclusions that long-continued application of heat leads to the occurrence of cancer and, more important still, to the obvious error of the Cohnheim hypothesis of the origin of cancer in cell rests, since, so far as he could determine, cancer of the abdominal skin was confined to those natives who wore the Kangri basket, and it is unlikely that these were the only men who had skin rests in the abdominal wall. He also wrote a great deal about betel-nut chewing and buccal cancer. But to Bashford, as to all the early workers in cancer, soot and tar and Kangri baskets were remote causes of the disease: they promote or precipitate cancer but play no further part.

The First Experimental Tumour

The first tumour produced experimentally was by the application of *x* rays to the root of the tail of the rat. This was done by Clunet, in France. It is of course only the deliberate application to a small animal of the same process which the pioneers of radium and *x* rays did unwittingly to themselves in the course of their professional work. Nobody could suppose that the tumour incidence was independent of the *x* rays; and nobody could believe that the *x* rays continued to act after the tumour had started or, in the case of the rat, could have any effect on the long succession of tumours in an unlimited series obtained by grafting small pieces in healthy rats. Bashford especially, and the contemporary workers incidentally, always distinguished sharply between what I have usually described as remote or adjuvant causes—chemical, physical, or parasitic—which lead to cancer and the intracellular continuing cause. This continuing cause is related to the pathological nature of cancer and is, in the sense in which we say that the tubercle bacillus is the cause of tuberculosis, the cause of cancer.

But at this point comes the real difficulty of the subject. Bashford and his colleagues could not find anything bacterial or chemical in tumour tissue that could start a new tumour. Any extract, freed of tumour cells, failed to cause the formation of a new tumour; likewise any tumour even partially dried was inactive. The tumours, in Bashford's sense, appeared to be causeless—that is, there was no continuing cause. Any procedure, however mild, which destroyed the life of the cancer cell destroyed all activity of the tumour after its inoculation into an appropriate animal.

Tumour-grafting

These findings corresponded beautifully with the findings of the process of grafting tumours. When a small fragment of a mouse tumour is placed under the skin of another mouse it was found—and this has been confirmed by workers all over the world—that the non-malignant tissues, the blood and lymph vessels and connective tissue

*An Imperial Cancer Research Fund Lecture delivered at the Royal College of Surgeons on March 22.

which constitute the stroma of a tumour, die rapidly; the central cells of the implanted tumour mass die more slowly, the peripheral cells of the graft alone surviving; a new stroma is formed by the new host, and this stroma nourishes the surviving malignant cells, which grow and multiply and form the daughter tumour. Bashford concluded, after a vast amount of work, that "cancer can only be transmitted experimentally by processes which allow of the continued growth of the tumour of one animal in other animals of the same species." He could find no evidence, nor could anybody else, which upset this conclusion, and therefore it appeared to him that it is not permissible to seek for the cause of cancer outside the life processes of the cells.

This naturally led Dr. Murray, Bashford's first assistant, to study cells, normal and malignant, with the greatest care to determine whether there is any difference which might account for the properties of malignant cells. Murray, who had worked for several years with Boveri, was naturally interested in the speculations which might usefully be summarized under the term "mutation," but he could find no evidence which justified any belief or confidence in such speculations. Thus the Fund's scientific staff arrived at the position in 1914 that cancers are, in a phrase familiar at that time, just "personal experiences" with no cause except possibly some intracellular distortion which in our then state of knowledge was undiscoverable. The situation, one of extreme pessimism, is illustrated by Ehrlich's reply to a young man who wished to do cancer research: "I have wasted 15 years of my life in cancer research. Until some fundamental discovery is made revealing the nature of life itself not a step forward will be made in our knowledge of cancer."

The First Chicken Sarcoma

In 1910 there came into the possession of Dr. Peyton Rous, of the Rockefeller Institute, a hen bearing a tumour which histologically was universally diagnosed as a spindle-cell sarcoma. This diagnosis was changed the following year—not by Rous—when Rous proved that cell-free extracts contained an agent which was capable of starting the tumour afresh in appropriate chickens. Here was an entirely new fact. All the laborious efforts of workers to do just this with filtrates of mouse, rat, and guinea-pig tumours had failed. The first chicken sarcoma examined by Rous succeeded, and pathologists and surgeons interested in cancer at once denied the malignant nature of the chicken tumours. The agent which multiplies with the growth of the tumour is now recognized as a virus. Electron-microscope photographs have been taken, and the sizes revealed by such photographs are in fair harmony with sizes deduced from experiments with colloidion filters. The virus is more or less spherical and occurs in clumps in the tumour cells.

Now we know of several hundred filterable tumours of the domestic hen. They are of different structures: some, but very few, are simple spindle-cell tumours resembling the Rous sarcoma; others include chondromas, osteochondromas, fibrosarcomas, myxosarcomas, muscle-cell tumours, and so on.

There is one strange character possessed by all these tumours which must be mentioned. When a tumour is propagated by grafting with living cells it is to be expected that the daughter tumour, if derived entirely by growth and multiplication of the implanted malignant cells, would be of the same histological type as the original tumour. With the propagation of tumours by means of cell-free filtrates the new tumour is literally new; it is made up of the host's cells which have been infected by the inoculated virus. But it has universally been found that the inoculated

virus can start a tumour only of the kind from which it is derived. Thus a spindle-cell tumour always gives rise to a spindle-cell tumour, an endothelioma to an endothelioma, and so on. The virus is so specialized in its effects that it represents in itself all the qualities and properties of the cell from which it is derived. Nevertheless, an immune serum made by injecting virus from one tumour will neutralize the viruses from other types of tumour; which indicates a common antigenic element.

Filterable and Non-filterable Tumours

It will clearly be seen from this brief account of various sorts of tumours that there is a fundamental difference of a strange kind between filterable virus tumours and the non-filterable mouse tumours; the tumours of chickens, the adenocarcinoma of the frog, the Shope papilloma of the rabbit, and now the mammary cancers of inbred mice all have a virus as a continuing cause, whilst the vast majority of tumours of the small mammals, and presumably of man, apparently have no continuing cause.

If one dwells exclusively on the non-filterable tumours one is driven to theorize and even to accept almost impossible explanations of cancer and to dismiss the tumours from which a virus can be obtained as a disease *sui generis*. But this cannot be done convincingly; for the filterable tumours of chickens possess all the properties which everybody acknowledges as being those of cancer and none other except this one property of transmissibility with filtrates and dried tissue. Manifestly if mouse tumours which have hitherto resisted such cell-free propagation could be so propagated, and especially if newer methods had to be worked out to achieve this success, the apparent fundamental dichotomy of tumours would disappear and we should then be able to include such tumours among the filterable tumours without any strain in our minds. We should be able to regard tumours in general as being of one sort, as collections of cells carrying an active virus as the intrinsic cause.

My colleagues and I have considered cancer literature very carefully and have come to the conclusion that two facts discovered long ago are possibly the key to the general problem of cancer causation. I shall now deal with these in turn.

Transformation of Stroma of Epithelial Tumours

The first fact is the occurrence of sarcomatous transformation of the stroma of epithelial tumours. This was observed independently by Bashford and Ehrlich, and was worked out by the late Professor Haaland in our laboratories and by the late Dr. Apolant in Ehrlich's laboratory in Frankfurt. It was shown by histological methods that the spindle-cell tumour arises from normal stromal connective-tissue cells and is not, as sometimes happens in animal and human tumours, an alteration in shape and appearance of the epithelial malignant cells. In other words, the epithelial cells influence the normal well-behaved connective-tissue cells, which then assume malignant properties. Either something passes from the malignant cell to the normal cell, or the normal cell just imitates the neighbouring malignant epithelial cell. Can we assume that a virus passes and becomes gradually adapted to the normal cell? This becomes more probable when we consider the frequency of this occurrence in the inbred strains of mice.

It is now common knowledge that by brother-sister mating of selected mice it is possible to get homozygous strains of mice in which 90% or even more of the females develop mammary cancer. When one of these tumours is transplanted in the normal stock of the same strain the graft always takes; transplantation is easy and certain. It

was observed by Dr. Gorer at the Lister Institute in 1937 that transplants of mammary cancers in inbred strains show sarcomatous transformation very readily—in about 70%—whereas in ordinary non-inbred mice it is an infrequent occurrence. We have considered this fact very carefully and have adopted the working hypothesis that the process of inbreeding for cancer is quite different from inbreeding for unit characters such as coat colour; these are incidental to such breeding. We have looked upon the process as a means of selecting the most active or virulent strain of virus, which has now been shown in these tumours. Certainly unless the tumour incidence is carefully watched and a main cancer line is kept the incidence of tumours falls from 90% to 40 or even 20%; and this fall is independent of the brother-sister mating. It is due, as W. S. Murray has proved, to a change in what he calls the "tumour inciter"—i.e., the virus—which presumably loses some of its pathogenicity.

Effect of Cold

The second fact is one which was discovered by Ehrlich and published in 1907. It is this: that cancers, both sarcomas and carcinomas, can be kept at the low temperature of -10°C . for as long as two years, and when transplanted again give rise to the same tumour. With the exception of my late colleague Dr. W. Cramer, all who have repeated the experiment have concluded that the cancer cells have survived this prolonged freezing. Cramer was more cautious, and gave very cogent reasons for his doubts. It must be remembered that cells which are kept at a temperature of -10°C . are not just frozen *en bloc*. This temperature is cold indeed, but its action on cells is disintegrative. Water is turned to ice, but the salts in the cells do not come out with the water until a temperature of -21.1°C . is reached. Thus the internal medium of the cells is a strong solution—15 to 20%—of brine. The cells are "cured" as bacon is "cured"; nevertheless this experience of Ehrlich's has been uniformly, except by Cramer, interpreted as a marvellous example of the resistance of cancer cells to adverse conditions.

Further, it is quite well known that malignant cells which are kept at ice-box temperature, around 0°C ., lose their capacity to start tumours again in a few days' time. The truth is, of course, that the failure of the early workers to find a continuing cause in tumours bit so deeply into the mind that there was no inclination to look at facts dispassionately. Moreover, the experience of histopathologists of the nineteenth century, especially that of Ribbert, had an enormous influence on the thinking of experimental workers.

Reinvestigation

We have reinvestigated the whole subject. The first step was a comparison of the effects of cold on normal tissues. The method lay readily to hand. Several years ago Miss Mann investigated the growth of embryonic tissue in inbred mice. It was already known that embryonic tissue of laboratory stock mice survives for some days in mice which are not inbred; the embryo tissue and the tissues of recipient mice are different antigenically, and those reactions which are set up against foreign proteins come into play very soon and lead to the death and destruction of the implanted embryonic tissue. In homozygous mice in which the tissues are identical chemically no such reactions occur; the embryonic tissue is not resisted, and consequently it grows to form an embryoma which persists practically indefinitely. The cells are at home, and the recipient mice accept them readily. Consequently it is an easy matter to determine whether cold destroys normal embryonic tissue and to prove the effects by simple grafting of such treated tissues into the same homozygous strain.

After prolonged and repeated experiments Miss Mann has proved quite conclusively that normal embryonic tissue is killed after less than an hour's exposure to a temperature of -79°C ., whereas malignant tissues survive such a temperature for a period of more than one year—it may be, so far as we know, indefinitely.

The second stage in the investigation was an examination microscopically of grafts of malignant tissue that had been transplanted into a number of mice which were killed at various intervals from hours to days. This form of investigation is designed to find what happens to the graft, to see whether the tumour which forms after inoculation of refrigerated tumour tissue into appropriate animals is derived from surviving cells—i.e., a continuation of the tumour by growth and division of the original cells—or whether it is a new tumour of the recipient mouse's own tissues. This form of experiment I had already carried out 20 years ago, and the same results were noted in this repetition. With tumours which have been subjected to a temperature of -79°C . the whole mass of implanted tissue shows no normal cells; they stain yellow with picric acid after a sojourn of two days in a mouse, or a structureless pink with eosin; the cells and nuclei are structureless; the mass of necrotic tumour tissue becomes surrounded by reaction tissue, becomes shrunken, and eventually disappears.

With sarcomas the new tumour forms in the capsule of tissue which is derived from the host. Occasionally when refrigeration is very short, a matter of minutes, an obvious malignant cell, best recognized when carcinomas are used, is found in the mass, and from this cell a daughter tumour arises by growth and multiplication of such a surviving cell. This observation, made during the time Cramer was studying the effects of refrigeration, led to a sense of insecurity, especially to one who was Bashford's pupil. The final conclusion we have reached from these studies is that malignant cells, like embryonic cells, are readily killed by extreme cold, but that dead malignant cells are nevertheless capable of starting a new, strictly new, tumour in virtue of the intrinsic virus which they contain. Comparisons with normal tissues and histological studies, however, do not give the certitude which is necessary when the issues are as important as they are in this work.

Tumour Transmission

While some of these researches were in progress Dr. Craigie, with Dr. Begg, studied the effects of extreme division of the tumour tissue and of the action of various diluents on the finely divided tumour tissue.

Normally in cancer laboratories tumour tissue is broken up or emulsified by using fine scissors to make a mince which will pass easily through a needle of very wide bore. This was not good enough for the quantitative work needed in these researches. Dr. Craigie therefore designed and had made in the Fund's workshop a new and improved tissue-mincer by which suspensions of cells in diluents such as distilled water, glucose solutions, glycerin, saline, etc., could be obtained sufficiently finely divided to pass through the finest bore of needle (26 gauge). The results obtained by injection of such fine emulsions of frozen tissue cannot be interpreted simply as being the consequence of survival of cells on freezing. For example, many diluents such as distilled water, 40% glycerin, and 40% glucose which one would expect to be lethal to cells do not prevent tumour formation, and, contrariwise, diluents such as peptone which would not be expected to kill cells on freezing do inhibit tumour growth. The whole matter is difficult and complicated, and is an obvious subject for more research. But it can be stated quite definitely that

long-continued exposure to a low temperature, as long as one year, does not affect the capacity of tumour tissue to start tumours again when injected into appropriate animals. Indeed, since we know from histology that all or nearly all cells—we can never exclude the possibility of one cell escaping microscopical attention—are killed by keeping at a temperature of -79°C ., and also that tumours kept a year are often more active than corresponding fresh tumour tissue, it is in the highest degree improbable that the tumours which form after inoculation of refrigerated tissue are a mere grafting of living cells. They are much more likely to be strictly new tumours brought about by the infective action of the intracellular cause which has hitherto escaped attention.

But on this slightly uncertain note we could not end. We realized, none more definitely, that it was necessary to transmit a tumour with tissue which had been completely dried. To this we therefore turned our attention. During the past 14 years most of the mouse and rat tumours have been dried by one method or another and no success has been obtained in any single experiment. A new apparatus for drying small amounts of tissue, seldom more than a gramme, was designed by Dr. Craigie and made for us by the Glass Printers Company of Harrow. A description of it is in the scientific press. The essential improvement in this apparatus is the absence of any constriction in the path that water vapour must follow and in the power of the "backing pump." With this apparatus we have succeeded in propagating three mouse sarcomas with perfectly dry tissue. The tumours are as follows:

1. A sarcoma which was started in the inbred mice known as C57 Black by injecting about 1 mg. of the pure chemical compound methylcholanthrene. The chemical was injected in 1941; in the early months of 1942 tumours formed. From one of them transplants were made into six normal C57 mice. The tissue inoculated grew in all the mice; from one, a further transplant was made. This process of perpetuating the tumour has now been carried on for seven years; the serial number of the tumour is now nearly 200. Thus about 200 transmissions by grafting have been carried out, and during these years the tumour was dried and filtered again and again in attempts to propagate the tumour acellularly. All these attempts failed. Even tissues which were deliberately used before drying was complete and whilst the tissue was still damp failed to give rise to tumours.

2. A sarcoma of the inbred strain R3 which arose by infection of the connective-tissue stroma of a carcinoma. This is the most malignant of our transplantable tumours. It was separated from the carcinoma by rapid transfers by Dr. Ludford. It is made up of spindle cells and multinucleated giant cells; around the tumour there is usually a glairy fluid in which disintegrated cells can usually be found.

3. A sarcoma of the homozygous strain C3H. This again arose during the transplantation of a sporadic mammary carcinoma and was also purified by early transplantation of the carcinoma by Dr. Ludford.

Experimental Results

It is best to take the methylcholanthrene tumour first. It is the least malignant of the three but has been tested most often for transmissibility with cell-free extracts during the past seven years. Even now, using our most efficient drying apparatus, we have so far been unable to obtain active dry material of a tumour fresh from a mouse. To obtain active dry tissue it has been necessary to subject the tissue to the low temperature of -79°C for five to eight weeks before drying. Even then, as the following table shows, it is not yet possible to obtain regularly active material.

It will be observed that in one experiment of this group only one tumour developed in eight mice, and this did not appear until the 24th day after inoculation. This experience has been found in other experiments. In others again, in which variations have been made in experimental methods,

the results were entirely negative. But as a summary we can affirm quite definitely that it is possible to obtain perfectly dry tumour tissue which will start the tumour afresh.

Expt.	Tumour	Time of Freezing at -79°C .	No. of Mice	No. of Tumours	Time of Appearance
406	C48 in C57 black	35 days	7	6	17 days
G13B	" " "	52 "	18	13	16-31 "
434	" " "	53 "	12	9	16 "
G26	" " "	105 "	8	1	24 "

It is a matter of some importance to propagate tumours in series with dry tissue. This has been started, but owing to the time needed for refrigeration before drying we have succeeded so far only in carrying through two generations; the third generation has only just been tested.

When we come to the much more malignant R3 and C3H sporadic tumours we find that both of these are capable of yielding active dry tissue when fresh tumour is dried. In two successive experiments with the R3 tumour we obtained from dry tissue of fresh tumour (derived originally from frozen material) eight tumours out of ten mice inoculated, tumours appearing 14 to 33 days later; and one tumour out of six mice, the tumour appearing in 14 days. In an experiment with the C3H strain, fresh tumour tissue dried under the optimum conditions attained so far gave six tumours in 12 inoculations, the tumours appearing in 15 to 27 days.

Even with these very malignant tumours of the R3 and C3H strains of mice it has been found that preliminary freezing is helpful. For example, in three successive experiments with the R3 tumour it was found that tissue which had been frozen 28 days gave six tumours in six mice inoculated, the tumours being observed at 11 days; tissue frozen 34 days and then dried gave 10 tumours in 10 inoculated mice, the tumours being palpable in eight days and large on the 13th day; and tissue frozen 75 days, dried, and the dry powdered tissue then inoculated into six mice gave six tumours in 11 days.

We have no proof of numerous possibilities which may happen to tissue which is refrigerated for long periods of time. It is possible that low temperatures denature an inhibitory protein, thus liberating virus and making it available even after drying. The only merit this speculation possesses is that in the filterable tumours of chickens it is possible under favourable circumstances to demonstrate the presence of an inhibitory substance. This has been particularly well shown by J. B. Murphy, of the Rockefeller Institute. We have no complete proof of any of our numerous private speculations on this point; we are content merely to state what is the fact. It is possible that with more perfect methods these difficulties will vanish and speculation will become unnecessary.

At present the only conclusion from the whole work which is of vital and abiding interest is that it is no longer justifiable to put the normally easily filterable tumours of chickens in a separate class and turn a rather scornful eye on them. This work has now brought tumours of mice—average tumours found in all well-equipped cancer laboratories—into the same class. They have, as was to be expected, a continuing cause, and, since the continuing cause of chicken sarcoma is a virus, probably it is viral in nature. We have not yet transmitted other mammalian tumours with dried tissues, nor indeed have we seriously attempted to do so; but we think we are justified in making the contention that the negative results of the past are merely negative and have the value only of negative experiment which is the result of imperfect technique. If the pioneers of cancer research, men like Jensen, Ehrlich,

Bashford, and many others, had by chance succeeded in transmitting mouse tumours with perfectly dry tissue the situation in cancer-thinking would have been entirely different, and we should have been spared the confused speculations which now dominate cancer research.

There is one final point I wish to refer to—namely, whether viruses are of intrinsic or extrinsic origin. It is now almost the custom to consider the viruses of the filterable tumours as particles of chemical nature made by the infected cells. Indeed, this mode of thinking has now gone so far that the word "virus" has been objected to, though the grounds of objection do not appear to us to be valid. We are not wedded to a word; they may be called anything so far as we are concerned. It is more important to discover perfect ways of getting and handling them, to find out how they act, and to learn if possible how to control them. The problem of discovering the nature of viruses, whether made by cells or of extrinsic origin, is not confined to cancer viruses; it is a part of the general virus problem. There was a time when the bacteriophage was allied to cancer viruses as being a product of the bacterial cell, but that has now passed. The argument concerning biogenesis or abiogenesis of the Pasteur-Bastian period does not interest us, and its repetition now, with viruses taking the place of bacteria, seems to be sterile from the beginning.

Conclusion

The experimental evidence described above points to the conclusion that cancer has a continuing cause and that this, in mammals as in birds, is a virus.

LONG-TERM CARDIAC OBSERVATION OF CHILDREN

BY

R. KEMBALL PRICE, M.D., M.R.C.P.

(From the Cardiac Department, Royal Sussex County Hospital)

The proper assessment of cardiac signs found in a child is of considerable importance to its future. Many children in whom murmurs are heard have no disability, and the greatest danger is that their activities may be restricted and the seeds sown for the development of cardiac neurosis. In rheumatic heart disease a normal school life with games can often be allowed when the disease is inactive, even when valve lesions are known to be present. With the advance of cardiac surgery it is desirable that cases for which it may be necessary should be diagnosed as early and as accurately as possible and submitted for operation at the most suitable time.

The choice of a career which will not need to be changed on account of a disability that is likely to increase should be made before leaving school. It is difficult for one who has done nothing but manual labour to find a sedentary occupation in middle life, and this can be avoided by choosing suitable employment from the start. For these and other reasons there are great advantages in children with cardiac abnormalities being under regular observation at cardiological centres. There are many questions in cardiology which can be settled only by watching patients over a period of years. J. W. Brown (1939) has done much in this way to increase our knowledge of the natural history of congenital heart disease. By similar observation much may be added to our understanding of the course of rheumatic heart disease.

What happens in the years between the acute or sub-acute phase seen in the child and the young woman who is discovered to have mitral stenosis during pregnancy?

Is the development of the stenosis due to recurrent activity of the rheumatic infection, or, once started, does the narrowing progress steadily without evidence of further activity, or are both factors at work? What happens to the innocent murmurs so common in childhood? At what age do they disappear?

A Cardiac Clinic for Children

With these considerations in mind a children's cardiac clinic was started eighteen months ago, and a report of the first 200 cases is here given. Apart from its intrinsic interest, the appreciation of doctors, parents, and, not least, the children themselves is encouraging and makes the clinic a happy one. As an investigation of this kind requires observation over a period of many years it is desirable that the observer should start while young enough to allow continuity of at least twenty years. It is with the idea of encouraging others who may be interested to take up such work that this preliminary report is presented.

The work has been made possible by the willing co-operation of the local school medical authorities. An afternoon is set aside and four to six children are seen by appointment each week. The number attending on each occasion is kept small so that full and unhurried examination may be made. It also means that no one is kept waiting very long—a point which makes them more willing to come again when asked.

Children showing any cardiac abnormality whatsoever at routine school examinations are placed on a list and referred to the clinic. The net is purposely cast wide to include all conditions, ranging from innocent murmurs to serious heart disease. A careful history is taken, followed by clinical examination, electrocardiograms, and cardioscopy. Twelve-lead electrocardiograms consisting of V1-6 and augmented unipolar limb leads in addition to the standard leads are now being taken in all cases, and routine phonocardiograms are to be started shortly.

If the child is very nervous or fretful the blood-pressure reading, electrocardiography, and cardioscopy may be postponed until the next attendance, as it is considered important that its memory of the visit to the cardiac department shall not be unhappy. This greatly facilitates subsequent examination. Teleradiographs are taken in cases showing abnormality on screening. Blood counts and sedimentation rates are done in certain cases. A report is sent to the school medical authorities and to the patient's own doctor. Arrangements are made for re-examination after an interval of six or twelve months or sometimes earlier.

Report of First 200 Cases

The distribution of the 200 cases is shown in Table I. There were 96 boys and 104 girls. It will be seen that approximately 40% had, or were suspected of having,

TABLE I.—Distribution of Cases

Cases with Organic Heart Disease.	
I. Congenital malformations (44):	
Fallot group (one with congenital heart-block)	7
Pulmonary stenosis	10
Ventricular septal defect	6
Patent ductus arteriosus	3
Coarctation of aorta	3
Atrial septal defect	3
Congenital heart-block	1
Observation—congenital heart disease	11
II. Rheumatic Heart Disease (37):	
Mitral stenosis (six with active disease)	21
Mitral stenosis and aortic incompetence	2
Observation—mitral valvular disease or cardiac enlargement	14
Cases without Organic Heart Disease (119):	
Innocent murmurs	96
Physiological third heart sound	5
Depressed sternum	1
Splitting of first heart sound	1
Anaemia	1
Displacement (scoliosis, 1; pulmonary fibrosis, 1)	2
Irregularities (sinus arrhythmia, 1; extrasystoles, 2)	3
No abnormality detected	10