network of connective tissue with a round-celled exudation and a few tubercles with giant cells. A few blood-vessels were present in the outer part of the wall.

Judging from the thickness and density of the abscess wall it must have been present for a considerable time, probably starting about the onset of the otorrhœa. Striking features in the case were the toughness of the wall of the abscess, as even after it was removed considerable force was necessary before a needle could be made to penetrate it, and also the absence of symptoms referable to the abscess, notwithstanding that the temporo-sphenoidal lobe in which it was situated was destroyed to a very considerable extent. The clinical aspect pointed more to a meningeal infection.

Glasgow.

RESEARCHES INTO THE ETIOLOGY OF CARCINOMA: ON THE PRESENCE OF PLASMODIOPHORÆ IN CARCINOMATOUS TUMOURS AND THE SUCCESSFUL CULTURE OF THE PARASITES.

BY W. FORD ROBERTSON, M.D. EDIN.,
PATHOLOGIST TO THE SCOTTISH ASYLUMS;
AND
HENRY WADE, M.B. EDIN., F.R.C.S. EDIN.,
CONSERVATOR OF THE MUSEUM OF THE ROYAL COLLEGE OF SURGEONS OF EDINBURGH.

THE researches of which we wish briefly to record some of the results were undertaken specially with a view to testing the validity of the hypothesis that carcinoma is dependent upon the growth of a parasite of the same class as the plasmodiophora brassicæ, which is known to cause tumour growths in various members of the cruciferæ, as well as in other plants. This hypothesis has been strongly advocated in various forms by Behla, Podwyssozki, Roswell Park, Gaylord,[1] and others, but only a certain amount of presumptive evidence has hitherto been obtained in support of it. To most workers at the subject of cancer it has seemed so improbable that they have devoted themselves to entirely different lines of investigation. Our own researches seem to show that it is in accord with fact. We have at least succeeded in demonstrating the presence in carcinomatous tumours of bodies corresponding in form and in reaction to a special method to 14 phases in the developmental cycle of the plasmodiophora brassicæ and in growing from such tumours an organism which accurately represents several successive phases of a parasite of this class.

On the threshold of the inquiry we were confronted with the difficulty, already experienced by other workers, of obtaining a clear view of the plasmodiophora brassicæ in vegetable tumours. The ordinary staining methods were found to be useless for the purpose. We devised special staining processes by which the organism was made to retain certain basic dyes, but we found that when applied to carcinomatous growths they were of little value on account of the depth of the colour imparted to the tissue elements. Nevertheless, by these methods we were able to observe in carcinomata bodies having a very close resemblance to the plasmodiophora brassicæ and we were therefore encouraged to pursue the inquiry further. It occurred to us that in a case of this kind in which ordinary staining methods had failed to yield satisfactory evidence either in support of, or against, the presence of a protozoan organism success might be attained by the employment of metallic processes. The platinum method, as used for the nervous system, was first tried and it proved to be of some utility for the purpose in view. Among the other metallic methods which we tried was one of impregnating tissues with silver, which had been partially worked out for the nervous system some years ago, but which had been abandoned on account of certain practical difficulties it presented. These difficulties were soon overcome and it was found that the method gave a better demonstration of the plasmodiophora brassicæ than any staining processes we had employed. It was also found to be applicable to formalin-hardened tissues from the human subject. It has been chiefly by the use of this silver method and toning of the silvered sections with gold, platinum, and palladium (see appendix) that we have been able to recognise what appear to be the various stages of a plasmodiophora in carcinomatous growths, as well as to identify the organism which has appeared in our cultures.

Plasmodiophora brassicæ.—We have studied this organism as found in the tumours which occur upon the root of the turnip and which are generally referred to in this country as "finger-and-toe disease." In America the condition is commonly known as "club-root" and in Germany as "kohlhernie." The plasmodiophora brassicæ was discovered by Woronin in 1876 and described by him in 1878. At this date he predicted that certain malignant growths in man would eventually prove to be due to a parasite of the same nature. The various stages of the organism have also been described by Nawaschin, De Bary, Gaylord, and others. The several descriptions that have been given do not fully harmonise with each other and a perusal of them leaves the impression that the observers have been unable, owing to the difficulty that there is in staining the organism satisfactorily, to obtain a view of all of the stages through which it passes. With the aid of the ammonio-silver process we have succeeded in tracing most of the various stages of its developmental cycle. We find that this cycle is much more complicated than would appear from existing descriptions. Important stages seem to have passed unobserved. As will be seen, the stages to which we refer are of vital importance for the investigation of the question of the presence of plasmodiophoræ in malignant tumours.

From the descriptions that have been given one gathers that "swarm cells" having entered a root hair of the plant reach the interior of certain of the plant cells; here they proliferate and stimulate the plant cells to divide; after a time several of these amœbæ lying in the protoplasm of a cell coalesce to form a plasmodium in which spores subsequently develop; these spores reach the ground when the plant decays and with the advent of spring develop directly into the amœboid swarm cells, which may once more find their way into the root hairs of a young plant. We have endeavoured to trace the various stages of the parasite not only during its sojourn in the vegetable host but also during its ectogenous existence, which under natural conditions is passed in the ground. We have found that the phenomena of this period can be studied in turnips in which the plasmodiophoræ have reached the spore stage, if the roots are incubated for several weeks at a temperature of from 55° to 60° F.

To follow the various phases of this organism, even when it is well stained, is by no means an easy matter. Normal and degenerative tissue structures and granules complicate the histological pictures and give rise to many difficulties of interpretation. From this cause we have, in one particular at least, already been misled—namely, in regard to the bodies which were interpreted as hyaline spores. We have recently found these bodies in the cells of a perfectly healthy turnip and notwithstanding the readiness with which on first view they seem to fit into the cycle, we are forced to conclude that they have really no place in it. The genuine spore is a nucleated body and the immediately succeeding stage is not granular, as we were led to believe, but the amœboid swarm cell. We have also fallen into error in our former description[2] through not having had an opportunity of examining early vegetable tumours and by having assumed that the life cycle could be completed from the descriptions already given by others. As the granular stage appeared to arise directly from a spore, it seemed legitimate to infer that the amœboid swarm cell described by others represented a later stage. By the use of improved methods of toning the silvered sections we have been able to trace the development of the swarm cells directly from the nucleated spore and to observe that it is from these minute amœboid bodies that the granular stage takes origin. By the study of the earliest examples of kohlhernia that we could obtain[3] we have, we think, succeeded in tracing this stage and the immediately succeeding one on to the nucleated stage. Our description of the life cycle differs from that of others mainly in the recognition of the granular and post-granular stages, without a knowledge

[1] Fourth Annual Report of the work of the Cancer Laboratory of the New York State Board of Health, 1902-03.

[2] See report of demonstration given at the annual meeting of the British Medical Association at Oxford, THE LANCET, August 13th, 1904.

[3] For invaluable assistance in securing these we are indebted to Mr. James Calder of Halterburn, Roxburghshire.

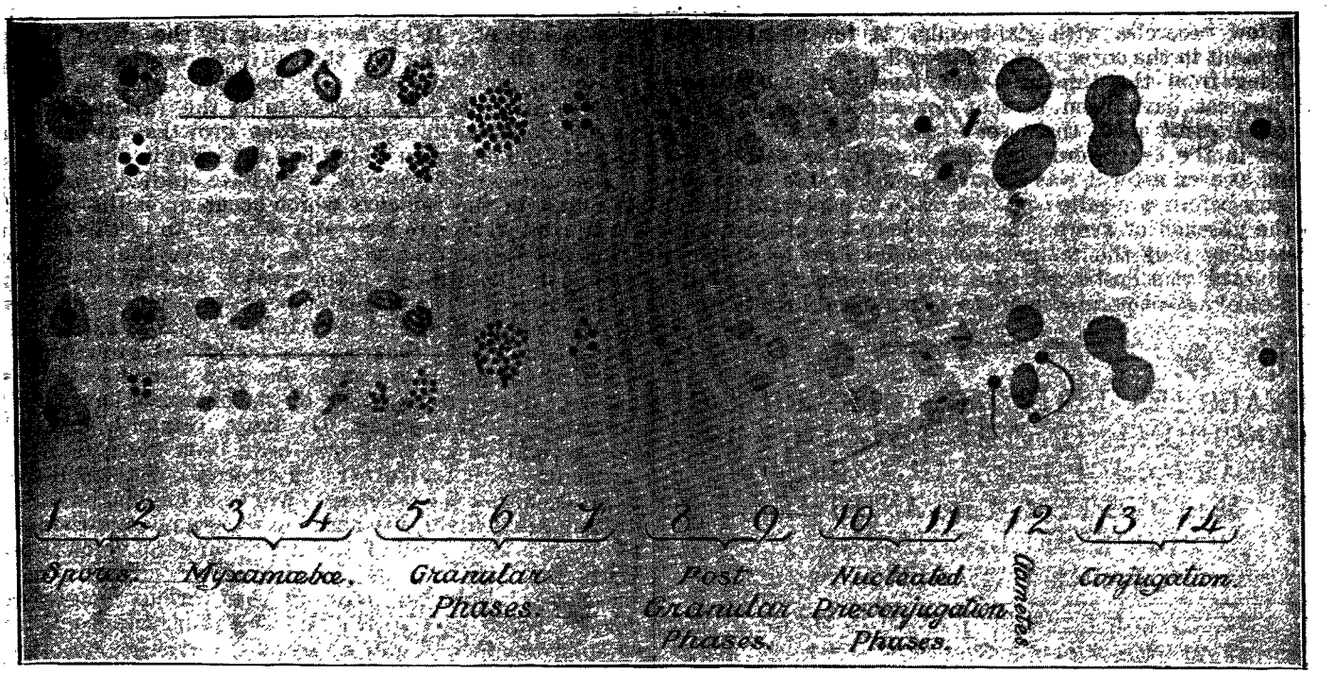

FIG. 1.

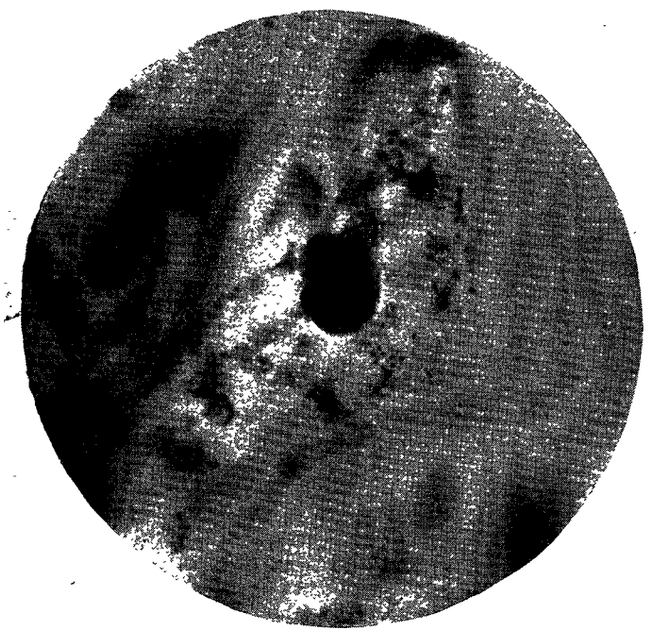

FIG. 2.—Plasmodiophora brassicæ. Early post-granular phase in protoplasm of turnip cell. Palladium-gold toning. (× 1000.)

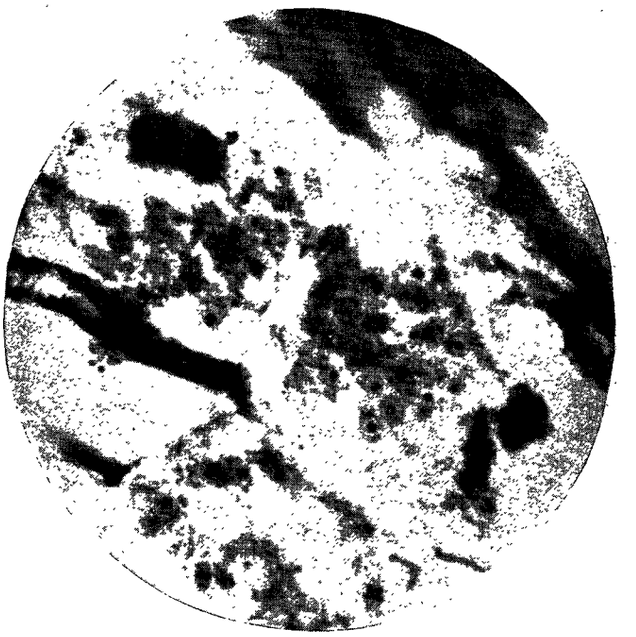

FIG. 3.—Plasmodiophora brassicæ. Nucleated pre-conjugation phase in turnip cell. Platinum method followed by methyl violet. (× 1000.)

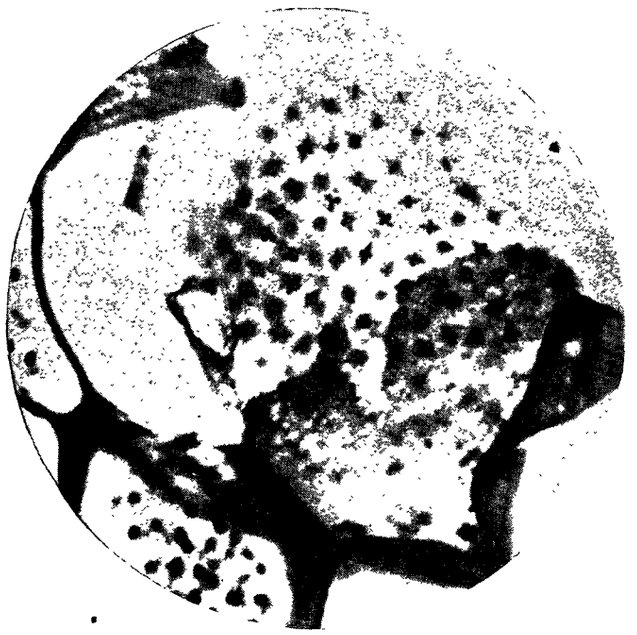

FIG. 4.—Plasmodiophora brassicæ. Nucleated pre-conjugation phase in mitosis. A carbol-fuchsin and carbol-thionin method. (× 1000.)

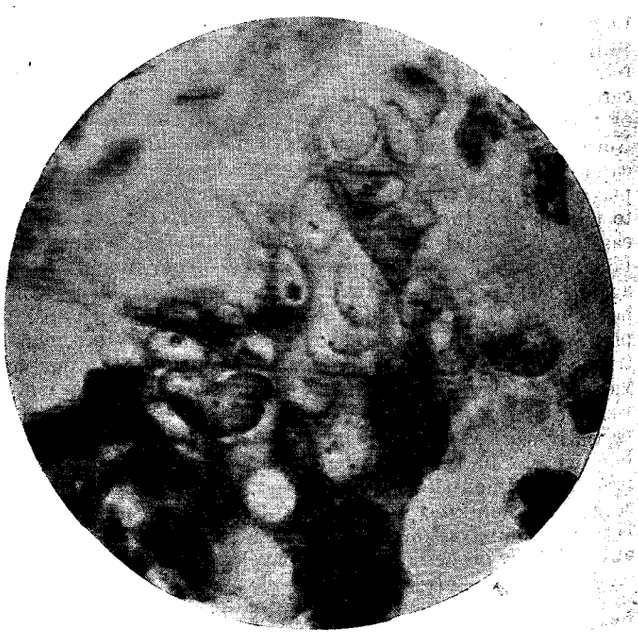

FIG. 5.—Plasmodiophora carcinomatis. Granular stage in nuclei of epithelial cells in a secondarily infected abdominal gland; intestinal carcinoma. Palladium-gold toning. (× 1000.)

FIG. 6.—Plasmodiophora carcinomatis. Post-granular phase in protoplasm of dividing epithelial cell. Mammary carcinoma. Gold toning. (× 1000.)

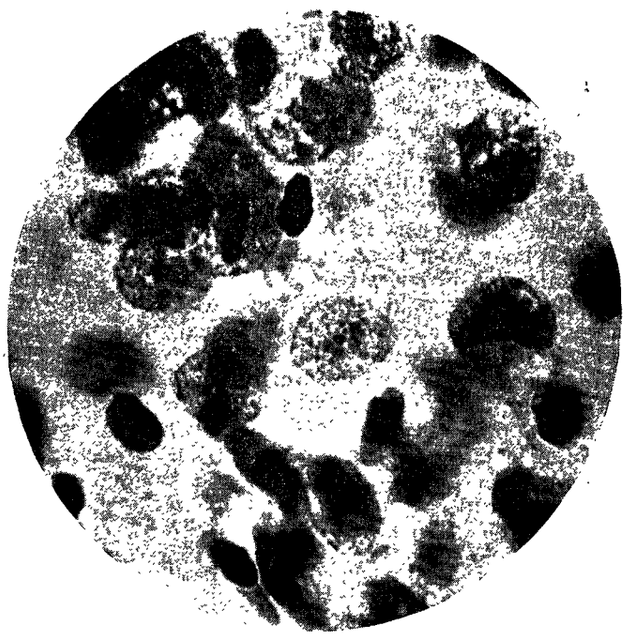

FIG. 7.—Plasmodiophora carcinomatis. Nucleated pre-conjugation phase in nucleus of epithelial cell. Mammary carcinoma. Platinum toning followed by methyl violet method. (× 1000.)

FIG. 8.—Plasmodiophora carcinomatis. Body which may represent a wandering gamete. Small dark body in centre of field with filamentous attachment. Intestinal carcinoma. Palladium-gold toning. (× 1000.)

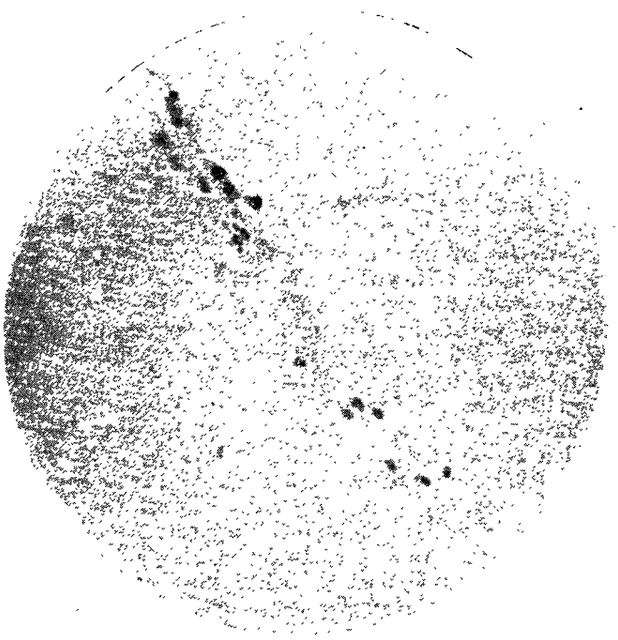

FIG. 9.—Plasmodiophora carcinomatis. Myxamœbæ in section of culture from a case of intestinal carcinoma. Palladium-gold toning. (× 1000.)

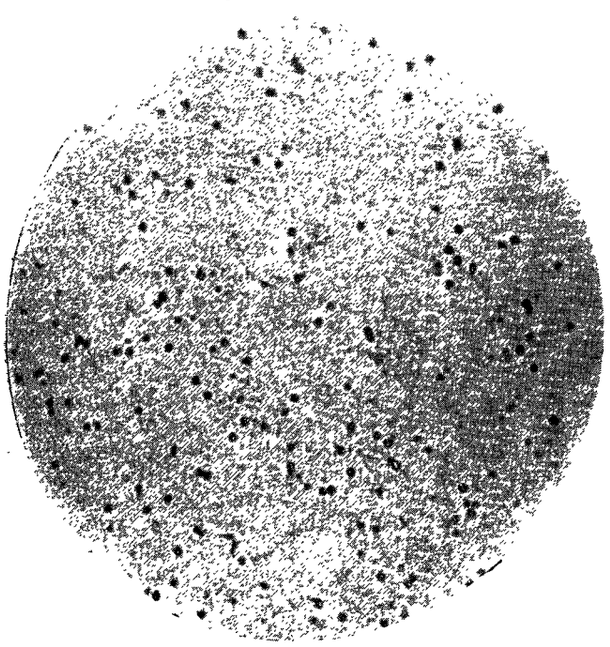

FIG. 10.—Plasmodiophora carcinomatis. Late post-granular phase in section of a culture from a case of intestinal carcinoma. Gold toning. (× 1000.)

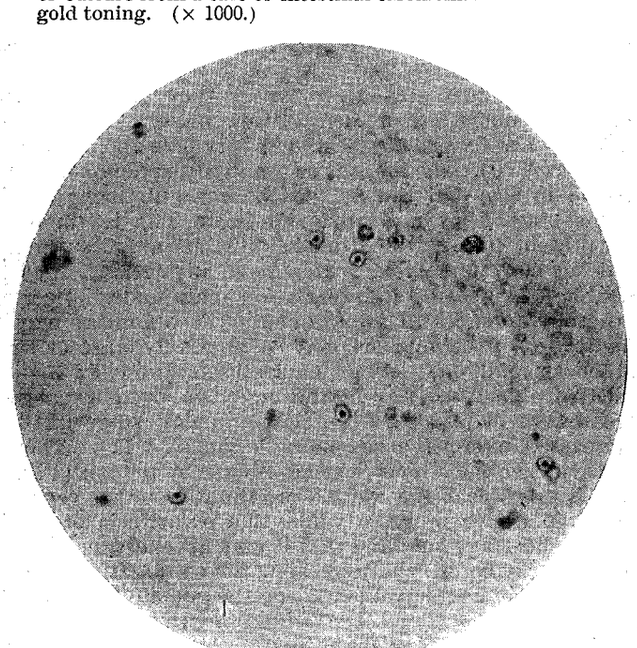

FIG. 11.—Plasmodiophora carcinomatis. Nucleated pre-conjugation phase in culture from a case of intestinal carcinoma. Gold toning following methyl violet. (× 1000.)

of which the question of the relation of carcinoma to plasmodiophoræ would inevitably remain obscure.

We begin with the spore which under natural conditions falls into the ground and lies dormant until early summer. It appears in silver-gold preparations of kohlhernia in turnips as a pale, round body from 1μ to 2·5μ in diameter, having a brown or black granular nucleus, measuring from 0·8μ to 1·4μ (Fig. 1, A 1). When this spore begins to germinate its nucleus undergoes some process of division which it is difficult to trace accurately. It appears to be initiated by the throwing out of a small body from the nucleus. Either two or four small dark bodies are ultimately formed (A 2). The immediately succeeding phase would appear to follow one or other of two courses, probably corresponding to a sexual differentiation. In one mode of development a pale reddish oval or pear-shaped non-nucleated body, from 1·8μ to 2·3μ in diameter, is formed (A 3). Within this there next appears an almost colourless oval central portion with well-defined margin and generally containing a minute dark particle (A 4). Then the clear central portion gradually enlarges and the dark granules within it multiply, whilst at the same time the marginal portion of the cell becomes attenuated (A 5). In the other mode of development the bodies resulting from division of the spore assume an oval form and a reddish, slightly granular appearance (A 3). More darkly stained particles, commonly four in number, soon become visible within them (A 4). There is next a gradual transformation into black granules indistinguishable from those resulting from the first mode of development. There is evidence that, prior to the development of the granules within them, both varieties of these bodies are motile. They very commonly invade the nucleus of the turnip cell and the second variety would appear also to invade the hyaline spheres which form a portion of the cell structure. They may even pass out of the vegetable cell in which they had origin. They are without doubt to be identified with the swarm cells or myxamœbæ described by others. In natural conditions they develop in the earth and having gained entrance to the root-hairs of a young plant travel by way of the vessels to various situations. Both varieties of myxamœbæ become transformed into the granular bodies which represent what we have termed the granular stage of the parasite (A 6). In silver sections toned with palladium and gold these bodies are generally deep black. The smallest of them are no larger than the most minute micrococcus. They are very frequently placed in rows of three and in these instances there is a suggestion of a delicate, unstained, uniting thread, the actual existence of which is, however, uncertain. These granules, which in natural conditions are now lying within the protoplasm of the cell of a young plant, gradually increase in size (A 7). At first they retain their dark appearance but this is gradually lost as they pass into what may be termed the post-granular phase (A 8). They now appear generally as pale reddish, almost homogeneous, round bodies with a sharply defined outline (Fig. 2). The protoplasm of the cell, which is more or less packed with parasites, becomes of a granular and opaque appearance, in consequence of which the outline of the organisms is obscured. The smallest of the parasites have at this stage a diameter of about 0·5μ, whilst the largest (A 9) equal the small forms of the next stage into which they gradually pass. This is the nucleated pre-conjugation stage. The nucleus, which is centrally placed, is at first very pale and difficult to recognise (A 10). The organisms gradually undergo a further increase in size and the nucleus becomes distinct (Fig. 3). At this stage they measure from 1μ to 3·4μ in diameter. They present at least five or six different aspects which as yet it has been impossible to arrange in any series. It seems probable that a sexual differentiation again underlies certain of these differences in appearance. The organism may be round or oval; the nucleus may be relatively large or small and occasionally a distinct nucleolus is present; and, as indicated above, the size of the organism, even when there is reason to believe that the post-granular stage has long been passed, exhibits a considerable range (A 11). There is clear evidence that the organisms divide by mitosis (Fig. 4). There is proof that the morbid proliferation of the vegetable cells is in active progress whilst the parasite is present in the granular stage only and that it may have advanced a considerable way before the nucleated pre-conjugation stage is reached. The next stage is that of the gamete (A 12). The nucleus disappears and the whole organism assumes an opaque, faintly granular aspect. All or most of the parasites in the cell undergo this transformation at the same time. Two adjacent cells then appear simply to fuse to form one (A 13). Simultaneously with this conjugation a distinct capsule forms round the zygote. The new cell has at first a finely granular, opaque appearance. Subsequently it diminishes in volume and becomes rounded in form. A remarkable alteration then occurs in consequence of which the body comes to have the appearance of four (sometimes more) round, pale globules, closely adherent to one another. Surrounded by its delicate capsule the organism has at this stage a very characteristic aspect. Very commonly several adjacent parasites appear to fuse and become enveloped by a common capsule. As, however, the final result is always the same—the formation of the peculiar bodies just described—it seems probable that there is a true fusion of only two individuals. At no stage of the process is there the formation of a multinucleated mass of protoplasm—i.e., a plasmodium. The term plasmodiophora brassicæ would therefore appear to be a misnomer. After a time the organism assumes the form of the nucleated spores already described and the cycle is completed. It will be noticed that in this cycle the large amœbæ figured by Woronin, and described also by others, have no place. It seems probable that what have been regarded as amœbæ were simply aggregations of the granules which arise in the swarm cells. Such aggregations may very commonly be seen within the vessels of the turnip at an early period of infection.

The bodies resembling plasmodiophoræ in carcinomatous tumours.—We have used for the purposes of our investigation chiefly carcinomatous tumours of the breast and malignant adenomata of the intestine (and secondarily affected abdominal glands) removed at operation. As controls we have used simple tumours and various inflammatory tissues, also removed at operation, as well as the tonsil, stomach, liver, and pancreas of the general paralytic and various nervous tissues.

The methods we have employed have been the same as those used for the study of the plasmodiophora brassicæ. It is not, however, to be thought that in these methods we have a means by which plasmodiophoræ can be specifically stained. Although they are capable of rendering such parasites visible they have the disadvantage that they at the same time colour almost everything else contained in the tissues. A partial exception is, however, to be made as regards the combined palladium and gold toning process with which we can claim to have made at least a beginning in the differential staining of certain stages of a plasmodiophora. To a greater or less extent in all the preparations the products of various pathological tissue changes, as well as some normal structures, simulate very closely many of the bodies which there are grounds for believing are plasmodiophoræ. The search for these parasites in carcinomata is therefore beset with difficulties and but for the fact that certain special structural characters can occasionally be recognised it would be impossible to derive any conclusive evidence either in support of, or against, their presence from the histological part of our investigation. There is, of course, room for difference of opinion as to the diagnostic value of any alleged special structural character. We can merely present the evidence as we have found it. The conclusions which it seems to us to warrant when taken in conjunction with that derived from cultures will be considered later.

The bodies in carcinomata, which we believe to be plasmodiophoræ, are considerably smaller than the plasmodiophora brassicæ. Both show a considerable range of size in all their stages and it is therefore somewhat difficult to estimate their average relative dimensions. Fuller investigation of the various phases of both parasites obliges us to regard the difference between them to be less than we have formerly estimated it to be. It may be stated that roughly the alleged plasmodiophoræ of carcinoma are about one-fifth of the volume of the corresponding phases of the plasmodiophora brassicæ. Bodies accurately corresponding in reaction and form to the resting spore of the plasmodiophora brassicæ may be observed, especially in palladium-gold preparations, lying between the epithelial cells, among the connective tissues, in the glandular spaces of adenomata, and occasionally within the nucleus or protoplasm of the epithelial cells. (Fig. 1, B 1.) The form of the protoplasm is not always rounded. Small dark bodies, devoid of any recognisable protoplasm, may also be observed and there are some grounds for supposing that they are really of the same nature as the nucleated bodies.

Forms representing the first phase in the transformation of the spore into myxamœbæ, though rare, may occasionally be seen (B 2). Bodies which may be young myxamœbæ (B 3) are common in the intracellular spaces and may occasionally be observed in the protoplasm or nucleus of the epithelial cells. This phase is, however, one lacking in any distinctive characters. The fully developed myxamœba in both its forms is represented by bodies which may occasionally be seen in the same situations (B 4). The clear oval central portion of one of these forms with its contained dark granule has a characteristic appearance which it is difficult to believe could be simulated by mere degenerative material. The development of these myxamœbæ into the granules is represented more rarely (B 5) but the fully developed granules (B 6 and 7) are often present in abundance. It is this stage of the organism which, both in kohlhernia and in carcinoma, we have found to be in a measure specifically stained by the palladium and gold process of toning. The method is, however, uncertain in its results. Its success appears to depend upon the fulfilment of certain precise conditions as yet not understood. It has failed almost completely when again applied to a batch of sections with which on first trial it yielded a satisfactory result. In our most successful preparations by this process granular bodies, having the exact appearance and arrangement of the granular stage of the plasmodiophora brassicæ, are present in considerable numbers in the nuclei of most of the epithelial cells and occasionally also in the protoplasm (Fig. 5). They occur generally in small groups, occasionally in masses. There are grounds for believing that in silver-gold preparations these bodies are often obscured by nucleolar matter which tends to deposit around them as they lie in groups within the nucleus of the cell. Similar bodies occurring singly or in groups may be observed in some of the tissue spaces, but as various forms of degenerative granules which tend to reduce silver salts are common in this situation it is impossible to attach any special significance to them. The post-granular stage (B 8 and 9) is also represented but less abundantly than the preceding one. The bodies corresponding in reaction and in form to this stage appear also to be present chiefly in the nuclei of the epithelial cells. As they stain much less deeply than the granules they are difficult to recognise in this situation. When, however, an epithelial cell containing them divides by mitosis they are temporarily thrown into the protoplasm and can then very commonly be recognised (Fig. 6). They may be observed in large numbers in cells in which the karyokinetic figure is normal in appearance, so that they can hardly be accounted for on the supposition that they are fragments of chromatin.

The nucleated pre-conjugation stage is still less abundant (B 10 and 11). It is represented by bodies of round, oval, or irregular form which may be seen either in the nucleus of the epithelial cells or in the protoplasm of a dividing cell, more rarely in the protoplasm of a resting cell. They measure 1·3μ to 1·5μ. When in the nucleus of an epithelial cell in a silver-gold preparation, bodies of this kind are obscured by the chromatin and nucleoli. We have, however, been able to see them clearly in silver preparations which, after toning with platinum, have been stained in a special way with methyl violet (Fig. 7). In these preparations the chromatin is darkened by the platinum, whilst the supposed parasites take up the methyl violet stain. We have a silver-gold preparation in which these bodies lying in the protoplasm of a dividing epithelial cell of a carcinoma of the breast appear to be undergoing mitotic division. More or less deeply tinted non-nucleated bodies, which correspond in characters to the gametes of the plasmodiophora brassicæ, are not uncommon within the epithelial cells and in the interstitial tissues (A 12). This form is, however, so lacking in distinctive morphological characters that it certainly cannot as yet be distinguished from many products of tissue disintegration. At the same time there are some grounds for believing that at this stage the plasmodiophoræ associated with carcinoma are actively motile and that they may therefore at times exhibit the morphological characters of wandering cells. In all silver-gold preparations of carcinomata, wandering leucocytes and connective tissue corpuscles constitute a striking feature. They generally stain deeply and are often thrown out into long narrow threads as they force their way between the masses of proliferated epithelial cells. For a long time we assumed that all such appearances were to be regarded as merely manifestations of the phagocytic activity of these cells, although we again and again noted appearances which it was difficult to reconcile with this view. These consisted especially in the occasional presence of very small deeply staining bodies stretched out into fine threads and terminating at one or both extremities in the form of a minute knob. We have recently been able to study these bodies to better advantage in the palladium-gold preparations (Fig. 1, B 12, and Fig. 8). They are only rarely present and a considerable search is generally required before one is found. The terminal knob measures generally about 0·5μ in diameter. The connecting thread or tail is extremely delicate. It may measure about 4·5μ. There is, of course, no proof that these bodies are wandering gametes. We simply direct attention to their presence and point out the possibility of their being of this nature. Hour-glass forms, which would represent the conjugation of the parasites, have occasionally been observed within the protoplasm of the epithelial cells and in the surrounding tissues. This again is a form which is too indefinite in character to be distinguished from various products of tissue degeneration. The phase of the plasmodiophora brassicæ which immediately follows conjugation has, on the other hand, very distinctive features. We have a preparation from a carcinoma in which there is lying at the edge of proliferating epithelial cells a body in which these features are exactly reproduced upon a slightly smaller scale (B 14). The next stage is that of the nucleated spores which has already been described.

It will be noted that as the granular phase is receded from the supposed parasites gradually diminish in number. In this respect there is a striking difference from what obtains in kohlhernia. We have, however, only to consider for a moment the comparative conditions under which the two parasites are living to find an adequate explanation. The vegetable host differs from the mammalian one in having no phagocytic cells. There is the most conclusive evidence that very active phagocytosis is constantly in progress within carcinomatous tumours. This action may be manifested in tumours in which, as far as can be seen, there are no dead or degenerated epithelial cells requiring to be removed. As already stated, the phagocytic cells may commonly be observed fixed in the act of moving about between the epithelial cells, being stretched out into long winding threads terminating at either extremity in a bulb. Appearances may not infrequently be noted which seem to indicate that one of these bulbs has penetrated into the protoplasm of an epithelial cell. That this actually occurs seems to be borne out by the fact that occasionally a leucocyte may be observed to be wholly imbedded in the protoplasm of such a cell. The phagocytic cells lying in the tissue spaces are commonly more or less loaded with granular débris, as well as with larger particles, some of which closely resemble the larger forms of the plasmodiophoræ. The evidence points strongly to the conclusion that the parasites are constantly being swept up by these cells, which are even capable of removing them from the protoplasm of the epithelial elements and that only those that remain within the shelter of a nucleus are safe from attack. If this is so, it is easy to understand why the number of plasmodiophoræ progressively diminishes as the granular phase is receded from. Notwithstanding that a rapid proliferation continues up to the time of the formation of the gametes the destruction of the parasites by phagocytic action is so great that only comparatively few survive to form spores. A very small number of these, however, are capable of starting another cycle. The motile myxamœbæ having penetrated into the nuclei of some of the epithelial cells will be able to give rise to an almost unlimited number of granules as the host cells undergo rapid proliferation. There is ample warrant for the belief that such penetration occurs in the demonstrable fact that the myxamœbæ of the plasmodiophora brassicæ are attracted to the nuclei of the vegetable cells.

The cultivation of an organism resembling the plasmodiophora brassicæ from carcinomatous tumours.—As already indicated, we have succeeded in growing from carcinomatous tumours an organism which accurately represents certain phases in the developmental cycle of a plasmodiophora. The technique which we now employ is based upon the results of many experimental attempts to obtain cultures from such tumours. It consists essentially in placing pieces of the fresh tissue upon the dry surface of an agar medium, various important details being attended to. Through the courtesy of Mr. F. M. Caird, Mr. David Wallace, and several other surgeons, we have been enabled to obtain the tumours immediately upon their removal from the patients. We

shall describe the procedure as carried out with a mammary carcinoma. The gland with the tumour incorporated in its substance is, immediately on its removal from the patient, received upon a thick layer of sterilised gauze swabs, an abundant supply of which is always obtainable in an operating theatre. The specimen is wrapped up in these and then further covered with a sheet of sterilised protective. It is at once removed to the laboratory at which the tissues arrive unchilled and alive. Tubes of ordinary sloped nutrient agar are in readiness. The preliminary preparation that they require consists in the removal of the water of condensation and liquefaction and re-sterilisation by heat in order that a fresh and yet somewhat dry surface, free from any condensed pellicle, may be provided for the reception of the piece of tumour. Glycerine agar has been successful in one case and we have also obtained growths upon some media of special composition which, however, did not appear to have any advantage.

A large area of the posterior surface of the specimen, which in many cases is the pectoralis major muscle, is cauterised to insure its sterility. With the aid of a pair of forceps and a scalpel, both of which have been sterilised in the flame of a Bunsen burner, the posterior surface of the tumour is exposed by dissection. Small portions are then as quickly as possible cut out with another sterilised knife and placed singly upon the surface of the agar in the prepared tubes. The tissue must on no account be allowed to fall to the bottom of the tube, as contact with even the small amount of water of condensation would almost certainly prevent a successful result being obtained. It should be placed about the middle of the agar surface. The tubes containing the pieces of tissue are transferred to the incubator with as little delay as possible. They must in the first instance be laid in a slightly inclined position, so that whilst the piece of tissue is not displaced the water of condensation does not flow over the surface. The temperature is kept at 37° C. By the second or third day the piece of tissue has become adherent to the agar. The tubes may then be placed in the erect position. To prevent undue evaporation the tubes should now be sealed by pushing the cotton-wool plug in about a quarter of an inch from the margin and filling the receptacle thus formed with melted hard paraffin obtained from the imbedding oven. In our later work with this technique we have been uniformly successful in preventing contamination of the medium. This we attribute to the favourable conditions under which we have been able to obtain the material, the extensive cauterisation of the posterior surface of the specimen, and the circumstance that we have worked in the still air of the laboratory of which we are the sole occupants. We have obtained cultures of an organism which we believe to be a plasmodiophora from four tumours—namely, one malignant adenoma of the intestine (a secondarily infected abdominal gland being used for making the cultivations) and from three mammary carcinomata. The growth does not develop at the surface but invades the subjacent medium. It appears as a faint greyish cloud in from two to seven days and generally continues to increase very slowly for about ten days more. It has as yet been found impossible to obtain a satisfactory view of the organisms in films made from these growths and stained by the ordinary methods employed in bacteriology. They are either stained in conjunction with granular matter in the medium without presenting any characteristic features or they are decolourised. It is therefore easy to understand how previous observers, when dealing with such growths, which certainly must occasionally have been obtained by some of them, have concluded that the faint cloud which formed was the result of the penetration of colouring matter or coagulable substances from the dead tissue. By hardening the medium in 5 per cent. formalin and making preparations by the silver-gold process we have been able to recognise not only that the cloudiness is essentially dependent upon the growth of an organism, but that this organism has characters which prove it to be a plasmodiophora. In the sections from the growths in agar obtained from the secondarily infected abdominal gland we have been able to demonstrate the presence of several successive phases of a plasmodiophora, corresponding exactly in form and reactions to the special method with the bodies we have described as present in sections of carcinomatous tumours. In one of the agar growths from this tumour a slight splitting of the medium had been accidentally produced by the pressure exerted in fixing the tissue on the surface. Upon the walls of the crevice so formed (which extends a considerable way into the medium) there are very numerous small bodies which have the exact characters of the myxamœbæ already described. They measure from 1·2 μ to 1·7 μ. Some are devoid of the pale centre, others present this feature, whilst others again show the black granules appearing in the centre (Fig. 9). Some of these myxamœbæ have penetrated a certain way into the subjacent agar. Masses of granules, exactly corresponding to those that develop from the myxamœbæ in the incubated kohlhernia, are also present, though only a few have been observed. Similar granules, many of them of considerably larger size, are present in abundance throughout the surrounding agar but they cannot be distinguished absolutely from the fine granular deposit which is caused by the circumstance that certain substances in the agar effect the reduction of the silver salt. The large and small post-granular phases are, however, very abundantly represented (Fig. 10). They are probably the chief cause of the faint opacity in the medium. With the aid of the silver-gold method alone we have not been able to trace them with absolute certainty into the nucleated stages, but by staining the silvered sections by a special methyl violet process this structure has been demonstrated in some of the largest forms (Fig. 11). If the presence of the immediately preceding and succeeding stages may be taken as further proof of the existence of the granular stages which intervene, then we have evidence of the presence of eight phases, or four different stages, of a plasmodiophora in this growth. In the growth from two of the mammary carcinomata we have been able to recognise only the post-granular stage but the examination of them was rendered difficult by the circumstance that the agar turned out to be of too soft a consistence for the obtaining of sections that could be manipulated with facility. The examination of the growths from the fourth carcinoma is not yet completed, although we have already satisfied ourselves of the presence of the post-granular stage of the organism. For the purpose of making control observations, we have treated in the same way portions of tissue obtained from two cases of interstitial mastitis, one intra-abdominal testicle removed for torsion of its pedicle, and one simple fibro-adenoma of the breast. In no instance has any change taken place in the medium similar to that observed in the other tubes.

The bearing of the observations made upon the question of the etiology of carcinoma.—The observations that we have made can, we believe, be shown to harmonise with, and to help to explain, many otherwise unintelligible facts already recorded in the literature of the pathology of carcinoma. Our object for the present is, however, simply to record our own observations which, depending upon the results given by a new histological process and breaking fresh ground, are capable of standing by themselves. It is obvious that if, as alleged, plasmodiophoræ are present in carcinomatous tumours many of the intracellular bodies described especially by Russell and San Felice as blastomycetes, and of those interpreted by numerous others as protozoa, are simply various phases of this species of myxomycetes, for it is hardly to be believed that more than one pathogenic organism would commonly be present. The plasmodiophora theory of carcinoma has been advocated by the workers already mentioned for many years, but the evidence that has hitherto been adduced in support of it cannot be said to be conclusive. It has certainly served to convince very few. Can the new evidence that we have obtained be regarded as serving to establish the validity of this hypothesis? In our researches we have traced what appear to be three parallel lines—namely, those of (1) the life-cycle of the plasmodiophora brassicæ, (2) a series of bodies found especially within the cells of carcinomatous tumours, and (3) the stages of an organism which can be grown from such tumours. The last line is incomplete, but through eight successive phases, or four separate stages, the parallelism is exact. With regard to the histological evidence it may be affirmed that all the stages of a plasmodiophora are accurately represented in carcinomatous tumours and that it has been found impossible to construct a similar series from control tissues. At the same time many of the bodies which correspond in form and reaction to certain of the stages of a plasmodiophora are indistinguishable from certain tissue granules. The presence of any number of tissue granules does not of course logically exclude the presence of micro-organisms which are known

to have a certain resemblance to them. All that can be demanded from the histological evidence in support of a hypothesis like this is that the presence of bodies morphologically identical with the parasite as it is known in cultures or elsewhere should be demonstrated. If the supposed parasites presented no very distinctive morphological feature the evidence would certainly have very little weight. But in this case there can be demonstrated several such features of a very unusual and striking nature. The two forms of myxamœbæ, already known as they exist in the life-cycle of the plasmodiophora brassicæ, are definite forms that could not easily be simulated by the products of tissue disintegration. These forms have been observed in carcinomata and the presence of black granules in the originally clear centre of the first variety has also been noted. The bodies which morphologically represent plasmodiophoræ in the nucleated pre-conjugation stage have been observed to be undergoing what is to all appearance mitosis. The minute wandering cells which we have described cannot be identified as any cellular form known in the human subject but would exactly represent the gamete of a small plasmodiophora. Lastly, a body has been found in a carcinoma which exactly conforms to the peculiar morphological characters of that phase of the plasmodiophora brassicæ which can be shown to follow conjugation.

The evidence derived from the study of the cultures is not subject to the same ambiguity of interpretation. In the sections of the growths in agar tissue cells are absent and an organism is present which has obviously been multiplying rapidly. In one instance, at least, this organism has been found to be present in four distinct forms which harmonise exactly with four successive stages in the developmental cycle of the plasmodiophora brassicæ, as well as with that which has been found in sections of carcinomatous tissue. In cultures from three other cases of carcinoma there has also been observed an organism which is identical with the post-granular stage, as observed in the other case, and this phase is one which, in cultures at least, has a very characteristic appearance. Included in the four stages observed in the cultures from the first case are the specially characteristic forms of the myxamœbæ and the nucleated pre-conjugation stage. This evidence seems to us to be incompatible with any other conclusion than that in these cultures we are dealing with organisms which are plasmodiophoræ. Taken in conjunction with the histological evidence of the presence of similar bodies in carcinomatous tissues, it points to the conclusion that plasmodiophoræ are constantly associated with carcinomatous growths of the kind from which the cultures were made.

We have not yet had an opportunity of examining cultures from a squamous epithelioma. We have, however, examined tissues from three cases by the ammonio-silver process and whilst we recognise the uncertainty attaching to histological evidence in a question of this kind we can say that, as far as such evidence justifies the expression of an opinion, this form of carcinoma is also associated with the presence of plasmodiophoræ in the cells. We have as yet no observations to record bearing upon the question of the presence of plasmodiophoræ in sarcomatous malignant growths. We strongly dissent from the recent assumption that all malignant growths must necessarily have the same origin. To discuss this question here would, however, be to go beyond the purpose of our paper. If future observations confirm those that we have here briefly recorded and it be thus established that plasmodiophoræ are present in carcinomatous tumours the etiological relationship of the organism to these growths will hardly require further proof, although this would probably be obtainable from inoculation experiments, and if a protozoan organism of this nature, which can be grown through an important part of its life cycle, is the essential etiological factor in carcinoma it may be confidently anticipated that it will be found to be possible to produce antibodies, by the therapeutic use of which the normal protective agencies of the body will be enabled to overcome the parasites in such growths.

In concluding, we have to express our indebtedness to the Fellows of the Royal College of Surgeons of Edinburgh and to the General Board of the Laboratory of the Scottish Asylums for the facilities they have afforded us for the carrying out of the researches recorded in this paper.

APPENDIX.

The technique of the ammonio-silver process and toning methods.—The ammonio-silver process was described in the *Review of Neurology and Psychiatry* in July of last year. The following general directions for carrying it out are for convenience given here:—

1. Wash thin slices of formalin-hardened tissues (from 5 to 10 per cent. solution of formalin in water; change on the second day) for about 24 hours in a bowl of tap water. Change the water at least once.
2. Transfer to an ammonio-nitrate of silver solution, prepared by adding to a 1 per cent. solution of silver nitrate in distilled water a 5 per cent. solution of ammonia in distilled water, drop by drop, until the precipitate which forms is nearly but not entirely dissolved, and then filter. In place of this silver solution a saturated solution of silver carbonate in ½ per cent. ammonia in distilled water may be used with advantage. Two days at least should be allowed for saturation. The fluid should measure at least 50 times the volume of the tissues. Cork the bottle or specimen tube and put it in the dark. The pieces are ready for examination in from three to ten weeks.
3. Place a piece of the impregnated tissue in a bowl of water (500 cubic centimetres) to which about 2 cubic centimetres of 5 per cent. ammonia have been added. Remove the surface deposit as far as possible. This is best done simply with the aid of the fingers whilst the piece of tissue is held under water. Transfer the piece to a second bowl of ammonia and water. Renew the fluid after about an hour and leave the tissue in this for three or four hours longer.
4. Transfer to dextrine solution (dextrine five ounces or 140 grammes, water ten ounces or 280 cubic centimetres; dissolve by boiling; filter the solution through cotton wool while still hot; after it has cooled add 1 per cent. of carbolic acid) to which ammonia has been added in the proportion of ten drops of a 5 per cent. solution to one ounce, immediately before use. Allow the tissue to remain in this for from 12 to 24 hours.
5. Cut thin sections on the freezing microtome. Transfer them from the knife to a bowl of water to which about ten drops of 5 per cent. ammonia have been added. After about five minutes transfer the sections to another bowl of ammonia and water, and after a similar period give them a third wash.
6. Transfer the sections to a bowl of water to which there have been added from five to ten drops of a saturated solution of citric acid in water, and allow them to remain in this for four or five minutes.
7. Place the sections in a bowl of tap water and after a few minutes transfer them to a second bowl of water. They are now ready for toning.
8. To ten cubic centimetres of a ¼ per cent. solution of gold chloride in distilled water add a single drop of a 1 per cent. solution of citric acid in water and filter the fluid, preferably into a flat-bottomed white porcelain dish. Transfer the sections from the water to this toning bath by means of a glass rod or platinum needle. Allow them to remain spread out for about one hour. About a dozen sections of ordinary size may be toned in this amount of the gold solution.
9. Place the sections for about ten minutes in a bowl of tap water and then transfer to a bowl of water to which ten drops of 5 per cent. solution of ammonia have been added, where they should remain for two or three minutes.
10. Transfer to a bowl of tap water.
11. Dehydrate the sections with absolute alcohol, clear in equal parts of turpentine and benzole; remove turpentine with pure benzole; mount in benzole balsam.

The process of toning the sections permits of being varied in an almost endless number of ways, with corresponding variations in the result. Not only may gold chloride be variously combined with other substances but certain other heavy metals may be made to displace the silver deposit. We have especially experimented with salts of platinum, palladium, iridium, ruthenium, and vanadium. It is also possible to follow the toning by staining with aniline dyes. In the meantime it must suffice to indicate briefly the details of two special toning processes which we have found to be useful.

Platinum toning of the silvered sections may be carried out as follows. To one ounce of freshly prepared ½ per cent. solution of potassium chloroplatinite in water add ten drops of a 5 per cent. solution of phosphoric acid in water (citric acid may be used instead). Filter this solution and place the sections in it for about half an hour. Wash the sections shortly in water and then clear them in a watch-glassful of 1 per cent. solution of platinum bichloride ($PtCl_4$) in water for about ten minutes. Wash well in water, dehydrate, clear, and mount in balsam.

The conditions upon which depends the success of the combined palladium and gold toning process have not yet been definitely ascertained. The best results have been obtained with sections of a formalin-hardened tumour which had been for two weeks in an ammonio-silver solution prepared with 5 per cent. silver nitrate and 5 per cent. ammonia solutions. The sections were placed for half an hour in a strong solution of palladium chloride slightly acidulated with citric acid, and after having been washed shortly in water they were further treated for a similar period with ½ per cent. gold chloride rendered faintly acid with citric acid. The sections were then simply washed in water, dehydrated, cleared, and mounted in balsam. In using palladium chloride for the toning of sections it is important that the fluids should be slightly acid throughout, as black granular precipitates may be formed in the presence of alkalis.

Edinburgh.

THE RESULTS OF TWO CASES OF INJECTION OF PARAFFIN.

BY F. C. WALLIS, F.R.C.S. ENG.,
SURGEON TO CHARING CROSS HOSPITAL, ST. MARK'S HOSPITAL, AND THE GROSVENOR HOSPITAL.

CASE 1.—A single woman, aged 32 years, was sent to me by Sir Francis Laking on Nov. 2nd, 1904, because of considerable and increasing difficulty in getting the bowels to act. The history given was that the present condition had been gradually arrived at and the patient was getting thinner. The difficulty of procuring any action of the bowels