Section of Tropical Diseases and Parasitology.

President—Dr. R. T. Leiper, F.R.S.

Some Features of Crown Gall in Plants in Reference to Comparisons with Cancer.

By Wilfrid Robinson, D.Sc.

(Professor of Botany, University College of Wales, Aberystwyth.)

From time to time comparisons have been made between tumour-like growths in plants, and tumours, benign or malignant, in animals and man. Such comparisons, when carefully analysed, have usually proved to be more superficial than real, if only because of the profound differences in the cellular construction and manner of growth in animals and plants. In recent years, however, the bacterial disease of plants known as *crown gall* has been very persistently compared with malignant tumours in man. The disease has been the subject of much work and of many papers by Dr. Erwin Smith in America, who first isolated the causal organism *Bacterium tumefaciens* and experimentally produced the disease by the re-inoculation of plants from pure cultures.

Dr. Smith soon formed the opinion that the growths characteristic of this disease are comparable to malignant tumours. In his earlier work the evidence for the far-reaching comparisons with cancer was derived from artificially-produced growths which, in addition to having primary swellings near the region of inoculation, showed others, described as secondary tumours, which were connected with the former by so-called tumour-strands. The parallel was thus drawn with the origin of secondary tumours in malignant diseases in animals by metastasis from the primary tumour.

Though able to isolate bacteria from the tumours, Smith failed to locate the former in the diseased tissues by direct microscopic observation. More recently Blumenthal and others working with him in Germany have isolated from the margins of human tumours a bacterium which gives cultural reactions almost identical with those of *Bacterium tumefaciens* (E. F. Smith). Further, at least, one of the strains isolated from human tumours has proved capable of producing, on sunflowers, galls identical with those of crown gall. In addition Blumenthal claims to have produced tumours in healthy animals by inoculation with the same organism.

I shall re-describe briefly some studies carried out by Mr. Walkden and myself at Manchester, on crown gall, using a strain of *Bacterium tumefaciens* isolated by Mr. Walkden from diseased plants of chrysanthemum found in gardens. From our results I shall endeavour to give reasons why Smith's comparisons cannot be regarded as valid, also, in my opinion, why the work of Professor Blumenthal and his colleagues cannot be accepted as proving that an organism similar to *Bacterium tumefaciens* is responsible for malignant diseases in animals and man. Finally, I hope to offer some suggestions as to a possible fundamental resemblance between crown gall and animal diseases in which cell-proliferation is the dominating feature. In doing this I shall have to refer to the recent illuminating researches of Warburg, in Berlin, in support of the view that, while the comparisons of Smith, Blumenthal, and others, cannot fruitfully be carried further, the study of the intimate cellular physiology of proliferating growths in both plants and animals gives promise of an ultimate solution of many of the problems of malignant disease.

54 Robinson: *Crown Gall in Plants in Reference to Cancer*

Our work on crown gall will now be dealt with, and it must be emphasized that the so-called secondary tumours described by Smith do not occur naturally on plants but result from artificial inoculations. The naturally occurring galls on the common garden marguerite (*Chrysanthemum frutescens*) are globular swellings with a rough surface bounded by superficial layers of dead cells. Such galls have the bacteria present in abundance on the surface. In our work the method adopted was the inoculation of the cut ends of shoots with pure cultures of the bacterium, since this organism can only produce galls after wounding.

The bacteria enter the intercellular spaces of the tissues as well as the open ends of the vessels passing down the shoot, for a smaller or greater distance. Adjoining the lines of invading bacteria, cells and tissues, normally inactive, are stimulated to repeated division—subsequently either continuing their growth or differentiating into irregularly arranged woody tissue. Control shoots cut similarly but not inoculated gave no such results.

It was thus demonstrated that the presence of the bacteria on the exterior of galls and in the air spaces between the cells (they have never been recognized within the living cells) influence these cells to active proliferation. The possible nature of the influence exerted will be referred to below. In such rough-surfaced galls, which correspond to the primary tumours described by Smith, the later development of the gall is partly due to the active presence and multiplication of *Bacterium tumefaciens* on the rough external surface of the gall. It was found possible to repeat most of the work of Smith on the so-called secondary tumours, but the results obtained show that the facts will not bear Smith's interpretation.

In the marguerite the so-called secondary tumours arise when the inoculations are made by needle-pricks into the vicinity of the growing points or apical meristems of actively growing shoots. A number of leaf-rudiments as well as the meristematic tissues of the stem are affected by the needle-prick, and by the bacteria carried in by it; and as the growth of the plant proceeds the injured parts which develop galls are widely separated from one another by the growth of the shoot. Many such galls have a rough exterior, but others are smooth, and these latter correspond to Smith's secondary tumours. They are not, however, due to the intrusion of tumour-strands as he suggested, but have been shown in our work to be due to the presence of bacteria in the air spaces between the tissues. These bacteria have been proved either to be carried by the growth extension of the tissues to a distance from the original point of entry, or to migrate actively in the intercellular spaces. Although regions infected by the migrating strands of bacteria superficially resemble invasive tumour strands, it has been demonstrated that cells are merely altered *in situ*, there being no migration of the cells of the diseased plant. The bacteria, on the other hand, move within the spaces as a zoogloeal thread.

This migration of the bacteria can be observed particularly easily in tobacco plants, if the young flowering shoots are cut across and the wounded surface then inoculated with *Bacterium tumefaciens*. A rough gall arises at the cut surface and a string of smooth galls, with healthy tissues between, occurs for some distance below the cut surface. As cell-proliferation proceeds around the loci formed by the invasive zoogloeal threads some of the pre-existing tissues may be somewhat displaced by inequalities of growth, but there is never any true metastasis.

Other features of crown gall, such as the production of apparent teratomata and the apparent transformation of the structure of one organ into another as a secondary effect of the bacterial activity, have been shown to be equally invalid as the bases for any far-reaching or real comparison of crown gall with malignant tumours.

Experimental work by Riker in America, and by Kuster in Germany, has independently led these investigators to conclusions similar to our own; others, in

Germany and France, approaching the subject from the point of view of malignant disease, have come to the extraordinary conclusion that *Bacterium tumefaciens* or organisms very similar to it occur in tumours in man, and that when isolated these organisms prove capable of producing not only galls on plants but tumours on animals. I find it difficult to accept Blumenthal's results as conclusive, for several reasons. In no case in which tumours occurred in animals after inoculation with the strains isolated from human tumours did Blumenthal succeed in re-isolating the inoculated bacteria from the tumour in the diseased animal. Until this has been done the causal connexion between the organism inoculated and the growth produced cannot be regarded as established. Blumenthal himself explained the discrepancy in the evidence by suggesting that an ultramicroscopic virus is associated with the bacterium in those cases in which inoculated animals develop tumours. In this way Blumenthal possibly foreshadowed the ultramicroscopic theory of Gye and Barnard.

Feggins and Funk in France have claimed more recently that they have isolated from a uterine tumour a bacterium, which, on inoculation into pelargonium plants, proved in one case capable of causing the development of galls as large as a hazel nut.

The fact that it should be possible to isolate organisms similar to *Bacterium tumefaciens* from malignant tumours is in itself not surprising in view of the well-known presence of a variety of bacteria on the necrosing margins of tumours. Such bacteria have of course no causal connexion with the tumours. It may therefore not be without significance that Blumenthal's isolations were made from the secretions of the marginal regions of tumours subjected to a special treatment with sun's rays, and that several other species of bacteria, in addition to the strains similar to *Bacterium tumefaciens*, were isolated.

There thus appears to me to be little, if any, justification either for the comparisons instituted by Smith, or for the conclusions arrived at by Blumenthal regarding a possible connexion between the causal agent in crown gall and malignant tumours. There are, however, certain features which the plant and animal diseases share in common. For example, in both cases, cells and tissues are stimulated to active atypical proliferation, and I have suggested elsewhere that a real insight into the nature of the changes taking place under the influence of *Bacterium tumefaciens* might throw light on the changes occurring as the result of unknown causes in the cells of animal tumours. The recent work by Warburg, mentioned earlier, bears directly on this aspect of the subject.

Rejecting the theories of Gye as well as those of Smith and Blumenthal on the ground of the lack of clinical evidence of the infectious nature of malignant tumours, Warburg holds that the problem is essentially one in cellular physiology. He has studied, by exact experimental methods, the energy-liberating reactions in rat sarcomata and carcinomata, and in fowl sarcoma, measuring the fermentative changes in the tumour tissue. He finds that this fermentative activity resulting in lactic-acid production is a property of all non-necrosing tumour tissue whether from animals or from man, and that such tissue shows this property irrespective of the presence or absence of oxygen. In the absence of oxygen all body cells show fermentation, but Warburg has proved that only embryonic tissues, e.g., of the hen's egg, show a fermentative activity, in the absence of oxygen, at all comparable in magnitude to that of tumour tissue. In the presence of oxygen this is masked in the healthy embryonic tissues by active respiration. Thus Warburg concludes that in tumour tissues the oxygen respiration of growing cells is electively injured, while the fermentative activity still proceeds. This can be illustrated experimentally by placing an embryo for some time in nitrogen and then once more in oxygen. The respiration is injured, but the fermentation is unaffected, i.e., the metabolism characteristic of tumour tissue persists. Poisons and

other injurious substances act similarly and, *in vitro*, tumour cells occasionally arise. Usually, however, in experiments, as in the body, the effect of injuring respiration is to cause the death of the cell.

In many cases in plants it is well known that lack of oxygen for a time may result in cell-proliferation, and it may be significant in crown gall that *Bacterium tumefaciens* is an organism which is very strongly aerobic. Thus it is possible that in the intercellular spaces it modifies the oxygen relations of the cells so that the internal changes resulting in proliferation are set up. Whether the tissues in crown galls show a similar type of metabolism to that which Warburg has found in animal tumours will be seen from further work. It certainly appears possible that in their cellular physiology the plant galls may have at least some points in common with the animal tumours.

[*May* 4, 1927.

The Development of *Schistosoma mansoni*.

By Dr. P. H. J. LAMPE, Surgeon to the Dutch-Indian Army.

(*Communicated from the Pathological-anatomical Laboratory of the Military Hospital of Paramaribo, Dutch Guiana.*)

DURING an investigation into the development of *Schistosoma mansoni* at the beginning of this year, undertaken more out of interest in the cause of a disease endemic here than with the intention of discovering anything new in connexion with it, a few details were observed, some not yet mentioned in the literature of the subject and some not entirely in keeping with facts therein stated.

Therefore, notwithstanding the excellent work of Leiper on the development in the intermediate host, and that of Yamagiwa, Lütz, Faust, and others, on the development in the final host, I venture to mention briefly certain observations of my own in Surinam on the development of this highly interesting trematode.

(I) THE DEVELOPMENT IN THE INTERMEDIATE HOST.

The full-grown parasites of this species of Schistosoma live in the hepatic portal vein and its branches. The parasites—paired—swimming against the blood-current, enter the narrower veins, where the thinner female, quitting the male, deposits its ova.

The ova, distinguished by a lateral spine, measure about 165 microns, and the spine about 26 microns. In order to continue the species, a number of the ova penetrate the intestinal wall and reach the intestinal canal.

The part played by the lateral spine is not quite understood. Possibly the ovum is forced back against the intestinal wall by the blood-current and remains sticking to it by means of this lateral spine. With the fæces the ova are ejected.

If the ovum be placed in a hypotonic medium (e.g., water, or very diluted eosin-solution, added to the fæces) the embryo, if alive, visible through the egg-shell, starts moving, while, after an interval—depending on light, temperature (optimum 35°) and unknown influences—the shell ruptures laterally somewhat behind one of the poles.

The miracidium slowly creeps out, and, escaping from the shell, swims away. I have never observed in the case of *Schistosoma mansoni* the dilatation of the egg-shell as described by Brumpt for *Schistosoma hæmatobium*—"L'œuf se gonfle."

The duration of the life of the free swimming miracidia—estimated at two to three days—is shown in Surinam (laboratory temperature 33° max.) to be forty hours at the longest: according to Faust, in China, three days (*Schistosoma japonicum*); according to Christopherson, in the Sudan, at the most nine hours