clinical, pathological, comparative, and experimental methods of investigating cancer. They are really complementary to one another and those who have studied the publications of the Imperial Cancer Research Fund must have perceived that every effort has been made to give each line of inquiry its due importance, and to coördinate them as the respective parts of one extensive investigation. The purely clinical and pathological aspects of cancer have received a large share of attention. The statistical investigations of the Imperial Cancer Research Fund are based on the most numerous and reliable clinical and pathological data yet tabulated. In certain investigations on the earliest stages of squamous-celled carcinoma of the tongue, the ripe clinical experience of Mr. H. T. Butlin was supplemented by experience of a different kind, gained in purely pathological and experimental studies on human and animal material, with most important clinical results.¹⁶

We beg leave to conclude that the comparative, the biological, and the experimental investigation of cancer is based not only on sound foundations, but that it is advancing our knowledge³¹ of the disease more rapidly than any other line of inquiry at the present time.

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ON OPSONINS AND IMMUNITY.¹

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IT has for long been a matter of uncertainty to which of the constituents of the blood its germicidal properties are due. That such properties exist is, of course, undoubted, and some of the earliest experiments on the subject showed that bacteria when injected into the blood stream rapidly disappear. It was shown later that defibrinated blood could destroy various kinds of micro-organisms, although after a time this same blood lost its germicidal properties and became an excellent medium on which the bacteria could grow. The germicidal powers of fresh blood must, of course, depend either on some substance contained in the serum or in the corpuscles, or on some actively destructive action of the latter. The white corpuscles, or phagocytes,

have been shown to possess the power of taking up and digesting bacteria, probably by the aid of some intracellular ferment, and the method of which I am speaking—the opsonic method—depends on our ability to estimate the relative capabilities of the phagocytes to take up bacteria (known as phagocytosis) when incubated with and acted on by different blood sera.

Phagocytes are either of the macrophage—i.e., fixed—variety, consisting of endothelial cells, some connective tissue cells, and some cells of the spleen pulp and bone marrow, or of the motile variety, which includes most of the polynuclear and mononuclear leucocytes. When micro-organisms are introduced into the body the phagocytes, both fixed and motile, seize upon the intruders and by means of their pseudopodia absorb them into their interior and digest them. Where the phagocytes are naturally absent or scanty, as in the anterior chamber of the eye and cornea, the invading micro-organisms stimulate the animal's protective mechanism to hurry forward an army of defending phagocytes of the motile kind which give immediate battle to the intruders. This attraction of the phagocytes is due to what is known as positive chemiotaxis, and is most marked in those cases where the natural immunity is greatest—indeed, the greater the natural immunity of the organism to infection the greater is the positive chemiotaxis. When the bacteria have been taken up into the phagocyte they are either digested by some ferment action or destroyed by some chemical poison possibly related to nucleic acid, but this action is not immediate and the bacteria are not instantly killed. This is shown by injecting into an animal phagocytes containing bacteria, and it is found that by doing this we can induce infection in the susceptible animal. If the bacteria are very virulent and the phagocytes are placed under disadvantageous conditions the bacteria may get the better of the phagocytes and kill them, or, if spores have been taken up by the phagocyte, these may develop within the phagocyte into bacteria and destroy the phagocyte.

The influx of these white corpuscles, or phagocytes, to an infected area means that the organism is resisting the invading forces which threaten disease. The phagocytes are aided in their struggle by poisonous substances circulating in the fluids of the body, secreted possibly by the white corpuscles, which act upon the bacteria in such a way as to prepare them for digestion by the phagocytes. These bacteriotropic substances, or opsonins, make ready or prepare the bacteria for the further action of the phagocytes. The word "opsonin" is derived from *ὀψωνέω*, meaning "I cater for." That opsonins have this action is proved by the following experiment of Wright and Douglas. Two tubes are incubated for a quarter of an hour at 37° C., the one containing three volumes of normal serum *plus* one volume of staphylococcus emulsion (previously heated to 75° C. and cooled), the other containing three volumes of serum which had been heated to 60° C. *plus* one volume of staphylococcus emulsion (previously heated to 75° C. and cooled). On removal from the incubator the first tube (containing normal serum) is heated to 60° C. and cooled. The contents of each tube are then added to three volumes of white blood corpuscles and the number of staphylococci taken up per phagocyte is counted. It is found that the number of staphylococci taken up in the second case is only one-seventh as compared to that taken up in the first case: in other words, the opsonin acts directly on the bacterium.

The opsonic index of a patient to any one micro-organism is simply the expression of the relative power of the patient's opsonins to influence the taking up of the micro-organism by phagocytes, as compared to that of a healthy individual. For instance, 50 phagocytes can when incubated with a tuberculous patient's serum take up 100 tubercle bacilli, while 50 of the same strain of phagocytes when incubated with the serum of a healthy person take up 150 bacilli in the same time. The patient's opsonic index is then the ratio of 100 to 150, or $\frac{2}{3}$, or 0.6.

The actual estimation of the opsonic index can only be carried out in a laboratory, but all that the physician in charge of a case has to do is to fill a specially prepared tube with blood and forward it to the bacteriologist and in 24 hours he can be advised what the patient's opsonic index is and what dose of vaccine should be administered. The method of estimation is as follows:—

(a) Two specimens of blood are obtained (by pricking the finger and allowing the blood to flow into a glass tube), one from the patient and one from a healthy person. These

¹ In part a lecture delivered at University College, London, on Feb. 5th, 1907.

tubes are sealed up by placing the ends of the tubes in a flame and the blood is allowed to clot, forming a clear layer of serum.

(b) 0.5 cubic centimetre of blood is drawn from the finger into a tube containing 10 cubic centimetres of sterile 1.5 per cent. sodium citrate solution and shaken up. Sodium citrate combines with the calcium salts of the freshly shed blood and prevents clotting. The tube is placed in the centrifugal machine for five minutes until the corpuscles have sunk to the bottom and the fluid is pipetted off. 10 cubic centimetres of normal saline solution are placed in the tube and the tube is shaken to wash the corpuscles and then replaced in the centrifugal machine for five minutes. This is repeated twice again and the fluid above the corpuscles is finally removed. A layer of white corpuscles free from serum is now available.

(c) An emulsion of the bacterium in salt solution (1.5 per cent.) is made by pouring the latter over a 24 hours' growth on agar and shaking. The fluid is removed and centrifuged until the right density of emulsion is obtained.

The two specimens of serum, the washed phagocytes and the emulsion of bacteria, are now ready for use and are labelled and placed in a rack or vessel containing wet sand. Two sterile capillary pipettes fitted with rubber nipples are marked at a distance of one centimetre from the end with a blue pencil, and into each are drawn up the following fluids. Into the first, equal volumes of healthy serum, washed blood cells, and bacterial emulsion. Into the second, equal volumes of patient's serum, washed blood cells, and bacterial emulsion. The contents of each pipette are thoroughly mixed and drawn up again into the respective pipettes. The two pipettes are sealed at the capillary ends and both are placed in an incubator at blood heat for 15 minutes. Of course, if the sera of more patients have to be examined at the same time similar pipettes, each containing one patient's serum, washed blood cells, and bacterial emulsion, are placed in the incubator also for 15 minutes and examined against the control. At the end of 15 minutes the two pipettes are taken out of the incubator; a blood film is prepared from each, stained by Leishman's method, and examined under a $\frac{1}{2}$ -inch oil immersion lens, using a mechanical stage. The number of bacteria contained in 50 polymorphonuclear cells is counted in each case, and since normal serum is taken as having an opsonic index of unity, the number of bacteria contained in 50 cells on the patient's slide, divided by the number in 50 cells on the control slide, gives the opsonic index of the patient.

Fallacies may, of course, occur in the hands of inexperienced workers, especially if the tubes be incubated for different periods, or the serum be heated, or the films imperfectly prepared, or the volumes incorrectly measured. But by practice a wonderful degree of accuracy can be attained.

Having determined the patient's opsonic index we have now to see whether we can raise it, if low, by the employment of bacterial vaccines. It is well known that many living organisms produce, during their growth, substances inimical to themselves. For instance, yeast grown on grape sugar produces alcohol, and this alcohol finally stops the growth of the yeast. Many bacteria in the same way produce during their growth substances (antibodies) which are injurious to themselves. Tuberculin contains such antibodies and is hence employed in tuberculous lesions, but in the old days much too large doses were employed and there was no method of checking its effects. Now, very small doses are given and we can tell with great accuracy what the results of each dose are. Nevertheless, tuberculin treatment must still be combined with other methods, and only under such conditions can bacterial vaccines be expected to give the best results.

The employment of sterilised bacterial cultures in the treatment of disease is based essentially on the assumption that by this means we can increase the germicidal substances of the fluids of the body and bring about a more or less marked artificial immunity. It is well known that immunity to snake poison can be induced by giving repeated small non-fatal doses of the toxin. 20 years ago it was shown that pigeons could by this means be made immune to the poison of the rattlesnake. This acquired immunity is, of course, to be distinguished from both natural and inherited immunity and may be either active or passive. Active immunity is brought about in the body of the animal as a result of the stimulus produced by the injection of bacterial cultures; passive immunity is the result of directly injecting

substances which can combine with and neutralise toxic bodies in the infected organism. Thus, in the case of diphtheria, active immunity can be produced in the horse by the injection of gradually increased doses of diphtheria toxin until its serum contains a considerable quantity of diphtheria antitoxin. This serum when injected into a patient suffering from diphtheria can neutralise a certain proportion of the toxin by virtue of the antitoxin which it contains, and so prevent some of the pathological changes in the patient's cells which would otherwise be brought about by the diphtheria toxin. By the injection of such antitoxin passive immunity is said to be produced in the patient.

Natural immunity to poisonous substances is possessed in various degrees by different animals. Thus large quantities of antimony and arsenic can be consumed by pigs without ill-effects, and the vapour of formalin, which is, of course, a strong antiseptic, is not only without effect on certain insects but they actually appear to thrive on it. Typhoid fever, so fatal to man, cannot be induced in the lower animals by inoculation with the typhoid bacillus, nor does the disease occur naturally in them, any more than do measles, scarlet fever, and leprosy. On the other hand, certain species of animals are susceptible to anthrax, such as sheep and cattle, whereas many other of the lower animals are quite insusceptible to the disease. But although some animals are immune to certain diseases and cannot be inoculated with the micro-organisms which produce these diseases, they are, nevertheless, susceptible to doses of the toxin produced by these micro-organisms and injection of typhoid toxin is fatal to many of the lower animals which cannot contract the disease. The toxin of cholera is harmless to the larva of a certain beetle, and if the same dose of cholera toxin is given to one of these and also to a small frog of equal weight the latter dies while the former is unaffected. If, however, the two are inoculated with the bacillus of cholera the frog escapes but the beetle dies. It is evident that this may apparently be explained on the assumption that the leucocytes of the beetle are able to get the better of the cholera toxin but not of the bacilli, and *vice versa* in the case of the frog, but what is not so easy to explain is why the beetle is unaffected by the cholera toxin introduced from outside, while it succumbs to that manufactured in its own body.

The mode of entry is often of the greatest importance in the future development of an invading organism. Thus the organism of Asiatic cholera is extremely fatal when taken into the alimentary canal, although it is almost harmless when injected subcutaneously. Tetanus bacilli and anthrax bacilli, however, when injected subcutaneously are very fatal, but if taken into the healthy alimentary canal they are practically harmless. The bacillus coli is normally found in the human intestinal canal and under such conditions produces no ill-effects because its toxic material is locked up inside the cell, but if the bacillus escapes into some position where by phagocytic or other action its intracellular toxin is set free pathological effects are speedily produced. The mode of entry of the tubercle bacillus and the nature of the bacillus which can cause tuberculous lesions in the human being are subjects which have been dealt with at length by the recent Royal Commission, and we are only concerned here with methods which can arrest the progress of disease in an already infected organism.

An increase of the opsonic content of the blood in disease is brought about by the injection of the appropriate vaccine—e.g., of tuberculin in tuberculosis, of staphylococci in boils and acne, and of gonococci in gonorrhoea. When possible the micro-organism should be grown directly from the patient's lesion and a small quantity of the pure culture, whether staphylococcus, tubercle bacillus, or other organism, triturated with normal saline solution to which $\frac{1}{4}$ per cent. lysol is added. The mixture is heated to 60°C. for an hour in order to kill the bacteria and make the vaccine sterile. The number of bacteria in a given volume is now calculated by mixing one volume with an equal volume of blood and two volumes salt solution and counting the relative number of bacteria and red corpuscles. It is known that normal blood contains about 5,000,000 red cells per cubic millimetre and the number of bacteria per cubic millimetre is therefore readily calculated and a vaccine of the required strength is easily prepared by dilution. An initial dose of 300,000,000 staphylococci, or $\frac{1}{3500}$ milligramme of tuberculin, is given with a sterile hypodermic syringe subcutaneously. The injection should cause no rise of temperature and no general or appreciable local reaction. The amount of the dose given

at subsequent injections and the intervals between the doses must depend on the influence produced by the preceding dose on the opsonic index.

If the right dose has been given a short negative phase will be followed by a well-marked positive phase, during which the anti-bacterial power of the blood is increased and the resistance of the patient to the disease increased. After tubercle inoculations this improved power of resistance may be maintained for several weeks, but, as a rule, it falls back again after ten days or a fortnight, and a further dose of the vaccine is necessary. Only the minimal dose must be employed which will produce a satisfactory response and this must not be increased until it ceases to produce its effect. The dose, moreover, must not be repeated until the effect of the preceding dose is passing off. If it were possible to produce a summation of positive phases it would be advantageous to give successive doses at short intervals, so as to produce a maximal favourable effect, but such summation is not possible in the case of tubercle. Each inoculation, therefore, produces its effect independently and it remains to take the greatest advantage possible of the increased elaboration of protective substances which the artificial stimulus has produced.

The results of treatment of some dermatological cases have already appeared in a paper by Pernet and myself in the October, November, and December numbers for 1906 of the *British Journal of Dermatology*. Some cases were also included in a paper of mine in THE LANCET of Jan. 19th, 1907, p. 144. So many other successful cases have now been recorded that the employment of bacterial vaccines must in the future prove of the greatest service in the treatment of disease when controlled by the method which Wright and Douglas have so ably elaborated. In conclusion, I have much pleasure in thanking Professor Sidney H. C. Martin for his kindness, encouragement, and advice.

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A NOTE ON TESTS FOR ACETONE IN THE URINE.

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A FEW words on the tests for acetone in the urine may not be out of place at the present juncture when the conditions of recurrent vomiting with acetonuria are attracting so much attention. The tests ordinarily employed are: (1) the iodoform test; and (2) the sodium-nitroprusside test. The first mentioned is simple and, I believe, yields absolute evidence (with certain exceptions) together with microscopical examination of the iodoform crystals. The smell of iodoform produced when performing this test is unreliable, as a similar smell to that of iodoform is produced when iodine and alkaline solutions are added to urine irrespectively of the formation of iodoform. If acetone is present in small quantities distillation of the urine is advisable.

The sodium-nitroprusside test, which consists in the darkening which takes place on the addition of acetic acid to a solution of sodium-nitroprusside, urine, and liquor potassæ, is, I think, rendered more simple, delicate, and accurate by a slight modification—namely, the substitution of strong ammonia for the liquor potassæ and the elimination of the use of acetic acid. In the ordinary test when the solution of potash is employed there is a uniform reddening of the whole solution which on the addition of acetic acid in the presence of acetone becomes darker when the acid comes in contact with the urine, the darkening passing down as the acid sinks. Therefore, with this test the addition of liquor potassæ to the urine and sodium-nitroprusside yields a red colouration whether acetone is present or not, and it is only after the final addition of acetic acid that the presence of acetone is recognised and then only when there is a fair amount of the latter present. The detection of the presence of acetone is therefore dependent on the darkening of an already darkish red fluid by the final reagent acetic acid, and the degree of darkening, when acetone is present only in small quantities, is not altogether easy to determine. If, however, strong ammonia replaces the potash solution the test for acetone is as follows. On adding the ammonia to a solution of sodium-nitroprusside and urine the ammonia solution remains on the

top; thus there is a clear solution uppermost with the urine solution below. Should acetone be present, even in minute quantity, a well-marked and absolutely characteristic ring of magenta (or petunia) appears within from one to three minutes at the junction of the two fluids and gradually *spreads upwards* pervading the whole of the ammonia solution if acetone is present in considerable amount. In the absence of acetone usually no colouration occurs, but sometimes an orange-red ring forms at the junction of the urine and ammonia solution, but this bears no resemblance to the "acetone ring" described above, hence the presence or absence of the latter is easily ascertained by the method which I believe has not been generally recognised.

The strength of the solution of sodium-nitroprusside is not important, but it is important that it should be prepared fresh and a few crystals dissolved in a test-tube of water are sufficient.

Clinical Notes:

MEDICAL, SURGICAL, OBSTETRICAL, AND THERAPEUTICAL.

A CASE OF RUPTURE OF A VERY EARLY EXTRA-UTERINE GESTATION; LAPAROTOMY; RECOVERY.

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THE following case is somewhat unusual owing to the early age of the extra-uterine conception and the entire absence of signs and symptoms preceding the rupture.

On Oct. 28th last I was asked by Dr. M. Aston Key of Southsea to see a young married woman in consultation who was presenting signs of some severe abdominal lesion. The patient was a healthy young woman, aged 23 years, who was married as recently as August 29th, 1906. Shortly after this date she had a regular menstruation period lasting for one week; at the end of September another regular period took place and on Oct. 22nd she again began to menstruate. This last period was rather shorter than usual and ended on the 26th. Otherwise she was in excellent health and attended a concert on the 27th. There had been no history of dyspepsia, hæmatemesis, or melæna. Early in the morning of the 28th on going downstairs she felt very faint and experienced severe pain in the hypogastrium rather to the right side; she revived and fainted again and this recurred several times during the morning. Vomiting occurred once in the afternoon and showed no peculiar characteristics. As she was getting much worse her medical attendant was summoned. When I saw the patient about 6.30 P.M. she was lying on her left side, moaning and in a very collapsed condition. Her skin was blanched; the pulse at the wrist was only feeble, flickering, and uncountable; the pupils were dilated; the abdomen was tumid, not distended, and below the umbilicus it was dull to percussion. She was anaesthetised with ether and an incision was made to the right of the linea alba through the right rectus abdominis muscle below the umbilicus. On opening the peritoneal cavity about two quarts of dark blood and clots escaped. The right Fallopian tube and ovary were normal, but in the region of the isthmus of the left tube there was a small swelling of the size of a hazel nut, from a small opening in which was presenting a small bunch of chorionic villi, which came away during handling and was unfortunately mislaid. The left Fallopian tube was then removed, considerable difficulty being experienced in securing all bleeding points on the uterine wall, some of which had to be included in passing the ligatures. The left ovary was normal. The lower half of the peritoneal cavity was then cleansed with absorbent pads and the wound was closed by peritoneal, muscular, and cutaneous sutures. The patient was in a profound state of collapse and so two pints of saline solution were infused into the right arm intravenously. She rallied soon after the operation, however, and made an uneventful recovery, being able to get up in three weeks' time.

The situation of the pregnancy in the isthmus of the Fallopian tube undoubtedly led to its early rupture. The