7. The volatile oils have no direct insecticidal effect. In a moist vapour of oil of wintergreen, oil of cloves, oil of caraw y, oil of turpentine, oil of eucalyptns, oil of thyme, etc., lice live for many hours at body temperature, and can be revived after immersion in these oils.

8. Over solid substances, such as iodoform, camphor, and paraform, and in contact with them, and in contact with garments impregnated with sulphur, borax, black hellebore, alum, etc., lice appear to remain practically unaffected.

9. The hungry louse feeds on the human body previously anointed with sulphur ointment, balsam of Peru, mercury oleate ointment, chrysarobin ointment, staves-acre ointment, and hellebore ointment. The louse certainly prefers the clean body, but it can feed on the body thus anointed and thereafter survive.

10. It has still to be determined whether some of these bodies that have been shown not to be actively insecticidal may not have, when rubbed on the body or placed in clothing, a useful repellent effect on body vermin.

PRACTICAL APPLICATION.

For practical purposes it has been found that destruction of lice and nits is best secured by immersion of verminous garments and bedclothes in a petrol or benzene bath. Danger from fire and waste of petrol are avoided by using such a bath and extractor as are employed in a dry-cleaning apparatus. In such an apparatus 90 per cent. of the petrol or benzene is recovered for future use. A petrol or benzene bath is necessary, especially for uniforms and woollen garments generally. Where the clothing is such that it is not injured by immersion in water, steeping the garments for half an hour at 12° C. (54° F.) in a soap solution containing 2 per cent. of trichlorethylene or 10 per cent. of tetrachlorethane secures destruction of lice and nits. It is only fair to say that the only soap solutions that I have so far experimented with are those sold as "Westropol" and "Westoran." Steeping for half an hour in a 5 per cent. solution of cyllin in water maintained at 65° C. (149° F.) is also effective, and this temperature has no injurious shrinkage effect on woollen articles.

For economical reasons the chlorine derivatives of ethane and ethylene cannot at present be used in a dry cleaning process, but their soap preparations are of value. Petrol has a wide application and is readily obtained.

For cleansing the body itself bathing or sponging with soap solutions containing 2 per cent. of trichlorethylene or 10 per cent. tetrachlorethane gives the best results.

In view of the known insecticidal action of these chlorine derivatives of ethylene and ethane it is probable that good results would be obtained by shampooing verminous heads with their soap preparations, and it is also probable that a 25 per cent. solution of trichlorethylene in vaseline would form an efficient insecticidal pomade.

It is almost certain that lice would not continue to live on the human body if anointed daily with a 25 per cent. solution of trichlorethylene in vaseline, or on the body anointed twice daily with a solution of petrol in vaseline of similar strength. The odour of such an ointment is not unpleasant. But living under verminous conditions constant precautions would have to be taken and every method of destroying vermin would require to be employed.

Means for the destruction of lice are available. Any attempt to render an army free from vermin in war time would require that all men occupying the same quarters at the same time, or for alternating short periods of time, would be regarded as a single unit for which a receiving station with cleansing apparatus would be provided. Such an attempt would also require that the movements of the men off duty were controlled. It would be limited by immediate military necessities.

There is reason to believe that vermin are responsible for the transmission of the infection of typhus fever,[3] and Nicolle[4] has shown that the louse can convey the infection. Epidemics of typhus fever come and go, and the amount of any epidemic will be influenced by the verminous conditions prevailing. In dealing with typhus patients vermin must first be destroyed by some of the methods above described. Ambulance men and receiving nurses are to be similarly protected, or, if available, a staff of ambulance men and receiving nurses already immune by a previous attack of the disease are to be employed.

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THE WASSERMANN TEST:

A METHOD NOT NECESSITATING THE USE OF GUINEA-PIGS AS THE SOURCE OF COMPLEMENT.

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WHEN the writer came first face to face with the necessity of doing Wassermann tests two main difficulties appeared. First, it was found well-nigh impossible to learn the technique in default of personal instruction, and in the light of experience this appears to have been due to omission of details in many of the published descriptions, making it impossible to sit down and perform the test from the description. The second difficulty was the matter of extra space required and extra work entailed in housing and feeding guinea-pigs for the supply of complement-containing serum.

It is thought that these two hindrances, especially the latter, must be a source of trouble to workers in some other laboratories, possibly at the present time to some of those attached to military hospitals at home and abroad.

The first — and obvious — solution was to practise Fleming's modification, which is at once short, simple, and avoids the necessity of keeping guinea-pigs. But without entering into the discussion of the value of this "modification," one may perhaps say there are several valid reasons, well supported by recognized authorities, for preferring a method which includes the main principles of the original Wassermann method as now practised. These principles are:

1. Deviation of complement by syphilitic serums in the presence of antigen.
2. Inactivation of serums to be tested (and controls).
3. Use of added haemolytic amboceptor and complement in previously determined and sufficient quantities, the same always for every tube and every batch of tests.

The last two are not included in Fleming's test.

It is claimed for the technique described below that—

1. It does include the three main principles of the Wassermann test as now practised, and is, in fact, a Wassermann test as opposed to a modification.
2. It avoids the use of guinea-pigs.
3. Comparatively small amounts of blood and materials are required, which means economy.
4. The time required is shortened to about 1½ hours.
5. The technique is efficient, and as simple as it can be made, having full regard to efficiency, and has stood the test in practice during the past two years.

The most important of the points in which the technique differs from the original method is the use of fresh normal human serum for the supply of complement, in place of guinea-pig's serum, the amount of the complement present being titrated for each batch of tests and 2 units (minimum haemolytic doses = m.h.d.) added to each tube, the serums tested having been inactivated. It is found in practice that serum obtained regularly from the same person will usually contain the same amount of complement, probably 3 to 6 units in a volume. This amount appears to vary more on account of the greater or less ease with which different samples of sheep's corpuscles will haemolyze, rather than in different samples of serum from the same person tested against one sample of sheep's corpuscles. So far as the writer is aware, human complement is used for the Wassermann test in four "modifications" only, namely, those described by Stern, Hecht, Fleming, and d'Este Emery; but in none of these is the amount estimated or regulated in any way, that present in each unheated serum to be tested being used; no attempt is made to have the same number of units for each serum to deviate. An objection may be urged on the ground that haemolytic amboceptor v. sheep's corpuscles is present in uncertain (and varying) amount in most human serums, and

that this addition may tend to deviate complement and vitiate the results. If this is considered a serious objection, the amboceptor can be easily absorbed by mixing up washed sheep's corpuscles with the serum and allowing it to stand at 0° C. in the ice-chest for one hour, after which the mixture is centrifugalized and the clear serum—now containing complement but no amboceptor—pipetted off. The writer prepared complement by this method for several months of experimental work, till a series of comparative tests appeared to show that this was an unnecessary refinement.

The second point of difference is the use of small volumes, as recommended by Fleming and d'Este Emery, the total bulk used being 120 c.mm. in small test tubes as described by Fleming. It is convenient, but not absolutely necessary, to use measured volumes, so long as all volumes used in one batch of tests are the same. The advantages are economy of materials and simplification of technique.

The third point of difference is the use of a water-bath for incubating the tests in place of the hot-air incubator. This method was recommended by d'Este Emery, and has the great advantage of reducing the time for incubation to one-third, and that for the whole test to about one and a half hours.

The description of the actual technique will be given under four headings: Preparation of apparatus and materials, standardization of materials, the actual test, some possible difficulties.

A. *Preparation of Apparatus and Materials.*

1. Prepare and sterilize about 1 litre of 0.85 per cent. sodium chloride (pure) in distilled water. This will keep fit for use for several weeks if kept sterile, and is to be used for all dilutions.

2. Antigen. Fresh sheep's heart, 5 grams, is weighed out carefully, minced small with a sharp knife, placed in a clean mortar (a large one is best) and pounded and pressed vigorously for several minutes, after which about 1 c.cm. of absolute alcohol is added and the pounding repeated, with the occasional addition of a further 1 c.cm. of alcohol until about 10 c.cm. have been added. About this period the muscle should appear finely divided, and alcohol may then be added up to a total of 45 c.cm. It is all-important to add the alcohol in very small quantities at first, till the muscle is finely divided. The whole is now poured into a sterile bottle and shaken up occasionally during the next hour, and then allowed to stand till morning, when it is filtered through a coarse filter paper (not best Swedish) into a sterile, well-stoppered bottle. This is the antigen or extract, and will keep fit for use for several weeks at room temperature, though some workers recommend making a fresh extract once each week. The formation of much precipitate is possibly an indication to make a fresh sample.

3. Sheep's blood must be secured fresh once each week from the slaughter-house. It is collected into a clean bottle, defibrinated by shaking with bits of glass-rod or beads, and kept in an ice-chest till wanted for use.

4. Rabbit's immune serum v. sheep's corpuscles (haemolytic amboceptor) can be obtained from most large hospital laboratories. The writer obtains his ready titrated from the Lister Institute of Preventive Medicine. When kept sterile, this remains efficient for several weeks.

5. *Serums.*—A syphilitic (positive control) and a normal blood (negative control) should be drawn into Wright's capsules, the serum drawn off into a small capsule, sealed and inactivated for twenty minutes at 56° to 58° C. in a water-bath. Patients' serums are treated in the same way.

6. *Apparatus.*—Any water bath may be used that can be kept at 36° to 38° C. A convenient and cheap form is an ordinary enamelled bowl (8½d.), holding about 4 pints, supported on an iron tripod over a Bunsen flame. Wire or glass tubing is bent to form a carrier for the plasticene tray in which the small test tubes are to be embedded, and a thermometer is fixed against the inside by a lump of plasticene. The level of the water has to be arranged to cover the fluid in the tubes, but not to flow over the tops, and the temperature is regulated from the Bunsen flame. Small test tubes are made from glass tubing of ¼ cm. bore by drawing this out in the gas-blower flame at 5 cm. lengths (2 in.), and sealing off. For use a nick is made in the middle with a glass-cutting knife (or file), and the tube broken across; we have thus two small test tubes. A stock of these should be made and kept ready. All mixing work is done with the rubber teat and Wright's opsonic pipettes. A number of these must be made with a rather wide bore capillary tube, and from these two types are to be fashioned. The "diluting pipette," for diluting extracts and suspensions of sheep's corpuscles, is made by marking off 10 equal volumes on the capillary stem by aid of a volume of mercury of 10 c.mm. (or about) and a glass-writing pencil. The "test pipette" is made by graduating 4 equal volumes of 20 c.mm. in the same way; the capillary stem is then bent at almost a right angle in the peep flame. A number of each type should be made at a sitting, and the marks made permanent by burning carefully in a Bunsen flame. The writer uses "automatic pipettes" for the graduations to ensure equal volumes on all pipettes of the same type. This is convenient in case of breakage during a test.

7. *Complement.*—Known normal human blood is drawn overnight from a clean finger into a glass capsule, the body of which is 5 to 6 cm. long and about ¼ cm. bore. The serum will be ready for use on the following day, when it should be drawn off into another capsule free of blood corpuscles. In drawing samples of blood, a band is wound twice round just behind the distal joint, and a good sharp prick made with a needle. A slight prick necessitates much pressure, and the serum will then usually appear haemolyzed. A slightly larger quantity of serum will be obtained if the capsule, when sealed, is put into the air-incubator for one to two hours. As mentioned above, the haemolytic amboceptor may be absorbed from the serum before use, if so desired.

B. *Standardization of Materials.*

1. Sheep's corpuscles must be "washed" fresh each day tests are performed; ½ to 1 c.cm. are shaken up with 10 to 15 c.cm. of the 0.85 per cent. saline, centrifugalized, the supernatant fluid pipetted off, and the process repeated four times. After standing for at least ten minutes, the last drops of saline are pipetted off to ensure a standard suspension, since all units in the test depend on this suspension—this is the starting-point in standardization. With a teat and diluting pipette, 94 volumes of saline, 1 volume of haemolytic serum (or amount to give 5 to 10 m.h.d. to each 2 volumes of suspension), and 5 volumes of the washed corpuscles are filled into a 1 c.cm. vaccine bulb. Mix thoroughly, and the "5 per cent. sensitized suspension of sheep's corpuscles" is ready for use.

2. *Complement.*—Set up four small test tubes in a row on a plasticene tray (a lid of a tobacco tin filled in with plasticene). With a teat and test pipette take up 4 volumes of saline and run out 1 volume into each tube. Take up 1 volume of unheated normal human serum, mix into tube 1, carry 1 volume over to tube 2 and mix, finally discarding 1 volume from tube 4; this gives four dilutions of the serum: 1-2, 1-4, 1-8, and 1-16. To each add 3 volumes of saline and 2 volumes of corpuscle suspension and incubate in the water-bath for twenty minutes. The last tube from left to right giving complete haemolysis contains 1 unit of complement—for example, tube 2 gives complete haemolysis, and therefore serum diluted 1-4 contains one unit, and two units will be contained in 1 volume of serum diluted 1-2. The haemolytic serum is titrated in a similar manner, but unsensitized 5 per cent. suspension of corpuscles must be used, and only one unit of complement added to each tube. Total bulk is always to be made up with saline to 4 volumes without corpuscles, and 6 volumes when corpuscles have been added. The result is usually obtained with a dilution of 1 in 1,000 or 1 in 2,000 of the serum.

3. *Antigen* (extract of sheep's heart).—Put eight 1 c.cm. bulbs in a row on a plasticene tray. From left to right fill in 95, 90, 85, 80, 75, 70, 60, and 50 volumes of saline, and make each up to 100 volumes by adding extract in the following manner: Take out from the left hand bulb about 20 volumes into a diluting pipette, leave an air bubble, and take up 5 volumes of extract; then expel into bulb and mix up and down rapidly. The emulsion should be as little opalescent as it can be made. In the stronger dilutions mix in the extract 5 or 10 volumes at a time. The method of making these dilutions is very important. We now have dilutions of extract of 5, 10, 15, 20, 25, 30, 40, and 50 per cent.

Now place nine small test tubes in a row on another plasticene tray. With a teat and test pipette fill in the left tube with 3 volumes of saline and 1 volume of appropriately diluted normal serum containing two units of complement. The remaining eight tubes are filled in with 2 volumes of the dilutions of extract, in order from left to right, 1 volume of saline, and 1 volume of complement. All ingredients must be filled into tube 2, then tube 3, and so on. Incubate in water-bath for twenty minutes, add 2 volumes of 5 per cent. sensitized corpuscle suspension, mix, and incubate a further twenty minutes.

The left-hand tube is a control, and should show complete haemolysis. The rest are examined from left to right until the tube is found giving complete inhibition of haemolysis—that is, a precipitate of corpuscles and a colourless supernatant fluid. One-third of the strength of extract in this tube is the strength to be used for the tests—for example, if 25 per cent. extract completely inhibits haemolysis, 8 per cent. is the strength to use in the tests. This 8 per cent. dilution is made fresh for each batch of tests from the stock undiluted extract.

C. *The Actual Test.*

The materials required having been prepared and standardized, the following procedure is convenient:

1. Prepare the 5 per cent. sensitized suspension of sheep's corpuscles.
2. Titrate complement and incubate.
3. Dilute extract to required strength for use.
4. Examine result of complement titration and dilute so that each volume contains 2 units of complement.

Place in a plasticene tray three rows of two small test-tubes for controls, and one row for each patient's blood to be tested. Put an extra tube to each row to dilute serums in, and as each serum is tested dilute it 2 volumes to 3 volumes of saline (2–5 dilution). Between filling in each row the pipette must be rinsed with distilled water and dried in a cool Bunsen flame and allowed to cool. The tubes are filled in as shown in Table I.

TABLE I.

	Left Tube.	*Right Tube.*
1st pair: No serum.	4 vols. saline.	2 vols. saline 2 vols. extract
2nd pair: Normal serum; negative control.	2 vols. saline 1 vol. heated serum (2–5 dilution) 1 vol. complement (2 units).	2 vols. extract 1 vol. heated serum (2–5 dilution) 1 vol. complement (2 units).
3rd pair: Syphilitic serum; positive control.	2 vols. saline 1 vol. heated syphilitic serum (2–5) 1 vol. complement (2 units).	2 vols. extract 1 vol. heated syphilitic serum (2–5) 1 vol. complement (2 units).
4th pair: First patient's serum.	Ditto, using first patient's serum heated and diluted (2–5).	Ditto, using first patient's serum heated and diluted (2–5).

Incubate in the water-bath for twenty minutes, add to each tube two volumes of the 5 per cent. sensitized suspension of sheep's corpuscles, mixing well first the right and then the left tube of each pair (to avoid carrying over complement), and just rinsing the test pipette with saline between each pair of tubes, but not drying it in the flame. Incubate a further twenty minutes, and read off the results as soon as the supernatant fluid can be seen in the tubes with no haemolysis. The result should be as in Table II.

TABLE II.

	Left Tube.	*Right Tube.*
1st pair ...	No haemolysis	No haemolysis.
2nd pair ...	Complete haemolysis	Complete haemolysis.
3rd pair ...	Complete haemolysis	No haemolysis.
4th pair (patient)	Complete haemolysis	Haemolysis complete="negative." Haemolysis nil="positive."

The method may be made quantitative by adding four extra tubes to each "patient's" pair and "titrating" one volume of the serum (2–5) through these four extra tubes as for titration of complement, and adding extract and complement as for the other right hand tubes.

In explanation of the number of volumes taken, it may be added that the calculations have been made so that the total bulk of six volumes, with corpuscles, corresponds to a total of 1½ c.cm. in the larger techniques, each volume being the equivalent of ¼ c.cm. The dilution of serums to be tested and the quantities are the equivalent of 0.1 c.cm. used in the ordinary techniques, and in the quantitative method the amounts of serum in the tubes containing extract are equivalent, from left to right, of 0.1, 0.05, 0.02, 0.01, and 0.006 c.cm. of the heated serum.

D. *Some Occasional Difficulties.*

The most common cause of difficulty is to be found in the sheep's corpuscles. Occasionally a sample will either not haemolyze at all, or only in very slight degree. Some few samples may haemolyze too readily, especially if not kept in the ice-chest, or if used when too old. The only remedy, if two extra washings make no difference, is to obtain a fresh sample. Serums when septic may lead to complete inhibition of haemolysis in both tubes. Frequently the serums will react satisfactorily if centrifugalized and then reheated for an hour at 56° to 58° C. If this has no effect, a fresh sample must be obtained. An extract inefficiently pounded, or carelessly diluted for use, is another source of trouble.

It will, however, seldom be necessary to repeat the tests owing to a "bad result" if the directions given above are carefully noted and followed, and if each step is carefully performed before going on to the next. The "no serum," "negative," and "positive" controls will show immediately if anything is wrong; if these controls are in order, the result shown in the "patient's" tubes may be trusted implicitly.

The Wassermann is an extremely important test, and the results given far-reaching. No stage of the working should ever be left to an unskilled worker, or undertaken otherwise than seriously.

ARTERIO-VENOUS ANEURYSM OF FEMORAL ARTERY SUCCESSFULLY TREATED BY OPERATION.

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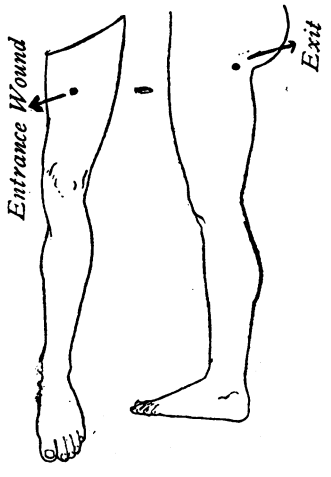
THE following case of arterio-venous aneurysm of the left femoral artery, due to bullet wound, came under my care at the Queen Alexandra Military Hospital, Grosvenor Road, London, and I report it as there are very few cases on record of successful operation for arterio-venous aneurysm in this particular region. Sir William Osler urged me to publish the case as statistics are required to aid military surgeons in the choice of operations for this lesion.



Private G. (cavalry), aged 25, received a bullet wound through the left thigh at Zellebeke on November 15th, 1914. He was sent to the Duchess of Westminster's Hospital in France for fourteen days, and then to the South Devon and Cornwall Hospital, Plymouth, for another fourteen days. He went home for twenty-eight days, and was then attached to another cavalry regiment. He was put to work in the cook-house. He felt all right and had no pain in the thigh until April 15th, 1915, when he was put on mounted parade for six hours. The action of riding brought on pain, and an arterio-venous aneurysm formed at once. The bullet evidently injured the femoral artery and vein at about the level of 10 in. above the knee-cap.