RECENT ADVANCES IN THE TREATMENT OF GONORRHOEA.

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It is universally realized that gonorrhoea presents a problem which medical science has hitherto failed to solve. The authors of this article do not claim to have produced a certain and rapid cure, but they do claim that research work at Woolwich during the past three or four years has opened up an entirely new field for investigation, and may go far towards revolutionizing vaccine treatment as a whole.

To the brilliant brain of Major Lyn Dimond, R.A.M.C. (T.) is due the whole credit for the inception of the theory, and for the bacteriological and biochemical side of the work.

The history of these discoveries begins with the failure of kataphoresis as a treatment for gonorrhoea.

Major Dimond had concentrated on this method and, when it was found that, although theoretically sound, practically it was unworkable, he concentrated on the metabolism of the gonococcus and its biochemical reactions. As a result of his researches in this direction he evolved the theories set forth below and, further, carried them into practice.

Major E. C. Lambkin, who at first carried out the practical work on cases of gonorrhoea in conjunction with Major Dimond, published two papers—one in the JOURNAL OF THE ROYAL ARMY MEDICAL CORPS in March, 1925, and another in the British Journal of Venereal Diseases in January, 1926, and also read a paper before the War Section of the Royal Society of Medicine in the summer of 1926. Otherwise nothing further has been made public. The present authors having tested the treatment in 1,018 cases of gonorrhoea for a period of over two years, consider they are justified in reporting their results in order that others may work on the same lines and confirm or reject the conclusions come to.

Work has largely, and of necessity, been done chiefly with the gonococcus, but vaccines have been prepared by this method from other organisms and have been used with most excellent results. Notable amongst these are: Pneumococcus, of all types, tubercle bacillus, streptococci, staphylococci, M. catarrhalis, B. coryza segmentosa, etc.

The original aim was to produce a vaccine which, whilst free from toxin, was at a maximum in antigenic power. It was known that heat
destroyed toxin, but at the same time destroyed antigen to a somewhat less degree. It was realized early that the best way to overcome this difficulty would probably be to prepare a vaccine which was entirely free from organisms—a solution of antigen alone—this is in fact what was actually accomplished.

Work was commenced with exhaustive and extensive considerations of the biochemistry and metabolism of the gonococcus, and it was found that what was applicable to this organism was equally so to others.

It is a well-known and established fact that all organisms have a minimum, maximum and optimum temperature for growth, but Major Dimond went further; his researches forced him to the conclusion that there was also an optimum alkalinity of the medium. The optimum for the gonococcus corresponds to a pH of 7.2. When grown on a medium suitably buffered to remain at this pH, the gonococcus is in its vegetative form, it is luxuriant in growth and very virulent, but is most vulnerable to destruction either by chemical means or phagocytosis.

If the medium, either in vivo or in vitro, is allowed to become acid, say to a pH of 6.8, the organism tends to lose its kidney-shaped diplococcal form, divides in all planes and forms morulae of 4, 8, 16, 32, etc. The gonococcus also becomes more basophilic in its staining reactions and, what is much more important, forms round itself a mucin envelope. This envelope consists of a scleroprotein, is chemically a glucosamine, and is identical with the chitin of lobster and crab shells; it is insoluble in anything but the strongest mineral acids. In this form the gonococcus is virtually encysted, and although inactive is very difficult to destroy, either by heat, chemicals or phagocytosis. This would largely account for the chronicity of the disease, and for the fact that a case may be infective even after apparent cure.

On the other hand if the medium is allowed to become alkaline to a pH of 8 the gonococcus begins to swell and large forms appear; this would probably account for the occurrence of the so-called “giant gonococcus,” previously considered to be a separate entity. In an alkaline medium also the organism commences to autolyse, thus releasing its endotoxin and accounting for the toxic symptoms which are liable to occur during the course of the disease.

A word here may not be out of place regarding the endotoxin. This substance would appear to be an end-product of metabolism—an excretion, in fact. Biochemically it is a deutero-albumose, the last phase in protein metabolism, before breaking down into purin bases and amino-acids.

As a result of early research, the theory was evolved that the bacterial antigen was a histone, or, if not actually a histone, was very closely allied to it. Body immunity was considered largely to depend on the reticulo-endothelial system. It is believed that the large macrophage cell is the true phagocyte of the body and that immunity is largely cellular rather than chemical.
Recent Advances in the Treatment of Gonorrhoea

It was discovered that when the gonococcus, or for the matter of that, any other organism, was grown on a medium rich in nucleo-protein, it tended to produce polar bodies which were loosely attached to the organism; these bodies stain black with Neisser's stain, and are easily distinguishable. They were found to consist of equal parts of α-nucleo-protein and β-nucleo-histone, the latter portion being soluble in 2 per cent salt solution.

Whilst working with this special medium it was also discovered that the histone present in the medium united with the gonococcus protein and fixed it in an insoluble form at the meta-protein and primary albumose stage, thus preventing the formation of the deutero-albumose or endotoxin.

The question now arises, how are these facts to be made use of clinically? It would appear that the first thing would be to bring the patient's body fluids to a pH of 7.2, and keep them there so as to cause the gonococcus to be most vulnerable. It is interesting to note also that at a pH of 7.2 the body is at its optimum to withstand infection, phagocytosis being at a maximum; on the acid side phagocytosis is at a minimum.

As an index of the alkalinity of the body fluids urine tests were employed and it was found that the average in a series of untreated gonorrhoea cases was pH 6.8. In an attempt to alkalize the patients a number of salts were tried. Any alkali containing CO₂ is contra-indicated as this is utilized by the gonococcus in its metabolism and in the formation of the mucin envelope—a condition to be avoided at all costs. Finally the alkaline sodium phosphate was chosen and the authors see no reason for changing it.

There are three phosphates of sodium—the basic salt or trisodium phosphate which is rarely, if ever, used in medicine; the acid salt or sodium dihydrogen phosphate, commonly used as a diuretic and, in conjunction with urotropin, as a urinary antiseptic; and the alkaline salt or disodium hydrogen phosphate. It is the latter salt which is used.

Large doses are required and as a routine half an ounce is given four times a day; this dose is just soluble in six ounces of water. No ill effects resulting from these large doses have been noted with the exception that at first a few patients complained of diarrhoea; the addition of ten minims of tr. hyoscyamus to the dose was found to be sufficient to prevent this complication.

On these doses—two ounces per diem—the average man's urine remains at a pH of 7.2. Individuals differ and some require slightly more or less; a check is kept by roughly estimating the urinary pH every morning; 0.2 per cent phenol red is used for this purpose.

In a normal individual, the mineral content (chiefly calcium) of the body is kept at a more or less constant level and any excess is excreted chiefly by the kidneys. Calcium is required in body metabolism and is closely bound up with the vitamins and with immunity. A simple test as to calcium sufficiency is by urine examination.

It was found that patients on large doses of alkaline sodium phosphate failed to excrete calcium in the urine, and the natural supposition was that
they were being "demineralized" by excretion of calcium and magnesium, etc., salts through the bowel. It was therefore apparent that these salts must be replaced. This can be accomplished in a cheap and efficient manner by saving all the water in which the vegetables for hospital diets are boiled and making it up into a palatable soup with marmite or stock. Patients like this and, given twice a day, it supplies all the mineral salts in the correct proportion required by the body.

Having brought the patients' urine to a pH of 7.2 it now remains to deal with the infecting organism. This can be accomplished either directly by local application of chemicals or indirectly by stimulating the tissues to destroy them.

The urethral mucous membrane is a very delicate structure and the gonococcus buries itself very deeply in it. It naturally follows that no chemical can destroy the organism without also destroying the mucous membrane; this is not only useless but harmful. The authors have formed the opinion that the majority of irrigating fluids are used in too great strength and further that a large proportion of cases of posterior urethritis are due to the administration of anterior irrigations only. In treatment in this clinic nothing stronger than \( \frac{1}{3000} \) potassium permanganate is used and posterior irrigations are insisted on. The irrigations are merely used as a mechanical means of cleansing the urethra by clearing out discharges. By this method it has been found possible to commence irrigations on the first day in the most acute cases, with excellent results.

So far nothing has been said about the actual destruction of the gonococcus; the procedure described above has merely been that necessary to place the body in an optimum position to deal with the infecting organism and it only remains to describe the steps taken to destroy it.

The culture media used—described in detail in an appendix at the end of this paper—were found to be of exceptional value. The growth of all organisms, even the tubercle bacillus, is extraordinarily prolific and rapid. As an instance it may be stated that the gonococcus has been kept alive without subculture for eighteen months and two years—a statement which may appear incredible to those workers who know the difficulty of growing the organism at all. Moreover all vaccines at present used are prepared from two strains which were isolated in the early days and are still growing strongly.

The routine preparation of the vaccine is as follows: Large 6 X \( \frac{3}{4} \) inch tubes of the nucleo-protein medium are inoculated with gonococcal culture and are incubated for twenty-four hours. In order to ensure uniform growth, the same sized tubes, sloped to the same angle, and a standard inoculating loop are used. After twenty-four hours, each tube is washed off with 1.5 cubic centimetres of 2 per cent saline carbolized with 0.5 per cent phenol—a separate pipette is used for the addition of the saline and for removal of the emulsion from the tubes, thus ensuring that the stock carbolized saline is not contaminated with gonococcal bodies which might autolyse on standing and release their endotoxin.
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The emulsion is put up in vaccine bottles in quantities of twenty-five cubic centimetres. Periodical counts show the average content to be $7,000 \times 10^6$ per cubic centimetre. These bottles are put, with the least possible delay, in a high speed centrifugæ giving 9,000 revolutions a minute and are "swung" for about four minutes. After centrifugalization it will be noted that the contents have separated into three layers, a lower greyish layer consisting of the bodies of the gonococci, a middle cream-coloured layer of the $\alpha$-nucleo-protein element of the polar bodies and a clear supernatant fluid which is a saturated solution of the $\beta$-nucleo-histone in a two per cent carbolized saline. This clear fluid is pipetted off and put in vaccine bottles ready for use. It is not heated. The solid portions might be removed by sedimentation, but high-speed centrifugalization is resorted to because it has been found that the shorter the period the diluting fluid is left in contact with the organism, the less likely is autolysis to take place. Vaccine prepared in this manner will keep well for considerable periods and under varying conditions of temperature, still retaining its antigenic power. The dosage is two cubic centimetres and the optimum interval between doses is six days.

The method of administration is of some interest. We believe that body immunity depends largely on the stimulation of the lymphatic system and that, if a vaccine be given subcutaneously, intramuscularly or intravenously, a large proportion will be excreted before it has been able to exert its antigenic power. Moreover there is a definite danger of thrombosis when saturated solutions of nucleo-proteins are introduced into the blood-stream direct.

For these reasons the vaccines are given intradermally. A dose of two cubic centimetres is, however, too big to administer by this route in one site, it is, therefore, distributed over seven areas as follows: inner and outer wall of the axilla on each side, inner side of both thighs and into the skin at the base of the penis. Provided a fine, sharp needle is used no difficulty is experienced and very little pain felt. The results of the vaccine are a slight local reaction and enlargement of lymphatic glands.

It is interesting to note that large macrophage cells increase in the urethral discharge and differential blood-counts show an increase in the large hyaline cells from a normal of 4 per cent to 40 per cent or 50 per cent as a result of these inoculations.

During the course of the disease no instrumental interference is resorted to unless symptoms appear to warrant it. In fact the majority of primary cases are not touched until before being discharged from hospital, when they are examined by the urethroscope or straight sound and by prostatic massage.

Under this treatment it has been found quite unnecessary to keep patients in bed on milk diet, and, unless complications are present, they are all treated as "up" patients and put on ordinary diet.

To summarize shortly the routine treatment:

On admission to hospital a smear is taken and if positive the following procedure is carried out:
(1) Half an ounce alkaline sodium phosphate is given four times a day.
(2) Two posterior irrigations a day are given with pot. permang.
(3) Two cubic centimetres polar body vaccine are injected intradermally in seven places. This is repeated every seventh day for as long as required.
(4) Daily smears are examined and the approximate number of pus and endothelial cells and gonococci are estimated (the numbers are recorded as approximately the number per field, i.e., "G.C. 2/1, P. 4/1, E.P. 1/10").
(5) A rough estimate of the urinary pH is made daily.
(6) The patient is kept "up" on ordinary diet with the addition of vegetable soup twice daily and four pints of barley water (made without lemons).
(7) Two or three times weekly the patients are assembled in the treatment room and their urine examined by the two-glass method. It has been found that, in view of the large amount of phosphate present, glacial acetic acid is frequently required to clear it. It is not suggested that the two-glass method is infallible but, if one method is adhered to, after some experience a good deal of information can be obtained.
(8) Immediately the discharge begins to lessen, irrigations are cut down to one a day and later discontinued. Patients are put on light fatigues at the earliest possible moment.
(9) When a patient has been "dry" for seven consecutive days on no treatment and on light fatigues he is examined with the urethroscope or straight sound and by prostatic massage. If no evidence of disease is discovered he is discharged from hospital to duty but is kept under observation for six weeks to two months, reporting at first weekly, later fortnightly.
(10) If, during the course of treatment, complications, such as acute posterior urethritis, develop, the patient is put to bed on a low diet with the appropriate treatment. Irrigations are stopped but the vaccine is continued. In fact the treatment of complications is the same as heretofore with the addition of vaccines and sodium phosphate.

In the early days much work was done with the gonococcus endotoxin. It was, moreover, originally thought that only about a third of the gonococcus strains were capable of producing polar bodies on suitable media. Improvements in the media and the technique now make it apparent that in all probability all strains can be made to produce these bodies, although some strains are far more prolific than others.

To produce a solution of endotoxin a strain of gonococcus giving a poor yield of polar bodies is selected and this is grown for a week on the ordinary isolation medium without nucleo-protein. The growth is then washed off with normal saline and this is standardized to contain $250 \times 10^6$ per cubic centimetre. The emulsion is repeatedly frozen and thawed to ensure complete autolysis. To this solution, which is an opalescent fluid, is added a small proportion of chemically prepared colloidal silver as a preservative.
It was realized that this was the most reliable test of cure yet produced. One cubic centimetre instilled into the urethra has no effect on the normal man or on a patient suffering from a non-gonococcal urethritis, but if there are any living gonococci present it will precipitate an acute attack in three to nine days. It is in fact reliable and selective in action.

Observation on the use of endotoxin as a test of cure in a large number of cases goes to show that, whereas if no reaction occurs within ten days of its exhibition the case may reasonably be considered free from gonorrhoea, if reaction occurs, the resultant attack is resistant to treatment and is liable to become chronic, the toxin is very virulent and throws the patient into what is apparently a negative phase. For this reason the authors do not recommend the use of endotoxin as a routine test of cure in the ordinary cases of gonorrhoea, but consider it is most valuable in the “end” or “marriage test” when some months or years have elapsed after the actual attack of gonorrhoea.

A word is necessary as regards nomenclature. The polar bodies are identical with those described by Babe and Neisser, and have therefore been called “Babe bodies.” The original name of “endotoxin” is a good one, as this is a true endotoxin, but the name of “exotoxin” for the vaccine is misleading as it is in no sense a toxin. Ecto-antigen might be a better name.

Conclusions.

Without being unduly optimistic, the authors and all who have worked with this method of treatment are convinced of its efficacy. So far it has only been used in male gonorrhoea; it is, however, a form of treatment eminently suited to the cure of the disease in the female, a condition in which no satisfactory method has yet been evolved.

When any new treatment is introduced in the Army the acid test is a comparison of the average number of days in hospital. At Woolwich the average stay for all cases of gonorrhoea for the year 1928, was forty-seven days: this included a number of old chronic cases transferred from other stations whose stay in hospital was one hundred days and over.

Sufficient of the vaccine is now being prepared in the central venereal laboratory at Woolwich to supply the whole Army, and the treatment is being carried out in India and the Colonies as well as at home by specialist officers who have had previous experience of its use at Woolwich.
APPENDIX "A."

Diagram to show the growth of the Gonococcus, the formation of endotoxin and polar bodies, and the detoxication which occurs during culture on special media.

Gonococcus Culture

\[ \text{Gonococcus phospho-protein} \rightarrow \text{Meta-protein} \rightarrow \text{Proto-albumose} \rightarrow \text{Hetero-albumose} \rightarrow \text{Deutero-albumose (secondary albumose)} \]

Soluble 90 per cent

- Glucosamine (1 mol.)
- Acetic acid (2 mol.)

Insoluble 10 per cent

- Leucin
- Protophospho-protein
- Mouse filtrate

Insoluble mucoid envelope

- Nucleo-protein
- a-Nucleo-histone
- b-Nucleo-histone

Primary albumoses

- Protein
- Histone
- Nucleic acid

Medium contains

- Protamine
- Histone
- Free nucleic acid

Insoluble, inactive compound.
# APPENDIX "B."

**TABLE SHOWING H-Ion CONCENTRATION (pH) OF URINE AND THE RESPONSE OF THE BODY TO INVASION BY THE GONOCOCCUS.**

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### Normal range of health:

- No phagocytosis of Gonococcus.
- Polymorphonuclear leucocytes in pus nearly 100 per cent. Mononuclear leucocytes (clasmocytes—reticuloendothelial cells) almost nil.
- Gonococci as tetrads due to growth at low pH, invade follicles, the contents of which are at low pH, due to H-ions from glucose.
- Maximum production of gonococcus mucoid envelope which as a capsule protects the tetrads from phagocytosis.
- Gonococci almost entirely extracellular.
- Urethral exudate = True pus.
- Little or no follicular mucin present.

### Range due to the administration of acid or alkali:

- Range due to acid:
  - Maximum polymorphonuclear phagocytosis of the gonococcus.
  - Maximum reticulo-endothelial phagocytosis of polymorphs (alone and with intracellular G.C.).
  - Maximum number of reticuloendothelial cells, including plasmocytes, monocytes, clasmoocytes, lymphocytes.
  - Gonococci are all uniform diplococci; no tetrads, figures of eight, ovoids or spheres present.
  - Minimum development of protective gonococcus mucoid envelope (alkali insoluble).
  - All gonococci intracellular.
  - Maximum secretion of true follicular mucin.
  - Exudate = Muco-pus.
- Range due to alkali:
  - Moderate polymorph and slight mononuclear leucocytosis.
  - Endotoxin production maximum.
  - In exudate, gonococci are present as acidophylic stained.
  - Diplococci of irregular size and spheres, figures of eight, ovoids or spheres present.
  - At pH 7.6 autolysis is more and more marked in exudate gonococci.
- Range due to acid:
  - Maximum polymorph and slight mononuclear leucocytosis.
  - Endotoxin production maximum.
  - In exudate, gonococci are present as acidophylic stained.
  - Diplococci of irregular size and spheres, figures of eight, ovoids or spheres present.
  - At pH 8.4 alkali reserve in the blood is maximum and no ammonia is present.
  - Urethral exudate = Watery mucopus.
  - Moderate polymorph and slight mononuclear leucocytosis.
  - Endotoxin production maximum.
  - In exudate, gonococci are present as acidophylic stained.
  - Diplococci of irregular size and spheres, figures of eight, ovoids or spheres present.
  - At pH 8.4 alkali reserve in the blood is maximum and no ammonia is present.
  - Urethral exudate = Watery mucopus.
APPENDIX " C."

TABLE SHOWING H-ION CONCENTRATION OF CULTURE MEDIUM AND GONOCOCCAL MORPHOLOGY AND CULTURAL CHARACTERISTICS.

<table>
<thead>
<tr>
<th>pH</th>
<th>6.5</th>
<th>6.6</th>
<th>6.7</th>
<th>6.8</th>
<th>6.9</th>
<th>7.0</th>
<th>7.1</th>
<th>7.2</th>
<th>7.3</th>
<th>7.4</th>
<th>7.5</th>
<th>7.6</th>
<th>7.7</th>
<th>7.8</th>
<th>8.0</th>
<th>8.2</th>
<th>8.4</th>
<th>8.6</th>
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<tr>
<td>pH</td>
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</tbody>
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The gonococcus forms large, refractile tetrads, figures of eight, sixteen, thirty-two, etc., embedded in a large amount of alkali insoluble gonococcus mucin. Gonococcus tetrads, etc., are deeply basophilic and highly refractive—due to lipoid content.

Cultures are raised from the medium owing to division of the organism in three dimensions and no marginal spread of the culture is observed. The culture is milky and opalescent.

Discrete colonies are opaque circular, with a central conical nipple, and may reach 4 mm. in diameter.

No polar bodies have been observed in the tetrads (they have been seen, however, in meningococci, Type II tetrads).

The tetrads tend to retain Gram's stain, i.e., are Gram-positive to graduated dehydration.

Range of optimum culture

Gonococci are uniform diplococci, all of the same size, are Gram-negative and tend to basophilic staining. Are non-refractive (= no lipoid).

Autolysis of culture is at a minimum. Minimum amount of gonococcus mucin formed.

Culture is transparent with wavy, extending margin due to marginal spread of the culture over the medium in two dimensions. It is moist and viscous with a tendency to be drawn into threads at the higher optimum pH's.

Discrete colonies are transparent, dew drop and not exceeding 0.5 to 0.75 mm. in diameter, centre of colouring slightly yellow with transmitted light.

Optimum polar-body development takes place.

The gonococcus forms spheres, ovoids, diplococci, figures of eight. Giant forms appear.

Staining is acidophilic in proportion to rise of pH and the amount of autolysis.

Maximum autolysis at pH 7.8.

Gonococcus mucin only present in small amount.

Opacity of cultures due to autolysis of gonococci.

Culture is opaque and with rising pH becomes more and more pasty.

At pH 8.2–8.4 it is dry, opaque, pultaceous mass, only removed from the medium with difficulty.

Colonies may reach 3–4 mm. in diameter. No polar bodies are developed.
APPENDIX "D."

ROUTINE FOR THE PREPARATION OF THE SPECIAL MEDIA AS USED AT THE ROYAL HERBERT HOSPITAL, WOOLWICH.

INTRODUCTION.

The greatest care must be exercised to carry out the whole process of production under the strictest aseptic precautions as sterilization by heat will only result in the destruction of several important properties.

Apparatus.

(1) Test tubes. The most convenient size is 6 inches × $\frac{3}{4}$ inches. These are boiled in the following solution:

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<tbody>
<tr>
<td>Potassium chromate</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Sulphuric acid, commercial</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Tap water</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

for two hours and then brushed and rinsed in hot water three or four times and left to soak in water for two hours; next dried in a hot air oven, plugged and sterilized at 160° C. for one hour.

(2) Filler. This is sterilized at 120° C. for one hour.

(3) Flasks. Fill with strong solution of potassium permanganate and leave for twenty-four hours, wash with hot water followed by commercial hydrochloric acid. When all stains are removed the flasks are rinsed in hot water to render them acid free, dried in the hot-air oven, plugged and sterilized at 160° C. for one hour.

Media.

There are two types of media employed: (1) Isolation medium. (2) Nucleic acid medium.

The isolation medium is essentially a nutrient serum agar, whilst the nucleic acid medium contains in addition a thymus nucleic acid base. They both contain an alkaline autolysate of ox heart.

A. Alkaline Autolysate of Ox Heart.

Obtain the hearts fresh, free them from all trace of fat and fibrous tissue, cut the meat into small cubes and mince in a sterile mincer. Weigh the minced heart and place in a sterile pan. To every gramme of meat add 1 cubic centimetre of distilled water and, in the case of fresh hearts, 40 cubic centimetres of N/1 NaOH to every litre of water added. In the case of frozen hearts 42.5 cubic centimetres of N/1 NaOH.

Leave the whole in the ice chest overnight to macerate. Next morning strain through gauze in 500 cubic centimetre bulks into litre flasks. Steam for quarter of an hour to allow coagulation to take place.

Strain again and steam for fifteen minutes on the first day, five minutes on each of three successive days.
B. Agar Base.

Witte's peptone ... ... ... ... 6 per cent
Agar fibre ... ... ... ... 6 "
Sodii chlorid ... ... ... ... 1 "
Sodii phosphat ... ... ... ... 1 "
Aqua dist ... ... ... ... q.s.

This base is made up in 200 cubic centimetre bulks in 500 cubic centimetre flasks and is placed in a steamer until the agar is melted, then to each add 0·3 per cent (0·6 grammes) of glucose and steam for a further fifteen minutes.

The base is made up the day before the batch is to be tubed off.

C. Herring-roe Extract.

Take ripe sperm of herring, grind it into a paste with distilled water, adding to every gramme of roe 1 cubic centimetre of water. Strain through gauze, steam for half an hour on three successive days.

In an emergency tinned roes may be used (Noel and Sons are the best), but the resulting yield of bodies is not good.

D. Nucleic Acid Base.

Desiccated thymus ... ... ... ... 4 gm.
Nucleic acid ... ... ... ... 1 "
Aqua distillata ... ... ... ... 100 c.c.

Mix in 250 cubic centimetre flask and steam for fifteen minutes. Adjust the pH to 7·2 as follows:—

Add 15 cubic centimetres of 0·02 per cent phenol red solution and 100 cubic centimetres of herring roe extract (C. above) then add N/1 NaOH until the yellow colour turns to brownish yellow, steam for a further ten minutes. Note.—If it is heated too much there is apt to be an alteration in the pH and consequent loss of efficiency of the medium.

(1) Ordinary Isolation Medium.

Steam the agar base (made as in B above, but without glucose) until the agar has melted. Steam the heart extract (A above) for five minutes. Mix 200 cubic centimetres of agar base with 200 cubic centimetres of heart extract. Tube off ten cubic centimetres to a tube, add 0·5 cubic centimetre of human serum to each tube and slope.

(2) Standard or Nucleic Acid Medium.

Take agar base 200 cubic centimetres and steam until melted. Heart extract fifty cubic centimetres and steam for five minutes, nucleic acid base 200 cubic centimetres and steam for fifteen minutes.

Mix all together, tube off fifteen cubic centimetres to a tube and to each tube add 0·5 cubic centimetre of human serum. Slope.

The following brands of ingredients have been found to give the best results:—

Witte's peptone, must be Rostock German brand. Sodium phosphate, British Drug Houses, pure. Nucleic acid, Martindale's. Thymus, desiccated, Willows, Francis, Butler and Thompson.