(2) the stage of depression, or the depressor stage; (3) the stage of equilibrium, when active abnormal afferent impulses are in abeyance.

We must now consider the mechanism of this reversal.

It has been shown that every sensory nerve contains pressor and depressor fibres to the vasomotor centre; stimulation of the pressor fibres only, induces reflex vasoconstriction and cardiac acceleration, while stimulation of the depressor fibres only, induces reflex vasodilatation, and at the same time inhibits vaso-constrictor impulses. Stimulation of such a mixed sensory nerve usually induces domination of the pressor group. Domination of the depressor group only occurs (in so far as the present study is concerned) when the pressors are fatigued.

Applying these principles to the results obtained in Chart 28, we conclude the following: The first stage of shock in that operation consisted in a rise of blood pressure and an increase in the pulse-rate, consequent on the domination of the pressor impulses. At a pressor fatigue commenced. Further stimulation initiated a domination of the depressor impulses, the cessation of these impulses being marked by a passive return of the blood pressure to an intermediate level, due to the cessation of active depressor domination.

Death from Shock.

Prolongation of the stimulus during the depressor stage leads to: (1) Continuous lowering of blood pressure—this renders inadequate the blood supply to the cardiac muscle through the coronary arteries, and this fact, combined with the lack of peripheral resistance, leads to delirium cordis; and (2) anaemia and starvation of the central nervous system—if this is carried to a sufficient degree, the recovery of the centres is rendered impossible.

REFERENCE.

1 *Annals of Surgery*, 1910.

A METHOD OF ESTIMATING THE STRENGTH OF A VACCINE BY A STANDARD BACTERIAL EMULSION.

BY

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THE following account deals with a method of counting an emulsion of bacteria of unknown strength by comparison with an emulsion of known strength—that is, a “standard”—using a differential stain (Gram’s method), and a “standard” of opposite staining properties to the bacterial emulsion whose strength we wish to estimate. Thus, if an emulsion of staphylococci has to be estimated, a Gram-negative “standard” (*B. coli*) is used, and vice versa; and by the formulae:

Gram + : Gram - :: $\times$: standard emulsion,

or

Gram - : Gram + :: $\times$: standard emulsion,

the strength is readily worked out.

The “standard” emulsion is obtained by applying the principles used in the estimation of the number of bacteria per cubic centimetre in water or sewage, and the apparatus needed is very simple, and in fact the simple forms of glassware to be mentioned shortly are much more convenient than any more elaborate. The requirements are: A well-spread twenty-four hours old agar culture of *B. coli communis*, a platinum loop, small white sterile glass beads in sterile saline, tubes of sterile normal saline, sterile test tubes, a litre measure, three or more 1 c.cm. pipettes graduated in one-tenths, a Wright’s pipette with a glass pencil mark $\frac{1}{2}$ in. from the tip, two glass-stoppered “half Winchester” bottles containing 999 c.cm. of water, three or four 6-oz. flat-sided medicine bottles containing 15 c.cm. of nutrient gelatine (12 per cent. to 15 per cent.) suitably plugged with wool and sterilized, two deep saucepans or water baths. The 1 c.cm. pipettes (rolled up in paper) and the “half Winchesters” with their contents are sterilized.

The culture of *B. coli* is now taken, and an emulsion made by pouring about 2 c.cm. of sterile saline over the agar, and then rubbing off the growth into the saline with the platinum loop. The resulting emulsion is now transferred to a sterile test tube, and some half-dozen of the sterile glass beads added.

The next step is to obtain a “convenient strength” of this emulsion to work with in the subsequent diluting experiments that are the most important part of the process. After many trials we have found that a strength roughly estimated at between 1,500 and 2,000 millions of bacteria per cubic centimetre is a “convenient strength” to use. The opacity of the emulsion which represents roughly this strength is easily obtained either by previous experience, or by allowing three to four drops of milk to mix with 5 c.cm. of water, and then diluting the emulsion from the agar down to the opacity represented by this mixture. Having diluted down the agar emulsion to this “convenient” strength, the open end of the test tube is sealed off in the flame, and the tube shaken for half an hour or more to ensure complete separation of the individual bacteria.

It is now necessary to ascertain the true strength of this “convenient emulsion.” The two water baths are next heated, one to boiling the other at 26° C., and the medicine bottles containing the gelatine put into the latter. With a graduated pipette take 1 c.cm. of the emulsion of “convenient strength” and put it into the first “half Winchester” bottle (A), stopper the bottle and shake vigorously. Place the tube containing the emulsion of “convenient strength” into the boiling water bath for a few seconds, then remove and add two drops of formalin. Take next 1 c.cm. of (A) and drop it into the second “half Winchester” bottle (B), and shake well. Use separate sterile 1 c.cm. pipettes for each bottle. From (B) with the third pipette take 1 c.cm. and carefully drop 0.5 c.cm. of this into the first medicine bottle (C), 0.25 c.cm. into the second bottle (D), and 0.1 c.cm. into the third bottle (E). Mix the contents of these bottles and lay flat on their broad side to solidify; when solid, incubate in the cool incubator. At the end of seventy-two hours rule out squares of suitable size with a grease-pencil on the bottle and count the colonies. The dilutions being known, the true strength of the “convenient emulsion” is known, an average being taken of the plates, though, as a rule, the plates come out in practically exact multiples of one another. For example, in (A) the “convenient strength” is diluted 1,000 times, and in (B) 1,000,000 times, there being already 999 c.cm. of sterile water in each “half Winchester.” Thus, if the “convenient strength” had happened to be 1,500 millions of bacteria per cubic centimetre, then 0.5 c.cm. of (B) will give 750 colonies (C), 0.25 c.cm. of (B) will show 375 colonies (D), 0.1 c.cm. of (B) 150 colonies (E).

This emulsion of 1,500 millions per cubic centimetre is now a “standard emulsion” of Gram-negative (*B. coli*) bacteria, and may be used to estimate the strength of an “unknown” Gram-positive emulsion by the following method: Having for convenience diluted the “unknown” emulsion down to approximately the opacity of the “standard” emulsion, a slide is thoroughly cleaned and passed through the flame several times to remove any traces of grease. With the Wright’s pipette equal volumes of the Gram-negative “standard” and the Gram-positive “unknown” emulsions are taken and thoroughly mixed. One end of the clean slide is slightly warmed and a portion of this mixed drop is put on this part of the slide. Using the pipette as a “spreader” a film is made, blowing across the film as it is spread. The result of the warmth and the blowing is that the film dries evenly and instantaneously. The film thus obtained is carefully fixed by passing three times through the flame, is stained by Gram’s method and counterstained by neutral red (2 per cent. aqueous). A large-sized “counting square” having been dropped into the eyepiece of the microscope, successive “squares” are counted with the one-twelfth objective, carefully noting on paper the Gram-positive and Gram-negative organisms in each “square.” When 200 to 300 of the “standard” bacterium have been counted, the total Gram-positive and Gram-negative organisms are carefully and separately added up. Knowing the strength of the “standard” emulsion the “unknown” emulsion is readily found by proportion. Thus, supposing 250 Gram-negative organisms and 200 Gram-positive organisms have been counted and the strength of

the "standard Gram-negative" emulsion is 1,500 millions per c.cm., then: — $200 : 250 :: \times : 1,500 = 1,200$ millions per c.cm. as the strength of the Gram-positive emulsion. This Gram-positive emulsion may be used as a "standard Gram-positive" emulsion, but if used as such more than 200 of the bacteria should be counted to ensure great accuracy, about 500 of each bacterium being counted before the calculation is made. Of course plate estimations can be made, using staphylococcus instead of *B. coli*, the process being similar to that just described, and a "Gram-positive standard" obtained in this way; but the obtaining of the "Gram-positive standard" from the "Gram-negative" standard saves two series of dilution experiments.

Having now obtained two "standard" bacterial emulsions (which keep well from the addition of the formalin, and are readily and thoroughly mixed from the presence of the glass beads), we are in a position to estimate the strength of any unknown emulsion by simply mixing it with equal parts of a "standard" of opposite Gram staining properties, making a film, and counting by the methods already fully described. The only precaution to be observed with regard to the "standard" emulsions is to cap them suitably to prevent evaporation.

This method has now been used for the past fifteen months in private and hospital practice, and it was found from the first as rapid as Wright's method, and after practice more rapid than this method. From numerous observations on Wright's method, the Thoma-Leitz counting slide method, and the method under consideration, it has been found that the lowest estimations of any bacterial emulsion are given by Wright's method and the highest by this method now described, while the Thoma-Leitz method is intermediate between the two. It is probable that the method under consideration is much more accurate than those commonly employed, and for the following reasons: (1) A constant "standard" obtained under the most accurate conditions possible is used; (2) one is comparing objects of approximately equal size; and (3) one is able to use a one-twelfth objective. In Wright's method the "standard"—that is, the red corpuscular count—is a fluctuating quantity, and ought to be re-estimated at each estimation of the strength of a vaccine, and, further, the standard is many times the size of the bacterium with which it is compared. In the Thoma-Leitz method one must allow thirty to sixty minutes to elapse from the making of the drop preparation to allow the bacteria to settle sufficiently, and one is unable, moreover, to use a one-twelfth objective.

To now briefly summarize the process:

1. Obtain a Gram-positive and Gram-negative "standard" by the diluting and plating out processes described. These "standards" keep good for at least six months in our experience.
2. Take equal parts of the emulsion of "unknown" strength and a "standard" of opposite Gram staining properties and mix thoroughly.
3. Make a film and fix it carefully.
4. Stain by Gram's method, using a counterstain.
5. Count 200 to 300 bacteria, using a "counting square" and a one-twelfth objective and noting the Gram-negative and Gram-positive organisms in the squares counted.
6. Knowing the strength of the "standard" and the proportion of Gram-negative to Gram-positive organisms, the strength of the unknown emulsion is easily worked out by proportion.

In conclusion, I should like to thank Mr. F. R. Chopping, senior laboratory assistant to the hospital, for his invaluable assistance and suggestions in the early part of our investigations and repeated co-operation in the subsequent laborious counting tests so necessary to prove the value of the method.

THE second Shakespeare commemoration service held at Southwark Cathedral, formerly the church of the parish in which Shakespeare was a resident, was held on April 23rd, when Mr. F. R. Benson gave an address on "Shakespeare and the fuller life of the people." It is announced that the fund, of which Dr. R. W. Leftwich is the honorary secretary, for the Shakespeare memorial in the cathedral, has been completed by a gift of £180 by Mr. Sanford Saltus, of New York.

A CASE OF TUBERCULOUS POLYSEROSITIS.

BY

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MULTIPLE inflammation of serous membranes is a somewhat uncommon condition, and the case here reported presents features of unusual interest, particularly in its exceptional onset, its abnormal course, and the final *post-mortem* proof of its tuberculous nature.

The patient was a married woman aged 46, with a healthy family history. She had no personal record of any serious illness until ten years ago, when she had an attack of pneumonia, involving the lower lobe of the right lung, and terminating in complete recovery after suppuration, and coughing up of the pus. No abnormal physical signs could be found at the commencement of the illness recorded in this paper.

On November 11th, 1909, the patient was taken ill with the symptoms of influenza, including headache, pain in the back and limbs, and severe prostration. She did not, however, seek medical advice until November 25th, when she was found to be in a state of high fever and nervous excitement, her temperature being 103.4°. She had been performing her household duties intermittently during the previous fortnight. During the following week she was kept in bed, and treated with quinine and simple antipyretic medicine. There were no arthritic symptoms, and no abnormal signs in the chest or abdomen.

On December 2nd the patient left her bed for a few minutes and fainted, and on examining her shortly afterwards she was found to be cyanosed and almost in a state of collapse, her pulse being 145 per minute. A very faint friction sound could now be detected near the base of the heart, and two days later there were definite signs of general pericarditis. Sodium salicylate was administered on the assumption that the pericarditis might be of rheumatic origin, but was discontinued after a few days, on account of its depressing effect. Treatment with quinine and stimulants was resumed.

On December 13th pleurisy developed over the lower lobe of the left lung, and signs of effusion were found on the following day.

On December 20th the dull area was explored for fluid, but none was found, for the probable reason that the needle was inserted too near the middle line, considering the position of the patient, who was lying on the affected side, so that the lung, no doubt, sagged down towards the axilla, and was wounded by the needle.

The erroneous conclusion was drawn, however, that the dullness was due to solidification, and a second exploration was not made at the time. The temperature continued febrile, ranging from 99° in the morning to 101° to 102° in the evening, while signs of scattered bronchopneumonia became perceptible in the right lung, and the signs of pericardial friction disappeared.

On January 4th, 1910, Dr. Brown, of Ipswich, saw the patient in consultation with me, and the chest was again explored—this time with success—and 52 oz. of clear serous fluid was withdrawn. Respiration was, of course, greatly relieved by the operation, but the fluid again accumulated with great rapidity, and paracentesis was again necessary on January 10th, 15th, and 19th, more than two pints being withdrawn on each occasion. The fluid was clear each time, and was examined for tubercle bacilli, as was also the sputum, with negative results.

The skin of the back was now oedematous, and scattered crepitations were heard over the right lung. On January 21st the temperature became normal and remained so for nearly a month, though the sputum, during the weeks immediately previous and subsequent to this, was deeply bloodstained.

Paracentesis thoracis was again done on January 31st, February 16th, March 4th, and March 21st, the patient always being greatly relieved for a day or two after each operation. A consultant who saw the patient on February 22nd considered the case one of polyserositis of rheumatic origin, and negated the previous diagnosis of tubercle. Potassium iodide and caffeine were now given, but the symptoms of iodism and depression were so marked during its use that it was discontinued after a week.

On March 23rd oedema of the legs became very troublesome, and Southey's tubes were inserted for its relief. They were removed after two days, and again used on several occasions during the remainder of the illness.

Enlargement of the liver and ascites now began to be very obvious, and the abdomen was tapped on April 11th, 5 pints of fluid being withdrawn. The operation was repeated on May 10th, June 2nd, and June 18th.

From July 16th the temperature became regularly hectic, and continued so during the subsequent progress of the case, though emaciation was not so marked as might have been expected.

On July 20th the chest was again aspirated and 3 pints of clear serous fluid were withdrawn.

On August 20th a third consultant saw the case and considered it was not tuberculous, on the ground that emaciation and debility were less than would be expected in the presence of tubercle. Von Pirquet's reaction was then tried, with a