

STUDIES ON PNEUMOCOCCUS GROWTH INHIBITION

IV. A SIMPLIFIED AGITATOR FOR GROWTH INHIBITION TESTS WITH SERUM-LEUCOCYTE MIXTURES; AND CERTAIN MODIFICATIONS IN THE TECHNIQUE OF THE TEST.

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PLATE 19.

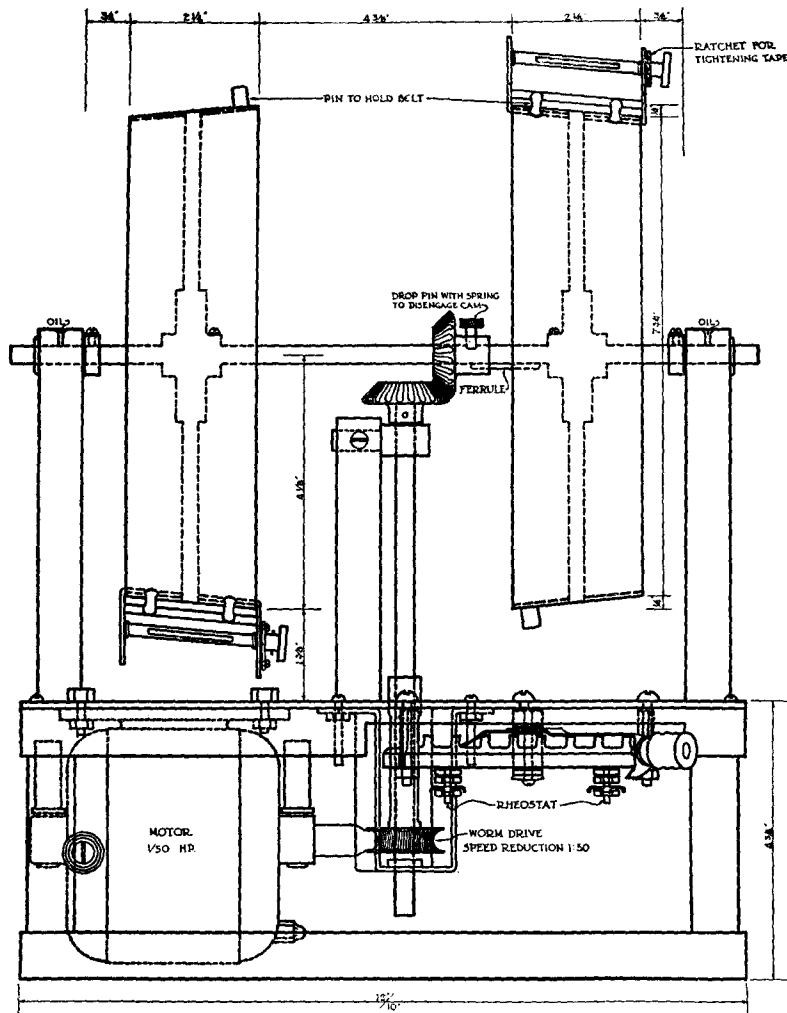
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Since the publication of the second study of this series¹ a much simpler and more compact agitating apparatus has been devised. Several changes have also been made in the technique of the growth inhibition test which have eliminated occasional irregularities in the outcome that had necessitated discarding the results of the individual experiment. Furthermore, it has been found that some of the test ingredients may be prepared the day before, thus shortening considerably the time required for the actual setting up.

Agitator.

The agitating apparatus pictured in Text-fig. 1 and Fig. 1 is made entirely of metal. It consists of two brass wheels with rims made in the form of truncated cones. The outer rim is $\frac{1}{4}$ inch smaller than the inner. A 1/50 h.p. motor, fixed to the base plate, drives the apparatus by means of a vertical shaft with a worm gear which gives a speed reduction of 1/50. A rheostat makes possible further slowing of rotation rate. The small gear wheel on the main (horizontal) shaft can be disengaged by pulling out the drop pin. The leather tube belt, cut from a pattern, is secured on the wheel by means of three pins and a small perforated shaft with a ratchet which takes up the slack tape and holds the belt firmly to the rim surface. In order to avoid undue strain on the gearing, the motor is started with the wheels free-running. Rotation is then initiated by hand and the sliding

¹ Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1924, xxxix, 219.



TEXT-FIG. 1. Diagram of agitator drawn to scale.

gear pushed into lock while moving. The apparatus in operation causes no appreciable vibration or heating of the incubator.

The rotation and oscillation given to the contents of the small tubes by this agitator bring about just as effective a mixing as when these two motions are carried out at different rates of speed, for results of duplicate tests, run simultaneously, with the two types of apparatus, are identical. A speed of 20 to 25 revolutions per minute is usually employed.

Modifications in Technique.

Pneumococcus Suspension.—In experiments with rabbit serum and leucocytes, it has occasionally happened that the control serum-leucocyte tubes, seeded with 0.0000001 cc. of the standard suspension (of high virulence for rabbits) grew out very slowly or failed to show any growth in spite of the fact that the dextrose blood broth controls grew well and blood agar plates yielded the usual number of pneumococcus colonies. The results of such a test were, of course, worthless. It seemed evident that the pneumococci had been injured sufficiently during suspension to render them susceptible to destruction by the normal rabbit serum-leucocytes although their ability to grow in a highly favorable medium was unimpaired. Investigation of the cause of this injury proved it due to prolonged suspension of the organisms in high dilution in the gelatin-Locke's solution.² When the pneumococcus suspension was delivered into the serum-leucocyte tubes soon after the dilution had been completed, the control tubes always showed abundant growth after 15 to 18 hours incubation. It was found possible to lessen greatly or to prevent entirely this deterioration of the pneumococcus suspension by adding 2 per cent of a M/15 balanced phosphate mixture, pH 7.8, to the gelatin-Locke's solution. However, it would seem wisest, even with phosphate added to the suspension fluid, to distribute the organisms into the serum-leucocyte tubes as soon as possible after the dilutions are completed. If a delay is necessary, the pneumococci should be allowed to remain in concentrated suspension. Once the organisms are seeded into the serum-leucocyte mixture, further standing for several hours at room temperature does not appear to retard growth after incubation begins.

Leucocytes.—A series of experiments on keeping washed rabbit leucocytes for varying lengths of time before use has shown that when suspended in their own serum, the leucocytes may be preserved in the ice box for a period as long as 48 hours without showing any deterioration in their activities as concerns pneumococci. In one test leucocytes obtained 5 days previously gave the same results

² Controls on the reaction of the suspension showed that injury to the pneumococci could not be attributed to variations in the H ion concentration. It is probably due to a toxic salt action.

as freshly prepared ones. Results with cat leucocytes are less conclusive and will need further investigation. The above finding with rabbit leucocytes makes it possible to prepare the normal serum-leucocyte mixture the day before the test is to be set up, which is a considerable advantage if the experiment includes a large number of tubes. In order to prevent the very occasional formation of fibrin in the gelatin-Locke's cell suspension, it has been found advisable to wash the leucocytes and red blood cells twice in gelatin-salt solution. If the cells are to be kept, the supernatant fluid is completely removed after centrifugation in gelatin-Locke's solution. The red blood cells are next added to the leucocytes in the usual amount. Then a quantity of the serum of the same animal sufficient to provide for the number of serum-leucocyte tubes required in the test, with 0.2 cc. of serum for each tube, is well mixed with the cells and the whole placed in the ice box. To keep the total fluid volume the same as that regularly used, an amount of gelatin-Locke's solution equivalent to 0.05 cc. for each serum-leucocyte tube is added to the serum-leucocyte mixture before it is distributed into the small tubes.

Normal Serum.—Numerous observations have shown that, in general, immune serum is effective in higher dilutions when used with normal serum and leucocytes obtained from the same animal. Hence fresh serum sufficient for the entire test should be secured, if possible, from the animal providing the aleuronat exudate.

Small Glass Tubes.—In our earlier work little attention was paid to the quality of glass in the small tubes used. However, the not infrequent occurrence of early hemolysis in a few or many tubes of an individual test, sometimes intense enough to disturb the results, pointed to impurities in the glass as a possible cause of blood cell injury. The use of Pyrex tubes has entirely eliminated this source of error.

SUMMARY.

A simplified and compact agitator for growth inhibition tests with serum-leucocyte mixtures has been described.

Several modifications have been made as well in the technique of the test, which have eliminated occasional irregularities that

necessitated discarding the results of the individual experiment. Such irregularities were found to be due chiefly to injury of the pneumococci brought about by prolonged suspension in gelatin-Locke's solution which resulted in failure of the organisms to grow in the control serum-leucocyte tubes. This deterioration of the pneumococcus suspension may be greatly lessened or entirely prevented by the addition of a small quantity of a balanced phosphate mixture to the gelatin-Locke's solution. The use of small tubes made of Pyrex glass has also eliminated the former, not infrequent, occurrence of early hemolysis which was sometimes intense enough to disturb the results of the test.

It has been found that washed rabbit leucocytes, suspended in their homologous serum, may be kept in the ice box for as long a period as 2 days without showing any apparent diminution of their functional activity in the serum-leucocyte test.

EXPLANATION OF PLATE 19.

FIG. 1. Simplified agitator used for growth inhibition tests with serum-leucocyte mixtures.

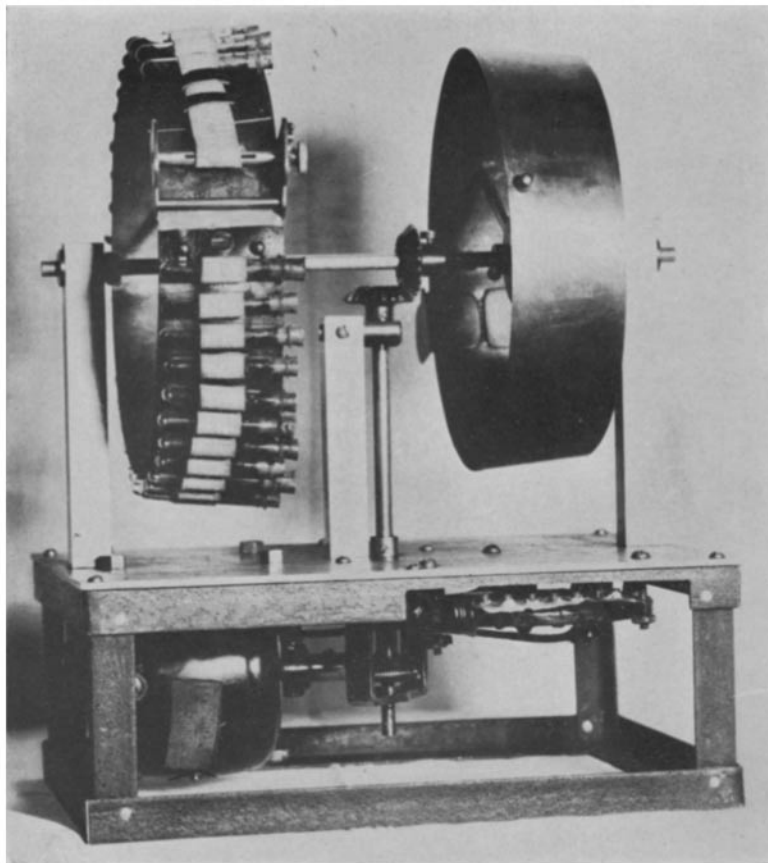


FIG. 1.

(Robertson, Woo, and Cheer: Pneumococcus growth inhibition. IV.)