AN IMPROVED METHOD OF MEASURING THE AMOUNT OF PRECIPITUM IN CONNECTION WITH TESTS WITH PRECIPITATING ANTISERA.

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Further experience with the method of measuring the precipitum obtained when experimenting with precipitating antisera has shown that *finer* degrees of reaction cannot be satisfactorily determined by means of the apparatus described by Nuttall[1]. In the latter apparatus capillary tubes were used in which to measure the precipitum therein deposited. It is true that capillaries as far as possible of the same calibre were used, nevertheless we have found that different readings were obtained when experimenting with tubes of varying calibre, variations evidently due to physical causes which prevent an equal degree of "packing" of the deposit in tubes of unequal width. We have modified the method after finding that calibrated tubes of uniform bore gave more constant results, and have devised a simple apparatus which greatly facilitates the making of measurements.

The Calibrated Tubes.

Several lengths of wide thermometer tubing are selected, the calibre being such that ·05 c.c. of fluid occupies about 20 mm. of the bore of the tube. This size was found to be a convenient one when working with ·5 c.c. of a 1 : 21 dilution of serum to which ·1 c.c. of antiserum of average strength was added[2].

[1] Nuttall (5. iv. 1902), *Brit. Med. Journ.* vol. i, pp. 825—827, reprinted in Nuttall (1904), *Blood Immunity and Blood Relationship etc.* pp. 315—318.

[2] A fine piece of glass rod, drawn out so that it tapers gradually, serves as a convenient cone for measuring the calibres of the tubing.

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The thermometer tube having the desired calibre is drawn out and cut in the form represented in Fig. 2, the uniform part of the tube (*D*) measuring about 11 cm., the tapered part (*F*) about 7 cm. so as to be conveniently introduced into the small test-tube (*E*) containing mercury, to the bottom of which it reaches. The tapered extremity moreover enables the operator to completely drain the contents of the small test-tubes in which precipitation has previously been allowed to take place. The object of the mercury in the small test-tube is to prevent the escape of the fluid (containing the precipitated matter in suspension), when the calibrated tube is stood upright. The tapered end of the tube should be as strong as possible and have a bore of about ·5 mm.

Each tube is carefully calibrated, the lower mark *B* being on the shank just above the shoulder. The space between graduations *B* and *A* above represents ·05 c.c. Each tube is marked with a number at *C*, sets of tubes being ordered according to the uniformity between the graduation marks *A* and *B*, the variations in calibre being noted.

Method of using the Tube.

Having added antiserum to serum dilution in the usual manner the mixture is shaken. The test-tubes are allowed to stand for 24 hours to permit the precipitum to accumulate at the bottom. (Further details will be found in the publications by Nuttall already referred to.) The greater part of the supernatant fluid is now drawn off, and the residue, together with the precipitum, is taken up into the calibrated tube. This is accomplished by holding the test-tube in the left hand, the tube in the right with its tapered extremity reaching to the bottom of the fluid in the test-tube, suction being gently applied by means of a piece of rubber tubing applied loosely to the end *C* of the calibrated tube. By applying the rubber tubing loosely a certain amount of air enters from without at the joint and gentle suction can be applied without fear of drawing up the fluid and precipitum suddenly into the rubber tubing thus vitiating the experiment. When the test-tube has been nearly emptied, the finger is applied at *C*, both tubes are inclined gradually, the finger regulating the inflow of the remaining fluid, until finally the former is on a higher level than the calibrated tube, into which the contents of the test-tube almost completely drain. The calibrated tube is now held horizontally, the finger applied to end *C* and the fluid allowed to flow along the tube,

by tilting it, until the lower meniscus is just above the graduation mark *B*.

The tube, held in the manner described, is now placed vertically, nozzle downward in the small test-tube containing mercury, and the tubes are placed on the rack, as shown in Fig. 1 (1). After standing

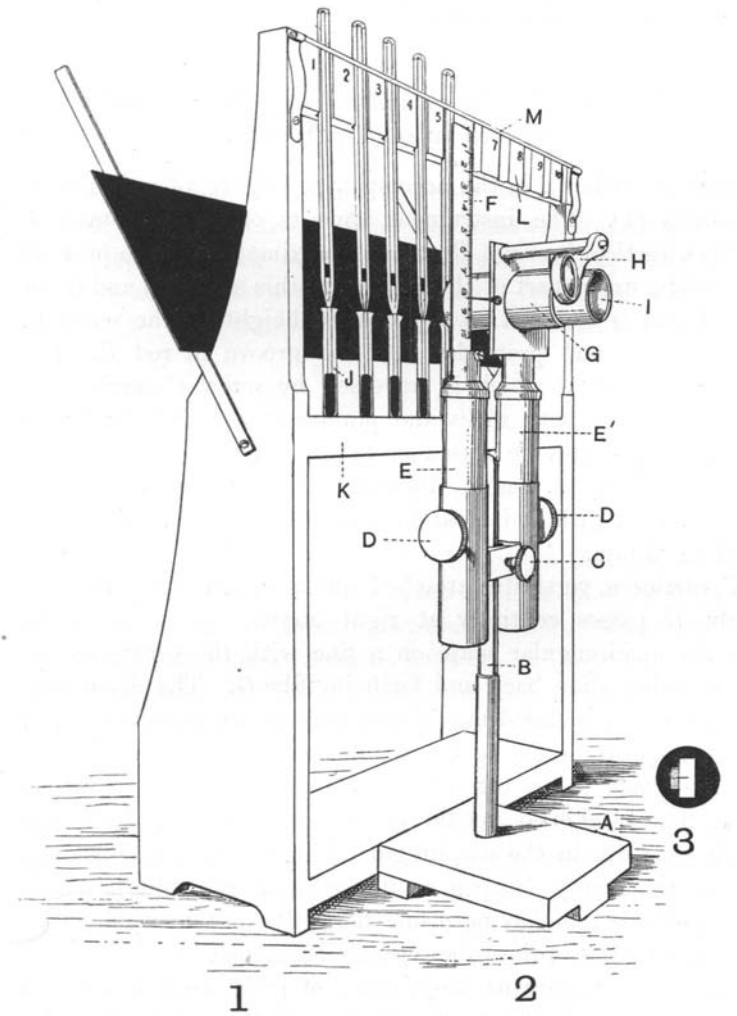

Fig. 1.

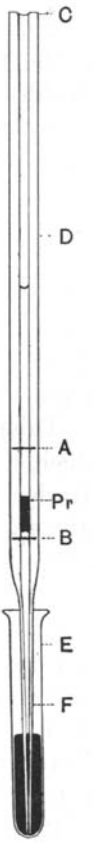

Fig. 2.

two clear days at a temperature sufficiently low to inhibit bacterial growth, the precipitum will have packed sufficiently to be measured in

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that portion of the tube which is calibrated between the marks *A* and *B*. (See Fig. 2, Pr., also represented in black in Fig. 1.)[1]

After the calibrated tubes have been used the contents are washed out forcibly under the tap, a short piece of rubber tubing connecting the end *C* with the tap. When not in use the tubes, at any rate in cold weather, can be kept in running water, being rinsed out before use with distilled water and drained.

Apparatus for measuring the amount of precipitum deposited in the calibrated tubes (Fig. 1, at 2).

This apparatus is shown in the accompanying figure, placed in front of the tube-rack (1). The instrument consists of an iron stand *A* provided with a vertical iron rod *B* grooved proximally so as to prevent the rotation of the upper part of the apparatus, this slides up and down along the rod and is clamped at any desired height by the screw *C*. Screw *C* is rounded and protrudes into the groove in rod *B*. The horizontal piece of metal which is traversed by screw *C* carries two vertical tubes provided with racks and pinions at *D* and *D'* by which inner tubes *E* and *E'* can be raised or lowered independently of one another, a play of 5 cm. being allowed. Tube *E* carries a steel 10 centimetre scale *F*, which is held in a vertical position. The scale is graduated in ·5 mm.

Tube *E'* carries a vertically attached quadrangular plate through which a tube *G* passes centrally at right angles. An indicator is attached to the quadrangular plate on a line with the centre of the tube *G*. Two tubes slide back and forth in tube *G*. The distal one, nearest the tube-rack, is closed by a diaphragm (shown separately at 3) provided with a quadrangular aperture having a width corresponding to that of the glass tubes containing the precipitum about to be measured; a fine blackened needle protrudes horizontally into the centre of this aperture in the diaphragm. The second tube *I*, sliding within tube *G* proximally, is open at its proximal end, but provided distally with a diaphragm having a fine horizontal slit traversing the centre. The inner parts of the tubes etc. are blackened.

By means of this apparatus the volume of precipitum within the glass tubes contained in the rack can be rapidly measured. The

[1] When, as sometimes happens, the precipitum does not settle entirely to the bottom, but becomes lodged in part in the upper portion of the tube, it can be made to sink by introducing a horse-hair which is twirled about and then withdrawn.

footboard of the rack (1) is placed in contact and parallel with the base *A* of the apparatus. The height of the apparatus is roughly adjusted by loosening the screw *C* and raising or lowering the upper part on the rod *B*, tightening the screw *C* again when the desired height is reached. The tube bearing the needle-carrying diaphragm is brought as near as convenient to the calibrated tubes without touching them. By racking at *D'* and looking through tube *I* the needle point is brought in position with regard to the lower meniscus of the fluid and precipitum in the glass tube. By racking at *D* the scale *F* is brought into suitable position with regard to the indicator, say at a centimetre graduation. Releasing screw *D* and racking again at *D'* in an upward direction the upper level of the precipitum is soon reached, and the indicator shows the distance travelled upon the scale *F*, the reading being facilitated by means of the small magnifier *H* which is carried at a suitable distance from the scale upon a rod attached to the quadrangular plate above described.

The Tube Rack (Fig. 1 at 1).

To facilitate the measurement of precipita contained in a series of tubes, we have devised a rack such as is illustrated in the accompanying Figure 1 (1). The rack is constructed of a convenient height with regard to the measuring apparatus (2), so that the calibrated portion of the glass tubes contained in the rack shall be on a level with eye-piece *I* when the apparatus is in a medium position. The rack is intended to fix the tubes in a vertical position, as near as possible to the diaphragm which carries the needle in the apparatus (3). The small test-tubes *J*, containing mercury, rest in conical depressions bored near one margin of board *K*, corresponding to numbered vertical grooves in board *L* above. The calibrated tubes rest on the bottom of the mercury tubes and fix the latter in position when held in the grooves above. These grooves are triangular in cross-section so as to hold the glass tubes correctly in position when pressed into the grooves by means of the horizontal rod *M*. The rod runs flush with the top of board *L*, and is held in place by a light spring at either end. The rod *M* feathers easily, and holds a series of tubes neatly in position. The tubes should be inserted between the groove and rod from below upward. A strip of blackened cardboard, resting on two light metal supports attached to the sides of the rack, serves as a background which facilitates the measuring of the precipita in the calibrated tube.

The base board of the rack is flush with the sides, and, being straight, serves as a guide when sliding the rack across the line of vision (through the apparatus 2). This sliding can be rendered accurate and easy by placing the rack upon an oblong glass plate of suitable length the sides of which are provided with guides. Beginning with tube 1 the readings are made one after the other by sliding the rack in one direction across the track of the apparatus.

The apparatus, which may be useful for other purposes, was made for us by Messrs W. G. Pye & Co., Granta Works, Cambridge.