SURFACE TENSION OF SERUM.

VI. The Study of Immune Serum. Time-Drop and Initial Value of Surface Tension.

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Plate 11.

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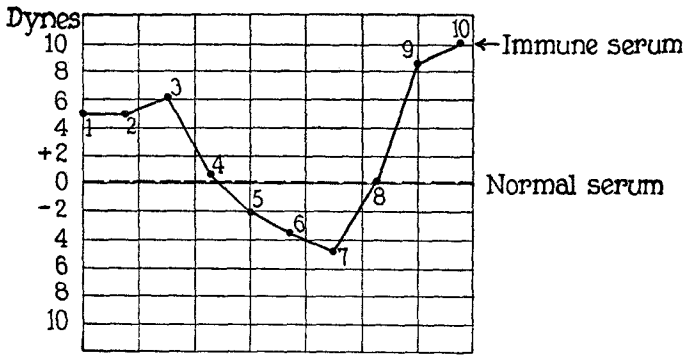
As stated in previous papers,[1-4] the four essential conditions under which the presence of antibodies in immune serum can be detected and followed by means of what might be termed the tensiometric method are: first, the dilution of the serum to 1:10,000; second, the use of strictly clean vessels; third, the care in handling the solutions, which must absolutely not be disturbed between the two measurements; and fourth, the study of the *time-drop*,[2] and not merely of the initial value of the surface tension. The first condition is a consequence of the numerous experiments reported in Papers I,[1] II,[2] IV,[3] and V.[4] The second has been emphasized in the same papers, and will be referred to again in the present paper. The third condition is satisfactorily fulfilled by the device represented in Fig. 1. However, the main object of this paper is to explain more extensively the reason of the fourth condition.

1. Time-Drop and Initial Value.—The results of a series of ten experiments on rabbits are reported in Text-fig. 1. From its mere inspection, it will be clear that the values of the initial surface tension of the solution of immune serum (1 part in volume of serum to 10,000 parts of saline 0.9 per cent) are sometimes higher and some-

[1] du Noüy, P. L., *J. Exp. Med.*, 1922, xxxv, 575.
[2] du Noüy, P. L., *J. Exp. Med.*, 1922, xxxv, 707.
[3] du Noüy, P. L., *J. Exp. Med.*, 1922, xxxvi, 547.
[4] du Noüy, P. L., *J. Exp. Med.*, 1923, xxxvii, 659.

times lower than the values of the solutions of normal serum. Hence, from these values alone, nothing could be gathered as to whether or not the serum contained antibodies. In order to make the chart clear, the initial value of the normal serum (before immunization) was taken as equal to 0, thus representing the axis of abscissæ, and the differences between the values of surface tension of the serum of the same animal, before and after immunization, were plotted with respect to this axis.

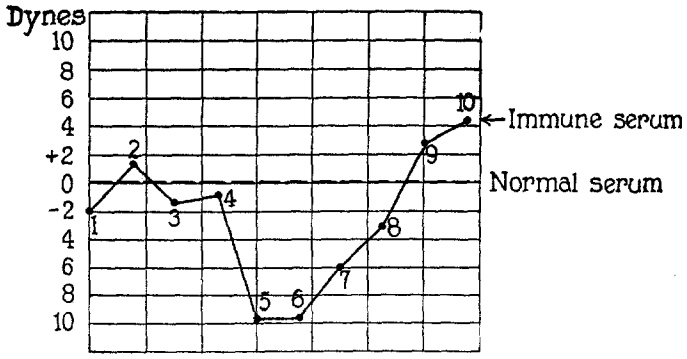
In Text-fig. 2, which represents the values of the surface tension of the solutions after standing for 2 hours, the same procedure was followed; *i.e.*, the values of the normal serum were taken as axis of 0,



TEXT-FIG. 1. Differences between the initial value of surface tension of normal and immune serum. Normal values are represented by the axis of abscissæ so that the differences are plotted in magnitude and sign.

and the differences between these values and those of the immune serum were plotted. For example, let us take two normal rabbits, A and B, whose serum solutions gave an initial value of surface tension of $A = 70.0$ dynes, and $B = 68.0$ dynes. After immunization, the initial value of the surface tension of the serum solution was $A_{imm.} = 73.0$ dynes, and $B_{imm.} = 66.0$ dynes. Then, if the normal values are used as the axis of abscissæ (ordinate = 0), the differences $A_{imm.} = +3.0$ dynes, and $B_{imm.} = -2.0$ dynes are plotted. In this way, the normal values will be represented by a straight line on both sides of which the differences between the values of the surface tension will be very obvious.

Consequently, if these two charts are brought together, however arbitrary the distance may be between the two parallel abscissæ representing the initial value of the normal serum, and the value after 2 hours, the ratio between their distance and the distance between the points representing the surface tension of immune serum in the two charts will express the ratio between the values of the so called time-drop in both cases, normal and immune serum. This was done in Text-fig. 3. It is clear that, in general, the variations in the value of the surface tension of immune serum after 2 hours



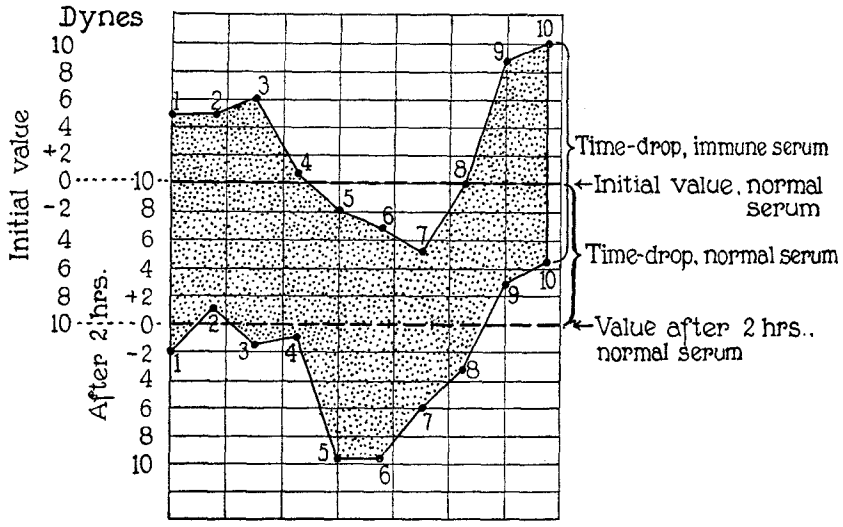
TEXT-FIG. 2. Differences between the values of surface tension of normal and immune serum after 2 hours. Normal values are represented by the axis of abscissæ so that the differences are plotted in magnitude and sign.

follow the fluctuations of the initial value more or less closely, so that, whether they are higher or lower, the time-drop is always greater for immune serum.

By joining the values of the surface tension of immune serum, two broken curves are obtained. The area between them may be integrated mechanically (Text-fig. 3, stippled area). By comparing the area thus measured (which is proportional to the time-drop for immune serum) to the area comprised between the two parallel lines (which is proportional to the time-drop of normal serum), a ratio is obtained from which the mean increase per cent in the time-drop of serum after immunization can be computed. In the present chart (Text-fig. 3), the increase is approximately 45 per cent. Now, all these experiments were by no means perfect; in other

words, they include measurements which were not made at the time when the maximum drop occurred, due to the presence of antibodies; *i.e.*, 13 days. Some were made too soon, some too late. Only two of them were made at the exact time, and they show an increase of 78 per cent (Nos. 3 and 5). A previous experiment[4] even showed an increase of over 100 per cent.

2. Normal Time-Drop.—Since the publication of the first paper on the action of antibodies on the time-drop, a number of data have been gathered concerning the time-drop of normal rabbit serum.



TEXT-FIG. 3. Text-figs. 1 and 2 brought together. The stippled area represents the time-drop of immune serum, in other words the differences between the initial value and the value after 2 hours, and the white area comprised between the two parallel lines corresponds to the time-drop of normal serum.

Comparison of the figures showed that, in the instances studied at least, the general value of the time-drop of the serum solution of a healthy, normal rabbit varied between 5 and 8 dynes in 2 hours. A higher drop, let us say above 10 dynes, always indicated that the rabbit was not absolutely normal. In that case, the increase in time-drop after immunization was smaller. Later, it was found that an epidemic of snuffles had developed in the cages in which the animals were kept. Subsequently, in order to obtain constant

results, all animals whose time-drop was above 10 dynes were discarded as not absolutely normal.

3. Details of the Technique.—It is necessary to lay particular emphasis on the cleaning of *all* vessels in which the serum, its solutions, and the distilled water are to be contained. They should be boiled for 2 hours in a concentrated solution of sulfuric acid, to which have been added 15 cc. of a saturated solution of potassium dichromate per liter. Just as important are the freshness and purity of the distilled water, and the cleanness of the sodium chloride to be used. Ordinary C.P. NaCl should be washed in a separating funnel. After 24 hours, the solution is collected from the bottom without stirring, a large amount of the supernatant liquid being left in the funnel. The solution is then recrystallized and used, great care being taken to prevent dust particles from falling on the crystals. The watch-glasses should be washed only 2 or 3 days at most before they are to be used; otherwise the liquid will not wet the glass perfectly. All the pipettes while in the hot cleaning solution must be washed inside by aspirating the acid up and down many times, by means of a rubber bulb and tube.

If this method is followed, the surface tension of the saline solution may be checked over and over again and will always give the same reading, within 0.05 dyne; otherwise, the results will vary greatly.

As a test for the cleanness of the vessels and the purity of the water and salt, allow the pure NaCl solution to crystallize; the crystals should be similar to the pictures published in Paper II, and should leave no rings on the glass.

As the position of the maximum time-drop in function of concentration obviously depends on the size of the watch-glass, that is on the ratio $\frac{\text{Total surface of liquid}}{\text{Volume of liquid}}$, it is important always to use the same size watch-glass and the same amount of liquid; for maximum drop at 10^{-4}, diameter of free surface of liquid equals approximately 4 cm. and volume of liquid equals approximately 2 cc.

CONCLUSIONS.

1. The initial surface tension of serum or serum solutions is not affected systematically by the presence of antibodies in the serum.

On the contrary, the time-drop in 2 hours is always increased, from 25 to 100 per cent.

2. It is extremely important, in order to demonstrate these phenomena, that the greatest care be taken regarding the cleanness of the vessels and the purity of water and NaCl.

3. It is equally important to use a device, for example such as is pictured in this paper, capable of preventing the jarring and shaking of the liquid.

4. The value of the time-drop of a normal, healthy serum is never higher than 10 dynes. Should it be higher, the serum must not be used for immunity experiments.

EXPLANATION OF PLATE 11.

FIG. 1. Turntable on ball bearings used in connection with the tensiometer to prevent jarring of the solution. The watch-glasses are brought successively under the platinum loop and raised by means of a screw.

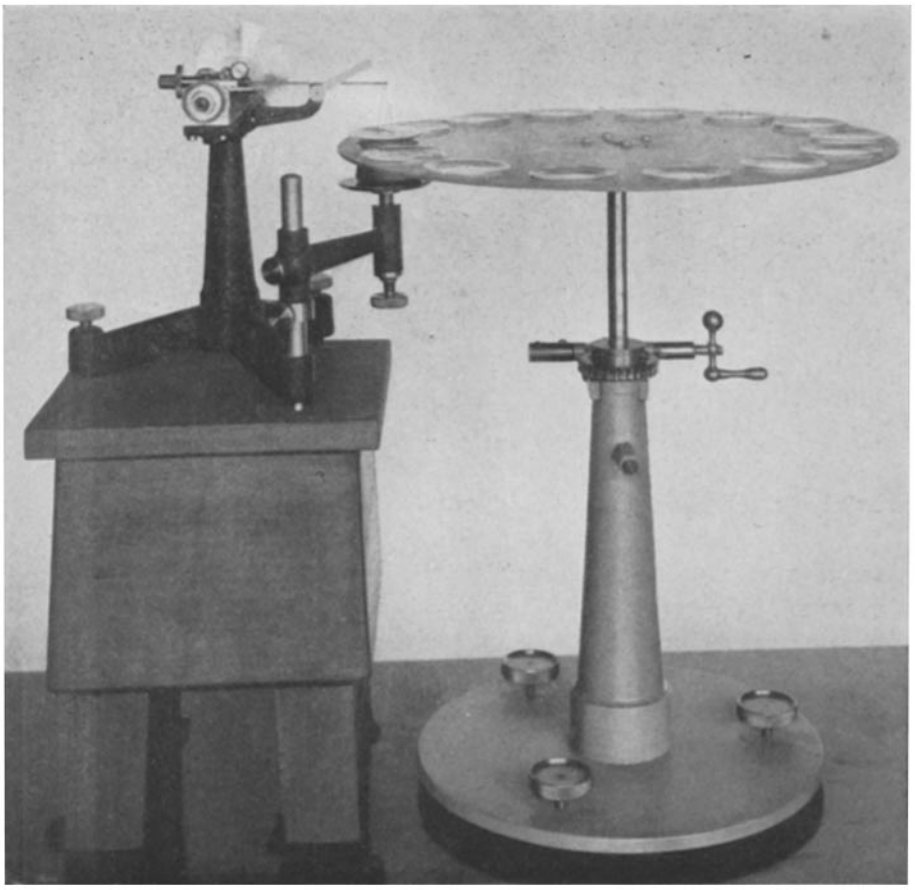


FIG. 1.

(du Noüy: Surface tension of serum. VI.)