THE VISCOSITY OF BLOOD SERUM, AS A FUNCTION OF TEMPERATURE.

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The viscosity of colloidal solutions is not easily determined by the methods which involve the use of a glass capillary. The technical difficulties encountered are important, and when they are successfully surmounted they still require a relatively large amount of liquid. Moreover, they do not permit the continuous observation of the variations which may take place in a solution as a consequence of a reaction, or under the influence of temperature, or of time. These were the main reasons which led us to establish a viscometer which would escape the above limitations. Such an instrument was described a few years ago.[1] Suffice it to say that it is based on the principle of two coaxial cylinders; the outside cylinder or cup, which contains about 1 cc. of the solution to be studied, is rotating at constant speed, and the inside cylinder, or bob, is suspended by means of a galvanometer wire. A mirror, supported by the suspension, a lamp and scale for the readings and a good thermostatic arrangement, are provided.

The purpose of the present paper is to report the results of roughly 167 series of experiments, representing about 17,000 readings of viscosity, performed with normal blood serum (rabbit, dog, horse), and to discuss the interpretation of some of the data obtained, with especial reference to the hydration of the serum proteins.

EXPERIMENTAL.

In order to save the reader's time, and to make him visualize a series of experiments at a glance, only charts will be published. The experiments chosen for publication are representative of the others, as no radical departure from the mean has been observed so far.

[1] du Noüy, P. L., *J. Gen. Physiol.*, 1923, v, 429.

The experiments were carried on in the following way: the serum was poured into the cup (1 cc.) and the bob lowered into the serum. The zero being checked, the motor was started, and a first reading made at the starting temperature (between 20° and 25°C.). After checking the zero again, and repeating this measurement at least 3 times, with an interval of 5 minutes between each reading, the heat was put on. (A current of 1 ampere through a resistance immersed in the oil surrounding the cup brought the temperature up from 20° to 70° in about 45 minutes.) Then the readings were taken, and recorded simultaneously with the temperature. A telescope placed alongside of the scale makes this possible. As a rule, unless some critical point was neared or some unforeseen phenomenon occurred, readings were taken every 2°. The current was left on until the temperature of 70°C. was reached, in the first series of experiments (Figs. 1 and 2); in all other experiments reported in this paper the heat was stopped after a certain temperature had been reached (50°, 55°, 56° to 60°) and the oil allowed to cool by itself. As a rule, in order that the cooling from, say, 55° to 20°, should take about the same time as the heating from 20° to 55°, cold water was made to circulate in the double wall of the oil bath, and the rate controlled accordingly.

The first set of experiments is shown in Fig. 1. The ordinates express the readings on the scale, and therefore are arbitrary figures, but proportional to the absolute viscosities. In order to give an idea of the order of magnitude, the curve expressing the viscosity of water as a function of temperature is drawn below.

A simple glance at this chart immediately reveals the presence of a critical point, corresponding to an absolute minimum of viscosity, at a temperature near 56°. Had we not known beforehand that this was a critical temperature for the serum, from a biological standpoint, we could not have failed to notice it from these curves.

Fig. 2 illustrates the same phenomenon. The turning point may vary, from 56° to 58°. But in the great majority of cases, the viscosity reaches its minimum value between 56° and 57°, stays constant up to 58°, sometimes up to 59°, and begins to increase more rapidly than it decreased before. From 62° to 65° the increase becomes very rapid, and it sometimes happens that the spot has left the scale before 70° is reached. This is usually the case with horse serum (which is normally more viscous than rabbit serum). It happens rarely in the case of rabbit serum. Fig. 2 shows that it was necessary to keep the temperature at 70° for 2 minutes in order to send the spot off the scale. It is to be noted that when fresh serum is used, important fluctuations

are frequently observed around 45° to 55°. These were *never* observed when the serum had been submitted to a heat of 55°, even for 5

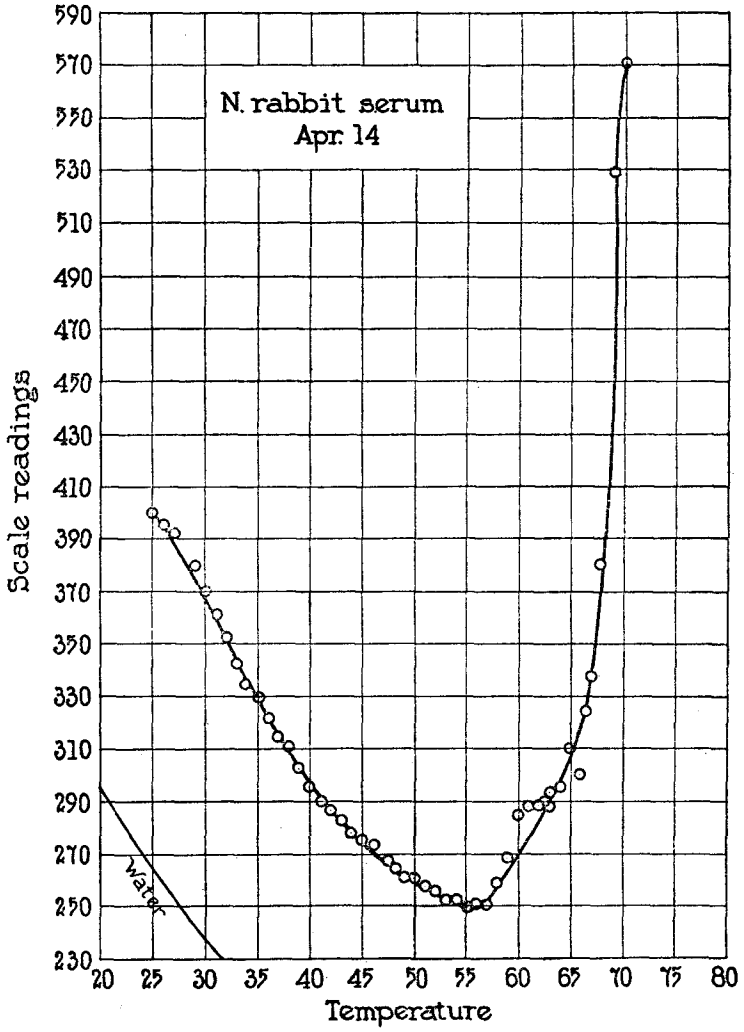

FIG. 1.

minutes, or when it was old. It appeared to us that it might be interesting to follow the phenomenon more closely, and to try and de-

termine whether the temperature played a specific part in the changes of viscosity, or whether the time of heating was the capital factor. In other words whether heating for 15 minutes at 55° would produce the same increase in viscosity as 5 minutes at 60°, for example. The first experiment was made with a serum heated at 55° for 10 minutes in a sealed tube. It is obvious from Fig. 2 that there is no fundamental change in the curve: the two control curves (white and black circles) almost coincide with that of the heated serum.

Another similar experiment is shown in Fig. 3. However instead of carrying the heating on up to the time when the spot goes off the scale, it was stopped, and the liquid was allowed to cool, according to the technique previously described. The white circles express the values of the heated serum 1 hour at 50°. The black circles are the values taken by the heated serum, on cooling, after having been kept at 57° for 5 more minutes. In general, no difference can be detected between heated and unheated serum when the serum is not heated above 55°. Up to 50° the curve expressing the viscosity of serum as a function of temperature is parallel to that of pure water, and its proteins play no part at all, or rather act only by their bulk, to displace the curve as a whole. From 50° on, a slight departure is observed; it goes on increasing until the minimum value is attained around 56°. Heating for 1 hour at 50° fails to alter permanently the viscosity of serum. The phenomenon is entirely reversible, as in the preceding case (Fig. 3).

Heating for 15 minutes at 55° acts in the same way. But as the heat was brought up to 56° in one series of measurements, while it was stopped at 55° in the other (Fig. 4) a small but marked difference could be detected between the two cooling curves. Taking evaporation into consideration, the slight increase in viscosity of the serum heated up to 55° can be accounted for, but the difference between this sample and the sample brought up to 56° is due to something else. Here the phenomenon is no longer reversible; the relative viscosity reaches 1.70. The same experiment was repeated with the same serum, heated for 15 minutes at 56° in a sealed tube. The mean viscosity was 1.70. The increase is small, but constant with this serum. It was not always observed with other sera. We can therefore state that, from our experiments, it appears that 56° is the lowest

temperature at which an irreversible phenomenon affecting its viscosity occurs in rabbit serum, in 15 minutes. However, an exception to this rule was found once. But if the heat is kept for 30 minutes at 56° (Fig. 5) the mean value of η climbs up to 1.77 and higher still on cooling. On the other hand in certain cases, 5 minutes at 56° (dog serum) may bring forth no change at all.

Another serum, the viscosity of which was normally high, heated up to 58° and cooled immediately after that temperature was reached, showed no modification. Serum 3 (η = 1.63) heated at 58° for 15 minutes, gave similar curves with η = 1.80. Half an hour at 58° brings the value of η up to 1.95.

Another serum heated for 1 hour, and 2 hours, at 58° (Fig. 6) reaches a viscosity of 1.85 (mean value) with a maximum of 1.90. 1 hour's heating of this serum at 60° fails to affect it more than 1 hour at 58°.

Fig. 7 is self-explanatory. The serum was heated in the cup, for 5 minutes, in all cases. As the temperature goes up, the different samples show a similar behavior. But on cooling, the differences between the different curves, according to the temperature reached, go on increasing. However, up to 62° no sign of structure in the liquid was found. The zero checked perfectly, and what was measured was true viscosity.

Fig. 8 summarizes for one animal a whole series of observations, showing which combinations of temperature and of time determined the same increase in viscosity. The question then arises as to what the increase in viscosity is due to, and how it can be interpreted.

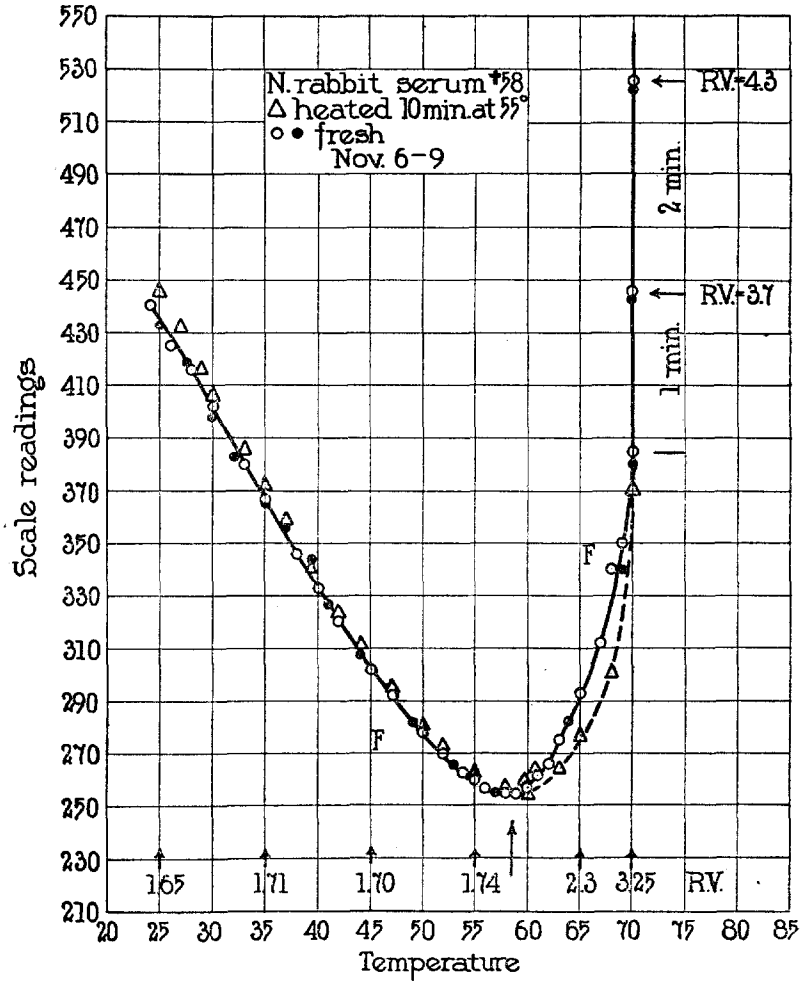
N. rabbit serum +58
△ heated 10min. at 55°
○ ● fresh
Nov. 6-9
R.V.=4.3
R.V.=3.7
2 min.
1 min.
F
F
Scale readings
Temperature
1.65
1.71
1.70
1.74
2.3
3.25
R.V.

FIG. 2.

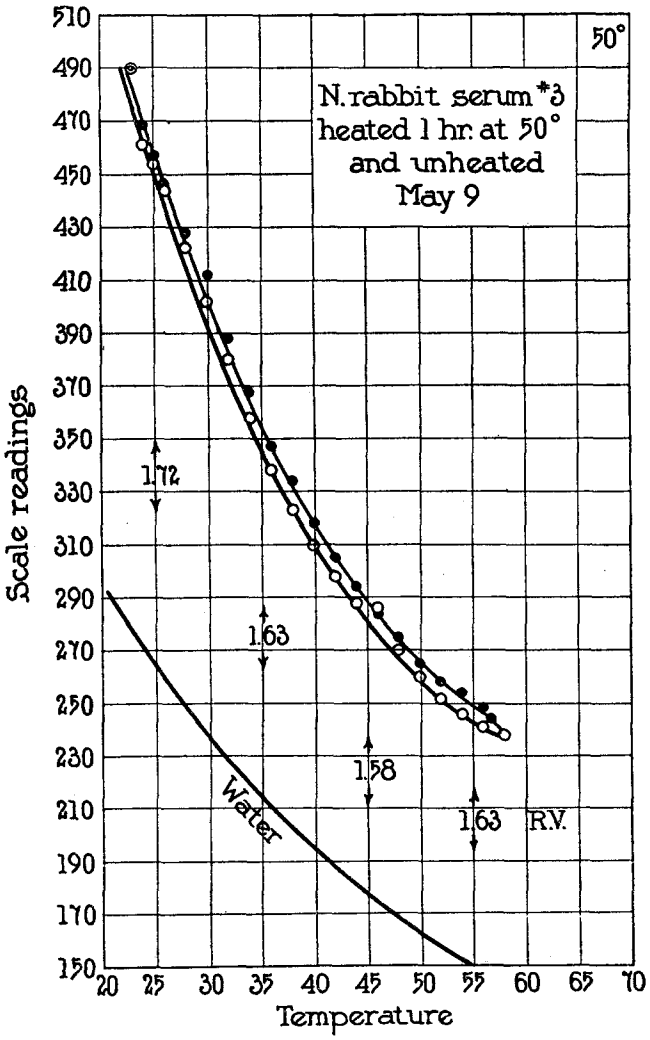
510
490
470
450
430
410
390
370
350
330
310
290
270
250
230
210
190
170
150
20 25 30 35 40 45 50 55 60 65 70
Temperature
Scale readings
50°
N. rabbit serum *3
heated 1 hr. at 50°
and unheated
May 9
1.72
1.63
1.58
1.63 R.V.
Water

FIG. 3.

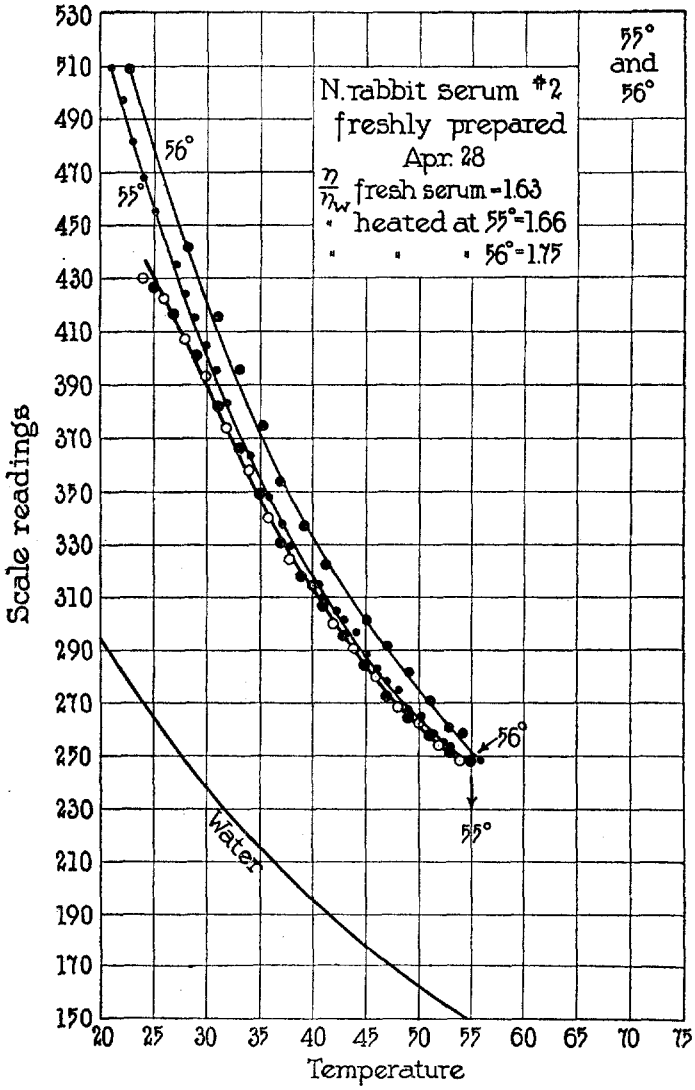
N. rabbit serum #2
freshly prepared
Apr. 28
η/η_w fresh serum = 1.63
" heated at 55° = 1.66
" " " 56° = 1.75
55° and 56°
56°
55°
56°
55°
Water
Scale readings
Temperature
530
510
490
470
450
430
410
390
370
350
330
310
290
270
250
230
210
190
170
150
20 25 30 35 40 45 50 55 60 65 70 75

FIG. 4.

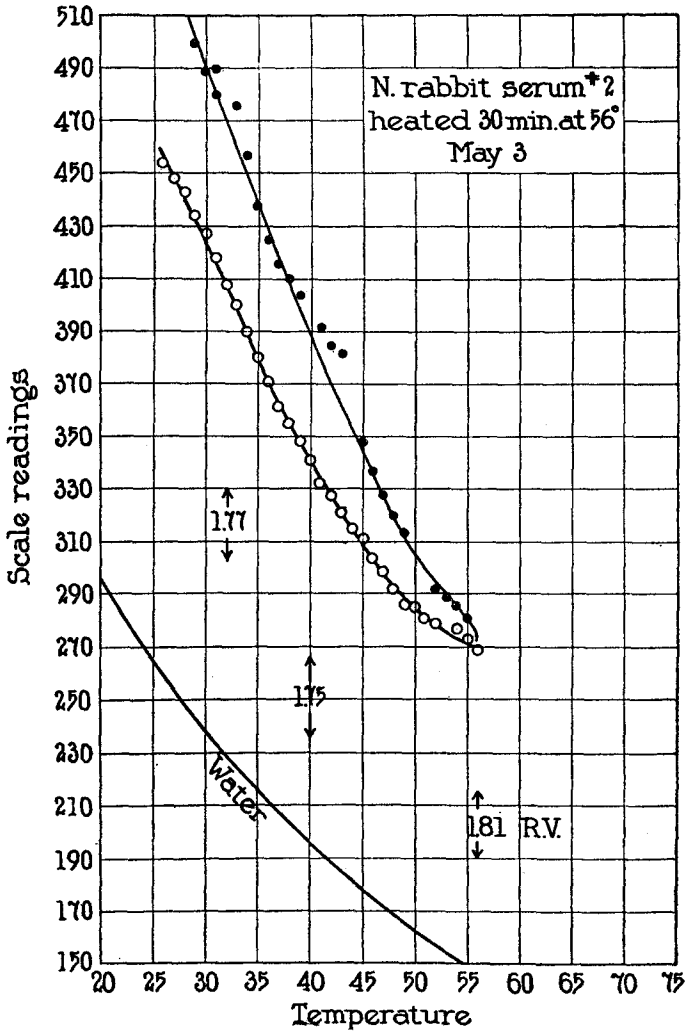
N. rabbit serum + 2
heated 30 min. at 56°
May 3
1.77
1.75
1.81 R.V.
Water
Scale readings
Temperature
510
490
470
450
430
410
390
370
350
330
310
290
270
250
230
210
190
170
150
20 25 30 35 40 45 50 55 60 65 70 75

FIG. 5.

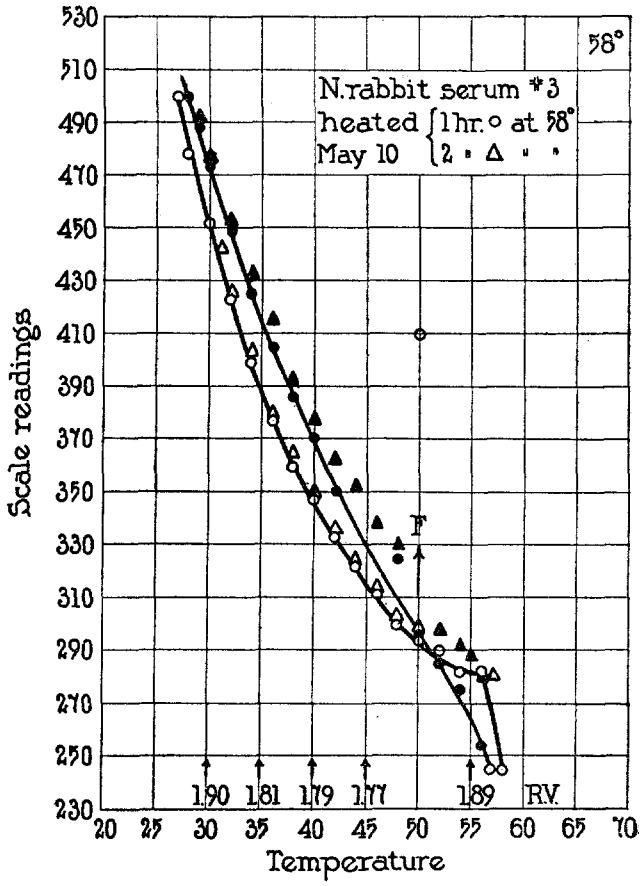
530
510
490
470
450
430
410
390
370
350
330
310
290
270
250
230
Scale readings
58°
N. rabbit serum #3
heated { 1 hr. ○ at 58°
2 " Δ " "
May 10
F
190
181
179
177
189
RV.
20
25
30
35
40
45
50
55
60
65
70
Temperature

FIG. 6.

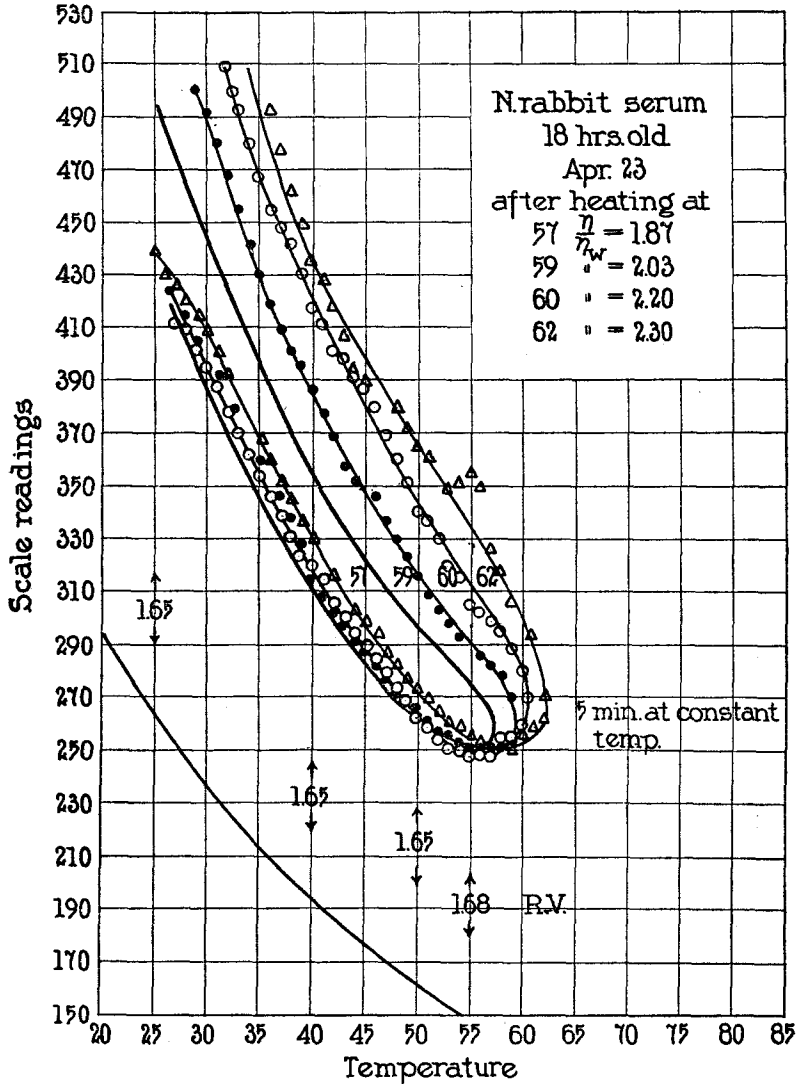
N. rabbit serum
18 hrs. old
Apr. 23
after heating at
57 η/η_w = 1.87
59 " = 2.03
60 " = 2.20
62 " = 2.30
5 min. at constant temp.
1.65
1.65
1.65
1.68 R.V.
57
59
60
62
Scale readings
Temperature
530
510
490
470
450
430
410
390
370
350
330
310
290
270
250
230
210
190
170
150
20 25 30 35 40 45 50 55 60 65 70 75 80 85

FIG. 7.

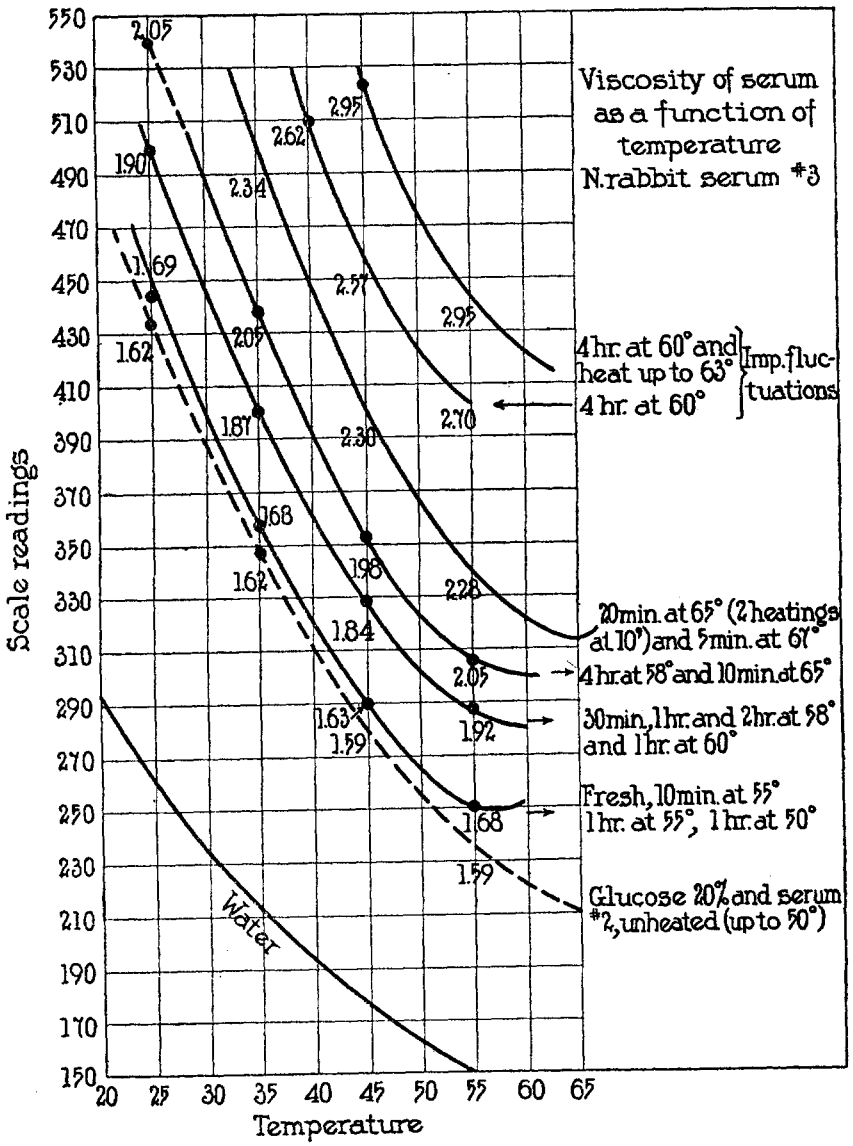
Viscosity of serum as a function of temperature
N. rabbit serum *3
4 hr. at 60° and heat up to 63°
4 hr. at 60°
Imp. fluctuations
20 min. at 65° (2 heatings at 10') and 5 min. at 61°
4 hr. at 58° and 10 min. at 65°
30 min., 1 hr. and 2 hr. at 58° and 1 hr. at 60°
Fresh, 10 min. at 55° 1 hr. at 55°, 1 hr. at 50°
Glucose 20% and serum *2, unheated (up to 50°)
Water
Scale readings
Temperature
550
530
510
490
470
450
430
410
390
370
350
330
310
290
270
250
230
210
190
170
150
20 25 30 35 40 45 50 55 60 65
2.05
1.90
1.69
1.62
2.34
2.09
1.87
1.68
1.62
2.62
2.57
2.30
1.98
1.84
1.63
1.59
2.95
2.95
2.70
2.28
2.05
1.92
1.68
1.59

FIG. 8.

DISCUSSION.

In our experiments the concentration remains constant, yet the viscosity increases. On the other hand, Einstein[2] states that the degree of dispersion, *i.e.*, the size of the molecules or particles is immaterial, and that viscosity can be expressed as a linear function of the volume fraction of the dispersed substance, according to the formula

$$\eta = 1 + 2,5\,\varphi \tag{1}$$

which is an approximation of the expression

$$\eta = \frac{1 + 0.5\,\varphi}{(1 - \varphi)^2} \tag{2}$$

where η stands for the relative viscosity of the suspension, and φ for the volume occupied by the dispersed substance expressed as a fraction of the total volume of the solution. These equations, however, fail to express the experimental facts. The reason for this lack of agreement is not clear. But Kunitz has recently proposed an empirical formula which applies remarkably well to a number of widely different cases, including lyophilic and lyophobic sols, and within large limits of concentration. This formula is[3]

$$\eta = \frac{1 + 0.5\,\varphi}{(1 - \varphi)^4}. \tag{3}$$

The problem we are facing can be stated as follows: Assuming that Einstein's view concerning the part played by φ as defined above is correct, and that the degree of dispersion is immaterial, we can use formula (1), and compute the values of φ, and consequently determine $\frac{\varphi}{C}$ which expresses the specific volume of the solute (proteins). These values divided by the specific volume of the dry proteins should give the amount of hydration of the proteins in solutions, at temperatures up to 55°, and its increase as a function of temperature. But we know that this formula does not apply to lyophilic sols at high concentration, consequently, we cannot rely on the figures at all.

[2] Einstein, A., *Ann. Physik.*, 1906, xix, 289; 1911, xxxiv, 591.
[3] Kunitz, M., *J. Gen. Physiol.*, 1926, ix, 715.

On the other hand we can apply Kunitz's formula (Table I) which we know fits the experimental facts very satisfactorily but then the main assumption of Einstein concerning the rôle of the degree of dispersion, which is a consequence of his mathematical derivations, may not hold any longer. Therefore, it does not seem possible, at present, to decide whether the figures computed in this way express quantitatively the increase due to hydration alone or whether some other

TABLE I.

Viscosity of Rabbit Serum 3, Heated at Different Temperatures, and Values of φ *and* $\frac{\varphi}{C}$ *Computed from Kunitz's formula (see Figs. 7 and 8).*

C = mean concentration of proteins in the serum = 6.5 per cent.

1	2	3	4	5	6	7
Relative viscosity $\frac{\eta}{\eta_0}$	φ	Specific volume $\frac{\varphi}{c}$	Specific volume of dry proteins	Ratio $\frac{\text{Column 3}}{\text{Column 4}}$	Increase per cent of specific volume (Hydration ?)	Increase in hydration due to heating
	per cent				*per cent*	*per cent*
1.65	10.7	1.645	0.785	2.09	109	Unheated
1.69	11.2	1.720	"	2.19	119	
1.88	13.3	2.030	"	2.58	158	39
2.03	14.8	2.275	"	2.90	190	71
2.30	17.2	2.650	"	3.37	237	118
2.62	19.8	3.050	"	3.88	288	169
2.95	21.8	3.350	"	4.27	327	208

phenomenon is also responsible for it to a certain extent. However, column 6 has been tentatively designed as "hydration."[4]

[4] The influence of the charge of colloidal particles on the viscosity of the sol has been taken in consideration, especially by von Smoluchowski (*Kolloid-Z.*, 1916, xviii, 194.) who enlarged Einstein's formula which became:

$$\eta_s = \eta_M \left\{1 + 2.5\,\varphi \left[1 + \frac{1}{\lambda\,\eta_M\,r^2}\left(\frac{D\,\zeta}{2\,\pi}\right)\right]\right\}$$

however our results are not in accord with this formula, which should lead to a lower viscosity as the size of the particles increases. We observe the contrary, and there seems to be no doubt that after heating the size of the particles increases, since coagulation is the limit of the phenomenon. Perusal of Freundlich's excellent discussions of the subject in general (Colloid and capillary chemistry, New York, pp. 367 and following; 539, etc.) will repay the reader.

All that can be said is that it expresses the increase per cent of the specific volume of the serum proteins. It is interesting to note that the figures expressing "hydration" obtained from Einstein's formula (3) are larger (3.71 times) than those obtained from Kunitz's formula (1), and that this ratio remains constant up to a viscosity of 2.03. From this figure up the discrepancy begins and increases rapidly with increasing viscosity.

Fig. 8 suggests another observation: the dotted curve (viscosity 1.62) was obtained with a sugar solution (glucose 20 per cent). The concentration is 3 times that of the serum. Hence, serum may be said to behave exactly like a true solution, as a function of temperature, up to nearly 55°. It is surprising that such a concentrated solution of proteins should have such a low viscosity. When, by diluting the serum, its specific volume is made equal to that of the sugar solution, its viscosity is much inferior to that of the latter.