COMPLEMENT ACTION IN REGARD TO SURFACE TENSION.

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CONTENTS.

Introduction.

In recent years immunity reactions have come to be regarded more particularly from the physico-chemical standpoint. Many of the phenomena occurring in immunity reactions are shown to be of the nature of colloidal chemical processes, and the laws which govern such processes have been found to hold, at least partially, for reactions occurring in sera, which belong to the class of colloidal protein solutions. Thus the application to immunity reactions, of such conceptions as viscosity and surface tension, has afforded a more reasonable explanation of these reactions. In the following paper special reference will be made to the surface tension, changes of which play an important part in all immunity reactions.

Traube (1908) showed, that if fresh guinea-pig serum was heated for half an hour at 55°, its surface tension was diminished. Thus the inactivation by heat caused the production of substances, which lower the surface tension. Addition of fresh serum had the effect of restoring the complement action and causing the return of the surface tension to its original value after 20 hours. Traube showed further, that if the serum is only heated to 40°–50° the diminution of the surface tension is less marked and complete restoration may occur spontaneously. Also the ageing of a serum is associated with inactivation and a loss of surface energy. These bathotonic substances, produced in the serum by thermoinactivation, must be adsorbed by the surface of any second phase, which may be added. Thus in the case of red corpuscles, according to Traube, a thickening of their lipoid sheath would occur owing to the adsorption of such substances present in an inactive serum, the result being an increase in their resistance to the haemolytic influence of the anchored amboceptor. These substances act therefore like antihaemolysins by weakening the effect of specific haemolysins. Now, according to Traube, complements are substances which annul this antihaemolytic resistance by destroying the lipoid sheath of the red cells, thus rendering the action of an haemolytic amboceptor effective. Traube supposes the anticomplementary power of some active sera to be due to the presence of peptone, which is a strong bathotonic substance by itself. With regard to the objection that the addition of sera, which have stood for a long time, produces the same effect on thermoinactive sera as the addition of fresh active serum, the author mentioned, that a thermoinactive serum, even in a dilution of 1 in 30, had still a well-marked anticomplementary action, although the value of its surface tension was scarcely to be distinguished from that of an equally concentrated active serum. His conclusion is therefore, that the smallest amount of bathotonic substances, too small to be traced by a stalagmometer, may produce anticomplementary action.

Mario Segale (1911) confirmed by his experiments those of Traube in regard to the alteration of the surface tension occurring in thermoinactivation and its restoration to the original value after 20 hours by adding fresh active serum. By means of the ultramicroscope Segale was able to show the existence of micelli produced by the action of heat on serum, but further experiments showed that the alteration of the surface tension is not due to the existence of these micelli. He found also that the complementary function of a mixture of equal parts of active and thermoinactive serum was diminished to 50 %, although the value of the

surface tension was quite normal, and *vice versa*, after centrifugalising the micelli out of a thermoinactive serum and adding them to a fresh serum, he was able to obtain a lowering of surface tension without an alteration of the complementary function of the serum. He concludes therefore that the bathotonic substances, which were produced either by the action of heat or of age, have nothing to do with the complement action of the serum.

In the following paper I give a record of a series of experiments undertaken to elucidate the possibility of an association between the value of the surface tension of a serum and its complementary function.

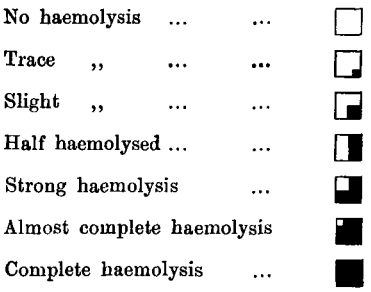
Technique of experiments.

The value of the static surface tension of a liquid was measured by the number of drops produced by the same volume of liquid under equal conditions. The number of drops does not give an absolute measurement of the surface tension, which latter is to be expressed by dyn./cm., but the observed values of the number of drops can be compared with each other and with that of water, because the formation of drops is, under equal conditions, a function of the surface tension. The measurements were made by a special stalagmometer, designed by Traube and manufactured by C. Gerhardt, Bonn. For my experiments I used three of them, which gave different numbers of drops for distilled water at the temperature of 20° C. (57·62, 58·89, 57·2). In order to obtain a better comparison of the values obtained by these different stalagmometers, I calculated the number of drops, which would be produced by a normal stalagmometer giving 100 drops for a certain volume of distilled water at the temperature of 20° C. If *Dw* indicates the number of drops for distilled water and *Df* the number of drops found for the liquid in question, I give in the following experiments the values of *D* as calculated by the equation

$$D = \frac{100}{Dw} \cdot Df.$$

Only the haemolytic complement of guinea-pig serum has been examined. Its complementary function was tested by its haemolytic power in combination with sensitized, three times washed sheep red corpuscles of which a 5 % emulsion in 0·85 % saline solution was used. The amboceptor was inactivated rabbit serum, the single lysing dose of which was 0·00125 c.c. by using 1 c.c. of the red cells emulsion and 0·1 c.c. of the complement-containing serum. In the following haemolytic tests

always the double amount of the above mentioned amboceptor dose was taken. To illustrate the different degrees of haemolysis the following scheme is used:



Alteration of the surface tension by storage.

First of all I was able to confirm the observations made by Traube and Segale that in complement-containing sera, which have been kept for some time, an alteration of the surface tension takes place. This alteration occurs sooner the higher the temperature at which the serum is kept, just as the complementary power of serum disappears under similar conditions of time and temperature. But the alteration of the surface tension is relatively small and never reaches values, which correspond to those obtained by the action of heat (55°–56°), and further the inactivation occurring in old complement-containing sera is in most cases not complete, for it is possible to observe a more or less strong complementary action occasionally even after the serum has been kept for weeks, if the complement dose is 0·1 c.c. or higher and strongly sensitized red corpuscles are taken. K. Hara (1913) lays special stress on the bacterial growth as causing the loss of complementary power and the formation of anticomplementary properties, which some sera develop if kept for a long time. How far the bacterial growth in old sera is responsible for the lowering of the surface tension is still a question for experiment. In any case, according to Hara, serum, if kept under sterile conditions, preserves its complement action much longer than sera for which sterility has not been observed. I found that neither freezing nor thawing altered the surface tension more than may be due to the time during which the serum is kept under these conditions. It is well known that low temperature has a preserving influence on immune bodies as well as on complement, and Ito (1912) was recently able to show that even the temperature of liquid air did not destroy the complementary function of a serum. This author found that, if serum is

kept a long time at a low temperature, it is disposed in layers such that the layer of serum at the bottom of the tube has a much stronger complementary action than the supernatant fluid. It would be interesting to know how far this phenomenon is due to surface energies.

Alteration of surface tension by dilution.

In most serological experiments the complement-containing serum is employed in a dilution of 1 : 10 in 0·85 % saline solution. Saline solution increases the surface tension of pure water in a slight degree, the "surface tension-concentration" curve being a straight line (Freundlich, 1909, p. 60). For an 0·85 % saline solution I obtained $D = 99{\cdot}59$. The surface tension of a serum increases with its dilution in saline solution, but here the "surface tension-concentration" curve does not follow a straight line, but is represented by a parabolic curve (Iscovesco, 1911 *a*) of which I give the figures in the following table:

EXP. I. *Alteration of the surface tension of fresh guinea-pig serum effected by dilution in* 0·85 % *saline solution.*

Concentration	Serum I D	Serum II D
1 : 1	110·98	110·37
1 : 1·25	—	108·76
1 : 1·5	—	107·92
1 : 2	107·75	106·78
1 : 3	106·53	105·58
1 : 4	106·09	104·94
1 : 5	—	103·99
1 : 7	105·61	—
1 : 8	—	103·55
1 : 10	105·05	103·07
1 : 20	104·88	102·07
1 : 40	103·91	101·24
1 : 80	102·52	100·72
1 : 320	100·54	—

For comparison I give in the following table figures representing the number of drops which have been obtained by the dilution of peptone with distilled water.

These figures if plotted give a curve of a similar type. According to Freundlich (1909, p. 65) such a curve can be represented in a first approximation by a general parabolic equation of the form

$$S_m - S_l = K \cdot C^{\frac{1}{n}}$$

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in which K and n indicate constants, S_m the surface tension of the pure solvent and S_l that of the solution. The value of the surface tension can be calculated from the number of drops by a proceeding mentioned by Traube (1904 *b*). From these figures it may be concluded, that a fresh serum itself contains bathotonic substances (protein? salts of fatty acids? free gallic acid?), the tendency of which to lower the surface tension must decrease with their dilution.

EXP. II. *Relation between the surface tension and the concentration of peptone.*

c.c. of a 5% peptone soln. in aqu. dist. added to 20 c.c. of distilled water	Resulting concentration of the peptone soln. %	D
0	0	100·0
0·05	0·0125	103·62
0·05	0·024	109·79
0·05	0·037	112·15
0·1	0·061	115·33
0·1	0·086	118·67
0·1	0·110	118·88
0·2	0·157	120·61
0·2	0·203	120·74
0·5	0·316	123·72
1·0	0·526	125·48
2·0	0·893	128·28
2·5	1·27	130·29

Alteration of surface tension by temperature.

Further I was able to confirm the observations of Traube and Segale, that a serum, if heated to 56° for half an hour, has its surface tension diminished by a considerable amount. If for instance the number of drops given by an active serum diluted in 1:10 by saline solution is 102·25, the same serum after being heated for half an hour to 55°–56° gives 107·29 drops. The surface tension of an undiluted active serum giving 110·73 drops, is so diminished by the effect of heating at 55° for half an hour, that 114·24 drops are obtained. If such a heated, undiluted serum is diluted by saline solution its surface tension will increase, following the same curve as mentioned above, and from the following figures it can be demonstrated, that the number of drops belonging to a concentration of 1:20, does not differ from that corresponding to the same concentration of an active serum.

EXP. III. *Influence of dilution on the surface tension of active and thermoinactive serum.*

Concentration	*D* of active serum	*D* of inactive serum 30 mins. 56°
1 : 1	110·73	114·24
2	106·63	109·37
3	105·24	107·48
4	103·97	106·49
5	103·69	106·19
6	103·46	105·35
7	103·27	—
8	103·07	104·57
10	103·02	104·50
20	102·08	102·72
40	101·41	101·64
80	100·80	101·05

Traube (1908) has already mentioned these facts and said that the difference in complementary power is due to such a small amount of bathotonic substances, as cannot be measured by an ordinary stalagmometer. I think it is evident that in this case the difference in the complement action has no association whatever with the surface tension of the serum. If a concentrated fresh active serum gives about the same number of drops as the same serum gives if diluted to 1 : 2 and inactivated by 30 minutes exposure to 55°, it is difficult to construct an association between surface tension and complementary function of the serum.

Now the loss of surface tension due to the effect of a temperature of about 55° takes place much sooner than in half an hour, as can be shown in the following experiments.

EXP. IV. *Alteration of surface tension in relation to time of exposure at* 55°.

Undiluted complement-containing serum was exposed to 55° for varying times. It was then diluted 1 in 10 with saline solution and tested with regard to surface tension and complement action. (1·0 c.c. of the diluted serum in contact with 1 c.c. of the sensitized red cell emulsion.)

Time of exposure to heat	Haemolysis	*D*
0 mins.	■	102·24
6	□	105·01
12	□	105·12
18	□	104·96
24	□	105·07
30	□	104·98

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The result shows that the value of the surface tension which is obtained by heating the serum to 55° is nearly reached in the same time in which the complement action of the serum is destroyed. (However after that short time the complement action can be restored either by adding fresh end-piece or by splitting the complement into the two fractions and mixing these together again, but after the influence of heat for half an hour at 55° C. such restoration is no longer possible, cf. H. Schmidt, 1913.) But if a serum which has been heated at 55° C. is subjected to the influence of a higher temperature, a further fall of surface tension occurs. A 1:10 diluted serum becomes an opalescent fluid if heated in boiling water and the colloidal state of the denaturated protein is of a very stable nature. The alteration of the surface tension in such a serum may be shown by the following figures:

EXP. V. *Alteration of the surface tension of a* 1:10 *diluted active serum at the temperature of boiling water.*

	D
1 : 10 diluted serum active	103·13
Do. after half an hour's exposure to 55° C.	108·93
Do. after heating in boiling water ...	112·04

I found in almost every serum which I examined, that the value of its surface tension, if it is heated only during 6 minutes to 55°–56°, is nearly the same as that which is reached by the effect of the same temperature in half an hour. Also the same short period of 5–6 minutes was sufficient to inactivate the serum, and the following experiment shows that the concentration under which the serum is heated does not influence the time necessary for the thermoinactivation.

EXP. VI. *Concentration of the serum in regard to the time of thermoinactivation.*

1·0 c.c. of fresh serum was exposed to 56° in different concentrations varying between 1:1 and 1:10 during different periods varying from 0 to 5 minutes. The serum was then diluted to 1:10 with saline solution and its complementary power tested. The result was that after three minutes no haemolysis occurred in any case, the control however giving good haemolysis.

The same astonishingly short time was found sufficient to render a serum inactive by heat in the recent experiments of Husler (1912). With regard to a paper of Noguchi and Bronfenbrenner (1911), however, who mentioned that sera treated in such a way are not completely

inactivated and that it is possible by using much larger doses than the usual 0·1 c.c. to demonstrate that the exposure of the serum to 55° even after half an hour is not sufficient to render it completely inactive, I give in the following the record of an experiment.

EXP. VII. *Influence of time in thermoinactivation.*

Haemolysis of 1·0 c.c. sensitized red cells by

c.c....	1·0	0·5	0·25	0·15	0·1	0·0
1 : 10 serum active	■	■	■	■	■	□
1 : 10 serum treated 5 mins. at 55°	□	□	□	□	□	□
1 : 10 serum treated 30 mins. at 55°	□	□	□	□	□	□

I.	0·15 c.c. 1 : 10 active serum	■
II.	Of the thermoinactive serum (5 mins. at 55°)	
	0·15 c.c. 1 : 10 — Same dose as in I ...	□
	0·15 c.c. 1 : 5 — Twice the dose in I ...	□
	0·15 c.c. 1 : 10 — 10 times the dose in I	□
	0·3 c.c. 1 : 1 — 20 times the dose in I	□
	0·6 c.c. 1 : 1 — 40 times the dose in I	□
	0·9 c.c. 1 : 1 — 60 times the dose in I	□
III.	Of the thermoinactive serum (30 mins. at 55°)	
	0·15 c.c. 1 : 1 — 10 times the dose in I	□
	1·5 c.c. 1 : 1 — 100 times the dose in I	□

From these figures I conclude that at least in the case of guinea-pig serum the thermoinactivation has been complete after an exposure of 5 minutes to 55°, but these data are probably not transferable to the sera of other animals.

Surface tension in regard to reactivation of thermoinactive serum.

By adding fresh serum to a thermoinactive serum a complete haemolytic effect can be obtained. The amount of fresh serum necessary for this effect depends upon the anticomplementary action of the thermoinactive serum, which may occasionally be so great, that the haemolytic action finally obtained by adding fresh serum, is merely due to the absolute amount of the latter, so that one cannot speak of a real restoration of the complementary action of thermoinactive serum.

I have been able to confirm the experiments of Traube and Segale, that after the loss produced by the heat, the surface tension reaches its normal level again after 24 hours by adding fresh active serum. But in order to recognise how far the haemolytic action produced in thermoinactive serum by addition of fresh serum agrees with the alteration of the surface tension I instituted certain experiments, of which the following may be quoted:

EXP. VIII, 1. *Alteration of surface tension occurring in thermoinactive serum by adding fresh active serum, compared with the haemolytic effect thus produced.*

1·0 c.c. sensitized red corpuscles plus

Of	Relative amount of active ser.	c.c. 1·0	0·75	0·5	0·25	0·15	0·1	0·0	*D*
I. 1:10 active serum ...	1:10	■	■	■	■	■	◪	□	103·33
II. 1:10 thermoinactive ser. (30 mins. 56°)	0	□	□	□	□	□	□	□	106·29
III. 22·0 c.c. of II + 0·2 c.c. 1:1 active ser.	1:100	◪	◪	□	□	□	□	□	105·88
IV. III + 0·2 c.c. active ser.	1:51	■	◨	◪	◪	□	□	□	106·23
V. IV + 0·3 c.c. ,, ,,	1:26·7	■	■	■	■	◨	◪	□	105·94
VI. V + 0·4 c.c. ,, ,,	1:15·5	■	■	■	■	■	◪	□	106·10

After the addition of active serum in III–VI, 2 c.c. of the mixture were taken off each time for the haemolytic test.

EXP. VIII, 2. *Instead of undiluted active serum, a* 1 *in* 10 *dilution was taken, but otherwise no alteration of the technique took place.*

1·0 c.c. sensitized red corpuscles plus

Of c.c....	1·0	0·5	0·25	0·15	0·0	*D*	Relative amount of active serum in the various mixtures
I. 1:10 active serum ...	■	■	■	◪	□	102·89	1:10
II. 1:10 inactive ser. (30 mins. 56°)	□	□	□	□	□	105·85	0
III. 20·0 c.c. of II + 0·5 c.c. 1:10 active ser.	□	□	□	□	□	105·30	1:410
IV. III + 1·0 c.c. 1:10 act. ser.	◪	□	□	□	□	105·33	1:130
V. IV + 2·0 c.c. ,, ,,	■	◪	◪	□	□	105·36	1:55·7
VI. V + 3·0 c.c. ,, ,,	■	◪	◨	◪	□	105·08	1:31·4
VII. VI + 3·5 c.c. ,, ,,	■	■	◪	◨	□	104·96	1:22
VIII. VII + 3·0 c.c. ,, ,,	■	■	■	◪	□	104·89	1:17·7

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Setting aside the question whether the haemolytic effect obtained by the addition of fresh serum to thermoinactive serum, is entirely due to the action of the active serum, after the anticomplementary power of the thermoinactive serum has been neutralized, or to a genuine restoration of the complementary power of the inactive serum, the experiment VIII shows, that haemolytic action can be obtained before the surface tension is altered in any effective degree. The value of the surface tension approached the original value very closely after about 20 hours (but not in all cases), the small difference still existing in the following figures being probably due to the influence of age.

Exp. VIII.	$\frac{1}{10}$ active serum	103·33
	$\frac{1}{2}$ hour 56°	106·29
	2·2 c.c. inactive serum + 1·1 c.c. + act. ser.	106·10
	After about 24 hours	103·92

The bathotonic substances produced by the thermoinactivation are naturally in a relatively higher concentration on the free surface. If therefore a large separating funnel is filled with an inactive serum, the surface tension may be expected to increase, if the lower part of the liquid is run out from time to time so as to permit the formation of a new surface (*v.* Exp. IX)

Exp. IX.	$\frac{1}{10}$ inactive serum (30 mins. 56°)	107·88
	Lowest portion	106·05
	Mixed together again ...	108·02

Experiment IX shows that a procedure of this kind does not produce any very appreciable increase of the surface tension. I have not observed any restoration of the complementary power of the serum by this treatment.

Alteration of surface tension in serum effected by addition of peptone.

The anticomplementary action of some sera (for instance in cases of uraemia) is believed by Traube (1908) to be due to the effect of small amounts of peptone. Peptone Witte lowers the surface tension of water considerably. As already mentioned (Exp. II), I found that the number of drops corresponding to a 1·27 % peptone solution in water was 130·29. If therefore the inactivity of a serum is in direct relationship with the low surface tension, it may be possible to render an active serum inactive by adding some peptone, the more so, because the loss of surface tension

produced by peptone is much greater than can be obtained by thermo-inactivation.

The following experiment shows the action of peptone on serum with regard to surface tension.

EXP. X. *Influence of peptone on surface tension and activity of serum.*

D of a 2 % peptone solution in 0·85 % NaCl solution = 134·59.

	Haemolysis after...	¼ hr.	½ hr.	1 hr.
1 c.c. complement + 1 c.c. saline sol. + 2 c.c. sensitized red blood cells emulsion		■	■	■
1 c.c. complement + 1 c.c. 2 % peptone + 2 c.c. sensitized red blood cells emulsion		□	■	■
1 c.c. saline sol. + 1 c.c. 2 % peptone + 2 c.c. sensitized red blood cells emulsion		□	□	□

In the following experiment instead of 0·85 % saline as diluent, peptone in a dilution of 2 % in saline solution was taken as dilution medium of the complement-containing serum.

	c.c. of 1 : 10 complement...	1·0	0·5	0·25	0·15	0·0	D
1.	1 c.c. active complement + 9·0 c.c. 2 % peptone in 0·85 % saline solution + 1 c.c. sensitized red blood corpuscles	■	■	■	◪	□	105·05
2.	1 c.c. active complement + 9·0 c.c. 0·85 % saline solution + 1 c.c. sensitized red corpuscles	■	■	■	◪	□	134·05

In spite of the differences in the surface tension no difference in the action of the complement can be observed. In mixture No. 1 a 1·6 % peptone solution results, but nevertheless no anticomplementary action is observed.

In the following experiment instead of a 2 %, a 20 % peptone solution is taken.

20 % peptone solution in 0·85 % NaCl		0·85 % NaCl solution		$\frac{1}{10}$ Compl. serum		Sens. red corpuscles	
0·2	+	0·8	+	1·0	+	1·0	■
0·4	+	0·6	+	1·0	+	1·0	◪
0·6	+	0·4	+	1·0	+	1·0	□
0	+	1·0	+	1·0	+	1·0	■
0	+	0	+	0	+	3·0	□

These strong peptone solutions show an anticomplementary effect.

1 : 10 active compl.		20 % peptone saline solution	D	
15 c.c.	+	0	102·93	In all tubes *Haemolysis complete*, but a slight dimness remained.
15 c.c.	+	0·1 c.c.	117·97	
15 c.c.	+	0·2 c.c.	121·53	
15 c.c.	+	0·4 c.c.	123·75	
15 c.c.	+	0·8 c.c.	123·96	

Experiment X shows that it is impossible to render a serum inactive by adding some peptone and thus lowering the surface tension. A relatively large amount of peptone is required in order to exhibit anticomplementary action.

From the experiments above mentioned it is evident that the value of the surface tension does not permit one to form any conclusions with regard to the complement action.

Surface tension with regard to inactivation by adsorption.

The following experiments deal with the behaviour of the surface tension in response to certain other procedures which deprive a serum of its complementary action. Many authors (see Sachs, p. 870) have shown the possibility of removing the complement by digestion with suspensions of organic cells or inorganic substances. The adsorption of the complement by kaolin is especially strong, as the experiments of Landsteiner and Stankovic (1906) have shown, the results of which are confirmed by Friedberger and Salecker (1911), who found that a contact of 2·0 c.c. normal guinea-pig serum with 0·2 c.c. kaolin for half an hour is sufficient to adsorb the complement completely. After centrifugalising a serum which has been treated with kaolin the supernatant fluid is found to contain a poisonous substance, which, if intravenously injected, is able to kill guinea-pigs in a very short time (Mutermilch, 1913). There is still some dispute as to the identity of this substance with the so-called anaphylatoxin. Friedberger, Salecker and also Mutermilch found that kaolin did not produce this poison, if the serum has been previously inactivated by heat at 56°. Mutermilch showed further that the serum became the more poisonous for guinea-pigs the larger the amount of kaolin which was employed to remove the complement, and he took the quantity of removed complement as a measure of the toxicity of the serum. By employing sulphate of barium (3 g. $Ba(SO_4)_2 + 8$ c.c. undiluted serum) as adsorbent, he observed neither loss of complement nor any formation of the presumed poisonous substance. The following experiment shows the alteration of the surface tension produced by kaolin in complement-containing serum.

These figures show, that the treatment by kaolin has produced in all three sera such an increase of the surface tension as to render the latter about equal to that of water, but the supernatant fluid in 1 and 2 was of a yellowish colour compared with the water-clear fluid in 3. Only the latter proved to be free of protein and no change occurred on

reheating, while by this procedure in the fluids (1) and (2) a well-marked fall of surface tension was observed and protein could be traced by boiling and adding acetic acid. Segale (1911), as already mentioned, found that the thermoinactivation of a serum was associated with a decrease of the degree of dispersion of the colloidal protein substances and the

EXP. XI. *Change of surface tension produced by kaolin.*

	Before the treatment	*After* by kaolin	Centrifugalised and then exposed for ½ hour to 56°	Centrifugalised and then exposed for ½ hour to boiling temp.
	D	*D*	*D*	*D*
1. 1:10 diluted active serum	102·25	100·02	107·29	108·43
2. 1:10 diluted serum ½ hr. exposed to 56°	108·16	101·02	—	107·99
3. 1:10 diluted serum boiled	112·04	100·42	100·42	100·5

same occurs on diluting a serum, according to P. Schmidt (1912). Now it is known (Sachs, 1913, p. 871) that the degree of dilution is an important factor in the adsorption of complement by any adsorbent, in the sense that the concentration is inversely proportional to the adsorption.

This dependence on concentration appears to be due probably to the lower degree of dispersity of the colloid in diluted sera, but the experimental data quoted above show that in thermoinactive serum as well as in active serum, kaolin does not remove all proteins. This effect took place only in the case of boiled serum, the degree of dispersity being then very small. Now the fact that in inactivated serum, there is still some protein left after the treatment by kaolin, renders the statement of Mutermilch and Friedberger, that such serum has lost its toxic property, the more interesting, especially if the surface tension is considered, which in both cases is rendered higher than that of the original serum. (Undiluted serum gives the same phenomenon, only the proportions of the figures being changed.) Friedberger (1911) found that the amboceptor is not removed from an immune serum by kaolin. This latter fact, taken in conjunction with the statement that kaolin removes all albumin, led him to suggest the possibility of proving the non-protein nature of the amboceptor. From my experiments, however, I think this suggestion to be very improbable.

In order to compare the adsorption of complement by kaolin with that obtained by $Ba(SO_4)_2$ or a suspension of red cells, the following experiment was undertaken.

EXP. XII. *Change of surface tension in inactivation by means of mechanical adsorption.*

1. 1 c.c. guinea-pig serum + 9 c.c. 0·85 % sal. sol. + 0·7 g. $Ba(SO_4)_2$.
2. 1 c.c. ,, ,, + 9 c.c. ,, ,, + 0·7 g. kaolin.
3. 1 c.c. ,, ,, + 9 c.c. of a 7 % red cell emulsion.
4. 1 c.c. ,, ,, + 9 c.c. 0·85 % sal. sol. ($D = 103·18$.)

$Ba(SO_4)_2$ and kaolin were previously purified and neutralized.

These mixtures were kept at 37° for 1 hour and shaken from time to time, then centrifugalised and the supernatant fluid tested with 1 c.c. sensitized red corpuscles, all tubes being filled up to 2 c.c.

c.c....	1·0	0·75	0·5	0·25	0·15	0·0	*D*
1. $Ba(SO_4)_2$	■	■	■	■	◨	□	102·79
2. Kaolin	□	□	□	□	□	□	101·73
3. Blood cells	■	■	■	■	■	□	103·69
4. Control	■	■	■	■	■	□	104·96

The experiment shows that only in the case of kaolin is the serum completely inactivated and the highest value of surface tension reached. While the power of adsorbing complement is common to most cell suspensions, the red corpuscles form an exception, only their stromata being able to adsorb complement (Sachs, 1913). The relatively low surface tension, which is observed in the case of the red cells, is probably due to the slight amount of haemolysis, which occurred in the mixture and which causes lowering of surface tension (Iscovesco, 1911 *b*).

I mention in this connection that I found neither mid- nor end-piece able to reactivate the supernatant fluid of a kaolin-treated serum and further that this fluid obtained by the treatment either of an active or of an inactive serum by kaolin has no anticomplementary action, if added to a haemolytic system.

Surface tension in regard to inactivation by mechanical agitation.

A further method of inactivating a serum is the treatment by means of mechanical agitation, a method inaugurated and known by the experiments of Jakoby and Schuetze (1910). Reference is made to this method in a special paper (H. Schmidt, 1913) and therefore I refrain from referring to the many recent works devoted to this subject, and I give here in this connection only a record of my experiences in connection with the inactivating of complement by mechanical agitation in so far as surface tension is concerned.

If a serum is shaken a more or less stable froth is formed. The formation of a froth is due to two circumstances: first of all, to the presence of a substance, which lowers the surface tension of the water and accordingly has the tendency to aggregate in the free surface, and secondly to the effect of surface viscosity, such as occurs in the case of bathotonic substances forming membranes on the surface (like peptone for instance). (Metcalf (1905), Freundlich (1909), p. 303, Ramsden (1804, 1904).)

If therefore water containing bathotonic substances, which can produce membranes on the surface, is shaken in air, the free surface of the liquid against air is enormously enlarged by the froth thus formed and a relatively larger amount of the bathotonic substances are to be found in the froth than in the liquid. By removing the froth and repeatedly shaking the liquid several times it may be possible to extract the bathotonic substances out of the liquid and to concentrate them in the centrifugalised froth. On this point I give the following experimental data obtained with complement-containing serum.

EXP. XIII. *The surface tension and complement activity of the froth compared with those of the remaining fluid.*

Fresh active complement-containing serum diluted 1 : 10 is shaken and from time to time the froth removed and centrifugalised. Afterwards both, froth and serum, were tested by the stalagmometer and with 1 c.c. sensitized red corpuscles in regard to their complement action, each tube being filled up to 2 c.c.

	D	c.c. 1·0	0·5	0·25	0·15	0·1	0·0
1. 1 : 10 active serum	102·85	■	■	■	■	◪	□
2. Froth	103·81	■	■	◪	■	◪	□
3. Remaining fluid	102·57	■	■	◪	■	◪	□
4. Mixture of 2 and 3 (equal parts)	102·67	■	■	■	■	◪	□

This experiment XIII shows that the froth contains a larger amount of bathotonic substances than the remaining liquid, but although I made a series of various experiments I never succeeded in detecting the complement either in the froth or in the residual liquid. The collected and centrifugalised froth had in all my experiments the same complementing power as the residual fluid. With regard to surface tension however it can be shown that an experimental error occurs, if for the measurement of the surface tension the froth is not taken into account.

I give in the following a series of experiments with regard to the alteration of surface tension occurring in sera which have been inactivated by shaking them at 36°.

Exp. XIV. *Alteration of surface tension in shaken sera.*

I. $\frac{1}{10}$ diluted serum was shaken 4 hours at 36°. As control the same serum in equal concentration was kept for the same time at 36°.

Haemolysis was tested with 1 c.c. sensitized red corpuscles.

c.c. of $\frac{1}{10}$ compl. sol....	1·0	0·75	0·5	0·25	0·15	0·1	0	*D*
Untreated serum ...	■	■	■	■	◻	□	□	101·79
Control serum ...	■	■	■	■	◻	□	□	103·85
Shaken serum ...	□	□	□	□	□	□	□	103·57
After shaking, heated $\frac{1}{2}$ hour to 56°	□	□	□	□	□	□	□	110·37

II. $\frac{1}{10}$ diluted serum was shaken in equal tubes of 20 c.c. volume but in different quantity, thus varying the intensity of agitation. Time of shaking was $5\frac{1}{2}$ hours at 36°.

Haemolysis was tested with 1 c.c. sensitized red corpuscles.

		Tubes containing serum in amounts of				
		Untreated serum	Control serum	I 15 c.c.	II 10 c.c.	III 8 c.c.
D (stalagmometer) ...		101·90	102·57	102·36	102·22	102·31
Haemolysis of 1 c.c. compl.		■	■	■	◪	□

III. Equal quantities of complement-containing serum was placed in equal tubes of 20 c.c. volume shaken in different concentration, at 36° during 4 hours.

	Untreated serum	Control serum	I	II	III	IV
Concentration	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{6}$	$\frac{1}{10}$
D, before shaking	103·18	103·18	107·41	105·00	103·88	103·18
D, after shaking	—	104·31	106·76	104·21	103·48	104·26

Haemolysis of 1 c.c. sensitized red corpuscles produced by complement, each tube filled up to 2 c.c.

Complement in c.c.	0·1	0·05	0·025	0
Control serum ...	■	■	◨	□
Concentration $\frac{1}{2}$ (I)	■	■	◻	□
Do. $\frac{1}{4}$ (II) ...	■	◪	◻	□
Do. $\frac{1}{6}$ (III) ...	■	◪	◻	□
Do. $\frac{1}{10}$ (IV) ...	◪	◻	◻	□

From these experiments one may conclude, that the surface tension of a shaken inactivated serum lowered, but generally this loss of surface energy does not differ much from that occurring in the control serum

and is at least partly due to the effect of the temperature of 36°. If the serum is shaken at room temperature (16°) the loss of surface tension which can be observed is very small, but here it is difficult to get the serum completely inactive. To obtain the following data a serum has been shaken at 16° during 6 hours.

Exp. XV.	D
$\frac{1}{10}$ diluted serum ...	102·58
Shaken 6 hours at 16°	103·10
,, 5 hours at 37°	103·41
Control serum ,,	103·2

If the serum has been inactivated before by heating it to 56° for half an hour and also by boiling it, the following experiment shows that such sera do not suffer an alteration of their surface tension, if shaken a long time either at room temperature or at 37°.

Exp. XVI.	D
$\frac{1}{10}$ diluted serum active	102·58
,, ,, inactive (30 mins. 56°) ...	107·45
,, ,, ,, 6 hours shaken at 16°	107·57
,, ,, ,, 5 ,, ,, 37°	107·86
Control serum at 37°	107·34
$\frac{1}{10}$ diluted serum boiled	109·65
,, ,, ,, 6 hours shaken at 16°	109·56
,, ,, ,, 5 ,, ,, 37°	109·73
,, ,, ,, Control at 37° ...	109·48

If such a shaken and completely inactive serum is exposed to a temperature of 56° for about 5–10 minutes or longer, the surface tension will be promptly lowered. The same occurs if a shaken thermoinactive serum is brought to the temperature of boiling water.

The only author who took the surface tension into consideration in experiments undertaken to destroy complement by mechanical agitation was Bertolini (1911). This author shook sera 8–10½ hours at a temperature of 18–20°. He could not obtain a complete inactivation of the shaken sera, but he saw the formation of micelli, as Segale did in inactivated sera. After removing the micelli out of the shaken serum, he showed that the surface tension which was lowered during the agitation, increased to its original value. He concludes that these alterations of the surface tension have nothing to do with the complement function of the serum. The micelli found by Bertolini are very probably the same as Ramsden's coagula and not identical with Segale's micelli found in thermoinactivated sera. The coagulation by shaking is,

according to Ramsden (1894), different from that produced by the effect of high temperature, by the different behaviour of the coagulated protein against KOH and HCl. It takes a much longer time to get a coagulation in thermoinactive sera by shaking them, in fact, it is a phenomenon which I have very rarely observed, but in boiled sera I never saw any change occurring by mechanical agitation. This formation of coagula is always to be observed, before a serum is rendered inactive by shaking, but *vice versa* a serum cannot be considered as inactive when this coagulation has occurred. With regard to surface tension it is evident that the removal of a part of that substance, which lowers the surface tension, must be followed by an increase of the latter, and if this increase cannot be observed, it must have been annulled by other influences such as the temperature of 37° (the effect of which is indicated by the control), and also possibly by local increase of temperature due to inner friction in the shaken tube. Such local increase of temperature may be sufficiently effective to influence the surface tension but not detachable by an ordinary thermometer.

Conclusions.

1. By keeping fresh guinea-pig serum a long time a loss of surface tension occurs, but the loss is small and does not correspond with the inactivity, which is generally not complete. It is possible that bacterial growth influences the surface tension.

2. Dilution with saline solution increases the surface tension, whether the serum is active or inactive. An alteration of the surface tension in high dilution is scarcely to be detected.

3. Exposure to heat causes a well-marked lessening of the surface energy, and the time necessary to produce it is nearly the same as that required for inactivation by heat.

4. This time is about five minutes and does not alter with the concentration of the serum. In spite of the shortness of the time the serum proved to be completely inactive.

5. Exposure to the temperature of boiling water causes a further diminution of the surface tension.

6. The reactivation of thermoinactive serum by adding fresh serum takes place before the surface tension has altered in any effective degree.

7. Peptone Witte produces a well-marked fall of surface tension, if added to fresh serum in such amount that the serum action is not inhibited.

8. Kaolin if added to serum and centrifugalised increases the surface tension of the serum, active serum being rendered completely inactive. In active and in thermoinactive serum kaolin does not remove all protein, so that further exposure to heat lessens the surface-tension. Only in boiled sera is all protein removed. The supernatant fluid can not be reactivated by either fraction of the complement.

9. Sulphate of barium also produces an increase of the surface tension, but no inactivation of the serum, if employed in the same way as kaolin.

10. In the inactivation of a serum by means of mechanical agitation no fall of surface tension occurs; the observed slight loss is due to the effect of the temperature of 37°.

11. The froth produced by the shaking must be taken into account in estimating the surface tension, for it contains a relatively larger amount of bathotonic substance. But no attempt to obtain the complement in the froth when separated from the remaining fluid was successful.

GENERAL CONCLUSION.

From these facts one may conclude that the surface tension of a serum does not permit any inference to be drawn as to its complement-action. Further if any relation does exist between the surface energy and the complement-function, they are not directly associated.

REFERENCES.

BERTOLINI, A. (1911). *Folia clin. Chim. e Microscop.* III. 351; ref. *Zeitschr. f. Immunitätsf.* 1911, 1151.
FREUNDLICH, H. (1909). *Kapillarchemie.*
FRIEDBERGER AND P. SALECKER (1911). *Zeitschr. f. Immunitätsf.* XI. 587.
HARA, K. (1913). *Zeitschr. f. Immunitätsf.* XVII. 209.
HUSLER, J. (1912). *Zeitschr. f. Immunitätsf.* XV. 157.
ISCOVESCO (1911 *a*). *Compt. rend. Soc. Biol.* LXX. Nos. 3, 10.
—— (1911 *b*). *Compt. rend. Soc. Biol.* LXX. No. 1.
JAKOBY AND SCHUETZE (1910). *Zeitschr. f. Immunitätsf.* IV. 730.
LANDSTEINER AND STANKOVIC (1906). *Centralbl. f. Bakt.* XLI. 108.
METCALF (1905). *Zeitschr. f. physikal. Chem.* LII. 1.
MUTERMILCH (1913). *Ann. Inst. Past.* 83.
NOGUCHI AND BRONFENBRENNER (1911). *Journ. Exper. Med.* XIII. 229.

RAMSDEN (1894). *Arch. f. Anat. u. Physiol. Abt. f. Physiol.* 517.
—— (1903). *Proc. Roy. Soc.* LXXII. 156.
—— (1904). *Zeitschr. f. physikal. Chem.* XLVII. 336.
SACHS (1913). *Handbuch der pathogenen Microorganismen*, II. 871.
SCHMIDT H. (1913). *Journ. Hygiene.*
SCHMIDT, P. (1911). *Zeitschr. f. Hyg.* LXIX. 513.
—— (1912). *Zeitschr. f. Chem. Industr. Kolloid.* X. 3, XI. 5.
SEGALE, MARIO (1911). *Instit. di Patolog. generale R. Università Genova. Patol. An.* I. 709; ref. *Zeitschr. f. Immunitätsf.* 1911, 385.
TETSUDA ITO (1912). *Zeitschr. f. Immunitätsf.* XV. 97.
TRAUBE, J. (1904). *Pflüger's Arch. f. Physiol.* CXV. 541.
—— (1904). *Grundriss der physikal. Chem.* Encke Stuttgart, 146.
—— (1908). *Biochem. Zeitschr.* 374.