CX. THE HAEMOLYTIC ACTION OF THE SOAPS.

BY ERIC PONDER.

From the Department of Physiology, Edinburgh University.

(Received June 26th, 1924.)

IN nearly every text-book of physiology the soaps are referred to as haemolytic substances, but the literature contains no quantitative information regarding their action. To supply this deficiency the following investigation was carried out.

METHODS AND MATERIAL EMPLOYED.

(i) *Soaps.* The results recorded in this paper were obtained from the examination of two specimens of sodium stearate, one of potassium stearate, two of sodium palmitate, two of potassium palmitate, three of sodium oleate, and one of potassium oleate. The specimens employed were selected for their purity. The use of impure samples of soap may give rise to very fallacious results, common impurities being alkali and even saponin, the presence of either of which will completely vitiate any results obtained. The soaps were dissolved, in suitable concentration, in 0.85 % NaCl. Experiments were carried out as soon as possible after the preparation of the solutions.

• (ii) *Cell suspension.* The cells used were human erythrocytes, collected into citrate, and thrice washed with 0.85 % NaCl. The cells contained in 1 cc. of blood were suspended in 20 cc. of 0.85 % NaCl., the strength of the suspension thus formed being the same as that usually employed in the writer's researches.

(iii) *Methods.* The time-dilution and time-temperature curves for the various specimens of soaps were determined in the manner described in a previous paper [1921]. The curves for percentage haemolysis were obtained by the use of the apparatus already described [1923, 2]. The action of serum on the lysis produced by the soaps was investigated by the methods previously used in connection with the inhibitory effect of serum on saponin and taurocholate haemolysis [1923, 1]. The methods employed for the determination of alterations in size of the cells under the action of the soaps have also been previously described [1922; Ponder and Millar, 1924]. Estimations of the surface tension of the solutions used as lysins were made by the capillary rise method in the usual way.

RESULTS.

The most convenient way in which to present the results is to consider each soap separately. In cases where more than one specimen was investi-

gated, it is to be understood that no discrepancy was found in the results yielded by the various specimens.

It may be as well to point out from the commencement that the soaps are very unsatisfactory as lysins when any data of a quantitative nature regarding their action are required. Being semi-colloids, they exhibit all the phenomena characteristic of their class. Their physical properties show a great tendency to alteration from time to time, the time-factor having to be taken into account in all determinations of their surface tension, their conductivity, and even of their haemolytic activity. As the circumstances which determine their physical conditions are not fully known, it is almost impossible to make two solutions of the same soap which are identical as regards physical state. This difficulty is sufficiently well marked when the soaps are in aqueous solution—when solutions in saline are dealt with, the difficulties are much greater, as the soaps tend to salt out, this change increasing with passage of time. This unsatisfactory state of affairs is perhaps responsible for the absence of any quantitative researches from the literature. The experiments recorded in this communication, are, of course, subject to the same difficulties, and must be judged accordingly. It is, however, possible to obtain a very good idea of the action of these substances, and to make certain quantitative determinations, if care is taken to use freshly prepared solutions, and to take into account the time which elapses between the preparation of the solutions and the performance of the experiments.

Sodium stearate. This soap forms a clear solution when hot, and gels on cooling. In a concentration of 1 in 500, an opalescence appears at 58° in saline solution—at 50° the solution is quite opaque. Gel formation becomes marked at about 40°. This results in great difficulty in making dilutions for haemolytic experiments, and in getting the red cells to mix with the lysin, for, in the cold, even solutions as dilute as 1 in 5000 show gelation.

The following table shows the relation between the dilution of sodium stearate and the time taken for the production of complete haemolysis of 0.4 cc. of cell suspension at 25°. The quantity of lysin used is 2 cc., the dilution being calculated after the addition of the cells.

Dilution	Time in minutes
1000	1
2000	2
4000	4.5
6000	20

From these figures it may be concluded that the haemolytic activity of sodium stearate is of the same order as that of sodium taurocholate.

At low temperatures, haemolysis is very slow—the fact that gelation occurs renders it impossible to form any accurate estimation of the haemolytic activity. At temperatures above 15° it becomes quite possible to estimate the rate of haemolysis, which steadily increases with rise of temperature. The relation between the temperature and the time taken for the production of complete lysis by any concentration of stearate appears to be the hyperbolic

one met with when saponin or the bile salts are used [1921]. Owing to the tendency to progressive opalescence and change of physical state alluded to, it is not easy to obtain either time-dilution curves or time-temperature curves for this soap. Irregularities, which can only be explained by changes in the activity of the haemolysin attributable to its tendency to physical change, are frequently met with, and interfere with the results of these haemolytic experiments.

Owing to the opalescence referred to, which occurs even in dilute solutions, it is impossible to obtain a percentage haemolysis curve by means of the radiometer.

The action of serum appears to be to inhibit the haemolysis produced by sodium stearate, if the serum be added to the soap before the addition of the cells. This action, however, is by no means well marked. If the serum be added to the soap after the latter has been in contact with the cells, no acceleration of haemolysis is to be observed as a rule—in a few cases there was a suspicion that there was an acceleration, but the result is so inconstant and ill-marked that no importance can be attached to it.

Care was taken to look for "periodicity phenomena"—that is, haemolysis in high dilutions when there is no haemolysis produced in more concentrated solutions of the lysin,—such as are observed with sodium taurocholate and human cells, and, according to Pickering and Taylor [1923], with saponin and the cells of the sheep, guinea-pig, and white rat. No such phenomena are observable with sodium stearate.

The alterations in form which the cells undergo are in general similar to those produced by saponin and sodium oleate, but somewhat less marked. Owing to the tendency of the soap to gel, it is very difficult to obtain information on the point, as there is great difficulty in making the necessary photomicrographs: solutions which do not gel at ordinary temperatures are too slow in their haemolytic action to give good results.

It may be mentioned that solutions of sodium stearate in saline tend to become less haemolytic on standing. This is probably associated with the progressive alteration in physical state which they undergo. As a result, freshly prepared solutions must be employed, and even in these, irregularities are met with.

Potassium stearate. This soap possesses properties very similar to those of the sodium soap. It gels in the same way in the cold, and forms clear solutions above 60°. Its haemolytic activity is of the same order as that of the sodium soap, from which it differs in no important way. It is impossible to obtain a percentage haemolysis curve for potassium stearate, owing to the opalescence which appears in solutions of the necessary concentration. The action of this soap is inhibited by serum. No periodicity phenomena are observable, and there appears to be little or no acceleration produced by adding serum to the lysin after it has been in contact with the cell suspension. The loss of haemolytic power on standing is well marked.

Sodium palmitate. This soap shows less tendency to opalesce and to gel than does the stearate, but these properties are sufficiently well marked to make haemolytic experiments very difficult. Solutions are clear above 60°, and show opalescence about 55°. The time-dilution relation at 25° is as follows:

Dilution	Time (min.)
1000	0.5
2000	1.0
4000	2.5
6000	9.0
8000	35.0

The palmitate of sodium is therefore a little more haemolytic than is the stearate. At low temperatures the usual retardation of haemolysis is observed, the rate increasing as the temperature increases. It is not possible to obtain a curve for percentage haemolysis, owing to the opalescence in the solutions used. Serum retards the haemolytic action of the soap, but not to a very great extent—the inhibition produced, moreover, is somewhat variable, so that it is not possible to make a quantitative study of the inhibitory phenomena. This variation appears to be connected with the general instability of physical state which the soap shows. On standing, solutions lose their lytic power considerably.

The alterations in form of the cells are similar to those met with under the action of other soaps: the same difficulty as is encountered in the case of sodium stearate renders the making of photographic measurements very difficult, and rather unreliable.

Potassium palmitate. The results of experiments on this soap may be summarised by stating that its action is almost identical with that of sodium palmitate. Potassium palmitate is no easier to work with than is the sodium salt, as its tendency to opalescence and gelation is as great, if not greater.

Sodium oleate. This soap is the only one of those investigated with which it is possible to obtain satisfactory results, and even in this case considerable irregularity is met with. Solutions in saline of concentrations between 1 in 500 and 1 in 10,000 exhibit a well-marked opalescence, especially after standing for a little while—no gelation, however, occurs in the dilutions required for haemolytic experiments. This opalescence is accompanied by a loss in the haemolytic power, which loss becomes very noticeable after the lapse of some time. Care must therefore be taken to compare samples which have stood for the same length of time, and to make that time as short as practicable.

At 25° the time-dilution relation is

Dilution	Time (min.)
5,000	0.5
10,000	1.5
20,000	3.5
30,000	8.0
40,000	20.0

These figures show that the haemolytic activity of this soap is nearly as great as that of saponin, and considerably greater than that of the other soaps examined, with the exception of potassium oleate.

The time taken for complete haemolysis increases as the temperature is reduced, lysis being very slow at temperatures below 5°. As the temperature rises, the rate of haemolysis increases in a regular manner, the relation between the temperature and the time taken for complete lysis being of the usual hyperbolic type. Some irregularities may be met with at low temperatures if solutions of soap which have undergone alterations of physical condition as a result of standing are employed: if fresh solutions are used, the time-temperature curves are very regular.

The appearance of opalescence in solutions of sodium oleate renders it a difficult matter to obtain a percentage haemolysis curve. Curves which are approximately correct can be obtained, however, with suitable concentrations of soap and cell suspension, and at suitable temperatures, which is more than can be done with any other soap. With a dilution of 1 in 20,000 at 14°, acting on 20 cc. of a cell suspension one-quarter the strength of that described above, the radiometer gave the following relations:

Time (min.)	% haemolysis	Time (min.)	% haemolysis
0	0	5	57
1	1.5	6	76
2	12	7	87
3	21	8	94
4	35	9	100

The corresponding curve is of the usual sigmoid type, and exhibits a slight skewness, the tendency being for a slight acceleration of the reaction towards its completion. The skewness is, in this case, about 0.12, a small amount for curves of percentage haemolysis. In accordance with the general principles considered in a previous paper, we may deduce that serum and haemoglobin have, if added to sodium oleate after this soap has been in contact with red cells, an accelerating effect on its haemolytic action, but that this effect is not well marked. If more concentrated solutions of soap than that mentioned in the above experiment are employed, the skewness in the percentage haemolysis curve becomes almost negligible, so that the curve appears to be a symmetrical one. This also, is what is to be expected from general considerations.

The haemolytic action of sodium oleate is markedly inhibited by small amounts of serum or plasma, if these be added to the soap before the latter comes in contact with the erythrocytes to be haemolysed. The inhibitory action of serum on the soap is not so well marked as it is on saponin, nor is the amount of inhibition produced by a given amount of serum so easily estimated, as considerable irregularities occur. Haemoglobin also has a very slight inhibitory effect. If the serum be added to a mixture of soap and red cells, the inhibition is replaced by an acceleration, as in the case of sodium taurocholate and sodium glycocholate [Ponder, 1923, 2]. This acceleration is not so well marked, however, as in the case of the bile salts. If the serum be added to the mixture of soap and cells immediately after this mixture has been made, the acceleration is very slight; if, on the other hand, the soap be allowed to complete the lysis of about 50 % of the cells in the absence of serum, the

addition of a small quantity (0.1 cc.) of serum or of haemoglobin solution causes an almost immediate haemolysis of the remainder. The effect of haemoglobin is slight compared with that of serum, or with the acceleration produced by haemoglobin on taurocholate haemolysis. These facts explain the positive skewness of the percentage haemolysis curve for sodium oleate, and also show why this skewness should be relatively slight: it is to be assumed that the haemoglobin released from the cells first haemolysed acts as an accelerating agent, and hastens the lysis of the remaining cells to a slight, but appreciable, extent.

No periodicity phenomena are to be observed with sodium oleate.

The alterations in form which erythrocytes undergo during haemolysis by sodium oleate may be observed readily enough, and are fully dealt with in another communication [1924]. These changes are similar to those met with when saponin is used as a haemolysin—the cells, immediately before lysis, become spheres without an alteration of volume; thereafter the diameter increases slightly, and the cell haemolyses.

Potassium oleate. This soap acts in a manner almost identical with that of sodium oleate, except that it is more difficult to work with, on account of the opalescence developed.

Surface Tension Experiments.

Since it is often suggested that the soaps are haemolytic because they lower surface tension, some experiments on this point were carried out, and may be briefly mentioned here.

Sodium oleate. Estimations of the surface tension of solutions of sodium oleate in saline solution, and in solution in water, confirm the observations (i) that it is very difficult to obtain concordant results, and (ii) that there is no finite value for $d\sigma/dc$ [Milner, 1907].

Sodium palmitate. This soap is stated not to lower the surface tension of water [Bottazzi, 1912]. Estimations made by us do not confirm this observation, for we find that a 1 in 20,000 solution has a surface tension of 53 dynes at 20°, and that slight lowering of surface tension is shown by solutions between the concentrations of 1 in 5000 and 1 in 50,000. Owing to gelation, figures for concentrations greater than 1 in 5000 cannot be obtained.

Sodium stearate. This soap also lowers surface tension. This is shown in the table below, where the soap was in saline solution, and the determinations made at 20°.

Concentration (c)	σ , dynes
1 in 5,000	51.3
1 in 10,000	52.1
1 in 20,000	51.8
1 in 40,000	57.4
1 in 80,000	60.4
1 in 160,000	63.4
Saline	63.4

This table shows that, as in the case of sodium oleate, there is no finite value for $d\sigma/dc$. The surface tension is almost the same for all concentrations above

about 1 in 30,000, and thereafter becomes greater, reaching that of water at a concentration of about 1 in 100,000. The fact that the surface tension of the soaps bears no definite relation to the concentration is an argument of considerable weight against the lytic action being due to a lowering of surface tension, as is frequently stated.

SUMMARY.

Summarising the general results of this investigation, the following conclusions may be drawn. (i) The stearates, palmitates and oleates are haemolytic, the stearates being the least so, and the oleates the most. (ii) There is little difference between the activity of the sodium and the potassium soaps. (iii) The time-dilution curves for these substances are of the usual logarithmic type. (iv) The time-temperature curves are of the usual hyperbolic type. (v) The percentage haemolysis curves cannot be investigated satisfactorily, except in the case of sodium oleate, owing to the opalescence which exists in haemolytic concentrations of the soaps. In the case of sodium oleate, this curve is of the usual sigmoid type, and shows, when suitable concentrations are used, a slight positive skewness. (vi) Serum or plasma, and also solution of haemoglobin, show an inhibitory effect on the lytic action of the soaps, especially on the action of the oleates. (vii) This inhibitory effect may be replaced by an acceleration, if the serum, plasma, or haemoglobin be added to the soap after this has been allowed to act on the cells. Such acceleration is not a very marked one. These facts explain the form of the percentage haemolysis curve for the oleates. The acceleration effect is very ill marked, or even absent, in the case of the stearates and the palmitates. (viii) No periodicity phenomena are observable with the soaps. (ix) The changes of shape which erythrocytes undergo when acted on by these soaps are very similar to the changes produced by saponin and by the bile salts. (x) Solutions of the soaps tend to lose their haemolytic power on standing. (xi) All indications point to the action of the soaps being of the same type as that of sodium taurocholate, and it is probable that they have their effect by attacking the protein components of the erythrocyte.

I have to thank Dr J. A. Hewitt for certain of the specimens of soap used in this investigation.

This research was carried out during the tenure of a Crichton Scholarship in the University of Edinburgh.

REFERENCES

- Bottazzi (1912). *Atti R. Accad. Lincei*, 365.
Milner (1907). *Phil. Mag.* 13, 96.
Pickering and Taylor (1923). *Biochem. J.* 17, 916.
Ponder (1921). *Proc. Roy. Soc. London*, B, 92, 285.
— (1922). *Proc. Roy. Soc. London*, B, 94, 102.
— (1923, 1). *Proc. Roy. Soc. London*, B, 95, 42.
— (1923, 2). *Proc. Roy. Soc. London*, B, 95, 382.
— (1924). *Quart. J. Exp. Physiol.* 14.
Ponder and Millar (1924). *Quart. J. Exp. Physiol.* 14, 67.