LXXXIX. STUDIES ON THE KINETICS OF HAEMOLYTIC SYSTEMS.

IV. A FURTHER STUDY OF THE SERIES OF RYVOSH.

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THE second paper of this series [Ponder, 1927] was concerned with an investigation, by quantitative methods outlined in the first paper of the series [Ponder, 1926], of the statement that the order of resistance of the red cells of various animals to saponin is the reverse of the order of resistance to hypotonic saline. The result of the study was to confirm the conclusion of Ryvosh [1907] that the following series holds for hypotonic saline, the most resistant species occurring first: man, guinea-pig, rat, rabbit, dog, pig, cat, ox, goat, sheep, and that the order of resistance to saponin is nearly the reverse. The same results have been more recently obtained by one of us [Yeager, 1928] and the facts thus seem to have been placed beyond question.

The explanation of the facts, however, is a very much more difficult matter. To make this clear, we shall tabulate the various suggestions which have been advanced.

(i) The resistance of red cells to saponin is stated to be determined by the quantity of cholesterol contained in them, and the lytic action of saponin to be due to its power of combining with this substance ([Yagi, 1911] and others).

(ii) Port [1922] has suggested that the phosphoric acid content of the cell is the essential factor in determining the resistance, a statement which is accepted by Orahovats [1926] and borne out by the recent analyses of Kay [1928].

(iii) Brinkman's theory regarding the resistance of cells to lysis by hypotonic saline implies that the resistance becomes greater as the cholesterol/lecithin ratio becomes greater [Brinkman, 1920], from which we may deduce that the resistance to saponin haemolysis becomes greater as this ratio decreases. This statement is incompatible with (i). Further, the conclusion of Meyer [1908] that red cells are the more resistant to saponin the higher the cholesterol/lecithin ratio, directly contradicts Brinkman's explanation.

(iv) In the second paper of this series we have suggested that saponin reacts with the cell proteins, and that the high negative correlation between

phosphoric acid content and resistance to saponin is due to there being a correlation of $+0.79$ between resistance and protein content (Abderhalden's figures [1895], not particularly reliable in this connection), and one of -0.75 between phosphoric acid content and protein content.

(v) The resistance of red cells to digitonin haemolysis is stated to be parallel to the cholesterol content of the cell [Fabre and Simonnet, 1926]. This statement corresponds to (i), made for saponin.

(vi) Kofler and Lázár [1927] have shown that the order of resistance of various cells to saponin is by no means the same as the order to digitonin and to other lysins. A new series, in fact, arises with each lysin. This is certainly true so far as the bacterial haemolysins are concerned [Ponder and McLachlan, 1927], and one of us [Yeager, 1928] has shown that the resistance series for sodium taurocholate is quite different from that for saponin.

The difficulty associated with any of these suggestions as to the cell component with which the lysins combine is due to the obvious fact that if (vi) is correct, no one of the above suggestions can be used to explain the action of more than one haemolysin. For example, if (vi) is true, either (i) or (v), or both, must be wrong, for it is impossible for the action of two lysins to be determined by the quantity of the same component and yet for the two lysins to be uncorrelated with each other with respect to their activities. Similarly, if we explain the action of saponin in terms of (ii), and if (vi) be correct, the same explanation cannot be used to account for the action of the related lysin digitonin, or of any other lysin which gives rise to a resistance series other than that obtained with saponin.

THE EXTENSION OF THE RYVOSH SERIES.

The following table shows the resistances of the washed red cells of various mammals to saponin and to hypotonic saline. The measurements of resistance were carried out exactly as described in the second paper of this series, and might properly be added to Table I of that paper, as the suspensions contained the cells from an equal volume of blood.

Table I.

Animal	Saponin R	Saline %	Animal	Saponin R	Saline %
Marmoset ...	0.57	0.40	Macaque ...	1.55	0.22
Baboon ...	0.59	0.18	Chimpanzee	1.70	0.28
Lemur ...	0.64	0.42	Armadillo	2.10	0.31
Squirrel ...	0.90	0.27	Raccoon ...	3.0	0.32
Green monkey	0.90	0.43	Kangaroo	3.5	0.32
Man ...	1.00	0.32	Skunk ...	3.7	0.39
Opossum ...	1.10	0.35	Wallaby ...	4.2	0.38
Spider monkey	1.24	0.28	Black buck	6.0	0.46
Sapajou "	1.42	0.38	Buffalo ...	7.0	0.42
Elephant ...	1.5	0.28	Elk ...	8.0	0.42

As can be seen from a mere inspection of the figures, there is no obvious relation between the resistance to saponin and the resistance to hypotonic saline. The coefficient of correlation, in fact, is -0.50 , and that of rank corre-

lation even lower, about -0.3^1 . It thus appears that the conclusions of Ryvosh cannot be extended to the red cells of the mammalia in general, although they are correct for the animals included in the original series. We must consider how this can come about.

The essential point is that the ordinary theory of correlation applies only where large numbers are concerned and where random sampling is approached. Suppose, for the sake of argument, that the red cells of the various mammals are tabulated with respect to their resistance to saponin and to hypotonic saline, and that there is zero correlation between these two attributes, we shall, nevertheless, by a judicious choice of animals, be able to pick a small series which shows a high positive (or negative) correlation between the resistances referred to. For instance, from the 29 animals included in the above table and Table I of the previous paper, we can select the following series for resistance to saponin and to hypotonic saline respectively (least resistance first).

Hypotonic saline: sheep, goat, ox, cat, pig, dog, rabbit, rat, guinea-pig, man.

Saponin: rabbit, rat, man, guinea-pig, dog, pig, cat, goat, ox, sheep.

The rank correlation shown here is -0.88 ± 0.071 . Or we may select the following series (least resistance first).

Hypotonic saline: green monkey, lemur, marmoset, sapajou, racoon, man, armadillo, elephant, macaque.

Saponin: marmoset, lemur, green monkey, man, sapajou, elephant, macaque, armadillo, racoon.

The rank correlation shown here is $+0.70 \pm 0.17$, a result almost exactly opposite to that of the first series.

These two coefficients are different in sign, and both cannot possibly indicate the true state of affairs in the population they purport to describe; so it must, therefore, be possible for either to arise by chance from the particular population under consideration. The question is whether we can assess the chance. Where small numbers are concerned, there is no way of estimating the chance of a high correlation coefficient, positive or negative, arising from a large population showing zero correlation, for the usual tests, in which we compute the number of times the standard error is exceeded, fail altogether in cases such as this, where the true standard error of the coefficients is unknown. The standard errors of ± 0.07 and ± 0.17 , attached to the correlation coefficients above, are, in fact, quite misleading, for they are calculated on the erroneous assumption that the numbers are large; if they were correct, the chances of the two coefficients arising by chance from a population showing

¹ Calculation of coefficients of correlation, real or rank, from such a small number of cases as those dealt with in this paper is, of course, unsound statistical practice, and the coefficients have very little meaning. They are useful, nevertheless, for expressing a relation between two attributes, when the relation is not evident by simple inspection. As describing such a relation, even with small numbers, such coefficients are quite satisfactory: the danger in their use arises from the fact that we cannot assume them to be efficient statistics for a population including any cases additional to those from which they were obtained.

zero correlation would be one in millions. The very fact that, out of a series of 29 animals, two such coefficients can be selected, shows that no significance can be attached to either as describing the true state of affairs in all the animals.

The series of Ryvosh accordingly appears to constitute a special case, involving the animals which, for economic reasons, are usually used in laboratories. This does not mean, of course, that there is actually zero correlation between resistance to saponin and resistance to hypotonic saline, for, were sufficiently extensive series examined, a significant negative correlation might still be found; on the existing evidence, however, we cannot say that such a correlation exists, and we may go even further and say that it is probable that it does not.

THE CELL COMPONENT DETERMINING RESISTANCE TO SAPONIN.

The fact that the cells of different animals vary some twenty times in resistance to saponin has suggested to many investigators that it may be possible to show that the varying quantity of some one substance accounts for the variation in resistance. The substances which have been suggested are (i) cholesterol, (ii) phosphates, and (iii) lecithin together with cholesterol, *i.e.* the cholesterol/lecithin ratio.

(i) The hypothesis that saponin produces lysis by combining with cholesterol rests upon several pieces of evidence: (a) that there is a high correlation between the cholesterol determinations of Abderhalden and resistance to saponin; (b) that digitonin, a substance allied to saponin, precipitates cholesterol quantitatively; (c) that Ransom [1901] and others have stated that the inhibitory action of serum on saponin haemolysis is due to the contained cholesterol uniting with the saponin and rendering it inert. We may place against these observations the following. (a) The analyses of Abderhalden give figures for total cholesterol, including esters which are not precipitated by saponin or digitonin, and are therefore rather misleading in this connection. (b) Kofler and Schrutka [1925] have measured the affinity of eleven kinds of saponin for cholesterol, and have shown that the combining power of saponin for this substance is by no means parallel to the lytic power. (c) Several observers have shown that the inhibitory action of serum on saponin haemolysis is due principally to its contained proteins [Ponder, 1923; Kennedy, 1925].

(ii) The hypothesis that saponin produces lysis by reacting with the phosphates (or with phosphate-containing substances) rests upon the fact that the coefficient of rank correlation between phosphoric acid content and resistance to saponin is very high (-0.83 , with Abderhalden's figures, or -0.92 with Kay's figures). The appearance of the negative sign, however, completely eliminates the possibility of the lysin reacting with the source of the phosphoric acid, or of using the phosphoric acid content as a guide to the mode of action of the lysin, for the following reason. The whole of recent work indicates that haemolysis by saponin can be treated quantitatively on the assumption that,

to produce lysis, a certain quantity of saponin, x , must be used up in combining with the cell membrane. The method of measuring resistance is such that the resistance of the cell increases as x increases, and hence as the substance attacked by the lysin increases. If the quantity of any substance in the cell membrane decreases as x increases, and if it is contended that the quantity of the substance determines lysis, the correlation coefficient has a negative sign; the meaning of the sign is that, whatever substance be attacked by the lysin, it is not this one.

(iii) The conflicting statements regarding the cholesterol/lecithin ratio have already been remarked upon.

The experiments hitherto done in this connection have generally failed to conform to one or all of three important conditions. (i) The usual methods for determining the resistance of cells to saponin really determine only the resistance of the most resistant cells, whereas chemical analyses determine the amount of substances contained in cells of average composition. Ideally, we should compare the content of the average cell with the resistance of the average cell population. (ii) The suspensions of the cells of various animals used in determining resistance must all contain the same number of cells, for, if the number is not the same, comparison of the results becomes almost impossible¹. The quantity of a given substance obtainable from this number of cells can, however, easily be calculated from analyses on any known number of cells. (iii) It is important that resistance and chemical composition be determined for the cells of the same animal, and that analyses of the cells of one individual of the species shall not be compared with the resistance of the cells of another individual of the species, for the variation is great. It is not necessary, however, to employ large numbers of animals of the same species, for the resistance of the cells of one individual depends (by hypothesis) upon the amount of a given substance in its own cells, and certainly not, on any conceivable hypothesis, on the amount of the substance in the cells of other individuals.

With these points in mind, we have carried out a series of experiments in the following way. A set of dilutions of quillia saponin in 0.85 % sodium chloride, from 1 in 1000 to 1 in 560,000, was made in the usual way and stored in a refrigerator, being removed only when actually to be used. Blood was obtained from the animal (sheep, ox, horse, man, dog, pig, rat, rabbit and guinea-pig), the cells washed three times in 0.85 % NaCl and then made up to the original volume of the blood from which they were obtained. This suspension was used for the estimation of the following substances: (a) cholesterol (method of Myers and Wardell), (b) inorganic phosphates (Benedict and Theis), (c) total acid-soluble phosphates (Fiske and Subbarow) and (d) lecithin-phosphorus (Bloor, Benedict and Theis). The results were then reduced to quantities present per 0.5×10^6 cells/mm.³

¹ The reason for this is that increasing the number of cells does not result in an equal increase in the quantity of saponin apparently used up. ~~The increase in the latter figure is proportional to that in the former, but the proportionality constant is difficult to determine.~~

A small portion of the suspension was used for a cell count and a part of the suspension was afterwards diluted so as to give a suspension containing 0.5×10^6 cells/mm.³, which is of a convenient strength for the determination of resistance to saponin. The resistances were determined by finding the quantity of lysin necessary to produce 50 % haemolysis after 7 hours at 25°. All the experiments performed were completed within 2 weeks, thereby ensuring that the activity of the lysin remained unchanged.

The results may be described briefly as follows. (a) The coefficient of rank correlation between resistance to saponin and quantity of cholesterol is small and negative (-0.6), and there is no evidence that the resistance increases with the quantity of contained free cholesterol. The results, in fact, lead to the same conclusions as those of Kofler and Schrutka. (b) The figures for total phosphates agree excellently with those of Kay. The correlation between the quantity of phosphates and resistance to saponin is again high but negative (-0.75). Although the quantity of contained total phosphates may be a satisfactory guide to the resistance of the cell, the negative sign precludes the possibility of the haemolytic reaction being dependent, except indirectly, on the quantity of phosphates. (c) The coefficient correlating inorganic phosphates and resistance is also high but negative (-0.80). (d) The cholesterol/lecithin ratio is not significantly correlated with resistance to hypotonic saline and therefore cannot be correlated positively or negatively in a significant manner with resistance to saponin ($+0.45$ to saline, -0.30 to saponin). The coefficients of rank correlation above given are, of course, of no absolute value, but will serve as an incomplete description of the relation between the two attributes concerned.

THE HAEMOLYTIC ACTIVITY OF RELATED LYSINS.

The hypotheses hitherto discussed in this paper, *i.e.* those of Yagi, and Fabre and Simonnet regarding cholesterol, of Port regarding phosphates, and of Meyer and Brinkman regarding the cholesterol/lecithin ratio, have in common the fundamental assumption that the total quantity of some substance contained in the red cell membrane determines the resistance to lysis—that is, the minimal quantity of lysin required for complete lysis. If we accept this idea, and also suppose that chemically related haemolysins act upon the same component, it then follows that the cells of a series of animals arranged in the order of their resistance to one of the lysins are also of necessity arranged in order of their resistance to all the other lysins.

The only data on the subject of closely related lysins are those of Kofler and Lázár, and these agree with the result of observations on chemically unrelated lysins in showing that quite different resistance series arise when different lysins are used. The animals in Kofler and Lázár's experiments were sheep, ox, horse, man, dog, pig, white rat, rabbit, and guinea-pig, and Table II shows the order of resistance to different lysins, the figure 1 indicating the greatest resistance.

Table II.

	Saponin	Digitonin	Smilacin	Helleborein	Senegin	Gypsophila saponin
Sheep ...	1	9	8	3	2	1
Ox ...	2	8	9	4	5	3
Horse ...	3	4	7	7	9	2
Man ...	4	3	2	5	1	5
Dog ...	5	2	5	1	7	4
Pig ...	6	7	3	2	8	7
Rat ...	7	1	1	6	4	9
Rabbit ...	8	5	4	8	6	6
Guinea-pig	9	6	6	9	3	8
Coefficient of rank correlation		- 0.34	- 0.57	+ 0.52	+ 0.7	+ 0.88

As can be seen from inspection of the figures, and as is indicated by the coefficients of rank correlation between the resistance to saponin and to each of the other lysins, there is no uniform order of resistance.

In view of the importance of this conclusion, we have repeated the experiments using quillia saponin, digitonin and smilacin, and have determined the concentration required to produce 50 % haemolysis of a suspension containing 0.5×10^6 cells/mm.³. The orders of increasing resistance are shown in Table III, bracketed animals having as nearly the same resistance as can be determined experimentally; to Table III is also added the resistance series obtained with sodium taurocholate [Yeager, 1928] and with the lysins produced by *B. tetani* and by *B. histolyticus* [Ponder and McLachlan, 1927].

Table III.

Saponin	Digitonin	Smilacin	Sodium taurocholate	Lysin of <i>B. tetani</i>	Lysin of <i>B.</i> <i>histolyticus</i>
Rabbit	Sheep }	Sheep	Guinea-pig	Rabbit	Sheep
Pig	Rabbit }	Horse	Sheep	Ox }	Ox
Rat	Ox	Ox	Dog	Horse }	Rabbit
Man	Man }	Pig }	Rabbit	Man }	Man
Guinea-pig	Pig }	Dog }	Rat }	Sheep	Horse
	Cat }	Rabbit }	Ox }		
Dog	Rat	Rat }	Man }		
Horse	Dog }	Cat }	Cat		
Cat	Guinea-pig }	Guinea-pig	Pig		
Sheep		Man			
Ox	Horse				

The results in the first three columns confirm the conclusion of Kofler and Lázár, and show, as do the results in the last three columns, that the resistance series is different with each lysin. It should be observed that in the case of the first three lysins the order of resistance of the cells of the different animals is not identical with that found by those investigators; this is no doubt due to the fact that two samples of quillia saponin, smilacin and even of digitonin are often not entirely comparable¹, owing to their uncertain composition, and the same remark might well apply to two preparations of the bacterial lysins. The failure to obtain the same resistance series as Kofler and Lázár, however, leads to the conclusion that different orders are obtained not only with different lysins, but sometimes even with different samples of what is thought to be the same lysin.

¹ Different specimens of digitonin, although stated to be chemically pure, have, for example, different effects on the heart.

Since there is conclusive evidence that the cells of animals arranged in order of their resistance to one lysin are not also arranged in order of their resistance to other lysins, it follows that the haemolytic action of different lysins cannot be determined simply by the varying quantities of a single cell component, *e.g.* we cannot imagine the action of quillia saponin, digitonin and helleborein to be determined by the quantity of free cholesterol, cholesterol ester, phosphate, or any other substance contained in the cell membrane. It should be particularly observed that the nature of the cell component whose quantity is imagined to determine the resistance is of no importance, for the same argument would apply whatever its nature and whatever the affinity of the haemolysin may be for the substance, since varying affinities would not change the order of resistance.

If this conception is rejected, as it has to be, there remains the possibility that the resistance to a given haemolysin depends on the total quantity of a cell component, but that for each lysin a different component must be postulated. This explanation, more particularly, demands that there shall be as many components as there are resistance series, and it is possible to imagine such a number only by postulating that such compounds as "protein" are really made up of numerous different proteins, each one of which reacts specifically with each one of the many lysins. Speculations in this direction are fruitless at the present time.

The subject is much more profitably approached in the following way. In studies of the kinetics of haemolysis by simple haemolysins, the only postulate which has been found necessary is that the lysin acts on a cell component S and transforms a portion of it S_1 , sufficient to make permeability to haemoglobin complete¹. The resistance is measured in terms of this quantity S_1 , or rather in terms of a quantity which is proportional to it, the quantity x of lysin used up in the process. This quantity x is directly obtainable from the figure for the smallest concentration (or the greatest dilution) required to produce a given amount of haemolysis, say 50 %, and, like the quantity S_1 , has no necessary or even probable relation to the total quantity of S , except that S_1 cannot be greater than S .

It follows at once that there is no necessary or even probable relation between resistance and the total quantity of any cell component which may be mentioned. The suggestions of Port, Yagi, Meyer, Fabre and Simonnet, Brinkman and others really imply that S_1 always is equal to S , or is a simple function of S . The idea that such a relation exists is not only unsupported by evidence, but, as we have seen, leads to the conclusion that every haemolytic reaction is unique, in the sense that every lysin which presents a new resistance series must be imagined to act on a different substance.

When a lysin acts on S and transforms S_1 of it, the new compound is

¹ In the discussion which follows, it should be noted that the hypothesis advanced differs from all others in assuming that all lysins attack only the one component S in any given type of cell; the nature of S , however, differs from one type of cell to another.

proportional in quantity to S_1 , but its nature depends on (a) the nature of S_1 (which may be imagined to be a protein, different in different animals), and on (b) the nature of the lysin concerned in the transformation. The new substance S_2 which has resulted from the action of the lysin on S_1 is now a component of the membrane, just as S_1 was previously, and the permeability of the membrane depends upon (a) the quantity of S_2 and (b) its nature.

To make clear the results which follow from those considerations, let us imagine four cases. (a) When destruction of semi-permeability is produced, the compound formed when saponin acts on human cells may be 20 micrograms in quantity, and its nature may be denoted by H-saponin, (b) the compound formed with ox cells may be 80 micrograms in quantity, and in nature O-saponin, (c) the compound formed with human cells and digitonin may be 80 micrograms in quantity and in nature H-digitonin and (d) the compound formed with ox cells and digitonin may be 20 micrograms in quantity, and in nature O-digitonin. Then the same result, *i.e.* destruction of semi-permeability is produced by 20 micrograms of H-saponin in human cells, but by 80 micrograms of H-digitonin in the same cells. This difference is easily understood when we remember that the compounds H-saponin and H-digitonin are different, and may, therefore, affect permeability to an unequal extent. But further, the same result is produced in ox cells by 80 micrograms of O-saponin and by 20 micrograms of O-digitonin. This difference is equally easily understood, for the two compounds are different. The figures for ox cells, furthermore, are not necessarily the same or in the same order as those for human cells, for not only is H not the same as O, but the human cell in which the compounds H-saponin and H-digitonin are formed is different in many other respects from the ox-cells, and the effect on permeability cannot, therefore, be expected to be the same in the two cases, even for the same lysin. *A fortiori*, it cannot be expected to be the same for two different lysins.

In short, the condition demanded by the existence of different resistance series is that the resistance of any cell to any lysin, whether denoted as a figure in micrograms, or as a relative resistance R , shall be a function both of the type of cell and of the nature of the lysin, *i.e.* $R = f(\text{cell, lysin})$. The hypothesis proposed above meets this condition, for it states that $R = f(S_2)$ and $S_2 = f(\text{cell, lysin})$. On the other hand, a hypothesis which assumes that lysis is determined by the total quantity of some one cell component, *e.g.* cholesterol, if extended to cover more than one lysin, fails to meet the required condition because it assumes that $R = f(S_1)$, the substance acted on, rather than $f(S_2)$, the substance formed as a result of the action, and, although S_1 is a function of the type of cell, it is not a function of the type of lysin. Furthermore, the hypothesis proposed above has the advantage that the kinetics of the haemolytic reaction postulated would be essentially similar for all lysins, as is in fact the case.

Since the lytic effect is supposed to depend, by this hypothesis, upon the formation of a compound whose nature and effect vary with (a) the type of cell

protein and (b) the type of lysin, it is interesting to find (by calculation from Table III of Kofler and Lázár's paper) that the variation of resistance resulting from a variation in the lysin used is 3-4 times as great as that resulting from a variation in the type of cell. This is a result quite in accordance with expectation.

SUMMARY.

1. The conclusion of Ryvosh that resistance to hypotonic saline varies inversely with resistance to saponin in the red cells of mammals, although substantiated for the animals included in the original series, does not appear to hold when the cells of other mammals are studied, and it is doubtful whether this inverse relation is in any way a general rule.

2. Simultaneous chemical analyses and determinations of resistance to various lysins do not reveal any one substance whose total quantity can be said to determine the resistance of the cell.

3. When the cells of various animals are arranged in order of their resistance to various lysins, a different order is found to be necessary for each lysin. This finding confirms the results of Kofler and Lázár, and, although inexplicable on the assumption that the resistance of the cell is determined by the quantity of some one substance in all cells, is completely in accordance with the theory upon which studies of the kinetics of the haemolytic reaction are based.

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REFERENCES.

- Abderhalden (1895). *Z. physiol. Chem.* **25**, 67.
 Brinkman (1920). *Biochem. Z.* **108**, 35.
 Fabre and Simonnet (1926). *Ann. Physiol.* **2**, 389.
 Kay (1928). *J. Physiol.* **65**, 374.
 Kennedy (1925). *Biochem. J.* **19**, 318.
 Kofler and Lázár (1927). *Wien. klin. Woch.* 16.
 — and Schrutka (1925). *Biochem. Z.* **159**, 327.
 Meyer (1908). *Beitr. chem. Physiol. Path.* **11**, 257.
 Orahovats (1926). *J. Physiol.* **61**, 447.
 Ponder (1923). *Proc. Roy. Soc. Lond. B* **95**, 42.
 — (1926). *Biochem. J.* **20**, 507.
 — (1927). *Biochem. J.* **21**, 56.
 — and McLachlan (1927). *Brit. J. Exp. Path.* **8**, 267.
 Port (1922). Höber's *Physikalische Chemie*, p. 593 (5. Auflage).
 Ransom (1901). *Deutsch. med. Woch.* **27**, 194.
 Ryvosh (1907). *Pflüger's Arch.* **116**, 229.
 Yagi (1911). *Arch. exp. Path. Pharm.* **64**, 144.
 Yeager (1928). *J. Gen. Physiol.* **11**, 779.