LX. A NOTE ON THE INFLUENCE OF p_H ON THE ACTION OF HAEMOLYTIC COMPLEMENT.

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THIS problem, which is fundamental to any work on haemolytic complement, does not appear to have received the attention which it deserves. Fuchs [1930] showed that bubbling carbon dioxide through plasma for a short time (6 minutes) increased its complement activity, while bubbling for a longer time (30 minutes) decreased it. He found that the action was increased by carbon dioxide, as long as the reaction was alkaline or neutral to litmus and that it was reduced immediately it became acid to that indicator. It is now thought that CO_2 may have a specific effect on complement, *i.e.* an effect other than through p_H . Andreesco [1930] found that anticomplementary sera had a higher alkaline reserve than normal. This may conceivably be the p_H effect.

Michaelis and Skwinsky [1910] used a series of mixtures of $N/7$ primary and secondary phosphates of sodium and found that specific haemolysis had its optimum at a slightly alkaline reaction corresponding with that of blood. An increase in alkalinity retards haemolysis but not to the extent that the increase in acidity does. Eagle and Brewer [1929] varied the reaction of the medium by the addition of $N/7$ NaOH and $N/7$ HCl. They found the optimum reaction for haemolysis to be between p_H 6.5 and 8. At p_H 5.3 and in the alkaline range there is an irreversible inactivation, complete at 4.8 and 8.8 respectively.

Unpublished observations indicate that relatively small variations in osmotic pressure affect the speed of complement action. It was therefore decided to repeat these observations with a stricter control of the osmotic pressure of the solution than that of previous workers. A method described by the author was used [Osborn, 1933]. This gives greater accuracy than the serial tube method, used by other observers in this field. It should be noted that this method measures the speed of complement action, whereas the former measures the end results of varying volumes of serum.

In earlier observations an isotonic phosphate buffer was used following Sørensen's method [1909]: this was discarded in favour of a mixture giving a wider range of p_H , *viz.* the "universal buffer mixture" of Prideaux and Ward [1924], as supplied by the B.D.H. Ltd. This mixture—consisting of phosphoric, boric and phenylacetic acids—was made up in twice the concentration recommended by the formula, adjusted to the required p_H by the addition of $0.4N$ NaOH (this too, being twice the formula strength) and adjusted to approximately the osmotic pressure of corpuscles by the addition of water. Determinations of p_H were made by means of a double hydrogen electrode and of osmotic pressure by measurement of the depression of freezing-point, all solutions being adjusted to within 0.02 % of 0.9 % NaCl.

In determining the speed of complement action ($3.5-x$) volume of buffer, x volumes of diluted serum and 0.5 volume of a 3 % suspension of sheep's

corpuscles sensitised with 5 units of amboceptor were mixed and placed in a bath at 37° , and the time for the complete haemolysis of the corpuscles was measured. Several different samples of guinea-pig's serum were used in varying concentrations. The guinea-pig's serum and the sheep's corpuscles were supplied by the S.A. Institute for Medical Research; the details of the preparation of the latter have been described previously [Osborn, 1933].

Examples of the results obtained, giving the extreme cases, are recorded graphically in Fig. 1. It will be seen that the optimum p_H is in the region of 7.5

Fig. 1. Showing variation of speed of complement action with p_H .

and that the actual position of the optimum point and the exact shape of the curve vary in different sera, but agree in showing some degree of slowing between p_H 6 and 7, and a marked slowing in the region of p_H 5.5 on the acid side, and a slowing between p_H 8 and 9 and complete inhibition at p_H 9.7 on the alkaline side. These results are in accordance with those of Michaelis and Skwinsky [1910] and Eagle and Brewer [1929]. It is unlikely that more consistent results will be obtained, until it is possible to separate the complement complex from the other constituents of the serum.

Several interesting facts were observed in the course of this work. The buffers themselves retarded haemolysis, for the optimum time with a buffer was at least twice that with normal saline. This retarding influence is common to a wide variety of inorganic and organic ions. (Unpublished observations.)

An attempt to investigate the alkaline range with borate buffers was frustrated by the fact that this ion may be haemolytic to both sensitised and unsensitised corpuscles. The effect appears to be roughly proportional to the hydrogen ion concentration and disappears at about p_H 8. The presence of sodium chloride and other ions protects the corpuscle from this haemolysis.

The observation of Coulter [1921], that heat-inactivation is most rapid in alkaline medium, was confirmed. The haemolytic activity of serum standing at room temperature for 24 minutes at p_H 8.6 was reduced by 44 %, while it suffered no demonstrable change at p_H 7.5, 6.5 or 5.5. That this was a true heat-inactivation was shown by the fact that a control at p_H 8.6 standing in ice-water showed no change.

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