CXXX. THE RESPONSE OF THE ISOLATED UTERUS OF THE GUINEA-PIG TO HISTAMINE.

By HAROLD JOHN PHELPS.

From the Physiology Institute, Cardiff.

(Received March 26th, 1935.)

RESEARCHES carried out in the last few years have led to the view that true adsorption of weak electrolytes from aqueous solutions by neutral adsorbing surfaces proceeds through the medium of unionised molecules. Studies of adsorption by charcoal and fuller's earth have shown clearly the distinction between true adsorption and the apparent adsorption resulting from the interaction of dissolved ions with polar groups in the adsorbing surface [Fromageot and Wurmser, 1924, 1, 2; Phelps and Peters, 1929; Phelps, 1929; 1931, 1, 2].

It is obviously most pertinent from the biological standpoint to enquire whether the living cell takes material from its surroundings by true adsorption processes or by a mechanism of ionic exchange. It is of course very probable that the transfer of material from the fluid surrounding the cell to the fluid within the cell takes place by a migration of ions, and indeed the Donnan theory of membrane equilibria provides a theoretical basis for such a migration and, in general, gives a quantitative explanation of the observed facts. Such a scheme, however, takes no account of any specific concentration at the cell boundaries which may result from adsorption processes. Since many of the chemical reactions taking place in the living cell appear to be surface reactions it is reasonable to suppose that they must be preceded by adsorption processes.

This point can plainly be tested experimentally with certain pharmacologically active weak electrolytes, by comparing their pharmacological activities in acid and alkaline media. It is to be expected, for example, that a pharmacologically active base which ionises over the physiological range of p_H will, if it is taken up by the cell by a process of true adsorption, show greater pharmacological activity in alkaline media, in which, of course, the proportion of unionised molecules will be higher. From an acidic electrolyte the reverse result would be expected. Experiments on these lines have been reported from time to time. In particular may be mentioned the work of Andrus [1924] which demonstrated that adrenaline and tyramine had more powerful effects on the auricles of the rabbit heart at p_H 8 than at p_H 7. On the other hand choline and its esters which are presumably potential acids, were more actively depressant in acid media. Clark [1928] has reached a similar conclusion in the case of the action of acetylcholine on the frog's heart and has provided a technical explanation of the opposite result obtained by Voss [1926].

More recently Trevan and Boock [1927] have carried out a comprehensive study of the influence of p_H on the action of certain local anaesthetics on the eye. The correlation between anaesthetising action and the concentration of unionised molecules, as calculated from the known ionisation constants, is very striking. Labes [1922] has shown that atropine and cocaine are markedly less toxic in acid solutions and Werner and Blanchard [1929] have reported a similar result with strychnine. Zipf [1927] finds that frogs' livers take up alkaloids and basic dyes more readily from alkaline than from acid solutions. The same author

[1928] also finds that the contracture of frog's muscle produced by caffeine, quinine, Nile blue *etc.*, is increased by increased alkalinity. Remarkably enough Zipf concludes from these results that the uptake of basic substances by tissues is a simple chemical replacement of free basic groups in the surface.

It is of course plain that any of these results considered individually is capable of two interpretations. Either it may be assumed that the neutral molecule of the stimulating substance is specifically active, or, on the other hand one must assume, as does Zipf, that increasing alkalinity induces some change in the cell surface itself which increases its responsiveness by increasing its adsorbing power or otherwise. When the results are considered collectively however the second interpretation seems most improbable. Trevan and Boock, for example, find that the anaesthetising actions of a number of substances in media of different reaction are in every case proportional to the concentration of unionised molecules as calculated from the known ionisation constants of the anaesthetics. If one may not assume that it is the unionised molecule which is adsorbed by the anaesthetised tissues, one must assume that the cell surfaces are so altered by change in reaction that their adsorbing power for each one of a number of different substances is also changed in such a way that in each case the change in adsorption happens to follow exactly the change in ionisation of the substance concerned, which surely is to assume an impossible degree of coincidence.

If however it is legitimate to argue from the analogous results obtained in the study of adsorption by charcoal and fuller's earth, it is surely reasonable to say that Zipf's results indicate rather a true "apolar" adsorption process.

In view of the strong presumptive evidence provided by these and similar investigations in favour of the view that the walls of the living cell take up substances from their surroundings by a mechanism of true "apolar" adsorption, it seemed desirable to study the influence of p_H on the pharmacological activity of some substance the adsorption of which by neutral adsorbents had already been studied. The influence of p_H on the adsorption of histamine has already been studied [Phelps and Peters, 1929]. From these results it appears that the adsorption of histamine increases rapidly over the range of reaction p_H 6-9. Since histamine is well known to have a marked pharmacological action on uterine smooth muscle it was thought that interesting results might be obtained by observing this action in media of varying reaction. It is true that Evans and Underhill [1923] have not found the action of histamine on the guinea-pig uterus to be influenced by changes in p_H , but as will appear in this paper, these workers did not employ a technique likely to disclose the small differences which might be expected to exist.

Work has been in progress on this problem for the last few years, and two brief reports have previously been published [Peters, 1929; Phelps, 1931, 3].

EXPERIMENTAL.

The guinea-pig uterus was chosen as providing suitable material, and the tests were carried out by a technique essentially similar to that described by Dale and Burn [1922] in their Medical Research Council Report on the Standardisation of Pituitary Extracts. The bath and fittings employed were of the modified design described by Gulland [1933].

The tests are beset with grave technical difficulties some of which may be mentioned. In the first place it was found (in confirmation of Dale and Burn [1922]) that not only do uteri from different animals show great differences in their response to histamine, but also that in any one preparation the relationship

between response and dose is qualitative rather than quantitative and further that the sensitivity of the preparations generally varied considerably in the course of a series of tests. The Ringer solutions used have been made up to the composition recommended by Dale and Burn. Various methods of adjusting the reaction of the Ringer have been tried. It was found in the first instance that the maximum range of reaction which the guinea-pig uterus would tolerate without becoming subject to violent contractions in the absence of histamine was roughly p_H 7–8.5. Three obvious methods suggest themselves: (a) the use of definite buffer solutions such as phosphate or borate, (b) aeration of the testing bath with appropriate mixtures of oxygen and carbon dioxide, (c) adjustment of the p_H of the Ringer with dilute hydrochloric acid and reliance on the buffering effect of the bicarbonate in the solution to keep the reaction reasonably constant.

Although many successful experiments have been carried out using Ringer solutions buffered with phosphate mixtures to p_H 7.2 and p_H 8, this method has certain disadvantages. The Ringer solution as made up without phosphate has a reaction of about p_H 8.4, but the solution was buffered to a reaction somewhat less alkaline since there was a marked precipitation of calcium in more alkaline solutions in presence of phosphate. It has been established independently that diminution of the calcium content of the Ringer *per se* lowers the sensitivity of the uterus (Sir H. H. Dale tells me that this agrees with his own experience) so that the precipitation of calcium might mask in some degree the effect of change in reaction. The use of phosphate buffers was therefore abandoned.

It was not found possible to keep the isolated guinea-pig uterus alive for any considerable period in borate buffers.

Aeration of the testing bath with O_2/CO_2 mixtures was found to render the uterus preparation very unstable. This was very possibly due to the diffusion of free CO_2 gas into the tissues. On the other hand preadjustment of the bicarbonate balance by the addition of the requisite quantity of hydrochloric acid was found very satisfactory. A similar result could be produced by bubbling CO_2 into the Ringer before use, but more accurately reproducible results could naturally be obtained by adding measured quantities of acid. The actual procedure which was found most satisfactory was to make up a "stock" Ringer solution to the formula given by Dale and Burn [1922], calcium and magnesium chlorides being omitted (a trace of phenol red being added to facilitate subsequent adjustment of p_H). For use the necessary amounts of strong solutions of these salts were added. To the acid Ringer sufficient $N/10$ HCl was added to bring the reaction to approximately p_H 7.15. To the alkaline Ringer sufficient NaCl was added to make $[Cl^-]$ the same as in the acid Ringer.

The acid Ringer so prepared became more alkaline after aeration owing to loss of CO_2 but it was generally found possible to make histamine tests before the reaction had changed significantly, as indicated by the phenol red. It is assumed that, as an average, the more acid histamine tests were carried out at p_H 7.2.

By the use of the two-way tap at the bottom of the testing bath, the latter could be filled with the appropriately buffered solution. When the preparation had become stable a suitable dose of histamine was added as histamine acid phosphate in normal saline in a concentration of 1/50 mg. per ml. When the bath was normally filled the volume of the testing solution was about 80 ml.

The preparation was first sensitised by a dose of histamine more than enough to produce maximum contraction (usually 0.02–0.04 mg.). The difference in response to be expected between p_H 7.2 and p_H 8 is small, the amount of unionised histamine increasing over this range in the ratio of 1/1.3 approximately. The first step therefore was to discover the minimum dose of histamine that

would produce a response in alkaline solution. In general this was 0.005–0.01 mg. in 80 ml. Minimum doses were then administered alternately in acid and alkaline media. As the experiment proceeded the sensitivity of the preparation usually altered somewhat and corresponding changes had to be made in the dosage. It was not to be expected that such small differences in response could be demonstrated in every experiment with such a tissue as the excised uterus, or indeed with any biological preparation. In the course of about thirty experiments however no single preparation has consistently given a greater response in acid than in alkaline solutions¹. In many experiments no difference in response could be detected with certainty; in the majority however there was a definitely greater response to histamine in alkaline media. Tables I and II are protocols of

Table I.

Phosphate buffering; 369 g. guinea-pig.

Test	Histamine vol. solution 1/50 mg. per ml. ml.	p _H	Result
A	0.2	7.8	Large contraction
B	0.2	7.2	No contraction
C	0.2	7.8	Fair response
D	0.4	7.8	Good contracture
E	0.4	7.2	Practically equal to "D"
F	0.4	7.8	Equal to "D" and "E"
(doses probably too large)			
G	0.3	7.8	Good contracture well maintained
H	0.3	7.2	Some contraction not maintained
J	0.3	7.8	Good contracture, maintained
K	0.1	7.2	Very slight contraction not maintained
L	0.1	7.8	Contracture much greater than "K"
M	0.1	7.2	Smaller contracture less maintained

Table II.

Bicarbonate buffering; 279 g. guinea-pig.

Test	Histamine vol. solution 1/50 mg. per ml. ml.	p _H	Result
A	0.5	8.4	Large contraction
B	0.5	7.2	No response
C	0.5	8.4	Slight contraction
D	1.0	7.2	Fair response, delayed
E	1.0	8.4	Large response
F	1.0	7.2	Slight irregular contraction
G	0.5	8.4	Large contracture
H	0.5	7.8	No response
J	0.5	8.4	Large response delayed
K	0.25	7.2	No response
L	0.25	8.4	Slight response
M	0.25	7.2	No response

two typical experiments which seemed free from technical fault; one with phosphate buffering and the other in which the reaction was adjusted with hydrochloric acid and in which the minimum dose fortunately was adjusted very exactly.

¹ It is of interest that the most stable preparations have been obtained in the summer months.

DISCUSSION.

It may reasonably be concluded from the results that the guinea-pig uterus shows an increased response to histamine in alkaline solutions, which might be expected if the pharmacological activity of histamine were due to the apolar adsorption of unionised molecules by cell surfaces.

The results of Evans and Underhill who failed to find any difference in the response and those of Hemingway [1926] who finds that the general responsiveness of living tissues is enhanced by alkali *per se* must be considered in the light of the present work. Since it has been observed that the difference in response is so small over the range of reaction in which the tissue may be regarded as in any way normal and can, in fact, only be demonstrated by administering minimum doses, it seems possible that the effect might have escaped the notice of Evans and Underhill entirely owing to the administration of doses in excess of the minimum dose.

Hemingway found that in general, uterus, the plain muscle of blood vessels and intestine in strongly alkaline solutions were pharmacologically hypersensitive. The effect persisted for some time in neutral media and probably depended on an alteration of the internal reaction of the cells, brought about by the large excess of alkali. These conditions are hardly comparable with those of this investigation. Further it was invariably the case that the preparations which I used were less stable and more responsive to purely mechanical stimulation at p_H 7 than at p_H 8, which adds support to the assumption that any increase in response found in alkaline solution must be a property of the stimulating agent rather than of the responding tissue.

It seems therefore that the guinea-pig uterus provides a further example of a tissue which responds to pharmacological reagents in the unionised form and of a tissue of which the cells take up at least some substances by a process of simple apolar adsorption.

In conclusion the author expresses his grateful thanks to Prof. R. A. Peters and Dr A. Hemingway for much valuable advice and to Dr H. M. Carleton for the loan of certain apparatus.

REFERENCES.

- Andrus (1924). *J. Physiol.* **59**, 361.
Clark (1928). *J. Physiol.* **64**, 123.
Dale and Burn (1922). *Med. Res. Coun. Sp. Rep. Series*, No. 69.
Evans and Underhill (1923). *J. Physiol.* **58**, 1.
Fromageot and Wurmser (1924, 1). *Compt. Rend. Acad. Sci.* **179**, 972.
— (1924, 2). *Compt. Rend. Acad. Sci.* **179**, 1404.
Gulland (1933). *Biochem. J.* **27**, 1216.
Hemingway (1926). *J. Physiol.* **62**, 81.
Labes (1922). *Biochem. Z.* **130**, 14.
Peters (1929). *J. State Med.* **37**, 1.
Phelps (1929). *J. Chem. Soc.* 1724.
— (1931, 1). *Proc. Roy. Soc. Lond. A* **133**, 155.
— (1931, 2). *Proc. Roy. Soc. Lond. A* **131**, 18.
— (1931, 3). *Chem. Ind.* **50**, 1043.
— and Peters (1929). *Proc. Roy. Soc. Lond. A* **124**, 584.
Trevan and Boock (1927). *Brit. J. Exp. Path.* **8**, 302.
Voss (1926). *Arch. exp. Path. Pharm.* **116**, 367.
Werner and Blanchard (1929). *Amer. J. Physiol.* **90**, 83.
Zipf (1927). *Arch. exp. Path. Pharm.* **124**, 259.
— (1928). *Arch. exp. Path. Pharm.* **140**, 56.