LXXIV. THE INFLUENCE OF CALCIUM ON THE RATE OF DIFFUSION OF SUGARS THROUGH SURVIVING INTESTINE.

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(Received March 9th, 1931.)

In previous work on this subject [Macleod *et al.* 1930; Auchinachie *et al.* 1930], the fluid bathing the surviving segments of intestine was always sugar-free Tyrode's solution. In view of the well-known effects of electrolytes on the physiological activity of living cells, it was decided to determine whether the selective properties of the intestinal epithelial cells would be affected by varying the composition of the outer fluid.

The procedure was the same as in previous experiments except that, as it was necessary to prepare the segments in sugar-free but otherwise normal Tyrode (called "normal Tyrode" in text), each segment was thoroughly washed in Tyrode of the composition to be used for the test, so as to ensure that all traces of fluid, except that whose influence was to be studied, would be removed from the intestine. Rabbit's small intestine was used, and its generally greater permeability in an aboral direction was ruled out by suitable selection of segments for control experiments. The volume of outer fluid was 50 cc., and 4 cc. 0.75 *M* sugar solution were generally placed in the lumen.

RESULTS.

When the rates of diffusion of 0.75 *M* glucose through the walls of segments bathed in normal and in Ca-free Tyrode respectively were compared, the rate was found to be greater in the former case, as the following figures from a typical experiment show:

Sol.	45	90	135 mins.
Ca-free Tyrode	29	103	185} mg. glucose per
Normal Tyrode	49	137	275} 100 cc. fluid.

The segments bathed in Ca-free Tyrode showed scarcely any movements; whereas those bathed in normal Tyrode contracted vigorously and undoubtedly increased the pressure of the lumen contents. This factor was not, however, responsible for the more rapid diffusion, because inhibition of the movements by continuous slow addition of a weak adrenaline solution did not alter the nature of the result. By starting with all the segments immersed in Ca-free Tyrode and then adding the usual amount of CaCl₂ to one series of beakers,

after a suitable interval, the accelerating effect of the CaCl_2 on diffusion was always elicited (see Fig. 1). When the diffusion was allowed to proceed into

normal Tyrode for a period and the Ca then precipitated by the addition of an equivalent amount of oxalate to one beaker a slowing of the rate of diffusion occurred (see Fig. 1). Similar results were obtained by adding an equivalent amount of citrate instead of oxalate. This result shows that the accelerating effect of calcium on the diffusion of glucose is due to calcium ions and not to un-ionised calcium, since the citrate, while it removes Ca^{++} , leaves un-ionised calcium in solution.

We are not aware of any reason why Ca^{++} should increase the permeability of the intestinal wall. Indeed, a contrary effect would not have been surprising in view of the discussion of the literature in an earlier paper [Magee and Reid, 1927]. The foregoing results having suggested that Ca^{++} was necessary for the physiological phenomena manifested by surviving intestine, it became all the more necessary to perform experiments testing this conception more stringently.

Effect of temperature. Adjacent segments containing the same volume of 0.75 M glucose were suspended in Ca-free and in normal Tyrode respectively and kept at 0° for a period. After withdrawing samples from the outer fluid the temperature was suddenly raised to 40° . Fig. 2 shows a typical result. It will be seen that after 30 minutes at 0° the amounts of glucose that diffused out were approximately equal, whereas at 40° the rate of diffusion was much more rapid in the case of the segment bathed in normal Tyrode. Since all physiological activity was in abeyance at 0° the equality in the rates of diffusion may be taken as proof that Ca^{++} would not affect the diffusion of glucose, either on

account of attraction of sugar by Ca^{++} in the outer fluid, or through an action on the texture of the gut wall. The more rapid diffusion in the case of the segment bathed in normal Tyrode at 40° , therefore, indicates that the physiological activity evoked by the rise in temperature was only possible in the

Fig. 2.

———— Ca in outer fluid. - - - - - No Ca in outer fluid.

presence of Ca^{++} . The results given below show that it made little difference to the rate of diffusion whether the Ca^{++} were present in the inner or outer fluid.

Inner fluid	Outer fluid	30	60	90 mins.	mg. glucose per 100 cc. outer fluid
4 cc. 0.75 M glucose	Ca-free Tyrode	17.1	43.4	80.5	
4 cc. 0.75 M glucose in 0.02 % CaCl_2	Ca-free Tyrode	21.0	50.3	101.2	
4 cc. 0.75 M glucose	Normal Tyrode	16.6	50.9	107.0	

Comparison of glucose and xylose. Previous work showed that, when equimolecular solutions of glucose and xylose were placed in adjacent loops, glucose generally passed into the outer (normal) Tyrode more rapidly than xylose. On repeating these experiments with Ca-free Tyrode no evidence of selective uptake of glucose could be demonstrated, but instead xylose passed out more rapidly than glucose, as was found in the case of dead intestine. This result again shows that the surviving intestine requires Ca^{++} to enable it to discriminate between the physiological sugar, glucose, and the foreign sugar, xylose.

Different concentrations of sugar. When equal volumes of M and 0.75 M glucose were placed in adjacent loops of surviving intestine, suspended in Ca-free Tyrode, diffusion was more rapid in the case of the M solution. This result, which is in accordance with the diffusion laws, is contrary to those previously obtained for diffusion into normal Tyrode. In this case diffusion was maximal with 0.75 M solutions.

It will, therefore, be seen that when the surviving intestine is examined by the above-described methods it fails to display in the absence of Ca^{++} those selective properties which are shown in the presence of Ca^{++} . Further experiments suggest, however, that Ca^{++} is not the only ion whose presence is

necessary for the manifestation of this selective action. Thus, when the diffusion of glucose was compared with segments bathed in normal and K-free Tyrode, the rate was greater in the former case.

Sol.	30	60	90 mins.
Normal Tyrode	39	91	154) mg. glucose per
K-free Tyrode	38	87	139) 100 cc. outer fluid

Similarly, diffusion was more rapid when the outer fluid was normal Tyrode of p_H 7.4–7.6 than when this was of p_H 5.4. The promotion of diffusion by the higher p_H was also shown to be independent of the concentration of HCO_3^- . But further work will be necessary before the rôles played by these ions in absorption can be clearly stated.

This investigation as a whole also shows that the passage of glucose through the wall of surviving intestine into an outer environment of Tyrode's solution is, to some extent, of the nature of an active transport of sugar molecules by the activity of the epithelium as well as a process of simple diffusion. But, as stated in an earlier paper, the effect of the surviving activity of the cells is liable under severe test to be masked by the purely physical phenomena.

SUMMARY.

In experiments with surviving rabbit's intestine it has been found that glucose diffuses more slowly from the lumen into Ca-free than into Ca-containing outer fluids, the process in the former case being a physical one but in the latter partly of the nature of a selective action.

The presence of calcium ions in the environment is indispensable for the manifestation of selective action of the intestinal wall towards sugars placed in the lumen.

We desire to express our thanks to Professor Macleod for advice and criticism.

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