paradoxic growth-acceleration of certain bacteria in the presence of homologous specific immune serum.[1]

8375 C

Evidence of Permeability of Tissue Cells to Potassium.

J. I. THALER. (Introduced by W. O. Fenn.)

From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester.

It is often stated, most recently by Hastings and Eichelberger,[1] that tissue cells are impermeable to potassium. Evidence to the contrary has been presented by Fenn and Cobb[2] from experiments on frogs. This paper reports certain experiments on cats leading also to the conclusion that the cells are permeable to potassium.

If successive blood samples are withdrawn over a period of 3 to 4 hours from the common carotid artery of a cat, and analyzed for potassium* a steady rise in potassium content of the plasma is ob-

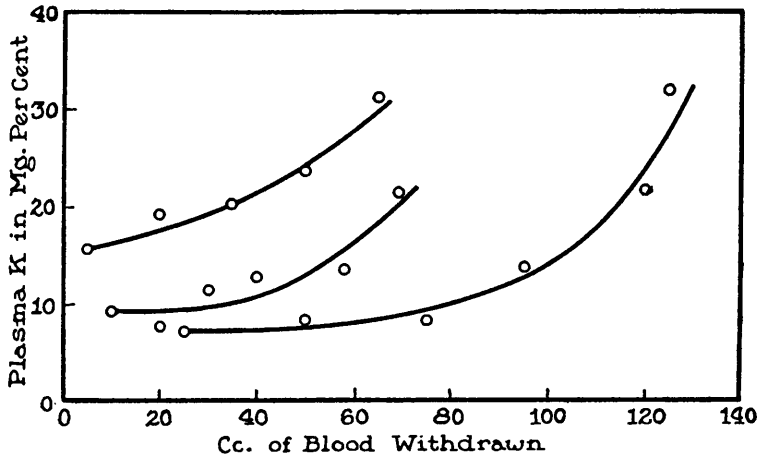

FIG. 1.

Results of 3 experiments showing the progressive rise in plasma potassium as successive samples of 10-25 cc. of blood were withdrawn for analysis.

[1] Nicolle, M., and Césair, E., *Ann. Inst. Pasteur*, 1926, **40**, 43.

[1] Hastings, A. B., and Eichelberger, L., *J. Biol. Chem.*, 1935, **109**, xli.

[2] Fenn, W. O., and Cobb, D. M., *J. Gen. Physiol.*, 1934, **17**, 629; *Am. J. Physiol.*, 1935, **112**, 41.

* The blood was dry-ashed in a muffle furnace at 500°C. and analyzed by the method of Shohl and Bennett (*J. Biol. Chem.*, 1928, **78**, 643) except that the precipitate of potassium was separated by centrifuge.

served as indicated by the graphs of Fig. 1. In this way 40 to 50% of the total blood volume can be withdrawn, bringing the potassium concentration to approximately 30 mg. % before death ensues. Since the completion of these experiments a similar result has been published by Baetjer.[3] She found further that any procedure which reduced the circulation by 80% of its normal value caused a similar rise in plasma potassium. This potassium apparently comes from the tissue cells after the tissue spaces have given up to the blood all the fluid they can spare, but the physico-chemical mechanism is not clear. If the cells are normally impermeable to potassium it might be regarded as the result of an injury to the cells accompanied by an increase of permeability. That this is not correct is indicated, however, by experiments in which blood or saline was reinjected after the potassium had been increased by a previous hemorrhage, with the result that the plasma potassium returned to normal. *i. e., potassium diffused back into the cells.* Were it assumed that there is a return of permeability to a normal condition of impermeability to potassium, this reversal would be impossible. Data from 2 of 4 similar experiments of this sort are shown in Table I. The figures

TABLE I.
Effect of Blood and Saline Injection on Plasma Potassium.

Time	Blood drawn	r.b.c.	Whole blood	Plasma	r.b.c.
min.	A. Ureters not ligated. Wt. of cat = 2.8 Kg.				
	cc.	%	mg. potassium per 100 cc.		
0	10	54.1	12.9	10.4	15.0
65	30	(incubated at 37° C.)			
95	10	37.5	16.0	16.4	15.2
110	30	(reinjected intravenously)			
130	10	43.2	11.7	9.0	15.3
	B. Ureters ligated. Wt. of cat = 3.2 Kg.				
0	10	35.3	14.6	11.0	21.2
35	40	(drawn and discarded)			
75	10	35.0	17.5	15.4	21.4
95	40	(normal saline injected intravenously)			
120	10	28.5	13.5	10.6	20.7

The animals were under Dial-urethane anesthesia.

show the results of analyses made on successive samples of blood, together with the time of bleeding and the amount drawn.

In the first animal (A) the plasma potassium was increased from 10.4 to 16.4 mg. % after removal of 40 cc., but returned to 9.0 mg. % after reinjection of 30 cc. of blood. In the second animal (B), the ureters were ligated to prevent loss of potassium into the

[3] Baetjer, A. M., *Am. J. Physiol.*, 1934, **109**, 3.

bladder. After raising the plasma potassium from 11.0 to 15.4 mg. % by removal of 50 cc. of blood, 40 cc. of saline were injected to bring up the blood volume, and the plasma potassium decreased to 10.6 mg. %. In the last column, the potassium content of the cells is calculated showing that the potassium content of the cells did not change, *i. e.*, the whole-blood potassium shows similar changes.

These potassium changes cannot, therefore, be ascribed to the blood cells, to the kidneys, or to the tissue spaces which must contain potassium at nearly the same concentration as the plasma. Some tissue cells must be concerned. It seems simplest to assume, therefore, that these cells are normally permeable to potassium and that shifts of potassium are the result of disturbances in the physico-chemical membrane equilibrium such as might be produced by an acid-base change either in the blood or the tissues, or both.

Another attempt has been made to drive an excess of potassium into the tissue cells. With the ureters ligated to eliminate loss through the kidneys, 45 cc. of 0.2 M KCl were injected intravenously; 45 minutes after completion of injection, the plasma potassium had increased from an initial value of 13.1 mg. % to 61 mg. %, while the corresponding values for striated muscle were 422.4 mg. % and 426.6 mg. %, respectively. This small increase in the muscle can readily be accounted for by an increased concentration of potassium in the tissue spaces parallel to the increase in the plasma. Even if the cells are permeable to potassium, the potassium must enter either by exchange with hydrogen ion (or some other cation), or as undissociated potassium hydroxide. It cannot enter with another anion since muscles are apparently anion-impermeable. Any entrance of potassium into the cells is therefore probably limited by the alkalinity which results and was imperceptible under the conditions of this experiment.

Another procedure which increases the blood potassium is injection of histamine. In one experiment the plasma potassium rose from an initial concentration of 10.8 mg. % to 16.5 mg. % after an intravenous injection of 0.05 mg. % of histamine per kilo of cat.

D'Silva[4] has also reported an increase in plasma potassium after hemorrhage. He suggested that the rise is not due solely to stimulation of the sympathetics resulting in a secretion of epinephrine, for while the first effect of injection of epinephrine is an increase of potassium in the blood this rise is all over in 5 minutes. My experiments also show that 5 minutes after injection of 1/10,000

[4] D'Silva, J. L., *J. Physiol.*, 1933, **80**, 7P; 1934, **82**, 393.

epinephrine no significant change in plasma potassium can be observed.

Conclusions. The concentration of potassium in the plasma increases as the circulatory volume decreases. This is a reversible process since the potassium concentration returns to normal if the circulatory volume is increased subsequently by reinjection. Saline is as effective as whole blood in causing the decrease of potassium concentration in this way. The tissue cells are reversibly permeable to potassium ions. The injection of epinephrine has only a transitory effect on the plasma potassium but histamine causes a more lasting increase.

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8376 C

On the Absence of Thiolhistidine in Insulin.

VINCENT DU VIGNEAUD, ROBERT H. SIFFERD AND GAIL MILLER.

From the Department of Biochemistry, School of Medicine, The George Washington University, Washington, D. C.

Considerable evidence has pointed to the possibility of the presence in insulin of sulfur other than that attributable to cystine, in which form the major portion of the sulfur is present. That thiolhistidine might account for part of this sulfur was a possibility to be considered. We therefore undertook studies to ascertain whether or not this amino acid is a constituent of the insulin molecule. On the basis of reports in the literature that the sulfur of thiolimidazoles could be split off as sulfate by boiling with ferric chloride and that the sulfur of cysteine was stable, we attempted to test for the presence of thiolhistidine. However, in testing this reaction with cystine, we found that ferric chloride will oxidize the sulfur of cystine to sulfate.[1] This reaction could not, therefore, be utilized to detect thiolhistidine in the presence of cystine in an insulin hydrolysate and some other method of approach was necessary.

In Barger and Ewin's early paper on the betaine of thiolhistidine, ergothioneine it was shown that the sulfur of this compound was

[1] Sifferd, R. H., and du Vigneaud, V., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 332.