Biochimica et Biophysica Acta, 1000 (1989) 431–432
Elsevier

Commentary by

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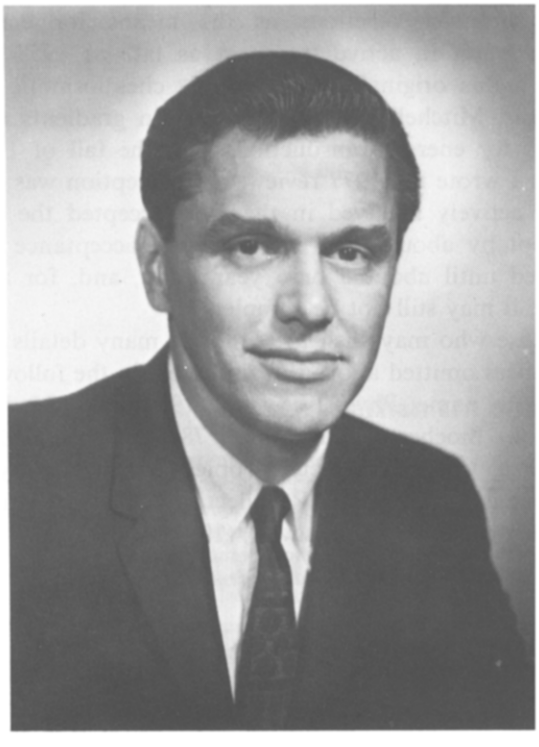
on 'On the mechanism of the intestinal absorption of sugars'
by R.K. Crane and S.M. Krane
Biochim. Biophys. Acta 20 (1956) 568–569

The everted sac technique of Wilson and Wiseman, which provided an easy way to test a compound for its active transport by the intestine, had been recently published. Glucose analogues of appropriate structure left over from a study of the specificity of brain hexokinase with Alberto Sols were sitting on a shelf in my laboratory. Putting the two together for a definitive test of the phosphorylation-dephosphorylation hypothesis was a rather simple idea that came to me suddenly while I was sitting in the departmental library half-listening to Melvin Cohn's persuasive arguments on the advantages of bacteria over animal tissues as a tool for studying membrane transport. I thought that Mel was basically right, though I don't know whether I would have tried to follow his advice. The question was made moot by my having such immediate good luck with the intestine. I continued to have good luck and it is now historical fact that the first clear proposal of ion–substrate cotransport as the driving force for membrane active transport derived from studies with hamster intestine and not from studies with bacteria. But I still think Mel was right.

When I got the idea for the experiments I was little more than a half-dozen years out of the Ph.D. Today, I



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am well into my third year of definitive retirement from science. To get myself back to where I was in 1956 I have had to cut through 32 years with a personalized version of a question that has had some recent currency in American politics, "What did I know and when did I know it?". It believe I did not know very much. As those who have read my reviews may agree, I always had a strong interest in and rather enjoyed placing experiments in historical perspective, but the perspective was achieved only after the reading in the literature had been done. Characteristically, the reading came after the experiment, not before. When I wrote my review on intestinal absorption in 1959, I was surprised by some of the findings in several papers on the phosphorylation-dephosphorylation hypothesis which were already published but which I did not know about in 1956. The source of the idea was surely not in what I knew. And, if not, then it must have been in the focus of my attention. The success of the test of the phosphorylation-dephosphorylation hypothesis which Stephen Krane and I made certainly focused my attention on the mechanism of active transport. The test, itself, may have sprung to mind because my attention was focused on the Wilson-Wiseman sac technique by a hint from Carl Cori that Tom Wilson might become a member of the department beginning in the fall of the year.

By 1956, the phosphorylation-dephosphorylation hypothesis for the active transport of glucose had already been extant for 20 years, albeit with considerable revision and not a few published doubts. The hypothesis was of wide-spread interest in biological science because it purported to explain how the energy provided to the cell by metabolism may be transduced into supporting the performance of work, the work in this case being to establish a gradient of substrate concentration across a cell membrane. The hypothesis was clean, clear and satisfying. It fitted the temper of the times when most seemed to believe that energy, as for active transport, not only is, but must be transduced by way of group transfer reactions. The hypothesis preceded by several years Fritz Lipmann's famous review of 1941, but it fitted beautifully with Lipmann's concepts and probably, thereby, gained more life than it deserved solely on the basis of experiment. After all, its creator, Lundsgaard, had already given up on it by the time it was taken up by others.

The test which Stephen Krane and I made of the hypothesis showed it to be wrong, but it was not destroyed. Others, to my knowledge, kept up a search for 'the phosphorylated intermediate'. And our results had no perceptible impact on the general belief in group transfer as the basis for active transport, except, perhaps, for myself. Having recently completed $2\frac{1}{2}$ years of work on the hexokinase reaction with Alberto Sols in the environment of Carl Cori's department, so conspicuously devoted to studies of glucose metabolism, I could claim some expertise in the subject. I knew of no other metabolic conversions of glucose which could, in my mind, take the place of phosphorylation-dephosphorylation. Nonetheless, Stephen Krane and I went on to search for some that were plausible, if not probable, and that were approachable within our experimental capabilities of the time. We found none and I stopped, entirely, believing in the possibility of any chemical, metabolic reaction of glucose as the driving force for its active transport. This was early in 1958. I turned my attention elsewhere and ended up 2 years later with the hypothesis, based largely on work done with Ivan Bihler, that glucose active transport in the intestine was energized by cotransport with sodium ion.

The message from the experiments that I did with Stephen Krane, and later with Bihler, was clear enough to me, but it seems not to have been clear to many other biochemists. Even Peter Mitchell was still championing group transfer reactions as the means for energy transduction in active transport as late as 1958 and 1959 in his original version of the chemiosmotic hypothesis. Mitchell actively pursued ion gradients as a means for energy transduction from the fall of 1960. When I wrote my 1977 review, my perception was that those actively involved in the field accepted the new concept by about 1970, more general acceptance was delayed until about 5 or 6 years later, and, for all I know, it may still not be complete.

Those who may wish to know the many details and references omitted above can find them in the following under my name; Physiol. Rev. 40 (1960) 789–825; Rev. Physiol. Biochem. Pharmacol. 78 (1977) 100–159; Semenza, G. (ed.) Selected Topics in the History of Biochemistry: Personal Recollections (Comprehensive Biochemistry, Vol. 35, Elsevier) (1983) 43–69.

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On the mechanism of the intestinal absorption of sugars[1]

The current hypotheses on the mechanism of "active" transport of sugar in the intestine postulate an enzymic modification of the sugar molecule at either carbon atom 1 or 6 by phosphorylation[2] or at carbon atom 1 by mutarotation[3]. With respect to the hypothesis of phosphorylation, the recent experiments of SOLS[4] on the substrate specificity of the hexokinase of intestinal mucosa clearly demonstrate that this enzyme cannot participate directly in "active" transport. However, by the methods used, the participation of a different enzyme of phosphorylation could not be disproved.

It occurred to us that direct test of the validity of these hypotheses could be made by the use in absorption experiments of model compounds lacking the hydroxyl function at carbon atoms 1 or 6 and incapable of undergoing phosphorylation or mutarotation. Accordingly, 1-deoxy glucose, 1-deoxy mannose, and 6-deoxy glucose were chosen as test compounds. Glucose and

3-methyl glucose, both of which have previously been found to be actively transported by the intestine[5,6], were chosen as controls. The experiments were carried out with WILSON's elegant method[6,7] in which everted sacs of the intestine of the golden hamster are used.

The results of these experiments are given in Table I. The data show that 1-deoxy glucose and 6-deoxy glucose were concentrated to a high degree on the serosal side of the preparation as were glucose and 3-methyl glucose, whereas 1-deoxy mannose, consistent with the observations of WILSON AND VINCENT with mannose[6], was not.

TABLE I

THE ABSORPTION OF SUGARS BY THE ISOLATED, EVERTED HAMSTER INTESTINE

Expt. No.	*Substrate*	*Concentrations; μM/ml*		
		inital	*final*	
		both sides	*mucosal*	*serosal*
1	1-deoxy glucose	8.0	6.8	19.8
	glucose	8.0	1.0	26.8
	1-deoxy mannose	8.0	8.0	8.5
	1-deoxy glucose	8.0	6.5	20.4
	glucose	8.0	3.0	25.2
	1-deoxy mannose	8.0	7.5	9.2
2	6-deoxy glucose	4.0	0.65	13.2
	3-methyl glucose	4.0	2.26	7.90
	6-deoxy glucose	4.0	1.79	10.1
	3-methyl glucose	4.0	3.25	5.65

The techniques for use of hamster intestine described by WILSON *et al.*[5,6] were followed in all respects. Segments of about 3 cm in length were cut, beginning at the upper jejunum. The substrates in each experiment are listed in the order of the successive segment used. Equal concentrations of substrate were placed inside and outside the sacs. Incubation was for 1 hour at 37° C. Analyses were made on barium hydroxide-zinc sulfate filtrates of the external medium and of the contents of the sac. The 1-deoxy sugars were assayed by SALO's periodate method[8] with appropriate small correction for reducing sugar. The latter were assayed by the SOMOGYI method[9]. The 1-deoxy compounds (also known as 1,5-sorbitan and 1,5-mannitan) used were the gift of Dr. N. K. RICHTMYER and contained no reducing sugar. The 6-deoxy glucose was the gift of Dr. E. HARDEGGER and contained about 0.5% of glucose, as judged by enzymic assay.

Inasmuch as WILSON AND VINCENT have reported that galactose is also actively transported, the list of compounds which undergo "active" transport now includes a glucose derivative varying from the parent compound in substitution or configuration at carbon atoms 1, 3, 4, or 6. From the standpoint of the chemical reaction which a transported compound might undergo, further studies on model compounds differing from glucose at carbon atom 2 and in the position of the ring are indicated. The fact that 1-deoxy glucose and 6-deoxy glucose are actively transported by the intestine renders untenable the hypotheses that phosphorylation or mutarotation are involved in intestinal absorption.

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[1] Aided by a grant from the National Science Foundation (U.S.A.).

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Received March 22nd, 1956