BBA 73012

Temporal stability of intestinal transport systems

Rapid renewal of the mucosa of the small intestine has been well established. It is conceivable, however, that certain parts of these cells are stable once formed and do not undergo significant metabolic alteration until sloughing occurs. Evidence in support of this can be found in the work of CROSBY[1] on iron persistence in the duodenal epithelium, and in the studies of LIPKIN AND QUASTLER[2] on protein stability during migration up the villus. There is accumulating evidence that intestinal transport systems are involved with specific proteins (for example, the systems are under genetic control); the degree of stability of the transport event might therefore be used as an indicator of the stability of intestinal proteins. Temporal stability of intestinal transport was therefore tested.

Everted gut sacs (three per hamster) from the entire length of the small intestine were made according to the method of WILSON AND WISEMAN[3]. Transport of $5 \cdot 10^{-6}$ M and $1 \cdot 10^{-3}$ M L-[^{14}C]methionine, 10^{-4} M L-[^{14}C]lysine, or 10^{-3} M [^{14}C]-glucose against a concentration gradient *in vitro* was determined by using liquid-scintillation counting. Sacs were filled with 1 ml of solution (compound in pH 7.4 Krebs–bicarbonate buffer) and incubated in 5 ml of identical composition for 1 h at 37° after gassing with 95% O_2 + 5% CO_2. Adult male hamsters were employed in all of the experiments and nine sacs studied per compound. Puromycin–HCl and actinomycin D were dissolved in phosphate buffer (pH 7.4). 25 mg of puromycin or 200 μg of actinomycin D were injected intraperitoneally, 1 h apart in two divided doses. Everted sacs were made 30 min after the last injection. Control animals received saline. To study the effects of hyperglycemia, 2 ml of guinea-pig insulin antibody serum[4] were injected intraperitoneally; sacs were prepared at intervals of 2 to 18 h after injection. Control animals were injected with serum from guinea pigs which had not received any insulin. Blood glucose concentrations were determined on all animals at the time of sacrifice. Only animals whose blood sugar concentrations were at least twice the control values were studied. In the experiments on glucose transport, the final fluids were chromatogramed in the ascending direction (Whatman No. 1 paper) in butanol–acetic acid–water (4:1:1, by vol.).

There was no inhibition of glucose, L-lysine or L-methionine transport following administration of puromycin (transport by experimental animals/transport by control animals = 1.0 $\pm$ 0.15). Actinomycin D also did not produce any alteration of L-methionine, L-lysine or glucose transport. For example, at $1 \cdot 10^{-3}$ M L-methionine, the serosal fluid gained 2.5 μmoles/g wet tissue weight; after actinomycin D the value was 2.4 μmoles/g. Insulin antibodies led to a sustained hyperglycemia in the hamsters, which appeared as early as 1 h and lasted as long as 18 h. During the period of hyperglycemia there was no alteration of either L-methionine or D-glucose transport against a concentration gradient. Chromatography of the glucose solutions after transport revealed the same relative percentage of glucose remaining in both controls and animals given puromycin or actinomycin D.

CRANE[5] reported that alloxan-diabetic rats transported more glucose than normal controls, but this effect was manifested only after 72 h; a similar increase in the intestinal transport of amino acids also took several days to be apparent[6], and human diabetics have accelerated glucose transport[7] The lag period[5,6], as well as

present inability to increase transport after 18 h of hyperglycemia, suggests that altered transport might be related to a modification of newly forming cells.

Lack of effect of inhibitors of protein synthesis on intestinal amino acid[8,9] and carbohydrate movement (although other organs may be affected)[10] does not mean that all intestinal transport systems are stable once formed, since a time factor might be involved. Ca^{2+} transport is not adversely affected by inhibition of protein synthesis, but the increase in its transport brought about by vitamin D can be blocked by actinomycin D[9,11]. We must likely begin thinking of intestinal transport systems with inducible components. The transport systems possess at least short-term stability.

Actinomycin D was kindly supplied by Merck, Sharp and Dohme Research Laboratories. This research was supported by Grants CA06519, AM09429 and AM08870 from the U.S. Public Health Service. H. J. B. received a special Fellowship, Grant 1-F3-AM28399, from the U.S. Public Health Service.

Departments of Medicine and Radiology,
Yale University School of Medicine,
New Haven, Conn. (U.S.A.)

HENRY J. BINDER
HOWARD M. SPIRO
RICHARD P. SPENCER

1 W. H. CROSBY, *Blood*, 22 (1963) 441.
2 M. LIPKIN AND H. QUASTLER, *J. Clin. Invest.*, 41 (1962) 646.
3 T. H. WILSON AND G. WISEMAN, *J. Physiol. London*, 123 (1954) 116.
4 B. H. B. ROBINSON AND P. H. WRIGHT, *J. Physiol. London*, 155 (1961) 302.
5 R. K. CRANE, *Biochem. Biophys. Res. Commun.*, 4 (1961) 436.
6 D. MANDELSTAM, *Federation Proc.*, 75 (1966) 695.
7 I. E. VINNIK, F. KERN, JR. AND K. E. SUSSMAN, *J. Lab. Clin. Med.*, 66 (1965) 131.
8 S. M. SABESIN AND K. J. ISSELBACHER, *Science*, 147 (1965) 1149.
9 H. E. HARRISON AND H. C. HARRISON, *Proc. Soc. Exptl. Biol. Med.*, 121 (1966) 312.
10 L. F. ADAMSON, S. G. LANGELUTTIG AND C. S. ANAST, *Biochim. Biophys. Acta*, 115 (1966) 355.
11 A. W. NORMAN, *Biochem. Biophys. Res. Commun.*, 23 (1966) 13.

Received July 22nd, 1966
Revised manuscript received December 22nd, 1966

BBA 73014

Actinomycin D inhibition of amino acid transport in *Streptococcus faecalis*

Actinomycin D has been reported recently to inhibit respiration and glycolysis in human leukemic leukocytes, suggesting that the inhibition of DNA-directed RNA synthesis may not be the only metabolic effect of this substance[1]. The reversal by glucose of actinomycin D inhibition of protein synthesis in ascites cells may reflect a related activity[2]. While investigating the amino acid transport systems of *Bacillus subtilis*, we found several years ago that the usefulness of actinomycin D as an inhibitor of amino acid incorporation into protein was limited in such experiments by its inhibition of amino acid transport. This effect has been examined further, and, in view of the current interest in additional metabolic effects of actinomycin D, we des-