Effects of Colchicine on Intestinal Mucosal Dehydrogenases

II. Biochemical Observations


We have reported certain histochemical observations relating to the action of colchicine on various tissues. The findings showed a definite alteration of pattern and apparent decrease in activity of the dehydrogenases studied, namely nicotinamide adenine dinucleotide (reduced) dehydrogenase (NADH dehydrogenase—DPNH), nicotinamide adenine dinucleotide phosphate (reduced) dehydrogenase (NADPH dehydrogenase—TPNH), succinic dehydrogenase (SDH), glucose-6-phosphate dehydrogenase (G-6-PDH), and lactic dehydrogenase (LDH). These effects were obtained with doses of colchicine ranging from 0.24 to 1.5 mg./kg. body weight.

As quantitative histochemical observations are liable to several errors and since biochemical technics are readily available for the study of the dehydrogenases, it was necessary to correlate the histochemical observations with quantitative biochemical assay of at least one of the above group of enzymes.

A possible explanation of the histochemical data might be cellular death resulting from the antimitotic action of colchicine. Since cell degeneration and death are often accompanied by an increase in the specific activity and free activity of lysosomal enzymes, it was of interest to examine the level of one of these (e.g., β-glucuronidase) following small and large doses of colchicine.

It has further been demonstrated that colchicine administration in antimitotic doses results in lowered oxygen uptake in liver slices. Since a decrease in dehydrogenase level occurred even after small doses of colchicine, it was of interest to determine whether a direct in-vitro effect of colchicine on the oxygen consumption of the mouse small intestine could be demonstrated.

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To further substantiate the impression that colchicine has a direct effect on enzyme activity rather than an indirect effect through inhibition of protein synthesis and thus secondary effects on levels of enzymes, the effect of puromycin at inhibitory doses on the LDH level in the intestine over a period of 60 hr. was studied.

METHODS

White inbred Swiss mice fed on the same standard Purina chow* and water were used in the experiments. Experimental and control mice were kept in separate cages. For the enzyme assays following colchicine administration, 2 groups of mice at each dose level (0.24 and 1.5 mg./kg. body weight) were used. These were further subdivided into 6 groups of six mice receiving sham injections of saline and injections of colchicine 10 min., 3 hr., 24 hr., 48 hr., and 96 hr. before sacrifice by neck dislocation. Portions of the jejunum about 4 in. in length were taken from each mouse and homogenized in 0.25M sucrose at 0°C with an Omni-mixer† for 30 sec. The homogenate was filtered through two layers of cheesecloth, and aliquots were used for enzyme assay and protein analysis according to Lowry's method.7

Lactic dehydrogenase was assayed by the estimation of unchanged pyruvic acid following incubation.8 Results are expressed as Wroblewski units per gram protein.

Beta glucuronidase was assayed according to the method of Fishman.9 Homogenates were prepared as above but also contained 0.1% of Triton X100‡ to relase any latent activity. No attempt was made to distinguish between free and latent activity of the enzymes.

In the puromycin experiments a group of 5 mice received a total dose of 0.4 mg./100 gm. body weight over a period of 60 hr., and a corresponding control group received sham injections of saline.6 The animals were sacrificed 2 hr. after the final dose of puromycin, and the LDH of the intestine was assayed as before. In another experiment a group of 6 mice received a total dose of 12.0 mg./100 gm. body weight of puromycin over a 4-hr. period, and a control group received sham injections of saline. The animals were sacrificed 1 hr. after the final dose of puromycin, and LDH was assayed as before.

Respiration experiments on strips of intact intestine were carried out in the Warburg apparatus with 100% oxygen as the gas phase, Krebs-Ringer phosphate, pH 7.4, as buffer, and 0.1M succinate as substrate. Incubations were carried out for 1 hr. at colchicine concentrations of 0.3 and 0.03 mg./ml. Following the experiment the tissue was removed from the flask and protein determined by the Lowry method on an aliquot of homogenate.

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