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An Antihemolytic Action of Pyridoxal.* (20890)

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In studying the action of pyridoxal on the concentrative uptake of amino acids by the Ehrlich mouse-ascites tumor cell, an antihemolytic action toward the contaminating red blood cells was accidentally noted. The cause of the spontaneous hemolysis in the ascitic fluid *in vitro* is unknown, but samples to which mM pyridoxal had been added escaped red cell breakage. Although we have not been able to demonstrate at will a protective action of pyridoxal at this level, an antihemolytic action can readily be shown at a 10 mM level.

Method. The procedure used was much like that of Wilbrandt(1). Blood was taken by venipuncture from persons in the postabsorptive state, and the blood treated with heparin. Aliquot portions were added to 2 samples of 10 volumes of the medium of Raker *et al.*(2). In one of the 2 portions of medium 10 mM per L of pyridoxal, or of sodium indoleacetate, replaced 5 or 10 mM per L of sodium chloride, respectively; in this way the osmotic pressure was kept uniform. The suspensions were then shaken for one hour at 37°C in an atmosphere of 95% oxygen and 5% carbon dioxide. Aliquot portions were then added with stirring to 10 to 20 volumes of hypotonic sodium chloride, buffered at pH 7.4 with 15 mM sodium phosphate. The suspensions were stirred occasionally for an hour, then centrifuged and the extent of hemolysis determined by measuring spectrophotometrically the optical density of the supernatant hemoglobin solutions. The time intervals were not critical; the same results were obtained after 15 minutes of exposure to hypotonicity. In calculating the osmolarities of the hypotonic solutions account was taken of the volume of the Raker medium and plasma included in the mixture.

Results and discussion. The results are illustrated in Fig. 1. The shift of the position of the curve was by 5 to 10 milliosmoles per liter. This effect would be expected if pyridoxal caused a displacement of cell solutes or a shrinkage of the cells. With the carcinoma cells of the Ehrlich ascites tumor, pyridoxal causes large amounts of potassium to move out of the cells; this shift is partially compensated by chloride exodus, and partially by sodium entrance into the cells(3). The

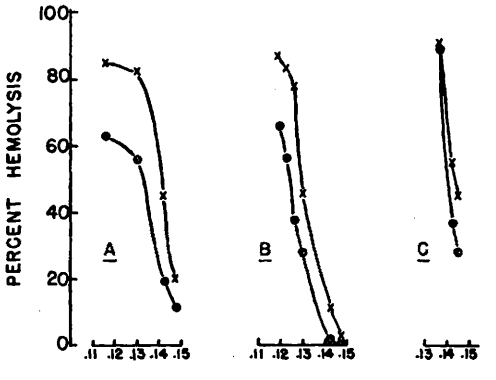

FIG. 1. Antihemolytic action of 10 mM pyridoxal (A and B) and indoleacetate (C). The encircled points represent the degree of hemolysis when the chemical agent was present, the crosses, when it was absent.

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cells shrink considerably. Erythrocytes show a similar but smaller shift both of electrolytes and water.

Indoleacetic acid also causes shrinkage of the tumor cells, and loss of potassium chloride. With erythrocytes the effect was quite small, although definite shrinking was seen(3). In correspondence the indoleacetate ion showed a smaller effect than pyridoxal in the present experiments (Fig. 1).

Evidence obtained with tumor cells from pyridoxine-deficient mice implicates pyridoxal in the normal process of amino acid accumulation by these cells(3,4). A close relationship to the transfer of potassium into cells is also indicated(3).

Summary. Pretreatment of erythrocytes with 10 mM pyridoxal makes the cells subsequently more resistant to lysis by hypotonic solutions. Indoleacetic acid shows the same behavior to a smaller degree. These effects are attributed to a net loss of cell solutes in the form of potassium and chloride which the agents produce.

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Effect of Isoniazid on Vitamin B_6 Metabolism; Its Possible Significance in Producing Isoniazid Neuritis.* (20891)

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Peripheral neuritis occurs as a side-effect of isoniazid (isonicotinic acid hydrazide, INH) therapy in tuberculosis, particularly when large doses are employed(1-6). This usually consists of paresthesia and numbness of the fingers and toes, advancing centripetally, with muscle soreness and weakness in some cases if medication is not discontinued. Vibratory sense may be impaired, and patchy hypesthesia has been seen. Reflexes may be diminished or exaggerated. The symptoms usually disappear within a few weeks if INH is promptly discontinued at the onset; however, late residuals such as burning feet, atrophy, fasiculation, and paresthesia may persist for months. In our experience, using a dose of 20 mg/kg/day of INH, neuritis has appeared in a predictable percentage of patients within definable limits of time. The similarity of this side effect to the neuritis induced by the vit. B_6 antagonist, desoxypyridoxine(7), has led us to investigate vit. B_6 metabolism in patients who were receiving INH for tuberculosis. The data to be reported indicate that INH does interfere with B_6 metabolism, and that the neuritis may be prevented with large doses of B_6.

Materials and methods. Subjects. All subjects studied were adult patients with active tuberculosis either on the wards of the Cincinnati General Hospital, or at Dunham Hospital, Cincinnati. During the study, all were receiving streptomycin (SM), one g daily or twice weekly. All patients were given the regular hospital diet. The various aspects of the study were carried out concurrently. Leading questions concerning symptoms of neuritis were avoided in evaluating the course of the patients, in order to eliminate the factor of suggestion. Any remarks by the patient, on the other hand, possibly connected with the onset of neuritis were pursued carefully. Neurologic testing was performed, but served no useful purpose in detecting the onset of neuritis. *Incidence of*

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