INTENSIFICATION OF THE CELLULAR ACCUMULATION OF AMINO ACIDS BY PYRIDOXAL*

by

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In a search for factors which might form a part of the apparatus by which amino acids are transferred into cells against concentration gradients, pyridoxal has been tested on the Ehrlich mouse-ascites carcinoma cell. In spite of the very strong concentrative activity which these cells already show, the addition of pyridoxal at appropriate levels increased by 65 to 75% the extent to which they accumulated glycine (Fig. 1). At higher levels pyridoxal became inhibitory. The stimulating effect has been obtained almost equally with cells from mice on stock diets, and on pyridoxine-deficient and pyridoxine-fortified diets. Even preincubation of the ascitic fluid-cell suspension for an hour in the presence of 0.5 millimolar pyridoxal did not abolish a subsequent stimulating effect of pyridoxal when the ascitic plasma was replaced by Krebs' Ringer-bicarbonate solution. Apparently what produces the stimulation is a definite level of the pyridoxal in the extracellular phase. A molecule-for-molecule entrance of pyridoxal and amino acid into the cell together is not the explanation, since when glycine was added at a 25 millimolar level the number of micromoles of extra glycine entering the cells was five times as great as the total quantity of pyridoxal added.

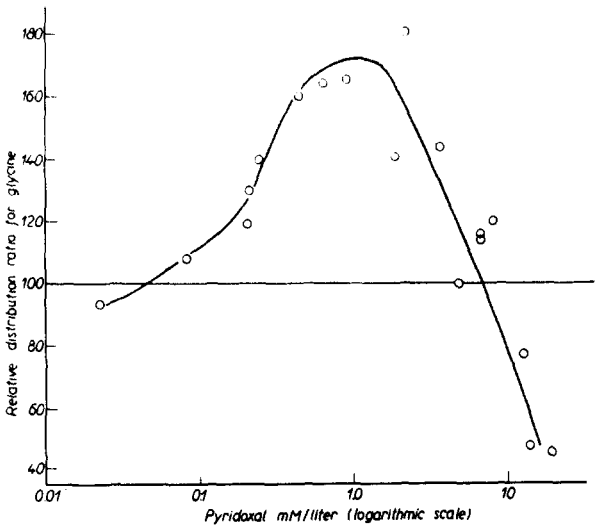

Fig. 1. Effect of added pyridoxal upon the accumulation of glycine by the tumor cell. Glycine added, 2 micromoles per ml of suspension. Incubation time, one hour. The distribution ratio is the ratio of the cellular glycine level to the extracellular level, each expressed in millimoles per kilogram of water. The average value for the controls was 12.5. Relative distribution ratio = 100 × (distribution ratio in the presence of added pyridoxal/distribution ratio in the absence of added pyridoxal).

Pyridoxal phosphate was if anything less effective than pyridoxal in the above respect. No stimulating effect of pyridoxamine, pyridoxine, riboflavin, thiamine, niacin or pantothenic acid could be detected. The concentration of L-α, γ-diaminobutyric acid by the cells was also stimulated.

METHODS

The cells from 2 ml of ascitic fluid, freshly collected, were resuspended in 2 ml of Krebs' Ringer bicarbonate solution containing pyridoxal, and also glycine at two to 30 millimolar concentration (two millimolar in the experiments of Fig. 1). The conditions of incubation, centrifugation and analysis have already been described[1].

DISCUSSION

In its coenzyme function pyridoxal phosphate is considered to become linked to the amino group of the amino acid, perhaps to form an N-pyridoxylidene derivative. The subsequent reaction

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in a few cases occurs at a fairly remote point in the molecule[2–5]. Therefore it seems likely that the formation of such derivatives may be a fairly general way by which amino acids are "grasped", perhaps for translocation as well as for structural modification.

Apparently it is the simultaneous presence of the amino acid and a definite level of pyridoxal in the extracellular fluid, rather than an adequate cellular supply of pyridoxal, which brings about the stimulation. One of the requirements of the "carrier" hypothesis of active transport (see for example ROSENBERG[6]) is that the carrier must be maintained at a higher concentration at the outer than at the inner limit of the membrane phase.

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BIOSYNTHESIS OF MILK PROTEINS IN THE FASTING GOAT

by

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Estimations of the amount of amino acid taken up by the mammary gland based on arterio-venous differences have suggested that the free amino acids of the blood could not be the major precursors of the milk proteins since they accounted for less than 40% of the milk protein (SHAW AND PETERSON[1]). According to GRAHAM *et al.*[2] milk protein nitrogen is chiefly derived from blood globulin, and REINEKE *et al.*[3] observed that the mammary gland of lactating goats removed a glyco-protein fraction from the blood. Recent studies with intravenously injected labelled amino acids, however, have shown that in rabbit (CAMPBELL AND WORK[4]) as well as in goat (BARRY[5], WORK *et al.*[6], ASKONAS *et al.*[7]) the isolated whey and casein proteins showed higher radioactivity than the plasma proteins. This suggested that the free amino acids of the blood represented the major precursors of the milk proteins. The apparent contradiction between these two sets of results may be attributed to the difficulty of determining the blood flow and small differences in the amino-acid levels in arterio-venous balance experiments (FOLLEY[8]).

REINEKE *et al.*[3] claimed from arterio-venous balance experiments that in the fasting goat there was no uptake of amino acids by the mammary gland in spite of the continued secretion of milk. This has been taken to indicate that the blood amino acids are not essential for the synthesis of milk proteins. If this were true it would indicate that the mechanism for synthesis of milk proteins in the fasting animal was different from that in the normal animal.

In order to test this possibility the following experiments were carried out with a lactating goat. The goat was given approximately 50 μc. of ^{35}S-methionine (6 mg) by intravenous injection immediately after milking dry, and the animal was milked at frequent intervals during the following 6 h. The milk proteins were fractionated into casein and whey protein fractions (CAMPBELL AND WORK[4]) and the activity of the fractions determined. The experiment was then repeated on the same goat after it had been starved for 48 h prior to injection of the methionine. Posterior pituitary extract was used to ensure complete drainage of the gland at the last milking.

The activities of the milk proteins from the two experiments are compared in the table. In both the normal and the fasting animal the activity of the milk proteins far exceeds that of the plasma protein, suggesting that in both cases the mechanism of synthesis was the same and therefore that the blood proteins are not the major precursors of the milk protein. It will be observed that whereas in the control animal the radioactivity of the milk proteins reached its maximum value 1¾ hours after the injection of the ^{35}S-methionine, in the fasting goat maximal activity was longer delayed