DYNAMICS OF AMINO-ACID COMPOSITION OF THE MEDIUM IN ISOLATED ORGAN CULTURE BY THE CONTROLLED-PERFUSION METHOD

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The dynamics of the amino-acid composition of the perfusion fluid was investigated during adequate perfusion of isolated dog organs (the thorax and a complex consisting of the thoracic organs, kidneys, and liver). The concentration of amino acids such as histidine, lysine, and alanine in the perfusion fluid 6 h after the beginning of perfusion of the organ complex was higher, whereas that of arginine, serine, aspartic acid, threonine with glutamine, isoleucine, proline, leucine, and valine was much lower than initially. In experiments on the isolated thorax the dynamics of the amino-acid composition of the medium was studied during perfusion for 4 h. The concentration of alanine, lysine, and histidine in the medium increased, whereas those of serine, aspartic acid, isoleucine, tyrosine, and phenylalanine decreased.

KEY WORDS: amino-acid composition; isolated dog—organ culture; controlled perfusion.

Maintenance of the viability of isolated mammalian organs by means of an artificial circulation is an essential technique for the study of the autoregulatory powers of the intact organism and is important for the solution of a number of practical problems in medicine – preservation of the functional activity of organs before transplantation. Despite progress made in this field [1] there are still great difficulties in providing organs with an adequate artificial circulation because of inadequate information on the metabolism of the supported tissues. Several investigations have been carried out to study amino-acid metabolism in tissue cultures [2-4, 6-8].

The object of this investigation was to study the dynamics of the amino-acid composition of the medium during perfusion of the isolated thorax of a dog and also of an organ complex consisting of the thoracic organs, kidneys, and liver. Such a combination of organs in one artificial circulation circuit (multiorgan culture) provides an experimental model with which to study regulation of hematopoiesis in vitro and is based on the concept of the role of the kidney and liver in erythropoietin production [5].

EXPERIMENTAL METHOD

Isolated organs of dogs age 3-5 years and weighing 18-20 kg were used. The technique of the operation to remove the organs, the apparatus for controlled artificial circulation, and the method of assessing viability and the functional state of the organs have been described previously [1]. The preparation was perfused with a mixture of autogenous plasma, stabilized with heparin (10 units to 1 ml plasma), and medium No. 199 in the ratio of 2:3. Amino acids were determined with the KLA-3B (Hitachi, Japan) automatic amino-acid analyzer. The medium for testing was first deproteinized with 1% picric acid and passed through a Dowex 1 × 8 column in the Cl-form to remove traces of picric acid. All experiments were carried out in 6-8 repetitions.

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TABLE 1. Changes in Concentrations of Amino Acids in Medium during Perfusion of Multiorgan Culture (kidney, thorax, and liver) for 6 h (M ± m)

Amino acids	I	K	K/I (in %)
Histidine	69,8±4,4	200,3±17,2*	286,0
Lysine	256,4±12,9	491,7±17,6*	191,7
Alanine	488,3±10,0	708,6±74,7†	145,1
Arginine	181,6±11,3	0*	—
Serine	249,1±6,8	33,1±7,5*	13,3
Aspartic acid	150,1±10,7	20,5±6,1*	13,6
Threonine and glutamine	529,2±11,1	115,9±24,2*	21,9
Isoleucine	96,4±3,6	52,6±8,3*	54,5
Proline	245,2±6,5	139,1±14,2*	56,7
Leucine	461,8±8,5	308,9±16,4*	66,8
Valine	269,6±11,3	190,7±26,6*	70,7

Legend: I) Concentration of amino acids in initial medium (in moles/liter medium), K) concentration of amino acids in medium 6 h after beginning of perfusion (in μmoles liter medium).

* $P < 0.01$; † $P < 0.5$ (compared with figures in column "I").

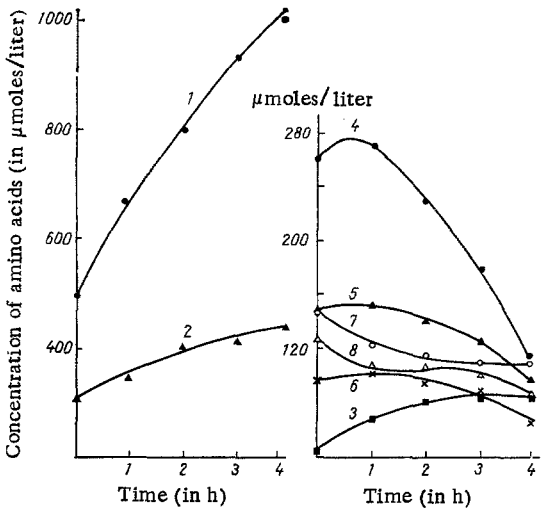

Fig. 1. Dynamics of amino-acid composition of medium during perfusion of dog thorax for 4 h: 1) alanine; 2) lysine; 3) histidine; 4) serine; 5) aspartic acid; 6) isoleucine; 7) phenylalanine; 8) tyrosine (concentration of amino acids given in μmoles/liter medium).

EXPERIMENTAL RESULTS

In experiments with normothermic perfusion of the organ complex (thorax, kidney, and liver) for 6 h the concentration of amino acids was measured in the original medium and 6 h after the beginning of perfusion. All the amino acids studied were divided into three groups depending on the character of their metabolism (Table 1). Group 1 included amino acids whose concentration in the medium increased during perfusion (lysine, histidine, alanine). Group 2 included amino acids (arginine, serine, aspartic acid, theronine with glutamine, isoleucine, proline, leucine, and valine) whose concentrations 6 h after the beginning of perfusion were much lower than initially; arginine disappeared completely from the medium during perfusion. Group 3 included amino acids showing negligible, inconsistent, or otherwise not significant changes. This group evidently contained amino acids whose exchange between the organ and medium was negligible or varied with time, together with amino acids present in excess in the standard medium. Further analysis of this heterogeneous group calls for experiments with modified concentrations of amino acids in the medium.

In experiments on the isolated thorax the dynamics of the amino-acid composition of the medium were studied. For this purpose samples of perfusion fluid taken from the artificial circulation circuit 1, 2, 3, and 4 h after the beginning of perfusion were analyzed (Fig. 1). In this case, just as in the experiments with multiorgan culture, the concentrations of alanine, lysine, and histidine were increased. The concentrations of serine, aspartic acid, isoleucine, tyrosine, and phenylalanine in the medium were reduced. Changes in the concentrations of glycine, methionine, proline, cystine, valine, leucine, glutamic acid, arginine, and threonine with glutamine were inconsistent.

The results thus reflect active amino-acid metabolism in isolated organ culture and, in addition, they provide evidence that the medium used for perfusion was unbalanced in its amino-acid composition: histidine, lysine, and alanine were present in excess, but other amino acids, notably arginine, serine, threonine with glutamine, and aspartic acid were deficient.

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