DYNAMICS OF AMINO ACID COMPOSITION OF THE MEDIUM DURING CULTURE OF ISOLATED LIVER AND KIDNEYS BY THE CONTROLLED PERFUSION METHOD

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The dynamics of the amino acid composition of the medium was investigated during perfusion of the dog liver and kidney for 6 h with a mixture of autogenous plasma and medium No. 199 in the ratio of 2:3. During culture of the kidney for 6 h the histidine concentration in the medium increased by 2.2 times compared with initially, the concentration of glutamic acid by 1.7 times, and of alanine and lysine by 1.6 times, whereas the concentrations of arginine, serine, and aspartic acid fell by 3.3 times and those of glutamine with threonine by 2.5 times. During perfusion of the liver the concentration of glutamic acid rose by 2.9 times, of alanine by 2.3 times, cystine by 2.0 times, and glycine by 1.5 times. The concentration of tyrosine fell by half, and that of phenylalanine and serine by 1.4 times. The arginine concentration fell so quickly during perfusion of the liver that by the second hour after the beginning of perfusion no arginine could be found in the medium. The method of amino acid analysis during organ culture as described can be used as a method of developing and correcting culture media.

KEY WORDS: method of perfusing the dog kidney and liver; amino acids; culture media.

When developing methods of long-term preservation of the functional activity of organs before transplantation [1, 2], it is necessary to know the specific features of metabolism of the surviving tissues and, in particular, of their amino acid metabolism. The amino acid exchange between organ and perfusion fluid is an indicator of the functional state of the organ and a measure of its integrity. Under these circumstances, it is important to supply the tissues of the organ in culture with amino acids which they do not synthesize themselves. Investigations have been carried out [3-5] in which, besides purely theoretical problems of amino acid metabolism, practical problems of medium optimization have been examined. The liver itself has been shown to be unable to regulate the normal plasma level of all free amino acids [6]. The conclusion drawn for the liver evidently is valid for the kidney also, which has a much less important role to play in the overall amino acid metabolism of the organism.

The object of this investigation was to study the dynamics of the amino acid composition of the medium during culture of isolated dog organs (kidney and liver).

EXPERIMENTAL METHOD

Organs of dogs aged 3-5 years, weighing 18-20 kg, were used. The technique of the operative removal of the organs, the apparatus for controlled artificial circulation, and the method of assessment of viability and the functional state of the organs were described previously [1]. The medium used for per-

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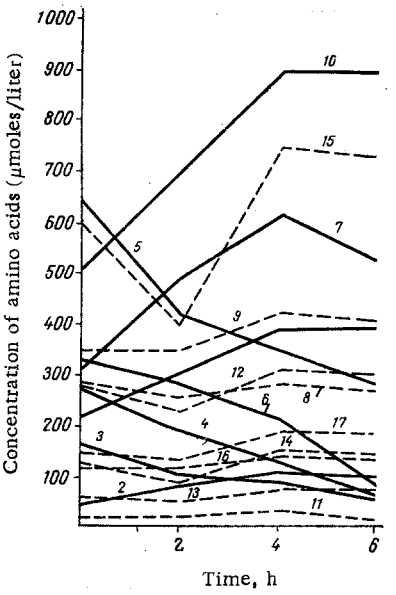

Fig. 1

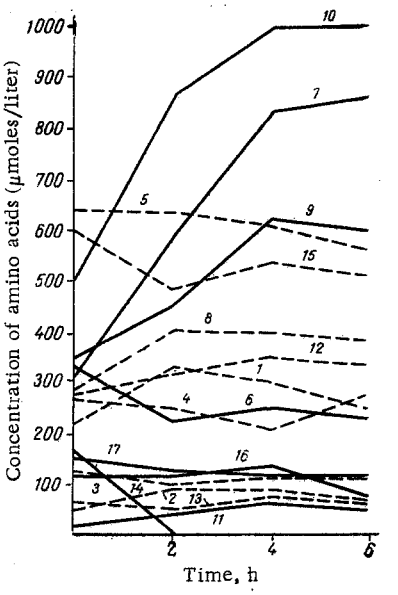

Fig. 2

Fig. 1. Dynamics of amino acid composition of medium during perfusion of dog kidney for 6 h: 1) lysine; 2) histidine; 3) arginine; 4) aspartic acid; 5) glutamine with threonine; 6) serine; 7) glutamic acid; 8) proline; 9) glycine; 10) alanine; 11) cystine; 12) valine; 13) methionine; 14) isoleucine; 15) leucine; 16) tyrosine; 17) phenylalanine. Amino acids between whose extreme values differences are significant are shown by a continuous line; the rest by a broken line.

Fig. 2. Dynamics of amino acid composition of medium during perfusion of dog liver for 6 h. Legend as in Fig. 1.

fusion consisted of a mixture of autogenous plasma stabilized with heparin (1 unit to 1 ml plasma) and medium No. 199 in the ratio of 2:3. Amino acid analysis was carried out by means of the KLA-3B (Hitachi, Japan) automatic analyzer. The medium for analysis was first deproteinized with a 1% solution of picric acid and passed through a column of Dowex 1 × 8 in the Cl^- form.

EXPERIMENTAL RESULTS

Certain regular patterns of change in the amino acid content of the medium during perfusion were discovered (Figs. 1 and 2). These changes were the result of simultaneous breakdown of tissue proteins, their resynthesis, and processes of deamination, reductive amination, and transamination of the free amino acids of the medium, and synthesis of biologically active substances from amino acid precursors.

In the choice of optimal media for perfusion, particular attention must be paid to those amino acids whose concentration changes most strongly during culture. In the present experiments with perfusion of the isolated dog kidney, the chief members of this group of amino acids were histidine, glutamic acid, alanine, lysine, arginine, aspartic acid, serine, and glutamine with threonine. For example, the histidine concentration in the medium 6 h after the beginning of perfusion was 2.2 times higher than initially, and the level of glutamic acid 1.7 times and of alanine and lysine 1.6 times higher; meanwhile the concentration of arginine, serine, and aspartic acid fell by 3.3 times and that of glutamine with threonine by 2.5 times. During perfusion of the liver the glutamic acid concentration rose considerably – by 2.9 times, and that of alanine by 2.3 times, cystine by 2.0 times, and glycine by 1.5 times; meanwhile the concentration of tyrosine fell by 2.0 times and that of phenylalanine and serine by 1.4 times. Arginine disappeared from the medium very quickly. No arginine could be found in the medium in the second hour after the beginning of perfusion. Consequently, medium No. 199 is deficient in arginine and special correction is required for liver culture.

Standard medium No. 199 is thus unbalanced in its amino acid composition as regards the metabolic requirements of the organs studied. Because of marked differences in protein composition, the creation of a universal medium suitable for all organs is evidently impossible. A medium adequate to the specific features of its amino acid metabolism must be provided for the culture of each organ.

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