with nonhistone proteins and newly synthesized RNA. On the other hand, DNA replication evidently begins with active genes [7], but DNA replication in fact begins in the chromatin fraction firmly bound to the matrix [2, 5], which is rich in unique sequences [2]. Comparison of the present results with the facts described above suggests that the chromatin fraction firmly bound to the matrix is enriched with actively transcribable genes.

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UREA SYNTHESIS IN HEART MUSCLE

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Urea formation by the isolated rat heart was studied during perfusion with NH_4Cl (10 mM), mixtures of NH_4Cl (10 mM) and L-aspartic acid (10 mM) and L-ornithine (2.5 mM) with L-arginine (10 mM), L-glutamine (10 mM), L-alanine (10 mM), L-leucine (5 mM), and pyruvate (5 mM). The most effective activator of urea synthesis is NH_4Cl and L-arginine; L-leucine and pyruvate have an inhibitory action. It was shown with the aid of the isotope ^{15}N that ammonia fixation can take place in the heart tissue through the formation of urea. The quantity of ^{15}N incorporated into urea increases with an increase in the concentration of ammonium-^{15}N acetate in the perfusion fluid from 1.6 to 3.4 mM. Isoproterenol necrosis of heart muscle leads to a significant increase in ^{15}N incorporation into urea.

KEY WORDS: urea; synthesis; ammonia, amino acids.

Information in the recent literature on the concentration of urea in the heart muscle and on its role in cardiac activity is extremely limited. Most conclusions of the urea balance in the heart have been based on the study of the coronary arteriovenous difference [2-4, 10]. The following rule was observed in this case: In the presence of high concentrations of urea in coronary blood (> 1 mM) it was assimilated by the heart, whereas in the presence of low concentrations it was eliminated. Hence it can be concluded that the exchange of urea between the blood and heart is determined by gradient.

On the other hand, there is evidence that urea can be formed in the heart itself [4, 13]; activity of enzymes of the ornithine cycle (arginase, orthithine carbamoyltransferase, arginine-succinase) has been found in homogenates of the heart of man and animals [1, 2, 14, 15].

The connection of urea with metabolism of other nitrogen-containing compounds in the heart has been studied in [4, 5]. Investigation of nitrogen metabolism in a group of patients with ischemic heart disease showed

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TABLE 1. Effect of Medium on Urea Concentration (in μg/g tissue) in Perfusion Fluid and Tissue of Rat Heart (M±m)

Perfusion medium	Number of experiments	Perfusion time, min				
		3	6	12	24	36
KHS	6	63,4±7,3	64,6±4,5	65,8±6,5	71,0±4,1*	74,2±5,1*
KHS + NH_4Cl (10 mM)	5	70,4±8,4	82,6±8,5*	100,5±10,0*	116,7±6,2*	119,8±6,5*
KHS + NH_4Cl (10mM), L-ornithine (2.5 mM)	4	68,3±5,4	73,8±10,0	95,6±14,3*	93,4±7,4*	86,4±8,3*
KHS + NH_4Cl (10mM), L-ornithine (2.5 mM) L-aspartic acid (10 mM)	4	72,4±10,0	87,4±6,5*	92,5±6,5*	90,5±9,6*	86,5±9,8*
KHS + NH_4Cl (10mM), L-aspartic acid (10 mM)	5	70,6±7,5	76,8±6,3*	83,4±9,3*	87,5±8,3*	98,0±6,5*
KHS + L-alanine (10 mM)	3	65,5±5,5	67,8±8,0	70,5±6,7	78,3 ± 8.7	83,7±12,5*
KHS + L-arginine (10 mM)	4	78,2±7,4*	87,4±9,0*	91,0±6,2*	100.8 ± 8.9	115,8±7,3*
KHS + L-glutamine (10 mM)	3	62,4±6,3	66,0±5,4	72,0±8,3	84 ± 9.0	86,5±6.0*
KHS + L-leucine (5 mM)	3	58,3±5,7	62,0±6,3	64,0±7,2	69.4 ± 8.2	69,8±4,3
KHS + Pyruvate (5 mM)	4	56,4±6,3	63,8±6,0	59,4±7,8	48.3 ± 11.4	62,3±8,0

Legend. KHS) Krebs–Henseleit solution.

*P < 0.05.

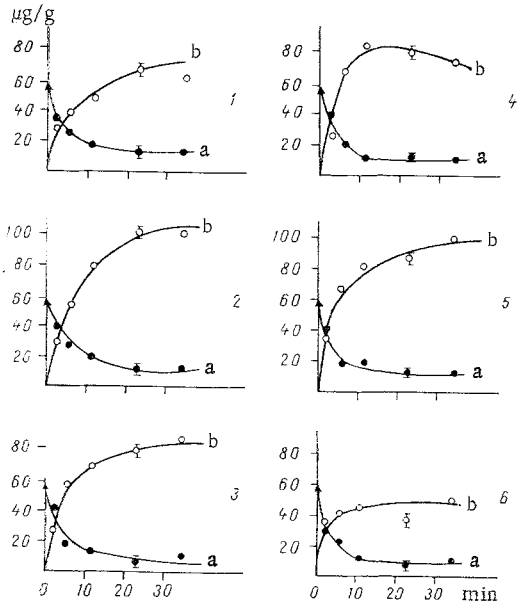

Fig. 1. Urea concentration in tissue of rat heart (a) and medium (b) as a function of perfusion time. Perfusion medium: 1) Krebs–Henseleit solution; 2-6) Krebs–Henseleit solution with NH_4Cl (10 mM), NH_4Cl (10 mM + L-ornithine (2.5 mM), NH_4Cl (10 mM) + L-aspartic acid (10 mM), L-arginine (10 mM), and pyruvate (5 mM) respectively. Filled circles denote urea concentration in rinsed heart. Abscissa, perfusion time (in min); ordinate, urea concentration (in μg/g tissue).

that the ammonia of the myocardium is fixed and excreted into the blood stream through the formation of glutamine, urea, and alanine. Urea formation in the heart may perhaps be an additional pathway for ammonia fixation [11].

The object of this investigation was to study the possibility of urea synthesis from ammonia and various amino acids by the isolated heart. Comparison of urea formation from ammonia in the normal heart and in experimental necrosis also appeared interesting.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250 g. The heart was removed from the anesthetized animals and performed by Langendorff's method with recirculation under a pressure of 60 mm Hg. Krebs–Henseleit solution containing NH_4Cl (10 mM) or a mixture of it with L-ornithine (2.5 mM) and L-

TABLE 2. Incorporation of ^{15}N into Urea after Perfusion of Rat Heart for 30 Min with Ammonium-^{15}N Acetate* (M ± m)

Concentration of ammonium-^{15}N acetate in perfusion fluid, mM	Intact heart	Heart with experimental necrosis	Number of experiments
1.6	0,062±0,005	2,080±0,005	3
2,2	0,078±0,005	0,126±0,005	4
2,8	0,098±0,006	0,128±0,007	3
3,4	0,123±0,005	0,154±0,007	3

Legend. *Excess atomic percentage of ^{15}N – differences between atomic percentage of ^{15}N in sample and in standard (0.365).

TABLE 3. Urea Concentration (in μg/g tissue) in Tissue of Rat Heart and Perfusion Fluid after Perfusion for 30 Min with Ammonium Acetate (M ± m)

Concentration of ammonium acetate in perfusion fluid, mM	Intact heart	Heart with experimental necrosis	Number of experiments
0	74,5±5,1	83,5±4,0	10
1,6	83,5±4,1	89,7±4,2	4
2,2	86,5±3,1	95,5±5,0	5
2,8	92,3±4,0	101,0±6,4	3
3,4	95,5±5,0	104,5±3,8	4

aspartic acid (10 mM), or with L-arginine (10 mM), L-alanine (10 mM), L-glutamine (10 mM), L-leucine (5 mM), and pyruvate (5 mM) served as the perfusion medium. The urea concentration in protein-free tissue extract and in the perfusion fluid was determined photometrically by the reaction with thiosemicarbazide [8].

Urea synthesis from ammonia-^{15}N was studied in the intact rat heart and in the heart with isoproterenol-induced necrosis. The heart was perfused with Krebs–Henseleit solution containing ammonium-^{15}N acetate in concentrations of 1.6, 2.2, 2.8, and 3.4 mM. Urea was isolated from the protein-free tissue extract and perfusion fluid on a column (2.5 × 60 cm) with Amberlite CG-120 III resin on a Beckman M-121 amino acid analyzer. Elution with lithium-citrate buffer (pH 2.8) took place at the rate of 260 ml/h at 40°C. The urea fraction was purified from components of the buffer by passage successively through the cationic exchange resin M-72 and the anionic exchange resin Dowex 2 × 8. The eluate was evaporated to dryness in vacuo at 40°C. The CHN analyzer was used for preparative isolation of urea nitrogen. The isotope composition of the nitrogen was determined on the MI-1305 mass spectrometer.

EXPERIMENTAL RESULTS

The first step was to determine the urea concentration in the rat heart before perfusion with Krebs–Henseleit solution. It was 60.5 ± 6.3 μg/g wet weight of tissue.

During perfusion of the heart with Krebs–Henseleit solution without additives, or with ammonium chloride, amino acids, mixtures of amino acids, and pyruvate the changes in the urea concentration were similar in character (Fig. 1): accumulation in the medium and a decrease in its concentration in the heart tissue with the course of time. Evidently the small size and the electrical neutrality of the urea molecule under physiological conditions facilitate its ready diffusion through cell membranes and its outflow along the concentration gradient. As Fig. 1a shows, perfusion led to the establishment of a "stationary" urea concentration in the muscle tissue after about equal times. Its level was relatively independent of the nature of the medium and amounted to 10-15 μg/g tissue. The presence of pyruvate and of L-leucine in the medium reduced the urea concentration in the perfusion fluid (Fig. 1: 6b).

The greatest stimulation of urea synthesis was found when NH_4Cl and L-arginine were used as nitrogen donors (Table 1). Addition of L-alanine, L-glutamine, and mixtures of ammonium chloride with aspartic acid and L-ornithine to the perfusion fluid was less effective. L-leucine inhibited urea synthesis and pyruvate completely blocked the formation of this substance. Similar results were obtained during perfusion of the liver, and in experiments on tissue slices, isolated mitochondria, and suspensions of liver cell [6, 7, 9, 12].

Urea formation in the heart tissue was confirmed by transfer of ^{15}N from its precursor (ammonium-^{15}N acetate) (Table 2). An increase in the degree of enrichment of the urea, both in the intact and the infarcted heart, and with the ^{15}N isotope was observed with an increase in the ammonium-^{15}N acetate concentration in the perfusion fluid.

In isoproterenol necrosis the quantity of urea formed by the heart during perfusion increased on average by 10% (Table 3). Using the data on enrichment of urea with the ^{15}N isotope and its concentrations in the heart and perfusion fluid, the total quantity of ^{15}N incorporated into urea during perfusion with ammonium-^{15}N acetate was calculated (Fig. 2). The quantity of ^{15}N incorporated into urea increased with an increase in the ammonium-^{15}N acetate concentration in the perfusion medium. Experimental necrosis led to a significant increase in ^{15}N incorporation (by 30-90%) compared with normal.

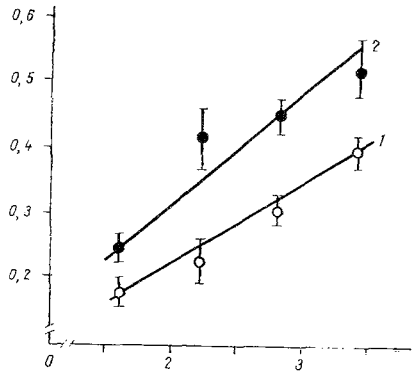

Fig. 2. Content of ^{15}N in urea of intact rat heart (1) and heart with experimental necrosis (2). Abscissa, concentration of ammonium-^{15}N acetate in perfusion fluid (in mM); ordinate, ^{15}N content (in μg-atoms ^{15}N/g tissue $\times 10^2$).

The similarity of slope of the curves representing the concentration of ammonium-^{15}N acetate in the perfusion fluid and incorporation of ^{15}N into urea is in good agreement with the earlier hypothesis [1] that urea synthesis in heart muscle is a response to elevation of its ammonia level. The important point is that urea synthesis is activated in a heart with experimental necrosis.

It can be concluded from the experimental results described above that reactions of urea synthesis take place in heart muscle. This is shown by the formation of large quantities of urea during perfusion of the isolated heart with ammonia and amino acids, significantly higher than the urea concentration in the myocardium before the experiment, and also by the enrichment of urea with heavy nitrogen of the precursors – ammonia-^{15}N. Urea synthesis is used by the heart to fix excessive quantities of ammonia, as is shown by the similarity of slope of the curves reflecting the quantity of urea synthesized and the concentration of ammonia-^{15}N introduced. The role of urea synthesis as a mechanism of regulation of the tissue ammonia concentration assumes greater importance during necrosis of the heart muscle.

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