CHANGES IN INCORPORATION OF AMINO ACIDS INTO PROTEINS AFTER DEATH

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Incorporation of glycine-C^{14} into proteins of liver and heart slices taken from the cadaver 5 and 240 min after death is virtually identical. The proteins of a pancreatic mince taken 5 min after death incorporate much more glycine than 240 min after death. The content of sulfhydryl groups in all investigated tissues was lower 240 min than 5 min after death.

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Investigations have shown that incorporation of various labeled amino acids into proteins takes place in surviving tissue slices and homogenates. This process is regarded an an index of synthesis of protein molecules in these biological systems [3, 5, 6].

Since protein synthesis is a fundamental assimilatory process intimately connected with phenomena of life, it is important to determine at what period after death of the organism the tissues lose their ability to carry out this process in the dying systems and what environmental factors limit it.

In the present investigation the effect of the time elapsing after removal of an organ from the body after termination of its vital activity on the incorporation of amino acids into proteins of surviving tissue slices and minces was studied.

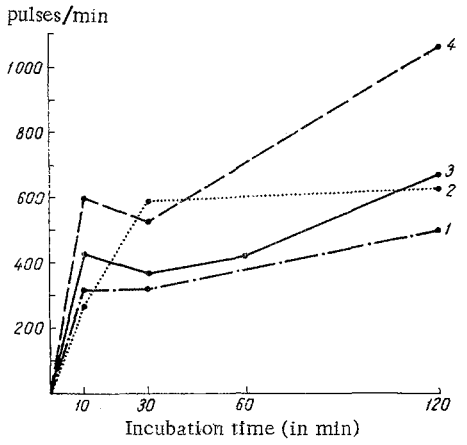

Fig. 1. Rate of incorporation of amino acids into proteins of dying tissue systems taken 5 and 240 min after death of the animal. 1,2) Liver slices taken 5 (1) and 240 (2) min after death; 3,4) heart slices taken 5 (3) and 240 (4) min after death.

EXPERIMENTAL METHOD

The test objects consisted of slices of liver and heart and mince of the thigh muscles and pancreas. The tissue samples were taken 5 and 240 min after sacrifice of the animal by decapitation. The animal's cadaver was kept at room temperature the whole time. The sample of tissue for investigation taken for the experiment was usually 150 mg. Krebs–Henseleit buffer, pH 7.3 was used. The tissue samples were placed in small flasks with ground-glass stoppers containing 25 ml buffer solution and the labeled amino acid glycine-C^{14} ($4 \cdot 10^6$ pulses/min per sample). One batch of experimental samples was saturated with oxygen. All samples were incubated for various times at 37° with shaking in a water thermostat. Proteins were then precipitated with TCA (final concentration 10%). The precipitates thus formed were treated in the usual manner [1]. Radioactivity of the protein samples obtained was determined. In addition, to identify changes in structure of the proteins in the experimental samples, the content of sulfhydryl groups was determined [4]. The content of total SH-groups was estimated in homogenates of the test organs, and the content of nonprotein SH-groups estimated in the filtrate after precipitation of proteins by sulfosalicylic acid; the difference gave the content of protein SH-groups in the samples.

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TABLE 1. Incorporation of Labeled Amino Acid into Proteins of Dying Systems in Relation to Time after Death (Mean Data)

Organ	Time of removal of organ after animal's death (in min)			
	5		240	
	with O_2 saturation	without O_2 saturation	with O_2 saturation	without O_2 saturation
Liver (slices)	96±26	79±24	89±19 ($P>0,05$)	70±20
Heart (slices)	81±15	70±20	64±18 ($P>0,05$)	67±10
Pancreas (mince)	595±42	395±30 ($P<0,05$)	207±35 ($P<0,010$)	153±15 ($P<0,05$)
Thigh muscles (mince)	60±10	40±15	77±17 (P>0,05)	53±20

TABLE 2. Changes in Content of SH-groups in Tissues 5 and 240 Min after Death (Mean Data)

Tissue	Time of removal of tissue (in min)	Content of SH-groups			Change (in percent)	P
		total	non-protein	protein		
Liver	5	1,35	0,21	1,14±0,16	—60	<0,05
	240	0,80	0,23	0,57±0,05		
Heart	5	0,93	0,13	0,80±0,07	—45	<0,05
	240	0,60	0,14	0,46±0,01		
Muscle	5	1,08	0,05	1,03±0,02	—42	<0,05
	240	0,650	0,045	0,60±0,04		

EXPERIMENTAL RESULTS

Incorporation of the radioactive amino acid into proteins of liver slices was of the same order 5 min and 240 min after death of the animal (Table 1). A similar picture was obtained when this process was investigated in slices of heart muscle and in a skeletal muscle mince. Incorporation of the amino acid into proteins of a pancreatic mince, on the other hand, was considerably reduced in samples taken 240 min after the animal's death, being only 34% of that found in sample taken 5 min after death.

The results given in Table 1 thus show that incorporation of amino acids into proteins of the liver, heart, and skeletal muscle tissues persists for a comparatively long time after death. The closest relationship between this process and the time elapsing after the animal's death was observed in the case of pancreatic tissue.

In another series of experiments the rate of amino-acid incorporation into proteins of liver and heart slices removed 5 and 240 min after death of the animal was compared. In sections of liver tissue taken 240 min after animal's death, the velocity of this process continued high during the first 10 min of incubation (Fig. 1), the radioactivity being almost the same as in the sample taken 5 min after death. Incorporation thereafter continued to increase, flattening out on a plateau after 30 min. Incorporation increased more slowly in samples taken 5 min after death, and had not reached the plateau even after 2 h.

Investigation of this process in heart slices revealed a parallel trend between the level of glycine incorporation during incubation of tissue samples taken 5 and 240 min after the animal's death; radioactivity in this case had not reached the plateau even after 2 h.

The indices of the velocity of incorporation of free amino acids into proteins also demonstrate that this process undergoes no substantial change in the liver and heart tissues during the 240 min after death, although in this same period, judging from changes in the content of SH-groups, considerable structural changes occur in the proteins of the tested organs. In all tissues taken 240 min after death, the content of protein SH-groups was much lower than in tissues taken 5 min after the animal's death (Table 2). At the same time, no change occurred in the content of nonprotein SH-groups.

The effect of saturation of the sample with oxygen on the incorporation of amino acid into tissue proteins of the liver and heart, and also of the pancreas, taken at various times after death was also studied. The incorporation of glycine-C^{14} into proteins of heart and liver tissue slices was unaffected by this procedure (Table 1). Incorporation into pancreatic tissue proteins was activated by oxygen saturation, the

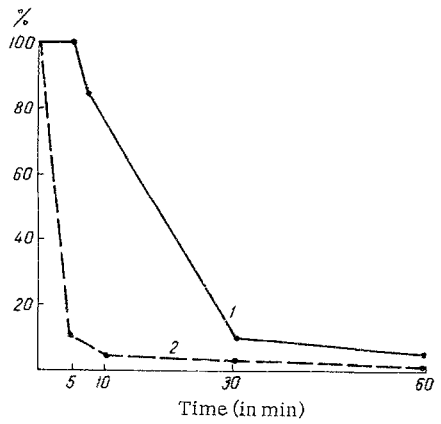

Fig. 2. Changes (in %) in incorporation of labeled amino acids glycine-C^{14} into heart (1) and liver (2) proteins of rabbits as a function of time after death. Glycine-C^{14} injected via artificial circulation.

sensitivity of the tissue to saturation being of the same order both 5 and 240 min after the animal's death.

Previous experiments [2] on dogs and rabbits showed that during perfusion of the animal's body after death incorporation of amino acids into liver and heart proteins virtually stops after 90 min. This sharp decrease in the velocity of protein synthesis after death, despite perfusion of the whole body, took place although the concentration of free labeled amino acids in the tissues remained at a high level throughout the experiment. Meanwhile the level and rate of incorporation of amino acids into proteins of analogous tissues taken 5 and 240 min after death remained virtually unchanged when incubated in vitro.

The differences described above (Fig. 2) suggest that protein synthesis is reversibly inhibited in the body after death. With a change in the environmental conditions and if the tissues are placed in suitable buffer solutions, protein synthesis is restored.

The results of these investigations demonstrate the unique resistance of the process of amino-acid incorporation into the proteins of tissue slices and minces during incubation for several hours. Ability to incorporate labeled amino acids into proteins continues at a high level in the liver and heart tissues even 4 h after death, and differences in the course of this process characteristic of each organ remain undisturbed.

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