GLYCOGEN METABOLISM IN ISCHEMIZED ORGANS

L. P. Selivanova and L. M. Saburova

UDC 616-005-8-07:616-008.934.586.3-074

Acute ischemia was produced by application of a tourniquet to the limb or a clamp to the pedicle of the kidney for a time corresponding to the critical level of metabolism in the test tissue. Restoration of the blood flow in the ischemized kidney led to accumulation of an excess of high-molecular-weight glucose polymer of glycogen type. The character of branching in its molecule, determined from the spectrum of its complexes with iodine, points to changes in processes of glycogen biosynthesis. For the needs of glycogenesis, lactate of the ischemized kidney can be used. It is shown that this abnormal glycogen is actively utilized by kidney tissue. Accumulation of glycogen in muscle tissue after acute ischemia does not exceed the normal level and its structure is unchanged.

KEY WORDS: rat kidney and limb; glycogen; ischemia.

Previous investigations showed that at the critical level of metabolism in ischemized grafts of kidney and small intestine, after recovery of the circulation glycogen accumulates in excess, far higher than the level during life [1-3]. These investigations were carried out on models of organ transplantation. Considering the very many nonspecific factors acting on the tissue under these circumstances (acute ischemia, decentralization, etc.), it was decided to study the role of acute ischemia in isolation in the development of this "glycogenosis" of the organ, with estimation of its ability to utilize the abnormal glycogen in cases of repeated oxygen deficiency.

EXPERIMENTAL METHOD

Comparative experiments were performed on two organs — the limb and kidney — in which acute ischemia was produced for a time long enough to assume that the test tissue would be in a state of the critical level of metabolism. Acute ischemia was induced by clamping the neurovascular muscle of the left kidney without disturbing the anatomical connection of the organ with the body. The clamp was removed after 1 h and the experiment ended 3 h after restoration of the circulation. Intact and contralateral kidneys served as the control. Acute ischemia of the rat limb was produced by applying a tourniquet at the middle of the thigh. The tourniquet was removed 3-5 h later and the experiment ended 3 h after restoration of the circulation. Intact and symmetrical limbs served as the control.

In all the experiments glycogen was extracted with alkali from the kidney or muscle tissue and, after acid hydrolysis, glucose was determined by an enzymic method [14]. The structure of the glycogen was studied by Krisman's method [5]. Simultaneously with determination of the glycogen level in the tissues the lactate concentration was studied by Hochorst's method [6]. In the experiments with ischemia of the kidney glycogen was isolated and its ability to be utilized was studied *in vitro* in Krebs–Ringer medium for 3 h at 37°C.

EXPERIMENTAL RESULTS

Restoration of the blood flow in the ischemized kidney led to surplus biosynthesis of a high-molecular-weight polymer of glucose of glycogen type. The spectrum of complexes of this glycogen with iodine differed significantly from that of the corresponding complexes of glycogen from intact kidneys (Fig. 1). The absorption maximum of glycogen from the ischemized kidneys occurred at 270 nm, whereas that of glycogen from the intact kidneys occurred at 400 nm. Differences in the profile of the curves are evidence that on restoration of the

Laboratory for Transplantation of Organs and Tissues, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, V. V. Kovanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 10, pp. 414-416, October 1978. Original article submitted February 16, 1978.

0007-4888/78/8610-1299$07.50

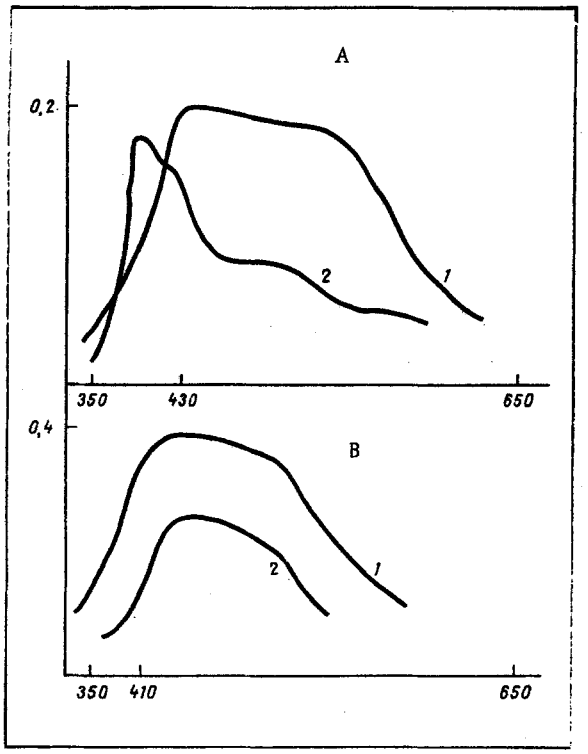

Fig. 1. Absorption spectrum of iodine complexes of glycogen isolated from ischemized kidneys and limbs before (1) and after (2) restoration of blood flow. Abscissa, wavelength (in nm); ordinate, relative optical density. A) Kidney; B) limb.

TABLE 1. Lactate Utilization by Rat Kidney Homogenate

Experimental conditions	Statistical index	Lactate concentration	
		before incubation	after incubation
Incubation with endogenous lactate	$M \pm m$	10,50±0,86	1,70±0,91
	P	—	<0,05
Incubation with exogenous lactate	$M \pm m$	9,30±1,03	2,30±0,36
	P	—	<0,05

blood flow modifications took place to the processes of glycogen biosynthesis which led to disturbance of the character of branching of the molecule of this polysaccharide. Comparison of the structure of the glycogen isolated after restoration of the circulation, both with that from the completely isolated grafted organ and from the organ ischemized without disturbance of its anatomical connections with the rest of the body, showed that the changes in profile were similar in character. The main factor in the development of "glycogenosis" was evidently acute ischemia itself. However, the change in structure of the glycogen did not affect its ability to be utilized in metabolism.

The model experiments showed that the polysaccharide, isolated in experiments with restoration of the circulation in the ischemized kidneys, was utilized by the tissue (Table 1). Consequently, under certain conditions (the critical level of metabolism) kidney tissue becomes capable of accumulating a high-molecular-weight polymer of glucose, which can be utilized in an emergency. Lactate is evidently one source for glycogen biosynthesis in kidney tissue. Kidney tissue both *in vivo* and *in vitro* becomes capable of glycolysis with the accumulation of lactate, but restoration of the blood flow leads to a marked decrease in the lactate concentration in the tissue (Table 2). The decline in the excess of lactate in the kidney tissue could be simulated *in vitro*. The addition of endogenous lactate to the incubation mixture in quantities corresponding to its concentration in the ischemized tissue enabled a decline in the lactate concentration to be recorded after 3 h of incubation. The ischemized kidney tissue was the source of the endogenous lactate. A similar effect was observed when

TABLE 2. Level of Metabolites in Ischemized Limb and Kidney after Restoration of Blood Flow (M ± m)

Organ	Metabolite, μmoles/g	Intact tissue	Toward end of period of ischemia	After restoration of blood flow
Limb	Glycogen	27,0±2,1	9,0±1,0*	16,4±2,5†
	Lactate	13,1±1,2	37,6±1,18*	26,5±0,3†
Kidney	Glycogen	7,36±0,64	0,84±0,14*	32,5±2,8†
	Lactate	4,06±0,16	9,9±0,31*	0,48±0,04†

Legend. *) $P < 0.05$ compared with control; †) $P < 0.05$ compared with ischemized tissue.

exogenous lithium lactate was added to the incubation mixture in the same concontrations. If compared with the observed accumulation of glycogen in the kidney tissue, the results of these experiments suggest that lactate in the ischemized kidney can be used for the needs of glycogenesis.

Investigaton of the glycogen content inmuscle tissue after restoration of the blood flow in the ischemized limb showed that far from exceeding its level during life, it was actually below the initial value. Excess accumulation of glycogen was observed in only 2 of 41 cases. The spectrum of its iodine complexes was the same as that of the control (Fig. 1).

Similar differences in the tissue response were observed previously by the writers during an investigation of glycogen metabolism in grafts [2]. Muscle tissue during evolution has developed the ability to create "long-term energy reserves," for it is frequently in a state of oxygen debt. To maintain functional activity the carbohydrate reserves are utilized. The enzyme system of ischemized muscle tissue remained capable for a long time of regenerating reserves of glycogen similar in structure to that in the intact muscle. The accumulation of carbohydrate reserves in the form of glycogen is not characteristic of kidney tissue. However, an extremal situation such as acute ischemia causes changes in coordination of the activity of the enzyme system responsible for biosynthesis and breakdown of carbohydrates, leading to excessive accumulation of glycogen. The creation of carbohydrate reserves in response to previous ischemia is a typical metabolic reaction for all tissues.

1. L. M. Saburova, N. I. Mosin, and V. A. Bukov, Byull. Éksp. Biol. Med., No. 5, 47 (1974).
2. L. M. Saburova and L. P. Selivanova, Byull. Éksp. Biol. Med., No. 2, 17 (1975).
3. L. M. Saburova, "Metabolic processes in grafts during acute ischemia," Author's Abstract of Doctoral Dissertation, Moscow (1975).
4. M. E. Preobrazhenskaya, in: Modern Methods in Biochemistry [in Russian], Vol. 2, Moscow (1968), p. 347.
5. C. R. Krisman, Analyt. Biochem., 4, 17 (1957).
6. H. J. Hochorst, Biochem. J., 112, 149 (1957).