A BIOCHEMICAL ANALYSIS OF BIOLOGICAL PREPARATIONS STIMULATING REGENERATION OF THE MYOCARDIUM IN RATS

V. L. Mant'eva

From the A. N. Severtsov Institute of Animal Morphology of the AN SSSR, Moscow

(Received March 11, 1958. Presented by Active Member AMN SSSR S. E. Severin)

In order to restore the lost powers of regeneration of the limbs of the tailless amphibia, with a view to stimulating destruction and dedifferentiation, the method of traumatization has been used [3]. The regeneration and development of animal organs are also readily stimulated by biological preparations obtained from the tissues of homologous organs [2, 5, 6, 8]. In our experiments, after causing injury to the myocardium of rats by means of diathermy coagulation, and the subsequent injection of the animals with hydrolyzates and tissue extracts of the rat's heart, lysis of the old, necrotic tissues was accomplished $2\frac{1}{2}$ times more quickly, dedifferentiation of the cut ends of the muscle was stimulated and regeneration of the young muscles in the focus of injury was intensified [4].

M. P. Tushnov [7] believed that the active principle of his organ preparations was the polypetides and peptides.

Protein-free extracts of the mammalian heart are nowadays used abroad in the treatment of various forms of cardiac insufficiency [9, 10]. There are indications that their active components are adenylic acid, histamine and choline [11].

The aim of our research was to ascertain the biochemical composition of a hydrolyzate and tissue extract of the rat's heart used for the purpose of stimulating the regenerating power of the myocardium.

EXPERIMENTAL METHOD AND RESULTS

Believing that the principal active factors of the preparations were proteins and their decomposition products, we made a preliminary determination of these in respect to the following constituents: 1) nitrogen – total, protein, nonprotein, amino-; 2) the number and character of the fractions revealed by amide black during paper electrophoresis; 3) the number of stains appearing with ninhydrin during paper chromatography; 4) the dry weight and pH.

In the 1956 experiments the hydrolyzate of rats' hearts was obtained by means of an acid 87% glycerol extract of rabbit's liver, but in the 1957 experiments, by means of cathepsin from the spleen [2]. Fresh rats' hearts, washed free from blood, were ground up and hydrolyzed at 37° in a citrate buffer at pH = 4.4 for 18 hours, by the action of cathepsin, the course of the hydrolysis being controlled by the biuret test. The product was filtered and vacuum-dried at room temperature. Before use, the hydrolyzate was diluted to contain 0.0086 mg/ml (as dry weight) in 0.85% NaCl solution. The animals were treated with 7 subcutaneous injections of a dose of 0.5 ml every other day.

The tissue extract of the rats' hearts was prepared from fresh rats' hearts, washed free from blood. The hearts were ground up, and a cold 0.85% solution of NaCl added until 28% and 14% extracts of the crude tissue

TABLE 1

Characteristics of the Hydrolyzate and 14% Extract of Rats' Hearts

Preparation	Dry wt., mg/ml	pH	Nitrogen, mg/ml			
			total	non-protein	protein	amino-
Hydrolyzate of rats' hearts	22	5	1.102	0.812	0.290	0.471
Extract of rats' hearts	13.2	7	0.560	0.168	0.392	0.005

TABLE 2

Characteristics of the Extract and Hydrolyzate of Rats' Hearts Injected into Each Experimental Rat

Preparation	Preparation injected into rat, mg	Nitrogen injected into rat, mg			
		total	non-protein	protein	amino-
Hydrolyzate of rats' hearts	0.03	0.0015	0.0011	0.0004	0.0006
Extract of rats' hearts	99	4.200	1.260	2.940	0.037

were obtained. The extract was allowed to stand for 1 hour at a temperature of +2° to +4° and filtered through paper; 5 injections of the 28% extract and 5 injections of the 14% extract were given subcutaneously every other day. The general features of the hydrolyzate investigated in a dilution of 22 mg/ml and of the 14% extract of the rats' hearts are described in Table 1.

The total and nonprotein nitrogen were determined by the micro-Kjeldahl method; the protein nitrogen was obtained by subtracting the nonprotein from the total nitrogen; the amino-nitrogen was determined by Van Slyke's method, and the pH colorimetrically. In Table 2 are shown the characteristics of the extract and hydrolyzate of rats' hearts injected into each rat (in the course of the experiment).

For separation of the biological preparations we used the paper electrophoresis method. Solutions of the preparations were poured on to strips of "slow" filter paper of Soviet manufacture, 34 cm long and 3.5 cm wide. The experiment was conducted in a humid chamber for 16-19 hours at room temperature. The experimental conditions were as follows: voltage applied to the strip 130 v, current along the strip 0.75 ma, phosphate buffer pH = 7.4. The electrophoregrams were developed with amide black [1].

In the heart extract 3 components were found to be stained by amide black: two clearly defined components moved toward the anode, and the third component was ill-defined at its margins and remained at the point of application. When the electrophoregrams of the extract were compared with the electrophregrams of the blood serum of rats, obtained under the same experimental conditions, we found that the mobility of the components of the extract was close to the mobility of the albumin and the α_2 - and γ-globulins of the serum.

Electrophoresis of the hydrolyzate of the hearts was carried out in the same conditions. In the hydrolyzate 2 components were found: one moved toward the anode, the other had ill-defined edges and remained at the point of application. The mobility of the components was close to that of the albumin and γ-globulin of the blood serum of the rat.

For the preliminary chromatographic analysis of the free amino acids of the enzymic hydrolyzate and the extract of the rats' hearts, we used "slow" chromatographic paper of Soviet manufacture. The preparations for analysis were purified from proteins with alcohol and concentrated in a vacuum drier. The amino acids were dispersed in a mixture of n-butyl alcohol – glacial acetic acid – water for 10-12 hours (descending linear chromatogram). The chromatograms were at first dispersed in a 4 : 1 : 5 mixture (upper layer), and then, after slight drying, in a 50 : 15 : 5 mixture (one or two phases).

In the hydrolyzate 11 stains were found, belonging to lysine, arginine, asparaginic acid, glycine, glutaminic acid, alanine, proline, tyrosine, valine, phenylalanine and leucine; in the extract there were 10 stains, among which the same amino acids were found, with the exception of arginine, proline and tyrosine. Two stains in the extract could not be identified. It is possible that the hydrolyzate also contained peptides, which coincided in position with the amino acids.

In our experiments the hydrolyzate of rats' hearts was more active than the extract. The hydrolyzate produced intensive destruction and dedifferentiation of the muscle stumps, accelerated the processes of lysis of the necrotic tissues and stimulated the growth of the newly formed fibers. In the dilutions which we employed it contained very small amounts of protein, nonprotein substances and amino acids. We believe that the polypeptides liberated in the process of hydrolysis could stimulate dedifferentiation of the muscle stumps, since the extract hardly stimulated destruction and dedifferentiation of the muscles nor lysis of the necrotic tissues at all. The extract did, however, considerably stimulate the processes of cell proliferation and in particular, growth of the newly formed muscle fibers. Evidently one of its component parts must have had this effect on the processes of growth and cell proliferation. The different biological activity of our preparations must have depended on their chemical composition and dosage.

The hydrolyzate contained little native protein and many of its decomposition products; the extract contained much protein and few decomposition products; the ratio of amino- nitrogen to total nitrogen for the hydrolyzate was about 40%, and for the extract, about 1%. The active part of the hydrolyzate may be the compounds liberated in the process of hydrolysis, which in the extract are present in a combined state. Hydrolyzate was used and showed its activity in small doses, extract in large; during the whole period of treatment of a single rat in the experiment, 0.03 mg of the dry substance of the hydrolyzate was injected, as compared with 99 mg of the dry substance of the extract, i.e., by dry weight, 3300 times less hydrolyzate than extract was administered.

The object of further research will be to define more precisely the chemical composition of the preparations and to discover the active fractions which stimulate regeneartion of the myocardium as a whole and the different phenomena of this biological process (destruction and dedifferentiation of tissues, cell proliferation and growth).

SUMMARY

For stimulation of regeneration of rat's myocardium the authors used a hydrolyzate and an extract of rat's heart. It was shown by the method of electrophoresis on paper that the hydrolyzate consists of 2 components similar by their motility to the albumin, and γ-globulin of the blood serum of rats. The extract contains 3 components similar by their motility to the albumin, α_2- and γ-globulin of the blood serum of rats. The chromatographic analysis of the free amino acids of the hydrolyzate demonstrated the presence of arginine, lysine asparaginic acid, glycine, glutamic acid, alanine, proline, tyrosine, valine, phenylalanine and leucine. The same amino acids except arginine, proline and tyrosine were revealed in the extract. The quantity of nitrogen, the dry weight and pH were also determined in the preparations. We consider that proteins, nucleoproteids and the products of their disintegration comprise the active part of the preparation.

LITERATURE CITED

[1] M. F. Bondarenko, Byull. Éksptl. Biol. i Med., 36, No. 10, 70-73 (1954).

[2] N. D. Zelinskii, Zhur. Obshchei Biol., 7, No. 3, 161-165 (1946).

[3] L. V. Polezhaev, Trudy Inst. Tsitol. Gistol. i Embriol., AN SSSR, 2, 2, 3-129 (1948).

[4] L. V. Polezhaev, L. V. Abakhadze, N. A. Zakharova and B. L. Mant'eva, Doklady Akad Nauk SSSR, 119, No. 5, 1039-1042 (1958).**

[5] L. V. Polezhaev, G. P. Ramenskaya, Doklady Akad. Nauk SSSR, 70, No. 1, 141-144 (1950).

[6] G. D. Tumanishvili, K. M. Dzhandieri, I. K. Svanidze, Doklady Akad. Nauk SSSR, 107, No. 1, 182-184 (1956).

[7] M. P. Tushnov, Collection of Papers on the Study of Histolyzates, 1, pp. 3-30, Kazan',1931 [In Russian].

*See English translation.

[8] D. M. Fedorov, Doklady Akad. Nauk SSSR 38, No. 1, 52, 56 (1943).

[9] G. Bassignana, Profilassi, 1954, v. 27, N. 1.

[10] A. Dussert, Thérapie, 1954, v. 9, N. 1.

[11] F. C. Lu, M. G. Allmark, Rev. canad. biol., 1953, v. 12, pg. 423-427.

[12] I. S. Fruton, M. Bergmann, J. Biol. Chem., 1939, v. 130, p. 19.