Experiments with the donors' peripheral blood confirmed the previous observations in experiments *in vivo*, showing that treatment with glycerol affects the immunologic activity of the lymphocytes through its selective action on the T lymphocyte population.

The new property of glycerol, revealed by these experiments, of reducing the homotransplantation activity of T lymphocytes thus suggests that conserved bone marrow has advantages over freshly prepared marrow because it weakens the GVHR and possesses higher proliferative activity. In addition, if conserved bone marrow is transplanted from several donors, there is no risk of inactivation of the stem cells by nonsyngeneic lymphocytes and exogenous colony formation in the recipient is enhanced.

LITERATURE CITED

1. L. A. Danilova, A. G. Fedotenkov, R. V. Petrov, et al., Probl. Gematol., No. 12, 21 (1978).
2. R. V. Petrov and L. S. Seslavina, Dokl. Akad. Nauk SSSR, 176, No. 5, 1170 (1967).
3. R. V. Petrov et al., in: Progress in Science and Technology. Series: Immunology [in Russian], Vol. 7, Moscow (1978), p. 201.
4. A. G. Fedotenkov, L. P. Ignasheva, and L. A. Danilova, Reducing the Homotransplantation Activity of Lymphocytes of Hematopoietic Tissue. Preprint [in Russian], Khar'kov (1980).
5. R. M. Khaitov, Byull. Éksp. Biol. Med., No. 6, 88 (1980).
6. C. Bianco et al., J. Exp. Med., 132, 702 (1970).
7. C. M. Clarce and A. Lengerova, Folia Biol. (Prague), 8, 335 (1962).
8. M. Jondol et al., J. Exp. Med., 136, 207 (1972).
9. B. Nakic and D. Volf, Proc. Yugoslav. Immun. Soc., 1, 46 (1969).

ANALYSIS OF BLOOD SERUM AFTER INJURY TO THE SUBMANDIBULAR SALIVARY GLAND IN RATS

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The writers have shown that sialotomy of the submandibular salivary gland (SMSG) activates proliferation from a distance in the corneal epithelium [6]. If the phenomenon were specific for SMSG, it could be postulated that it is attributable to high-molecular-weight polypeptide growth factors synthesized by the gland: epidermal (mol. wt. 74,000), endothelial (mol. wt. 80,000-86,000) [2], and mesodermal [14]. The present writers also found hyperplasia of the corneal epithelium after trauma to the liver [6]. It is worth mentioning that other nonspecific mitogens also exist.

The object of the present investigation was to compare changes in the protein, glycoprotein, enzymic, and antigenic composition of the blood in the course of time after partial resection of SMSG in order to detect any possible regeneration mitogens.

EXPERIMENTAL METHOD

Experiments were carried out on 129 Wistar rats weighing 140-160 g, of both sexes. Trauma to SMSG was produced by the method described previously [6]. Serum protein fractions were studied by electrophoresis in agarose gel. The seromucoid content was determined by the modification in [5]. Proteins of the traumatized and contralateral SMSG were detected in the blood by double diffusion in agar as in [4]. A saline extract of SMSG tissues obtained from 140 intact rats was used as the antigen. Antisera were obtained by immunizing six mature rabbits 9 times. The antiserum was exhausted of antibodies against blood proteins by fractional absorption with

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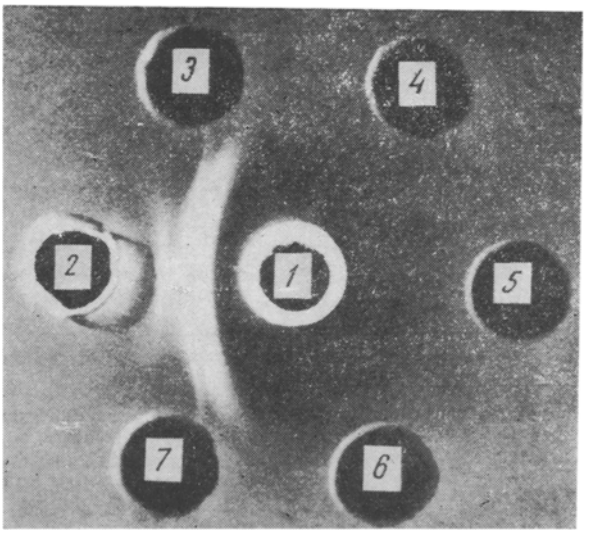

Fig. 1

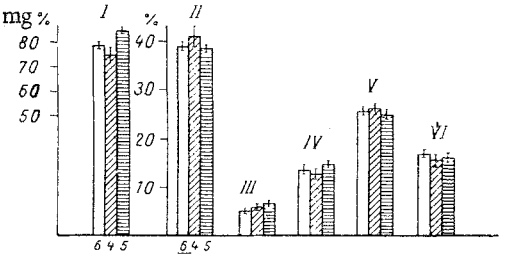

Fig. 2

Fig. 1. Determination of tissue antigens in blood at different times after resection of SMSG in rats. 1) Antiserum against SMSG; 2) extract of SMSG; 3) serum of intact rats; 4) rat serum 3 h after sialotomy; 5) 24 h after sialotomy; 6) 48 h after sialotomy; 7) physiological saline.

Fig. 2. Composition of protein fractions and seromucoid in rats after resection of SMSG. First columns — normal state; second columns — 24 h after sialotomy; third columns — 48 h after sialotomy; 6, 4, 5) number of animals in group. I) Seromucoid, II) albumin, III) α_1-globulin, IV) α_2-globulin, V) β-globulin, VI) γ-globulin.

TABLE 1. Effect of Injury to SMSG on LDH Isozyme Spectrum in Gland Tissue and in Blood

Test object	Time of investigation	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5	n
Blood	Normal state	10,1±0,2	8,9±0,3	10,5±2,0	8,8±1,8	61,7±0,8	30
	12 h after trauma	15,1±0,1***	11,6±0,6**	6,2±0,8	7,4±1,2	59,6±0,6*	3
	24 h after trauma	15,6±0,2***	10,4±0,8	6,0±0,7*	8,0±0,6	59,9±0,9	4
Tissue of SMSG	Normal state	4,6±0,2	15,6±1,6	18,3±1,0	26,4±1,0	36,0±1,0	10
	12 h after trauma	0***	6,6±0,7***	15,9±0,8	33,3±1,7**	44,2±1,3***	4
Tissue of contralateral SMSG	12 h after trauma	0,4±0,1***	8,8±0,6**	20,4±2,0	30,5±1,2*	39,8±1,9	4
Tissue of SMSG	24 h after trauma	0,2±0,2***	6,1±0,8***	17,9±0,7	34,0±0,9***	41,7±0,8***	5
Tissue of contralateral SMSG	24 h after trauma	2,3±0,4***	9,6±1,8*	17,5±1,0	31,8±1,1*	38,8±1,6	5

Legend. *P < 0.05; **P < 0.01; ***P < 0.001; n) number of animals.

lyophilized rat blood serum under control of the precipitation test [10]. The lactate dehydrogenase (LDH) isozyme spectrum was studied by the method in [7]. The ratio between the isozyme and blood protein fractions was determined as a percentage after densitometry on the Statron 301 E integral densitometer (East Germany). The significance of differences was estimated by Student's t test.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that the reaction between antiserum and normal SMSG tissue revealed three precipitation lines. No antigenic components of SMSG were found in the blood 3, 24, and 48 h after resection of the gland. These results were confirmed by a repeated ex-

periment on other rats. It was also shown that the antigenic composition of the tissue proteins of the contralateral gland was unchanged after trauma. Considering the high resolving power of the gel diffusion test, whereby about 1 mg/ml protein can be recorded [1], and our own calculations based on those in [3], it can be postulated that a positive reaction would be observed if as little as one-thousandth of the protein of the necrotized mass of the gland entered the bloodstream. Consequently, no breakdown products of SMSG with antigenic properties could be found circulating in the bloodstream. It must be borne in mind that the times of sacrifice of the rats did not correspond to discharge of the whole mass of antigens into the bloodstream. For instance, it has been shown [12] that new antigens appear in the blood 2 weeks after denervation of the gland. The present investigation was based on the fact that mitogens appear in the blood immediately after trauma, for otherwise it is difficult to explain activation of proliferation on the 3rd day of the experiments.

The results of a study of the biochemical properties of the blood serum are illustrated in Fig. 2. It was found that the quantity of the seromucoid fraction was increased by 7% 48 h after trauma ($P < 0.01$), just as after other forms of trauma [5]. The content of individual protein fractions was not significantly changed at any time.

The LDH_1 fraction in the traumatized gland had completely disappeared 12 h after the operation (Table 1) and the content of the LDH_2 fraction was reduced by 44%. At these same times and 24 h after trauma, the blood levels of LDH_1 and LDH_2 showed a sharp increase (by 50%, $P < 0.001$, and by 30%, $P < 0.01$, respectively). High-molecular-weight cytoplasmic LDH isozymes are known to appear in the blood only after injury to cell membranes. The cause of this in the present experiments was evidently circulatory hypoxia in the gland undergoing resection, and this was confirmed by an increase in the LDH_5 content and disappearance of LDH_1 in SMSG tissue, characteristic of an anaerobic type of metabolism. By the end of the first day after the operation a tendency was noted for the relative percentages of LDH fractions to be restored in the injured gland.

The results agree with the view that triggering of proliferation is nonspecific. Entry of the cells into mitosis can take place under the influence of various factors; in particular, nonspecific activation of T lymphocytes after trauma to the liver and SMSG [11] is linked with hypoxia [9].

In the early stages after trauma to SMSG antigens of the injured gland, which could be nonspecific mitogens of post-traumatic regeneration, are thus not found in the bloodstream, although the conditions exist for their release into the bloodstream (as shown by the data for LDH). Of course, the selective character of changes in vascular permeability in SMSG after trauma [8] cannot be ruled out. The results are evidence that high-molecular-weight proteins of the injured tissue play no part in the triggering of post-traumatic regeneration.

LITERATURE CITED

1. G. I. Abelev, in: Methods of Developmental Biology [in Russian], Moscow (1974), pp. 436-437.
2. A. G. Babaeva and E. A. Shubnikova, Structure, Function, and Adaptive Growth of Salivary Glands [in Russian], Moscow (1979), pp. 94-95.
3. P. P. Gusak, Byull. Éksp. Biol. Med., No. 7, 99 (1980).
4. A. I. Gusev, in: Immunochemical Analysis, ed. L. A. Zil'ber [in Russian], Moscow (1968), pp. 99-117.
5. A. B. Denisov and A. A. Belopol'skii, Byull. Éksp. Biol. Med., No. 4, 478 (1980).
6. A. B. Denisov and V. V. Mikhailov, Byull. Éksp. Biol. Med., No. 3, 364 (1981).
7. F. I. Komarov, B. F. Korovkin, and V. V. Men'shikov, Biochemical Investigations in Clinical Medicine [in Russian], Leningrad (1976), pp. 30-34.
8. Yu. A. Petrovich and N. F. Trusova, Patol. Fiziol., No. 1, 80 (1981).
9. M. V. Popugailo, "Study of the role of lymphoid cells in regulation of hematopoiesis during exposure to extremal factors," Author's Abstract of Candidate's Dissertation, Chelyabinsk (1979).
10. M. I. Khramkova, in: Immunochemical Analysis, ed. L. A. Zil'ber [in Russian], Moscow (1968), pp. 5-20.
11. N. V. Yudina, "Reaction of lymphoid tissue to injury and recovery of organs with different powers of regeneration," Author's Abstract of Candidate's Dissertation, Moscow (1980).
12. A. Mofty and C. Scheyer, Experientia, 30, 950 (1974).
13. D. W. Moss and P. Butterworth, Enzymology and Medicine, London (1974), pp. 228-253.
14. J. M. Taylor, S. Cohen, and W. M. Mitchell, Proc. Natl. Acad. Sci. USA, 67, 164 (1970).