DETECTION OF STAGE-SPECIFIC ANTIGENS IN REGENERATING MUSCLE TISSUE

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A considerable number of papers [2, 8, 9, 11] have been devoted to a study of the antigenic properties of developing tissues taken at different stages of ontogenesis, while immunological data concerning regenerating tissues has been presented only in a few reports in which observations were made on lower vertebrates. In these works antigenic properties and the periods of the appearance of individual contractile muscle proteins [10, 14, 16, 17] were studied during regeneration. The authors were chiefly interested in quantitative changes in the antigenic composition of tissues which occur during regeneration. However, the qualitative character of the antigens of regenerating tissues is important since there is data [13] concerning the presence in tissues during periods of the most accelerated developmental processes of specific antigens which are not found in normal definitive tissue [1, 2, 13]. The development of such antigens in muscle tissue during ontogenesis has been shown by a series of authors [1, 3, 4, 6]. Similar material was obtained also in regard to regenerating liver tissue [12]. We have not found any work on the detection of similar antigens in regenerating muscle tissue of mammals.

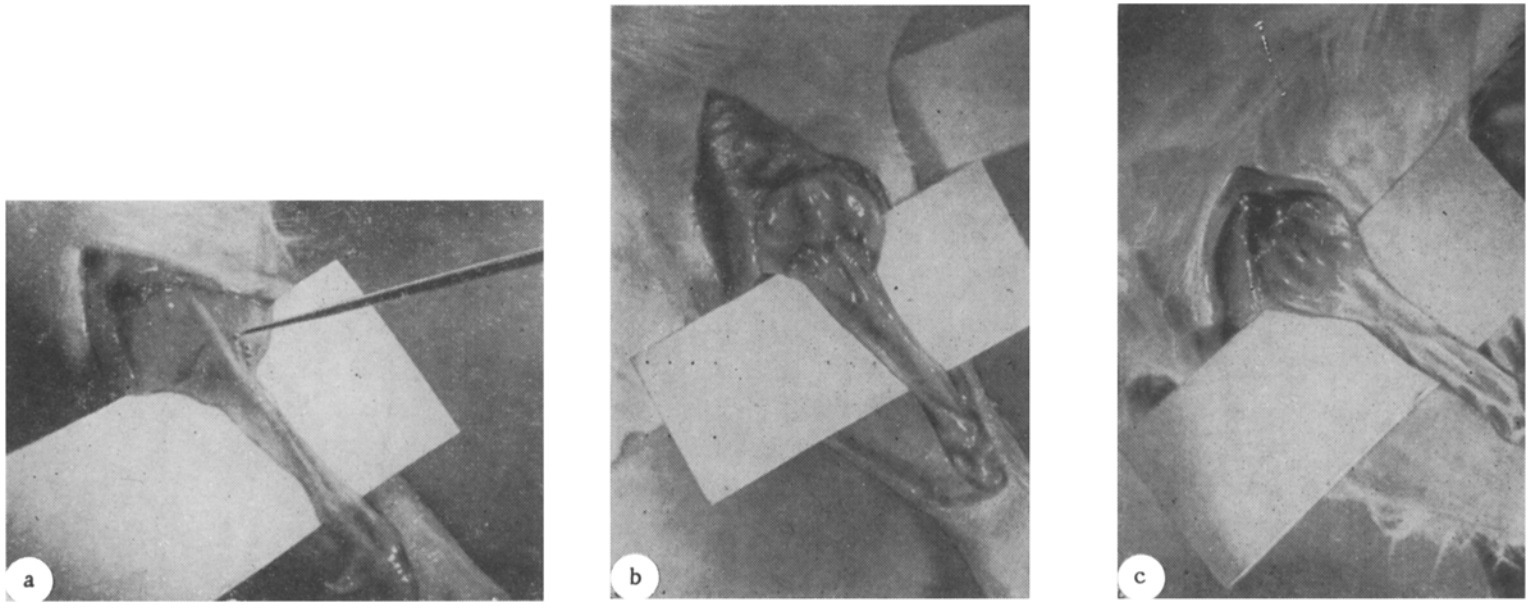
In the present article the development of the antigenic properties of muscle tissue of white rats during regeneration was studied.

METHODS

The investigations were carried out using the anaphylaxis desensitization reaction in guinea pigs. The possibility of using this reaction for qualitative characterization of antigenic properties of the tissues under investigation during regeneration was shown earlier [7].

The skin of white male rats weighing 110-130 g was cut under ether along the inner sides of the shin [5]. The skin was separated at an angle from the underlying muscle covering, and the gastrocnemius and the vascular nerve bundles were dissected off. Further across the longitudinal axis of the muscle two marks were made under the perimysium with a sterile solution of India ink (the upper—at the level of the entry of the tibia nerve, the lower—somewhat removed from the place where muscle passes into the Achilles tendon). The gastocnemius was removed at these marks so that the marks remained in the area of the cut. To prevent hypertrophy of the remaining muscles of the tarsal complex, they were also removed. After this, an unbroken silk suture was applied to the muscle covering and skin. After the elapse of fixed periods (see below) the regenerates were removed somewhat away from the marks on the remaining stump of the amputated limb (see figure, a). The regenerates were freed from fibers and nerves, placed in sterile ampoules and lyophilized, after which they were used as antigens for sensitizing guinea pigs for the anaphylaxis-desensitization reaction.

Each guinea pig was subcutaneously injected with 0.2 ml of a suspension of regenerates taken 10, 30, 45, and 90 days after the operation (see figure, a, b, c). To prepare the suspension, the lyophilized muscle was ground in a sterile porcelain mortar, to which sterile physiological solution was gradually added to obtain a homogeneous mass (1-1.2 ml of physiological solution to 60-80 mg lyophilized muscle). Desensitization was carried out on the 21st day after sensitization: the guinea pigs were injected intraperitoneally at night with 0.6 ml of an aqueous salt extract of definitive muscle. The extracts were prepared according to the calculation of 4.5 ml physiological solution per 100 mg lyophilized muscle tissue. Extraction of the pulverized definitive muscle tissue was carried out for



External appearance of the regenerates at different post-operative periods. a) After 10 days; b) after 30 days; c) after 90 days.

Anaphylactic Reaction in Guinea Pigs Sensitized with Regenerated Gastrocnemius Muscle Suspension of White Rats in Response to the Injection of Extracts from Normal and Regenerated Gastrocnemius Muscle Tissue

Guinea pig number	Time after operation muscle tissue taken for sensitization (in days)	Dose (in mg of dry weight)	Desensitization to extracts from normal unoperated muscle tissue		Verification of the completeness of desensitization to extracts from normal unoperated muscle tissue		Permissible injection of extracts of regenerated muscle tissue		
			Dose (in mg of protein)	Reaction	Dose (in mg of protein)	Reaction	Time after operation tissue taken (in days)	Dose (in mg of protein)	Reaction
1	10	16	36	++	12	—	10	12	++
2	10	16	36	++	12	—	10	12	+
3	10	16	36	+++	12	++	10	12	
4	10	16	.	.	.	.	10	12	+++
5	10	16	.	.	.	.	10	12	+++
6	. . . . .	.	.	.	.	.	10	12	—
7	. . . . .	.	.	.	.	.	10	12	—
8	30	17	36	+++	12	—	30	12	+++
9	30	17	36	++	12	—	30	12	++
10	30	17	36	++	12	—	30	12	+
11	30	17	.	.	.	.	30	12	+++
12	30	17	.	.	.	.	30	12	+++
13	. . . . .	.	.	.	.	.	30	12	—
14	. . . . .	..	.	.	.	.	30	12	—
15	45	12	36	+	12	—	45	12	+
16	45	12	36	+	12	—	45	12	+
17	45	12	36	+	12	—	45	12	+
18	45	12	.	.	.	.	45	12	++
19	45	12	.	.	.	.	45	12	++
20	. . . . .	.	.	.	.	.	45	12	—
21	. . . . .	.	.	.	.	.	45	12	—
22	90	9	24	+	12	—	90	12	+++
23	90	9	24	++	12	—	90	12	+++
24	90	9	24	+	12	—	90	12	+++
25	90	9	.	.	.	.	90	12	+++
26	90	9	.	.	.	.	90	12	+++
27	. . . . .	.	.	.	.	.	90	12	—
28	. . . . .	.	.	.	.	.	90	12	—

Symbols: + tremor, chewing motions, twitching of nose and whiskers, bristling of the fur, panting, frequent urination and defecation; ++ same symptoms exhibited more sharply, the guinea pig sneezes often; +++ same symptoms exhibited even more sharply, spasms, cough, spasms of the vocal cords, lateral position. The animal survives; • injections not carried out; — no reaction.

Note: In guinea pig No. 3 verification of the completeness of desensitization was done six times, but complete desensitization was not obtained.

18-20 h at 0°, then it was centrifuged at 3000 rpm for 25 min. The supernatant liquid was used in the experiments. The concentration of protein in the extracts which were injected into the guinea pigs was first determined by Lowry's method [15] with subsequent equalization of the amount of protein in all inoculates.

Twenty four hours after the intraperitoneal injection of the guinea pigs with the extract from definitive muscle completeness of desensitization was verified by injecting the guinea pigs intravenously with an extract of definitive muscle using the same dose as on the night before (0.6 ml). A positive reaction indicated the absence of desensitization and therefore after two hours the same procedure was repeated. The guinea pigs showed desensitization to the extract from normal definitive muscle after three 0.6 ml injections of the extract with two hour intervals. Two hours after the last injection an injection of the permissible antigen dose (extract of regenerated muscle tissues) was made, equal in amount of protein to the last desensitizing dose. The anaphylactic reaction was carried out by the standard method.

RESULTS

Data obtained from the anaphylactic reaction in guinea pigs sensitized with regenerated muscle tissue taken at different post-operative periods is presented in the table.

As seen from the table, all guinea pigs, sensitized with suspensions from regenerated muscle tissue taken 10-90 days after the operation, in response to an intravenous injection of 0.6 ml of an extract from muscle of normal unoperated rat exhibited anaphylactic shock, which indicates the presence in regenerated rat muscle tissue in these post-operative periods of antigens similar to the antigens of definitive unoperated muscle. After attaining complete desensitization to an extract from definitive muscle and injection of a permissible dose of extract from regenerated muscle tissue the guinea pigs again had an anaphylactic reaction. This indicates the presence in regenerated muscle tissue of antigens which are not found in definitive muscle.

Two groups of control animals were used in each series of the experiment: 1) guinea pigs which were not desensitized but on the 21st day were injected intravenously with the permissible dose of antigen (verification of the effectiveness of the dose selected for sensitization); 2) unsensitized guinea pigs which were injected with an amount of antigen equal to the permissible dose (test of the toxicity of the injected extracts).

As seen from the table the sensitizing dose was selected correctly, since after intravenous injection of the extract from regenerated gastrocnemius muscle tissue of the appropriate periods (10-90 days after operation), the non-desensitized guinea pigs showed a clearly expressed anaphylactic reaction (+++, ++). In testing the toxicity of the injected extracts, reactions in the animals were absent.

Thus, using the anaphylaxis-desensitization reaction in guinea pigs we were able to establish that the 10th, 30th, 45th, and 90th day after operation in regenerated muscle tissue, besides the antigens closely related to the antigens of definitive muscle, there are antigens which are absent from this muscle, the so-called stage-specific antigens, suggested by O. E. Vyazov [2]. Our material agrees with the data of some authors [12], that in regenerating livers an antigen is also detected by the immunoelectrophoresis method which is absent from unoperated rat liver.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
