A STUDY OF THE ANTIGENIC PROPERTIES OF REGENERATING MUSCLE TISSUE

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Numerous publications have been devoted to immunological studies of morphogenic processes taking place during embryogenesis [1]. However, the process of regeneration has not been sufficiently studied immunologically. We were able to encounter only a single publication by Italian workers [6] on an immunoelectrophoretic study of proteins from regenerating rat liver. In addition to this more studies were made on lower vertebrates [5, 7, 9, 11]. In these the authors studied the antigenic properties and the periods of appearance of different contractile muscle proteins during the process of regeneration of the tail and of extremities in the triton. The opinions of the authors differed on the times of the appearance of antigens in the course of the regenerative process: some authors thought that the appearance of antigens in the course of regeneration precedes the appearance of transverse striation in the muscles [5], others have shown that specific muscle proteins appear simultaneously with myofibrils (i.e., together with the transverse striation) [7], and still others have stated that during the course of regeneration, the formation of certain proteins precedes the appearance of others (e.g., actine precedes myosine) [9]. It has also been said [11] that during the regeneration of an amputated organ, such as the tail of the triton, quantitative changes occur more rapdily than qualitative. This lack of agreement of results is probably due to the fact that antigenic properties were studied in whole organs (extremities, tail), taken in toto, and consisting of different tissues (muscle, bone, vascular, nervous, etc) which differ from each other antigenically.

We have attempted to follow the changes in antigenic properties during the process of regeneration of a single muscle, the gastrocnemius muscle of the white rat.

METHODS

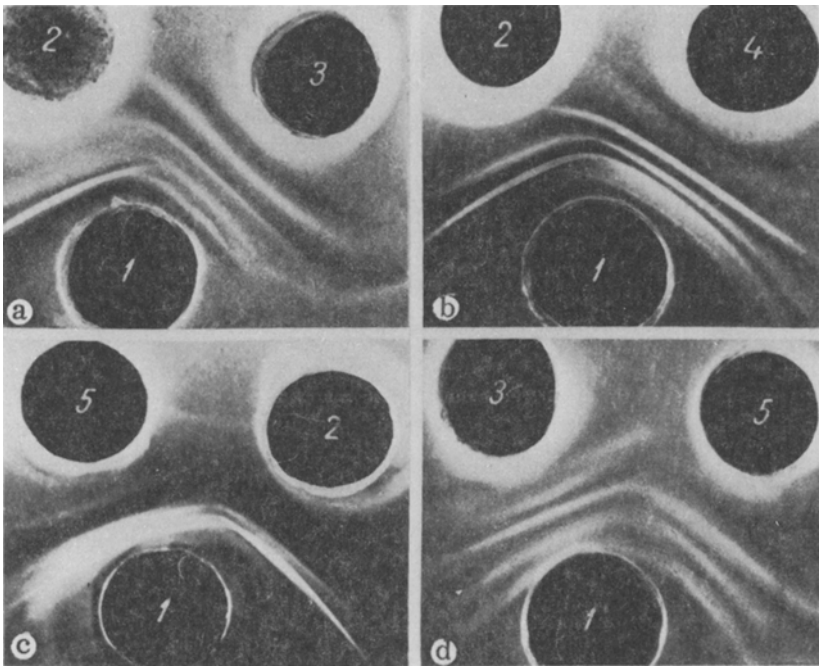
Male white rats weighing 110-130 g were anesthetized with ether, and a subtotal resection of the gastrocnemius muscle of the left hind leg was made [4]. In one of the experimental variables the minced muscle tissue was replaced in the area from which it was taken, and in the other one it was not.

The regenerating muscle was removed 7, 10, 14, 21 and 30 days after the operation, it was freed of blood vessels and nerves, placed in a sterile ampule and lyophilized. These lyophilized muscles were used to prepare extracts in normal saline, in a proportion of 50 mg lyophilized tissue per 1 ml of normal saline. To prepare the extracts, the lyophilized regenerating muscles were ground in sterile porcelain mortars and extracted for 18-20 h at 0-4°C. Then the material was centrifuged at 3000 rpm for 25 min.

Actomyosine was extracted from rat muscle according to the method described by Ivanov and Yur'ev [3], a actine, according to the method described by Straub [3]. The muscle proteins and the saline extracts of muscle were used to immunize rabbits. Ten rabbits weighing 2-2.5 kg were immunized with each antigen. Several schemes of immunization were used. The most effective one was that in which Freund's adjuvant was used (15 g vaseline oil, 5 g of anhydrous lanolin and 50 mg BCG). The immunization cycle consisted of the following. The antigen (0.5 ml) was introduced into a marginal ear vein, and simultaneously 0.5 ml of a mixture of equal volumes of the adjuvant and of antigen was injected subcutaneously in the region of inguinal and axillary lymph nodes. The injections were repeated 3-4 times with 10 day intervals. Blood was obtained 7-10 days after the last injection.

Results of Agar Gel Diffusion Experiments with Antisera and Extracts of Normal Muscle and Those of Regenerating Muscle Taken at Different Periods Following Operation (Minced muscle tissue was not placed at the site of excision)

Antiserum	Antiserum No.	Number of precipitation lines					
		no. of days following operation					normal muscle
		7	10	14	21	30	
Against saline muscle extract	851	4	6	4	3	3	2
	832	5	6	4	3	3	2
	2943	3	3	3	2		1
	2920	4	3	3	3	3	2
Against actomyosine	2976	5	4	4	3		2
	2978		6	4		4	3
	2081		6	3			2
Against actine	2062	3	3		2		1
	2060			4	4		2
	2044		5			4	2
	2018		4	3	3		2
	2936				3	2	1
	2949	5		4	3	3	2



Precipitation in agar gel between antiserum and saline extracts of regenerating muscle at different periods of regeneration and saline extract of normal muscle. 1) Antiserum; 2) extract of normal muscle tissue; 3) extract of regenerating muscle on the 10th day following operation; 4) on the 14th day; 5) on the 30th day.

Against each type of antigen 3-6 active antisera, with titers high enough for experimental purposes, were obtained. Antisera were used only if they gave distinct lines of precipitation with antigens in agar gel in dilutions of 1:16 and 1:32. To increase the titers of antisera they were concentrated according to the method of McErlean [8].

One of the methods used was Ouchterlony's precipitation reaction in agar gel in Petri plates [10] and a mod - ification of this method for precipitation on microscope slides [2]. Saline extracts of lyophilized non-operated right hind gastrocnemius muscles were used as controls.

RESULTS

The antisera obtained produced different numbers of precipitation lines with muscle extracts made from muscles taken at different periods following the operation with antisera against a saline extract of normal muscle (Nos. 851, 832, 2943, 2920), against actomyosine (Nos. 2976, 2978, 2081) and against actine (Nos. 2062, 2060, 2044, 2018, 2936, 2936 and 2949).

It will be seen from the table that saline extracts made from regenerating muscle tissue produced more precipitation lines with all the antisera used than with the saline extract of normal muscle (figure a, b, c). Sometimes in a few cases it was the reverse: there were more precipitation lines with normal muscle extract than with extract of regenerating muscle, but this was probably due to the formation of a connective tissue scar at the site of the regenerating muscle.

It was also found that extracts of regenerating muscle, taken at early periods following operation (7, 10 and 14 days) produced more precipitation lines than extracts of regenerating muscle taken 30 days following operation (figure 1d).

The results of experiments in which the minced tissue was placed at the site of the excised muscle and in which it was not, were the same.

Thus the results of our studies on antigenic properties of regenerating muscle tissue have shown that regenerating muscle tissue differs antigenically at different periods following operation (7, 10, 14, 21 and 30 days) from normal muscle tissue. In addition to antigens present in the normal muscle tissue, there are present other antigens in regenerating muscle tissue, as witnessed by a larger number of precipitation lines in agar gel diffusion plates in which extracts of regenerating muscles were used.

Our results are in accordance with those of other authors [6] who studied the antigenic properties of regenerating rat liver. They also found that in the regenerating liver there is an antigen not present in normal liver.

It is difficult to make definite conclusions from the data presented above, because the antisera, with which extracts of regenerating muscle gave more precipitation lines than extracts of normal muscle, were obtained against muscle proteins isolated from normal muscle. Nevertheless, it may be assumed that during the process of regenera - tion there occur changes in the total number of soluble proteins, i.e., antigens. There are more of them in regenerating muscle tissue than in normal muscle tissue. During regeneration the number of readily soluble antigens gradually decreases. In a saline extract of normal muscle these soluble antigens might have been present in concentrations too low to be discerned in the immunological reaction used by us. However, the immunization of rabbits with extracts of normal muscle could have evoked the production of antibodies against all the proteins present in the extract, so that the minimal amounts of easily soluble protein antigens could have been sufficient to produce antibodies against them. These antibodies could produce precipitation lines with corresponding antigens.

SUMMARY

The precipitation in gel test after Ouchterlony was used to study the antigenic properties of regenerating muscle tissue in albino rats. It was found that antisera against muscle tissue proteins in the rat form with regenerate extracts a large number of precipitation bands on the 7th, 10th, 14th, 21st and 30th day after the operation than with normal muscle extracts. In addition, it is shown that extracts from regenerates obtained at earlier times after the operation forms more precipitation bands than in the case of later regenerate extracts.

It is supposed that soluble antigens decrease in their number during regeneration.

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