COMPARATIVE STUDY OF ANTIGENIC PROPERTIES OF TISSUES OF ANIMALS OF DIFFERENT SPECIES AT DIFFERENT STAGES OF DEVELOPMENT

PART I

COMPARATIVE STUDY OF ANTIGENIC PROPERTIES OF MUSCLE TISSUE OF FROGS (RANE RIDIBUNDA) AND NEWTS (TRITURUS CRISTATUS)

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I. L. Krichevsky [1], applying the reaction of binding of the complement, was able to show that tadpoles have different antigenic properties to both frogs and newts. The differences between the tadpoles and the adult forms were, however, smaller than between the two adult forms. On this basis the author concluded that recapitulation of ancestral antigenic properties occurs during larval development. Our researches differ from those of I. L. Krichevsky in that we studied muscle tissue only, and not the whole animals.

We prepared immune serums against aqueous saline extracts of frog femoral muscle (AML) and of newt tail muscles (AMT).* The muscle was comminuted and ground thoroughly in a porcelain mortar, taking 9 ml of saline per g of tissue. The suspension was shaken for 20 minutes, and then centrifuged for 15-20 minutes at 2000 r.p.m. The supernatant was pipetted off, and used as antigen in the reaction of binding of the complement and for production of immune serums.

We took 6 healthy rabbits, weight 3-3.5 kg, for immunization. Three rabbits were given daily intravenous injections of 1 ml of saline extract of frog muscle, and 3 rabbits received similar doses of newt muscle extract. Active immune sera were obtained from all 6 rabbits by the 11th day after the last injection.

These serums, together with the antigens from frog muscle and from tail muscle of tadpoles and newts, were taken for complement-binding reactions, using the usual technique. Control systems with non-immune serums were also set up (Norm.)

The results are presented in Table 1.

As is evident from Table 1, frog muscle antiserum reacted in the complement binding reaction with frog muscle antigen at a dilution of 1/320, and with tadpole muscle antigen at a dilution of 1/80. The difference in titers, and in the intensity of the reaction of the frog muscle antiserum with both these antigens is evidence of the existence of considerable differences between the antigenic properties of the muscles of tadpoles and frogs. The least reaction was between frog muscle antiserum and newt muscle antigen.

The Table also shows that newt muscle antiserum reacted at the highest dilution with newt muscle antigen, at a lower one with tadpole muscle antigen, and at the lowest with frog muscle antigen.

* We found in a preliminary experiment that the femoral and caudal muscles of a single animal did not differ as to their antigenic properties.

TABLE 1

Results of 25 Reactions of Binding of the Complement, Using Antiserums Against Frog and Newt Muscles, and Antigens from Frog, Tadpole, and Newt Muscles

Serum						Muscle		
1/20	1/40	1/80	1/160	1/320	1/640	Frog	Tadpole	Newt
AML						++++	+++	++
	AML					++++	++	++
		AML				+++	+	H
			AML			++	±	H
				AML		+	.	.
					AML	H	.	.
						Newt	Tadpole	Frog
AMT						++++	+++	++
	AMT					++++	++	+
		AMT				++++	+	H
			AMT			+++	±	H
				AMT		+++	H	H
					AMT	++	H	H
Norm.						H	H	H
	Norm.					H	H	H
		Norm.				H	H	H
			Norm.			H	H	H
				Norm.		H	H	H
					Norm.	H	H	H

Note: The results of the reactions of binding of the complement are expressed in the usual way; ++++ total absence of hemolysis, +++, ++, +, and ± intermediate degrees of hemolysis, H total hemolysis. A dot (·) indicates that the reaction was not tested.

A comparison of the titers and intensities of reaction of frog and newt muscle antiserums with all three antigens showed that the difference between the antigenic properties of tadpole and newt tissues was much smaller than that between the antigenic properties of newt and frog muscles.

Since, however, we used muscles from different parts of the body (femoral muscle of frogs and caudal muscle of newts), we thought it necessary to do a control experiment. For this purpose we compared the antigenic properties of axolotl* femoral and caudal muscles.

Guinea pigs weighing 300-350 g were sensitized by giving them subcutaneous injections of antigen prepared from femoral and caudal muscles of axolotls, equivalent to 16 mg of muscle per guinea pig. After 21 days the animals were desensitized by intraperitoneal injection of axolotl femoral muscle antigen.

Two days after desensitization to femoral muscle antigen a challenging injection of tail muscle antigen was given, at a dosage of 60 mg of muscle per guinea pig.

The responses are indicated as follows: + tremor, chewing movements, rubbing of the nose and ears, ruffling of fur, asthma, frequency of micturition, slight fall in temperature; ++ the same symptoms, in a more acute form, and also often sneezing; +++ still more acute symptoms, with convulsive movements and cough; the animal lies on its side; ++++ all symptoms very severe, and the animals die; – no symptoms of anaphylactic shock; (·) no injections given.

* We used axolotls because for this experiment it was necessary to use relatively large amounts of muscle.

The results are presented in Table 2.

TABLE 2

Anaphylactic Reaction of Guinea Pigs Sensitized with Axolotl Muscle Antigen Prepared from Different Muscle Groups, in Response to a Challenging Injection of Axolotl Tail Muscle Antigen

Guinea pig No.	Sensitized (subcutaneously)			Desensitized (intraperitoneally)			Check of completeness of desensitization (intravenous)			Challenging injection		
	source of antigen	dose (mg)	reaction	source of antigen	dose (mg)	reaction	source of antigen	dose (mg)	reaction	source of antigen	dose (mg)	reaction
1691	Axolotl tail muscle	16	–	Axolotl femoral muscle	100	+	Axolotl femoral muscle	60	–	Axolotl tail muscle	60	–
1693	Ditto	16	–	Ditto	100	+	Ditto	60	–	Ditto	60	–
1723	Axolotl femoral muscle	16	–	Ditto	100	+	Ditto	60	–	Ditto	60	–

We see from Table 2 that guinea pigs sensitized with axolotl tail muscle antigen and desensitized with axolotl femoral muscle antigen do not give any anaphylactic reaction in response to a challenging injection of axolotl tail muscle antigen. It hence follows that the antigenic properties of axolotl femoral and tail muscles are identical.

The question arises, in connection with the results of Table 1, as to whether the difference between the antigenic properties of tadpole tail muscle antigen and frog muscle antigen is due to the acquisition during ontogenesis of a new antigenic properties, or whether tadpole tail muscles contain antigens which are specific for this stage of ontogenesis, and which subsequently disappear. In order to answer this question we performed a similar experiment to the preceding one, involving sensitization to a given antigen, followed by desensitization with the other antigen, followed by a challenging injection of the first antigen. The results are presented in Table 3.

TABLE 3

Anaphylactic Reaction of Guinea Pigs Sensitized with Tadpole Muscle Antigen, and Desensitized with Frog Muscle Antigen, in Response to a Challenging Injection of Tadpole Muscle Antigen

Sensitized (subcutaneously)			Desensitized (intraperitoneally)			Check of completeness of desensitization			Challenging		
source of antigen	dose (mg)	reaction	source of antigen	dose (mg)	reaction	source of antigen	dose (mg)	reaction	source of antigen	dose (mg)	reaction
Tadpole muscle	16	–	Frog muscle	60	+	Frog muscle	60	–	Tadpole muscle	70	++++
Ditto	16	–	Ditto	60	++	Ditto	60	–	Ditto	100*	+++
"	16	–	"	60	++	"	60	–	"	60	++++
"	16	–	"	60	++	"	60	–	"	60	++++
"	16	–	"	60	+	"	60	–	"	60	++
"	16	–	.	.	.	"	.	.	"	40**	++
"	16	–	.	.	.	.	.	.	"	70	++++
"	16	–	.	.	.	.	.	.	"	70	++++
.	.	.	.	.	.	.	.	.	.	.	–
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* 40 mg introduced, followed after 15 minutes by 60 mg.

** Part of the antigen was introduced subcutaneously.

It can be seen from Table 3 that all the guinea pigs sensitized with tadpole tail muscle antigen and desensitized with frog muscle antigen responded with anaphylactic shock to a challenging dose of tadpole tail muscle antigen. As a control, guinea pigs sensitized with tadpole muscle antigen, but not desensitized, were given challenging doses of tadpole muscle antigen; severe anaphylactic shock resulted in all cases. As a further control, tadpole muscle antigen was injected into a group of normal, unsensitized guinea pigs; the reaction was in all cases negative.

The results obtained show that tadpole muscle contains an antigen which is absent from frog muscle.

It thus follows from our experiments that, firstly, tadpole muscle differs significantly from frog muscle in its antigenic properties, and, secondly, that this difference depends on the presence in tadpole tail muscle of "stage-specific" antigens, which are absent from frog muscle. Finally, the difference between the antigenic properties of tadpole and newt muscles is smaller than between those of newts and frogs; this suggests that at that stage of development of tailless amphibia at which they resemble more closely tailed amphibia in their morphology, they also have more similar antigenic properties.

LITERATURE CITED

[1] Kirchevsky, I. L., Zbl. f. Bakt., 1914, Vol. 72, pp. 81-94.