

## THE EFFECT OF LIVER TISSUE HOMOGENATE ON GROWTH IN THE LIVER OF TADPOLES

L. K. Ramanova

From the Laboratory of Growth and Development (Head - Prof. L. D. Liozner), the Institute  
of Experimental Biology (Director - Prof. I. N. Mal'sky), Acad. Med. Sci. USSR

(Received September 21, 1956. Presented by Prof. N. N. Zhukov-Verezhnikov, Active  
Member of the Academy of Medical Sciences USSR)

Specific growth stimulation in the organs of chicken embryos (liver, kidneys, heart) has been obtained by many researchers [1,3,4,5,9] by the administration of a water-salt extract made from the tissue of the same organs. Some authors [3,5,8] propose that substances are formed in the living cells which specifically affect the processes of growth and tissue differentiation and the presence of autoregulation of organic growth and development due to their action [3,8].

According to H. Telf [7], the tissue suspension contain substances which stimulate mitoses and influence cell differentiation. McJukin and Breuhais [6] observed mitoses to increase in the liver of mice after partial hepatectomy, with the intraperitoneal injection of liver tissue.

The only work which has studied the problem of specific stimulation of the regenerating process in the liver of amphibians with the injection of an extract from the same is not convincing [2]; it lacks reliable criteria by which to judge the regeneration of the liver.

The purpose of this work was to ascertain how widely the rule regarding organic growth stimulation effected by extracts from tissue of corresponding organs applies, and how specific the effect of the extracts is. The relation of the specific effect to the age of the donor of the organ from which the homogenate was prepared and to the age of the recipient was also studied.

### EXPERIMENTAL METHODS

The studies were done on *Rana temporaria* and *Rana ridibunda* tadpoles.

The animals were divided into 2 groups. The first group (4 series of experiments) contained 197 tadpoles, and 157 tadpoles were used as the control.

The second group (also 4 series of experiments) contained 51 tadpoles, and 42 tadpoles were used as the control.

In the experiments on the first group, *R. temporaria* and *R. ridibunda* tadpoles, at the I, II, and III stages of metamorphosis (according to L. Ya. Blyakher) were intraperitoneally injected with a suspension of liver tissue from *R. temporaria* at stage V.

In the experiments of the second group, *R. ridibunda* tadpoles, at stages II and III, were intraperitoneally injected with liver tissue from the same species of tadpole at the 1st or an even earlier stage of metamorphosis, before digital differentiation had occurred.

The liver tissue suspension was prepared immediately before the injection. The liver tissue was ground

in a quartz mortar together with a 0.75% solution of NaCl, using a wooden pestle, to a homogeneous mass (ratio by weight of liver tissue to NaCl solution = 1:2).

The animals of the first group were injected once with 8-10 mg of the liver tissue homogenate, the animals of the second group, with 10-15 mg. The control tadpoles were injected intraperitoneally with the same amounts of a 0.75% solution of NaCl. After 3-6 days, the tadpoles were killed, and the entire body of the animal, the liver, pancreas and anterior extremity were weighed.

### EXPERIMENTAL RESULTS

In the experiments of the first group, the liver tissue suspension from the *R. temporaria* stage V tadpoles specifically stimulated growth in the liver of the *R. temporaria* and the *R. ridibunda* Stage I, II and III tadpoles; the relative weight increase of the liver = average of each series = was 0.12%, 0.28%, 0.32% and 0.19%. When the material was statistically processed according to Student's formula, corresponding values of  $t$  were obtained: 2.4, 2.2, 4.5, 3.8, i.e., the changes observed were proven statistically (Table 1).

TABLE 1

Change in Relative Weight of Liver, Pancreas and Anterior Extremity in *R. temporaria* and *R. ridibunda* Tadpoles Caused by Injection of Liver Homogenate from *R. temporaria* Stage V, Tadpoles

Animal group	Recipient	Stage	Duration of experiment in days	Number of tadpoles	Relative weight			$t$ , of Student's formula for relative wt., in exp. and control	P, according to Student's tables
					of liver	of pancreas	of anterior extremity		
Experiment	<i>R. temporaria</i>	I-II	3	70	0.93	—	—	2.4	0.0164
Control				43	0.81	—	—		
Experiment	The same	III	3	50	2.61	0.270	0.460	2.3	0.0214
Control				49	2.33	0.200	0.410		
Experiment	<i>R. ridibunda</i>	II	3	18	1.48	0.420	0.059	4.5	0.0000
Control				19	1.16	0.390	0.057		
Experiment	The same	0-I	4-5	59	1.41	0.390	0.045	3.8	0.0001
Control				46	1.22	0.400	0.058		

However, there was no statistically verified increase in the weight of the pancreas or anterior extremity in one of the first group series. In two series, the average increase of relative weight of the pancreas was 0.01% and 0.03%, but in another series, the relative weight of the pancreas, as compared with that in the control animals, even decreased (experiment = 0.39%, control = 0.40%).

The stimulating effect of the liver homogenate was observed even 5 days after its injection. For example, in one series of experiments, the average relative weight of the liver after 5 days was 1.41%, as opposed to 1.22% in the control;  $t$  in Student's formula was 3.8, i.e., the difference between the experiment and the control was not accidental.

In the second group of experiments, the relative weight of the liver tended to decrease (Table 2).

In three series, the average decrease of relative liver weight in the experiment as compared with the control was 0.56%, 0.18% and 0.25%. The corresponding values of  $t$  were 2.7, 0.7 and 1.1, i.e., the difference between the experiment and control was statistically proven in only one series.

In the fourth series, some increase in relative liver weight was observed in the experiment, the difference between the experiment and the control (0.11%) was not, however verified statistically ( $t = 0.6$ ).

The inhibition of liver growth caused by injecting a homogenate from tadpoles at earlier development stages than the recipients was also specific. There was no statistically proven decrease in the relative weight of the pancreas or anterior extremity in the experiment, as compared with the control, observed in any of the second group of experimental series. In two series, the relative weight of the pancreas in the control exceeded that in the experiment (0.028%, 0.021%), while, in the other two series, the relative weight of the pancreas was slightly greater in the experiment than in the control (differences of 0.021% and 0.036%, not statistically proven).

TABLE 2

Change in Relative Weight of Liver, Pancreas and Anterior Extremity of *R. ridibunda* Tadpoles Caused by Injection of Liver Homogenate from *R. ridibunda* Tadpoles at Stages 0-1.

Animal group	Recipient	Stage	Duration of experiment in days	Number of tadpoles	Relative weight			t of Student's formula for relative wt. of liver in exp. and in control	P, according to Student's tables
					of liver	of pancreas	of anterior extremity		
Experiment	<i>R. ridibunda</i>	II	3	12	1.81	0.447	0.073	0.6	0.5485
Control				13	1.70	0.426	0.079		
Experiment	The same	II	4	12	2.23	0.505	0.119	2.7	0.0069
Control				10	2.79	0.533	0.143		
Experiment	" "	III	3	16	2.96	0.469	0.274	0.7	0.4839
Control				12	3.14	0.433	0.260		
Experiment	" "	III	6	11	1.74	0.376	0.108	1.1	0.2713
Control				7	1.96	0.397	0.121		

Therefore, the proposal that the tissue extracts received from donors younger than the recipients have an inhibiting effect cannot yet be definitely accepted. This possibility needs further verification on a larger number of animals.

It was interesting to note that, in two series of the first experimental group, liver tissue from *R. temporaria* tadpoles specifically stimulated liver growth in the *R. ridibunda* tadpoles. The difference between the experiment and the control was quite substantial ( $t = 4.5, 3.8$ ).

Therefore, it can be assumed that interspecies stimulation is possible, i.e., stimulation of organic growth by proteins not specific to the given species. Apparently, the only necessary condition for the effective action of the extract is that organ-specific proteins be present in it.

Further research is needed to confirm this hypothesis.

#### SUMMARY

Two groups of experiments were conducted on *Rana temporaria* and *Rana ridibunda* tadpoles.

In the first group *Rana temporaria* and *Rana ridibunda* tadpoles of the I, II and III stages were injected intraperitoneally with a liver suspension of tadpoles (*Rana temporaria* of the V stage) in a 0.75% NaCl solution. In the second group, tadpoles (*Rana ridibunda*) of the II and III stages were injected with a liver suspension of tadpoles of the 0 and I stages of the same species.

In the first group the growth of the liver was specially stimulated by the homogenate, in the second group, a tendency to diminution of the relative weight of the liver was noted.

#### LITERATURE CITED

- [1] G. D. Tumanishvili, K. M. Dzhandieri and I. K. Svanidze, *Doklady Akad. Nauk SSSR*, 1956, Vol. 108, No. 6, pp. 1107-1109.
- [2] G. D. Tumanishvili, K. M. Dzhandieri and I. K. Svanidze, *Doklady Akad. Nauk SSSR*, 1956, Vol. 107, No. 1, pp. 182-184.
- [3] G. Andres, *Journ. Exper. Zool.*, 1955, Vol. 130, No. 2, pp. 221-249.
- [4] L. D. Ebert, *Proc. Natl. Acad. Sci. U.S.*, 1954, No. 5, Vol. 40, pp. 337-347.
- [5] G. Levander, *Nature*, 1945, 155, pp. 148-149.
- [6] F. A. McJukin and H. C. Breuhain, *Arch. Path.*, 1931, Vol. 12, pp. 900-908.
- [7] H. Teir, *Nord. Med.*, 1953, Bd. 50, N 28, S. 982-982.
- [8] P. Weiss, *Science*, 1952, Vol. 115, No. 2992, pp. 487-488.
- [9] P. Weiss and H. Wang, *Anat. Rec.*, 1941, Vol. 79, No. 3, pp. 62-63.