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Cause of Death of Toads after Destruction of their Lymph Hearts.

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Destruction of the 4 lymph hearts of batrachians produces marked disturbances in lymph circulation, leading to rapid death[1, 2] as we have repeatedly demonstrated in some 500 toads (*Bufo arenarum* Hensel) and frogs (*Leptodactyllus ocellatus* (L.) Gir.). The anterior and posterior lymph hearts are easily destroyed by dorsal route approach under ether anesthesia and cauterizing with a thermocautery. The operation requires 2 to 3 minutes and when the effects of anesthesia have worn off, the animals appear entirely normal, and may be put in sinks kept damp by running water.

All the toads in which the 4 lymph hearts were destroyed at one operation died before the 4th postoperative day in winter, and before the 3rd in summer. Mortality during the first 2 days was 87% of all operated animals. Longer survival seems due to incomplete operation.

The weight of the toads was found to increase progressively from operation to death at the rate of 20% daily. At necropsy, an enormous accumulation of fluid was observed in the lymph spaces, the peritoneal cavity, and the tissues. This fluid retention seems to be enough to account for the increase in weight of toads (Table I).

Presence of a single lymph heart seems sufficient to maintain toads for an indefinite period of time without apparent symptoms. It was observed that if 3 of the lymph hearts were destroyed at one

TABLE I.
Change in Body Weight and Mortality of Toads without Lymph Hearts and of Normal Controls Kept in Sinks Moistened with Running Water.

	Avg time of death of							
	38 toads without lymph hearts				28 normal control toads			
At end of day	1	2	3	4	1	2	3	4
Daily mortality %	21	66	10	3	0	0	0	0
Increase in wt (% of initial wt)	20	38	60	—	0	0	0	0

1 Foglia, V. G., *Rev. Soc. Argent. Biol.*, 1939, **15**, 97; *Compt. rend. soc. biol.*, 1940, **133**, 153.

2 Foglia, V. G., and Gerschman, R., *Rev. Soc. Argent. Biol.*, 1939, **15**, 113; *Compt. rend. soc. biol.*, 1940, **133**, 155.

operation and the fourth a week later, survival was more prolonged than when all 4 hearts were destroyed simultaneously. In one group of 7 toads, operated upon in 2 stages, 2 animals continued to live for 2 months while the others survived for 2, 5, 9, 11, and 12 days respectively. This prolonged survival was accompanied by little or no increase in body weight. It is possible that in these cases the ciliated funnels of the peritoneum, well studied by Rugh,[3] permitted the establishment of an active circulation between lymphatics and veins.

The studies of Pascualini[4] on absorption of substances injected under the skin, have demonstrated that the reabsorption of fluid takes place by way of the lymph hearts and blood capillaries, the former being more important. Substances of high molecular weight such as inulin and hemoglobin pass into the circulation by way of the lymph hearts. On the other hand, those of low molecular weight such as glucose, sodium chloride, and strychnine pass both by way of the blood capillaries and the lymph hearts. Destruction of the lymph hearts therefore impedes the absorption of substances of the first group only.

After paralysis of the posterior lymph hearts by cutting their nerves, accompanied by cauterization of the anterior lymph hearts, toads die in the same period of time and with the same signs as when there is destruction of all 4 hearts. Mechanical obstruction of the lymphatic circulation, due to operation does not, therefore, produce the disturbances, but the cessation of contraction of the hearts is the cause of death.

It was not found possible to prolong life of toads with all the lymph hearts destroyed by keeping them in a dry or a humid atmosphere, or by submerging them in distilled or tap water or in solutions of sodium chloride in concentrations varying between 8 and 10%.

In toads with all lymph hearts destroyed, profound alterations in blood, lymph, and tissues occur. These begin at the time of operation and increase progressively until death. Our observations on these changes are summarized in Table II.

Observed changes in the blood seem to depend directly upon the enormous loss of water and salts (chlorides, sodium, and potassium). Their loss causes (a) a diminution in blood volume; (b) the appearance of hemolysis which increases progressively post-operatively; (c) marked hemoconcentration as evidenced by an increase in the hematocrit, red cells per cubic millimeter, hemoglobin, and total

[3] Rugh, R., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 717.

[4] Pascualini, R., *Rev. Soc. Argent. Biol.*, 1939, **15**, 438.

TABLE II.
Chemical Changes Observed in Toads (*Bufo arenarum* Hensel) Kept in Moist Sinks Before and After Destruction of the 4 Lymph Hearts. Average Values of Several Groups of 40 Toads Each.

	g%		mg%					
	Water	Total nitrogen	Chlorides	Sodium	Potassium	Hematocrit, %	Hemoglobin, g%	Erythrocytes per mm³
Blood before	84.4	1.52	263	233	145	33.4	8.6	
24 hr after	76.8	3.33	202	152	175	51	16.8	
36 " "			121	100	135	68.6		
Plasma before		0.55	335	305	13			
24 hr after		0.59	282	250	21			
36 " "			198	198				
Erythrocytes before		1.53	157	120	370			710,000
24 hr after		3.33	128	73	254			810,000
36 " "			92	80	150			1,250,000
Lymph, 36 hr after	97.5	0.3	187	167	21			
Liver before	68.8	2.3	131		420			
24 hr after	70.2	2.1	67		229			
Muscle before	77.1	3	60		305			
24 hr after	81.5	2.5	49		290			

nitrogen (which is due especially to the increase in concentration of hemoglobin). As a consequence of these alterations in the blood, it is observed that (a) the mean arterial aortic pressure decreases about 30%; (b) the glomerular and capillary circulation of the kidney is sluggish; and (c) urinary output is only a third of that of the controls.

In the tissues (liver and muscles), there is an important loss of chloride and potassium. The increase in water content and decrease in protein content observed in the direct analysis of muscle must be interpreted as resulting from interstitial edema. The enormous amount of lymph which accumulates consists of 97% water. There is a protein content of 2.05%. The salts (chlorides, sodium, and potassium) are in a concentration equal to or slightly less than that of blood. These mineral elements come from blood and tissues.

In summary, acute destruction of the lymph hearts of the toad (*Bufo arenarum* Hensel) and the frog (*Leptodactyllus ocellatus* L. Gir.) leads to death within 4 days. During this time, body weight increases and edema fluid appears in the lymphatic spaces. In their development, the disturbances of the venous lymphatic circulation produced by the abrupt cessation of contraction of the lymph hearts play a primary rôle, for paralysis of the lymph hearts by

denervation causes death. These disturbances can be overcome and death avoided by destroying the hearts in 2 stages separated by an interval of at least a week which gives time for the development of new collateral pathways. The existence of a single intact lymph heart prevents the development of all disturbances, including death. As a result of the destruction of the lymph hearts, the lymph which transudes from the blood vessels collects in the lymph spaces and cannot return to the blood stream. Water, salts, and proteins coming from blood and tissues remain in the lymph. Water in large amounts comes from the external environment. The decrease in the content of water, salts, and possibly protein in the blood and the changes in the chemical composition of the tissues cause the death of the animal.

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Transmission of St. Louis Encephalitis to the Hamster.

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The virus of St. Louis encephalitis has been found to be pathogenic for relatively few laboratory animals. Muckenfuss, Armstrong and McCordock[1] first established the virus in monkeys, but it was difficult to maintain in this animal. Webster and Fite[2] showed that Swiss mice could be infected easily by intracerebral and intranasal inoculation. All types of albino mice as well as house mice,[3] field mice and meadow mice[4] are susceptible.

Smith[5] has shown that the virus of St. Louis encephalitis persists in the brain of rats and guinea pigs for 8 or 9 days after intracerebral inoculation and slight anatomical lesions are demonstrable. These animals, however, showed no symptoms of disease. The

1 Muckenfuss, R. S., Armstrong, C., and McCordock, H. A., *Pub. Health Rep.*, 1933, **48**, 1341.

2 Webster, L. T., and Fite, G. L., *Science*, 1933, **78**, 463.

3 Harford, C. G., Sulkin, S. E., and Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 331.

4 Greutter, J. E., Fulton, J. D., Muether, R. O., Hanss, E. V., and Broun, G. O., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 253.

5 Smith, M. G., *J. Infect. Dis.*, 1939, **64**, 307.