injection, and was evoked as well by single doses of 1 mg/kg as by 3 mg/kg. While procaine had an inappreciable effect on toxicity of digitalis under the conditions of the experiment, it might be of at least transient beneficial effect under other conditions.

Summary. 933F, 883F, procaine and quinidine do not increase tolerance of cats to lethal effects of digitalis. Ventricular fibrillation induced by digitalis overdosage is of a different type than that elicited by epinephrine or related agents.

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Fatal Loss of Plasma Volume After Lymph Heart Destruction in Toads.

R. L. ZWEMER AND V. G. FOGLIA.

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York City, and Institute of Physiology, University of Buenos Aires, Argent.

In certain cases of shock and after severe burns there is a tendency toward blood concentration. Determinations of total red cell volume or count per cubic millimeter when compared with determinations of plasma proteins and other plasma constituents suggest that the hemoconcentration is due to loss of whole plasma from the blood vessels into the tissue spaces, where it remains manifest as an edema. Normally, the lymphatic system returns any excess intercellular fluid to the blood circulation.

In batrachians, lymph flow is aided by 4 lymph hearts. In both toads (*Bufo arenarum* Hensel) and frogs (*Leptodactyllus ocellatus*) the 2 anterior and 2 posterior lymph hearts can be easily destroyed by thermocautery. Foglia and his collaborators[1,2,3,4] found that if the operation was complete, death ensued in 4 days. Marked changes were found to occur in the water and electrolyte concentrations of blood, lymph and tissues, beginning at operation and continuing until death. There was an enormous increase in body weight due to water retention (60%). These animals were kept in sinks moistened by dripping water and the water absorbed through the skin was retained in the tissue spaces. Since the interstitial fluid failed to reach the blood stream it could not be eliminated by kidney excretion. A complete bibliography of the problem will be found in the paper by Braun-Menendez and Foglia.[3]

During the course of the experiments by Foglia and Gerschman,[2] some animals without lymph hearts were kept in a chamber with high humidity but no liquid water. These toads died with no gain or loss in body weight.

We thought it worth while to investigate the fluid distribution and concentration in this type of experiment since with diminished urine excretion, most of the changes would involve an internal redistribution of water.

Material and Methods. The toads (*Bufo arenarum* Hensel) were operated in one stage under ether anesthesia. All 4 lymph hearts were destroyed by electrocautery using the technic described in previous papers.[1,3] In operated controls an equal connective tissue and muscle area was burned in a nearby region.

Red cell volume was determined in a 10 cm capillary tube with freshly drawn blood, by centrifugation at 3500 r.p.m. for 20 minutes. Coagulation was prevented by a few grains of dry heparin (Connaught Labs.).

Specific gravity of plasma and lymph were determined by the falling drop technic of Barbour and Hamilton.[5] This was converted

1 Foglia, V. G., *Rev. Soc. Argent. Biol.*, 1939, **15**, 97; *C. R. Soc. Biol.*, 1940, **133**, 153.

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

3 Braun-Menendez, E., and Foglia, V. G., *Arch. Inter. de Pharm. et Therap.*, 1940, **64**, 273.

4 Foglia, V. G., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 598.

5 Barbour, H. G., and Hamilton, W. F., *J. Biol. Chem.*, 1926, **69**, 625.

to grams of protein by subtracting 1.0054 for water and salts from the density and multiplying by 340.1.

Potassium was determined in plasma by the micro method of Truszkowski and Zwemer[6] and in tissues by the method of Marenzi and Gerschman.[7]

Tissue water was studied by drying a chopped mass from many animals, or by small pieces rapidly weighed on a micro torsion balance and then dried to constant weight, and also in a few cases by recovery of water distilled off from a mass of chopped tissue.

Tissue sections were made by frozen and paraffin methods and stained with hematoxylin and Sudan III or eosin.

Constant humidity was maintained in a large wide-mouthed, glass bottle fitted with a 2-hole rubber stopper. Air was evacuated through one tube connected to an ordinary water suction pump. Air entering the chamber through the other tube was first bubbled through water, thus ensuring constant saturation regardless of temperature. By placing the container on its side, a larger number of toads could be accommodated.

Discussion of Results. The first requirement was confirmation of the constancy of body weight, since this was the factor that determined the fact of an *internal* redistribution of water. The weights of individual toads in the chamber was so constant that individual listing would be an unnecessary waste of tabular space so group weights are given in Table I. In the presence of liquid water, absorption through the skin produced large gains and the animals are listed separately.

In the blood and lymph studies (Fig. 1) the animals were tested for packed red blood cell volume, plasma and lymph protein and plasma and lymph potassium. The figures given are averages of separate determinations in 3 to 5 animals. The most important change appears to be the steady increase in red cell volume. For some hours before death it was extremely difficult to obtain samples from the heart or aorta. One can deduce the plasma-free state and red cell congestion in the peripheral vessels.

The water content of the liver and muscle tissue of operated animals in constant humidity

TABLE I.
Body Weight of Toads After Lymph Heart Destruction.

Controls In humid chamber Group weights (g)			After lymph heart destruction: In humid chamber* Group weights (g)			After lymph heart destruction: In the presence of running water Individual weights (g)		
16 ♂	4/28	5/5	4 ♂	5/6	5/7	3/31	4/1	4/2
	2335	2320		550	550	215	255	265
						165	200	240
16 ♂	5/7	5/10	8 ♂	5/7	(8 hrs later)	155	180	—
	1840	1880		1130	1120	125	150	160
5 ♂	5/10	5/13	10 ♂	5/10	5/13	7/3	7/4	7/5
	560	570		1180	1180	102	127	140
						105	120	130
16 ♂	5/15	5/19	8 ♂	5/19	5/21	104	122	135
	1810	1800		970	960	105	122	130
						110	130	145
4 ♂	6/26	6/28				103	115	120
	372	370				85	110	122
						106	125	130
						100	123	125
						100	122	125
						72	95	95
						All toads gained wt within 24 hr of operation.		

* Individual animal weights of controls and operated animals in humid chamber showed remarkable constancy.

[6] Truszkowski, R., and Zwemer, R. L., *Biochem. J.*, 1937, **31**, 229.

[7] Marenzi, A. D., and Gerschman, R., *Rev. Soc. Argent. Biol.*, 1932, **8**, 38.

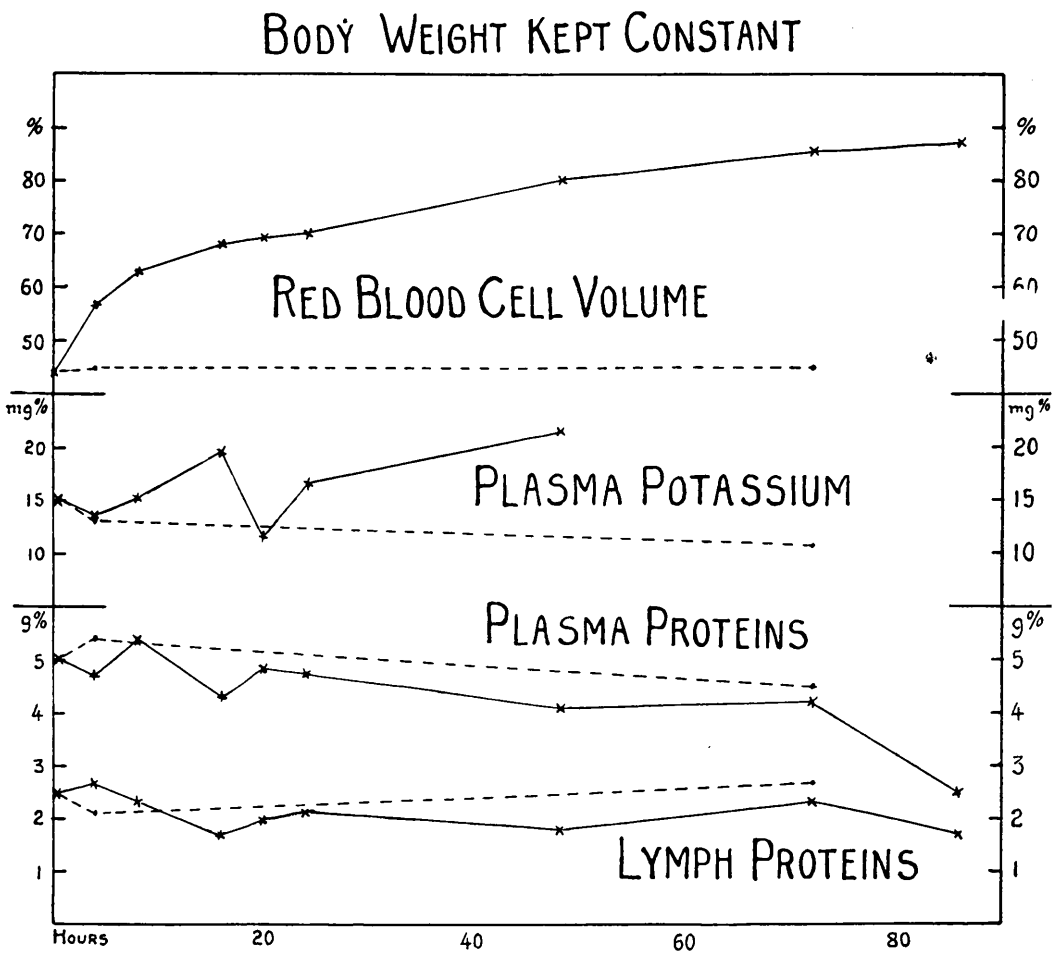

FIG. 1.

Blood and Lymph Changes After Lymph Heart Destruction.

In the graph each point is an average of separate determinations in 3 to 5 animals. Solid lines represent data from experimental animals. Broken lines, data from "dummy-operated" controls run under identical conditions.

did not change significantly (Table II), whereas in the presence of water there was an increase in the per cent of tissue water.

The plasma protein concentration remained constant, thus indicating that whole plasma was lost through the capillary walls. The fact that the lymph protein did not rise can be explained on the basis of proportion of plasma extravasated to the total amount of tissue fluid already present in the animal. A 100 g toad with 6% blood (of which 55% is plasma) would have 3.5 cc of plasma. When the plasma volume falls to 15% of the blood (Fig. 1) there has been a calculated loss of 2.6 cc. This would give a negligible change in the total lymph protein concentration.

However, in the presence of running water there was a marked fall in the protein content of both plasma and lymph. This is in accord with the great water retention noted by Foglia[4] and confirmed by the present experiments.

The plasma and lymph potassium showed a tendency to increase in constant humidity. This increase reached approximately 100% when the animals were in contact with water, the potassium being derived from erythrocytes and other cells as found by Foglia.

Sections of tissues by both frozen and paraffin technics showed no significant differences in cell size between the controls and the operated animals either humid or in contact with liquid water.

TABLE II.
Water Content of Tissues.

Group	Time, hr	Liver	Muscle
Control			
A	0	68.8	77.1
B	0	67.7	75.2
C	0	—	77.6
Without lymph hearts, humidity constant			
B	48	68.5	75.9
C	48	70.3	75.3
C	86	71.7	74.9
Without lymph hearts + water present			
A	24	70.2	81.5
C	30	72.6	83.4
B	48	72.4	80.6
B*	48	68.7	78.0
C	48	73.7	80.8

A. Data from Foglia and Gerschman.

B. Data from Dr. Mazzocco on mixed samples from many animals.

B*. Special water recovery method.

C. Data averaged from many single determinations in present experiments.

Conclusions. Toads without lymph hearts kept in constant humidity showed no change in body weight, whereas other animals kept in the presence of water showed a gain in weight of as much as 20% daily. With a constant body weight the fluid redistribution can be assumed to be internal. In both series of experiments there was a marked increase in red cell volume to a point where flow even in large vessels was impeded.

The fall in plasma and lymph protein, and the rise in body fluid, potassium content and tissue water are much greater when water is absorbed through the skin by contact.

When the normal flow of fluid from plasma to tissue space to lymph channels to vascular system is blocked by lymph heart destruction, there is a marked increase in the interstitial fluid at the expense of the plasma. The failure of this edema fluid to return to the blood vessels in these experiments is a primary and uncomplicated cause of death.

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The Effect of Phosphatides on Utilization of Vitamin A and Carotene.

CHARLES A. SLANETZ AND ALBERT SCHARF. (Introduced by H. D. Kesten.)

From the Department of Animal Care, Columbia University, New York.

The problem of the utilization of carotene and vitamin A as influenced by the oil or fat in the diet has been investigated repeatedly during recent years.

Sherman[1] studied the effect of various oils on the utilization of carotene and found that the addition of 0.1 ml of cottonseed oil daily and especially soybean oil to the diet greatly improved the growth response of vitamin A deficient rats receiving 1 and 2 μg carotene daily.

Quackenbusch, Cox and Steenbock[2] reported that tocopherol promoted the response to carotene and concluded that tocopherol functioned in the intestinal tract as an antioxidant.

Sherman[3] reported that carotene was destroyed in the intestinal tract in the absence of tocopherol.

Quackenbusch, Cox and Steenbock[4] provided further evidence that tocopherol is essential for the utilization of carotene in the intestinal tract but reported even better results with a soybean oil distillate.

1 Sherman, W. C., *J. Nutrition*, 1941, **22**, 153.

2 Quackenbusch, F. W., Cox, R. P., and Steenbock, H., *J. Biol. Chem.*, 1941, **140**, civ.

3 Sherman, W. C., *Fed. Proc. Soc. Biol. Chem.*, 1942, **1**, 134.

4 Quackenbusch, F. W., Cox, R. P., and Steenbock, H., *J. Biol. Chem.*, 1942, **145**, 169.