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Blood Pressure and Blood Protein Determinations in the Frog.

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In a previous note,[1] the authors called attention to the low protein content of frog's blood. The low colloidal pressure of the blood is an important factor in determining the production of urine. The blood pressure of the frog is not nearly as high as in mammals, but if filtration is the basis of glomerular activity, there should be enough pressure to overcome the osmotic pressure of the colloids of the frog's blood.

It seemed desirable, therefore, to make some actual blood pressure determinations and determinations of the protein content of the blood on the same animal.

In order to make blood pressure determinations on the intact animal, the following apparatus was devised. A modified apparatus such as is used for determining arterial blood pressure in man was used. Small rubber cuffs were constructed from small rubber balloons, varying in size from one-half to one inch wide, and 3 to 5 inches long. A rubber tube was cemented into the open end of the balloon and by means of it the balloon was connected to a mercury manometer, an outlet valve, a Woolf bottle to act as a buffer, and a rubber atomizer bulb. The frog was placed in a suitably sized box with its right leg protruding through a small opening. The rubber cuff was then wrapped around the thigh or leg, the web of the foot placed under the low power of the microscope and as large an arteriole as could be found was brought into focus. A pulsating stream could be observed. The pressure in the cuff recorded by the mercury manometer was raised until pulsations in the arteriole could no longer be seen. The air was then gradually let out of the system and when pulsations first were observed, the mercury manometer was read for systolic pressure. As the pressure continued to fall slowly, it was noticed that between systolic peaks of pressure, the blood stream slowed to complete stoppage and sometimes to a reverse flow. When this reverse flow disappeared and the stoppage between systoles began to disappear, the diastolic pressure was taken. The readings obtained in this manner have been checked up with a mercury manometer attached directly to an artery in the abdominal

[1] Scott, F. H., and Bieter, R. N., PROC. SOC. EXP. BIOL. AND MED., 1922, xx, 120.

cavity and with a Hurthle manometer attached in the same manner, and the readings were found to be within the limit of error.

Blood pressure readings have been taken in a series of small frogs *(Rana pipiens)* and also in a series of large frogs *(Rana catasbiana)*, after which these animals were bled for plasma protein determinations.

The determinations of the protein content were made as follows: After the blood pressure determinations had been made, 5 mg. of heparin were injected subcutaneously into the large frogs and 1 mg. into the small ones. After waiting about half an hour, the animals were etherized, a cannula inserted into the coeliac axis in the large frogs and the frogs bled. In the case of the large frogs, the blood was taken in 2 parts; the first sample (2-5 cc.) came rapidly; the second sample of about the same amount came slowly, sometimes 20 to 30 minutes. In the case of the small frogs, the tip of the ventricle was snipped off and allowed to bleed into a small tube. The tubes were centrifuged and a definite volume of clear plasma removed with a pipette. This was placed in a weighed centrifuge tube and 10% trichloracetic acid added in excess. After standing, the tube was centrifuged, the supernatant fluid removed, more trichloracetic acid added, and recentrifuged. The supernatant fluid was removed and the same process repeated with 70% absolute alcohol. The tubes were then placed in an oven at 102° and dried to a constant weight.

The blood pressure of the frog was found to be quite variable at first. As a result of struggling, systolic pressures as high as 7 mm. Hg. have been observed. However, after a while, the pressures became fairly stable, ranging around 25-35 systolic to 18-28 diastolic and giving a pulse pressure of 7-10 mm.

The proteins (trichloracetic acid precipitate) of the plasma ran, ordinarily about 3.7% on the first or normal sample of blood, the variations being from 4.48% to 2.7%. As a result of the hemorrhage, the protein content of the second sample was frequently cut in half. Attention has been called by one of the authors[2] to the fact that the fluid entering the blood of mammals after hemorrhage is practically pure salt solution. The same fact is observed here. Little difference was observed in the blood pressures of the large or small frogs, but the protein content of the small *(R. pipiens)* frog was usually somewhat less than that of the large frog *(R. catasbiana)*. The highest figure given by Krogh[3] for the osmotic

[2] Scott, F. H., *J. Physiol.*, 1916, l, 157.

[3] Krogh, A., *Anatomy and Physiology of Capillaries*, 1922.

pressure of frog's blood is 40 mm. H_2O for 1% protein. White[4] gives practically the same figure. There is thus seen to be plenty of filtration pressure to produce urine in the frog, the systolic pressure being about 3 times the osmotic pressure of the colloids.

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Significant Difference in Response of Pernicious Anemia to Fetal Calf and Beef Liver Feeding.

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A marked increase in the number of circulating reticulocytes represents a well-known feature in the response of patients with pernicious anemia to the feeding of beef liver or of the Cohn-Minot liver extract. So constant is this response that the reticulocyte curve is being used in the testing of the potency of extracts. An equally constant but usually less pronounced feature is the pouring out of an increased number of erythroblasts into the circulation, which phenomena precedes the reticulocyte increase by a few days. Biologically both these phenomena are reliable indications of a certain lag in the complete cell maturation.

TABLE I.

Subject: Mrs. Q. U. Hosp. No. 43942. Diagnosis: Pernicious Anemia.

Day of observation	Erythrocytes millions per cu. mm.	Reticulocytes in % of erythrocytes	Hemoglobin	Remarks.
1	1.08	1.5	31	On liver extract, potency unknown.
3	1.33	1.8	33	
5	1.36	3.4	33	
7	1.24	2.4	33	
9	1.19	1.8	32	
11	1.20	1.3	35	From 10th day on powdered fetal liver.
13	1.34	1.6	39	
15	1.38	1.8	41	
17	1.40	1.6	40	
19	1.70	.6	42	
21	1.85	.4	43	
23	1.90	.8	47	
25	2.10	.6	51	

[4] White, H. L., *Am. J. Physiol.*, 1924, lxviii, 523.