by the method of Woolley, McDaniel and Peterson.[7] When this material was added at a level equivalent in biotin content to 2% of rice bran extract, no growth response was obtained. The same assay technic was used here as with the liver fractions; that is, the rats were fed the synthetic ration for 5 to 6 weeks, then given 100 γ per day of pantothenic acid, and subsequently the biotin concentrate. The ability of the animal to respond was finally proven by addition of one of the potent liver fractions described above. While this does not prove that the rat does not require biotin, it shows that this substance is not the sole remaining growth factor for this species.

It is not justifiable to ascribe the growth-promoting activity of the above liver concentrate solely to one substance. The experience of the past has emphasized the need for caution in attributing "filtrate factor" activity of concentrates to one compound. However, the above method is a simple and effective means of purifying the remaining unknown rat growth factors. The final answer as to whether only one substance is concerned must await crystallization of the active factor.

11195 P

Acute Circulatory Failure (Shock) Following Subcutaneous Injection of Hypertonic Sodium Chloride Solution.

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Intraarterial injection of hypertonic solutions of NaCl is followed by a marked rise in blood pressure which persists for several hours.[1,2,3] The present study is concerned with the effect of the subcutaneous introduction of hypertonic NaCl solutions upon the circulation of the blood, the purpose being to create an area of excessive osmotic pressure and thus induce a flow of fluid out of the capillaries into the site of injection.

Materials and Methods. Seven dogs, weighing from 5 to 8 kg,

[7] Woolley, D. W., McDaniel, L. E., and Peterson, W. H., *J. Biol. Chem.*, 1939, **131**, 381.

[1] Davis, H. A., Jermstad, R. J., and Choisser, R. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 144.

[2] Davis, H. A., *M. Ann. District of Columbia*, 1937, **6**, 344.

[3] Kendrick, D. B., *Surgery*, 1939, **6**, 520.

were anesthetized with sodium pentobarbital. Solutions of NaCl (25%) were injected subcutaneously into the left hind leg, the dosage being 25 cc per kg of body weight. The blood pressure was recorded in the usual manner after insertion of a cannula into the right carotid artery. Blood obtained from the right femoral vein before and at hourly intervals after the introduction of the solution was studied with respect to hemoglobin content, hematocrit, erythrocytes, non-protein nitrogen, sugar and NaCl.

Results. All the animals died within 5 to 10 hours. Immediately after the injection the systolic and diastolic blood pressures rose 10-15 mm Hg above the initial level, then fell to the original level, at which they were maintained for 1-3 hours. During this period the blood showed an increasing concentration. The heart rate was accelerated and the respirations became rapid and shallow. The blood pressure fell slowly to the shock level (80 mm Hg) and thereafter fell relatively rapidly. The mucous membranes of the mouth and tongue showed an increasing cyanosis, and the blood obtained by venipuncture was dark and appeared poorly oxygenated. The amount of urine formed during each experiment varied from 20 to 100 cc.

Edema developed at the site of injection and frequently extended to the abdominal wall and even to the outer aspect of the left thorax. Its extent seemed to bear a definite relation to the survival time and was greatest when the period of survival was longest.

The erythrocyte counts showed increases varying from 20 to 63%, the hematocrit reading rose 37 to 47% above the initial level, and the amount of hemoglobin was increased by 23 to 73%. These figures suggest that a considerable diminution of the plasma volume had occurred. The blood sugar was increased by 108 to 151%, the blood non-protein nitrogen by 76 to 137%, and the whole blood chloride (estimated as NaCl) by 31 to 50%. Representative data are shown in Table I.

The significant rise in the blood chloride level must be commented upon. In other forms of shock a fall takes place, and the rise in these experiments may be attributed to absorption of NaCl from the site of injection into the blood stream. A simple calculation will reveal that a total of only 2-3 g of NaCl was absorbed. The intravenous administration of similar or larger amounts in the form of hypertonic solution results in a definite hemodilution, which is in contradistinction to the hemoconcentration found in the present experiments. By altering the stated dosage of hypertonic NaCl solution it was possible to hasten or delay the time of onset of shock.

TABLE I.

Dog wt, kg	Blood pressure, mm Hg		Hematocrit, %		R.B.C., millions per cm		Hb, %		Blood sugar, mg %		Blood non-protein N, mg %		Blood NaCl, mg %	
	B.I.	A.I.	B.I.	A.I.	B.I.	A.I.	B.I.	A.I.	B.I.	A.I.	B.I.	A.I.	B.I.	A.I.
6.8	165	10	38	56	5.5	8.98	68	118	72	181	34.2	60	650	890
6.4	135	35	48	57	7.94	9.22	98	115	89	136	19.8	30	678	992
8.0	160	5	42	58	6.94	10.6	85	150	84	52	42.6	60	736	1110

B.I. = Before injection of hypertonic NaCl solution.
A.I. = After injection of hypertonic NaCl solution.

Conclusions. The subcutaneous injection of hypertonic NaCl solution is followed by acute circulatory failure, which is associated with hemoconcentration and with an increase in the amount of sugar, non-protein nitrogen, and NaCl in the blood. Other hypertonic solutions, such as hypertonic glucose solution, when given subcutaneously, might be expected to produce somewhat similar results.

11196 P

Relative Significance of Water and of Protein Loss in Dehydration Shock.

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The importance of loss of fluid at the site of injury has been emphasized in shock due to trauma or burns and associated with hemoconcentration.[1, 2] The chemical composition of this fluid is similar to that of blood plasma and its total protein is approximately 5.2 g per 100 cc.[3] The present study was undertaken to determine the relative significance of loss of the protein fraction and of the aqueous fraction of the blood plasma in the production of acute circulatory failure associated with hemoconcentration.

Materials and Methods. Shock was induced in 6 dogs which had been anesthetized with sodium pentobarbital, by the subcutaneous introduction into the left leg of 25% sodium chloride solution, the dosage being 25 cc per kg of body weight. Details of this method have been published elsewhere.[4] Determinations of the hemoglobin, hematocrit and erythrocytes were made upon blood withdrawn from the right femoral vein before and during the course of each experiment. After death each animal was bisected and the difference in weight between the halves of the body was determined. In estimating the amount of fluid lost at the site of injection, a correction was made by subtracting from the total weight the weight of the injected solution; 100 cc of 25% solution was found to weigh approximately 118.8 g, and the calculations were made upon this basis.

1 Blalock, A., *Arch. Surg.*, 1930, **20**, 959.

2 Parsons, E., and Phemister, D. B., *Surg., Gynec. and Obst.*, 1930, **51**, 196.

3 Harkins, H. N., and Harmon, P. H., *Ann. Surg.*, 1937, **106**, 1070.

4 Davis, H. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 354.