time.

Pathology. The lesions were the same in all animals with progressive symptoms, whether they died spontaneously or were killed by exsanguination. They consisted in diffuse degeneration of the ganglion cells throughout the brain, without any visible difference between different areas. In Nissl sections, the ganglion cells showed either vacuolar degeneration, with swelling of the cell, disappearance of Nissl bodies and increased colorability of the dendrites, or pyknosis. Only occasional, normal cells were found. In Weigert-Pal sections, no definite changes could be found.

Clusters of unidentified cells were observed close to the ependymal lining. Such clusters have been described in various species. We are unable to decide whether these clusters have any pathological significance. No alterations were found in the liver, aside from moderate hyperemia of the capillaries. In none of our cases was there any fatty degeneration, such as has been described as one of the most constant features of DDT poisoning after ingestion of the drug.

No alterations were observed in other organs.

Conclusion. Progressive neurological symptoms were produced in cats by the intramuscular injection of DDT. The signs evolved in the sequence of stiffness, tremor, clonic movements, and death.

An animal with chronic neurological symptoms was not obtained, either because death supervened or because the animal returned toward normality.

Nissl sections revealed diffuse damage to the ganglion cells of the brain, characterized by vacuolar degeneration or pyknosis. No alterations, other than capillary dilatation, were recognized in the liver.

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Some Effects of Depletion and Repletion in Proteins on Body Fluids in Adult Dogs.*

J. B. ALLISON, R. D. SEELEY, J. H. BROWN, AND F. P. FERGUSON.

From the Bureau of Biological Research, Rutgers University, New Brunswick, N.J.

A decrease in plasma volume and a nutritional edema accompanies hypoproteinemia in dogs.[1-3] The fall in concentration of plasma proteins is associated also with an increase in the utilization of nitrogen, the nitrogen balance indexes of proteins being greater in protein-depleted than in normal animals.[4] An analysis of these data suggests that there are regular shifts in body fluids and in the utilization of nitrogen as the protein stores of the animal are altered. Experiments were organized, therefore, to study changes in plasma and "available fluid" volumes and in the excretion of various forms of nitrogen in adult dogs during control, depletion, and repletion periods.

Methods. Four dogs were depleted by feeding a protein-free diet; 3 days of plasmapheresis being used initially to speed up the process of depletion. The details of this method of

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1 Allison, J. B., Anderson, J. A., and Seeley, R. D., *Bull. N. Y. Acad. Sci.*, in press.

2 Weech, A. A., Goettsch, E., and Reeves, E. B., *J. Exp. Med.*, 1935, **61**, 299.

3 Weech, A. A., Wollstein, M., and Goettsch, E., *J. Clin. Inv.*, 1937, **16**, 719.

4 Allison, J. B., Seeley, R. D., Brown, J. H., and Anderson, J. A., *J. Nutrition*, 1946, **31**, 237.

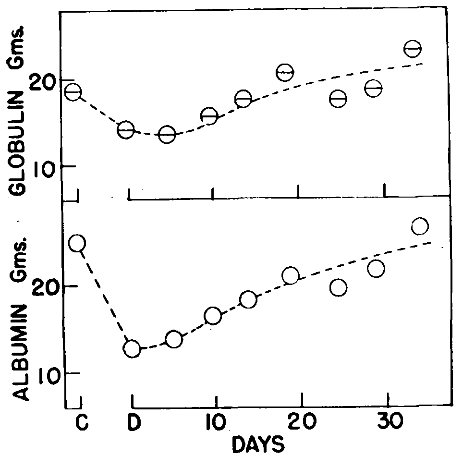

FIG. 1.
Total circulating plasma albumin and globulins in dog No. 68 before (C) and after depletion in proteins (D) and during 35 days of feeding 0.35 g of casein hydrolysate nitrogen/day/kilo of body weight.

depletion have been described previously.[5] During the repletion period 2 dogs were fed orally a casein hydrolysate, and 2 were fed a lactalbumin hydrolysate,[†] each dog receiving 0.35 g of nitrogen/day/kg of body weight. The plasma and "available fluid" volumes were determined according to the technics described by Gregersen and Stewart.[6] Nitrogen determinations on the blood and urine were made using the Pregl Micro-Kjeldahl. The albumin and globulin fractions were determined by the salt fractionation method of Howe.[7]

Urine ammonia and urea nitrogen were determined by the aeration method of Van Slyke and Cullen,[8] creatinine and creatine were determined by the alkaline picrate procedure of Folin,[9] and uric acid was analyzed by the indirect method of Folin.[10] Urine alantoin was determined by the procedure of Young and Conway[11] and the ninhydrin-carbon dioxide method of Van Slyke, MacFadyen and Hamilton[12] was used to determine the α-amino nitrogen.

Results. The data in Fig. 1 illustrate the

TABLE I.
Data Obtained on 4 Dogs Before and After Depletion in Proteins and After Repletion from Feeding 0.35 g of Nitrogen per Kilo of Body Weight for 30 Days. Dog 28 and 65 received a lactalbumin hydrolysate and Dogs 42 and 68 a casein hydrolysate during the 30-day repletion period.

Dog No.	Wt, kg	Plasma protein, g %	Plasma vol., (P) ml	Available fluid (A) ml	A/P
			Control.		
28	10.5	6.75	444	3100	7.0
65	10.8	7.14	475	3000	6.3
42	12.1	6.90	430	2600	6.0
68	12.0	7.04	625	3500	5.6
			Depleted.		
28	10.7	4.52	373	3500	9.3
65	11.1	4.50	410	4400	10.7
42	10.6	4.72	400	3600	9.0
68	11.1	5.20	502	4400	8.7
			Repleted.		
28	11.4	6.50	443	2800	6.3
65	11.7	6.51	626	3200	5.1
42	10.3	6.80	465	2600	5.6
68	11.4	7.07	600	3400	5.6

5 Seeley, R. D., *Am. J. Physiol.*, 1945, **144**, 369.

† These hydrolysates were prepared by Dr. Bacon F. Chow of the Squibb Institute for Medical Research. Details concerning these hydrolysates will be published elsewhere.

6 Gregersen, M. I., and Stewart, J. D., *Am. J. Physiol.*, 1939, **125**, 142.

7 Robinson, H. W., Price, J. W., and Hogden, C. G., *J. Biol. Chem.*, 1937, **120**, 481.

8 Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1916, **24**, 117.

9 Folin, O., *J. Biol. Chem.*, 1914, **17**, 469.

10 Folin, O., *J. Biol. Chem.*, 1933, **101**, 111.

11 Young, E. G., and Conway, C. F., *J. Biol. Chem.*, 1942, **142**, 839.

12 Van Slyke, D. D., MacFadyen, D. A., and Hamilton, P. B., *J. Biol. Chem.*, 1943, **150**, 251.

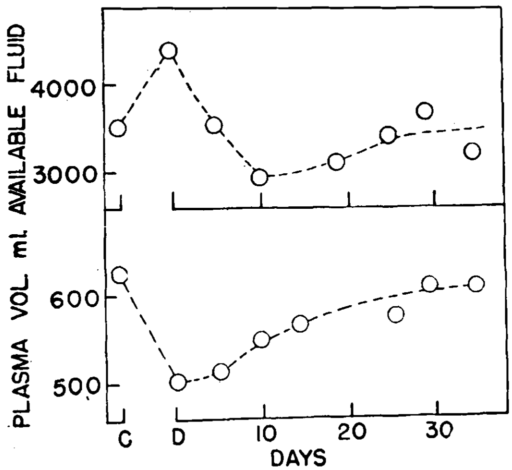

FIG. 2.
Available fluid and plasma volumes before (C) and after depletion in proteins (D) and after 35 days of feeding 0.35 g of casein hydrolysate nitrogen/day/kilo of body weight.

effect of depletion upon plasma proteins in one dog, typical of all 4. These data prove that plasma albumin decreases markedly while plasma globulins decrease slightly below control values in the depleted animal. The reduction in globulin fractions may represent a decrease in the protein stores of the lymphatic tissues. Certainly the α globulin fraction is not reduced, the reduction in globulins being restricted to the β and γ fractions.[13] Under these conditions, the dogs become very susceptible to disease, developing kennel sores and other skin disorders. Replenishment of protein stores by feeding a high-quality protein restores rapidly the natural resistance of the animal to these disturbances. Fig. 1 demonstrates the gradual return of albumin and globulin fractions from the depleted to the normal condition when the dog was fed a casein hydrolysate. Similar regenerations were found in the other 3 animals, one receiving the casein hydrolysate and the other 2 lactalbumin hydrolysate. A critical evaluation of the regeneration of the plasma proteins in these experiments has been made through the analysis of electrophoretic patterns which were obtained on these and a number of other dogs.[14]

The data in Table I record the effects of depletion and repletion in proteins on the plasma and available fluid volumes.

The plasma volume decreases below the control value in the depleted dog, returning toward normal during the repletion period. The available fluid, on the other hand, increases in the protein-depleted dog, returning to control levels upon repletion. Thus the ratio between the available fluid and plasma volumes increases in the depleted animals to above normal. This increased ratio detects a "nutritional edema" long before increased fluid in tissue spaces can be observed clinically.

The data in Fig. 2 illustrate graphically, in more detail than in Table I, the changes in available fluid and plasma volumes which accompany depletion and repletion in proteins. The rapid decrease of available fluid volume which occurred in all dogs soon after the repletion process started is especially noteworthy.

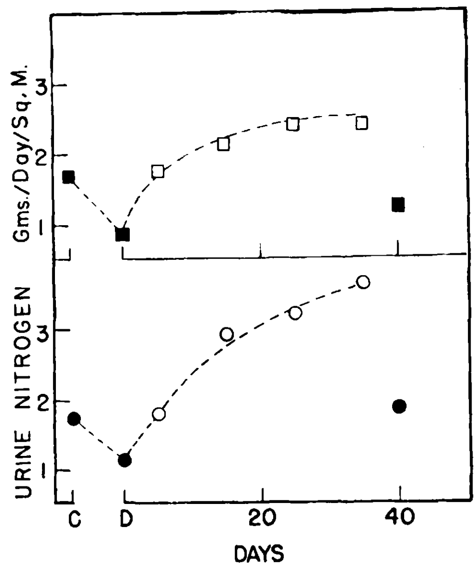

FIG. 3.
Total daily urine nitrogen excretion in dogs 28 □ and 68 ○ while on a protein-free diet before (C) and after depletion in proteins (D) and during 38 days of feeding 0.35 g of hydrolysate nitrogen/day/kilo of body weight. The last point at 40 days represents the daily excretion of nitrogen on a protein-free diet after repletion.

13 Chow, B. F., Allison, J. B., Cole, W. H., and Seeley, R. D., PROC. SOC. EXP. BIOL. AND MED., 1945, 60, 14.

14 Chow, B. F., Allison, J. B., Cole, W. H., and Seeley, R. D., in press.

TABLE II.
Daily Excretion of Compounds Containing Nitrogen During a 5-day Protein-free Feeding Period Before and After Depletion in Proteins and Repletion from Feeding for 35 Days 0.35 g of Hydrolysate Nitrogen per Day per Kilo of Body Weight.

Condition	Total N, g/day/sq.m.	Urea and Ammonia N, g/day/sq.m.	Creatine N, g/day/sq.m.	Creatinine N, g/day/sq m.	Uric acid N, g/day/sq.m.	Alantoin N, g/day/sq.m.	Amino N, g/day/sq.m.
			Lactalbumin Hydrolysate, Dog 28.				
Control	1.73	1.19	0.04	0.20	0.01	0.19	0.026
Depleted	0.84	0.46	0.02	0.13	0.01	0.19	0.031
Repleted	1.22	0.76	0.02	0.16	0.01	0.17	0.028
			Casein Hydrolysate, Dog 42.				
Control	1.35	0.84	0.03	0.17	0.01	0.24	—
Depleted	0.82	0.49	0.01	0.11	0.01	0.17	0.021
Repleted	1.60	1.06	0.04	0.14	0.01	0.20	0.022
			Casein Hydrolysate, Dog 68.				
Control	1.72	1.11	0.02	0.20	0.01	0.21	0.035
Depleted	1.13	0.70	0.02	0.16	0.02	0.16	0.025
Repleted	1.86	1.33	0.03	0.16	0.01	0.19	—

Fig. 3 illustrates data on urine nitrogen excretion obtained on 2 of the dogs, typical of all 4. The first point (C) records the urine nitrogen excretion of the normal dog while receiving the protein-free diet. The second point (D) records the excretion of urine nitrogen of the depleted dog while receiving the protein-free diet. The white squares and circles illustrate data obtained during the repletion process while dog No. 28 was receiving 0.35 g of lactalbumin hydrolysate nitrogen and dog No. 68 was receiving 0.35 g of casein hydrolysate nitrogen/day/kg body weight. The last black point records data obtained at 40 days, while the repleted dog was receiving a protein-free diet. These data prove that the excretion of body nitrogen on a protein-free diet is decreased below control values in the depleted dog. Upon repletion this excretion of body nitrogen returns toward control values, the body protein stores being replenished. The gradual increase in the excretion of nitrogen during the period of repletion is due to the gradual increase in body protein stores accompanied by a decrease in retention of dietary nitrogen. These data supplement those previously published where a reduction from control values in nitrogen excretion and an increase in nitrogen balance index of the dietary protein above normal was demonstrated in the depleted dog.[4]

Table II records the average daily urine excretion of compounds containing nitrogen during the protein-free feeding periods before and after depletion and after repletion in proteins.

These data prove that the decrease below control levels of urine nitrogen in the depleted dog is due primarily to a decrease in ammonia and urea nitrogen.

Summary. The plasma albumin decreases markedly while the total plasma globulins decrease slightly below control values in the protein-depleted animal. The dogs are very susceptible to disease in the depleted state, a susceptibility which is reduced upon repletion in proteins. The plasma volume drops and the available fluids increase as the total circulating plasma albumin is reduced by the process of depletion. This nutritional edema is corrected rapidly by repletion in plasma albumin.

The excretion of urine nitrogen gradually increases during the feeding of protein nitrogen in the repletion period. This gradual increase is the result of a decrease in the utilization of the protein and of an increase in the excretion of nitrogen from body stores. The decrease in urine nitrogen excretion below normal which occurs in the hypoproteinemic dog is primarily the result of a decrease in ammonia and urea nitrogen.