

As a control, a culture was grown in Long's medium without added PABA, and the norite eluate was prepared as described above. When it was fed at a level equivalent to 80 cc of the original filtrate per 100 g of ration, the growth and feathering obtained were somewhat better than on the basal ration, but did not approach the optimum.

The amount of "folic acid" supplied by the various preparations per 100 g of ration is also shown in Table I. Although in most cases there was fair correlation between the "folic acid" content of the ration and growth and feathering, band No. 3, which gave good results, supplied only 5 γ of "folic acid" per 100 g of ration, less than that supplied by band No. 2. This indicated that the response of the culture filtrates could not be attributed to their "folic acid" content.

These results show that compounds active for the chick are synthesized by a strain of *Mycobacterium tuberculosis* on a completely synthetic medium, and that the production of these vitamins is definitely stimulated by

high concentrations of PABA in the medium. This observation is interesting in light of our earlier suggestion³ that feeding PABA to chicks on the basal ration causes increased growth by stimulating the production of the unknown vitamins by the intestinal bacteria. This increased production of these vitamins may be a result of actual incorporation of the PABA into the vitamin molecules, or of stimulation of the bacterial metabolism in some manner to produce compounds distinctly different from the PABA.

Summary. Culture filtrates of a certain strain of *Mycobacterium tuberculosis* grown on a synthetic medium containing high concentrations of *p*-aminobenzoic acid, contain considerable amounts of the unidentified vitamins (B₁₀ and B₁₁) which promote growth and feathering in chicks. The synthesis of these vitamins by the microorganism is definitely stimulated by the presence of high concentrations of *p*-aminobenzoic acid in the medium.

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Effects of Desoxycorticosterone Acetate on Water and Electrolyte Content of Brain and Other Tissues.

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In a previous study on the antagonistic action between pitressin and desoxycorticosterone acetate in epileptic subjects, the authors¹ found the latter substance to have a striking anti-convulsive effect for both spontaneously occurring and pitressin-induced seizures. The principal experimental subject involved in that investigation, a young man with extremely severe epilepsy, has remained essentially free from convulsive attacks during the intervening 3 years while continuing to receive this synthetic hormone sublingually or by subcutaneous pellet implantation. The present experimental study was undertaken

with the hope of obtaining some information regarding the physiological or pharmacological mechanism responsible for this effect.

Our immediate objective was to determine the effects of the hormone on the water and electrolyte content of the brain tissue of normal animals. It has been abundantly demonstrated by Darrow and Miller^{2,3} and by Ferrebee and co-workers⁴ that the potas-

² Miller, H. C., and Darrow, D. C., *Am. J. Physiol.*, 1941, **132**, 801.

³ Darrow, D. C., and Miller, H. C., *J. Clin. Invest.*, 1942, **21**, 601.

⁴ Ferrebee, J. W., Parker, D., Carnes, W. H., Gerity, M. K., Atchley, D. W., and Loeb, R. F., *Am. J. Physiol.*, 1941, **135**, 230.

¹ McQuarrie, I., Anderson, J. A., and Ziegler, M. R., *J. Clin. Endocrinol.*, 1942, **2**, 406.

TABLE I.
Effects of Desoxycorticosterone on Water and Electrolytes of Brain and Muscle Tissue.

Tissue	Per kilo blood-free wet tissue			
	H ₂ O g	Cl m. eq.	K m. eq.	Na m. eq.
Brain				
Controls	776	33.72	77.30	49.73
"Doca"	776	33.24	60.58	52.45
Muscle				
Controls	750	11.84	75.43	31.98
"Doca"	751	12.90	60.67	33.65
		Per 100 g fat-free solids		
Brain				
Controls	605	26.28	60.25	38.75
"Doca"	602	25.77	46.96	40.66
Muscle				
Controls	341	5.38	34.28	14.53
"Doca"	335	5.75	27.08	15.02

sium content of skeletal muscle, as well as that of blood plasma, is greatly reduced and the sodium content is somewhat increased by daily injections of comparatively large doses of desoxycorticosterone acetate. Heart muscle and liver showed much less, or (in some animals) no alteration.³ Data regarding the brain were not reported by these authors. In the present study, changes in skeletal muscle, liver and heart muscle were determined for the purpose of comparison.

Eighty young hooded rats (initial weights, 150 to 200 g each) maintained continuously from the time of weaning and throughout the experiments on a standard rat diet containing 0.605 g of K, 0.565 g of Na, and 1.155 g of Cl per 100 g, were divided into two equal groups. The 40 experimental animals were each given daily doses of 1 mg of desoxycorticosterone acetate, one sub-group receiving it for a period of 7 days only, others for 23, 28, 35, and 41 days respectively. This was administered subcutaneously in sesame oil. The 40 control animals were subdivided into similar groups and were treated identically except for the omission of the steroid from the oil injected.

At the expiration of the various periods, the rats were decapitated and thoroughly bled without anesthesia. Fresh samples of the different tissues were then dissected out and immediately placed in stoppered weighing bottles. Aliquot portions of the minced tissues were analyzed for the following constituents: *water*, by drying in oven at 105°C: *total lipids* by first extracting with alcohol and ether

and then with petroleum ether; *hemoglobin* (method of Flink and Watson⁵)—for use in calculations of extracellular constituents; *potassium* (Shohl and Bennett⁶); *sodium* (Butler and Tuthill⁷), and *chloride* (Wilson and Ball modification of Van Slyke method⁸). The relative masses of extra- and intra-cellular phases of the tissues were calculated according to the method of Manery and Hastings.⁹

The results are summarized in Table I. Since variations in the effects of desoxycorticosterone acetate due to differences in length of periods of administration were insignificant, the values given represent the averages for all of the experimental animals on the one hand and for all of the controls on the other.

It is apparent from the data presented that desoxycorticosterone acetate caused a reduction of approximately 20% in the K content of the brain tissue, which was almost identical with the reduction found by previous workers and by ourselves in the case of skeletal muscle. The sodium was but slightly increased in both tissues. While heart muscle showed no decrease in K content, liver was found to

⁵ Flink, E. B., and Watson, C. J., *J. Biol. Chem.*, 1942, **146**, 171.

⁶ Shohl, A. T., and Bennett, H. B., *J. Biol. Chem.*, 1928, **78**, 643.

⁷ Butler, A. M., and Tuthill, E., *J. Biol. Chem.*, 1931, **93**, 171.

⁸ Wilson, D. W., and Ball, E. G., *J. Biol. Chem.*, 1928, **79**, 221.

⁹ Manery, J. F., and Hastings, A. B., *J. Biol. Chem.*, 1939, **127**, 657.

suffer a 30% loss in this element as a result of the steroid injections. There was no significant alteration in the water content of the brain or other tissues examined. Whether or not the anti-epileptic effect of desoxycortico-

sterone acetate previously referred to is in any way related to the observed decrease in brain potassium cannot be determined on the basis of our present knowledge.

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Effect of Low Fat Diet on Lipids of Erythrocytes, Plasma, Serum, and Whole Blood of Dogs.*

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Although many reports have been made concerning the relative amounts of lipid in plasma and red blood corpuscles in both human beings and lower animals,¹⁻⁸ relatively little information is available concerning the qualitative characteristics of the fatty acids involved, especially as these may be affected by dietary conditions. Hansen and Wiese^{9, 10} have reported that the fatty acids in the various lipid fractions of the serum were definitely less unsaturated in dogs maintained on diets extremely low in fat as com-

pared with those found in control animals receiving lard as the source of dietary fat. The study here reported concerns the character of the fatty acids in the red blood cells in dogs and the effect of dietary fat on these fatty acids, as well as on those in the plasma, serum and whole blood.

Material and Methods. Six dogs, 3 on a low fat diet and 3 on the same diet except for the isocaloric substitution of 28% of lard for sucrose, were maintained under conditions described by Hansen and Wiese.⁹ Blood was drawn from the jugular vein after a fast of 16-20 hours. The anticoagulant used was a saturated solution of ammonium oxalate, 0.024 cc per cc of blood. In the determination of the lipids of the erythrocytes, about 20 volumes of the usual 3:1 alcohol-ether mixture of Bloor were added rapidly to the red blood cells which had been laked with an equal volume of distilled water. The mixture was agitated, then heated in a boiling water bath. At times a solid gummy mass formed. This was ground with sea sand and returned to the extraction mixture. Simultaneous determinations indicated that the results were the same whether or not this mass formed. The extract was filtered through lipid-free filter paper into a volumetric flask, numerous additional extractions were made, the solution was cooled to room temperature and brought to volume. For the extraction of the lipids of whole blood, 3 cc of the oxalated blood were added slowly to the hot alcohol-

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¹ Mayer, André, and Schaeffer, G., *J. de physiol. et de path. gén.*, 1913, **15**, 984.

² Horiuchi, Y., *J. Biol. Chem.*, 1920, **44**, 345.

³ Sundstrom, E. S., and Bloor, W. R., *J. Biol. Chem.*, 1920, **45**, 153.

⁴ Iwatsura, R., *Arch. f. d. ges. Physiol.* (Pflüger's), 1924, **202**, 194.

⁵ Boyd, Eldon M., *Am. J. Dis. Child.*, 1936, **52**, 1319.

⁶ Bloor, W. R., *Biochemistry of the Fatty Acids and Their Compounds, the Lipids*, New York, 1943, Reinhold Publishing Corporation.

⁷ Erickson, B. N., Williams, H. H., Hummel, F. C., and Macy, I. G., *J. Biol. Chem.*, 1937, **118**, 15.

⁸ Bodansky, M., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 630.

⁹ Hansen, Arild E., and Wiese, H. F., *Proc. Soc. Exp. Biol. and Med.*, 1943, **52**, 205.

¹⁰ Wiese, Hilda F., and Hansen, Arild E., *Fed. Proc.*, 1944, **3**, 97.