shape than it does. Consequently, the 10° curve would have shown even greater relative variation when compared to the 15° culture. It is possible that the short death time of the 24- and 27-day cultures can be explained on the basis of reduced load of organisms tested.

It must be pointed out that since the 10° culture was studied at pH 8 and the 15° at pH 7.7 these 2 curves can be compared only as to shape, not as to magnitude.

The 2 incubation temperatures are believed to represent the optimum and the minimum because, of cultures grown at 5°, 7½°, 10°, 12½°, 15°, 17½°, and 20°, the 15° tubes clouded most rapidly. The 7½° showed no clouding.

These data indicate that for *Bact. salmonicida*—a Gram-negative psychrophile—organisms in the lag phase and the "logarithmic" death phase are more resistant to the action of 500 p.p.m. alkaline acriflavine solution at 10° than are bacteria in the phase of logarithmic growth. Organisms grown below the optimum temperature show a greater variation than those grown at the optimum.

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Production of Unidentified Vitamins by a Strain of *Mycobacterium tuberculosis* Grown on Synthetic Medium with p-Aminobenzoic Acid.*

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Mayer[1] reported the formation of a yellow pigment in cultures of a certain strain of Mycobacterium tuberculosis when grown in the presence of high concentrations of p-aminobenzoic acid (PABA). He suggested that the pigment might possibly be related to factors of the vitamin B complex. Since the solubility properties of the pigment were similar to those of vitamins B_{10} and B_{11}[2] which promote feathering and growth in chicks, and since PABA can partially replace these vitamins in chick diets,[3] it was decided to feed a culture filtrate of the organism grown in the presence of PABA to chicks on a diet deficient in the above vitamins.

The chick ration used (493K) contains, per 100 g: dextrin 61 g, alcohol extracted casein 18 g, gelatin 10 g, salts V[2] 6 g, soybean oil 5 g, l-cystine 0.3 g, choline 0.15 g, inositol 0.1 g, biotin 20γ, thiamin 0.3 mg, pyridoxine 0.4 mg, riboflavin 0.6 mg, pantothenic acid 2 mg, nicotinic acid 5 mg, 2-methyl-1, 4-naphthoquinone 50γ, and alpha-tocopherol 0.3 mg. Vitamins A (1200 I. U.) and D (120 I. U.) were fed by dropper weekly. Water and the experimental ration were given *ad libitum*. On this ration chicks grew slowly and feathered poorly, as shown in Table I. The addition of 2% liver fraction L (solubilized liver) or the Super filtrol eluate of liver fraction L[4] provided for rapid growth and good feathering.

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1 Mayer, R. L., *Science*, 1943, **98**, 202.

2 Briggs, G. M., Jr., Luckey, T. D., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1943, **148**, 163.

3 Briggs, G. M. Jr., Luckey, T. D., Mills, R. C., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 7.

4 Hutchings, B. L., Bohonos, N., and Peterson, W. H., *J. Biol. Chem.*, 1941, **141**, 521.

TABLE I.
Growth Results with Chicks.

Supplement to Ration 493K	No. of chicks	Avg wt at 4 weeks g	Feathers 0 = very poor 100 = very good	"Folic acid"* supplied/100 g ration, γ
None	11	145	33	0
Super Filtrol eluate of liver fraction L ≅ 5%	12	260	98	50
Norite eluate of culture filtrate ≅ 55 cc/100 g	6	230	85	16
" " " " " (no PABA) ≅ 80 cc/100 g	6	196	45	4
Band No. 1 of norite eluate ≅ 80 cc/100 g	6	130	30	0.4
" " 2 " " " ≅ 80 cc/100 g	6	184	50	7
" " 3 " " " ≅ 80 cc/100 g	6	245	85	5
Dialysis filtrate of norite eluate ≅ 80 cc/100 g	5	234	80	12
" residue " " " ≅ 80 cc/100 g	6	188	40	2.5

*"Folic acid" is measured with *Streptococcus lactis*.[6]
Figures are based on a standard having a potency of 40,000.[7]

In the bacterial work we employed the same organism that Mayer used, Strain No. 607 of the American Type Culture Collection. It was grown on Long's medium[5] plus 1 g PABA per liter. Long's medium contains per liter: asparagine 5 g, ammonium citrate 5 g, K_2HPO_4 3 g, Na_2CO_3 3 g, NaCl 2 g, $MgSO_4$ 1 g, ferric ammonium citrate 0.05 g, and glycerol 50 g. It is made up to volume with water and adjusted to pH 7.

The procedure for preparing the norite eluate of the culture filtrate was as follows: 4 liters of the medium were divided among 12 one liter Erlenmeyer flasks, which were then plugged, sterilized, inoculated, and incubated at 37° C for 14 days. After a few days growth the medium began to turn yellow, and later became brown. The flasks were autoclaved and the contents filtered through a Super Cel pad on a Buchner funnel. The filtrate (3300 cc) was adjusted to pH 3 with H_2SO_4 and the precipitate filtered off and discarded. Twenty-five g of Norite A were then added, the mixture stirred for 75 minutes and the norite filtered off. The filtrate was discarded. The norite was treated with 200 cc of elution mixture (50 cc ethyl alcohol, 10 cc NH_4OH, and water to 100 cc) for 45 minutes, filtered, and the elution repeated once. The combined eluates were concentrated *in vacuo* to 100 cc and made to pH 4. They were then extracted 20 times in a separatory funnel with 50 cc portions of ethyl ether, to remove the remaining PABA, and the residue was made alkaline to litmus with NH_4OH.

When the norite eluate, prepared as described above, was fed to chicks at a level equivalent to 55 cc of the original filtrate per 100 g of ration, good growth and feathering resulted, approaching that obtained with the Super Filtrol eluate of liver.

In an attempt to further purify the active material, a portion of the norite eluate was made to pH 5 and passed through an adsorption column of Super Filtrol and Super Cel (2:5), 2.5 x 50 cm. After developing with distilled water, 3 distinct yellow-brown bands were obtained, which were separated, eluted with the elution mixture, concentrated, and fed at a level equivalent to 80 cc of original filtrate per 100 g of ration. The results in Table I show that most of the activity was in band No. 3, the lower and least strongly adsorbed band. This band supplied 50 mg of dry matter per 100 g of ration, as compared with 100 mg supplied by the Super Filtrol eluate.

Another portion of the norite eluate was dialyzed through a Cellophane tube (Visking No. 232), and it was found that most of the activity passed through the tubing, as has been found to be true with vitamins B_{10}, B_{11} and "folic acid" with this tubing.

5 Wells, H. G., and Long, E. R., *The Chemistry of Tuberculosis*, 2nd Ed., 1932.

6 Luckey, T. D., Briggs, G. M., Jr., and Elvehjem, C. A., *J. Biol. Chem.*, 1944, **152**, 157.

7 Mitchell, H. K., and Snell, E. E., *Univ. Texas Pub.*, 1941, **4137**, 36.

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 5γ 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 suggestion[3] 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 (B_{10} and B_{11}) 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 authors[1] 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-workers[4] that the potas-

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

[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.