TABLE II.
Comparison of "Folic Acid" Production of *Aerobacter aerogenes* as Influenced by the Presence of Various Pterins when Assays Were Performed with *Strept. lactis R* and *L. casei* ε

Exp.	Medium	Test material	Turbidity	"Folic acid" produced: *Strept. lactis* assay mγ/ml	"Folic acid" produced: *L. casei* assay mγ/ml	Biotin produced μγ/ml
1	C	None	321	9.2	16.8	15
		100γ xanthopterin	325	2.5	5.4	15
		100γ leucopterin	320	9.0	17.0	17
		100γ isoxanthopterin	321	9.2	17.6	15

sented, large amounts of the antimetabolite might be expected deleteriously to influence the growth of the organism or nearly completely to inhibit the formation of the metabolite. Obviously, an adequate explanation for the depressant effect of xanthopterin on the production of "folic acid" by *A. aerogenes* cannot be offered at this time. It is suggested, however, that these experiments supply additional evidence for relating the structure of xanthopterin to that of "folic acid." Available data on the empirical analysis of crystalline folic acids[7,19] appear to substantiate this suggestion.

Summary. When xanthopterin was added to cultures of *A. aerogenes* only small amounts of "folic acid" were synthesized by the organism. Xanthopterin was without effect on the resulting turbidity of the cultures or on the amount of biotin produced. Several other purines, pyrimidines, and pterins were without effect on the growth of the organism or on the production of "folic acid" or of biotin. It is suggested that xanthopterin either may partially replace "folic acid" in metabolism or its structural similarity to an hypothetical intermediate in the microbiological synthesis of "folic acid" enables it to inhibit the synthesis or utilization of the intermediate.

[19] Pfiffner, J. J., Binkley, S. B., Bloom, E. S., Brown, R. A., Bird, O. D., Emmett, A. D., Hogan, A. G., and O'Dell, B. L., *Science*, 1943, **97**, 404.

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Muscle Glycogen as Influenced by Castration, Adrenalectomy, and Treatment with Testosterone and Desoxycorticosterone Acetate.

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Schumann[1] has reported that castration of male rats and rabbits leads to a depletion of muscle glycogen. Testosterone propionate or desoxycorticosterone acetate were said to be effective in restoring the muscle glycogen in either castrated or adrenalectomized animals to a level even above that found in unoperated controls. Furthermore, injections of testosterone into normal rats led to muscle glycogen values higher than those in the controls. His results, if they should be confirmed, would constitute the first clear evidence for the participation of the testicular hormone in carbohydrate metabolism in skeletal muscle. Previous attempts to link the secretion of the testis with general metabolic processes have been, as Koch[2] has pointed out, inconclusive.

[1] Schumann, H., *Z. f. d. ges. exp. Med.*, 1938, **103**, 117; *Ibid.*, 1939, **105**, 577; *Pflüger's Arch. f. d. ges. Physiol.*, 1940, **243**, 686; *Ibid.*, 1940, **243**, 695; *Klin. Wchnschr.*, 1940, **1**, 364.

[2] Koch, F. C., *Physiol. Rev.*, 1937, **17**, 153.

TABLE I.

Operation	Treatment	Glycogen concentration: No. of animals	Glycogen concentration: Avg muscle glycogen, mg %	Glycogen concentration: Difference from controls, mg %	Seminal vesicle, mg per 100 g body wt	Max. tension: No. of animals	Max. tension: Avg g/g
None	None	29	578		385	13	1674
"	Testosterone	13	616	38 ± 23.7*	701	7	1806
"	Desoxycorticosterone acetate	5	546	32	392	9	1591
Castration	None	36	531	47 ± 19.7	7	17	1576
"	Testosterone	26	613	35 ± 25.0	483	15	1562
"	Desoxycorticosterone acetate	7	530	48	6	6	1493
Adrenalectomy	None	16	422	156 ± 17.7	362	8	1498
"	Testosterone	20	446	132 ± 18.4	857	8	1532
"	Desoxycorticosterone acetate	7	453	125	307	8	1549

* Standard error.

It was therefore thought worthwhile to repeat some of these experiments, especially in view of the following factors in Schumann's report: 1. The normal rat muscle glycogen values reported were unusually low, about 400 mg %. 2. The rats were killed by a blow on the head, a procedure which gives inconsistent tissue glycogen values.[3] 3. The conclusions were based on only 5 rabbits, and an unstated number of rats.

Procedure. Male rats of the Sprague-Dawley strain, on a constant diet of Purina dog chow, were divided into 9 groups as shown in Table I. The animals were not fasted, but were all sacrificed about midday. Castration was performed at an average age of about 40 days; the animals were sacrificed at about 100 days of age. Three dosage levels of testosterone were used: 1. subcutaneous implants of 5-mg pellets of methyl testosterone* 2 weeks before the muscle was analyzed; 2. subcutaneous injections of 0.5 mg of testosterone propionate in 0.1 cc of sesame oil daily for one week; 3. similar injections, 1 mg daily for 3 weeks. These amounts are in excess of those shown by Greene *et al.*[4] to be adequate for maintenance of the accessory genitalia in the castrated male rat. Since there was no difference in the muscle glycogen values with the 3 levels of testosterone dosage, they have been combined for the purposes of this report (Table I). At autopsy, the seminal vesicles were dissected out and weighed to gauge the adequacy of the testosterone treatment. Desoxycorticosterone acetate was administered to some of the animals, in the form of 5-mg subcutaneous pellets, 2 weeks before sacrifice.

Bilateral adrenalectomy was performed in one stage by the usual dorsolateral approach, and the animals sacrificed 5 days later. It was found that the untreated adrenalectomized animals had lost, on the average, 13.9% of their body weight in the 5 days. Those treated with testosterone lost 15.9%, while those treated with desoxycorticosterone acetate gained a little more than 1% in weight. Gaunt *et al.* have also reported testosterone to be non-beneficial for adrenalectomized ferrets[5] and rats.[6]

For glycogen analysis, the right gastrocnemius was dissected out under nembutal anesthesia, and immediately dropped into ice cold 30% potassium hydroxide. The glucose content of the acid hydrolysate was determined by the Folin-Wu procedure, with reading in a Klett-Summerson photoelectric colorimeter. In some of the animals, maximum tension developed by the left gastrocnemius was measured by a method previously described.[7]

Conclusions. Schumann's results are not

[3] Cori, C. F., *Physiol. Rev.*, 1931, **11**, 143.

* Methyl testosterone and desoxycorticosterone acetate were obtained through the courtesy of Dr. Irwin Schwenk, Schering Corporation.

[4] Greene, R. R., Burrill, M. W., Oppenheimer, E., and Nelson, D., *Endocrinology*, 1942, **30**, 734.

[5] Gaunt, R., and Hays, H. W., *Am. J. Physiol.*, 1938, **124**, 767.

[6] Gaunt, R., Nelson, W. O., and Loomis, E., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 319.

[7] Winter, C. A., and Knowlton, G. C., *Am. J. Physiol.*, 1940, **131**, 465.

confirmed. From the data in Table I, it appears that: 1. Castration did not significantly alter muscle glycogen concentration. 2. Neither testosterone nor desoxycorticosterone, in the dosages used, affected the concentration of glycogen in the gastrocnemius muscle of the intact or the castrated male rat. 3. Neither testosterone nor desoxycorticosterone restored to normal the low muscle glycogen levels found in adrenal insufficiency. This finding, with regard to testosterone, though contrary to that of Schumann, is in agreement with the report of Gaunt *et al.*[8] 4. None of the experimental procedures led to any marked change in the maximum tension developed by the muscle.

[8] Gaunt, R., Remington, J. W., and Edelmann, A., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 429.

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Effects of Different Intakes of B-Complex Vitamins upon Neuromuscular Regeneration.*

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The frequency with which reports have appeared concerning the occurrence of peripheral nerve degeneration in various vitamin B deficiencies prompted us to investigate the role of vitamin B-complex in neuromuscular regeneration. This was done by making a comparative study of the rates of regeneration in animals subsisting on different levels of vitamin B-complex.

Experimental. The experiments were carried out on the gastrocnemius muscles and tibial nerves of albino rats. The animals were matched as to age, initial body weight and sex. Complete denervation of the gastrocnemius muscle of one limb was produced by crushing the tibial nerve. The non-denervated muscle and nerve of the contralateral limb served as a control. At specified times after operation measurements were made concerning the strength and weight of the regenerating and control muscles. The muscle strength was determined by measuring the maximum isometric tension which developed in response to volleys of adequate stimuli applied directly to the muscle and to its motor nerve. The technics that were employed for denervation and strength measurements have been described in detail elsewhere.[1] The quantity of functional reinnervation was estimated from the ratios of tension responses to direct muscle stimulation and to motor nerve activation. The tension responses of control non-denervated muscles to nerve stimulation were of the same magnitude as those elicited by direct muscle activation; while in the case of muscles undergoing reinnervation and regeneration the former was only a fraction of the latter.

In our studies the following basal ration has been used: sucrose 74%, purified casein 18%, salt mixture 3% and corn oil 5%. Each animal received in addition 2 mg alpha-tocopherol and 2 drops of halibut liver oil per week. The "control" group received the basal diet supplemented with 8% yeast. The "B-deficient" group received the basal ration without yeast or other B supplements. The diet designated as "B-excess" was prepared by adding to the basal diet 8% yeast and sufficient choline, calcium pantothenate, thiamine, pyridoxine, riboflavin, and rice bran concentrate (Vitab) to provide a ten-fold increase in the calculated vitamin B complex content of the diet. In view of the fact that

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[1] Hines, H. M., Thomson, J. D., and Lazere, B., *Am. J. Physiol.*, 1942, **137**, 527.