CCLXXVIII. IRRADIATED ADENINE SULPHATE AS A SOURCE OF VITAMIN B₁ FOR GROWTH¹.

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(Received September 25th, 1933.)

EVER since the early findings of Suzuki *et al.* [1912] and of Drummond and Funk [1914] that nicotinic acid is a constituent of the curative fraction of rice polishings, suggestions have come forth associating the antineuritic factor with substances having purine or pyridine structures. The latter idea was accepted as a working basis by R. R. Williams [1916]. Williams, however, believed that only certain isomerides of the hydroxypyridines were effectual in curing polyneuritis in pigeons and, hence, developed his theory of the rôle of isomerism in the physiology of vitamins. This work was not corroborated by other investigators.

In a recent preliminary communication Tschesche [1933] has called attention to the similarity between adenine hydrochloride and the vitamin B₄ crystals isolated by Barnes *et al.* [1932]. Heard *et al.* [1933], while admitting that vitamin B₄ crystals consist largely of adenine, stated that the latter did not exhibit vitamin B₄ activity. Neither did irradiated adenine produce a vitamin B₄ response. Contrary to the experience of the latter workers, Guha and Chakrovarty [1932] have reported in a preliminary communication sent by cable from India, that vitamin B₁ can be synthesised photochemically by the ultra-violet irradiation of adenine sulphate.

In our recent investigations of the protective action of vitamin B₁ against the toxicity of thyroxine, highly concentrated extracts prepared by the author were employed, which were potent to the extent that 1 mg. daily per animal produced a growth of 3 g. weekly, which is equivalent to the modified Sherman unit [Sherman and Spohn, 1923]; and 3 to 5 mg. per rat daily produced a growth of 12 to 15 g. weekly, which is equivalent to the Chick and Roscoe unit [1929]. Since in our recent hyperthyroidism work [Sure and Smith, 1933] we have employed pure crystalline thyroxine, it was desirable to follow such studies with as concentrated and as pure a source of vitamin B₁ as is obtainable. Attempts were made, therefore, to secure vitamin B₁ by the procedure of Guha and Chakrovarty. Since no details of the technique were given, adenine sulphate² was irradiated for periods varying from 1 to 30 mins. These time limits had been found adequate for various sources of vitamin D. The irradiated adenine sulphate was dissolved in water and the daily dose administered volumetrically with a finely graduated pipette.

In view of the recent report of Kinnersley *et al.* [1933] that as little as 0.1 γ (0.0001 mg.) of pure vitamin B₁ should be a sufficient daily dose for pigeons, a smaller daily allowance than 0.1 mg. for the albino rat was not tried. The daily

¹ Research paper No. 318, Journal Series, University of Arkansas.

² A pure product purchased from Eastman Kodak Company, Rochester, N.Y.

Table I. *Irradiated adenine sulphate as a source of vitamin B₁ for growth.*

Change in body weight is expressed in g. (D)=died.

Animal number and sex	Period of experimen- tation (days)	Daily dosage (mg.)	Period of irradiation				
			1 min.	5 mins.	10 mins.	16 mins.	30 mins.
♀ 9649	13	0.1	—	—	-9(D)	—	—
♀ 9675	13	0.1	—	—	-8	—	—
	7	0.5	—	—	-4	—	—
♂ 9676	13	0.1	—	—	-5	—	—
	7	0.5	—	—	-2	—	—
	7	0.1	-7	—	—	—	—
	14	0.5	-8 (D)	—	—	—	—
♀ 9677	13	0.1	—	—	-11	—	—
	7	1.0	—	—	-11	—	—
♀ 9678	13	0.1	—	—	-6	—	—
	7	1.0	—	—	-8 (D)	—	—
♂ 9694	13	0.1	—	-4	—	—	—
	7	0.1	—	—	—	—	-15(D)
♀ 9695	13	0.1	—	-6	—	—	—
	7	0.1	—	—	—	—	-4
	7	0.1	-2	—	—	—	—
	7	0.5	-4(D)	—	—	—	—
♂ 9696	13	0.1	—	-8	—	—	—
	7	0.2	—	—	—	—	-8
♀ 9697	7	0.2	—	—	-4	—	—
	7	0.2	—	—	—	—	-3
	7	0.1	-1	—	—	—	—
	28	1.0	-9(D)	—	—	—	—
♀ 9698	7	0.2	—	—	-1	—	—
	40	0.5	—	—	—	—	-17(D)
♀ 9707	7	0.2	—	—	+1	—	—
	7	0.2	—	—	—	—	-2
	8	0.2	-4(D)	—	—	—	—
♂ 9711	7	0.2	—	—	—	—	-5(D)
♀ 9718	7	0.2	—	—	-3	—	—
	7	0.5	—	—	-9	—	—
♀ 9719	7	0.2	—	—	-1	—	—
	7	0.5	—	—	-9	—	—
♂ 9720	7	0.2	—	-2	—	—	—
	7	0.5	—	-4(D)	—	—	—
♂ 9721	7	0.2	—	-7(D)	—	—	—
♂ 9725	13	0.1	—	-5	—	—	—
	7	0.5	—	-10(D)	—	—	—
♀ 9726	6	0.1	—	-6	—	—	—
	7	0.1	—	—	—	-2	—
	15	0.5	—	—	—	-6	—
	7	0.5	—	—	—	—	-7(D)
♀ 9727	6	0.1	—	±0	—	—	—
	7	0.1	—	—	—	-6	—
	7	0.5	—	—	—	-10(D)	—
♂ 9728	6	0.1	—	-7	—	—	—
	7	0.1	—	—	—	-3(D)	—
♀ 9729	6	0.1	—	±0	—	—	—
	7	0.1	—	—	—	-2	—
	15	0.5	—	—	—	-5	—
	18	1.0	—	—	—	-5(D)	—

Table I (*cont.*).

Animal number and sex	Period of experimentation (days)	Daily dosage (mg.)	Period of irradiation				
			1 min.	5 mins.	10 mins.	16 mins.	30 mins.
♀ 9730	16	0.1	—	—	—	+2	—
	11	0.1	-6	—	—	—	—
	19	1.0	-8(D)	—	—	—	—
♂ 9731	13	0.1	—	—	—	±0	—
	3	0.1	—	—	—	—	-3
	9	0.1	-9(D)	—	—	—	—
♀ 9733	13	0.1	—	—	-5	—	—
	7	0.1	—	—	—	—	-3(D)
♀ 9734	13	0.1	-4(D)	—	—	—	—
♂ 9735	13	0.1	+4	—	—	—	—
	3	0.1	—	—	—	—	-4
	4	0.1	-5(D)	—	—	—	—
♂ 9736	13	0.1	—	—	—	+3	—
	3	0.1	—	—	—	—	-1
	11	0.1	-7	—	—	—	—
	7	0.5	—	—	—	—	-10(D)
♀ 9737	13	0.1	—	—	—	-3	—
	15	0.1	—	—	—	—	-6
	7	0.5	—	—	—	—	-5(D)
♂ 9738	13	0.1	—	—	-2	—	—
	15	0.2	—	—	—	—	-14
	24	0.5	—	—	—	—	-5(D)
♀ 9739	13	0.1	—	—	-7	—	—
	7	0.2	—	—	—	—	-1
	11	0.1	±0	—	—	—	—
	13	0.5	—	—	—	—	-10(D)
♂ 9740	13	0.1	—	-4	—	—	—
	7	0.5	—	—	—	—	-9(D)
♀ 9741	13	0.1	—	+5	—	—	—
	3	0.5	—	—	—	—	-2
	24	0.1	-17(D)	—	—	—	—

dose varied from 0.1 to 1 mg. A total of 32 animals was employed, 19 females and 13 males. The experimental period lasted between 3 and 52 days.

The dietary technique employed has been recently described [Sure, 1933] by the writer. First, the animals were transferred from our stock diet No. 1 [Sure, 1926] to a ration deficient in the vitamin B complex, which consists of caseinogen (purified)¹, 20; agar-agar, 2; McCollum's salts No. 185, 4; filtered butter fat, 10; and dextrin, 64. On this ration depletion of reserves of vitamins B₁ and B₂ was secured in two and generally in not more than three weeks. The rats were then transferred to our vitamin B₁-deficient ration of the following composition: caseinogen (purified)¹, 10; McCollum's salts No. 185, 4; autoclaved dried round steak², 15; filtered butter fat, 10; dextrin, 61. On such a dietary regime, supplemented with 1 mg. daily of Sure's vitamin B concentrate [1932], a gain of 3 g. per week per animal was obtained, and with 3 to 5 mg. daily of such concentrate, a weekly gain, satisfying the Chick and Roscoe [1929] unit, was secured; yet, as much as 1 mg. daily of irradiated adenine sulphate as a source of vitamin B₁ resulted in total failure of growth. The complete results are summarised in Table I.

¹ With acidulated water and extraction with 25 % ethyl alcohol in the cold [Sure, 1933].

² Heated for 6 hours at 20 pounds pressure and dried.

SUMMARY.

Pure adenine sulphate, irradiated for periods ranging from 1 to 30 mins. and fed in doses of 0.1 to 1 mg. per rat daily proved to be a complete failure as a source of vitamin B₁ for growth.

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