Biotin Deficiency and Cryptorchidism in Rats.* (19061)

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A form of cryptorchidism attributed to biotin deficiency was recently reported in rats (1). The reproductive anomaly was produced in 8 rats in 2 experiments involving a diet rich (66%) in spray-dried egg white, containing avidin, a protein which combines with biotin within the intestine in a non-absorbable complex(2). The cause of the cryptorchidism was suggested as due to the contracture of the cremaster muscle which completely surrounds the testicle in the rat. Biotin deficiency symptoms have been described in detail in the rat(3) and fowl(4), and the biochemical action of this vitamin in carboxylase and deaminase systems has been reported in bacteria and mammalian tissues(5,6). Although other vitamin deficiencies, for example, A and E, have been involved in male reproductive disorders, the claim that biotin deficiency may specifically cause testicular ascent is of unusual interest. In the absence of details pertaining to biotin therapy, contractility of the cremaster muscle, or growth and weight changes in the experimental animals, we have attempted to repeat and elaborate on this work.

Five experiments, involving 98 male weanling albino rats, were conducted to test the specific effect of biotin deficiency in causing cryptorchidism, as well as possible factors mediating or counteracting the reproductive disorder. Rats of the University of Massachusetts and the Carworth Farms strains were used in all tests. The animals were isolated in wire screen cages with screen bottoms and fed weighed quantities of food: 10 to 15 g/rat/day or as much as each animal would eat, with water supplied *ad libitum*. Litter mate controls were used where possible. Weanling weights, however, varied considerably, between 29 and 48 g. The experiments and conclusions are summarized in Table I.

Exp. I. Rats, placed on a commercially-prepared biotin-deficient diet‡ 8 days after weaning, continued to gain weight and appeared normal for a period of 7 weeks. Following this, growth was subnormal, and finally the characteristic, but not unique, symptoms of biotin deficiency developed, including scaly eyelids, desquamation of the skin, falling hair, arched back, and, within 3 months, the anticipated cryptorchidism. These symptoms did not appear in controls fed Purina Laboratory Chow and were remedied in those animals returned to chow after 3 months on the experimental ration. A characteristic deficiency symptom—a marked protrusion of the penis—in these and the semi-starved rats (Exp. V) appeared shortly before death.

Exp. II. Animals placed on the basal biotin-free diet immediately after weaning became cryptorchid within 20 to 25 days, but the marked severity of this ration was demonstrated by its lethal effect in allowing a survival period of 36 days (average) after weaning. The symptoms of biotin deficiency which developed were similar to, and masked by, symptoms of severe inanition and starvation. The addition of excessive egg white to the basal diet, at various times after the initiation

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‡ S-7 formula of Welsh and Wright(7) prepared by General Biochemicals, vitamin test casein 18, hydrogenated vegetable oil 10, corn oil 2, sucrose 59.9, salt mixture 4, non-nutritive fibre 4, vit. A, D and E concentrate .08, choline chloride .10 (g); thiamine hydrochloride .2, riboflavin .4, pyridoxine hydrochloride .2, nicotinic acid 4, calcium pantothenate 4.4, p-aminobenzoic acid 4, inositol 8 (mg).

7. Welsh, A. D., and Wright, L. D., *J. Nutrition,* 1943, v25, 555.

TABLE I. Summary of Biotin-Deficiency Diets, Responses, and Effects of Supplements Administered to Weanling Albino Rats.

Group		Diet	Supplement	Response
I A	(6)*	Biotin-dr‡ (S-7) beginning 8 d post weaning		C¶ within 3 mo
B	(3)	Laboratory Chow (control)		Descended testes
II A	(4)	Biotin-dr (S-7)		C (<25 d) dead in 36 d
B	(9)	dr + 20% egg white, beginning 27th day		C (20-25 d) dead in 38 d
C	(7)	dr + 20% egg white, beginning 6th day		C (20-25 d) dead in 36 d
D	(8)	dr + 20% egg white, beginning immediately		C (20-25 d) dead in 33 d
E	(4)	dr	Biotin† fed 100 μg/d	C
F	(4)	dr	" inj. 150 μg, 3x/wk	C
III A	(5)	Biotin-dd‖ (9-A) + 20% egg white		Descended testes
B	(5)	dd + 40% egg white		" "
C	(5)	dd + 20% egg white	Biotin inj. 150 μg, 3x/wk	Descended testes. Increase in body wt
IV A	(6)	dr (S-7)		4 C. 2 testes small (40 d)
B	(4)	dr	Testosterone§ inj. 500 μg, 2x/wk	C
C	(10)	dr	Chorionic gonadotrophin§ 50 units, 2x/wk	Descended testes
D	(4)	Laboratory Chow (control)		" "
E	(4)	" " "	"	" "
V A	(5)	" " (½ ration)		Descended testes after delay
B	(5)	" " "	"	Normal testes

* No. of animals.
† The biotin (free acid) was obtained from General Biochemicals, Inc.
‡ dr = deficient ration.
§ Testosterone propionate (Oreton) and chorionic gonadotrophin (Pranturon) contributed by the Schering Corp.
‖ dd = deficient diet.
¶ C = cryptorchid.

of the experiment, caused little change except perhaps to shorten the life span (II, B,C,D). Two groups of animals administered biotin in the food (II, E) or by intraperitoneal injection (II, F) throughout the experiment showed no improvement whatever in the cryptorchid condition. Biotin injected rats, however, lived longer than animals not receiving biotin (46 compared with 36 days on the average). Because of the immediacy of response to this severe dietary regimen and the lack of effective biotin therapy, the tentative conclusion was drawn that inanition rather than biotin deficiency *per se* was the cause of the cryptorchidism.

Exp. III. The severity of the dietary response elicited in the previous experiments suggested the use of a milder biotin-deficient ration. As several of the components of Manning's diet(1) were unavailable, we used a diet previously shown to be biotin-free in extensive experiments with mice(8). On this ration,§ begun at weaning, our rats did not develop cryptorchid symptoms (III, A). To assure further the requirements of a biotin-free diet, the egg white content was increased from 20% to 40%, without producing crypt-

8. Wilson, J. W., Leduc, E. H., and Winston, D. H., *J. Nutrition*, 1949, v38, 73.

orchidism (III, B). However, vitamin deficiency was indicated by the increase in body weight toward normal in those rats injected with biotin (III, C).

Exp. IV. The usual association of cryptorchidism with hypophyseal malfunction suggested that this gland might be involved, particularly in relation to the secretion of gonadotrophin. A decrease in pituitary stimulation, as opposed to a reduction in responsiveness of the tissues, might be expected to result from such a dietary deficiency. Ten animals were placed on the severe S-7 biotin-deficient diet at weaning and given chorionic gonadotrophin intraperitoneally (50 units twice weekly) beginning at weaning. In all 10 rats the testes descended within 10 to 12 days (IV, C). The positive response to gonadotrophin was temporary, however, and within a week the testes began to reascend. While the gonadotrophin thus appeared to counteract or mask the cryptorchidism, therapeutic attempts with testosterone propionate (500 μg twice weekly) proved ineffective (IV, B). Laboratory Chow controls showed the same testicular development (IV, D) as did rats fed chow while receiving chorionic gonadotrophin (IV, E).

Exp. V. The effect of slow starvation with a reduced quantity of complete diet was tested by feeding Laboratory Chow in amounts up to one-half the normal intake While not unequivocal, these results indicate that testicular development may be retarded in semi-starved rats, smaller gonads descending 1-2 weeks after those of controls and then with signs of reduced spermatogenesis (V, A,B). Supplements of gonadotrophin administered along with this semi-starvation diet partially remedied the incipient cryptorchidism.

Some rats from each experimental group, while under nembutal anesthesia, were investigated for contractility of the cremaster musculature. Isotonic contractions recorded kymographically from the tip, the body, or from an excised strip of cremaster muscle showed no characteristic differences in the control and experimental animals other than those due to size of the animals. Weak responses in rats which had undergone severe dietary restriction and inanition indicated that lesser, rather than greater, contractility of the cremaster muscle occurred.

These results are interpreted to indicate that the cryptorchidism is brought on by a type of pseudo-hypophysectomy resulting from general inanition and is not specifically induced by biotin deficiency(9). Biotin lack may intensify the condition, but is not the sole cause of the cryptorchidism, nor is biotin therapy effective in remedying the situation.

§ 9-A ration of Wilson *et al.*(8): vitamin test casein 10, egg white 20, corn starch 28.5, Crisco 20, corn oil (Mazola) 4, cod liver oil (Squibb) 2, powdered brewer's yeast 8, salt mixture U.S.P. XII_2 7, Sulfaguanidine .5 (g) Egg white supplied by Henningsen Lamesa, Inc.; sulfaguanidine contributed by Lederle Laboratories.

9. Mulinos, M. G., and Pomerantz, L., *J. Nutrition,* 1940, v19, 493.

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An Attempt to Transmit Rheumatoid Arthritis to Humans. (19062)

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The etiology of rheumatoid arthritis remains an enigma. The suppressive effects of cortisone on the manifestations of the disease throw no light on its primary cause. Because of this, and because of the many features of the disease which suggest an infectious etiology, such as fever, leucocytosis, increased sedimentation rate, weight loss and the signs of joint inflammation, a return to the search for infection seems indicated. This search however, must be at the level of the ultramicroscopic organisms since many years of intensive research have excluded a bacterial cause. The recent isolation of pleuropneumonia-like organisms in Reiter's Syndrome, which closely resembles rheumatoid arthritis