bilateral nephrectomy, nor was there any increase in brain chloride concentration. (3) After nephrectomy, the serum chloride of rats falls markedly.

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Utilization of d-Biotinol by Microorganisms, the Rat and Human.* (19079)

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Following the synthesis of d-biotinol, the alcohol analog of d-biotin, by Sternbach, Kaiser and Goldberg(1), it was of interest to compare the biological activity of this derivative to that of biotin. Alcohol analogs of other vitamins are oxidized readily to the corresponding vitamin by animal organisms as shown by urinary excretion studies. For example, d-panthenol, the analog of d-pantothenic acid, and 3-pyridinemethanol, the analog of nicotinic acid, are converted efficiently by humans(2,3); panthenol has been shown to replace pantothenic acid in the nutrition of the rat(4,5). The experiments to be described concern the activity of biotinol for growth of microorganisms and the rat and include determinations of the excretion of biotin after administration of biotinol to the rat and human.

Experimental. (1) *Microbiological Activity.* The biotin activity of several biotinol preparations was determined for *Lactobacillus arabinosus*(6), *Lactobacillus casei* (7), and *Saccharomyces cerevisiae*(8), the organisms commonly used for assay of biotin. Each lot tested showed the same activity for all three organisms, but the activity varied from lot to lot, ranging from 0.2 to 2%. This variability suggested a probable contamination with small amounts of biotin. Separation of this biotin was achieved by passage of solutions containing 1 mg of biotinol per ml through a column of Amberlite IRA-400 as described by Weiss *et al.* for panthenol(9). Microbiological assays of the column eluates showed the biotin activity of biotinol *per se* to be less than 0.05%. This inactivity is analogous to the failure of panthenol to replace pantothenate in the nutrition of *Lactobacillus arabinosus and Saccharomyces carlsbergensis* (2). Recovery tests with 2% and 20% biotin added to the biotinol solution indicated complete removal of the added biotin by the resin. Quantitative recovery of the biotinol in the column eluate was demonstrated by rat excretion assays in which equal urinary excretions of biotin were found after oral dosage of resin-treated and untreated biotinol solutions.

(2) *Rat curative assay.* Biotinol was compared to biotin as regards activity in curing egg white-induced biotin deficiency. The 30% egg white diet, vitamin supplement and test conditions were the same as those reported by Rubin, Drekter and Moyer(10).

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TABLE I. Wt Gain of Biotin-Deficient Rats Fed d-Biotin or d-Biotinol.

Supplement	Dose (fed 3 times weekly), μg	No. of rats	Avg gain in 4 wk, g ±S.E.
None	—	3*	3 —
Biotin	.80	5	44 ± 4.3
Biotinol	.75	5	41 ± 7.3
Biotin	1.60	5	62 ± 6.2
Biotinol	1.50	5	85 ± 5.6

* 1 rat died.

Weight gains in the 4-week assay period are given in Table I and show biotinol to be as effective as biotin in promoting growth of these biotin-deficient rats. The fact that the response is greater with biotinol than with biotin at the 1.6 mcg level is not considered significant in view of the small number of rats involved and the individual variations found. Accompanying the weight gains of the test animals was a disappearance of the dermatitis and alopecia with both biotinol and biotin.

(3) *Rat Excretion Studies.* (a) *Basal Excretions.* Basal urinary and fecal excretions of biotin by 200-300 g rats on a Friskie dog chow diet were determined by assay with *L. arabinosus*(6). The average urinary excretion was 1.3 μg per day ± S.D. 0.45. Direct assays of the feces averaged 4.5 μg per day, but after autoclaving with sulfuric acid for 2 hours at 15 lb, the fecal biotin assay increased to 10 μg per day. No difference was found when 1, 2 or 4 N sulfuric acid was used for this hydrolysis.

(b) *Urinary Excretions after Low Doses* (Table II). After oral dosage by catheter of 50 μg or less of biotin or equivalent amounts of biotinol, 25 to 38% of the dose is excreted as biotin in the urine in 24 hours. The lack of significant difference between biotinol and biotin at the 3 levels fed indicates an efficient oxidation of biotinol to biotin by the rat.

After intramuscular injection of 50 and 158 μg of biotin or equivalent amounts of biotinol, the urinary excretion of biotin is greater after biotin than after biotinol (Table II), but at the 225 μg level there is no difference. Since only 4 rats were tested in a group at the 2 lower levels and the urines pooled for assay, the significance of the difference after biotin and biotinol cannot be evaluated. The equivalent excretions at the 225 μg level where more rats were tested indicates an efficient conversion of biotinol to biotin after intramuscular injection.

(c) *Urinary and Fecal Excretion after High Doses* (Table II). After oral dosage of 3.4 and 10 mg, a much higher percentage of the dose appears in the urine after biotinol than after biotin. In the latter case the fecal excretion of biotin is considerably greater than after biotinol so that the total of urinary and fecal excretions is only slightly lower after biotin. This difference in excretion may well arise from the fact that the solubility of biotinol in water is at least 5 times as great as that of biotin. This effect is not shown after intramuscular injection where the urinary excretion of the biotin is higher than after oral dosage, and of the same order of magnitude as after intramuscular biotinol.

(4) *Human Excretion Studies.* A preliminary investigation was made of the extra urinary biotin excretion after oral or intramuscular administration of biotin and biotinol. The subject was a 33 months old male whose urinary biotin excretion had returned close to the basal level of 4 μg per day after previous intake of biotin. One mg of biotin or biotinol was given daily for 2 days followed by 2 days without supplementation. In the first 2 test periods biotinol was given orally and then intramuscularly, and in the next 2 test periods the same procedure was followed with biotin. After oral administration, 20% and 48% of the two biotin doses and 51% and 59% of the biotinol doses were excreted in the urine as biotin in 24 hours as determined by *L. arabinosus* assay. After intramuscular injection, 54% and 87% were excreted in the 24-hour urines after biotin and 54% and 47% after biotinol. Although the limited nature of the data and the fluctuations in excretions preclude a quantitative estimation of the relative utilization of biotin and biotinol, it is evident that biotinol is converted rather efficiently to biotin by the human after both oral and intramuscular administration.

Summary. d-Biotinol does not replace d-biotin in the nutrition of *L. arabinosus, L. casei,* or *S. cerevisiae* but is fully as effective

TABLE II. Urinary and Fecal Excretion of Biotin After Administration of Biotin or Biotinol to Rats.

Dose (as d-biotin)	No. of rats		Extra excretion in 24 hr: d-Biotin		d-Biotinol	
				% of dose		% of dose
		Oral				
μg			μg		μg	
10	4	Urine	2.9	29	3.7	37
25	8	"	6.3	25	6.8	27
50	16–18	"	19	38	17	34
mg			mg			
3.4	2	"	1.6	47	3	88
		Feces	1.4	41	.14	4
10	6	Urine	2.2	22	7.2	72
		Feces	5	50	1.1	11
		Intramuscular				
μg			μg			
50	4	Urine	22	44	11.4	23
158	4	"	83	53	62	39
225	12–14	"	112	50	113	50
mg			mg			
5	4	"	3.5	70	2.8	56

as d-biotin in curing egg white-induced biotin deficiency in the rat. Comparison of urinary excretions of biotin after oral or intramuscular administration of biotin and biotinol shows the latter to be converted to biotin efficiently by both rat and human.

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Absence of Analgesia During Induced Hyperadrenalism.* (19080)

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Selye first ascribed anesthetic properties to hormonally active steroids(1-3) and others (4,5) have suggested that cortisone and ACTH produce analgesia if not anesthesia. Thus, we examined the threshold for pain in patients with rheumatoid arthritis before and during therapy with cortisone and ACTH.

There are probably two major types of superficial or cutaneous pain(6), both of which we have attempted to evaluate. Two categories of visceral pain have also been described, the so-called "true visceral pain," a diffuse poorly localized sensation, and

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