TABLE I.
Effect of Female Sex Hormones on Population Increase of *Trichomonas vaginalis.*

Hormone	Gammas per 10 cc of culture medium	Avg population per mm³ Day examined 1	2	3	4	5	6
Estradiol-3-benzoate	2.100	29	66	132	221	287	282
	0.210	30	63	119	198	279	272
	0.021	29	76	131	214	271	279
Control		26	67	125	203	276	273
Estradiol	21.000	38	94	190	251	327	322
	2.100	42	58	219	305	329	318
	0.210	33	97	199	306	329	326
Control		36	100	219	311	342	320
Theelol	21.000	36	79	165	231	327	315
	2.100	36	90	181	226	321	318
	0.210	34	85	170	228	324	314
Control		36	100	219	311	342	320

ments the female sex hormones here employed have no effect upon increase in population of bacteria-free cultures of *Trichomonas vaginalis.*

13376 P

Formation of an Antirachitic Product from Cholesteryl Sulfates.

ALBERT E. SOBEL AND SIDNEY P. GOTTFRIED. (Introduced by B. Kramer.)

From the Pediatric Research Laboratory, Jewish Hospital of Brooklyn.

This communication deals with a report of some of the conditions under which an antirachitically active product is obtained from some of the new cholesteryl sulfates recently prepared.[1]

Eck and Thomas reported the formation of an antirachitic product on heating ammonium or potassium cholesteryl sulfate at 220°-250° for 30 minutes. The yields were low, approximately 0.09 I.U. per mg of the ether extract of the heated product.[2] At first the conditions of Eck and Thomas were repeated with slight modifications using the newly prepared barium, pyridonium, silver, as well as the

1 Sobel, A. E., and Spoerri, P. E., *J. Am. Chem. Soc.*, 1941, **63**, 1259.
2 Eck, J. C., and Thomas, B. H., *J. Biol. Chem.*, 1937, **119**, 631.

previously known potassium cholesteryl sulfate.[3, 4] The results are presented in Table I. The yield of antirachitically active product (in terms of international rat units) was much higher in the case of the barium, pyridonium, and silver salts than in the case of the potassium salt. Our potassium salt gave a lower activity than that reported by Eck and Thomas, but of the same order of magnitude. When any of the salts were heated at their respective decomposition temperatures, no antirachitically active products were obtained.

In the next experiment the salts were suspended in tetralin and oxygen bubbled through. Under the conditions stated in Table I, the barium cholesteryl sulfate gave an antirachitically active product. All the other salts were negative in the amounts tested. When air was substituted for oxygen, none of the salts yielded an active fraction.

TABLE I.

Cholesteryl sulfate	No. of rats	Conditions	I.U. of vitamin D per mg of petroleum ether soluble fraction
Barium, lead, silver, potassium	2	Heated in air at their respective decomposition temp. 10 to 15 min.	Negative (fed 70 to 80 mg 10 days)
Barium	5	Heated in air at 250° 10 to 15 min.	.50 per mg cholesterol in starting material
Pyridonium	7	"	1.20
Silver	6	"	0.99
Potassium	5	"	.02 per mg cholesterol in starting material
Barium	5	Heated in tetralin at 200° 15 to 30 min. while bubbling through oxygen	1.11
Pyridonium, lead, silver, potassium,	2	"	Negative (fed 6 to 12 mg 5 days)
Pyridonium,	6	Heated in tetralin at 200° 15 to 30 min. but bubbled through air	Negative (fed 16 to 32 mg 5 days)
barium, silver, potassium, lead	2		
Barium	5	Heated in an evacuated tube at 250° 15 min.	1.6
Silver	5	"	2.8
Pyridonium	2	"	Negative (fed 23 mg 5 days)
Potassium	4	"	Negative (fed 50 to 200 mg 10 days)

[3] Mandel, I. A., and Neuberg, C., *Biochem. Z.*, 1915, **71**, 186.

[4] Natelson, S., Sobel, A. E., and Kramer, B., *J. Biol. Chem.*, 1934, **105**, 761.

Since we desired to study further the effect of oxygen on antirachitic activation, the barium, pyridonium, and silver salts were heated at 250° in an evacuated tube. The results are tabulated in Table I. Both the barium and silver salts gave a positive result. The pyridonium and potassium salts were inactive on repeated attempts.

It thus appears that oxygen is necessary for the antirachitic activation of the potassium and pyridonium salts but not for the barium and silver salts. It also appears that the nature of the cation plays an important rôle in the antirachitic activation under a given set of conditions.

Experimental. The cholesteryl sulfates employed were prepared according to the procedure of Sobel and Spoerri.[1] When the salts were heated in the dry state, the residue was extracted with petroleum ether (B.P. 35°-60°). When the salts were heated in tetralin, the latter was first evaporated under vacuum, keeping the temperature under 125°, and the residue extracted with petroleum ether. In both cases, the petroleum ether soluble residue was dissolved in 10 cc of Vitamin D-free maize oil and fed to the rachitic rats.

The method of bioassay was similar to that by Natelson and Sobel.[5] By means of a pipette, 0.1 cc daily feedings of maize oil solutions of the petroleum ether extract were carried out for 5 days. At the same time pure maize oil and maize oil solutions containing 3 units of Vitamin D in 0.1 cc were fed daily to several control rats. The rachitic rats receiving pure maize oil showed severe rickets with no healing. The rats that were fed a total of 15 International Units of Vitamin D in 5 days showed a typical 2 plus healing. The conversion of healing more or less than 2 plus in terms of 2 plus healing was accomplished according to the procedure of Bills, *et al.*[6] In other studies carried out in this laboratory, we have found the method of Bills to be quite reliable.

In preliminary bioassays which preceded the assays reported in the table, X-rays of the rats were taken at the end of 5, 7, and 10 days in order to obtain the approximate range for a more exact bioassay. During this period daily feedings of 0.1 cc of maize containing the petroleum ether extract took place.

---

[5] Natelson, S., and Sobel, A. E., *J. Biol. Chem.*, 1935, **109**, 687.

[6] Bills, C. E., Honeywell, E. M., Wirick, A. M., and Nussmeier, M., *J. Biol. Chem.*, 1931, **90**, 619.