TABLE I. Days Survival.

Treatment	Day of death 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Group avg
Control	1	5	1											3	5.6
Benadryl	2	4				1								3	5.8
Pregnenolone	1	2	3	1	1									2	5.1
Allopregnane	1	2	1	2	1			1						2	5.7
Cortisone	2	2	1		1	2								2	5.4

suggests the possibility that the observed proteolysis in peritonitis is not produced in a manner analogous to the production of fibrinolysin by specific antigens.

Conclusion. Benadryl, an antihistamine and three steroids, cortone, allopregnane and pregnenalone do not affect the course of experimental appendical peritonitis in dogs.

Received January 16, 1951. P.S.E.B.M., 1951, v76.

Effects of Methoxinine in the Mouse.* (18536)

JOHN J. TRAVERS AND LEOPOLD R. CERECEDO.

From the Department of Biochemistry, Fordham University, New York.

Roblin *et al.*(1) showed methoxinine to be a bacteriostatic agent for *E. coli* and *Staph. aureus*, and the growth inhibition caused by it to be reversed by L-, but not by D-, methionine. Shaffer and Critchfield(2) reported the alleviation, by methionine, of the toxic effects of methoxinine in rats. These latter investigators claimed that methoxinine exerted lipotropic activity, but they failed to take into consideration the effect of the caloric restriction imposed by the methoxinine. Best(3) warns that "another pitfall . . . is that of the 'pseudolipotropic' effect of a low caloric intake." In connection with other studies being carried out in this laboratory, it was of interest to study the effects of methoxinine in mice. Some of the data seemed to clarify the "apparent" lipotropic activity of methoxinine, and are reported here.

The basal diet used in these studies had the composition shown in Table I. All additions were made at the expense of sucrose. In the first experiment, young adult male mice of the Swiss strain, in groups of 4 each, were treated as shown in Table II. Male mice of the C3H strain, 5 to 6 weeks of age, in groups of 5 each,

TABLE I. Diet Composition (per kg of diet).

	g
Soy protein*	100
L-Cystine	2.5
Salts†	50
Ruffex	20
Hydrogenated vegetable oil‡	100
Lard	50
Sucrose	677.5
	mg
Thiamine	20
Riboflavin	20
Pyridoxin	20
Calcium pantothenate	40
α-Tocopherol	40
	units
Vitamin A§	67500
Vitamin D‖	5000

* Alpha-protein, The Glidden Co., Chicago, Ill.
† Hawk-Oser salt mixture.
‡ Crisco.
§ Vit. A concentrate, Nopco Chemical Co., Harrison, N. J.
‖ Drisdol, Winthrop-Stearns, New York.

* This investigation was supported (in part) by a research grant from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

1. Roblin, R. O., Jr., Lampen, J. O., English, J. P., Cole, Q. P., and Vaughan, J. R., Jr., *J. Am. Chem. Soc.*, 1945, v67, 290.

2. Shaffer, C. B., and Critchfield, F. H., *J. Biol. Chem.*, 1948, v174, 489.

3. Best, C. H., *Fed. Proc.*, 1950, v9, 506.

TABLE II. Effect of Various Supplements on Weight Change and Liver Lipids of Young Adult Swiss Male Mice.

Supplement, %	Initial wt, g	Wt change, g	Total food intake, g	Liver wt, g	Liver lipids % fresh wt
—	37.5	1.6 ± 1.45*	97.2	2.95 ± 0.11*	21.0 ± 4.13*
.5 choline	37.0	1.0 ± 1.82	87.5	1.82 ± 0.26	6.4 ± 0.56
.25 methionine	36.3	—0.8 ± 1.75	87.0	2.16 ± 0.19	9.2 ± 1.34
.25 methionine + .25 methoxinine	36.3	—0.8 ± 1.36	77.1	2.24 ± 0.07	8.6 ± 1.21
.25 methoxinine	37.5	—7.8 ± 2.57	58.0	2.25 ± 0.15	7.4 ± 1.74
Basal (restricted)	37.3	—8.6 ± 3.03	59.2	1.73 ± 0.17	7.2 ± 1.10

* All values are means ± standard errors. S.E. = $\sqrt{\frac{\Sigma d^2}{n\,(n-1)}}$.

TABLE III. Effect of Various Supplements on Weight Change and Liver Lipids of Young C3H Male Mice.

Supplement, %	Initial wt, g	Wt change, g	Total food intake, g	Liver wt, g	Liver lipids % fresh wt
—	18.9	—1.3 ± 1.50*	60.6	1.78 ± 0.22*	30.8 ± 2.99*
.5 choline	18.8	2.8 ± 0.51	65.4	1.32 ± 0.04	8.2 ± 0.28
.25 methionine	18.3	2.6 ± 1.41	63.3	1.50 ± 0.16	12.9 ± 1.49
.25 methionine + .25 methoxinine	19.3	—5.0 ± 0.76	41.3	1.08 ± 0.07	7.0 ± 0.50
.25 methoxinine	19.1	—7.2 ± 0.57	28.1	0.90 ± 0.07	5.3 ± 1.45
Basal (restricted)	19.1	—7.6 ± 0.41	30.6	0.74 ± 0.18	4.7 ± 0.75
.5 methoxinine	Survival 11.6 days (4-17 days)				

* All values are means ± standard errors. S.E. = $\sqrt{\frac{\Sigma d^2}{n\,(n-1)}}$.

were similarly treated (Table III), with an additional group receiving a higher level of methoxinine. With the exception of one group in each experiment which was restricted, on the basal diet, to the food intake of the methoxinine treated animals, the food was administered *ad libitum*. Body weights and food consumption were recorded daily. After 21 days the animals were sacrificed, and the livers removed and weighed immediately. The liver lipids were determined gravimetrically, in the first experiment via chloroform extraction, and in the second experiment by extraction with a 1:1 ethanol:ether mixture.

Results. From Tables II and III it can be seen that methoxinine is toxic for mice, and that its toxic effects are alleviated by the simultaneous administration of methionine. Each of the treatments caused a lowering of liver lipids; with methionine and choline, this has been accomplished without any effect on food intake. When methoxinine was added to the diet along with methionine, there resulted a lower liver fat value, and a reduced food consumption. When the basal diet was restricted, in amount comparable to that consumed by the animals on the methoxinine supplement, there resulted a weight loss and a liver lipid value comparable to those values obtained in the methoxinine treated mice. Calculated on the basis of average body weight during the experimental period, the methoxinine intake of the mice in each of the groups receiving only the methoxinine supplement at 0.25% of the diet was of the same order, that is, 20-22 mg per 100 g of body weight per day. When methoxinine was incorporated in the diet at the 0.5% level, the survival period was short. On an intake of 45 mg per 100 g of body weight per day, one mouse survived only 4 days; on an intake of 30-32 mg per 100 g of body weight per day, the survival times of three mice were 13, 14, and 17 days.

Discussion. Neglecting the differences between the diets used, the toxicity of methoxin-

ine is of the same order in both mice and rats. Since the same general picture was obtained in both experiments with mice, it seems reasonable to assume that the effect of the methoxinine is to reduce the caloric intake, which restriction alone is capable of reducing the liver lipids to a level as low as the values obtained with choline or methionine. In the Swiss animals, an equivalent quantity of methionine offsets the effect of methoxinine. In the C3H mice, more methionine seems to be required. This is in line with our observations(4) that the C3H mice do not respond as well as Swiss mice to diets that are low in methionine, suggesting a higher requirement of the former for methionine (or protein). The basal diet, while affecting only the liver in the mouse, produced the complete choline deficiency syndrome in the weanling rat, that is, fatty liver, hemorrhagic kidneys, and intraocular hemorrhages. Littermate rats, which received methoxinine, had normal kidneys and eyes on gross examination, even though the large yellow livers indicated higher liver lipid values than were seen in the animals on the basal diet. When other littermates were fed methionine, in addition to methoxinine, the livers appeared nearer to normal.

Conclusions. 1. In mice, methoxinine causes a reduction in the food consumption and a weight loss. 2. The toxic effects of methoxinine are alleviated by methionine. 3. Methoxinine is not lipotropic in mice, since the caloric restriction imposed by its toxicity can completely account for its "apparent" lipotropic activity.

4. Travers, J. J., and Cerecedo, L. R., unpublished data.

Received February 1, 1951. P.S.E.B.M., 1951, v76.

Effect of Locally Applied Vitamin A and Estrogen on Rat Epidermis.* (18537)

JOSEPH D. SABELLA, HOWARD A. BERN, AND RAYMOND H. KAHN.
(Introduced by Richard M. Eakin)
From the Department of Zoology, University of California, Berkeley.

Recent observations on the ability of intravaginally instilled vitamin A to partially counteract vaginal cornification induced by estrogen(1) have led us to reconsider the implication of McCullough and Dalldorf's suggestion(2) that local vitamin A deficiency is responsible for all epithelial keratinization (including that accompanying metaplasia). In particular, we were interested in investigating the possible role of local vitamin A inadequacy in normal epidermal keratinization and any interrelation between vitamin A and estrogen in this process. A relation between vitamin A and excessive epidermal keratinization has been established by observations on the syndrome of follicular hyperkeratosis accompanying vit A deficiency in man(3-5) and in the rat(6). In addition, a beneficial effect on wound healing has been reported both for vit A and for cod liver oil(7-10). However, more recently Brush

* Aided by a research grant from the University of California Board of Research. We are greatly indebted to Schering Corporation for the estradiol benzoate and to Dr. Philip L. Harris of Distillation Products Industries for the vitamin A alcohol used in this study. Thanks are also due Mr. Robert S. Levy for certain technical assistance and Mr. Victor Duran for the photomicrography.

1. Kahn, R. H., and Bern, H. A., *Science,* 1950, v111, 516.

2. McCullough, K., and Dalldorf, G., *Arch. Path.,* 1937, v24, 486.

3. Lowenthal, L. J. A., *Arch. Derm. Syph.,* 1933, v28, 700.

4. Frazier, C. N., and Hu, C.-K., *Arch. Derm. Syph.,* 1936, v33, 825.

5. Frazier, C. N., Hu, C.-K., and Chu, F.-T., *Arch. Derm. Syph.,* 1943, v48, 1.

6. Molt, F. H., *Arch. Derm. Syph.,* 1943, v47, 768.