cedures were followed throughout except for a group of 10 animals on 100 γ of estrone, where it was necessary to use 0.1 cc of oil owing to the relative insolubility of the material.

The intrasplenic unit is defined as that amount of estrogen required to produce positive smears in 50% of at least 10 rats treated as described. On the basis of this standard procedure the intrasplenic unit of alpha-estradiol* has been 50 γ, estrone about 70 γ, estradiol benzoate 24 γ, and estradiol dipropionate 5 γ. This study is being continued on these estrogens as well as stilboestrol, estriol, estradiol benzoate-butyrate, and estrone benzoate under various conditions.

These preliminary results lead to the following possible conclusions: (1) A large proportion (about 98%) of intrasplenically injected free estrogen is destroyed either in the liver or spleen. (2) The point of attack on estradiol is probably at the hydroxyl groups since partial protection of this estrogen is afforded by covering one hydroxyl group and greater protection by covering both hydroxyl groups. (3) The esters of estradiol are at least partially absorbed from the spleen as esters since the esterification afforded protection from destruction.

13211

Ineffectiveness of Thiamin Hydrochloride in Treating Late Weaning Paralysis of Vitamin E Deficient Rats.

ROGER TERRY. (Introduced by Karl E. Mason.)

From the Department of Anatomy, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

It has recently been reported by Holmes and Pigott[1] that orally administered thiamin hydrochloride is effective in curing the neuromuscular disorder which appears during late lactation in the offspring of rats receiving a restricted intake of vitamin E. These observations were based upon 3 litters in which paralysis appeared between

* We would like to thank: Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products for supplies of alpha-estradiol (Ovocylin) and estradiol dipropionate (Di-ovocylin), Dr. Erwin Schwenk of the Schering Corporation for quantities of estradiol benzoate (Progynon-B), and Dr. Oliver Kamm of Parke, Davis and Company for estrone (Theelin).

[1] Holmes, A. D., and Pigott, M. G., *Am. J. Physiol.*, 1941, **132**, 211.

the 20th and 22nd days of lactation. Ten offspring died from dystrophy or other causes before the remaining 10 afflicted rats were given vitamin B_1 treatment, supplemented by oral feeding of the E-deficient diet mixed with distilled water. Amounts of thiamin hydrochloride varying from 12 to 43 γ were said to have effected cures in the latter 10 rats. Since a certain number of such paralyzed rats can be expected to recover spontaneously[2] (a phenomenon noted by Holmes and Pigott in 3 other litters prior to the institution of B_1 therapy), it seemed desirable to investigate more fully the value of thiamin hydrochloride in the prevention and in the cure of this paralysis in suckling rats.

For this purpose 10 litters of paralyzed rats were used. The mothers of these litters, reared from the 14th day of life on a vitamin E-deficient diet (casein, 20; starch, 50; lard, 18; salts, 2.5; yeast, 7.5; cod liver oil, 2), were used in bioassay tests during their first pregnancy. A preliminary test was carried out on the litter of one rat given a low assay dose of rat liver. The 8 offspring showed paralysis at the 21st day of life, at which time daily administration of 0.5 mg of thiamin orally and 0.5 mg intraperitoneally was begun. Only one of the litter recovered, the others dying within 3 or 4 days.

The conditions employed for the production of the paralysis in the other 9 litters are summarized in Table I. It will be noted that the level of vitamin E dosage giving a positive response in 70% of assay tests permitted no lactation (group C), while 2 and 4 times this level resulted in satisfactory lactation (groups B and A). However, paralysis appeared in most of the offspring between the 17th and 20th days of life, which is somewhat earlier than in the litters studied by Holmes and Pigott. Approximately one-half of the members of each litter received 0.5 mg (1/20th cc of a 10 mg/cc solution) of thiamin hydrochloride orally, fed daily for a period of 4 to 5 days, the remaining members serving as untreated controls. When rats of either group appeared weak or paralyzed, they received a mixture of the E-deficient diet mixed with distilled water and fed by pipette at frequent intervals during each day and evening. The procedure was essentially the same as that used in the studies of Holmes and Pigott except for the fact that the amount of thiamin administered was greatly in excess of that found by them to be effective in curing paralysis. There is no reason to believe that the difference in the level of thiamin dosage in the two studies is of significance.

The first series of observations were made on the litters of group

[2] Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 273.

TABLE I.
Source and Lactation Record of Paralyzed Litters.

Group	Assay dose vit. E conc. mg	No. of assay tests	Positive responses, %	Litters allowed to suckle	Lactation record: Total No. at birth	Lactation record: Died before onset of paralysis	Lactation record: Age at onset of paralysis, days	Lactation record: Age treatment begun, days
A	7.5	10	100	5	44	3	18-20	18-20
B	3.75	10	100	4	34	7	17-18	15
C	1.875	20	70	6	46	46*	—	—
D	0.937	10	0	—	—	—	—	—

*Died 2 to 4 days after birth.

A, in which treatment was initiated as soon as hyperflexion of the forehand or other early evidence of paralysis appeared. It will be noted (Table II) that the proportion of survivors was essentially the same in the treated and the untreated animals.

It is generally recognized that the earlier in lactation the neuromuscular disorder appears, the more severe are its symptoms and the lower is the incidence of spontaneous recovery. Since it was evident that the litters of group B would show earlier and more severe paralysis than those of group A, the treatment described above was begun 2 to 3 days before the first signs of paralysis appeared in any of the members (Table I). Paralysis was very marked and the frequency of recovery somewhat less than in the litters of group A. However, as in the case of the latter group (Table II), the proportion of recoveries was not significantly different in the treated and the untreated animals. Although the pipette feeding undoubtedly prolonged the survival time and facilitated recovery of the afflicted rats, the results give no indication that vitamin B_1 therapy alone exerted any beneficial effect.

TABLE II.
Deaths and Survivals in Thiamin-treated and in Untreated Paralyzed Rats (9 Litters).

Group	No. of offspring	Killed*	Experimental animals: Total	Experimental animals: Died	Experimental animals: Recovered
Treated					
A	22	2	20	8	12
B	14	0	14	6	8
A and B	36	2	34	14	20
Untreated					
A	19	10	9	3	6
B	13	5	8	4	4
A and B	32	14	17	7	10

*These animals were sacrificed for metabolic studies after moderate or advanced paralysis had appeared.

The E-deficient diet used in the studies of Holmes and Pigott, and in those reported here, should afford an ample supply of vitamin B_1 for the mother and offspring. It is also of interest to note that this neuromuscular disorder first came to the attention of Evans and Burr[2] when they had put into effect their observations that the vitamin B requirements of the rat are greatly increased during lactation. In fact, with increasingly larger proportions of yeast in their diets there was a corresponding increment in the number of young reared, in the average weight at weaning, and in the number of rats showing paralysis.

Conclusions. It is concluded that, contrary to the claims of other investigators,[1] oral administration of vitamin B_1 (thiamin hydrochloride) supplemented by force feeding is ineffective in preventing or in curing the paralysis in suckling rats attributable to an insufficiency of vitamin E.

13212

Failure to Find Sodium Pregnanediol Glucuronidate in Bull's Urine.

HERBERT S. STRICKLER, M. EVELYN WALTON AND DONALD A. WILSON. (Introduced by William S. McEllroy.)

From the Department of Physiological Chemistry, School of Medicine, University of Pittsburgh, and the Endocrine Laboratory, Elizabeth Steel Magee Hospital, Pittsburgh.

The discovery by Marker, Wittle and Lawson[1] of large amounts of pregnanediols in bull's urine was made in extracts of the hydrolyzed urine. Also, though the paper of Marker and Hartman[2] appears to refer to presence of pregnanediol glucuronidate in pregnant cow's, pregnant mare's and bull's urine, examination of the papers they cite in this connection reveals no reference to actual demonstration of the presence of pregnanediols conjugated as glucuronidate.

The value given by Marker, *et al.*,[1] for the average amount per gallon of pregnanediol 3α, 20α in bull's urine is 100 mg. Dividing this by Venning's[3] factor 0.597, one finds that this is equivalent to

1 Marker, R. E., Wittle, E. L., and Lawson, E. J., *J. Am. Chem. Soc.*, 1938, **60**, 2931.

2 Marker, R. E., and Hartman, C. G., *J. Biol. Chem.*, 1940, **133**, 529.

3 Venning, E. H., *J. Biol. Chem.*, 1937, **119**, 473; 1938, **126**, 595.