both showed significant, if slight, antibody response falling off to undetectable levels 3 to 4 weeks after the initial injection with typical rapid secondary response in the animal reinjected on day 28.

Although the highest levels of antibody were achieved in the 2 animals receiving the highest dosage (1.5 ml of culture fluid—30,000,000 $TCID_{50}$), one of 2 animals receiving 1/100 as much virus (only 0.015 ml of culture fluid) responded equally well (#3015). It is apparent, however, that this is approximately the smallest dose capable of eliciting a maximal response.

*Discussion.* Studies on the immunologic relationship between strains of Adenovirus Type 12, and on their oncogenicity, will obviously be facilitated through development of an *in vitro* method of identification.

Such tests have been described for Poliovirus by McBride(9); and Rozee and Casey (10). McBride(9) has stated "for best resolution of differences among Poliovirus strains, it is best to use comparatively early antisera"; and it is quite possible that among Adenovirus strains also, maximum resolution will be attained with specific antiserum prepared against small quantities of virus as here described. With the present method also, antiserum of usable titer can be obtained as early as 7 days after injection, with relatively small amounts of virus, and blood can be drawn up to 28 days later without appreciable losses in titer. If higher titered antiserum is desired, a booster inoculation of low dosage (300,000 $TCID_{50}$) raises antibody titers to higher levels for a short time.

Additional studies are in progress to determine the antibody response of rabbits to single doses of other Adenovirus types, as well as other Type 12 strains, and to explore the possibility of strain differences within types.

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Received March 14, 1963. P.S.E.B.M., 1963, v113.

## Prevention of Ovarian Cyst Formation by Ethamoxytriphetol (MER-25).* (28329)

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Ethamoxytriphetol (MER-25), an estrogen antagonist(1-3) will counteract the enhanced ovarian sensitivity of the hypothyroid rat to chorionic gonadotrophin(4). Furthermore, the compound may be useful in treatment of human polycystic ovaries(5). However, no evidence has been presented to determine whether or not MER-25 will prevent any of the biochemical changes which occur in the ovary in association with ovarian cyst formation. Cystic ovaries of hypothyroid-chorionic gonadotrophin treated rats exhibit a characteristic subnormal concentration of both free and esterified cholesterol whereas the ovaries from euthyroid rats following chorionic gonadotrophin reveal a decrease in esterified cholesterol only(6,7). The present study involves the influence of MER-25 on rats subjected to an experimental regime for ovarian cyst induction. Adrenal cholesterol and hypo-

* Supported by grants from Nat. Inst. Health, USPHS and from Wm. S. Merrell Co.

TABLE I. Weights and Cholesterol Content of Organs from Hypothyroid-Chorionic Gonadotrophin-Treated Rats.

| | Control | Thiouracil 0.5% | Chorionic gonadotrophin | Thiou + C.G. | Thiou + C.G. + MER-25 |
|---|---|---|---|---|---|
| No. rats | 12 | 8 | 10 | 19 | 10 |
| Body wt, g | 162 | 117 | 147 | 101 | 96 |
| Ovarian wt, mg | 31.6 | 17.6 | 72.6 | 542.3 | 126.1 |
| Ovarian cholesterol, % | | | | | |
| Total | 1.04 ± .10 | 3.11 ± .45 | .71 ± .08 | .18 ± .03 | .94 ± .14 |
| Free | .23 ± .02 | .29 ± .02 | .21 ± .01 | .08 ± .01 | .29 ± .03 |
| Adrenal wt, mg | 22.2 ±1.1 | 10.1 ± .7 | 16.7 ±1.4 | 10.0 ± .4 | 12.8 ± .4 |
| Total adr'n'l cholest., % | 3.00 ± .28 | 3.65 ± .29 | 2.94 ± .36 | 1.27 ± .13 | 2.02 ± .26 |
| Pituitary wt, mg | 4.9 ± .3 | 5.7 ± .3 | 5.1 ± .3 | 9.2 ± .6 | 4.4 ± .4 |
| Thyroid wt, mg | 7.5 ± .4 | 49.4 ±4.4 | 7.8 ± .4 | 56.8 ±3.1 | 41.4 ±8.1 |

C.G. = Chorionic gonadotrophin (10 I.U./day). MER-25 = Ethamoxytriphetol (4 mg/day). Thiouracil fed for 30 days, C.G. injected last 20 days.

physeal weight changes are included.

*Materials and methods.* Long-Evans strain rats weighing 60-70 g were fed a purified diet containing 20% casein and 25% fat. Hypothyroidism was induced by incorporation of 0.5% thiouracil in the diet. Both euthyroid and hypothyroid rats were injected subcutaneously daily with 10 i.u. of human chorionic gonadotrophin (Follutein)† on the last 20 days of the 30 day experimental period. Ethamoxytriphetol (in oil) was administered subcutaneously throughout the 30 day period in 4 mg daily doses.

Ovaries, adrenals, thyroids and pituitaries were weighed. Ovarian and adrenal cholesterol concentrations were estimated following extraction with acetone:alcohol (1:1) and precipitation by an alcoholic solution of digitonin(8).

*Results.* Chorionic gonadotrophin administration to euthyroid rats increased ovarian weight 100% and decreased total ovarian cholesterol concentration 30%. In hypothyroid rats, chorionic gonadotrophin increased ovarian weight 15-fold, caused the formation of cystic follicles and reduced total ovarian cholesterol from 1.04% to .18%. Furthermore, free cholesterol was reduced from .23% to .08%. When MER-25 was administered to hypothyroid - chorionic gonadotrophin-treated rats, ovarian weights approximated those of euthyroid animals treated with chorionic gonadotrophin; total and free cholesterol concentrations simulated those of normal animals (Table I).

Hypophyseal weight was slightly greater in animals fed thiouracil and a further increase from 4.9 to 9.2 mg/100 g body weight was obtained in animals treated with chorionic gonadotrophin and thiouracil. Hypophyseal weight remained essentially normal, however, when MER-25 was administered during the period of attempted ovarian cyst induction. No effect of MER-25 was noted on body weight or thyroid weight.

Thiouracil alone or in combination with chorionic gonadotrophin caused a 50% reduction in adrenal weight but only when the gonadotrophin was administered to the hypothyroid rat did adrenal cholesterol concentration decrease significantly. Adrenal weight depression was not prevented by MER-25 but in gonadotrophin-treated hypothyroid rats a partial protection of the adrenal cholesterol decrease was obtained (Table I).

*Discussion.* MER-25 does not affect ovarian weight or cholesterol content in normal rats(4) and the antagonistic action of the drug is assumed to occur at sites of estrogen stimulation(1). Thus, heightened secretion of estrogen by gonadotrophin stimulated ovaries of hypothyroid rats may be involved in cystic ovary formation. Whether or not estrogen production is contributory to human polycystic ovary development is not known but steroid metabolism is abnormal(9-11).

Estrogen-induced pituitary hypertrophy can be inhibited by MER-25(1,3); hypophyseal enlargement is a concomitant of

† Human chorionic gonadotrophin (Follutein) was generously supplied by E. R. Squibb & Sons. MER-25 was kindly supplied by Wm. S. Merrell Co.

ovarian cyst induction in rats and is prevented by MER-25 as is the development of cystic ovaries. Whether the pituitary hypertrophy signals a contribution of this organ to ovarian cyst formation or is simply a reflection of ovarian cyst formation is not established. However, the pituitary is necessary for chorionic gonadotrophin to induce cystic ovaries in hypothyroid rats(12).

*Summary.* Chorionic gonadotrophin-treated hypothyroid rats develop cystic ovaries characterized by decreases in free and esterified cholesterol. Ethamoxytriphetol prevents cystic ovary formation and the associated cholesterol changes, blocks pituitary hypertrophy and partially protects against adrenal cholesterol depletion.

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Received March 14, 1963. P.S.E.B.M., 1963, v113.

## The Biological Activity of Di-α-Tocopherone.* (28330)

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Di-α-tocopherone is the trivial name of a major metabolite of vitamin E which has been recently isolated from mammalian liver, synthesized by $K_3Fe(CN)_6$-oxidation of α-tocopherol, and assigned the structure shown in Fig. 1(1,2). In the studies reported here, the biological activity of this compound as a substitute for Vit. E in the nutrition of rats and rabbits has been investigated. Also, the hypothesis that di-α-tocopherone might represent an "active form" of the vitamin whose formation *in vivo* is genetically blocked in animals exhibiting hereditary *Muscularis dystrophia*, was tested using a strain of mice affected with this disease.

*Methods. Rat fertility experiments.* Fifty-eight weanling female rats of the Sprague-Dawley strain were maintained for 8 weeks on the following Vit. E-deficient diet (%): Glucose (Cerelose) 70.4, 'Labco' casein 20.0, salts 446(3) 4.0, methyl linoleate (60%)† 3.0, cod liver oil 2.0, vitamin mixture in Cerelose(4) 0.5, choline chloride 0.1. The females then were mated to normal males, divided into 4 treatment groups, and assigned to one of the following treatments: (a) none (negative controls) (b) 25 mg di-α-tocopherone (c) 100 mg di-α-tocopherone (d) 25 mg d-α-tocopheryl succinate. In each case the supplement was dissolved in 1.75 ml methyl linoleate and incorporated into the diet in 3 equally divided doses given on the 8th, 11th and 14th days after the start of the 5-day mating period. All the females were killed on the 21st day and the uteri were examined for the presence of live fetuses and for evidence of resorption.

Indications that the dimer was poorly absorbed from the lumen of the intestine prompted a second study in which the compound was administered parenterally. The

* Supported by a grant from National Science Foundation.

† Nutritional Biochemicals Corp., Cleveland, Ohio.