of cholesterol degradation have taken place. Such a compound could conceivably be that encountered by us in our chromatographic experiments.

The physiological significance of our observations is that plant sterols, absorbed from the intestine, can be degraded and presumably excreted *via* the bile in the form of water soluble acids.

Summary. Oxidation of ergosterol-28-C^{14} and of ergosterol-U-C^{14} by mitochondrial preparations from rat or mouse livers has been investigated. Either substrate yielded $C^{14}O_2$. Ergosterol-U-C^{14} yielded radioactive neutral and acidic products, while ergosterol-28-C^{14} gave only neutral radioactive material. Among the acidic products obtained from ergosterol-U-C^{14} was a radioactive acid with R_f close to that of trihydroxycoprostanic acid. Similarities between the oxidation of cholesterol and ergosterol by liver mitochondria *in vitro* suggest that enzyme systems involved are closely related to each other, if not identical.

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Endocrine Studies of Chlordiazepoxide.* (26449)

ALFRED BORIS, JOSEPH COSTELLO, MARIE M. GOWER AND JOAN A. WELSCH
(Introduced by William Schallek)
Dept. of Pharmacology, Hoffmann-La Roche, Nutley, N. J.

Several reports in the literature indicate that psychotropic drugs of the phenothiazine and reserpine type have endocrine effects, particularly on the pituitary-gonadal axis (1-6). We therefore decided to study the endocrine effects of chlordiazepoxide, a member of a new chemical class of compounds possessing potent pharmacological activity in animals(7-8) and psychotropic effects in man(9).

* Librium®, Hoffmann-La Roche.

TABLE I. Endocrine Studies of Chlordiazepoxide.

Test	No. animals per group	Initial body wt (g)	Dosage	Response
Reduction in testes and prostate wt in immature rats	7-10	50-60	2.5 or 5 mg/day s.c. or 50 mg/kg/day p.o. × 10 days	Negative
Prevention of ovarian hypertrophy in parabiotic rats	4-7 pairs	50-60	5 mg/day × 10 days s.c.	"
Interruption of estrous cycle in rat	7	250-260	50 or 100 mg/kg/day p.o. × 5 days	"
Prevention of ovulation in rabbit in response to mating	6	2800-3000	25 or 50 mg/kg p.o. 1 hr prior to mating	"
Prevention of goitrogenic activity of thiouracil	9-10	220-230	10 mg/day × 14 days s.c.	"
Effect upon seminal vesicle and prostate wt in castrated rats	8	50-60	2.5 mg/day × 7 days s.c. and/or testosterone propionate .05 mg/day × 7 days s.c.	"
Effect upon uterine wt in ovariectomized rats	10	85-90	5 or 10 mg/day × 3 days s.c. and/or estradiol benzoate .0005 mg/day × 3 days s.c.	"
Progestational and anti-progestational activity in estrogen primed, intact rabbit	4	800-900	5, 10, or 20 mg/day × 5 days p.o. and/or progesterone 0.2 mg/day × 5 days s.c.	"

Methods. Rats were obtained from Charles River Breeding Laboratories, Brookline, Mass. Subcutaneous (s.c.) injections were given in sesame oil, while distilled water served as the vehicle in oral (p.o.) administrations. Female rats were ovariectomized and united parabiotically with an intact female partner on the day following ovariectomy. The ovariectomized parabiont received all injections. Rats used for estrous cycle studies were preselected for normal cycling. Vaginal smears were examined once daily during treatment. Thiouracil was administered in the drinking water as an 0.1% solution. Treatment of male castrates started one day after castration. Ovariectomized rats were used 4 days following ovariectomy. Progestational activity was determined histologically in rabbits primed for 5 days before chlordiazepoxide treatment with .006 mg estradiol benzoate per day s.c.

Results and discussion. Table I indicates that chlordiazepoxide failed to manifest endocrine effects in any of the procedures employed at dosages which produce significant non-endocrine pharmacological effects in rats and other species(7-8). The lack of effect upon the pituitary-gonadal axis is particularly evident, since chlordiazepoxide was inactive in 4 different tests utilizing 2 species. In parallel studies with potent gonadotropin inhibitors such as estradiol or testosterone, reduction in testes weight of 60 to 70% occurred with dosages of .005 mg and 0.1 mg/day, respectively, after 10 days administration to intact immature male rats.

The goitrogenic activity of thiouracil is dependent on increased output of thyrotropin from the pituitary, and thyroid enlargement does not occur in hypophysectomized, thiouracil-treated animals(10). Consequently, the failure of chlordiazepoxide to prevent thiouracil-induced thyroid enlargement indicates that chlordiazepoxide does not possess anti-thyrotropic activity.

Summary. Studies utilizing standard endocrine technics indicate that chlordiazepoxide does not possess gonadal hormone-like activity, anti-gonadal hormone activity, anti-goitrogenic activity, nor does it interfere with normal functioning of the pituitary-gonadal axis.

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Serological Relationships between Collagenase and the A_2-Isoagglutinogen-like Substance of Animal Parasites.* (26450)

JOSÉ OLIVER-GONZÁLEZ AND H. NAIM KENT

Departments of Microbiology, School of Medicine, San Juan, P. R., and Pathobiology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.

Recent studies have indicated that the A_2-isoagglutinogen-like substance (A_2IS) in animal parasites may be associated with a collagenase complex. Thus the sera from rabbits artificially immunized against the A_2IS agglutinate human erythrocytes of Group O treated with tannic acid and exposed to collagenase prepared from various sources(1). Also, collagenase, like A_2IS inhibits the a_2 isoagglutinins in human sera of Groups B and O. The A_2IS utilized in these studies was extracted from the cuticle of *Ascaris* and was used as a crude material. A purified A_2IS has been prepared which shows more activity than the crude material with respect to inhibition of a_2 isoagglutinins and which is also serologically related to collagenase.

Lewert *et al.*(2) have shown that collagenase-like enzymes are present in the larval forms of *Schistosoma mansoni, Ancylostoma caninum* and *Strongyloides ratti.* Collagen-like substances have also been described from various animal parasites(3,4) particularly from the cuticle of *Ascaris,* which constitutes a good source of A_2IS. Collagenase, therefore, is apparently present in animal parasites either as a component of their tissues or in their metabolic products.

Materials and methods. Animal inoculations. Male rabbits weighing from 1.5 to 2 kilos were artificially immunized with various substances as described previously(1). Dogs weighing 4.5 to 5 kilos were each injected intravenously with 2 to 3 mg of A_2IS, collagenase, pepsin and trypsin† per kilo of body weight. The animals were observed closely for reactions to the various substances and were bled before injection and immediately after death. Dogs were autopsied and tissues fixed for histopathological studies immediately after death.

Preparation of purified A_2IS. The crude and dry material obtained from *Ascaris* cuticle, according to the procedure described elsewhere(1) was reextracted with triple distilled water at 4°C, centrifuged for 30 minutes at 9000 × G (International refrigerated centrifuge PR-1) and the supernatant dialyzed exhaustively against triple distilled water in a cold room for a period of 2 days with several changes. The dialyzed solution was then lyophilized. The lyophilized material will be referred to as "purified A_2IS".

Total protein analyses were carried out by the method of Lowry *et al.*(5); total carbohydrate and cold trichloracetic acid-precipi-

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† Collagenase A—Nutritional Biochemical Corp., Cleveland, Ohio.

Collagenase B—Mann Research Laboratories, New York.

Pepsin and Trypsin—Difco Laboratories, Inc., Detroit, Mich.