amounts of lipid contributed to the E.K.G. changes by binding the freely available calcium. The possibility exists that the cations in the tisues and blood were utilized to neutralize the free fatty acids liberated by hydrolysis of neutral fats.

We have shown that convulsions occur in hamsters after fat meals, but not after similarly sized meals of highly unsaturated oils such as cod liver oil and safflower oil(4,5). The mechanisms of the seizures is not known, but is thought to be related to alterations in the circulation(1,2) and reduction in availability of oxygen in the brain(3) which occur after fat feedings. It is postulated that the permeability of brain capillaries is increased and free fatty acids, normally excluded from the brain, pass the blood-brain barrier in toxic or irritant amounts. No direct relationship can at present be established between the mechanisms of the seizures and E.K.G. changes. The fact that the changes noted above occur after fat meals and not after oil meals indicate that the metabolism of saturated fatty acids is significantly different, quantitatively or qualitatively, from the metabolism of unsaturated fatty acids.

Summary. Large cream and oil meals were fed to hamsters. At intervals after feeding electrocardiograms were made. After the fat meals prolongation of the QT interval, especially its ST segment occurred. These changes were absent after oil meals.

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Gonadotrophin Inhibiting and Anti-Fecundity Effects of Chloramiphene.* (26054)

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Modification of the chemical structure of chlorotrianisene (TACE®)(1) has led to a varied spectrum of biological activities. The specific biological effects produced by these modified substances are: (a) inhibition of cholesterol synthesis by triparanol (MER/29®)(2), and (b) estrogen antagonism by ethamoxytriphetol (MER-25)(3). The present paper pertains to a new structurally related compound, chloramiphene (1-[*p*-*β*-diethylaminoethoxy) phenyl] -1,2-diphenyl-2-chloroethylene).‖ Its major biological activity refers to pituitary gonadotrophin inhibition and anti-fecundity.

Materials and methods. Chloramiphene was tested as the citrate salt. It was administered in suspension in an olive oil vehicle unless otherwise indicated. Control animals received the vehicle alone. Rats utilized were of Sprague-Dawley strain, and were given tap water and stock laboratory diet (Rockland) *ad libitum. Pituitary gonadotrophin inhibition. Intact immature male rats.* Rats of approximately 70 g were divided into groups of 5 and were given chloramiphene in doses ranging from 0.3 to 70.0 mg/kg/day, by once

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‖ One of a series of structurally related compounds, synthesized by Allen, R. E., Feil, V. J., and Palopoli, F. P.

daily subcutaneous injection for 10 days. Autopsies were performed on the day after last injection. Organs were removed and weighed. *Intact mature male rats.* Rats weighing 310 to 350 g were divided into 6 groups of 5 animals each. Three groups received 1.0 mg/kg/day chloramiphene subcutaneously. At the end of each 5, 10 and 20 days, one group of control and one group of treated animals were sacrificed and autopsied. Testes, seminal vesicles, ventral prostate, and pituitary were removed and weighed. *Intact immature female rats.* Chloramiphene was given subcutaneously at 1.0 mg/kg/day. One control and one treated group (5/group) were autopsied after each 10 and 20 days of treatment. Ovaries, uteri, and pituitaries were removed and weighed. *Parabiotic rats.* Litter-mate female rats, 30-32 days of age, were parabiotically joined(4). One parabiont was ovariectomized. Chloramiphene was given to the castrate partner at doses ranging from 0.001 to 1.0 mg/castrate parabiont/day for 10 days. Each dose level was tested in 3 to 5 parabiotic pairs. Effectiveness was evaluated by both subcutaneous and oral routes. Ovaries of the intact parabiont and uteri of both animals were removed and weighed at time of autopsy. *Gonadotrophin content of pituitaries.* Pituitaries were removed from a group of 5 donor control animals and a group of 5 donor animals treated for 20 days with chloramiphene at 1.0 mg/kg/day. Pituitaries were pooled according to group, macerated and homogenized in isotonic saline. They were then administered subcutaneously to 22-day-old female recipient rats, once daily for 3 days in a dose equivalent to 1 pituitary/rat/3-day period. Pregnant mare serum§ (PMS, Gonadogen®) was used as a control standard. On the day after last injection the rats were sacrificed. Uteri and ovaries were removed and weighed. *Absence of antagonism to exogenous gonadotrophin.* Chloramiphene (3 mg/kg/day) was administered subcutaneously for 4 days to 22-day-old female rats (7/group). Exogenous gonadotrophin§ (PMS) was administered at a separate injection site for each of the last 3 days of chloramiphene treatment. On the day after last injection the rats were autopsied. Ovaries and uteri were removed and weighed. *Interruption of estrous cycles of rats.* Young, adult female rats were divided into control and experimental groups of 4 to 7 animals each. Daily vaginal smears were studied for at least 2 full cycles before start of drug. Chloramiphene was given either subcutaneously or orally at doses ranging from 0.01 to 3.0 mg/kg/day for 6 or 8 days. Vaginal smears were taken once daily. *Anti-fecundity activity.* Young, adult, female rats were divided into control and experimental groups of 3 to 7 animals per group. Chloramiphene was given to female rats, either subcutaneously or orally in doses ranging from 0.01 to 3.0 mg/kg/day. Fecundity was determined for the period during drug administration, the period immediately after stopping the drug, and period after return of normal sex cycling after stopping treatment. Treatment was daily for 6 days for the last experimental approach, 8 days for the second, and from 8 days before cohabitation and then during cohabitation through the second day after observation of sperm cells in the vagina, for the first test approach. Males were rotated during cohabitation.

Results. In *immature male rats* chloramiphene in doses from 1 mg/kg/day and greater yielded lower relative weights of testes, seminal vesicles, and ventral prostates (Fig. 1). Degree of lowering was dose dependent. Ventral prostates were lowered more than either seminal vesicles or testes. Rate of body weight gain was not altered at doses below 3 mg/kg/day. Increasing the dose above this amount caused a progressive decrease in rate of weight gain. Rats receiving the highest dose of 70 mg/kg/day gained 33 g in weight while control animals gained 65 g. In *mature male rats,* chloramiphene-treated rats had lower ventral prostate and seminal vesicle weights after 20 days of treatment with 1 mg/kg/day (Table I). These organs were about three-fourths of control weight. No difference was observed at 10 days. Pituitary weights in these mature male animals treated for 10 or 20 days were not altered by chloramiphene.

§ PMS dose: Total dose of 20 I.U./rat given in 3 days, e.g. 6.6. I.U./rat/day.

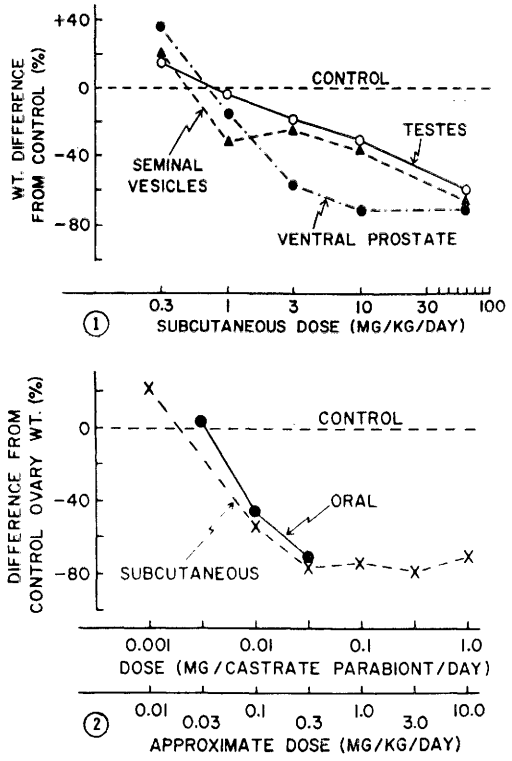

FIG. 1. Effects of chloramiphene on weights of testes, seminal vesicles, and ventral prostates of immature rats. Treatment was by subcut. inj. for 10 days.

FIG. 2. Gonadotrophin inhibiting activity of orally and subcut. administered chloramiphene to immature parabiotic littermate female rats (1 castrate : 1 intact). Ovarian wt was the index of response. Chloramiphene was given for 10 days to the castrate parabiont. Approximate dose is based on wt of castrate parabiont.

In intact immature female rats subcutaneous treatment at 1 mg/kg/day for 10 days produced a lowering of mean relative uterine weight. It was 59% of mean control weight (115 mg/100 g body weight). Ovarian weight was not altered (27 mg/100 g body weight). Subcutaneous administration at the same dose for 20 days resulted in a lowering of both uterine and ovarian weights to 62% and 29% below the controls, respectively. The same degree of lowering was obtained by 10-day oral administration of 2 mg/kg/day (uterus and ovary, 44% and 21% below controls, respectively). After 10 days and 20 days of subcutaneous treatment, mean relative pituitary weights were 13% and 19%, respectively, below control weight. The oral dose had no effect (2% above controls). In *parabiotic rats,* chloramiphene at a dose of 0.01 mg/parabiont/day (approximately 0.1 mg/kg/day on the basis of body weight of castrate parabiont) resulted in ovarian weights that were 50% of control (Fig. 2). Doses of 0.3 mg/kg/day reduced ovarian weight to a plateau of response approximating 20% of control. Oral and subcutaneous routes of administration were equally effective.

The *gonadotrophin content of pituitaries* from adult male rats was examined after 20 days of treatment with chloramiphene at 1 mg/kg/day. Pituitaries from control and treated donor rats produced comparable increases in ovarian (2.8- and 2.9-fold respectively) and uterine (3.5- and 3.7-fold) weights of the immature recipient test rats. Thus, the pituitary gonadotrophin content was not altered by treatment.

Chloramiphene *failed to block exogenous gonadotrophin* (PMS). When chloramiphene was given at 3 mg/kg/day along with PMS at 20 I.U./rat, mean weight of the ovaries was 96 mg. Rats receiving only PMS had a mean ovarian weight of 83 mg. The increased uterine weight (125 mg) induced by PMS treatment alone was, however, reduced to 78

TABLE I. Effect of Chloramiphene (1.0 mg/kg/Day Subcutaneously Once Daily) on Organ Weights of Mature, Intact, Male Rats.

Group	Length of treatment (days)	Body wt* Initial (g)	Body wt* Final (g)	Relative organ wt (mg/100 g body wt)* Testes	Seminal vesicles	Ventral prostate	Pituitary
Control	5	355 ± 9	373 ± 10	830 ± 51	94 ± 6	130 ± 6	2.7 ±.11
Treated	5	341 ± 12	344 ± 15	980 ± 31	84 ± 2	120 ± 6	2.5 ±.10
Control	10	342 ± 10	356 ± 12	900 ± 46	95 ±11	120 ±11	2.8 ±.12
Treated	10	343 ± 12	338 ± 9	950 ± 37	83 ± 4	120 ± 9	2.6 ±.05
Control	20	329 ± 9	349 ± 9	910 ± 21	92 ± 7	120 ± 8	2.8 ±.15
Treated	20	312 ± 7	303 ± 6	1060 ± 48†	68 ± 6†	94 ± 7†	2.7 ±.15

* Mean ± S.E.

† $.05 > P > .02$ by t test(6).

TABLE II. Interruption of Estrous Cycles by Daily Administrations of Chloramiphene to Young Adult Female Rats.

Dose (mg kg/day)	Route	Vehicle	Rats with interrupted cycles / No. of rats
Control	subcut.	oil	0/5
.3	"	"	5/5
Control	oral	"	0/15
.01	"	"	0/4
.1	"	"	4/4
.3	"	water	7/7
1.0	"	"	7/7
3.0	"	oil	5/5

mg by concurrent chloramiphene treatment. At this dose, chloramiphene alone doubled uterine weight from 29 to 59 mg, but had no effect on mean ovarian weight (17 mg).

Chloramiphene doses of 0.1 mg kg/day and greater *interrupted the estrous cycle* in young adult female rats (Table II). The lower dose of 0.01 mg/kg day was not effective. Interruption of the sex cycle at 0.1 mg kg/day was evident after second or third administration of chloramiphene. In this experiment first day of treatment was coincident with metestrus. Cells found in the vaginal smear during the cessation of cycling appeared to be characteristic of incomplete diestrus. The smear was a mixture of mostly leucocytes with only a few epithelial and cornified epithelial cells.

Anti-fecundity activity of chloramiphene was observed in female rats receiving during cohabitation, doses of 0.3 mg/kg day subcutaneously or 3.0 mg/kg day orally. None of the 10 treated animals became pregnant whereas 8 of 10 control animals became pregnant. Five orally treated and 3 of 5 subcutaneously treated animals copulated during period of treatment. The 2 non-copulating females did copulate after treatment was stopped. Average time from initial cohabitation to copulation was 4 days. This was equivalent to the time observed in control animals. When treated female rats were started in cohabitation with the male on the day after oral treatment was stopped with either 0.3 or 1.0 mg/kg/day, 6 of 7, and 7 of 7 rats, respectively, became pregnant.

Mean number of days from start of cohabitation to first day of pregnancy was 4.6 days for the control group, 14.8 days after 0.3 mg/kg/day, and 17.6 days after 1 mg/kg/day. In a separate experiment, female rats (3 or 4/group) were treated at 0.01 mg/kg/day or 0.1 mg/kg/day and then allowed to return to normal cycling. They were then cohabitated with males. All these prior-treated animals were fertile.

Discussion. Chloramiphene produced a dose dependent reduction in weight of reproductive organs of intact rats: ovaries and uteri in females, and testes, ventral prostates and seminal vesicles in males. It prevented the ovarian hypertrophy induced by joining castrate and intact rats in parabiosis. Such results are indicative of pituitary gonadotrophin inhibition. Chloramiphene, however, did not change pituitary gonadotrophin content and produced no change or possibly a slight decrease in pituitary weight. Estradiol, on the other hand, produces pituitary hypertrophy(5).

Administration of chloramiphene to mature female rats interrupted normal estrous cycles and produced a loss in fecundity. Both these effects were reversible after drug administration was stopped.

The 3.0 mg/kg/day dose of chloramiphene to immature female rats gave a doubling of uterine weight indicative of weak estrogen-like activity. This level of chloramiphene antagonized the indirect uterotrophic effect of PMS which suggests an anti-estrogen action of chloramiphene. Thus, the vaginal smear picture, the mild uterotrophic effect and antagonism to the uterine weight increase, indirectly induced by PMS, indicate that chloramiphene has secondary mild or atypical estrogen-like and estrogen-antagonizing effects. Pituitary inhibition, however, was the biological effect observed at the lowest dose: *e.g.*, 0.1 mg/kg/day prevented ovarian hypertrophy in immature parabiotic rats and interrupted estrous cycle in mature female rats.

Summary. Chloramiphene (1-[*p*-*β*-diethylaminoethoxy) phenyl] -1, 2-diphenyl-2-chloroethylene) is a pituitary gonadotrophin inhibitor. In immature litter-mate female parabiotic rats (1 castrate: 1 intact), 0.1 mg/kg/day inhibited ovarian weight gain by 50%. Larger doses yielded greater inhibition.

It was equally active by oral and subcutaneous routes. Doses of 0.1 mg/kg/day and greater interrupted the estrous cycles of young mature female rats. Treatment during cohabitation produced a loss in fecundity which was reversible after cessation of treatment. Pituitaries of chloramiphene-treated rats had approximately the same weight and gonadotrophin content as control rats. Chloramiphene did not inhibit exogenously administered gonadotrophin.

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Effect of Reserpine on Pituitary-Gonadal Axis.* (26055)

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Interference of tranquilizing agents, particularly chlorpromazine and other phenothiazine derivatives, with adrenal, thyroid and gonadal functions has been reported by several investigators(1-5). Most authors ascribe this interference to action of the drug on the hypothalamus. Since the hypotensive and tranquilizing effect of reserpine is presumed to result from its action on the diencephalon, and particularly on the hypothalamic region (6-7), reserpine might be expected to interfere with the normal functioning of the endocrine system.

Reserpine was reported to interfere with estrous cycles in mice(8), ovulation and menstruation in monkeys(9), prolactin secretion and discharge in rats, rabbits and women (10-12), oxytocin and prolactin secretion in lactating rats(13), and to effect recession of exophthalmus(14).

In this paper we wish to report on the effect of reserpine on the pituitary-gonadal axis.

Materials and methods. Reserpine stock solution was prepared according to the method described in Martindale's Extra Pharmacopoea 1959, and diluted to the required concentrations. Experiments were carried out on a total of 200 male and female infantile and adult albino rats and 10 male pigeons of the Columba strain. Vaginal smears and gonadotropin determinations were performed on 8 female patients.

The following aspects of the effect of reserpine on the pituitary-gonadal axis were studied: A. Vaginal pattern in infantile rats, adult rats and women; B. Ovarian and uterine development in infantile and adult rats; C. Testicular development in infantile male rats, adult male rats and adult male pigeons; D. Gonadotropin content in urine of menopausal women.

Results. A. Vaginal Pattern. 1. *Infantile female rats.* Twenty rats were injected subcutaneously with 0.05 mg/kg reserpine and another 20 rats with 0.2 mg/kg; 20 controls received the vehicle. In 10% of controls, vaginal opening was complete at age of 35 days, and in 50% at age of 44 days. After this date positive vaginal smears in control animals varied between 20-50%. At dose level of 0.05 mg/kg the stage of vaginal opening and incidence of positive smears closely resembled the pattern observed among the controls (see above). At dose level of 0.2 mg/kg a delay in vaginal opening was ob-

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