total blood O_2 and pH in femoral artery and vein and portal vein show marked parallelism of the response to histamine. Decrease of O_2 and pH after injection of histamine resembles stagnant anoxia due to other causes such as shock.

Injury to capillaries and to parenchymatous tissues of the area of portal circulation by hypoxia(8,9) may occur in histamine treated animals. Such injury associated with acid and peptic hypersecretion may contribute to the etiology of acute and chronic posthistaminic gastric lesions(2,3,4,5).

Summary. Blood from femoral artery and vein and from portal vein of dogs was sampled before and 10, 20 and 30 minutes after injection of histamine. Oxygen content and pH of the blood was determined. Marked decrease of both O_2 and pH of blood was found in all histamine treated animals as compared with untreated controls. The pattern of this reaction corresponds to one observed in stagnant anoxia and acidosis. Stagnant anoxia of splanchnic area which occurs after histamine is considered an important etiological factor responsible for the posthistaminic gastric lesions.

1. Dale, H. H., Laidlaw, P. P., *J. Physiol.*, 1919, v52, 355.
2. Kowalewski, K., Bain, G. O., *Acta Gastro-Enterol. Belgica*, 1954, v17, 539.
3. Kowalewski, K., *Canad. J. Bioch. & Physiol.*, 1954, v32, 553.
4. ———, *ibid.*, 1954, v32, 600.
5. Kowalewski, K., Lyon, R. K., Edwards, G. E., Shnitka, T. K., *ibid.*, 1958, v36, 977.
6. Haddy, F. J., *Am. J. Physiol.*, 1960, v198, 161.
7. Thomas, W. D., Essex, H. E., *ibid.*, 1949, v158, 303.
8. Moon, V. H., *Bull. N. Y. Acad. Med.*, 1950, v26, 361.
9. Barach, A. L., *ibid.*, 1950, v26, 384.

Received October 5, 1961. P.S.E.B.M., 1962, v109.

An Orally Effective Mammalian Antifertility Agent. (27138)

GORDON W. DUNCAN, JACOB C. STUCKI, STANLEY C. LYSTER AND DANIEL LEDNICER
Departments of Endocrinology and Chemistry, Upjohn Co., Kalamazoo, Mich.

Reproduction of the species represents a culmination of many intricate mechanisms, each potentially susceptible to modification by chemical agents. Of the numerous biological contraceptives available, relatively few can be considered practical for control of mammalian reproduction(1,2,3). This paper pertains to biological spectrum of U-11555A,* one member of a new series of 2,3-diphenylindenes, which on the basis of the following data is considered to be a potent, orally effective antifertility agent in laboratory mammals. Chemistry of these compounds has been reported elsewhere(4).

Methods and results. Sprague-Dawley rats, fed Purina Laboratory Chow *ad libitum* and maintained in an environment of 78 ± 2°F, were used.

Pregnancy inhibition. Proestrus 200 g rats were placed with males of proven fertility. The day sperm were identified in vaginal smear was considered Day 1 of pregnancy. On Day 8 females were sacrificed and uteri examined for implantation sites and gross abnormalities. Administration of U-11555A† from proestrus to Day 6, at 0.5 mg/kg/day and above, was 100% effective in inhibiting pregnancy (Table I). A single dose of 2.5 mg/kg, administered within 4 days post insemination, was equally effective in preventing pregnancy, whereas single doses up to 25 mg/kg, administered on Day 5, were ineffective in inhibiting pregnancy (Table II). Five females received 2.50 mg/kg/day from Day 5 through Day 8; at autopsy on

* Triethylamine, 2-(*p*-[6-methoxy-2-(*p*-methoxyphenyl)-inden-3-yl]-phenoxy) hydrochloride.

† In all experiments U-11555A was administered orally in Upjohn Sterile Vehicle No. 122, containing 0.25% methylcellulose.

TABLE I. Inhibition of Pregnancy Following Daily Oral Administration of U-11555A for 7 Days Initiated during Proestrus.

	Daily dose (mg/kg)					
	.125	.250	.500	1.00	2.50	5.00
No. of rats pregnant	5/5*	11/18	0/11	0/3	0/2	0/2
% inhibition of pregnancy	0	39	100	100	100	100

* No. pregnant/No. of treated rats.

TABLE II. Inhibition of Pregnancy Following a Single Oral Dose of U-11555A.

Dose (mg/kg)	Day of pregnancy compound administered					Implants per rat*
	1	2	3	4	5	
0	5/5†	5/5	5/5	4/5	5/5	10.7
.625	4/4	5/5	4/5	5/5	5/5	9.9
1.250	5/5	4/5	5/5	4/5	4/5	6.3
2.50	0/5	0/5	0/5	0/5	5/5	0
5.00	0/5	0/5	0/5	0/5	3/3	0
25.0	0/3	0/3	0/3	0/3	3/3	0

* Avg/pregnant rat treated prior to the 5th day.
† No. pregnant/No. of treated rats.

Day 12 an average of 10.0 (range 7-13) embryos was observed. Five females received 0.125 mg/kg/day throughout pregnancy. Average number of pups/litter was 10.7; average weight/pup was 6.4 g. An average of 9.9 pups, with an average weight of 6.2 g, was obtained from 10 control rats. Female pups from these treated litters were bred when sexually mature. Each conceived and delivered litters normal in gross appearance. Although minimum effective doses have not been determined, 10 mg/kg/day for 6 days following insemination inhibits pregnancy in mice, guinea pigs, and rabbits.

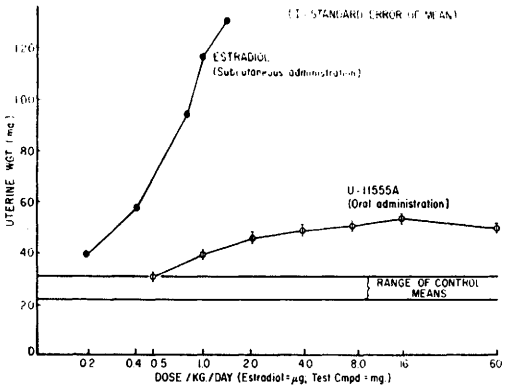

FIG. 1. Uterine response of immature rats following 10 days of drug administration.

Uterotropic activity. U-11555A was administered orally for 7 days to 55 g ovariectomized rats. Significant uterine stimulation occurred at daily doses of 1.0 mg/kg/day and above (Fig. 1). Maximum observed uterotropic activity was equivalent to that produced by 0.30 μg/kg of estradiol administered subcutaneously.

Anti-estrogenic activity. Administered concomitantly with estradiol for 7 days to 55 g ovariectomized rats, U-11555A inhibits uterine response to exogenous estrogen (Table III). Administered concomitantly with estradiol for 7 days to adult, castrate, female rats. U-11555A effectively inhibits estrogen-induced vaginal cornification (Table IV). Vaginal cornification was not observed following administration of U-11555A alone.

TABLE III. Inhibition of Uterine Response to Estradiol by U-11555A.

Preparation	No. of rats	Dose/kg/day	Uterine wt (mg ± S.E.)
Vehicle controls	10	.1 cc subq .5 cc oral	22 ± .7
Estradiol	10	.8 μg subq	88 ± 3.2
	5	.6 " "	77 ± .9
U-11555A with .6 μg/kg estradiol	4	1.0 mg oral	52 ± 1.4
	4	4.0 " "	49 ± 2.2
	2	16.0 " "	46 ± 4.5
	1	64.0 " "	41
U-11555A with .8 μg/kg estradiol	5	10.0 " "	61 ± 1.3
	5	20.0 " "	62 ± 2.5

TABLE IV. Inhibition of Estradiol-Induced Vaginal Cornification by U-11555A.

Preparation	No. of rats	Dose/kg/day	% of cornification
Controls	5	.10 cc CMC	0
Estradiol	5	.50 μg	100
U-11555A	5	12.50 mg	0
U-11555A*	5	.50 mg	0
	5	2.50 "	0
	5	12.50 "	0

* Concomitantly with .50 μg/kg estradiol.

TABLE V. Antigonadotropin Activity of U-11555A.

Preparation	No. rats	Dose, mg/kg/day	Testes wt, mg	Lev. ani wt, mg	Sem. ves. wt, mg	Adrenal wt, mg	B.W. gain, g
			10-day test				
Controls	5	.5 cc vehicle	1201 ± 57*	39 ± 2.4*	18 ± .5*	25	39
U-11555A	5	.35	1179 ± 45	26 ± .6	12 ± .7	23	34
	4	.70	1203 ± 99	19 ± 2.4	12 ± 1.3	26	31
	5	1.40	1263 ± 75	23 ± 1.2	13 ± .8	25	30
	5	2.80	1073 ± 43	14 ± .7	8 ± .8	25	18
			30-day test				
Controls	10	.5 cc vehicle	3296 ± 206	133 ± 7.9	133 ± 6.8	36	147
U-11555A	10	1.00	3324 ± 234	86 ± 5.3	103 ± 7.5	35	129

* ± S.E.

day of U-11555A for approximately 28, 28, and 40 days, respectively, during which time daily vaginal smears were continued. No alteration of the estrous cycle was observed in the 0.5 mg/kg/day group. When administration was stopped the day prior to breeding, 4 of 5 rats had normal implants at autopsy at Day 12 of pregnancy. Daily vaginal smears of the 2.5 mg/kg/day group reflected continued regularity of ovarian activity. The estrus smear, however, was modified by presence of considerable numbers of nucleated epithelial cells and leucocytes. All females copulated by the second expected estrus after introduction of males. Treatment stopped the day prior to insemination. Five rats had normal implants at autopsy on Day 12 of pregnancy. The group receiving 5.0 mg/kg/day exhibited similar modified vaginal smears for 23-31 days after which only leucocytes were observed in the smear for the remainder of the 40-day treatment period. Cyclic vaginal activity was reinitiated 12-19 days following cessation of treatment.

Progestational and antiprogestational activity. U-11555A at 0.5 and 2.5 mg/day for 5 days to estrogen-primed 1 kg rabbits caused no uterine proliferation. Progesterone, 0.20 mg/day, subcutaneous, caused a maximum uterine response in similar rabbits (McPhail assay(5)). Oral administration of 2.5 mg/day of U-11555A concomitantly with 0.2 mg/day of progesterone, subcutaneous, did not inhibit uterine response to progesterone. Three rabbits per dose were employed.

Gonadotropin inhibition. Intact, immature, male rats, 26-28 days of age, were administered U-11555A daily for 10 days at doses from 0.35 to 2.80 mg/kg/day. Approximately 24 hours after the last dose, animals were autopsied and testes, seminal vesicles, levator ani, and adrenals were weighed (Table V). Average testes weight of an additional 10 rats receiving 1 mg/kg/day of U-11555A for 30 days was 3324 mg *versus* an average of 3296 mg for 10 control rats. Decreased response of seminal vesicles and levator ani muscles at higher doses and after prolonged treatment may either represent direct interference with LH or androgen effects or be associated with diminution in body weight gains.

Discussion. U-11555A is an orally effective antifertility agent in 4 laboratory species. Efficacy in rats is restricted to the 4-day period immediately following insemination, which in the rat approximates the period of tubal transport of the fertilized ovum(6). Development of blastocysts in the uterus is not affected. Antifertility activity of U-11555A may be the result of a direct effect on the zygote during tubal transport, such as suggested for triphenyl ethanol derivatives(7,8), or the result of an alteration in tubal environment which alters transport mechanism. Uterine stimulation induced by U-11555A differs in character and magnitude from the uterotropic effect of frank estrogens. The maximum observed uterotropic activity with U-11555A was equivalent to that of 0.3 μg/kg/day of subcutaneously administered estradiol which represents 15% of the amount of estradiol required (2.0 μg/kg/day) for efficacy in rat pregnancy inhibition assays. Anti-estrogenic activity is apparent from the inhibition of uterine enlargement

and vaginal cornification following concomitant administration with exogenous estrogen and is also reflected by modification of vaginal cytology during continued administration to cycling female rats. Copulation during extended administration of up to 2.5 mg kg/day of U-11555A, and normal development of ensuing pregnancies if treatment is stopped just prior to breeding, suggests ovarian activity and psychic responses are adequate to support normal reproductive functions. On a mg/kg basis, doses of U-11555A comparable to the minimal effective antifertility dose in rats do not exhibit significant progestational, antiprogestational, antigonadotropic, androgenic, or estrogenic activities.

Summary. U-11555A, a derivative of 2,3-diphenylindene, is an orally active antifertility agent in laboratory mammals. In rats, its efficacy is limited to the period of tubal transport of the zygote. Single oral doses, administered any time within 4 days of breeding, effectively inhibit pregnancy. After the zygote has reached the uterus U-11555A is ineffective as a pregnancy inhibitor. At effective antifertility dosages, U-11555A does not exhibit significant progestational, antiprogestational, antigonadotropic, androgenic, or estrogenic activities. U-11555A inhibits the uterine and vaginal response to endogenous and exogenous estrogens.

The authors wish to express their appreciation to J. C. Cornette and A. D. Forbes for their technical assistance.

1. Jackson, H., *Pharmacol. Rev.*, 1959, v11, 135.
2. Sanyal, S. N., *Indian Med. Assn. J.*, 1960, v35, 552.
3. Tyler, E. T., *J. A. M. A.*, 1961, v175, 225.
4. Lednicer, D., Babcock, J. C., Lyster, S. C., Stucki, J. C., Duncan, G. W., *Chem. and Industry*, 1961, #51, 2098.
5. McPhail, M. K., *J. Physiol.*, 1934, v83, 145.
6. Asdell, S. A., *Patterns of Mammalian Reproduction*, Comstock Publ. Co., Ithaca, N. Y., 1946, p270.
7. Segal, S. J., Nelson, W. O., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 431.
8. Chang, M. C., *Endocrinol.*, 1959, v65, 339.

Received October 5, 1961. P.S.E.B.M., 1962, v109

Effects of Enzymatic Digests of DNA on Staphylococci.* (27139)

JAMES J. MCKEE AND WERNER BRAUN
Institute of Microbiology, Rutgers, The State University, New Brunswick, N. J.

Enzymatic digests of DNA have been shown to contain a factor that is capable of promoting the establishment of virulent mutants in initially avirulent cultures of a number of bacterial species(1-6). The active factor has been shown to be non-identical with known breakdown products of DNA, such as purines, pyrimidines, nucleosides, and nucleotides, and is obtained following exposure of DNA to DNAase regardless of the source of the nucleic acid. In the case of pneumococci the selective population change from non-smooth to smooth was found to be attributable to a selective stimulation of DNA synthesis and with it an increase in the rate of multiplication of smooth virulent types; non-smooth avirulent types did not respond to the DNA digest(4,5). The present report will discuss recent attempts to study influences of the DNA digest factor on population changes and growth of staphylococci of different virulence.

Materials and methods. Coagulase-positive and coagulase-negative strains of known relationship were employed. Most of these originated from cultures kindly furnished by Dr. John E. Blair, who had obtained them as primary isolates from human sources. DNA from calf thymus isolated by the procedure of Kay *et al.*(7), was used routinely, but DNA from bacterial sources also was employed. Pancreatic DNAase (Worthington) was used and the mixtures of DNA and

* This work was supported by NSF and Nat. Inst. Health, U.S.P.H.S. grants.