TABLE I.
Mitotic Activity in the Accessory Sex Glands of 20-day-old Rats Treated with Antuitrin-S.
(All the animals received colchicine.)

	Group I				Group II			
	Control		20 R.U. Antuitrin-S in 2 doses		Control		20 R.U. Antuitrin-S in 1 dose	
Time in hrs after 1st inj.	v.pr.	s.v.	v.pr.	s.v.	v.pr.	s.v.	v.pr.	s.v.
13					5.64	2.84	2.64	5.04
20	7.36	8.0	6.6	7.76				
23					3.72	8.28	4.8	8.88
28					11.52	7.36	4.64	27.64
28					—	3.32	0.56	26.56
30	3.16	2.12	2.96	63.64				
34					2.32	2.88	12.2	75.92
35	10.08	6.24	16.68	68.80				
43	11.88	5.64	37.2	69.16				
44					4.88	3.44	28.48	68.04

effect of gonadotropin is apparent by the sudden rise in the number of mitotic cells in the seminal vesicle at 28 and 30 hours. Increased mitotic activity lags in the ventral prostate; only slight increases occur at 34 and 35 hours but by 43 to 44 hours the increase becomes significant.

These limited data show clearly that a single 20 R.U. dose of urinary gonadotropin is effective and detectable. It is interesting to note that despite the indirect action of gonadotropin, via the testis, the effect of the Antuitrin-S is elicited several hours earlier in prepuberal rats than is testosterone injected into castrated young adults.[2]

Conclusions. The effect of a urinary gonadotropin is apparent in the accessory organs of reproduction of 20-day-old intact male rats by an increase in mitotic activity, arrested by colchicine; seminal vesicle response occurs earlier than prostate response —28-30 hours as compared with 35-43 hours. A single 20 R.U. dose is as effective as 2 equally divided doses.

13871

Effect of Benzophenone and Allied Compounds on Human Tubercle Bacilli *in vitro*.

B. L. FREEDLANDER. (Introduced by Ernest L. Walker.)

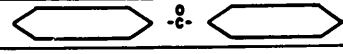
From the Department of Medicine, Division of Tuberculosis, University of California Medical School, and The Harold Brunn Research Institute, Mt. Zion Hospital, San Francisco.

1. *Bacteriostatic Action on a Rapidly Growing Avirulent Strain.* The bacteriostatic action of benzophenone (diphenyl ketone) and its derivatives was tested on an avirulent Novy strain of tubercle bacilli. This culture normally produces a heavy surface growth within 4 days. A liquid synthetic asparagin media, containing 6.% glycerine, was used, with pH 7.2. The chemical compounds, with a few exceptions, were dissolved in propylene glycol to make 1.% solutions. Subsequent dilutions were made directly into the media. Twenty-seven compounds, listed in Table I, were tested.* The amino derivatives were

* Most of the chemicals were obtained from the Eastman Kodak Co., Rochester, N.Y. Those which were impure were recrystallized. o-Chlorobenzophenone was synthesized by the Friedel and Crafts reaction with o-chlorobenzoyl chloride, benzene, and aluminum chloride. Promin was obtained from Parke, Davis & Co., Detroit, Mich.

Table I

Bacteriostatic Action on Growth of Avirulent Tubercle Bacilli *in vitro*

	Compound		Highest Bacteriostatic Dilution
1.	Benzophenone		1:10,000
2.	p-Hydroxybenzophenone	$C_6H_5 \cdot CO \cdot C_6H_4OH$	1:5,000
3.	p Methoxybenzophenone	$C_6H_5 \cdot CO \cdot C_6H_4 \cdot OCH_3$	1:20,000
4.	4,4' Dimethoxybenzophenone	$CH_3O \cdot C_6H_4 \cdot CO \cdot C_6H_4 \cdot OCH_3$	< 1:3,000
5.	p-Aminobenzophenone	$C_6H_5 \cdot CO \cdot C_6H_4NH_2$	1:5,000
6.	4,4' Diaminobenzophenone	$NH_2 \cdot C_6H_4 \cdot CO \cdot C_6H_4NH_2$	< 1:3,000
7.	Tetramethyldiaminobenzophenone	$(CH_3)_2NC_6H_4 \cdot CO \cdot C_6H_4N(CH_3)_2$	< 1:3,000
8.	Tetraethyldiaminobenzophenone	$(C_2H_5)_2NC_6H_4 \cdot CO \cdot C_6H_4N(C_2H_5)_2$	< 1:3,000
9.	p-Chlorobenzophenone	$C_6H_5 \cdot CO \cdot C_6H_4Cl$	1:40,000
10.	o-Chlorobenzophenone	$C_6H_5 \cdot CO \cdot C_6H_4Cl$	1:40,000
11.	2,4' Dichlorobenzophenone	$Cl\,C_6H_4 \cdot CO \cdot C_6H_4Cl$	1:100,000
12	4,4' Dichlorobenzophenone	$Cl\,C_6H_4 \cdot CO \cdot C_6H_4Cl$	< 1:3,000
13.	Phenyl-p-tolyl Ketone	$C_6H_5 \cdot CO \cdot C_6H_4CH_3$	1:20,000
14.	Di-p-tolyl Ketone	$CH_3C_6H_4 \cdot CO \cdot C_6H_4 \cdot CH_3$	< 1:3,000
15.	o Benzoylbenzoic Acid	$C_6H_5 \cdot CO \cdot C_6H_4 \cdot COOH$	< 1:3,000
16.	Diphenyl Sulfone	$C_6H_5 \cdot SO_2 \cdot C_6H_5$	< 1:3,000
17	Di-p-tolyl Sulfone	$CH_3 \cdot C_6H_4 \cdot SO_2 \cdot C_6H_4 \cdot CH_3$	< 1:3,000
18.	Promin	$(C_6H_4)_2 \cdot SO_2[NH \cdot CH \cdot (CHOH)_4CH_2OH \cdot SO_3NA]_2$	< 1:3,000
19.	Diphenyl Sulfoxide	$C_6H_5 \cdot SO \cdot C_6H_5$	1:5,000
20.	Benzhydrol	$C_6H_5CHOH \cdot C_6H_5$	1:5,000
21.	Tetramethyldiaminobenzhydrol	$(CH_3)_2N \cdot C_6H_4 \cdot CHOH \cdot C_6H_4 \cdot N(CH_3)_2$	1:5,000
22.	Benzanilide	$C_6H_5 \cdot CO \cdot NH \cdot C_6H_5$	< 1:3,000
23.	Benzil	$C_6H_5 \cdot CO \cdot CO \cdot C_6H_5$	< 1:3,000
24.	o-Chlorobenzoic Acid	$ClC_6H_4 \cdot COOH$	< 1:3,000
25.	p-Chlorobenzoic Acid	$ClC_6H_4 \cdot COOH$	1:3,000
26.	o-Chlorobenzoyl Chloride	$ClC_6H_4 \cdot COCl$	< 1:3,000
27.	p-Chlorobenzoyl Chloride	$ClC_6H_4 \cdot COCl$	< 1:3,000

dissolved in dilute HCl, as well as in propylene glycol. Promin was dissolved in water. Controls were run with all diluents. As many of the compounds formed varying amounts of precipitates in the aqueous media, the bacteriostatic actions are not strictly comparable. The results (Table I) show only the highest bacteriostatic dilution for each compound: this was taken as that dilution which showed no macroscopic growth within 6 days. The compounds are listed with their formulae in an order which illustrates the relation between chemical structure and inhibitory action.

The parent compound, benzophenone, showed a moderately high inhibitory effect in a concentration of 1:10,000. p-Hydroxybenzophenone inhibited to a lesser degree (1:5,000). p-Methoxybenzophenone gave a high bacteriostatic effect in 1:20,000 concentration: however, the di-p-methoxy derivative showed a decidedly decreased inhibitory effect, (less than 1:3,000). The p-amino derivative (no. 5) prevented growth only in 1:5,000 dilution. 4,4′-Diaminobenzophenone, as well as its tetramethyl and tetraethyl derivatives, were low in bacteriostatic effect (less than 1:3,000).

The chlorine derivatives showed the highest degree of inhibition of the compounds tested. Both the ortho and the para chlorobenzophenone inhibited growth in a dilution of 1:40,000, while the 2,4′-dichlorobenzophenone inhibited in a concentration of 1:100,000. The 4.4′-dichlorobenzophenone, on the other hand, prevented growth only in a dilution of less than 1:3,000. All the chlorine derivatives gave heavy precipitates in the media in the lower dilutions. Two alkyl derivatives (nos. 13 & 14) were tested. Phenyl-p-tolyl ketone showed a good inhibition, in concentration of 1:20,000; the dimethyl derivative (di-p-tolyl ketone) was bacteriostatic in dilution of less than 1:3,000. o-Benzoyl benzoic acid was the only carboxyl derivative tested: this did not inhibit in dilution of 1:3,000.

Benzhydrol and its tetramethyldiamino derivative (nos. 20 & 21), wherein the central ketone group is partially reduced, were bacteriostatic in concentration of 1:5,000. Benzanilide and the diketone, benzil, showed poor inhibition. Diphenyl sulfoxide prevented growth in dilution of 1:5,000. The diphenyl sulfone was slightly inferior to the sulfoxide, while di-p-tolyl sulfone and promin were no better. Both ortho and para chlorobenzoic acids were poor, as were also ortho and para chlorobenzoyl chlorides.

2. *Bacteriostatic Action of 2,4′-Dichlorobenzophenone on a Slow Growing Virulent Strain. A. In Liquid Media.* A modified Sauton's media was used, 100 cc to each flask. Six flasks were used for each dilution. The strain of tubercle bacilli employed was taken from the first transplant of a growth isolated from sputum. A heavy suspension was made in buffered saline (pH 7.0); 0.2 cc of this suspension, which contained approximately 20 bacilli per oil immersion field, was inoculated into each flask. The drug was tested in concentrations of 1:10,000, 1:30,000, 1:60,000. Controls were run with 1.% propylene glycol. Readings were made at the end of six weeks. The compound inhibited growth in all flasks, including the 1:60,000 dilution, which was the highest dilution tested. Growth in the controls was heavy.

B. In Solid Media. Growth was tested on Lowenstein's egg media. Dilutions of the drug were made in asparagin solution, the egg was then added and the mixture sterilized by heat. No bacteriostatic action was noted. Apparently sterilization of the compound in media with heat destroys its effect.

Summary and Discussion. Various derivatives of benzophenone inhibited the growth of tubercle bacilli. 2,4′-Dichlorobenzophenone was bacteriostatic in high dilution, ortho and para chlorobenzophenone to a lesser degree. These compounds were effective in spite of their relative insolubility in the aqueous media. Attempts are being made to increase the solubility of these compounds without decreasing their bacteriostatic effect. Other halogen derivatives are also being investigated. The para methyl and methoxy derivatives also inhibit growth. It is possible that other higher alkyl derivatives may show an increased effect. All of the di-para de-

rivatives (amino, methyl, methoxy, chloro) were inferior to the mono para compounds. Substitution of the hydrogen atoms of the amino groups with alkyl groups did not increase the effect. Benzil, benzanilide, and o-benzoyl benzoic acid did not inhibit in the lowest dilution. The sulfoxide and sulfone derivatives, including promin, were low in bacteriostatic action, although no analogous halogen or alkyl derivatives of these compounds were tested. 2,4'-Dichlorobenzophenone also inhibited a virulent strain of tubercle bacilli in high dilution in liquid media, but not on solid media.

13872

Lactogenic Hormone Prolongs the Time During Which Deciduomata May be Induced in Lactating Rats.*

ROBERT LYON. (Introduced by W. R. Lyons.)

From the Department of Obstetrics and Gynecology, University of California, San Francisco, California.

The ability to produce deciduomata is an indicator of corpus luteum activity. This functional test has been used as evidence that the corpora were active during lactation in the rat. Inasmuch as deciduomata can be produced as late as the sixteenth day of lactation,[1] the corpora function normally for approximately this duration. Although the corpora do not function for any appreciable period after this time, it has been possible by progesterone administration to prolong the period during which deciduomata may be produced.[2]

Recently[3] purified lactogenic hormone has been given hypophysectomized rats to produce deciduomata. This appears to demonstrate a gonadotropic function of this hormone. In these experiments the corpora lutea appeared large and healthy even when the follicles, as well as the interstitial tissue were below normal. Furthermore in this same work the effectiveness of lactogenic hormone in producing the growths in adrenalectomized rats was demonstrated.

Fifteen lactating rats were subjected to uterine stimulation as late as the twenty-fourth postpartum day and were thereafter injected subcutaneously with 100 I.U. of com-

TABLE I.
Prolongation of Luteal Function by Lactogenic Hormone.

Uterine stimulation postpartum day	Test rats	Deciduoma	Control rats	Deciduoma
20	3	3	2	0
21	3	3	2	0
22	3	3	2	0
23	4	3	2	0
24	2	1	—	—
	—	—	—	—
Total	15	13	8	0

* This study was aided by the Christine Breon Fund for Medical Research.

1 Lyon, R. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 151.

2 Unpublished experiments.

3 Evans, H. M., Simpson, M. E., Lyons, W. R., and Turpeinen, K., *Endocrinology*, 1941, **28**, 933.