TABLE I.
Fifty-seven Adrenalectomized Guinea Pigs with Subcutaneously Implanted Tablets of 3 Different Pregnenolones.

Steroid	Absorbed per day, μg	No. of animals	No. of animals dying on days indicated			% of animals surviving for more than 9 days	Longest survival, days	Loss of wt per day
			2-7	8-9	>9			
Pregn.	716-1466	10	9	1	0	0	9	113
3-acetate	733-2625	22	14	2	6	27	19	140
21-acetoxy	700-1250	25	5	0	20	80	162	65

Prgn. M.P. = 190°; 3-acet. preg. M.P. = 149-150°; 21-acetoxy-pregn. M.P. = 183°.

roids. But since 3-AP is more active than P one wonders whether the cortical action of 21-AOP might be further increased by esterification at C_3 also, *i.e.* using a diester of 21-oxypregnenolone.

Summary. Pregnenolone was unable to keep alive the adrenalectomized guinea pig. There was some cortical activity with 3-acetate-pregnenolone. But the activity of 3-acetate of pregnenolone was much less than that of 21-acetoxypregnenolone.*

* Our thanks are due to Dr. E. Oppenheimer, Ciba Pharmaceutical Products, Summit, N.J., and Dr. Carl Miescher, Ciba, Basel, for a generous supply of steroids.

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On the Antitumorigenic Action of Large Quantities of Pregnenolones.

RIGOBERTO IGLESIAS AND SILVIO BRUZZONE. (Introduced by A. Lipschutz.)
From the Department of Experimental Medicine, National Health Service of the Republic of Chile, Santiago.

Estrogen-induced abdominal fibroids can be prevented by different 3-keto-steroids,[1] of which progesterone is the most powerful.[2] But the original supposition that there is a correlation between progestational and antifibromatogenic action[2,3] had to be discarded when it was shown that enhancement of progestational activity by a substitution at C_{17} is not accompanied by an increase of antifibromatogenic activity; ethinyl- and ethyltestosterone are even less antifibromatogenic that testosterone.[4,5]

So far no steroid with an hydroxyl group at C_3 has been found to be antifibromatogenic; neither 21-acetoxypregnenolone (abbreviated 21-AOP),[3] nor 3-acetate-pregnenolone (abbreviated 3-AP)[6] prevented estrogen-induced fibroids even when quantities several times larger than those of progesterone were used. On the other hand, pregnenolone derivatives may show unexpected activity when administered in very large quantities; progestational[7] and cortical activity[8,9,10] and protective action on the sem-

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2 Lipschutz, A., Bruzzone, S., and Fuenzalida, F., *Cancer Research*, 1944, **4**, 179.

3 Lipschutz, A., Bruzzone, S., and Fuenzalida, F., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 303.

4 Iglesias, R., and Lipschutz, A., *Lancet*, 1946, **2**, 488.

5 Lipschutz, A., Iglesias, R., Bruzzone, S., Fuenzalida, F., and Riesco, A., *Fourth Internat. Cancer Research Congress*, 1947, 23; *Texas Reports of Biology and Medicine*, 1948, in publication.

6 Iglesias, R., unpublished work; see series LXXXI in Table I of the present paper.

7 Selye, H., and Masson, G., *J. Pharm. and Exp. Therap.*, 1943, **77**, 3.

8 Selye, H., *Science*, 1941, **94**, 94.

inal tubules have been observed.[11] For all these reasons we decided to use in experiments with estrogen-induced fibroids, much larger quantities of 21-AOP and 3-AP than those we have formerly used.

Experiments. Pellets of 21-AOP and 3-AP were implanted beneath the skin into castrated female guinea pigs; absorption per day varied according to the number of pellets implanted. A pellet of α-estradiol was also implanted into the same animals. Control animals were implanted with a pellet of α-estradiol only. Results are summarized in Table I.

With subcutaneously implanted pellets of α-estradiol an average fibrous tumoral effect of F.T.E.=5 to 6 was obtained in 90 to 99 days. The simultaneous absorption of 70 of 322 μg of 21-AOP per day did not alter, or altered only very slightly, this result.[3] There was still a remarkable tumoral effect with such quantities as 808 to 1241 (Table I), though the F.T.E. was somewhat diminished. There was no antifibromatogenic action at all with 114 to 506 μg of 3-AP per day; but with larger quantities of 3-AP there was a definite preventive action the F.T.E. dropping from 5 to 1.6 and 1.3. No animal receiving 552 to 1670 μg reached the average F.T.E. of the control group though some may approach it (see range); especially remarkable was the fact that the incidence of fibroids of about 3 to 6 mm in diameter (Class 2 and 3) dropped with large quantities of 3-AP from 2 to 0.1.

The fact that 3-AP was more antifibromatogenic than 21-AOP is all the more remarkable as the cortical activity at 3-AP was considerably less[10] than that of 21-AOP.[9]

Other comparative findings with large quantities of the 2 pregnenolones also may be mentioned. Uterine bleeding, which is induced in guinea pigs by continuous action of estrogens and prevented by 3-keto-steroids, occurred in the present experiments

TABLE I.

Seventy-two Castrated Female Guinea Pigs with Subcutaneous Implantation of Pellets.

No. of series and duration, days	Pregnenolone absorbed per day, μg	α-Estradiol absorbed per day, μg	No. of animals	Avg fibrous tumoral effect F.T.E.*	No. of animals with F.T.E. no less than 5*	Incidence of tumoral marks of classes 2 and 3 per animal	Avg uterine wt, g
XCVIII 92	0	17-47	10	5.1 (1.0-9.0)	5	1.7	5.3 (2.6-10.7)
XCVIII 89-91	21-AOP† 808-1241	30-55	14	3.0 (1.5-5.5)	2	0.9	4.2 (2.0-10.6)
CXXXI 96-99	0	51-79	10	6.4 (1.5-10.0)	7	2.0	6.5 (2.5-14.5)
LXXXI 90-93	3-AP‡ 114-506	36-72	19	5.2 (0.5-10.0)	8	1.8	4.8 (2.5-13.6)
CXXXI 93-96	3-AP 552-980	14-97	10	1.6 (1.0-4.5)	0	0.2	2.2 (1.2-3.5)
CXXXI 94-98	3-AP 1040-1670	40-103	9	1.3 (1.0-3.0)	0	0.1	2.1 (1.4-2.9)

* For the explanation of these units see especially 2.
† M.P. = 183°. Pellets 10 to 12 per animal. Surface of the pellet about 60 mm2. Absorption was of 1.4 to 1.7 μg per mm² (avg 1.5 μg).
‡ M.P. = 149-150°. Pellets 1 to 12 per animal. Absorption per mm² was of 1.5 to 2.8 μg per mm² (avg 2.2 μg).

[9] Bruzzone, S., Borel, H., and Schwarz, J., *Endocrinology*, 1946, **39**, 194.

[10] Bruzzone, S., and Lopez, H., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 578.

[11] Masson, G., *Am. J. Med. Sc.*, 1946, **212**, 1.

with large quantities of 21-AOP but not with 3-AP. There was only a slight and scarcely significant preventive action against the increase of the uterine weight when large quantities of 21-AOP were given; but with antifibromatogenic quantities of 3-AP the uterine weight dropped considerably.

With 3-AP growth of the mammary glands was rather enhanced, as in former work with 3-ceto-steroids.[12,13,14] There was some androgenic action with 1040 to 1670 μg of 3-AP per day as evidenced by the slight enlargement, of the clitoris in 4 out of 9 animals.

The antifibromatogenic action of large quantities of 3-AP was as unexpected as was the estrogenic action of large quantities of androgens.[15] It would be idle to speculate now about the mechanism of similar paradoxical statements. We wish only to mention that pregnenolone has been tentatively assumed to play a role in the biogenesis of steroid hormones[16] and has been subsequently found in the testicle of the pig.[17]

Summary. With quantities of 3-acetate-pregnenolone about 30 to 50 times those of the antifibromatogenic minimum of progesterone, estrogen-induced abdominal fibroids can be prevented in the guinea pig to a very considerable degree. This is the first statement of an antifibromatogenic activity of a steroid with an hydroxyl group at C_3. There was only a slight diminution of the fibromatogenic effect when quantities of 21-acetoxy-pregnenolone as large as 50 to 80 times those of progesterone were given.

Our thanks are due to Dr. Carl Miescher, Ciba, Basel, who suggested work with 3-acetate pregnenolone, for the generous supply of this steroid, and to Dr. E. Oppenheimer, Ciba Pharmaceutical Products, Summit, N.J., for 21-acetoxypregnenolone. Thanks are due to Dr. F. Fuenzalida for the determination of M.P. of the steroids used.

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Failure of Oral Saccharine to Influence Blood Sugar.

CARL A. BUNDE AND ROBERT W. LACKEY.

From the Department of Physiology and Pharmacology, Southwestern Medical College, Dallas, Texas.

A number of investigators have employed saccharine in examining the possibility that a sweet taste results in an alteration in blood sugar level. Various authors have claimed that an increase, [1,2] a decrease,[3] or no significant change[4,5] in blood sugar followed drinking a saccharine solution. The most recent report by Kun and Horvath,[3] and the only one presenting statistically significant data, shows a drop in blood sugar. We were interested in employing this technique as a means of maintaining a low blood sugar but in the face of earlier conflicting evidence, it was planned first to repeat the work under conditions duplicating as nearly as possible those described by Kun and Horvath.

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4 Althausen, T. L., and Wever, G. K., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 517.

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