TABLE V. Effect of Toxic Factor, Isolated by "Salting Out" with $(NH_4)_2SO_4$, on Cleavage of *Arbacia punctulata* Eggs. Coelomic fluids were partially purified by autoclaving, followed by centrifugation and dialysis.

	Partially purified toxic coelomic fluid saturated with $(NH_4)_2SO_4$		Partially purified normal coelomic fluid saturated with $(NH_4)_2SO_4$		
	Precipitate	Supernatant	Precipitate	Supernatant	Sea water control
	(Dialyzed)				
% of eggs cleaved	0	97	94	96	99

dialyzable toxic factor, capable of causing death when injected into normal worms. This toxic substance, originated from the cells of the coelomic fluid, has been partially isolated by saturation with ammonium sulfate. A semi-microbiological assay has been described.

1. Prinzmetal, M., Bergman, H. C., and Kruger, H. E., *J. Clin. Invest.*, 1946, v25, 781.
2. Chaet, A. B., *Biol. Bull.*, 1951, v101, 206.
3. ———, *ibid.*, 1955, v109, 356.
4. Helderweirt, G., Schievers, J., and Simonart, M. A., *Arch. intern. pharmacodyn.*, 1938, v60, 462.
5. LaFontaine, A., *ibid.*, 1946, v73, 113.
6. Simonart, M. A., *Bull. Acad. R. Med. Belg.*, 1947, v12, 116.
7. ———, *ibid.*, 1951, v16, 259.
8. Heilbrunn, L. V., *An Outline of General Physiology*, 3rd ed. W. B. Saunders Co., Philadelphia, 1952.
9. ———, *The Dynamics of Living Protoplasm*, Academic Press, N. Y., 1956.
10. Heilbrunn, L. V., Harris, D. L., LeFevre, P. G., Wilson, W. L., and Woodward, A. A., *Physiol. Zool.*, 1946, v19, 404.
11. Harvey, E. B., *Biol. Bull.*, 1953, v105, 365.

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Reduction of Mitotic Activity in Pinna Epidermis of Mice Given Cortisol or 9α-fluorocortisol.*† (22343)

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The inhibitory effect of cortisone upon mitotic activity in certain tissues seems to be established(1-4). In this study, antimitotic effects were noted for hydrocortisone (cortisol) and for 9α-fluorocortisol. In order to detect antimitotic effects occurring within the range of the daily mitotic rhythm, this work was carried out 1) under environmental conditions standardized for evaluation of 24-hour periodicity(5), 2) during the period of day of relatively high mitotic activity, 3) on a population of mice previously found to exhibit a marked mitotic rhythm in pinna epidermis(6).

Materials and methods. For 7 days prior to experiment, male ZBC mice, 8 weeks of age, were kept singly housed in a room maintained at 80 ± 1°F, illuminated from 6 A.M. to 6 P.M., and kept dark from 6 P.M. to 6 A.M. Purina Fox Chow and tap water were available to the mice *ad libitum*, from the time of weaning and throughout experiment. On the day of experiment, a first group of 11 mice was not handled, while 3 other groups, each composed of 12 mice, were given intraperitoneally 100 γ or 1,000 γ of cortisol acetate or 10 γ of 9α-fluorocortisol acetate. Each mouse was weighed prior to injection and the dose was adjusted to refer to 20 g of body weight. The treatments were given in rotation, in order to minimize the possible role of a time factor difference among treatments.

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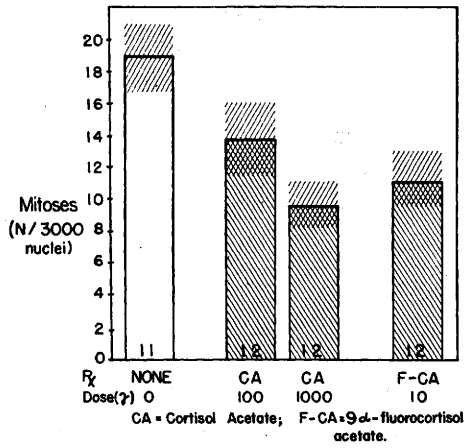

FIG. 1. Effects of cortisol and 9 α-fluorocortisol upon pinna mitoses of intact male ZBC mice (the height of the columns denotes the means, the shaded areas denote ± 1.0 S. E.).

The injections were made from 8:30 A.M. to 9:30 A.M. of the same morning, and the injected and uninjected mice were all sacrificed 4 hours later. The ears were clipped off by means of scissors, the margins were trimmed, and the ears were then placed into Bouin's fixative. The time elapsed from the moment of removal of a mouse from its cage until the immersion of its ear into the fixative did not exceed 10 seconds. Following embedding in paraffin, at least 20 sections of each ear were prepared. The sections, about 6 μ thick, were stained with Heidenhain's iron hematoxylin and were counterstained with eosin. In sections from each of the ears, 3,000 epidermal cells were examined under oil immersion and the total number of mitoses as well as their respective stages were recorded.

Results. The height of the columns in Fig. 1 shows the mean mitotic count for each of the groups studied, the shaded areas denoting ± one standard error. It may be seen that the mice given corticosteroid treatments had lower mean counts, as compared with untreated mice. As far as cortisol treatment is concerned, the group receiving 1000 γ had a lower mean count than that receiving 100 γ. Furthermore, the antimitotic effect of 10 γ of 9α-fluorocortisol compared favorably with the corresponding effect of 100 γ and unfavorably with that of 1000 γ of cortisol. The effects of both 1000 γ of cortisol and 10 γ of 9α-fluorocortisol were significant at the one percent level, as may be seen from Table I. The proportions of the 4 mitotic stages in the groups receiving either 10 γ of 9α-fluorocortisol or 1000 γ of cortisol were, however, roughly comparable to those in the untreated group, as shown in Table II.

TABLE I. Significance of Inter-Group Differences in Mean Mitotic Counts.

Difference studied*	DF	Difference* ± S.E.	t	P
9 α-fluorocortisol acetate 10 γ	21	7.7 ± 2.6	2.94	.008
Cortisol acetate 1000 γ	21	9.1 ± 2.3	3.90	<.001
" " 100 γ	21	4.8 ± 2.9	1.65	.115

* Mean mitotic count of "no treatment" group *minus* the corresponding value for treatment group listed.

The χ^2's for the slight differences in proportions of mitotic stages between the untreated group on one hand and the 10 γ of 9α-fluorocortisol and 1000 γ of cortisol groups, on the other hand, were 2.25 (P>.39) and 1.89 (P>.57), respectively. By contrast, the corresponding difference between the untreated group and the 100 γ of cortisol group was significant below the 5% level ($\chi^2 = 8.53$; P = .037). The latter group differs from the former groups primarily by a higher proportion of prophases and by a lower proportion of telophases. This difference in proportions may have been brought about by the shorter duration of the antimitotic effect of the lower dose of cortisol. Conceivably, in mice given 100 γ of cortisol, at 4 hours post-injection, cells may have entered prophase without inhibition, but as yet, they may not have ad-

TABLE II. Distribution of Mitotic Stages in Groups Investigated.

Treatment	Dose (γ)	% prophase	% metaphase	% anaphase	% telophase
None		26	43	11	20
9 α-fluorocortisol acetate	10	26	37	11	26
Cortisol acetate	1000	22	39	16	23
" "	100	33	44	13	10

vanced to telophase (Table II). This possibility, however, can not be supported without additional data obtained at earlier times after injection.

The main points of this report are the following: First, the administration of cortisol in 1000 γ doses, as well as that of 9α-fluorocortisol in 10 γ doses, results in a significant reduction in number of mitoses in pinna epidermis. Second, doses of the compounds which exhibit significant antimitotic effects do not change significantly the proportions of mitotic stages. Accordingly, these compounds may not interfere with the mitotic process *per se*, but rather, they may act upon some aspect of interphase. Third, a comparison of those doses of cortisol and of 9α-fluorocortisol which exhibit antimitotic activity supports the suggestion that the substitution of a fluorine atom in the 9α position results in a marked enhancement of the antimitotic activity of cortisol. The fluorine-substituted compound has more than 10 times the activity of cortisol, under the conditions of this study. This suggestion is in keeping with the enhancement of other biologic activities of cortisol by fluorine-substitution in the 9α position(7-13).

Summary. Mitotic activity in pinna epidermis of male ZBC mice was studied under environmental conditions standardized for evaluation of 24-hour periodicity, during the period of day of relatively high mitotic activity. Under these circumstances, antimitotic effects, exerted apparently during interphase, were noted for hydrocortisone and for 9α-fluorohydrocortisone. The latter compound had more than 10 times the antimitotic potency of the former.

1. Green, H. N., and Ghadially, F. N., *Brit. Med. J.*, 1951, v1, 496.

2. Studer, A., and Frey, Y. R., *Dermatologica*, 1952, v104, 1.

3. Bullough, W. S., *J. Endocrinol.*, 1952, v8, 265.

4. Teir, H., and Isotalo, A., *Ann. Med. Exp. Biol. Fenn.*, 1953, v31, 171.

5. Halberg, F., *J. Lancet* (U.S.A.), 1953, v73, 20.

6. Chaudhry, A. P., Halberg, F., and Bittner, J. J., *Fed. Proc.*, 1956, v15, 34.

7. Fried, J., and Sabo, E. F., *J. Am. Chem. Soc.*, 1953, v75, 2273.

8. Fried, J., *ibid.*, 1954, v76, 1455.

9. Borman, A., and Singer, F. M., *Fed. Proc.*, 1954, v13, 185.

10. Goldfien, A., Beigelman, P. M., Laidlaw, J. C., and Thorn, G. W., *J. Clin. Endocrinol. and Metab.*, 1954, v14, 782.

11. Liddle, G. W., Pechet, M. M., and Bartter, F. C., *Science*, 1954, v120, 496.

12. Callow, R. K., Lloyd, Y., and Long, D. A., *Lancet*, 1954, v267, 20.

13. Halberg, F., and Spink, W. W., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 222.

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Plasma Enzyme Actvity in Myocardial Infarction in Dog and Man.* (22344)

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Increases of plasma transaminase(1) and lactic acid dehydrogenase in liver disease(2) and myocardial infarction(3) suggested the probability of the release of other enzymes from the myocardium or the liver as a result of hypoxia or tissue necrosis. This laboratory has been interested in the immediate changes following experimental myocardial infarction in dogs. For example, a higher concentration of pyruvate in coronary sinus than in arterial blood (negative myocardial balance of pyruvate) was observed within the first thirty minutes following coronary embolization(4). This phenomenon has also been observed in hemorrhagic shock(5) and in ventricular fibrillation(4). It is likely that the reduction in coronary flow which immediately follows

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