ficity of the sedimentable and non-sedimentable CF antigen preparations were established. The non-sedimentable CF preparation was compared to myxovirus soluble antigen in the sense that this term was used to describe the internal CF antigen of the latter. The sedimentable rubella CF antigen was considered to be "soluble" antigen attached to or associated with cellular material.

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Mitosis-Stimulating Factor in Partially Hepatectomized Rats as Affected by Adrenalectomy and Dexamethasone.* (31946)

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The existence in plasma of partially hepatectomized rats of a factor that stimulates mitosis in the liver of normal rats was first demonstrated by 3 groups: Christensen and Jacobsen(1), Bucher *et al*(2), and Wenneker and Sussman(3). Subsequent work confirmed these findings(4-6) and also demonstrated that after partial hepatectomy mitosis is stimulated in other tissues as well as in the liver (6); this finding suggested a possible relationship between this mitosis-stimulating factor and the mechanism of control of normal cell division and organ size(7). These studies led to attempts to demonstrate and identify in the plasma of hepatectomized rats such a mitosis-stimulating factor or factors. The results reported in the literature are conflicting, with findings indicating that the administration of serum obtained from partially hepatectomized rats (a) stimulates mitosis in the liver of normal rats(4,5) or (b) has no effect at all(8).

The absence of important endocrine glands such as the pituitary does not prevent the appearnce of the mitosis-stimulating factor in cortisone-treated, partially hepatectomized

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rats(6). Based upon this finding and our own with regenerating rat liver(9-11), a study was initiated in which the numbers of mitoses, before and after partial hepatectomy, in the liver(12) and cornea, were determined.

Evidence will be presented to indicate that, under our experimental conditions, the rise in the corneal mitotic index that occurs after partial hepatectomy is absent in adrenalectomized rats and is restored in adrenalectomized rats treated with dexamethasone.

Methods and material. Female rats of the Wistar strain, with weight ranging between 120 and 140 g, were used in all experiments.

Partial (70%) excision of the liver was performed according to the technique of Higgins and Anderson(13). The mortality was low (5 to 10%) in the groups of animals with intact adrenals; subsequently in this paper the term hepatectomy will be used to mean partial (~70%) removal of the liver.

Bilateral adrenalectomy was carried out in groups of rats 3 days before hepatectomy, as indicated under *Results*. Post-adrenalectomy mortality was from 5% to 10% in the different groups. The mortality rate among the adrenalectomized animals that also were hepatectomized, but received no treatment with dexamethasone, varied between 20% and 40% in the different groups; most of the deaths occurred within the first 10 hours after hepatectomy. The surviving rats were in an apparent state of health comparable to that of the control groups(12). The mortality in the adrenalectomized-hepatectomized animals treated with steroid was low and comparable to that found with the normal hepatectomized controls.

All animals were kept on commercial complete diet *ad libitum*. Physiologic saline was given, *ad libitum*, instead of water, to all adrenalectomized rats.

The animals were sacrificed by decapitation, either 24 or 48 hours after hepatectomy. Control non-hepatectomized groups were sacrificed at the same time as some of the hepatectomized groups of animals. All surgery was carried out between 8:00 a.m. and 11:00 a.m.

The eyes were removed from the animals and fixed in Alfac (a mixture containing 85 ml of ethanol 80%, 10 ml of formalin and 5 ml of glacial acetic acid). Total corneas were mounted on glass slides, after staining with Schiff's reagent (Feulgen's reaction)(14). Mitoses were counted with the use of a microscope with a reticulum (125 μ side) ocular (8 $\times$) and oil-immersion lens (100 $\times$); 50 fields (125 $\mu \times 125$ μ) were counted in each cornea.

Treatment with dexamethasone was carried out in the doses and at the times described under *Results*.

Results and discussion. In agreement with the findings of other authors, a significant increase in the number of mitotic cells was found in the corneas of rats sacrificed 48 hours after hepatectomy, as compared to their normal controls. It is interesting, however, that in the group of rats sacrificed 24 hours after hepatectomy, the number of cells in mitosis was decreased to a small but significant ($P = 0.025$) degree, as compared to normal controls.

Whether this depression in the number of cells in mitosis, as found 24 hours after partial hepatectomy, is in any way related either to post-surgical fasting or to changes in the composition of the blood(15), or both, is not clear and requires further study.

On the other hand, the significant increase in the number of cells in mitosis observed in the corneas of the group of animals sacrificed 48 hours after hepatectomy does appear to be related to those aspects of "surgical stress" that are mediated through the adrenal glands and also to decreased inactivation of steroid hormones by the remnant of the liver. This is indicated by: (1) the presence of such a stimulus in hepatectomized rats, (2) the absence of such an occurrence in adrenalectomized-hepatectomized rats, (3) a significant increase in the number of cells in mitosis in the corneas of rats non-operated, but treated with dexamethasone, a potent glucosteroid, and (4) the lower intensity of the mitotic stimulus of the cornea of adrenalectomized rats treated with dexamethasone, as compared to that observed in adrenalectomized-hepatectomized rats treated with dexamethasone (see Tables I, II, and III). As the liver is probably the major site of mammalian catabolism

TABLE I. Mitoses/50 High-Power Fields in the Cornea of Adrenalectomized Rats.

Groups	No. of rats	Cells in mitoses ± S.E.*
Adrenalectomized	10	105 ± 9.4
" treated with saline†	8	139 ± 14.4
" treated with testosterone	8	168 ± 6.5
" treated with estradiol	7	169 ± 11.1
" treated with dexamethasone†	12	219 ± 14.2

Administration of dexamethasone (0.4 mg), testosterone (2.5 mg), estradiol benzoate (0.5 mg), and physiologic saline (0.1 ml) was done by the intraperitoneal route at 4:00 p.m. and all animals were sacrificed on the following day, at about 10:00 p.m.

* Standard error of the mean values.

† Student's test, done to compare these 2 groups, indicated a significant difference ($P<0.01$).

TABLE II. Mitoses/50 High-Power Fields in the Cornea of Normal Rats.

Groups	Before hepatectomy	After hepatectomy	
		24 hours	48 hours
Control	167 ± 11 * (7)†	122 ± 10 (5)	248.7 ± 12.5 (7)
Treated with dexamethasone	202 ± 10.4 (7)	—	305 ± 27 (8)

* Standard error of the mean values.

† ( ) number of animals in each experimental group.

of all steroid hormones, the resection of such a large proportion of its parenchyma (70%) necessarily would affect the inactivation of the steroid-hormones, as it does in states of liver insufficiency(16). Experiments with ^{14}C-labeled steroids will be done, in an attempt to establish a possible relationship between increased half-life of circulating glucosteroid in states of liver insufficiency(16) and the intensity of mitosis in tissues such as the cornea.

The fact that administration of a single dose of 0.4 mg of dexamethasone, 18 hours before sacrifice of the animals, stimulates mitosis in the cornea of non-operated rats and that such a response is obtained with dexamethasone and not with either estradiol or testosterone, used in the experiment as control steroid drugs, strongly suggests a specific glucosteroid effect. The data obtained in this experiment are presented in Table I.

On the other hand, the results that were of particular interest were those observed 48 hours after hepatectomy, when a clear and most intense mitotic rise was found; hence, all subsequent experimental groups were sacrificed either before, or at 48 hours after, partial hepatectomy.

We decided first to investigate whether the administration of dexamethasone to animals with intact adrenals could in any way alter the stimulation of mitosis found after hepatectomy. For this purpose, dexamethasone was administered to some animals, as a single intraperitoneal dose of 0.4 mg given between 11:00 p.m. and 12:00 p.m. These animals were sacrificed between 10 a.m. and 11 a.m. on the following morning. To a second group of rats the same dosage of dexamethasone was administered 1 hour before hepatectomy and, after surgery, this was repeated 2 times: at 24 hours, and at 36 hours. The rats were sacrificed 48 hours after the partial hepatectomy.

The results presented in Table II show that 48 hours after partial hepatectomy, there is a marked increase in the number of mitoses in the corneas of rats ($P<0.01$), as compared to their normal controls before hepatectomy. It is also shown that dexamethasone administration does not inhibit mitosis in the cornea of rats; in fact, the opposite effect was found. The increased number of cells in mitosis seen both before ($P<0.05$) and after hepatectomy in the corneas of the dexamethasone-treated rats, as compared to those observed in the control rats, suggests that the steroid exerts either a direct or an indirect stimulating effect upon mitosis.

To verify whether the post-hepatectomy mitoses stimulating factor observed in the untreated group of rats could reflect simply

TABLE III. Mitoses/50 High-Power Fields in the Cornea of Adrenalectomized Rats.

	Before hepatectomy	48 hr after hepatectomy
Adrenalectomized	105 ± 9.4*(10)†	126.1 ± 12.1 (10)
" treated with dexamethasone	99 ± 9.8 (7)	289 ± 23.7 (20)

* Standard error of the mean values.
† ( ) number of animals in each experimental group.

increased levels of corticosteroid, occurring after partial hepatectomy, experiments with adrenalectomized rats were carried out. Thus, in the following series of experiments, adrenalectomized rats, either treated or untreated, with dexamethasone, were studied both before and after partial hepatectomy. For this purpose, the adrenalectomized rats were divided into 4 experimental groups.

Group I received a single intraperitoneal dose of dexamethasone, 0.4 mg, at 11:30 p.m.; the animals were sacrificed 12 hr later. Group II received 3 doses of dexamethasone, each of 0.4 mg, at 1 hour before hepatectomy, and 24 hr and 36 hr after hepatectomy, respectively. These animals were sacrificed 48 hr after partial hepatectomy. The results obtained with treated and untreated adrenalectomized rats, both before and after partial hepatectomy, are presented in Table III.

The rise in the number of mitoses in the cornea 48 hours after partial hepatectomy, as observed in the control group presented in Table I, contrasts markedly with the absence of such a response in the untreated adrenalectomized group, as shown in Table III. This finding suggests that the adrenal glands, through secretion of corticosteroid hormones, are involved in the stimulation of mitotic activity in the corneas that results from partial hepatectomy in the rat. This hypothesis is strengthened by the striking effect obtained with dexamethasone, as indicated by the significant rise in the number of cells in mitosis observed in the group of adrenalectomized rats treated with this glucosteroid and sacrificed 48 hours after partial hepatectomy, as compared to the absence of such an effect in the untreated adrenalectomized-hepatectomized animals.

In conclusion, our results indicate that glucocorticoid secretion plays an important and determinant role in the appearance of the mitosis-stimulating factor, after the partial hepatectomy of rats. They also suggest that dexamethasone has a stimulatory effect upon the mitotic process that is independent of partial hepatectomy. Experiments with the repeated administration of dexamethasone and naturally occurring glucocorticoids, such as cortisone and cortisol, are in progress in both normal and adrenalectomized animals, to confirm and extend the results described.

Evidence is presented to indicate that, under the experimental conditions used, the increase in the number of cells in mitosis that occurs in the cornea of rats 48 hours after partial hepatectomy, does not occur in adrenalectomized rats. The data presented also indicate that treatment with dexamethasone re-establishes in adrenalectomized rats the response to partial hepatectomy seen in rats with intact adrenals.

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Effect of Nalorphine and Levallorphan on Brain Concentrations of Levorphanol in the Dog.* (31947)

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The remarkable antagonism of narcotic analgesic agents by nalorphine has stimulated recent efforts toward elucidation of the mechanism(s) of narcotic drug action and particularly the afore-mentioned antagonism. One possible explanation of the latter is alteration of CNS distribution of narcotic drug by nalorphine. Since the absolute amount of most narcotic agents present in the CNS is small after pharmacologic doses, studies on alteration of selective CNS distribution depend upon accurate and highly-sensitive methods of drug estimation. The latter requirement has now been met with the availability of radioactive-labeled narcotic drugs. One such radioactive synthetic analgesic, N-C^{14}-methyl levorphanol, has been prepared(1) and was utilized in the present study. This communication reports data obtained in experiments showing the effect of nalorphine or levallorphan on the selective distribution of radioactive-labeled levorphanol in the CNS of the dog after subcutaneous injection.

Methods. 1) Synthesis of C^{14}-methyl labeled levorphanol. Early experiments (Univ. of Michigan, 1959-60) utilized labeled drug prepared by the method of N-formylation and catalytic reduction described previously(1). The more recent studies (Univ. of Iowa, 1964) were done with C^{14}-labeled levorphanol prepared by a modification of the synthesis of N-C^{14}-methyl labeled morphine(2).

A mixture of 120 mg (0.2 mM) of levo-3-hydroxymorphinan, 5 ml of absolute-methanol, 15 mg of C^{14}-paraformaldehyde (0.5 mM, approximately 2 mc/mM) and 0.5 ml (13 mM) 97% formic acid were placed in a 15 ml flask and heated to reflux for 6 hours at 100°C (bath temperature). Then 15 mg of non-labeled paraformaldehyde was added and the mixture was again heated to reflux for another 6 hours. The alcohol and excess formic acid were removed under nitrogen and reduced pressure and the white residue was dissolved in 3 ml of 0.25 N HCl. The crude product was precipitated from the acid by addition of aq. NH_4OH. The mixture was centrifuged and the precipitate washed twice with 1 ml of ice-cold water and dried *in vacuo* in an Abderhalden apparatus for 2 hours at 56°C. The crude product weighed 120 mg (88% from the morphinan reactant) and contained about 10% impurity. A solution of the above impure product in 3 ml of absolute methanol was passed through a column (400 × 9 mm) containing 5 g of neutral alumina and eluted with 80 ml of absolute methanol. After the solvent was removed under nitrogen and reduced pressure in the absence of light, the residue was dissolved in 3 ml of 0.25 N HCl. Insoluble material was removed by passing the solution through filter paper and

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