the comparatively low ATP values in the "cirrhotic" red cells, particularly during storage of blood in the cold, is not clear. A partial inhibition in the phosphoglyceric acid kinase, for example, could result in the increase of DPG and the slightly lowered ATP concentrations. It is difficult to assess the possible influence of abnormal plasma components on the metabolism of the red cells.

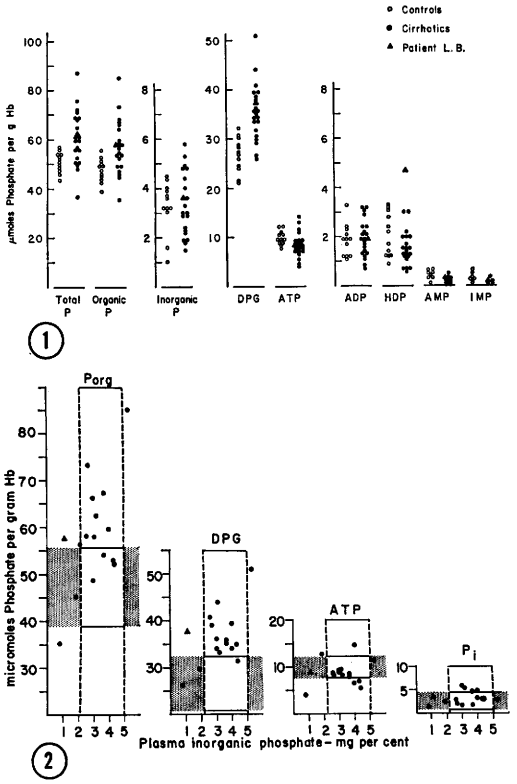

FIG. 1. Partition of acid soluble phosphate compounds in the erythrocytes from controls and from cirrhotics.

FIG. 2. Relation of plasma P_i to red cell phosphate compounds. The control ranges for the red cells and the plasma P_i are shown by the shaded area and by the dotted lines, respectively.

Summary. The concentrations of phosphorylated compounds of freshly drawn washed red cells of cirrhotic patients were determined. A significant increase in the values of P_{org} and DPG was observed in these cells and the remaining compounds, including ATP, were not appreciably affected. After storage of the bloods of cirrhotic patients for 14 and 21 days, the DPG levels were higher and the ADP levels were lower than those of the respective controls.

The technical assistance of Miss Anna Spicer and Mr. Harvey Snapp is gratefully acknowledged.

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Independence of Luteinizing Hormone and Adrenocorticotrophin Secretion in the Rat. (29788)

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Stresses of various sorts can decrease the weights of gonads and accessory sex organs in the rat(1). This has led to the view that stress may inhibit gonadotrophin secretion. Recently, Moore reported that sympatholytic and parasympatholytic drugs which blocked ovulation in rats also evoked adrenocorticotrophin (ACTH) discharge(2). When the rats became tolerant to the ACTH-releasing properties of these agents, they no longer blocked ovulation. Consequently he postu-

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lated that the ovulatory blockade in these rats might be related to the discharge of ACTH, and that there might be a reciprocal relationship between ACTH and luteinizing hormone (LH) secretion. No work on this problem has been reported using a specific assay for LH. Consequently, the following experiments were performed to determine if alterations in the titers of circulating adrenal and ovarian steroids would produce alterations in the secretion of both LH and ACTH or would modify only the secretion of the trophin specific for the particular target gland.

Methods. Adult, virgin female rats, weighing 180-220 g, at the beginning of the experiment, were housed in group cages in a constant temperature room (75°F) with 12 hours of light per day and fed Purina laboratory chow. All operative procedures were performed with the rat anesthetized with ether. Animals were given 0.1 ml of combiotic‡ intramuscularly at the conclusion of the procedure. Adrenalectomized rats were given 1% sodium chloride as drinking water. Rats were injected with various steroids as described in *Results*. At termination of the experiment, the animals were anesthetized with ether and 4-6 ml of blood were removed from the external jugular vein with a heparinized syringe. After centrifugation to remove the cells, the pooled plasma from a group of donor rats was injected intravenously (iv) into immature females of the Sherman strain which had been pretreated with gonadotrophins. The percentage ovarian ascorbic acid depletion was determined by a modification(3) of the method of Parlow(4). In some instances, 2 doses of plasma were used. In others, plasma was tested concurrently with 2 doses of the NIH LH S1 ovine standard.§ In all experiments, plasma from control and experimental donors was tested on the same day to eliminate day-to-day fluctuations in the sensitivity of the test rats as a factor in the results.

Organ weights were determined and the anterior pituitaries were ground in 0.5 ml of 0.9% saline with a stirring rod. Pituitaries from a given group were pooled, centrifuged, and the supernatant extract stored in the frozen state prior to assay. Pituitary LH was assayed using 3 or 4 point assays in Holtzman rats as described by Parlow(4). If pituitary ACTH was to be assayed, the glands were ground in 0.1 N HCl instead of saline. Assay was performed according to the method of Sayers *et al*(5) except that the rats were used 2 days after hypophysectomy; USP reference standard ACTH was the standard in those instances where potency estimates are reported.‖ Standard statistical methods were used for calculating potency.

Results. Plasma LH activity in ovariectomized rats with altered levels of adrenal and ovarian steroids. Ovariectomized rats were used in all experiments since previous studies have demonstrated high levels of plasma LH after removal of the negative feedback from ovarian steroids(6). As expected, significant plasma LH activity was found in ovariectomized controls (3-4 months post-ovariectomy) (Fig. 1, Tables I, II). If these ovariectomized rats were adrenalectomized 3 weeks prior to bleeding, LH activity was if anything slightly enhanced by adrenalectomy ($P<.05$).

The converse experiment, namely, artificially elevating the titer of corticoids in ovariectomized rats, was performed in two ways. First, 6 mg of cortisol acetate/100 g of body weight were injected intraperitoneally 4 hours prior to bleeding. Second, 5 mg of cortisol/rat were injected subcutaneously (sc) daily for 14 days with bleeding on the last day of injection. Slight but insignificant declines in plasma LH activity in these ovariectomized cortisol-treated rats were observed (Fig. 1, Table I). In terms of the LH standard, plasma LH was 3.9 (2.4-6.5)¶ and 4.7 (3.2-6.9) μg/100 ml in the ovariectomized controls and 3.3 (2.0-5.4) and 3.7 (2.3-6.2) in ovariectomized rats treated with single

‡ Penicillin and dihydrostreptomycin in aqueous suspension.

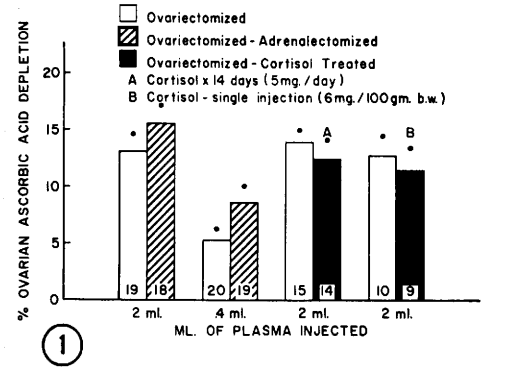
§ This standard was a gift from the Endocrinology study section, N.I.H.

‖ Kindly provided by Dr. Herman Cohen, Princeton Laboratories, Princeton, N. J.

¶ Mean (95% confidence limits).

and multiple injections of cortisol, respectively. Chronic treatment with cortisol produced the expected declines in body and adrenal weight (Table I). The latter was reduced to about 40% of normal, and was small both on an absolute scale and in terms of mg/100 g body weight.

As was to be expected(6), treatment of the ovariectomized rats with a daily dose of 20 μg of estradiol benzoate administered sc for 21 days produced a decline in plasma LH activity (Table II). There was an accompanying decline in body weight. Anterior pituitaries were markedly enlarged, and adrenal weight remained unchanged on an absolute basis. It was increased if calculated in terms of body weight.



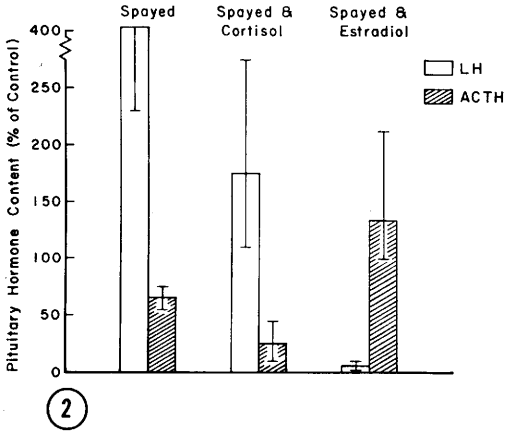


FIG. 1. Effect of adrenalectomy and treatment with a single intraperitoneal or multiple subcutaneous injections of cortisol on plasma LH in ovariectomized rats. Dots represent standard error of mean; numbers at base of columns indicate number of test rats injected with plasma.

FIG. 2. Effects of alterations in levels of ovarian and adrenal steroids on pituitary ACTH and LH. Values are expressed in terms of normal diestrous controls in the case of ovariectomized (spayed) rats and in terms of ovariectomized controls in the case of ovariectomized, steroid-treated rats. Bars represent 95% confidence limits.

TABLE I. Effect of Treatment with Cortisol (5 mg sc/Day) on Organ Weights and Plasma LH Activity in Ovariectomized Rats.

	Type of rat	
	Ovariectomized	Ovariectomized plus cortisol
Body wt (g)	263 ± 4.6 (12)*	181 ± 4.7 (12)
Anterior lobe wt (mg)	10.4 ± .2 (12)	10.3 ± .3 (12)
Adrenal wt (mg)	51.1 ± 3.6 (12)	22.0 ± 1.5 (12)
% Ovarian ascorbic acid depletion†	14.0 ± 1.1 (15)	12.4 ± 1.8 (12)

* Mean ± S.E.M. (No. of rats).
† 2 ml of plasma per assay rat.

TABLE II. Effect of Treatment with Estradiol Benzoate (20 μg sc/Day) on Organ Weights and Plasma LH Activity in Ovariectomized Rats.

	Type of rat	
	Ovariectomized	Ovariectomized plus estradiol
Body wt (g)	254 ± 4.2 (25)*	204 ± 3.2 (37)
Anterior lobe wt (mg)	10.0 ± .3 (24)	35.6 ± 1.9 (37)
Adrenal wt (mg)	60.4 ± 2.4 (25)	58.3 ± 1.5 (37)
% Ovarian ascorbic acid depletion†	15.3 ± 1.2 (23)	5.6 ± 1.7 (25)

* Mean ± S.E.M. (No. of rats).
† 2 ml of plasma per assay rat.

Pituitary LH and ACTH in rats with altered levels of adrenal or ovarian steroids. Pituitary ACTH was slightly lowered by ovariectomy. [Pituitary ACTH content of the ovariectomized controls was 100 (70-140) mU/gland (mean of 2 assays).] This is in marked contrast to the behavior of LH which has been shown repeatedly to rise in spayed rats(6) (Fig. 2). Chronic treatment of ovariectomized rats with cortisol as described above produced a borderline significant ($P<.05$) rise in pituitary LH on comparison with the ovariectomized controls. This same regime caused a marked decline in pituitary ACTH ($P<.01$). Treatment of ovariectomized rats with 20 μg of estradiol benzoate daily for 21 days resulted in either no change (1st assay) or a slight rise ($P<$

.05, 2nd assay) in pituitary ACTH content. Estradiol produced the expected pronounced fall in pituitary LH content. Since the pituitary was markedly enlarged in the estrogen-treated animals there was an actual fall in ACTH concentration and an even more dramatic fall in LH concentration to a level only 2% of that found in ovariectomized controls.

Discussion. Little support is provided by the above studies for a reciprocal relationship between the secretion of ACTH and LH. Rather, each trophic hormone appears to be affected specifically by an alteration in titer of steroid from its target gland. Thus, acute treatment with cortisol at a dosage which completely blocks adrenal ascorbic acid depletion(7,8) did not significantly lower plasma LH. Presumably, in this situation the release of ACTH is much reduced. Chronic treatment with cortisol in a dosage which produced marked adrenal atrophy and a decline in stored pituitary ACTH likewise failed to alter plasma LH and even produced a slightly elevated level of pituitary LH. Adrenalectomy was followed by an increase in plasma LH of borderline significance. By contrast to this borderline change, dramatic rises in plasma ACTH of the adrenalectomized, etherized rat have been demonstrated (9).

The results obtained with alterations in levels of ovarian steroids were more suggestive of a reciprocal relationship between the secretion of these 2 trophins. Ovariectomy did not increase pituitary ACTH, whereas it clearly elevates LH. Actually, the level of ACTH in the gland was somewhat lowered. Treatment of the ovariectomized rat with large doses of estrogen produced a lowering of both plasma and pituitary LH at a time when adrenal weight was maintained and even increased in terms of body weight. Pituitary ACTH showed a tendency to increase in this situation. Thus, with estrogen treatment LH secretion was inhibited, but ACTH secretion was maintained or even enhanced; a result which is in agreement with reports in the literature that estrogen can stimulate ACTH secretion(10).

Thus the feedback control of the secretion of ACTH and LH exerted by their target gland steroids appears to be relatively specific. Increases and decreases in corticoids cause reciprocal effects on ACTH secretion but have little influence on LH secretion. Decreases in ovarian steroids appear to stimulate LH secretion but not ACTH secretion. On the other hand, an elevation in estrogen may stimulate ACTH secretion while inhibiting LH secretion. This is the only real suggestion of a reciprocal effect on ACTH and LH. It appears that estrogen acts on sensitive hypothalamic elements to augment secretion of ACTH(11) and to inhibit that of LH(12). Incidentally, estrogen has also been shown to augment the secretion of prolactin (13,14) by a central action.

Stressful situations which are accompanied by a release of ACTH appear not to activate LH secretion since no ovarian ascorbic acid depletion is produced by a variety of stressful stimuli in both adult and immature rats which have been pretreated with gonadotrophins(3). Furthermore, ovulation which is accompanied by an outpouring of LH(15) is not associated with detectable release of ACTH as measured by adrenal ascorbic acid depletion(16). All in all, it appears that discrete mechanisms regulate the secretion of ACTH and LH. Recently, we have been able to show that the corticotrophin-releasing and LH-releasing factors in hypothalamic extracts are also separate entities(17). These releasing factors presumably constitute the link between the hypothalamic regulatory mechanisms and the adenohypophysis.

Summary. 1. Adrenalectomy of spayed rats produced a borderline rise in plasma LH, whereas acute or chronic treatment with large doses of cortisol failed to alter plasma LH. Chronic treatment with cortisol lowered pituitary ACTH and elevated pituitary LH slightly. 2. Treatment of ovariectomized rats with a large dose of estradiol benzoate lowered plasma LH while adrenal weight remained unchanged. Since body weight fell, the ratio of adrenal to body weight rose. Pituitary LH was markedly lowered by this treatment, whereas pituitary ACTH was slightly elevated. 3. It was concluded that alteration in target gland steroids produces

specific effects on LH in the case of ovarian steroids, and ACTH in the case of cortical steroids. Estrogen can stimulate ACTH secretion at the same time that it inhibits LH secretion.

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Further Tests in Hamsters for Oncogenic Quality of Ordinary Viruses and *Mycoplasma*, with Correlative Review.* (29789)

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The convincing evidence for primary role of viruses in neoplasia in animals together with the outstanding quality of ordinary nature of such viruses makes it expedient to examine the possible role of virus in carcinogenesis in man. During mid 1961, this laboratory undertook to examine the oncogenic potential for newborn hamsters of a wide variety of viruses which represent the major groups(1) of viruses of the human species and, in addition, some viruses of animal or avian origin with which man may have contact. In a first report(2), the findings in tests of 25 different viruses were recorded. In that report, the oncogenicity of types 12 and 18 adenovirus(3,4) was confirmed, the neoplastic potential for type 7 adenovirus was shown, and the appearance of tumor in 2 animals given Bryan strain Rous sarcoma was recorded. In a second report(5), the primary role of all of 3 newly isolated strains of type 7 adenovirus was shown and details relating to the virology, pathology and immunology of type 7 adenovirus carcinogenesis was presented. The present report records the failure of 25 additional virus or *Mycoplasma* strains to induce tumor when inoculated into newborn hamsters and discusses the relationship of oncogenicity to family, biophysical, biochemical and structural properties of the viruses studied.

Materials and methods. Virus preparations. Details relating to passage history, host tissue used to prepare virus for hamster inoculation, and the infectivity titer of each virus are presented in Table I. Certain of the viruses were freshly isolated from human specimens. Herpes simplex virus was obtained from Dr. E. H. Lennette; reovirus 1 and Coxsackie A10 and A21 from Dr. H. Wenner; arbovirus C strain Marituba from Dr.

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