extent of their secretion by the stomach, new determinations of the pK_B values and absorption maxima were made. A somewhat better correlation of pK_B and stomach:blood ratios was obtained by using commercial instead of pure samples of dyes. The distribution of the dyes between benzene and aqueous buffers of pH 2 and pH 6.73 was measured but no satisfactory correlation found. The method is limited by the tendency of compounds to dissolve 100% in either the buffer or the benzene.

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Inactivation of 11-Dehydro-17-Hydroxycorticosterone by Tissue Slices.* (19391)

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Numerous investigations carried out during the past several years have clearly demonstrated that the liver is the organ most concerned with the inactivation of estrogens, testosterone and desoxycorticosterone(1-3). However, when 11-dehydro-17-hydroxycorticosterone (cortisone) became available for study, it became apparent that degradation of such 11-oxygenated steroids might differ from that of estradiol, testosterone and desoxycorticosterone. The latter steroids are practically ineffective when given orally, whereas cortisone§ is approximately 80% as effective orally as parenterally. The following data however, strongly suggest that the 11-oxygenated steroids are rapidly inactivated by the body. A very small percentage of administered cortisone can be recovered from the urine of the patients. The finding of relatively high concentrations of 11-oxygenated steroids in the adrenal vein blood, and of very little in peripheral blood(4,5) is also suggestive of inactivation. deAndino, Fontan, and Pashkis (6) showed that intrasplenic injection of cortisone was ineffective in decreasing thymic weight unless huge doses were administered, thus implicating the liver in the inactivation of this steroid.

The purpose of this study was to determine which organs of the body might inactivate cortisone *in vitro.* In addition, experiments were devised to ascertain whether the inactivation mechanism could be modified.

Methods and materials. Rat tissue slices taken from normal male rats immediately after decapitation were used for studies of cortisone inactivation. Liver, kidney, spleen, muscle, and brain were sliced by hand; 300 to 350 mg of tissue were incubated in 5 ml of Krebs-Ringer solution (pH 7.4) with 100 to 250 μg of cortisone for 3 hours at 37°C. After incubation, the tissue slices were homogenized in a Ten Broeck homogenizer, kept at 4°C overnight and the whole homogenate bioassayed on the following morning as described below. Two types of control were used: 1) tissue slices which had been boiled previously for 10 minutes; 2) non-incubated controls to which the cortisone was added immediately before bioassay. This was performed to exclude the possibility of adsorption of the steroid to the tissue protein. Cortisone, in the form of the free alcohol, was prepared in 95% ethanol in the concentration of 1 mg/ml and 0.1-0.25 ml of this standard solution were

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added to the Krebs solution. The amount of cortisone remaining after incubation was determined by bioassay according to the method of Venning, Kazmin, and Bell(7) with certain modifications. Male mice (Rockland Farm strain) weighing 20 to 25 g and adrenalectomized 3 days previously, were used. They were fasted for 12 hours, and on the morning of the assay each mouse received 0.25 ml of the homogenate to be assayed plus 0.25 ml of 5% glucose in physiological saline at 2-hour intervals for 4 injections. Approximately equal numbers of control and experimental animals were used. One hour after the last injection, the animals were weighed and killed by severing the vertebrae. The livers were removed within 30 seconds and glycogen determined by the method of Nelson(8). Dose response curves for mouse liver glycogen were established at levels of 10, 25, 50, 75 μg of cortisone. With each experiment, cortisone was incubated with boiled tissue slices and the amount of cortisone recovered in this manner was used as the index for calculating the loss of activity of the cortisone incubated with the living slices. Occasionally, the recovery appeared to be greater than 100%; this indicated only that in the particular experiments the glycogen deposition was greater than the mean value obtained on the standard curve. Liver glycogen analyses, after the administration of cortisone and cortisone with boiled tissue slices were performed on 586 mice with an accuracy of ±20%.

TABLE I.

No. of assay animals (control + exp.)	Dose of cortisone incubated (per ml) μg	Recovery, μg: Controls	Recovery, μg: Fresh tissue	Inactivation, %
Inactivation of cortisone by liver slices				
15	20	25	14	44
12	20	15	7	55
12	20	15	6.5	57
12	20	24	4	65
7	20	25	0	100
9	20	15	0	100
10	.5 ml (Ad. cort. extr.)	50	14	72
Inactivation of cortisone by spleen slices				
11	20	26	3.5	87
11	20	40	5.5	86
14	42	52	16	69
25	50	63	13	79
16	50	42	30	29
16	50	58	28	52
16	50	100	34	66
Inactivation of cortisone by kidney slices				
12	20	20	4	80
14	20	20	5	75
8	20	17	9	46
12	20	16	8	50
10	42	32	14	56
11	.5 ml (Ad. cort. extr.)	41	18	56

TABLE II. Inactivation of Cortisone by Muscle, Brain and Blood Serum.

No. of assay animals (control + exp.)	Dose of cortisone incubated (per ml) μg	Recovery, μg: Controls	Recovery, μg: Fresh tissue	Inactivation, %
Muscle				
9	20	17	15	17
10	20	11	18	0
12	42	23	14	22
11	42	51	45	17
14	50	17	20	0
Brain				
13	20	20	37	0
12	42	70	38	46
14	42	15	20	0
13	50	54	49	9
Blood serum				
9	20	24	20	16
13	20	20	17	15
11	20	19	22	0
12	42	48	42	12

Results. I. Tissue slices. The results are presented in Tables I and II. Slices of liver, kidney, and spleen inactivate cortisone to approximately the same extent, under the conditions of this experiment. The average percentage of degradation by liver slices was 70%; kidney, 61%; spleen, 70%. Similar results were obtained using Upjohn's Aqueous Adrenocortical Extract (Table I). On the other hand, incubation with muscle, brain, and blood serum caused negligible inactivation of the cortisone (Table II).

II. Tissue homogenates. DeMeio, Rakoff, Cantarow, and Pashkis(9) and Coppedge, Segaloff, Sarett, and Altschul(10) demonstrated that while liver slices were capable of inactivating estradiol, homogenized liver lost most of this ability. They concluded that cellular integrity must be intact for the ef-

TABLE III. Inactivation of Cortisone by Homogenized Liver Slices.

No. of assay animals (control + exp.)	Dose of cortisone incubated (per ml), μg	Recovery			Inactivation	
		Controls	Homogenized tissue, μg	Fresh tissue	Homogenized, %	Fresh, %
7	42	32	35		0	
9	42	43	33		23	
14	50	70	40	24	43	66
13	50	41	24	12	41	71
11	50		29	10	42	80

ficient working of the enzyme system. The addition of certain cofactors (nicotinamide or diphosphopyridine nucleotide) to liver homogenates restored the inactivation system to normal. Similarly, incubation of homogenized liver instead of liver slices resulted in a definite decrease from 70% to 25% in the percentage of inactivation of cortisone, under these conditions (Table III).

III. Influence of other steroids. Pregnenolone, testosterone propionate, desoxycorticosterone, progesterone, and cortisone were administered to normal rats for a period of 10 days, and liver slices from these animals incubated with cortisone as above. This was done in an attempt to ascertain whether the enzyme system responsible for the inactivation of cortisone could be saturated by other steroids. None of these steroids exerted an inhibitory effect on the inactivation of cortisone. Under these conditions, the average percentage of inactivation was 71% as compared to 70% for the controls.

IV. Influence of stress. Liver slices from adrenalectomized rats, maintained for a week on 1% NaCl as drinking water, were also tested. No significant difference between the inactivation capacity of liver tissue from such animals as compared to normal ones was apparent. Normal rats were exposed to cold (4°C) for 24 hours and their livers tested. This procedure also produced no change in the percentage of inactivation.

V. Effect of incubation with glucuronidase. Recent investigations have demonstrated that the great bulk of urinary cortico-steroids are conjugated as glucuronides(11,12). Thus in order to break any possible glucuronide linkage which might be responsible for cortisone inactivation, 3,000 units of animal glucuronidase were added to each 5 ml homogenate after the usual 3 hours of incubation. They were then incubated again for 24 hours at 37°C. This procedure did not increase the recovery of cortisone. The percentage of inactivation after glucuronidase incubation was 69% while the values for the controls averaged 71%.

Discussion. Cortisone can be inactivated by kidney and spleen as well as liver slices. This is in direct contrast with the estrogens, androgens and desoxycorticosterone which appear to be degraded primarily by the liver. Although nothing is known of the enzyme system involved, it is clearly heat-labile, and certain cofactors are necessary for its efficient operation. This is evidenced by the fact that destruction of the integrity of the cells by homogenization markedly reduces the degree of inactivation of cortisone. Similar results have been obtained with other steroids(9,10).

Sayers(13) has postulated that under conditions of stress, there is greater peripheral utilization of adrenal steroids. It was thought to be of interest to determine whether under conditions of stress, tissues inactivated cortisone to a greater extent. Liver slices of rats exposed to cold exhibited the same degree of cortisone inactivation as normal or even adrenalectomized animals. The pre-administration of pregnenolone, testosterone, progesterone, desoxycorticosterone, and cortisone in an attempt to saturate the inactivation system was also ineffective.

Summary. 1. Slices of liver, spleen, and kidney incubated in Krebs solution for three hours with added cortisone caused approximately a 70% loss of cortisone activity. Blood serum and brain or muscle slices were inactive. Tissue homogenates were much less effective than slices. Incubation of the tissue-cortisone

mixture with the enzyme, glucuronidase did not alter the experimental findings. 2. There was no detectable difference in the capacity of tissues of stressed or adrenalectomized animals, as compared to tissues from normal rats, to inactivate cortisone. The inactivation mechanism could not be altered by the pre-administration of progesterone, pregnenolone, testosterone, desoxycorticosterone or cortisone.

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Relation of Stage of Cycle and Source of Luteinizing Hormone to Superovulation in Dairy Cattle.* (19392)

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In research to develop practical technics for egg transplantation in dairy cattle, the first problem is that of superovulation. This problem has already been investigated by various workers(2-10). When the research reported here was initiated, some workers(2,3,10) had injected cows or heifers with gonadotrophins during the luteal phase of the cycle without expressing the corpus luteum, and the evidence indicated, although not conclusively, that cows superovulated and bred under these conditions produced infertile eggs. Rowson(8) later reported that eggs, shed by cows which had been bred and which had a functioning corpus luteum, were infertile. Various sources of gonadotrophins have been employed by different workers, including unfractionated sheep pituitary gonadotrophin (USG) and human chorionic gonadotrophin (HCG) for intravenous injections to rupture the follicles. No one has compared these two gonadotrophins quantitatively.

This paper reports the results of two series of investigations with heifers and cows to study primarily a) the effect of the stage of the cycle upon ovarian response to gonadotrophins and b) the quantitative difference in response to USG and HCG.

Experimental. Study of stage of cycle, dosages of FSH and two sources of LH. The first series consisted of a factorial experiment. It was designed to study follicular development and number and fertility of ova as affected by: a) initiation of treatment on the fourth day of the cycle (luteal) versus sixteenth day (follicular), b) 3 levels of follicle stimulating hormone (FSH), namely 20, 30 and 40 g-equivalents (g.e.) of desiccated sheep pituitaries, and c) one g.e. of USG versus 2500 I.U. of HCG (Choriogonin) for the intravenous injection. In this experiment 12

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