taining 1.9 cc of Ringer's solution and 0.1 cc of immune serum had only slight inhibitory potency or none at all. The observations may have a relation to a finding already reported—namely that the effectiveness of normal guinea pig serum against 6C3HED lymphoma cells *in vivo* is enhanced upon admixture with an immune serum made in rabbits with the 6C3HED lymphoma cells as antigen(1)—and it may have other implications also. The possibility that one or another of the components of complement may participate in the reactions responsible for these *in vivo* effects has been discussed in a previous paper(1). The question is now being tested whether immune serum alone, or mixtures of normal guinea pig serum and immune rabbit serum, will bring about regression of established AKR lymphomas *in vivo*.

Summary. In the experiments here given, as in those previously reported, normal guinea pig serum, given in amounts of 1 or 2 cc intraperitoneally to C3H mice one hour following the implantation of 2 million 6C3HED lymphoma cells in their subcutaneous tissues, always markedly inhibited growth of the lymphoma cells, and when 2 cc of the serum was given on the day of implantation and again on the 2 succeeding days the inhibition was regularly complete. In striking contrast, normal guinea pig serum, even when given repeatedly in the largest feasible amounts, had little or no inhibitory effect *in vivo* on the cells of 7 AKR lymphomas recently transplanted in AKR mice of the line in which they had originated, and it manifested only limited effectiveness against the cells of a single AKR lymphoma that had been transplanted during about 2 years in laboratory-bred mice of the line in which it had originated with later transfer to AKR mice of another inbred line. An immune serum, prepared by injecting rabbits with the lymphoma cells of one of the AKR growths together with Freund's adjuvants, proved moderately to markedly inhibitory for the AKR lymphoma cells when injected intraperitoneally into mice one hour after implantation of the cells in the subcutaneous tissues, and mixtures of the immune serum with normal guinea pig serum were often completely inhibitory. The significance of the findings is briefly discussed.

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Secretion of 17-Hydroxycorticosterone by Adrenal of Hypophysectomized Dog: Effect of ACTH.* (21233)

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The rate of secretion of 17-hydroxycorticosterone by the adrenal of the hypophysectomized dog prior to and following injection of ACTH has been determined to elucidate the time-relationships concerned in the activation of adrenal secretion.

Methods. Male dogs weighing 11.3 to 19.5 kg were anesthetized with sodium pentobarbital and hypophysectomized via the oral approach. The animals were maintained under light sodium pentobarbital anesthesia throughout the remainder of the experiment. At 3.25 to 12.7 hours after the hypophysectomy the left lumboadrenal vein was cannulated

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for collection of adrenal venous blood. The adrenal vein and all collateral branches of the lumboadrenal vein were ligated. Seven to 10 ml of 1% heparin solution were injected intravenously. A small amount of heparin was added to each collection vessel. Control samples of adrenal venous blood were collected over 3 consecutive 10-minute intervals. ACTH‡ was then injected intravenously as follows: 1) 40 units were injected as the initial dose, 2) thereafter 8 units were injected every 10 minutes for 50 minutes. Adrenal venous blood was collected continuously during this period in samples representing 2-minute intervals for the first 10 minutes (with the exception of Exp. I in which the first post-ACTH sample was collected over a 10-minute interval) and 10-minute intervals thereafter. Each sample of adrenal venous blood was diluted with an equal volume of distilled water and extracted twice with a volume of chloroform equal to that of the blood-water mixture. Centrifugation was necessary to break the emulsion which formed during extraction. The residue from the chloroform extract was partitioned between 70% ethanol and hexane. Each 70% ethanol fraction was resolved by paper chromatography (48 hours) in a toluene-propylene glycol system(1). 17-Hydroxycorticosterone was located on the developed chromatograms with an ultraviolet lamp. The 17-hydroxycorticosterone was eluted with methanol and quantitatively analyzed by a modified Porter-Silber method(2). The results are expressed as μg 17-hydroxycorticosterone/kg body weight/minute. Recovery of 17-hydroxycorticosterone from chromatogram was determined and found to be consistently over 90%.

Results. The results of this study are summarized in Fig. 1. In 4 of the 5 experiments the post-hypophysectomy interval was greater than 4 hours and the control level of secretion of 17-hydroxycorticosterone was .06 $\pm$ .02 μg/kg/minute. This rate is approximately 1/10 of the average (.675 $\pm$ .2 μg/kg/minute) obtained from 4 intact anesthetized dogs bled under the same conditions(3). The low

‡ Armour "Acthar", 1.4 units/ml in .9% NaCl made slightly acid with HCl.

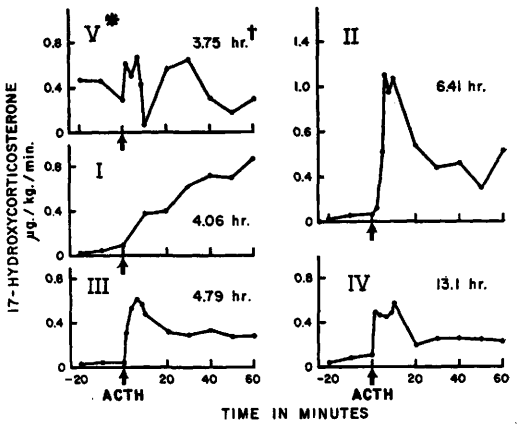

FIG. 1. Secretion of 17-hydroxycorticosterone by adrenal of hypophysectomized dog before and after intravenous ACTH as determined by analysis of adrenal venous blood.

* Exp. No. (see text).

† Time in hr between hypophysectomy and initial injection of ACTH.

level of secretion at 4 or more hours after hypophysectomy is in agreement with findings reported earlier from this laboratory(4). In Exp. V (3.75 hours post-hypophysectomy) the average control level of secretion was higher (.416 μg/kg/minute) than in the other experiments; the level declined in Exp. V during the 30-minute control period.

The rates of secretion of 17-hydroxycorticosterone 2 minutes after ACTH injection were significantly higher ($P < .05$) than the control rates. The over-all average for the 60 minutes following the initial injection of ACTH was .502 μg 17-hydroxycorticosterone/kg/minute, which when compared with the average of .675 $\pm$ .2 μg/kg/minute found in intact anesthetized dogs(3) indicates that a near-normal level of secretion was obtained; however, the adrenal secretory response varied markedly among the experimental animals. In 3 experiments (II, III, IV, Fig. 1), the response was a burst of 17-hydroxycorticosterone secretion followed by a decline to an approximately constant level. In one experiment (V) the response was biphasic, consisting of a primary increase immediately following the first ACTH injection and a secondary increase occurring at 20 minutes. By contrast, in one experiment (I) the response consisted of a gradual increase in the rate of secretion

over the 60-minute interval studied. The reasons for this variability in response are not clear at this time.

An important aspect of the problem of the response of the adrenal to ACTH is the relative role of increased *synthesis de novo* as compared to the *release of stored hormone.* The finding of a sudden increase of 17-hydroxycorticosterone secretion immediately after ACTH (Exp. II, III, IV, V) suggests the release of preformed 17-hydroxycorticosterone or the rapid conversion to this steroid of closely related precursors which may have accumulated in the gland during the period following hypophysectomy. Analysis of the steroid content of the gland during the post-hypophysectomy period may shed light on this important problem.

The data presented here permit an estimate of the speed with which the pituitary-adrenal system is capable of responding to a stressful stimulus. Increased pituitary ACTH secretion is known to occur within one minute after exposure of the animal to stress(5-7). The experiments reported here do not give an *exact* determination of the time-lag between the entrance of ACTH into the circulation and the beginning of increased secretion, but they indicate that it is less than 2 minutes. It seems evident that by 3 minutes (pituitary time-lag plus adrenal time-lag) after the application of a stressful stimulus the pituitary-adrenal system will have become activated to secrete increased amounts of corticosteroids. This finding is of interest in view of the work of Sayers and Sayers(8) who found a maximum decrease in the ascorbic acid content of the rat adrenal within 30 minutes after stress or ACTH. Our findings are also in agreement with those reported by De Gurpide(9) who found an increase in the 17-hydroxycorticosterone content of adrenal venous blood of the dog a few minutes after ACTH injection. They are also in agreement with the findings of Hechter *et al.*(10) who demonstrated an increased output of steroid by the isolated perfused bovine adrenal within 30 seconds after the addition of ACTH to the perfusate.

Summary. The rate of secretion of 17-hydroxycorticosterone by the adrenal of the dog as determined by chromatographic analysis of adrenal venous blood reached a low and apparently constant level within 4 hours after hypophysectomy. Two minutes after intravenous ACTH injection in these animals, the rate of secretion was significantly increased.

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