fat. Carbohydrates and proteins had no effect. 2. The same phenomenon was found in human beings and factors influencing alkaline phosphatase serum levels are discussed.

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Elaboration of Steroid Hormones by Surviving Adrenal Tissue Slices Obtained from Thermally Stressed Dogs.* (20736)

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In a previous communication(1), a rapid method was described for estimating the secretory activity of surviving adrenal cortical tissue slices. Essentially, the method consisted of incubating dog adrenal cortical tissue in the animal's own serum with added antibiotics in a Warburg apparatus for 24 hours. Following incubation, the hormones were extracted from the tissue and medium, purified by florosil column and paper chromatography methods and quantitatively estimated. The amount of the individual hormones elaborated by surviving adrenal cortical tissue from normal dogs was found to be fairly constant when the value was expressed as micrograms of hormone per milligram of adrenal cortical nitrogen.

Methods. Healthy adult male dogs were anaesthetized with nembutal, shaved, and 30% of the body surface burned by multiple exposure to a bank of 43 750-watt reflector photolamps for 0.8 second(2). This exposure was sufficient to cause a third or deep second degree burn. The dogs received no supportive therapy and were allowed food and water *ad libitum*. Four dogs were sacrificed 24 hours after burning and 4 others 72 hours after burning. The adrenal glands were incubated as described(1) except that two incubation vessels were used, one with and one without corticotropin.† Five dogs were given large doses of corticotropin‡ over a period of 24 hours and sacrificed immediately. Two dogs received the corticotropin intramuscularly in divided doses at 8-hour intervals, and three by continuous intravenous infusion of corticotropin dissolved in 5% glucose in water. Slices of adrenal tissue were similarly incubated.

Results. As may be seen in the Table, there was a marked increase in the amount of hormone produced by the adrenal slices obtained from dogs sacrificed 24 hours after burning compared with the values obtained by incubating adrenal slices from normal dogs. The dogs which received corticotropin for 24 hours prior to sacrifice also showed increased elaboration of steroid hormones, but the increase was less marked than in the burned animals particularly with respect to 17-hydroxycorticosterone (Compound F). There was no significant difference observed between the dogs which received the corticotropin by I.M. or I.V. injection. The adrenal slices from the dogs which were sacrificed 72 hours after burning showed

* The opinions expressed herein are those of the authors and should not be construed as necessarily representing the opinion of the Naval Service.

† Each vessel contained 10 mg of corticotropin (Armour lot No. 128-105R, potency 1.6 times that of U.S.P. standard) supplied by Dr. Irby Bunding, Armour Laboratories, Chicago.

‡ Approximately 7 U. S. P. units/Kg of ACTHAR®.

TABLE I. Steroid Hormones Isolated after Incubation of Dog Adrenal Tissue Slices. Figures in parentheses refer to No. of dogs per experiment.

Group	Condition of dog		Compound (μg/mg adrenal N.) F	E(?)	B	A	S
A	Normal (8)	Mean	2.6	1.6	.85	.95	.76
		S.E.	.17	.10	.07	.04	.08
B	24 hr after burning (4)	Mean	4.2	2.5	1.6	1.4	1.6
		S.E.	.08	.12	.14	.06	.19
		P_A	<.001	<.001	<.001	<.001	<.001
C	72 " " " (4)	Mean	1.8	1.8	.91	.85	.84
		S.E.	.03	.18	.13	.08	.22
		P_A	.01	—	—	—	—
D	ACTH treated (5)	Mean	3.4	2.3	1.6	1.4	1.3
		S.E.	.05	.22	.16	.10	.09
		P_A	<.01	<.01	<.001	<.001	<.01
		P_B	<.001	—	—	—	—

Corticotropin was added to incubating medium in these experiments. "P" values were obtained from standard formulae(9) and indicate significance of the differences from the other groups as indicated by the subscripts. They are omitted except when P<.02.

a return to control levels of hormone elaboration with the exception of the normally predominant hormone, Compound F, which was consistently lower than the untreated value.

All of the adrenal slices regardless of previous treatment responded to *in vitro* corticotropin. In our experiments, those flasks which contained corticotropin consistently showed a production of hormones about 60% greater than the controls incubated without corticotropin. We did not observe any one hormone that was particularly increased by *in vitro* corticotropin. These observations are in accord with the findings of Haynes, Savard, and Dorfman(3), and of Hofmann and Davison(4).

The identity of the compound whose rate of descent on the paper chromatograms corresponded to that of Compound E is in question because the compound recovered from the paper chromatograms failed to give the characteristic color with iodine and potassium iodide ordinarily obtained with authentic Compound E(5).§ Experiments are in progress to obtain sufficient amounts of this hormone for further analytical procedures.

Discussion. The increased production of adrenal cortical hormones after a stressing situation as evidenced by an increased excretion of 17-ketosteroid hormones in the urine has been observed by numerous investigators. In the present experiments, there was a slightly greater increase in hormone production after burning than that obtained by administering large amounts of corticotropin. This finding is of interest in the light of experiments of Selye(6) and Liddle *et al.*(7) who have presented evidence for the existence of an adrenal growth factor distinct from corticotropin. The present data suggest that stimulation of adrenocortical activity cannot be achieved by the particular dose of exogenous corticotropin employed to the same degree of intensity within a 24-hour period as a severely stressing situation.

It is of interest to note that there was no disproportionate rise in the elaboration of any particular hormone when the dog was traumatized by burning. The return of the hormone production to normal values in 72 hours is in agreement with the experiments of Weichselbaum, Margraf, and Elman(8) who found a somewhat similar condition in the 17-hydroxycorticosteroid levels of blood from patients subjected to surgical procedures. The disproportionate depression in the production of Compound F by the adrenal slices obtained 72 hours after burning in the face of normal values for the other hormones is of special interest. It is conceivable that such an alteration in the hormone pattern following severe stress may play a role in the etiology of the so-called diseases of adaptation.

Summary. Adrenal slices from dogs sacri-

§ The authors are indebted to Drs. G. L. Farrell and D. H. Nelson for suggesting this possible discrepancy.

ficed 24 hours after burning showed a marked increase in the elaboration of hormones compared with the values observed for adrenal tissue from normal dogs. Prior treatment of dogs with 7 U.S.P. units of corticotropin per kg also caused the production of more hormone than untreated controls, but the increase in Compound F was significantly less than in the tissue from burned dogs. Adrenal glands from dogs which were sacrificed 72 hours after burning showed a return to control levels of hormone production with the exception of Compound F which was significantly less than the normal value.

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A Simple Procedure for Separation of Prothrombin and Accelerator Globulin from Citrated Human Plasma.* (20737)

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In studies of the mechanism of blood coagulation, it is frequently desirable to make use of purified systems rather than plasma or serum from which one or more of the components have been removed. As more data become available on the various properties of the clotting components in different species, the advisability of working with systems derived from a single species become apparent. A number of practical difficulties are encountered in attempting to prepare purified fractions from human plasma by methods designed for use with bovine material. The relative lability of human accelerator globulin (Ac-G) and purified human prothrombin makes it necessary to use fresh plasma. This accentuates the difficulty of obtaining sufficient plasma to apply efficiently the methods previously used for large quantities of bovine plasma. In addition, many of the adsorption technics are applicable only to oxalated plasma, which precludes their direct use on citrated blood which is the most convenient source of large volumes of human plasma. The method described here makes it possible to prepare both purified prothrombin and partially purified plasma Ac-G from the same small (10-100 cc) samples of fresh citrated human plasma. The prothrombin is obtained in high yield and free of other known clotting factors. The procedure has the additional advantages of simplicity and rapidity; the preparation is easily completed in a single working day.

Assay methods. Prothrombin. Both the one-(1) and 2-stage(2) assays were used for measuring prothrombin. In the 2-stage method acacia was eliminated from the incubation mixture. With this modification nor-

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