experiment, for liver to exert its optimal effect.

Subsequent experiments were conducted in an effort to determine whether the greater swimming capacity of rats on the liver ration at a water temperature of 20°C was due to an increased production of adrenal cortical hormone(s). Eighteen female rats of the Long-Evans strain were selected at 23 to 25 days of age and fed *ad lib.* the basal ration plus B vitamin diet employed above. After 12 weeks of feeding the animals were divided into 3 groups and swimming tests were conducted at a water temperature of 20°C (6 animals per group). Each rat in group I received an intraperitoneal injection of 5 mg ACTH‡‡ 30 minutes before the test; each rat in group II received an intraperitoneal injection of 5 mg cortisone acetate§§ seven minutes before the test; rats in group III served as untreated controls. No significant difference was observed in the swimming performance of rats in the various groups. The average swimming time of animals in group I was 11.8 minutes; in groups II, 11.4 minutes; and in group III, 12.6 minutes. It would appear, therefore, that an increased production of ACTH or cortisone in response to stress was not the cause of the greater swimming capacity of rats fed the liver-containing diet at a water temperature of 20°C.

Discussion. Findings indicate that immature rats raised to maturity on a purified ration containing 10% whole liver powder swam for a significantly longer time at a water temperature of 20°C than rats fed a similar ration in which the B vitamins were provided in synthetic form. The beneficial effect of liver was apparently not due to its content of known B vitamins. Available data do not indicate, however, whether its protective effect was due to an unidentified factor or to its content of known nutrients. In view of the fact that military personnel and others may on occasion be forced to spend prolonged periods of time in water at a temperature of 20°C or below, it would appear that the identification of a factor which might be employed to increase resistance to this form of stress may have considerable practical value.

Summary. Immature rats raised to maturity on a purified ration containing 10% whole liver powder swam for a significantly longer period at a water temperature of 20°C than rats fed a similar ration containing the B vitamins in synthetic form. The protective factor in liver was distinct from any of the known B vitamins.

‡‡ ACTHAR, Armour and Co., Chicago, Ill. The material employed was diluted with saline solution to a concentration equivalent to 5 mg of the standard LA-1-A per cc.

§§ Saline Suspension of Cortone Acetate, Merck and Co., Rahway, N. J. Each cc contained 25 mg of cortisone acetate.

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Effect of ACTH on Whole Blood Coagulability.* (18825)

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Administration of ACTH in sufficient dosage induces numerous metabolic changes. Reports of the effect of this hormone on the coagulation time of whole blood, however, have been conflicting. Cosgriff *et al.* conclude that ACTH shortens the clotting time, induces hypercoagulability and a state conducive to thromboembolic complications(1). Smith *et al.*, on the other hand, reported a

* The author is indebted to Drs. Albert E. Renold and Peter H. Forsham for continued interest in this study, and to Dr. H. Cluxton, of the Armour Laboratories, Chicago, Ill., for the ACTH used in this work.

1. Cosgriff, S. W., Diefenbach, A. F., and Vogt, W., *Am. J. Med.*, 1950, v9, 752.

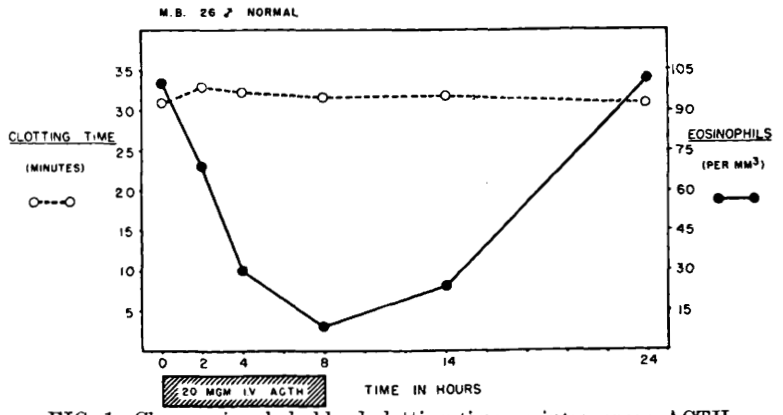

FIG. 1. Changes in whole blood clotting time on intravenous ACTH.

prolongation of the clotting time(2). Although these authors showed an increase in circulating heparin-like factors following ACTH administration, no change in protamine titration was found by the other group. Neither could find any significant alteration in 2-stage prothrombin values. Ac-globulin, prothrombin time or anti-fibrinolysin. These previous reports conflict primarily on interpretation of changes in the whole blood clotting time. This apparently simple test can be easily misleading. In the present experiments a carefully controlled and sensitive, modified Lee-White test of whole blood coagulability was applied in order to evaluate possible changes induced by ACTH administration.

Methods. The clotting time of whole blood was determined by a technic modified from that of Allen(3). Venepuncture is made with a No. 20 (or larger bore) needle and a dry syringe with minimal blood stasis. After releasing the tourniquet, at least 2 ml of blood is drawn into the initial syringe. This syringe is then removed from the needle and an oiled syringe, in which the "dead" space is filled with mineral oil, is substituted. Five ml or more of blood is then withdrawn gently, without difficulty and without air bubbles. Timing begins when blood enters this syringe. When the desired amount is obtained, the needle is quickly withdrawn from the arm and removed from the syringe and one ml of blood is run carefully into each of 5 test tubes. Ten minutes after blood withdrawal, the first tube is tilted horizontally and this is repeated at intervals of one minute thereafter until at least half of the blood is clotted, as observed by giving the tube a sharp snap through a 90 degree arc, when this becomes necessary. When the first tube has reached this end point the time is noted and the second tube is tilted one minute later and at similar intervals until its blood clots. This procedure is repeated until the end points for all tubes have been obtained. The clotting time recorded for the test is the average of the time recorded for tubes 4 and 5, the last 2 tubes. The normal range was 25-40 minutes, usually 30-35 minutes. by this method. Variations in clotting time done at the same time in 2 different sets of tubes did not exceed 3 minutes or 10% of the average value obtained. It should be noted that the test tubes measure 100 x 13 mm and are scrupulously cleaned with scrubbing and acid chromate before being repeatedly rinsed with distilled water. All tests in this study were carried out by the same person. The possibility existed that mineral oil in the second syringe might alter the contacting blood. However, when parallel experiments were carried out by taking blood directly into a third siliconed syringe, after removing sufficient blood in the oiled syringe, no difference in end point was observed between blood collected in either syringe. Blood collected each

2. Smith, R. W., Margulies, R. R., Brennan, M. J., and Monto, R. W., *Science,* 1950, v112, 295.

3. Allen, J. G., *Transactions of the Third Josiah Macy, Jr., Foundation Conference on Blood Clotting and Allied Problems,* New York, 1950, p. 213.

TABLE I. Short-Term ACTH Effect on Whole Blood Coagulation Time.

Patient	Dose, mg	Route	Coagulation time (min) Control	0-4 hr after end of intravenous infusion	hr after beginning intramuscular ACTH test 4	24	48	64
H.	20	I.V.	32	33				
B.	20	I.V.	35	34				
M.	20	I.V.	34	35				
F.	20	I.V.	35	34				
A.	20	I.V.	36	36				
P.	20	I.V.	39	39				
M.	55	I.M.	33		34	32	35	35
A.L.	55	I.M.	36			37	37	36
B.	55	I.M.	35		37			

way was also added to siliconed test tubes, but the normal clotting time in the silicone tubes was in the range of 2 hours and was deemed impractical for the present work.

Experimental. The possible effects of ACTH on the whole blood coagulation time were studied from the standpoint of (a) acute, short-term administration, (b) prolonged therapy, (c) withdrawal, (d) natural or artificially induced hypo- and hyper-adrenal states. Two studies with cortisone are included. ACTH was administered in 20 mg doses in 500 ml solutions by means of an intravenous infusion over an eight hour period (4). When the intramuscular route was used, 25 mg, followed by 10 mg every 6 hours, was given.

Intravenous ACTH administration to a normal subject caused an eosinophil fall to less than 5% of the control value, while the coagulation time remained unchanged (Fig. 1). Similarly, in 6 patients (Table I) no change in the coagulation time was observed at the end of the 8-hour administration period or within the following 4 hours, when maximal metabolic effects are present During intramuscular ACTH administration the coagulation time was determined at 0, 4, 24 and 48 hours and no significant change was observed.

Four patients were studied during 2-3 week periods of therapy. The findings are charted in Table II. No alteration or trend was noted during prolonged ACTH administration. It should be emphasized that these patients showed evidence of maximal adrenal stimulation, as evidenced both by clinical response and by increase in the urinary 17-ketosteroid excretion. These patients were also followed after cessation of therapy (Table II) with an inconsistent suggestion of prolonged clotting. This was not considered significant.

Studies were done in pathologic conditions which are characterized by abnormal adrenal cortical activity. Neither a patient with Addison's disease nor two patients maintained on cortisone following bilateral adrenalectomy showed unusual coagulation time (Table III). Patients with natural and induced Cushing's syndrome were also without abnormalities in the whole blood clotting time.

Two patients treated with large doses of cortisone, given by mouth and by intramuscular injection, showed no coagulation changes (Table II).

Discussion. In contrast to 2 previous but contradictory reports, the present investigation showed no significant change in the whole blood coagulation times in normal subjects and in patients receiving ACTH. Two differences contrast this and the previous reports. The intramuscular route of administration was utilized in the earlier work, while the intravenous route was primarily adopted in this study. However, the intramuscular technic was used in a few cases and no differences were observed. Also, the intravenous route, while using less total ACTH, has been demonstrated to be a more effective way of stimulating the adrenal cortex(4).

Further, the methods of determining the coagulation time in the previous investigations are somewhat different from ours. The value

4. Renold, A. E., Forsham, P. H., Maisterrena, J., and Thorn, G. W., *New Eng. J. Med.*, 1951, v244, 796.

TABLE II. Long-Term Effect of ACTH and Cortisone on Whole Blood Coagulation Time.

Patient	Sex	Diagnosis	Daily dosage (mg)	Drug route	Control	Day of therapy 1	2-4	5-7	8-10	11-15	16-21	Day after therapy 1	2	3	4-9	10-15
F.	M	Spinal cord tumor	20	A* I.V.	35	36		36	31	33		34	38		36	
M.	M	Rheumatoid arthritis	20	A I.V.	35	35				34	31	33		38	43	39
J.	F	Acute rheumatic fever	20	A I.V.	36					34		34		35	36	
G.	M	"	20	A I.V.	38	40			31		33					
J.	F	"	400	C* P.O.	33	34	31		33							
B.	F	Rheumatoid arthritis	500	C I.M.	32				33	30	30					

* A = ACTH. C = cortisone.

TABLE III. Whole Blood Coagulation Time in Abnormal Adrenal States.

Patient	Diagnosis	Clotting time (min)
C.	Addison's disease	34
H.	100 mg cortisone/day; bilat. adrenalectomy, 52 days post-operative	34
K.	25 mg cortisone/day; bilat. adrenalectomy, 75 days post-operative	35
A.	Cushing's syndrome	30
M.	After 10 mo continuous ACTH; dermatomyositis; Cushingoid syndrome	37

and pitfalls of the whole blood coagulation time determination have been discussed in some detail elsewhere(5-7). The need for elimination of tissue juice contamination should be especially emphasized. In 3 instances during this study blood did not flow freely into the second syringe and there was some question as to whether the open end of the needle was completely within the vein, having either incompletely entered or pierced it. Clotting times in each instance were less than 22 minutes and immediate repetition of the test using a good draw from another vein gave clotting times well within the normal range. Bubbles and dirty glassware may also accelerate the coagulation process. Oily test tubes may prolong clotting.

Summary. A reproducible sensitive test, which minimizes contamination with thromboplastically active tissue juices is outlined for determination of the whole blood coagulation time. No significant alteration of the coagulation time was found with the administration of ACTH to human subjects or in patients with hypo- or hyper-adrenocorticism when this method was used.

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5. Lee, R. I., and White, P. D., *Am. J. Med. Sci.*, 1913, v145, 495.

6. Quick, A. J., Honorato, R., and Stefanini, M., *Blood*, 1948, v3, 1120.

7. Barker, N. W., and Margulies, H., *Transactions of the Second Josiah Macy, Jr., Conference on Blood Clotting and Allied Problems*, New York, 1949, p. 106.

Studies on Estrogen Tri-*p*-anisylchloroethylene. (18826)

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Laboratory studies have demonstrated that tri-*p*-anisylchloroethylene (Tace*), like other estrogens, causes estrogenic changes in the mammary gland, uterus, and vagina, sensitizes the uterus to progesterone, produces abortions

* Trademark of The Wm. S. Merrell Co. Tace was prepared by M. G. Van Campen in the Organic Research Laboratories of this Company.