biochemical defects are apparently not caused or prevented by alterations in thyroid activity. This suggestion is supported by the finding of a normal basal metabolic rate in deprived animals on comparison with pair-fed controls(1).

Summary. On administration of desiccated thyroid in the diet, the syndrome of vit. B_6 deprivation in the rat was not markedly altered. One apparent effect of thyroid-feeding was to eliminate the customary elevation in fasting blood urea consequent to vit. B_6 restriction. This and an earlier study suggest that the biochemical defects of vit. B_6 deprivation in the rat are not caused or prevented by alterations in thyroid activity.

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Prolongation of Serum Prothrombin Time After Heparin. (21121)

SAMUEL LOSNER AND BRUNO W. VOLK.

From the Division of Laboratories, Jewish Sanitarium and Hospital for Chronic Diseases, Brooklyn, N. Y.

In the performance of the prothrombin consumption test the prothrombin time is determined 15, 30, 45, and 60 minutes following coagulation and centrifugation(1). The prothrombin time of *freshly* coagulated and centrifuged blood is not considered a true measure of prothrombin since considerable free thrombin occurs in the serum(1). The antithrombic effect of heparin was, therefore, expected to influence the prothrombin time of fresh serum. In order to confirm this hypothesis, a comparison of the prothrombin consumption tests was carried out before and after administration of heparin, with particular emphasis upon the prothrombin time of fresh serum. Further evidence of a thrombin effect as well as thromboplastin effect in fresh serum was obtained by the interaction of calcified serum upon plasma at various time intervals before and after administration of heparin.

Material and methods. Twenty normal individuals were divided into 2 groups of 10 each. Prothrombin consumption tests according to the procedure of Quick(1) were performed in all 20 individuals just before and one hour after the intravenous administration of heparin. The first group of 10 individuals received 50 mg of heparin while the second group received 75 mg of heparin each. Deprothrombinized oxalated plasma was obtained with barium sulfate according to Rosenfield and Tuft(2). In view of the emphasis in this study upon the prothrombin time of fresh serum the tests were not performed at intervals of 15, 30, 45, and 60 minutes following coagulation as described by Quick, but immediately after coagulation (zero-hour) as well as 30, 45, and 60 minutes later. A. *Prothrombin consumption test.* Five ml of venous blood were placed into a test tube in a water bath at 37°C. When coagulation was complete the tube was centrifuged for one minute at top speed. The prothrombin time of the

serum was determined immediately thereafter by adding 0.1 cc of the supernatant serum to a mixture of 0.1 cc of deprothrombinized human plasma, 0.1 cc of Difco thromboplastin and 0.1 cc of 0.02 M calcium chloride. Following this "zero-hour" determination, the procedure was repeated at the above intervals before and one hour after the administration of heparin. B. *Interaction time of calcium, serum and plasma.* Equal amounts of 0.1 cc each of serum, obtained at zero-hour, 30, 45, and 60 minutes after coagulation, and 0.02 molar solution of calcium chloride were incubated in a small cuvette (75 x 6 mm) at 37°C for 3 minutes. Similarly, homologous plasma was incubated. Subsequently, 0.1 cc of the plasma was swiftly blown into the cuvette which had been placed into the microadapter of a Coleman Spectrophotometer Junior Model 6A. At this time a stopwatch was clicked. When the galvanometer light-beam began to swing to the left indicating the onset of coagulation, the watch was stopped. The physical setup of this procedure was identical to that utilized in the photoelectric determination of the plasma prothrombin time (3). The only difference consisted in the fact that fresh serum was utilized instead of thromboplastin and that calcium was not added as a last step, but at an earlier phase of the procedure. The interaction time between calcium, serum and plasma was determined at the same intervals as described above, namely at zero-hour, 30, 45, and 60 minutes after coagulation. The procedure was carried out in a total of 20 individuals before and one hour after the intravenous administration of heparin, at which times serum and homologous plasma were derived from the same specimen of blood. Ten subjects received 50 mg of heparin while the other 10 received 75 mg of heparin. C. *Interaction of fresh serum and fibrinogen solution.* Normal serum obtained at zero-hour was added in the quantity of 0.1 cc to an equal amount of bovine fibrinogen (Chilcott-Warner), containing 300 mg % in physiological saline. Six determinations were carried out at 37°C. D. *Interaction of serum and homologous plasma.* Normal serum was added in the quantity of 0.1 cc to an equal amount of homologous citrated plasma. Six experiments were carried out at 37°C and repeated at 30, 45, and 60 minutes after coagulation.

TABLE I. Prothrombin Consumption Tests.

a) Before heparin admin.

Min. after coagulation	Mean value, 20 individuals (sec.)	Range (sec.)
*	2.3	1.8- 3.0
30	9.1	6.4-12.3
45	11.3	8.0-15.7
60	14.7	9.6-21.3

b) One hr after intrav. admin. of 50 mg heparin

Min. after coagulation	Mean value, 10 individuals (sec.)	Range (sec.)
*	8.1	5.2-13.0
30	14.5	11.8-16.0
45	18.1	16.0-21.3
60	20.9	17.6-26.5

c) One hr after intrav. admin. of 75 mg heparin

Min. after coagulation	Mean value, 10 individuals (sec.)	Range (sec.)
*	11.9	8.0-16.8
30	17.6	13.8-27.4
45	23.0	18.8-29.5
60	28.9	20.8-32.0

* Immediately (zero-hr).

Results. A. *Prothrombin consumption test.* As seen from Table I the prothrombin time obtained with serum of freshly coagulated blood increased from a mean value of 2.3 seconds at zero-hour to 9.1, 11.3, and 14.7 seconds at 30, 45, and 60 minutes after coagulation, respectively. While the prothrombin time at zero-hour had a range of 1.8-3.0 seconds only, the values were more widely scattered at 30, 45, and 60 minutes after coagulation. Following the intravenous administration of 50 mg of heparin the mean prothrombin time of fresh serum (zero-hour) increased to 8.1 seconds with a range from 5.2-13.0 seconds. Subsequent determinations 30, 45, and 60 minutes later, produced mean values of 14.5, 18.1, and 20.9 seconds, respectively. Upon intravenous injection of 75 mg of heparin, the mean serum prothrombin times increased further to 11.9, 17.6, 23.0, and 28.9 seconds at zero-hour, 30, 45, and 60 minutes after coagulation, respectively. B. *Interaction*

TABLE II. Interaction Time of Calcium, Serum and Plasma.

a) Before heparin admin.

Min. after coagulation	Mean value, 20 individuals (sec.)	Range (sec.)
*	9.4	6.8-12.0
30	12.6	10.0-17.0
45	14.3	11.2-20.0
60	15.8	12.0-20.9

b) One hr after intrav. inj. of 50 mg heparin

Min. after coagulation	Mean value, 10 individuals (sec.)	Range (sec.)
*	23.7	13.3- 35.7
30	36.4	15.8- 60.6
45	66.6	19.0-150.0
60	—	19.0- ∞

c) One hr after intrav. inj. of 75 mg heparin

Min. after coagulation	Mean value, 10 individuals (sec.)	Range (sec.)
*	58.9	31.0-120.0
30	—	40.0- ∞
45	—	50.0- ∞
60	—	70.0- ∞

* Immediately (zero-hr).

time of calcium, serum, and plasma. As seen from Table II the interaction time of calcium, serum, and plasma increased from a mean value of 9.4 seconds at zero-hour to 12.6, 14.3, and 15.8 seconds at 30, 45, and 60 minutes after coagulation, respectively. Following the intravenous injection of 50 mg of heparin the corresponding mean value increased to 23.7 seconds at zero-hour, 36.4 seconds at 30 minutes and 66.6 seconds at 45 minutes following coagulation. No mean value was obtained at 60 minutes following coagulation. One hour after intravenous administration of 75 mg of heparin the mean value of the interaction time of calcium, serum, and plasma increased to 58.9 seconds. It seems, however, of considerable significance that with this dosage the coagulum became exceedingly small, while the injection of 50 mg of heparin was devoid of such an effect. No mean values were obtained 30, 45, and 60 minutes following coagulation of blood obtained one hour after injection of 75 mg of heparin. C. *Interaction of fresh serum and fibrinogen solution.* Upon addition of fresh serum to a solution of bovine fibrinogen only a very minute and hardly visible coagulum was observed, the clotting time varying between 23 and 45 seconds in 6 experiments. D. *Interaction of serum and homologous* plasma. When fresh serum was added to an equal amount of homologous citrated plasma at zero-hour the mean clotting time was 9.0 seconds as obtained in 6 experiments, but rapidly lengthened to 43.5 seconds at 30 minutes, 60.3 seconds at 45 minutes and 70.0 seconds at 60 minutes following coagulation.

Discussion. The frequent failure of the Lee-White clotting time to reflect appropriately the action of heparin has been an incentive to search for other methods which may be applicable to its detection. A chemical assay for heparin in blood has been reported by Jaques(4). The fibrin appearance time of Weiner and Shapiro(5) may indicate a heparin effect provided a special apparatus with a rotating glass bulb is utilized. The citrate clotting time recently reported by the authors(6) indicates the anticoagulant effect of heparin with considerable sensitivity and does not require any extraneous agents besides a given suboptimal concentration of sodium citrate. While it appears that the citrate clotting time offers a more accurate indication of the anticoagulant action of heparin than the Lee-White clotting time, the procedure requires observation for prolonged periods of time.

The results of this study conclusively demonstrate that the coagulative power of fresh serum, as reflected in the very short prothrombin time at zero-hour, is substantially reduced by the therapeutic administration of heparin and in accordance with the dosage employed. It is of interest that the technic of the one-stage prothrombin time can be applied to the detection of the anticoagulant effect produced by heparin, not only within the first few minutes but long after its administration, provided serum and not plasma is used. It is obvious that this phenomenon is not adequately accounted for by the antiprothrombin action of heparin which is only of short duration and reflected in the plasma prothrombin time as reported by Laruelle(7) who demonstrated a prolongation of the one-stage prothrombin time varying with the concentration

of heparin per unit of plasma. Quick(8) similarly has shown that the prolongation of the prothrombin time is transient and most pronounced 5 minutes after the intravenous injection of heparin. It was, therefore, concluded that the prolongation of the serum prothrombin time, particularly at zero-hour as caused by heparin may be due to its effect either upon nascent thrombin, thromboplastin(9) or serum prothrombin conversion accelerator(10).

The assumption of free thrombin in fresh serum was not well supported by experiments in which serum at zero-hour was added to a fibrinogen solution. On the other hand, the solid coagulum obtained 9.0 seconds after the interaction of fresh serum and homologous plasma more strongly favored the assumption of the presence of free thrombin in fresh serum. The lengthening of this clotting time at later intervals pointed to a rapid neutralization of whatever amount of thrombin has been present after clotting. The instantaneous stabilization of the serum prothrombin time upon addition of sodium citrate also points to an extremely rapid neutralization of such thrombin content. On the other hand, the accelerating effect of calcium as shown in the interaction time of calcium, serum, and plasma (Table II) seems to be indicative of a definite and substantial thromboplastin effect of serum. In this procedure, serum, as an ingredient, assumes the role of thromboplastin as compared with the one-state Quick prothrombin determination.

The finding that bovine fibrinogen does not coagulate as readily as does homologous citrated plasma after addition of fresh serum, does not preclude the presence of thrombin. According to Laki(11), the clot formed in blood plasma is different from the clot formed from purified fibrinogen.

The prolongation of the interaction time of calcium, serum, and plasma following the administration of heparin appears to be due to both its antithrombic(1) as well as antithromboplastic actions(9). The assumption that it may be due to an effect upon serum prothrombin conversion accelerator (SPCA) seems to be difficult to reconcile with the finding that heparin added to serum plasma mixtures capable of lengthening the prothrombin time of plasma alone did not abolish SPCA activity (10).

Summary. 1. The one-stage prothrombin time of freshly coagulated and centrifuged blood is considerably prolonged after the intravenous administration of heparin. 2. The interaction time of calcium, serum, and plasma is similarly prolonged after intravenous injection of heparin. 3. The prolongation of the serum prothrombin time after administration of heparin is assumed to be due to both the antithromboplastic as well as antithrombic actions of the latter.

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