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Influence of Alloxan Diabetes on Coagulability of the Blood.*

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The possibility that the pancreas may influence the coagulation of the blood has been considered by several investigators. Boldyreff[1,2] has reported that pancreatectomy results in a temporary delay of the clotting time and that the establishment of a pancreatic fistula causes a significant and permanent prolongation of the clotting time as well as a bleeding tendency. Recently Hecht[3] suggested a relationship between the internal secretion of the pancreas and the coagulability of the blood. He observed that diabetes was more common in families in which cases of sporadic hemophilia occurred, the criterion of sporadic hemophilia being the absence of the disease in the 4 preceding generations. This observation led Hecht to study the effect of alloxan on the clotting time in rats. He found that the clotting time of recalcified plasma was delayed in those animals which responded to alloxan and developed hyperglycemia.

In the present paper the influence of alloxan on the coagulation process was studied by means of the coagulation time and by various other methods which are more specific in detecting the basic disturbances in the coagulation mechanism.

Experimental. The studies were carried out on 10 adult male rabbits. The alloxan monohydrate in a 4% neutral solution in physiological saline was injected intravenously. The dose was of 200 mg per kilo of body weight. To counteract the transitory hypoglycemia resulting from the drug, 5 cc of 50% glucose in water were given every 2 hours by stomach tube for the first 10 hours.

The blood sugar level was determined every 2 hours for the first 10 hours and then every 2 days using the method of Folin and Wu[4] on venous blood obtained from an ear vein. On the second, fourth, seventh, tenth and fourteenth day samples of blood were obtained from the ear artery of the animal in a syringe coated with Silicone (General Electric Dri Film 9987) through a needle also coated with Silicone. Care was taken to avoid foaming and contamination with tissue juice. Two 1 cc samples of blood were immediately transferred to serological tubes with a uniform internal diameter of 11 mm, kept in a water bath at the constant temperature of 37°C and the clotting time determined according to a modified Lee-White technic.[5] The remaining blood was decalcified by the addition of 0.1 M sodium oxalate in the ratio of one to ten. The prothrombin consumption was determined in the serum obtained from the samples of blood used for the determination of the clotting time according to the technic of Quick.[6] The oxalated plasma, obtained by centrifuging the oxalated blood for 10 minutes at 2,000 r.p.m. was used for the determination of the clotting time of recalcified plasma, prothrombin time, relative concentration of the "labile factor", antithromboplastin and antithrombin activities. Both prothrombin time and clotting time of recalcified plasma were determined by the method of Quick.[7] The

* This research was supported by a grant from the Division of Research Grants, National Institute of Health.

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1 Boldyreff, W. N., *The Tohoku J. Exp. Med.*, 1937, **31**, 469.

2 Boldyreff, W. N., *Erg. d. inn. Med. u. Kinderheilk.*, 1940, **59**, 29.

3 Hecht, E., *Acta Med. Scand.*, 1947, **129**, 311.

4 Folin, O., *J. Biol. Chem.*, 1929, **82**, 83.

5 Quick, A. J., Honorato, C. R., and Stefanini, M., *Blood*, 1948, **3**, 1120.

6 Quick, A. J., *Am. J. Med. Sc.*, 1947, **214**, 272.

7 Quick, A. J., The Hemorrhagic Diseases and the Physiology of Hemostasis, Charles Thomas, Springfield, Ill., 1942.

TABLE I.
Relationship Between Blood Glucose Level, Clotting Time of Whole Blood, Prothrombin Consumption, Prothrombin Time, Clotting Time of Recalcified Plasma and Relative Concentration of the "Labile Factor" of Prothrombin, 7 Days After Intravenous Injection of Alloxan Monohydrate (200 mg per kilo body weight).

Rabbit No.	Blood glucose level, mg per 100 cc blood	Prothrombin time, sec.	Clotting time,* min.	Clotting time of recalcified plasma, sec.	Relative conc. of "labile factor," %	Prothrombin consumption 1 hr sec.	2 hr
Control	117.4	6	12½	95	100	16	21
"	125.6	6½	11½	120	85	23	28
"	106.0	6	13	95	100	11	15½
"	112.8	7	11	115	95	10½	17
1	382.1	6½	15	120	100	13	21
2	122.4	6	12	135	90	12	18
3	402.7	7	17½	160	100	22	30
4	257.4	7	18	150	100	20	24
5	442.8	6	16½	150	80	10	16½
6	239.7	6½	20	165	100	14	19
7	108.3	6½	9½	95	100	11½	14
8	193.7	6	17½	120	95	17½	19
9	320.4	6	14	150	100	10	18
10	379.5	6	16	150	85	14	18½

* Arterial blood.

relative concentration of the "labile factor" and the antithrombin activity were determined according to technics recently described.[8,9]

Results. Two rabbits out of the 10 investigated failed to exhibit any modification of their blood sugar level. This is not surprising as occasionally animals have been found by other investigators[10] which resist the action of alloxan. The other 8 gave a typical response and the modification of their blood sugar level is presented in Table I. Two hours after injection the rabbits presented a transitory hyperglycemia followed, about 4 hours later, by a hypoglycemic phase which persisted until the eighth hour. On the second day the rabbits showed a sharp elevation of the blood sugar level, which reached its maximum level on the fourth day and then remained stationary. Correspondingly a marked glycosuria was noted.

Data showing the effect of alloxan on various constants of the clotting process are shown in Fig. 1 and Table I. The clotting time of whole blood and that of recalcified plasma appeared to be slightly delayed without any appreciable difference between the fourth and the fourteenth day of experimental hyperglycemia. The difference between these animals and normal controls is small but consistent. As observed by Hecht in rats, transitory reduction of the hyperglycemia to normal with injection of 4 units of insulin per kilo weight had no effect on the behavior of the clotting time. The prothrombin time was normal in all treated and untreated animals. The prothrombin consumption, taken one and 2 hours after the completion of clotting, showed wide variations both in normal and treated animals, without any direct relationship to the blood sugar level. Determination of the relative concentration of the "labile factor" showed no difference between treated and untreated animals.

The possible presence of an anticoagulant was ruled out. The addition of half volume of whole blood and plasma of normal and alloxan treated animals gave a clotting time close to the arithmetical average of the two. The possibility of the presence of an antithromboplastin was excluded as both normal and treated rabbit plasmas gave similar curves when their prothrombin times were determined with thromboplastin of varying

[8] Stefanini M., and Quick, A. J., *Fed. Proc.*, 1948, **7**, 191.

[9] Stefanini, M., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 22.

[10] Walpole, A. L., and Innes, J. R. M., *Brit. J. Pharm. and Chemiother.*, 1946, **1**, 174.

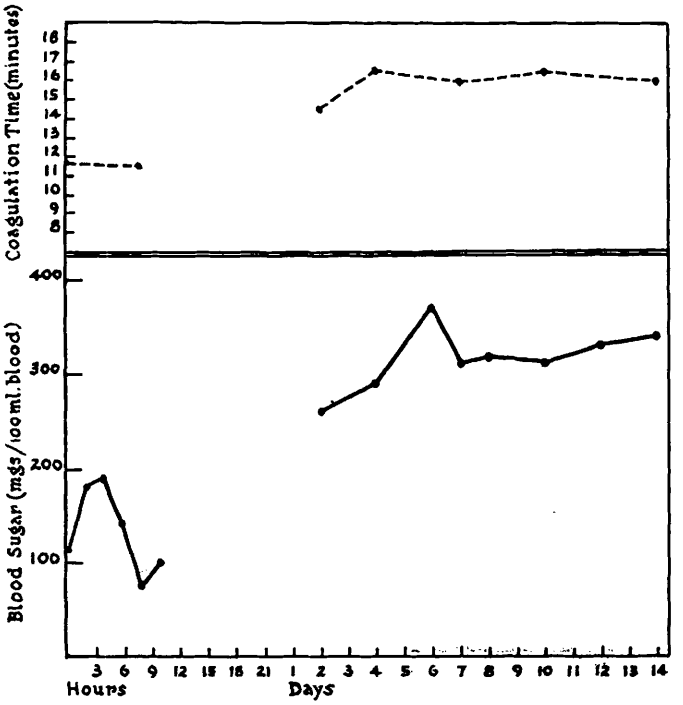

FIG. 1.

Behavior of the clotting time of whole blood and blood sugar level after the intravenous injection of alloxan monohydrate (200 mg per kilo body weight) in rabbits (average figures).

strength prepared by serial dilution in physiological saline. Normal and pathological plasmas also showed a similar antithrombin activity, when tested immediately or after incubation.[9]

Discussion. The results given in the paper show that after the administration of alloxan sufficient to cause an experimental condition of severe diabetes in rabbits, the coagulability of the blood was very slightly influenced. The clotting time of whole blood and recalcified plasma were slightly prolonged but the other tests that supply more specific information as to the manner in which the mechanism of the coagulation process is influenced, gave normal results. Normal activity of thromboplastin and prothrombin was demonstrated by the normal prothrombin consumption test and the normal prothrombin time. No antithrombin or any other anticoagulant could be demonstrated and the activity of fibrinogen appeared to be normal.

From these results one must deduce that the influence of alloxan diabetes on coagulation is not significant and the prolongation of the coagulation time is probably a non-specific effect, the nature of which remains to be investigated.

Summary. Blood of rabbits injected with doses of alloxan monohydrate sufficient to determine an experimental condition of severe diabetes shows a slight delay of the clotting time of both whole blood and recalcified plasma but not accompanied by any appreciable modification of other tests giving more analytical information of the clotting process. The slight hypocoagulability, which is probably non-specific, is not due to the hyperglycemia as it is not corrected by an amount of insulin sufficient to normalize transitorily the blood sugar level.