TECHNICAL PAPERS

Effect of the Injection of Glucose Into the Cerebrospinal Fluid

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The mechanism whereby the processes of carbohydrate metabolism are correlated with carbohydrate intake has been a subject of much investigation and discussion. One of the views which has received attention is that a control of insulin output of the islet tissue through the glucose content of the blood is an important factor. The evidence that a mechanism whereby increased amounts of glucose in the blood elicit increased insulin liberation involves the activation of centers within the brain stem is discussed by Macleod (*2*). In general, the experimental procedures which have been employed are of a laborious nature.

Recently, Marinelli and Giunti (*3*) have reported that injections of relatively small amounts of glucose into the cerebrospinal fluid by way of the cisterna magna result both in the dog and in man in a rapid and pronounced fall in glucose content of the blood, followed by a slower return to basal values. Marinelli and Giunti infer that the glucose content of the cerebrospinal fluid represents a direct chemical stimulation upon the glycoregulatory nervous centers, resulting in functional changes affecting the glucose level of the blood. They suggest that the blood-sugar curves look very much like those following insulin administration. If such responses consistently obtain following this procedure, which is easily carried out and which involves the introduction into the body of an amount of glucose so small as to be quantitatively insignificant in relation to the total glucose content of the body fluids, a number of experiments would be made practicable in further elucidation of the problem of regulation of blood-sugar levels.

In an attempt to corroborate the findings of Marinelli and Giunti, 6 normal, docile dogs were selected, and after a fast of 18 hours blood samples were taken. Without the use of an anesthetic, a needle was placed in the cisterna magna, 2 ml. of cerebrospinal fluid was withdrawn, and 2 ml. of a 5 per cent solution of glucose (Abbott's 5 per cent dextrose, pyrogen free) was injected. In each instance it was possible to carry out the procedure without signs of excitement on the part of the animal and without struggling. Blood samples for glucose determinations were taken at 5, 15, 30, 60, and 120 minutes after the glucose injection. Blood-sugar determinations were made in duplicate by the method of Hagedorn and Jensen (*1*). The results are shown in Table 1. It is observed that lowering of the glucose level of the blood was in no instance obtained following intracisternal injection of glucose solution. There appears to be a definite tendency toward an increase in glucose content of the blood during the second hour of the tests.

The experiment was repeated using 3 dogs anesthetized 15 minutes beforehand by the intravenous administration of pentobarbital sodium in a dosage of 30 mg./kg. of body weight. The results were essentially the same as with unanesthetized animals.

TABLE 1

BLOOD-SUGAR LEVELS FOLLOWING INTRACISTERNAL INJECTIONS OF GLUCOSE IN DOGS

(Blood sugar in mg./100 ml.)

Dog No.	Preinjection	Postinjection time in minutes				
		5	15	30	60	120
1	85	87	101	102	78	97
2	118	119	132	123	125	140
3	100	108	109	126	117	128
4	96	97	95	99	102	121
5	67	77	75	84	79	108
6	76	80	81	79	87	121

We are therefore unable to corroborate the findings of Marinelli and Giunti that the injection of small amounts of glucose into the cisterna magna results in a hypoglycemic reaction in dogs. Though the weight of glucose injected in our experiments was the same as that employed by Marinelli and Giunti, we may not have duplicated their procedure exactly, inasmuch as they make no statement as to the volume of solution in which the glucose was dissolved. The volume we used was chosen with a view to maintaining isotonicity.

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The Function of Ac-Globulin in Blood Clotting[1]

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It has been shown that a newly recognized plasma factor (*1, 2, 3, 5, 6*) accelerates the activation of purified prothrombin by thromboplastin. We refer to this factor as Ac-globulin (*6*). Because of its apparent importance we have concentrated our studies on its purification and its reactions.

Ac-globulin activity of plasma is quite different from that of serum. In serum the activity is comparatively more intense. Fig. 1 illustrates the difference. Curve A shows the activation rate with Ac-globulin of serum origin, and curve B, with Ac-globulin of plasma origin. In the former reaction the production of thrombin is far more rapid, but the final yield is the same as with Ac-globulin of plasma origin. For purposes of nomen-

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clature we refer to these substances as plasma Ac-globulin and serum Ac-globulin. The properties of the two are so similar that no difference has been detected, even though both have been obtained in concentrated form.

Thrombin is the substance which is responsible for the production of serum Ac-globulin, and calcium ion is not required for that purpose. The most active thrombin prepara-

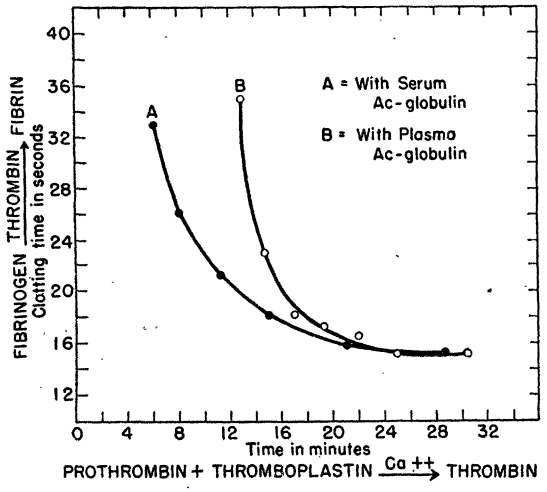

FIG. 1. Activation of purified prothrombin with serum Ac-globulin (curve A) and with plasma Ac-globulin (curve B) in the presence of excess thromboplastin and optimum calcium-ion concentration.

tions obtained to date have been added to large quantities of oxalated bovine plasma, and subsequently, concentrates of serum Ac-globulin have been obtained in quantity and quality equal to those obtained from bovine serum itself.

The function of Ac-globulin in the clotting mechanism can then be outlined by use of the following equations:

(1) Prothrombin + Thromboplastin $\xrightarrow{Ca^{++}}$ Thrombin

(2) Plasma Ac-globulin $\xrightarrow{\text{Thrombin}}$ Serum Ac-globulin

(3) Prothrombin + Thromboplastin $\xrightarrow[\text{Serum Ac-globulin}]{Ca^{++}}$ Thrombin

(4) Fibrinogen $\xrightarrow{\text{Thrombin}}$ Fibrin Clot

The clotting reaction is initiated by thromboplastin which comes from platelets and tissue juices. Some of the newly formed thrombin alters plasma Ac-globulin so that it becomes serum Ac-globulin. The latter intensifies the interaction of prothrombin and thromboplastin. Thrombin thus accelerates its own formation through an intermediate. This may be regarded as co-autocatalysis. These conclusions differ distinctly from those of Owren (*3*), but are in harmony with the old and well-known evidence presented in the literature to show that autocatalysis is involved in thrombin formation. This is, however, not autocatalysis but co-autocatalysis, because an intermediate is involved.

We have found that neither serum Ac-globulin nor plasma Ac-globulin can substitute for thromboplastin in the activation of prothrombin in the presence of optimum amounts of calcium ion.

The curves of Fig. 1 were obtained with the use of prothrombin prepared by $(NH_4)_2SO_4$ fractionation as described previously (*4*). This product possessed a maximum specific activity of 23,000 units/mg. of tyrosine. The activity was measured by the two-stage method (*7*). Plasma Ac-globulin was purified by the method briefly outlined (*5*). The same procedure was used for the preparation of serum Ac-globulin.

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The Action of Pteroylglutamic Conjugates on Man[1]

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In 1944, Leuchtenberger, Lewisohn, Laszlo, and Leuchtenberger (*4*) reported that a "folic acid concentrate" and a fermentation *L. casei* factor inhibited the growth of sarcoma 180 transplanted in female Rockland mice. Further studies by Lewisohn and his co-workers (*5*) in 1945 showed complete regression in about one-third of the single spontaneous breast cancers observed in three different strains of mice treated with daily intravenous injections of 5 μg. of fermentation *L. casei* factor. This substance was thought at that time to be folic acid; it is now known that it was a conjugate of folic acid, pteroyltriglutamic acid (*3*). Subsequent work showed that pteroylglutamic acid (folic acid), when tested under similar conditions, was not effective in producing regression of these breast cancers (*6*).

In 1944, Hutchings, *et al.* (*3*) reported the isolation of the fermentation *L. casei* factor. This compound was shown to be 60–80 per cent as active when assayed with *L. casei* and 2–6 per cent as active when assayed with *Str. faecalis* R as was the previously isolated liver *L. casei* factor, pteroylglutamic acid (*8*).

Degradative reactions have shown that the fermentation *L. casei* factor differs from pteroylglutamic acid in that the

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