THE EFFECT OF URINARY CORTIN-LIKE MATERIAL ON CARBOHYDRATE METABOLISM[1]

PREVIOUSLY we demonstrated that urinary extracts are capable of increasing the resistance of adrenalectomized rats to low environmental temperatures[2] as well as maintaining the lives of adrenalectomized rats.[3] Using glycogen deposition in the liver of the fasting adrenalectomized rat, we have now found that these extracts are able to influence carbohydrate metabolism in a fashion similar to that demonstrated for cortin and certain cortical compounds.[4,5]

The experimental procedure employed was a modification of the technique described by Reinecke and Kendall.[6] Adrenalectomized rats (140–160 gms) were fasted for 24 hours from the third to fourth day after operation. At the end of this period, the urinary extract was administered by stomach tube. Samples of the livers were removed and analyzed for glycogen by the method of Good, Kramer and Somogyi.[7] The urinary extracts used in this study are similar to those already described.[2,3]

In experiment A, the equivalent of 2 liters of urine dissolved in 1 cc of a 10 per cent. ethanol solution was administered once at 0 hours and again at 3.5 hours. The time of the termination of the 24-hour fast was reckoned as zero time. Samples of liver were removed between the seventh and eighth hours. In experiment B, the equivalent of 1.5 liters of urine dissolved in 1 cc of a 10 per cent. ethanol solution was administered at 0, 2.25, 4.5 and 6.75 hours. Samples of liver were removed between the eighth and ninth hours. All controls received 1 cc of a 10 per cent. ethanol solution at the hours indicated.

The results are presented in Table 1, in which the liver glycogen values are expressed in mgs of glycogen per 100 gms of liver (mg per cent.). The mean liver glycogen concentrations of those animals receiving urinary extracts were increased 5 and 10 times, respectively, for experiment A and B as compared with the controls. It is evident, therefore, that these urinary extracts influence carbohydrate metabolism in much the same way as do adrenal cortical extracts.

TABLE 1

THE INFLUENCE OF URINARY EXTRACTS ON THE DEPOSITION OF LIVER GLYCOGEN IN FASTING ADRENALECTOMIZED RATS

Experiment	Total urinary equivalent administered per rat in equal doses (liters)	Total volume of solvent (10 per cent. ethanol) administered (cc)	Times of administrations after completion of 24 hr. fast (hrs.)	Number of rats	Mean glycogen in liver (range) (mg. per cent.)
A	0	2.0	0 ;3.5	5	15.5 (13.9–19.0)
	4.0	2.0	0 ;3.5	5	75.4 (21.1–128)
B	0	4.0	0 ;2.25 ; 4.5 ;6.75	3	16.4 (12.5–19.3)
	6.0	4.0	0 ;2.25 ; 4.5 ;6.75	7	178 (60.9–308)

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

NON-VIRULENT FROZEN AND DRIED ANTIGENS FOR COMPLEMENT-FIXATION TESTS WITH CENTRAL NERVOUS SYSTEM VIRUS INFECTIONS

Two steps have already been taken in developing a specific, practical complement-fixation test for diagnosing central nervous system virus infections. The first was the production of antigens from infected brain tissue which, under stated conditions, gave specific and duplicable results;[1] the second was the rendering of the antigens non-virulent in order to make them safe for general use in hospital diagnostic laboratories.[2] We wish now to describe a third step, namely, the successful freezing and drying of small quantities of non-virulent antigens, thus permitting their distribution by central production laboratories and their use over relatively long periods of time.

Standard virulent antigens were prepared[1] by obtaining infected mouse brains, making them into a 10 per cent. suspension with diluent in a mechanical homogenizer, centrifugalizing the material at 2,500 r.p.m. in a horizontal centrifuge, freezing and thawing

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it, and finally centrifuging it at 5,000 r.p.m. in an angle-head centrifuge and discarding the sediment. These preparations were then rendered non-virulent by exposure to the rays of a mercury arc lamp for a determined period of time.[2]

Freezing and drying of these antigens are accomplished in the following manner. The irradiated antigen is freed of sediment by centrifugation in a horizontal centrifuge for 10 minutes at 2,500 r.p.m. To the clear supernatant, merthiolate in a dilution of 1:10,000 is added. The antigen is then pipetted in 2 or 5 cc quantities into glass ampoules, frozen quickly by immersion into a dry ice-alcohol mixture, and dried over a period of 20 hours in a Flosdorf-Mudd apparatus,[3] after which the ampoules are sealed. The ampoules containing the desiccated antigen in an air-free space are stored at 2° C. When needed for use in tests the ampoules are opened and 2 or 5 cc of distilled water added to the desiccated material. A similar method was employed by Smadel and Wall for preserving spleen lymphocytic choriomeningitis antigen.[4]

In the following table the results of one test with an ampoule of irradiated frozen and dried Western equine encephalomyelitis antigen are shown. The figures in the first column indicate that the titer of the complement was the same with frozen and dried as with standard virulent antigen or saline control (0.13 cc), showing that the dried material was not anti-complementary. Column 2 indicates that the titer of dried and virulent antigens was the same, 1:8, showing that there was no loss of antigenicity from irradiation or freezing and drying. Finally, column 3 indicates that the fixation titer was the same in hyperimmune serum, whether virulent or frozen and dried antigen was employed.

Similar antigens with St. Louis and West Nile viruses have been prepared and tested. They have proved practically identical to the virulent antigens in antigenicity and specificity. Our observation of their keeping qualities is limited thus far to 6 weeks, but we believe that in view of universal experience with lyophilized materials, these antigens will maintain their specific properties for long periods of time.

TABLE 1

EFFECT OF IRRADIATION AND FREEZING AND DRYING ON THE COMPLEMENT-FIXING ANTIGEN OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS

(Antigen irradiated for 100 minutes; tested for virulence by intracerebral inoculation into Swiss mice. Out of 12 mice inoculated none died).

Antigen	Anti-complementary power*	Antigenicity†							Specificity and titer of serum‡							
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
No antigen; saline control	0.13 cc								0	0	0					
Western equine encephalomyelitis, non-irradiated .	0.13 cc	4¶	4	4	4	0	0	0	4	4	4	4	4	4	1	0
Western equine encephalomyelitis, irradiated and lyophilized	0.13 cc	4	4	4	4	0	0	0	4	4	4	4	4	4	2	0
West Nile, irradiated and lyophilized	0.13 cc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Amount of guinea pig serum in dilution of 1:30 equivalent to one unit.
† Dilution of antigen reacting with a constant amount of Western equine encephalomyelitis mouse immune serum.
‡ Dilution of Western equine encephalomyelitis mouse immune serum reacting with a constant amount of antigen.
¶ 4 = Complete fixation; 0 = complete hemolysis; 1, 2 = intermediate degree of hemolysis.

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