Protein-Bound Hexosamine in Plasma and Erythrocytes of Normal and Diabetic Rats and Human Beings.* (26593)

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Protein-bound hexosamine, as a component of glycoprotein, has been found to vary greatly in a number of pathological conditions (1). Very little information, much of which is conflicting, is available concerning the relationship of these compounds to diabetes mellitus. Jacobs(2) reported that a mucoid fraction of blood plasma obtained by mild hydrolysis was increased in diabetic subjects and was decreased following administration of insulin in the same manner as glucose. Other investigators found the serum hexosamine content within normal range in diabetes uncomplicated by vascular degeneration(3, 4). In the diabetic rat, Spiro(5) reported no impairment in rate of synthesis of glucosamine in the liver, while in the skin, Schiller and Dorfman(6) observed a reduction in incorporation of C^{14}-labeled glucose into hyaluronic acid to one-third of that seen in the normal animal. There have been no reports on the hexosamine content of erythrocytes.

A preliminary study of protein-bound hexosamine concentrations in plasma and erythrocytes of normal and diabetic rats and human beings is reported here. The terminology used in this paper is according to Winzler(1).

Methods. Animals used in these experiments were male albino rats of the Wistar strain weighing 300-500 g. Alloxan diabetes was produced by intramuscular injection of 0.06 ml of a 10% solution of alloxan monohydrate in H_2O after a 48 hour fast. The animals were fed Purina rat chow *ad libitum* up to time of experiment.

Blood was collected from the tail into 1 ml teflon beakers, each of which contained 1 drop of dried Sequester-Sol as an anticoagulant. One ml of blood was transferred to a Wintrobe hematocrit tube by means of a Wintrobe pipette. Extreme care was taken to avoid hemolysis. The tubes were centrifuged at 2000 rpm for 30 min, after which the plasma was removed with a Wintrobe pipette and transferred to test tubes. The white cell layer was removed and discarded. The erythrocytes were washed 3 times with physiological saline, then diluted with saline to make a 1:1 suspension. The glycoprotein was precipitated by addition of 5 ml of 95% ethanol to 0.1 ml of the plasma samples, etc. and to 0.4 ml of the rbc suspensions in 12 ml centrifuge tubes. The tubes were centrifuged and the precipitate washed twice with 95% ethanol. Two ml of 3 N HCl were added to the precipitated proteins, the tubes fitted with air condensers and the contents hydrolyzed in a boiling water bath for 7 hours. One ml aliquots of the acid hydrolysates were neutralized with 4 N Na OH and salt concentration adjusted in the blank and standard tubes. Hexosamine determinations were made by the method of Elson-Morgan(7) as modified by Winzler(1). Optical density was determined at 530 mμ in a Coleman Jr. Spectrophotometer.

In some of the earlier experiments the hydrolysates were put through Dowex 50W ion exchange columns as described by Boas(8). It was found, however, that the hexosamine content of the plasma and red cells were essentially the same in the eluate from the ion exchange column as in the untreated hydrolysate (Table I). From these data it would appear either that little or no impurity was being measured when the Dowex column was not used, or that the Dowex column failed to remove any impurity which might have been present. In any event, the differences be-

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TABLE I. Recovery of Hexosamine after Passage through Dowex 50W Column.

		% of recovery*								
	Sample No.	1	2	3	4	5	6	7	Mean	S.D.
Glucosamine standard		101	97	91	102	100	97	99	98.1	3.7
Plasma hexosamine		101	99	102	99	101			100	1.4
Cell "		100	102	97	99				100	2.1
Plasma "	+ 125 μg glucosamine	94	94	99	106	98	99		99	4.9
Cell "	+ " " "	88	89	102	99	101	94		96	5.9

* Amount of material found in the aliquot analyzed without passage through the Dowex 50W column is in each instance arbitrarily assigned a value of 100%. Each percentage is derived by comparing the value obtained after chromatography with that found in the corresponding nonchromatographed specimen.

tween samples run through the column and those not so treated were not significant at the 5% level of confidence (Table I). Therefore, the use of Dowex 50W for determination of tissue hexosamine was abandoned. Our actions in this regard are in agreement with those of Rosenlund(9). No attempt was made to identify the particular hexosamine or group of hexosamines normally present in red cells.

Fasting human blood was collected from normal laboratory personnel and from known diabetic patients who were well controlled with diet and insulin or oral hypoglycemic agents.

Results. In Table II are shown the hexosamine content of plasma and erythrocytes and corresponding blood glucose values in normal and diabetic rats and human beings. The normal human plasma level was similar to that reported by Winzler(1). There was no significant difference between normal values and those of diabetic patients in either plasma or erythrocytes. Human hexosamine levels in both cells and plasma were lower than those in rats. The normal rats had significantly higher levels of hexosamine in both plasma and cells than the diabetic rats, which agrees with the work of Nichols *et al.*(10).

Discussion. In this study, the decreased hexosamine levels observed in cells and plasma of the diabetic rat differ from our own findings and those of others relating to diabetic patients, in whom either normal or increased serum mucoprotein concentrations were noted(3,4). However, a few cases of decreased serum mucoprotein in diabetic patients have been reported(12), although it was not stated whether or not other pathological conditions were present. In our hu-

TABLE II. Protein-Bound Hexosamine Levels in Plasma and Erythrocytes.

		Hexosamine, mg/100 ml	
Group	Glucose, mg/100 ml	Plasma	Packed erythrocytes
		Rat	
Normal	127	154	53.0
	107	172	46.5
	136	156	41.5
	110	154	56.0
	114	160	52.5
		159.2 ± 7.6*	49.9 ± 5.8
Diabetic	424	137	36.5
	386	138	32.5
	304	145	50.0
	356	134	46.0
	270	142	35.7
	894	114	31.1
	258	152	41.8
		137.4 ± 11.9	39.1 ± 7.1
		Human	
Normal	95	92.5	22.6
	76	76.0	17.8
	81	81.7	21.6
	64	68.0	18.4
	89	88.5	18.2
	88	128.8	22.3
	76	114.6	19.3
	66	115.3	18.4
		95.7 ± 21.6	19.8 ± 2.0
Diabetic	126	78.9	20.4
	80	109.6	27.3
	64	93.6	20.1
	79	112.0	19.4
	61	102.2	22.6
	50	61.4	17.3
	108	114.0	19.3
	84	67.8	28.6
	38	69.0	27.3
		89.8 ± 20.9	22.5 ± 4.2

* Mean ± S.D.

The 5 normal rat plasma levels compared to 7 diabetic rat samples by the Fisher "t" test(11) gave $P < 0.01$; a comparison of the cells gave $P < 0.02$.

man subjects, the diabetes was well controlled at the time of observation.

Reductions in serum mucoprotein have been most frequently associated with impaired liver or endocrine function(13,14); our finding is a decrease in the protein bound hexosamine. Since the liver has been implicated as the primary site of glucosamine formation(15), it may be possible that these alloxanized rats have impaired liver function, resulting in a decreased synthesis of these compounds. The decreases in serum proteins, particularly α_1 globulin and albumin fractions in diabetic rats observed by Nichols *et al.*(10) are indicative of altered hepatic activity.

It has been reported that the liver of the diabetic animal as the primary site of glucosamine formation can synthesize hexosamines at a rate similar to the normal animal (5). If this were the case, the lower levels in the diabetic rat may be a reflection either of decreased synthesis or release of these compounds by secondary sites, such as connective tissue.

Summary. In both cells and plasma, alloxanized rats had significantly lower concentrations of protein-bound hexosamine than normal animals. In diabetic patients under insulin control, values were normal. Concentration of protein-bound hexosamine was twice as high in plasma and erythrocytes of normal rats as in normal human beings.

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Different Hemostatic Responses of Dogs to Heparin Injection.* (26594)

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It is well known that the only effect which heparin has on hemostasis when it is injected intravenously in humans as in thrombosis is a drastic one on coagulation. The occurrence of rare accidents in heparinized patients has been interpreted as an individual susceptibility to hemorrhage while the blood remains incoagulable.

In our extensive observations on skin hemostasis in dogs, before and after injection of variable amounts of heparin, marked differences were noted not only with varying amounts of heparin, but also with a standard dose in different dogs. This difference in group response in dogs is described herein.

Methods. Unless otherwise stated the he-

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