RENAL CLEARANCES OF GRASS POLYSACCHARIDE: OBSERVATIONS ON GLOMERULAR POROSITY AND ON THE RELATION OF THIS FUNCTION TO PROTEINURIA IN RENAL DISEASE

By J. BEATTIE[1] AND A. C. CORCORAN

(From the Research Division of the Cleveland Clinic Foundation and the Frank E. Bunts Educational Institute, Cleveland, Ohio)

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Dr. D. J. Bell, Biochemical Laboratory, Cambridge, drew our attention in 1948 to a fructosan polysaccharide (levan) prepared from Italian rye grass. It is readily soluble in cold water, yields levulose on acid hydrolysis and is not metabolized. At that time, the fructosan was believed to be a homogeneous substance with a molecular weight somewhat greater than that of inulin, so that the suggestion was made that it might replace inulin in the measurement of glomerular filtration rate. It has since (see below) been shown to be inhomogeneous. Thus, this report deals with the excretion characteristics of this dispersed polymer in normal unanesthetized dogs and in normal and abnormal human subjects.

METHODS

1. Preparation of the grass polysaccharide

The polysaccharide was isolated from a pure strain of Italian rye grass grown at the Hannah Dairy Research Institute, Scotland. The grass was cut in June 1948, and dried immediately. Details of the extraction method are given by Palmer (1).

2. Determination of the polysaccharide in plasma and urine

The polysaccharide was estimated in diluted urine and in deproteinized plasma filtrates by an adaptation of the inulin method of Harrison (2). Preparation of suitable protein-free plasma filtrates was at first difficult. Recoveries of the material from filtrates prepared by the use of cadmium sulfate and sodium hydroxide or of barium hydroxide and zinc sulfate were irregularly incomplete, ranging between 80 and 100 per cent. The Folin Wu method was not satisfactory in other respects. We turned to the acid zinc sulfate sodium hydroxide method of Somogyi (3); 100 ml. of acid zinc reagent were diluted to 212 ml. with water. This gave satisfactory recoveries at plasma dilutions of 1–20. Recoveries ranged from 96–102 per cent at plasma concentrations between 20 and 40 mg. per cent. Appropriate corrections were made for plasma and urinary blanks.

[1] Present address: Physiological Laboratories, Cambridge, England.

3. Dog clearance techniques

(*a*) *Single injections:* Trained unanesthetized dogs were given 300 ml. of 0.3 per cent NaCl by stomach tube 30 minutes before the test. Test materials were injected intravenously at a rate of about 30 ml. per minute as 50 ml. of a solution containing 2 or 5 gm. each of polysaccharide and creatinine. Samples of blood and urine were collected at intervals, at first of five and 10 minutes and then at longer intervals over four to six hours. Plasma concentrations were calculated by interpolation to the mid point of each period of urine collection from a semilogarithmic graph of the determined concentrations, and these estimates were used in the calculation of plasma clearances.

(*b*) *Continuous infusion method:* A small plastic catheter was placed in the femoral vein and 30 ml. of priming solution, containing usually 1 gm. each of polysaccharide and creatinine and 4 gm. of mannitol, were injected rapidly. This was followed by a sustaining solution containing saline and given at a rate of about 1 ml. per minute. The bladder was rinsed with saline 15 minutes after completion of the priming injection; this was noted as zero time. Urine samples were collected at intervals of 10 to 15 minutes and combined with the saline bladder rinses (30–50 ml.). Samples of venous blood were collected near the mid points of each urine collection. This routine was varied in detail (concentration of polysaccharide, rate of infusion and intervals of collection) in some experiments.

4. Clearance techniques in human subjects

The methods, except for addition of polysaccharide to the priming and sustaining media in amounts of 3 and 5 gm., respectively, are those described elsewhere (4) for the determination of plasma clearances of mannitol (C_M) and p-aminohippurate (C_{PAH}). Some of the observations in patients include only the three periods of urine collection used in the measurement of PAH clearance at low plasma levels. PAH was omitted from most of the infusions used in normal subjects.

RESULTS

1. Experiments in dogs

(*a*) *Single injection method:* The ratio of polysaccharide (P) to creatinine (Cr) clearance (C) decreased progressively after injection. The poly-

TABLE I

Excretions of polysaccharide and creatinine in dog

Urine sample no.	Time after injection (*mins.*)	Urinary content (*mg.*)		Plasma concentration (*mg. per 100 ml.*)		Plasma clearance (*mg. per min.*)		Clearance ratio C_P/C_{Cr}
		P	Cr	P	Cr	P	Cr	
				(*a*) *Single injection*				
11-16-49								
1	10.0	1347	558	295	96	45.7	58.1	0.79
2	20.75	885	361	166	62	48.5	58.0	0.84
3	31.25	663	330	113	51	55.9	61.6	0.90
4	40.75	353	215	75	41	49.6	55.2	0.90
5	61.25	519	420	53	35	47.8	58.6	0.82
6	91.75	435	454	35	27.5	47.7	54.1	0.88
7	121.0	249	345	26	22	32.7	53.6	0.61
8	163.25	175	305	20	18.5	20.7	39.0	0.53
9	199.75	115	242	16	15.5	19.6	42.8	0.46
10	240.0	93	245	13.5	13.2	17.1	46.1	0.37
11	303.0	114	309	12.5	12.5	14.5	39.2	0.27
12	363.0	50	240	11.5	11.4	7.2	35.1	0.21
				(*b*) *Infusion method*				
11-3-48								
0	15							
1	25			61	22.7	24.0	39	0.62
2	35			60*	19.0*	17	40	0.43
3	49			59	17.4	14.5	44	0.33
4	59			57.5*	16.1*	14.9	46.6	0.32
5	69			55.5	15.5	12.3	45.7	0.27
6	79			52.5*	15.1*	11.4	41.3	0.28
7	89			48.5	15.2	11.0	39.7	0.28
3-24-48								
0	22							
1	32			64	18.6	27.5	43	0.55
2	42			56	18.5	24.6	46	0.53
3	60			47*	17.0*	20.3	37	0.54
4	70			41	15.3	20.6	41	0.46
5	79			38.5*	15.2*	48.7	43	0.44
6	90			36	15.0	17.8	42	0.43

Three experiments in dog 7–67, approximate weight 19 kg., the first by (*a*) the single injection method and the second by (*b*) infusion after a priming injection. Other measurements of filtration rate by the infusion method in this dog in late 1949 indicate a mean of about 55 ml. per minute, from which it seems that hydration and injection of polysaccharide do not disturb the rate of glomerular filtration. Contributing to the decrease of C_{Cr} in the latter periods of the first experiment were restlessness and repeated blood sampling. Plasma concentrations in (*a*) are obtained by interpolation from a smooth semilogarithmic curve of measured concentrations against time; interpolated concentrations in (*b*) are indicated by asterisk.

saccharide clearance (C_P) was about 80 per cent of the creatinine clearance (C_{Cr}) during the first periods of urine collection and decreased to less than 30 per cent by the end of six hours. The decline in the ratio was somewhat more rapid in dogs given smaller (2 gm.) than those given larger (5 gm.) amounts of polysaccharide. A representative experiment in a dog given 5 gm. of polysaccharide is shown in Table I(*a*).

(*b*) *Constant infusion method:* The aim of these experiments was to determine if the decrease in relative polysaccharide clearance observed after single injections could be related to the rapid fall in plasma polysaccharide concentrations. The experiments were carried on over one or two hours and at plasma concentrations ranging from 15–60 mg. per 100 ml. Plasma polysaccharide concentration was maintained at a constant level in some experiments and decreased or increased in others, as shown in Table I(*b*). The absolute and relative polysaccharide clearances could not be related to plasma polysaccharide concentration, to the direction or rate of change in this function or to urine flow. Instead, the decrease in rela-

tive C_P was a function of the time elapsed after the priming dose. Results of some 60 clearance periods (10 experiments in three dogs) indicate a range of the ratio C_P/C_{Cr} of 0.92 to 0.56 in the first 20 minutes after priming (approximate mean 0.74) and a range of 0.53 to 0.27 (approximate mean 0.34) 90 minutes later. The variability in the rate of change of the ratio with time is attributable in part to individual variability among the three dogs tested and possibly in part to the use of different lots of the polysaccharide in these experiments.

2. Human experiments

(*a*) *Normal subjects:* In these, as in the patients described below, the sustaining infusion was continued for about 90 minutes. Urine collections began 15 minutes after infusion of the priming dose and were made at intervals of 12–15 minutes. The ratio C_P/C_M was found to be constant in successive periods of urine collection (Table II), in contrast to the falling ratios found in dogs. The mean ratio in the 30 clearance periods was 1.12 (standard deviation ± 0.08). In our experience plasma mannitol clearance is about 10 per cent less than the simultaneous inulin clearance. Consequently C_P is at the level of inulin clearance or glomerular filtration.

(*b*) *Essential hypertension:* Observations were made in seven patients suffering from hypertensive disease of varying severity. In these, effective renal plasma flow (C_{PAH}) ranged from 156 to 491 ml., glomerular filtration rate ($C_M \times 1.1$) from 33–135 ml., filtration fraction from 0.25 to 0.37 and Tm_{PAH} from 21 to 77 mg. per minute, per 1.73 sq. M. body surface. The ratio C_P/C_M was constant in any patient over the period of observation. The mean of this ratio (0.98, standard deviation ± 0.07) is significantly different from that in normal subjects.

TABLE II

Clearances and clearance ratios in successive clearance periods in human subjects

Clearance period no.	Normal		Essential hypertensive disease		Primary glomerular disease	
	Means	No.	Means	No.	Means	No.
1	1.17	5	0.98	7	0.63	9
2	1.10	5	0.98	7	0.60	9
3	1.07	5	0.96	7	0.65	9
4	1.11	5	0.99	7	0.70	4
5	1.08	5	0.96	7	0.70	4
6	1.08	5	0.96	7	0.74	4
Means	1.10		0.97		0.67	

Means and number of observations in successive clearance periods and means of these means of the ratio C_P/C_M in each of the groups of human subjects demonstrating that this function in human beings is not depressed as the time after the priming dose in prolonged clearances measured by the infusion method. The end of period 1 corresponds to 15 to 20 minutes after priming; each period is of 10 to 12 minutes duration so that elapsed time to the end of period 6 is about 80 minutes.

(*c*) *Glomerular disease:* In five patients suffering from acute or chronic diffuse glomerulonephritis, the mean of the ratio C_P/C_M was 0.76 (standard deviation ± 0.12). The ratio did not correlate with absolute levels of other renal function tests; in one patient (Table III, No. 1) it in-

TABLE III

*Renal functional data in glomerular disease**

Diagnosis	No.	Date	R.P.F.	G.F.R.	F.F.	Tm_{PAH}	C_P/C_M	Periods
Ac. glom.-neph.	1	12-49	827	79	0.10	—	0.75	3
		1-50	827	93	0.11	—	0.67	3
		6-50	517	70	0.14	38	0.73	3
		7-12	673	89	0.13	41	0.74	3
		8-9	574	69	0.12	56	0.83	3
Chr. glom.-neph.	2	12-49	432	51	0.12	23	0.67	6
		1-50	228	26	0.11	—	0.64	3
Ac. glom.-neph.	3	12-49	597	60	0.11	61	1.07	5
Chr. glom.-neph.	4	5-50	322	50	0.16	32	0.81	3
Chr. glom.-neph.	5	9-50	—	6	—	0.1	0.80	6
Amyloidosis	6	1-50	545	91	0.17	81	0.70	6
Glomerulosclerosis	7	10-50	108	19	0.18	—	0.88	3

* Diagnosis (Ac. glom.-neph. = acute diffuse glomerulonephritis, Chr. glom.-neph. = chronic diffuse glomerulonephritis), renal functional data (R.P.F. = renal plasma flow from C_{PAH}, G.R.F. = glomerular filtration rate from $C_M \times 1.1$, F.F. = filtration fraction, Tm_{PAH}, mean ratio C_P/C_M) and number of polysaccharide clearance periods in patients with primary glomerular disease.

creased during recovery from acute glomerulonephritis. The ratio was depressed in one patient with renal amyloidosis (confirmed by biopsy) and one with intercapillary glomerulosclerosis. These observations are listed in Table III.

3. Observations on proteinuria

The decrease in the ratio C_P/C_M found in hypertensive disease and the still lower values of this function in patients with glomerular disease, in whom proteinuria is usually more severe than in hypertension, prompted us to examine the association between the ratio C_P/C_M and proteinuria. Proteinuria was measured during 12 or 24 hour periods by the Shevky-Stafford method immediately before or after the determination of relative polysaccharide clearance. The correlation coefficient of the ratio C_P/C_M (x) to proteinuria in gm. per 24 hours (y) was found to be -0.74 (standard error ± 0.23). This correlation coefficient is more than three times the standard error, from which it follows that, in these cases of renal disease, proteinuria and the ratio C_P/C_M are inversely associated.

DISCUSSION

The diffusion coefficient and sedimentation constant of the polysaccharide, assuming a partial specific volume of 0.65, yielded Ogston (5) an estimated molecular weight of 5,400. Dialysis for three weeks against water removed two-thirds of polysaccharide; studies on the residue indicated that its molecular weight was about 8,400. It would seem that the material was not homogeneous, but consisted of a large proportion of a polysaccharide with a molecular weight of about 5,000 and a smaller fraction of molecular weight about 9,000. More recently Palmer (1) obtained four fructosan fractions from Italian rye grass by serial precipitations from increasing concentrations of ethanol. The molecular weights of these as calculated from their glucose contents were 6,160, 5,380, 3,240, and 3,080. By the same method she found molecular weights of two fractions of dahlia inulin to be 7,290 and 6,800. Inulin has been considered to have a molecular weight of 5,100 (6). Thus, the polysaccharide we used is probably less homogeneous than dahlia inulin, to which it is similar in most of its properties; and its molecular weight is not very different.

In spite of these similarities, the clearance of this polysaccharide is notably different from that of inulin in the dog, in which creatinine and inulin clearance rates are identical. The ratio C_P/C_{Cr} in the dog is always less than unity and decreases as the experiment is prolonged. This inequality of C_P and C_{Cr} can hardly be attributed to tubular reabsorption of this inulin-like polysaccharide; the clearance ratio is independent of the direction of change in, or absolute level of plasma polysaccharide concentration and also of urine flow. Rather it seems that the polysaccharide mixture is, in part, not freely filterable through the glomerular capillaries. The decrease in the ratio with time can then be explained by assuming that some part of the polysaccharide mixture is perhaps filtered as freely as inulin, while another part can escape through perhaps only 20 per cent of the glomerular surface.

This assumption is based on the concept of glomerular porosity proposed by Yuile (7), and his demonstration that hemoglobin (molecular weight 68,000) in dogs passes through 3 per cent of the glomerular surface and myoglobin (molecular weight 16,400) through 20 per cent (8). Similarly, Marshall and Deutsch (9) have shown recently a rough correspondence between the molecular size of proteins and their clearances relative to creatinine in dogs. They also point out the importance of molecular asymmetry as a determinant of filterability. Thus, in dogs, ovomucoid (molecular weight 27,000) and ovalbumin (molecular weight 44,000) are cleared at similar rates. In human subjects, the passage of hemoglobin through the glomerulus has been estimated as occurring through 3 per cent (10) or 12 per cent (11) of the glomerular surface.

Lack of complete correspondence between molecular weight of proteins and their glomerular filterability seems to hold for fructosans. Taking Palmer's estimates of molecular weight, the freely filterable inulin is heavier than any fraction of the grass polysaccharide. Alternatively, using Ogston's estimates, and assuming the molecular weight of inulin to be 5,100, it seems that inulin is close to the limit of filterability through the dog's glomerulus, and that part of the polysaccharide exceeds this limit. On either basis, lack of complete filterability may result from inhomogeneity of the polysaccharide as to weight,

diameter and charge or from differences in elongation of the molecules. The observations in normal human subjects indicate that the clearance of the grass polysaccharide is substantially at the level of glomerular filtration, from which it seems that the human glomerulus is somewhat less retentive than that of the dog.

The 10 per cent decrease in the ratio C_P/C_M in patients with hypertensive disease can be explained by assuming that 10 per cent or more of the residual glomerular surface may have become damaged and thickened so that, while it transmits mannitol freely, it retains part of the polysaccharide. On this assumption one might expect that the ratio C_P/C_M would fall off in successive clearance periods as did the C_P/C_{Cr} ratio in the dog. We have no explanation why this did not occur. The observed decrease in the C_P/C_M ratio in hypertension, where some degree of glomerular damage can be anticipated, suggested that the ratio might serve as an index of the degree of glomerular disease. The low ratios observed in patients with diseases primarily affecting the glomeruli seem to justify this expectation. We envisioned use of the ratio as a clinical test of relative glomerular porosity. Unfortunately, a patient (Table II, No. 6) who had a history of hay fever developed mild asthma and severe urticaria during administration of the polysaccharide. Another who had had severe asthma, developed a very frightening asthmatic attack immediately after administration of the priming dose of polysaccharide. Consequently, the use of grass polysaccharide as a routine test for glomerular damage is unduly hazardous.

The observations on proteinuria bear on the debated point of glomerular versus tubular participation in the genesis of proteinuria. A decrease in the ratio C_P/C_M in renal disease could be of either glomerular or tubular origin. If it were tubular, one would have to make the rather bizarre assumption that the damaged tubule somehow newly acquires a capacity for active reabsorption of polysaccharide, so selective that it does not entail a similar reabsorption of such a close congener as inulin. This is unlikely. Much more likely is the possibility that tubular damage would result in passive back diffusion of substances from tubular fluid and, since mannitol diffuses more rapidly than polysaccharide, this would involve an increase rather than a decrease in the ratio C_P/C_M. We can therefore admit the assumption that the ratio C_P/C_M is a measure of glomerular function and that depression of this ratio is evidence of a glomerular abnormality which can be characterized as a decrease in glomerular porosity to molecules with the physical characteristics of the polysaccharide.

The rates of proteinuria in our patients with hypertensive and primarily glomerular renal disease show a significant inverse correlation with the function C_P/C_M. Hence, on the above assumption, the proteinuria is more probably due to glomerular than tubular disorder. It is paradoxical that passage of plasma protein through the glomerulus should seem to increase as porosity to polysaccharide (molecular weight less than 10,000) decreases. The explanation may lie in the physical characteristics of the substances. Thus, Brandt, Frank, and Lichtman (10) found that glomerular porosity to hemoglobin is not increased in proteinuric patients but may be decreased. The mechanism of a decreased porosity to molecules normally partially or wholly filterable, such as polysaccharide and hemoglobin, in the presence of increased proteinuria can perhaps be visualized if one likens the passage of the filterable molecules as akin to true filtration, and the escape of plasma protein as a leak in a filtering surface.

SUMMARY

Renal plasma clearances of a polysaccharide isolated from Italian rye grass with a mean molecular weight of about 5,400 were measured in dogs and human beings. The observations in dogs indicated that the material was not all freely filterable through the glomerulus. The ratio of polysaccharide clearance (C_P) to creatinine clearance (C_{Cr}) was less than unity and decreased in successive clearance periods, apparently because of the more rapid excretion of the more filterable component(s) of the polysaccharide.

In contrast, in normal human beings, the ratio of C_P to mannitol clearance (C_M) averaged 1.1, indicating that the polysaccharide was freely filterable with a renal clearance equal to that of inulin. The normal human glomerulus is apparently a less retentive filter than that of dogs.

Observations in patients with hypertensive disease yielded a mean C_P/C_M of 0.98, indicating that about 10 per cent of the residual glomerular bed had become impermeable to the polysaccharide, while still permeable to mannitol. In patients with primary glomerular disease still lower ratios were found. These were interpreted as resulting from decreased glomerular porosity and were considered to confirm by functional means, the severe anatomical changes which would be found in the glomerular capillaries in these conditions.

Assuming the ratio C_P/C_M to be a measure of relative glomerular porosity, glomerular porosity is found to decrease as proteinuria increases in hypertensive and primary glomerular renal disease. From this it seems to follow that the mechanism of such proteinuria is more probably glomerular than tubular.

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ADDENDUM

The rhizomes of the wild iris (*Iris pseudocorus*) contain a polysaccharide, irisin, which Ogston estimated had a molecular weight of 22,000–24,000 and which was apparently homogeneous in the ultracentrifuge. The polysaccharide was isolated by the method of Euler and Erdtman (12). When its clearance relative to creatinine was determined in dogs, we found that it behaved as did the polysaccharide from Italian rye grass. From this is seemed likely that this material, like grass polysaccharide, was also inhomogeneous.

Since allergy to iris is much less common than grass allergy, we had hoped that the iris polysaccharide might be adapted to clinical estimates of glomerular porosity. However, the ratios C_{Ir}/C_M in three normal human subjects were 0.73, 0.50, and 0.53; values of 0.72 and 0.65 were found in two patients with essential hypertension; in 11 observations in patients with glomerular disease, the ratios ranged from 0.29 to 0.65. The excretion of iris polysaccharide evidently does not reflect the interesting changes in glomerular porosity measured by grass polysaccharide.

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