Studies on Renal Excretion of Beryllium.* (19285)

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Considerable attention has been given in the recent literature to the problem of beryllium poisoning(1-6). The distribution and excretion of beryllium have been studied by several workers(7,8). In pursuing further knowledge regarding the interactions of beryllium in the body, it was of interest to study the mechanism by which this metal is handled by the kidney. By combining renal clearance measurements with plasma ultrafiltration studies, it was hoped to show whether beryllium is excreted by glomerular filtration or by means of tubular activity. While this work is by no means definitive, a strong suggestion has been obtained that the latter must play an important role.

Experimental. While the rabbit is not usually the animal of choice for renal clearance studies, rabbits rather than dogs were employed in the present work because of the high cost of the Be^7 isotope used. Although clearance values often vary widely from one rabbit to another and are extremely sensitive to differences in the rate of urine flow, the use of clearance ratios, where the clearance of the substance being studied is referred to the simultaneous clearance of a standard substance such as inulin, minimizes such difficulties(9). Young male rabbits were fasted for about 18-20 hours preceding the beginning of the experiments. About 2 hours before the experiments, water (30 ml/kg) was administered by stomach tube; this was repeated 3 times at half-hourly intervals. About 1 hour before the experiments, inulin (10 ml of a 20% solution in isotonic saline) was given subcutaneously, followed by a similar intravenous dose about 30 minutes before the experiments. Just before the start of the experiments, the rabbits were tied down on a rabbit board and catheterized. The Be^7 solution, containing about 5 mg of citric acid to keep the beryllium in soluble form before injection, and adjusted to physiological pH, was given intravenously. A few minutes after the isotope injection, the bladder was washed repeatedly with warm water, and the collection of urine was begun. Urine was collected for about 30 minutes, after which the bladder was washed repeatedly and the washings added to the urine sample. In most of the experiments, 3 such collection periods were run. Blood samples (about 2 ml) were obtained from the peripheral vein of the ear at 10-15 minute intervals throughout the urine collection periods. The blood was collected in heparinized tubes and centrifuged immediately. The plasma was removed, and together with the urine samples stored in the refrigerator prior to analysis. In several of the experiments, an additional blood sample of about 10-15 ml was withdrawn for the purpose of determining the ultrafiltrability of the beryllium. This sample was taken early in the experiment so that the Be^7 activity would be fairly high. The ultrafiltration was carried out immediately after collecting the blood sample. The latter was centrifuged, the plasma activity measured, and ultrafiltration was carried out using a centrifuge type of apparatus essentially the same as that described by Feldman *et al.*(10). These authors had studied the permeability of the cellophane membrane employed: it was found to be permeable to small colloidal particles such as inulin, but impermeable to beryllium hydroxide and protein; beryllium in 0.001 M hydrochloric acid readily passed through the membrane, as did beryllium in neutral solution in the presence of complexers such as citrate. Plasma and urine samples were counted with a Geiger-Muller counter equipped with a dipping tube. In all cases, sufficient counts were taken to give a counting error of less

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than 3%. Inulin determinations were carried out by Harrison's modification of the diphenylamine method(11). In tests of the method on analyses of plasma samples containing known quantities of inulin in concentrations similar to those encountered in this study, errors greater than 5% were never found.

Results. The results of all the clearance determinations are summarized in the accompanying table. It may be seen that in those cases where citrate was not added to insure the solubility of the beryllium prior to injection, the beryllium clearance was very small as contrasted with those cases where soluble beryllium was injected. This might suggest that the beryllium, once in an insoluble form, is resistant to the action of physiological complexers to the extent that it never, during the course of the experiments, assumed the same chemical form as did soluble beryllium. It is strange to note, however, as pointed out below, that even "soluble" beryllium becomes non-diffusible after equilibration with plasma. Further studies regarding the state of beryllium in the plasma will be required for an explanation of these findings.

A second observation of interest is that the addition of carrier did not markedly alter the beryllium clearance. It may be pointed out that while only 180 γ of carrier was added, this represents an increase of several orders of magnitude over the quantity of beryllium in carrier-free Be^7.

Third, it may be seen that there is little correlation between the inulin clearance and the beryllium clearance. The clearance ratio varies sufficiently widely, not only from one rabbit to another but also with the same rabbit in different urine collection periods, to suggest that beryllium is excreted by a mechanism different to some extent from that involved in inulin excretion. Errors in the methods used could account for only a small part of the variation. This suggests tentatively the idea of tubular activity in beryllium excretion.

The results of the plasma ultrafiltration study support this view. These results may be summarized by stating that in no case was any part of the plasma beryllium found to be ultrafiltrable. Plasma samples containing several hundred counts per minute per ml invariably yielded filtrates contain-

TABLE I. Summary of Clearance Data.

Rabbit No.	Description of dose	Inulin clearance (ml/min)	Beryllium clearance (ml/min)	Clearance ratio, C_{Be}/C_{inulin}
1	Carrier-free Be^7 without citrate*	6.6	1.8	.27
		8.7	1.8	.21
		7.8	1.6	.21
		8.2	3.5	.43
2	Same	12.1	.82	.07
		15.5	1.2	.08
		4.7	.26	.06
		1.3	0	0
3	Be^7 with 180 γ carrier and citrate†	11.6	6.6	.57
		14.2	14.1	.99
4	Carrier-free Be^7 with citrate†	7.6	7.1	.93
		14.9	22.3	1.5
		11.7	7.9	.68
5	Same	19.8	14.6	.74
		14.9	8.1	.54
		9.2	4.1	.45
6	Same and PAH and carinamide‡	8.3	6.8	.82
		11.4	8.1	.71
		2.6	2.8	1.1

* Citrate was not added to insure solubility of the beryllium before injection.

† Citrate, a potent complexer of beryllium, was added to the solution before the pH was raised to physiological value prior to injection; thus the beryllium was administered in the form of a true solution.

‡ In an attempt to demonstrate tubular secretion of beryllium, this rabbit was given 1 g of carinamide by stomach tube 1 hr before the experiment, and 1 g of p-aminohippuric acid intravenously repeated at 3 half-hourly intervals during the experiment.

ing no more than 5 or 10 counts per minute per ml, in other words, not significantly greater than background. In view of the studies of Feldman *et al.*(10) using the same type of apparatus, it is certain that the failure to find beryllium in the ultrafiltrate represents a true non-diffusibility and is not an artifact resulting from the apparatus employed. While it is not considered that the cellophane ultrafilter resembles in all regards the glomerular filtration mechanism of the kidney, such *in vitro* studies often lead to significant suggestions. Taken together with the clearance data, the ultrafiltration studies point toward the involvement of tubular activity in beryllium excretion. The clearance of a non-filtrable substance seems unusual, but there is insufficient data to discuss it further. Studies on the state of beryllium in the blood, now in progress, and further work on the renal mechanisms involved, will be required for elucidation of the problem.

As shown in the table, an attempt to demonstrate an inhibitory effect of p-aminohippuric acid and carinamide on possible tubular excretion of beryllium was not successful, but because of the variety of tubular secretory systems, negative findings in such an experiment prove little.

Summary. Carrier-free Be^7 injected in an insoluble form has a very low clearance as compared with the simultaneous inulin clearance. When citrate, a potent complexer of beryllium, is added to insure that the injected beryllium is in an insoluble form, the clearance is much greater, despite the fact that ultrafiltration studies indicate the soluble beryllium to assume a non-diffusible form after mixing with the blood. The addition of carrier does not markedly alter the beryllium clearance. The clearance ratio, *i.e.*, C_{Be}/C_{inulin}, varies widely enough to suggest that the two substances are not excreted by the same mechanism, and the possibility of tubular activity in beryllium excretion comes to mind. This idea is supported by ultrafiltration studies which show that beryllium exists in the plasma in a non-diffusible form. Complete clarification of the problem must await further work on the state of beryllium in the blood.

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Differential Rates of Diffusion of Mannitol from Phases of Extracellular Compartment in Edematous States.*† (19286)

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