Chromium-51 Ethylenediaminetetraacetate for Estimation of Glomerular Filtration Rate

Abstract. *Renal clearances of inulin and chromium-51 ethylenediaminetetraacetate were compared in sheep. In 41 clearance periods from five animals the mean clearance ratio of the chelate to inulin was 0.95 ± 0.03 (S.D.). Part of the difference between the two sets of results may have been due to binding of the metal chelate by plasma protein since 1.5 to 2 percent of chromium-51 ethylenediaminetetraacetate in plasma could not be removed by dialysis.*

Inulin is the standard substance for estimating glomerular filtration rate, but it is not particularly easy to measure, and there have been many attempts to find a suitable alternative. Labeled compounds have not been overlooked; C^{14}-carboxyl-labeled inulin, I^{131}-diatrizoate, and I^{125}-allyl inulin have been recommended (*1*) but expense or other disadvantages have limited their use. A compound labeled with a gamma-emitting isotope and excreted solely by glomerular filtration would greatly facilitate the study of renal function, and the work of Watkin *et al.* (*2*) drew attention to the possibilities of Co^{57}-vitamin B_{12}. Nelp, Wagner, and Reba (*3*) subsequently showed that the freely diffusible fraction of Co^{57}-vitamin B_{12} in plasma was excreted by the kidney in a manner similar to that of inulin, and several recent reports have been made on the use of the labeled vitamin for measuring the glomerular filtration rate in man (*4*). As a replacement for inulin, Co^{57}-vitamin B_{12} has two disadvantages: (i) it is appreciably bound to plasma protein (*5*), and (ii) it has a long biological half-life in the liver so that repeated use of the labeled substance in a patient with seriously impaired renal function would be undesirable.

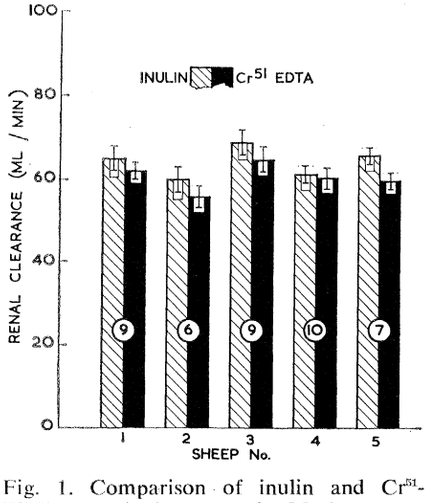

Fig. 1. Comparison of inulin and Cr^{51}-EDTA renal clearances in Merino ewes. Means and standard deviations are shown for five animals; the encircled numbers are the clearance periods from which the data were derived.

In initial work with sheep we have shown that Cr^{51}-EDTA (*6*) may fulfill requirements for estimation of the glomerular filtration rate. Inulin (*7*) and Cr^{51}-EDTA clearances were compared simultaneously in five trained, conscious, Merino ewes whose body weight was 35 to 40 kg (*8*). A steady diuresis was maintained by the administration of a water load through a rumen fistula; the animals were trained to urinate reflexly when their hindquarters were gently touched. Urinary flow rates varied somewhat from animal to animal; for the five ewes (Fig. 1) the mean values ranged from 2.5 to 11.5 ml/min. Inulin was measured by the resorcinol technique (*9*) modified for use with an Auto Analyser (*10*); Cr^{51} was assayed in a well-type scintillation counter. Figure 1 shows the clearance values for the two procedures. In each experiment there were between six and ten consecutive clearance periods of 15 minutes and the mean values for the experiments are shown by the heights of the bars in the figure. Altogether 41 clearance periods from five sheep are represented in Fig. 1, and the overall mean of the clearance ratio of Cr^{51} to inulin was 0.95 ± 0.03 (S.D.). With both techniques the coefficient of variation between clearance periods was 3 to 5 percent. This low variation can probably be attributed to the placid nature of the sheep. The animals were well accustomed to their environment and were unperturbed by the experimental procedures. In several experiments not reported here, similar clearances of Cr^{51} and inulin were also obtained when mannitol was used as a diuretic.

The clearance was not affected when the amount of Cr-EDTA in the plasma was grossly increased (Fig. 2). After cannulation of the ureters, bilateral renal clearances were estimated in an anesthetized sheep before and after the specific activity of Cr^{51} in the plasma was lowered by the infusion of a large dose of inactive Cr-EDTA. Diuresis was induced with mannitol. Initially the concentration of Cr was 0.004 mg/100 ml of plasma, and this figure was raised to a calculated level of 1 mg of Cr per 100 ml of plasma during the constant infusion of carrier Cr-EDTA. Since the excretion of the Cr^{51} was not affected by the excess of the carrier, it seemed clear that the renal clearance of Cr-EDTA was independent of the plasma concentration.

The metal chelate is negatively charged, and the slight difference between the inulin and isotope clearances could have been due, in part, to binding of Cr^{51}-EDTA to plasma protein. Plasma labeled with Cr^{51}-EDTA was dialyzed for 16 hours against 0.9 percent saline at 25°C, and 1.5 to 2 percent of the activity was nondialyzable.

Mellor (*11*) has emphasized that, regardless of the stability constant, metal complexes may be classed as labile or inert. Unlike most EDTA chelates used in biological work, the Cr(III) complex of EDTA is stable and inert (*12*), so it is unlikely that exchange reactions with other metal ions would occur in vivo. The deleterious effect of Ca-EDTA on the renal proximal tubules is well recognized (*13*); however it seems reasonable to assume that Cr-EDTA would not show similar effects because it is inert. Indeed this was

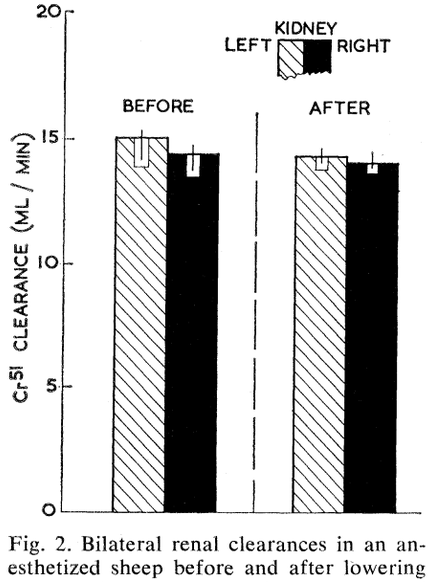

Fig. 2. Bilateral renal clearances in an anesthetized sheep before and after lowering the specific activity of Cr^{51} by infusion of Cr-EDTA. Block heights are the means of five consecutive clearance determinations; the ranges of the values are shown by the central vertical lines.

shown to be so in some recent experiments in our laboratory. Rats were injected for 25 days with unlabeled Cr-EDTA in amounts that would have been nephrotoxic had the EDTA been given as the calcium chelate. At the end of the experiment the animals were injected with a single dose of Cr^{51}-EDTA, and, since they excreted the labeled Cr as rapidly as normal animals did, their kidney function was presumably unaffected by the prolonged treatment with large quantities of Cr-EDTA. Also, histological examination of the kidneys failed to reveal any abnormalities.

After intraruminal administration very little Cr-EDTA is absorbed from the gut, and Downes and McDonald (*14*) have described how the complex may be used to advantage as a soluble rumen marker. Our results suggest that Cr^{51}-EDTA may also prove of value in the investigation of renal function.

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6. Initially we prepared Cr^{51}-EDTA from $Cr^{51}Cl_3$ by the technique of Downes and McDonald (*14*). Subsequently, supplies of Cr^{51}-EDTA became readily available at relatively low cost from the Isotope Division of the Australian Atomic Energy Commission, Lucas Heights, N.S.W., Australia. Storage of the labeled material (specific activity 0.5 c/g of Cr) for a period of one half-life of Cr^{51} (28 days) caused no obvious change in the renal excretion of Cr^{51}-EDTA.
7. Inulin for injection was obtained from Thomas Kerfoot & Co., Lancashire, England. The purity of the material was examined by paper chromatography of solutions to which were added tracer amounts of C^{14}-fructose. The only measurable component that could be separated from inulin was free fructose, and this was present in negligibly small amounts (<1 percent). We thank Dr. T. W. Scott for advice on these investigations.
8. After priming injections of inulin and Cr^{51}-EDTA, plasma levels were stabilized by constant intravenous infusion. The priming dose of Cr^{51} was 80 μc, and the sustaining infusion was 0.5 μc/min; plasma levels were 5 to 10 mμc/ml. Urine and plasma samples (3 ml) were counted in a NaI (Tl) crystal with an efficiency of 4 percent (count rates corrected for background were > 20 count/sec).
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10. The procedure with the Auto Analyser (Technicon Instruments Corporation, New York) was developed for the determination of inulin by the direct resorcinol reaction without alkali treatment. Details of the method are being prepared for publication by B. W. Wilson of C.S.I.R.O., Division of Animal Physiology, New South Wales, Australia. Estimates of the renal clearance of inulin were similar when the automated technique was compared with the manual method for measuring alkali-stable inulin. Recovery of inulin added to urine or plasma was 98.5 to 101 percent by autoanalysis.
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Urease Activity in Blue-Green Algae

Abstract. *The apparent enzymatic hydrolysis of urea has been detected in whole blue-green algae and in cell extracts. Urease is present as an intracellular component in cultures in which no bacterial contaminants are found. The activity in the cells was recovered from the extracts.*

During an investigation of the physical properties of normal and fully deuterated phycocyanins (*1*), it was observed that when urea was used as a structure perturbant and dissociating agent there was an increase in *p*H of the buffered protein preparations over a period of several hours. This finding raised the question whether the enzyme urease was present in the preparations. No previous report of urease activity in Cyanophyta has been found. There are conflicting reports about the use of urea as a nitrogen source by blue-green algae (*2*, *3*). In addition, the mechanism of urea metabolism in blue-green algae was obscure, for urease activity could not be detected. An investigation of the origin and nature of the degradation of the urea by the phycocyanin preparations was therefore undertaken.

A phycocyanin preparation from *Plectonema calothricoides* in the presence of urea was examined manometrically. A gas was evolved under both basic and acidic conditions in the approximate ratio of 2 : 1, which is the proper stoichiometry for NH_3 and CO_2 evolution from the urease reaction. This fact and the determination of increasing amounts of ammonia with time by a Nessler technique appeared to indicate definite urease activity.

Fresh cultures of *Plectonema calothricoides*, *Phormidium luridum*, *Porphyridium aerugineum*, and *Porphyridium cruentum* were obtained from the Indiana University culture collection and were maintained bacteria-free in appropriate media (*4*). Several cultures obtained from other sources were also used in these experiments; *Cyanidium caldarium* (M. B. Allen, Kaiser Foundation, University of California, Berkeley), and *Synechococcus lividus* (J. Middlebrook, Ling-Temco-Vought Research Center, Dallas, Texas). All the cultures were grown under fluorescent light on rotary shakers and, with the exception of the thermophilic *S. lividus* and *Cyanidum caldarium*, all were cultured at room temperature. Cells harvested by centrifugation were washed twice with phosphate buffer (*p*H 7.0, $\mu = 0.1$) and then were assayed whole or used to prepare cell extracts. Samples of the harvested cell cultures were inoculated into casein hydrolyzate semi-solid agar with thioglycollate (*5*) and incubated at 37°C for 72 hours and at room temperature for another 72 hours. If no growth appeared at the end of the incubation period, the cultures were considered as probably free of most contaminants. Examination of these cultures under high magnification with a phase-contrast microscope did not reveal detectable bacterial contaminants. If any contaminants were present they were extremely minute. Cultures found to be essentially bacteria-free by the thioglycollate test are listed in Table 1. Bacterial contaminants were evident in cultures of *Synechococcus lividus* and *Porphyridium cruentum*.

Cell extracts of *Plectonema calothricoides*, *Phoridium luridum*, and *Synechococcus lividus* were prepared by adding lysozyme and leaving the suspension at room temperature for 24 hours. The suspension was centrifuged at 18,000*g* for 10 minutes, the supernatant was decanted, and more lyso-