ENVIRONMENTAL RESEARCH 2, 1–10 (1968)

Renal Excretory Mechanisms of Heavy Metals
I. Transtubular Transport of Heavy Metal Ions in the Avian Kidney

J. VOSTÁL AND J. HELLER

Institute of Industrial Hygiene and Occupational Diseases, Praha, Czechoslovakia

Received June 6, 1967

Inulin, as a reference glomerular substance, was injected simultaneously with radioactive isotopes of copper, cobalt, chromium, manganese, molybdenum, mercury or lead ions, as well as with cadmium in the form of cadmium cysteinate hydrochloride, into the renal portal circulation of the chicken. Radioactivity in all studied metals, independent of valence or positive or negative charge of the ions, was demonstrated in fractionated urine collections before the first detectable traces of inulin. All studied heavy metals are therefore considered to be similar to sodium and other essential ions in being able to pass into the urine not only by glomerular filtration but also by direct transport across the tubular wall. The possible significance of this transtubular transport in the renal excretory mechanism of heavy metals, in relation to their plasma diffusible fractions, is briefly discussed.

The pharmacology of heavy metals, in view of their extensive use in industry and their increasing presence in the external environment, has attracted considerable attention. Results of recent studies have been summarized (Passow *et al.*, 1961). The general aspects of the mechanisms of renal excretion have not, however, been studied to any great extent so far, and in some respects even basic data are lacking. Knowledge of excretory mechanisms may be expected to contribute understanding of basic principles of evaluation of individual or population exposure and may even afford the possibility of therapeutic alteration of such regulatory mechanisms.

Although individual heavy metals differ considerably in their chemical reactivity, they are practically all able to bind with a number of organic molecules. The high degree of their interaction with ligands present in erythrocytes and/or plasma proteins, however, considerably limits the use of classical clearance methods (Smith, 1955) in the study of their renal excretion. If ultrafiltration through the glomerular membrane and/or permeation through the tubular wall in both directions be considered (the two most important general processes in the transport of any substance between blood and urine) then it may be agreed that the participation of glomerular filtration in the mechanism of renal excretion has been demonstrated for a number of metals (Wills, 1953 for uranium; Hursh *et al.*, 1960, for radium; Collins *et al.*, 1961, for chromium; Vostál, 1963, for lead, etc.) and predicted in others (Simmonds, 1942; Rodin and Crowson, 1962; or Gayer *et al.*, 1962, for mercury, etc.). On the other hand, the predominance of tubular secretory mechanisms in metal excretion by the kidney has been stressed for mercury (Bergstrand *et al.*, 1959; Passow *et al.*, 1961; Berlin and Gibson, 1963; Mam-

bourg and Raynaud, 1965, etc.) and the experiments carried out by stopflow technique in dogs by Gayer *et al.* (1962) tended to demonstrate tubular reabsorption of filtered mercury in the kidney. Similar behavior of lead ions as well was observed in acute exposure experiments on dogs (Vostál, 1963). The question, therefore, whether heavy metal ions are able to be transported across the cellular structures of the renal tubule seemed to be important for demonstrating tubular component participation in the general mechanisms of heavy metal excretion by the kidney.

Renal portal circulation in avian kidney has been used in this investigation to study the possibilities of transtubular transport of heavy metals.

EXPERIMENTAL APPROACH

The transport of sodium and other ions across the tubular wall not only from the lumen into peritubular fluid but also in opposite direction was demonstrated by Chinard and Enns (1955), after simultaneous injection of radioisotopes together with a glomerular substance directly into renal artery of the dog. A glomerular substance which does not penetrate the tubular wall can appear in urine only after ultrafiltration through the glomerular membrane. Earlier appearance of the simultaneously injected isotope must mean that this ion was able to pass

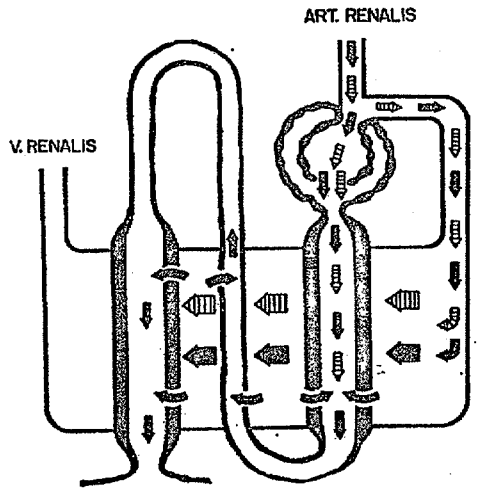

FIG. 1. Schematic drawing showing Chinard's technique of transtubular transport testing in the mammalian kidney. Tested substance (black arrows) is injected together with glomerular substance (striped arrows) simultaneously into the renal artery of the dog kidney.

through the tubular epithelium distal to the glomerulus i.e. by-passing, with the postglomerular blood, part or parts of the nephron more rapidly than the filtrate flowed through the tubular lumen and thus reached, via the peritubular capillaries, the outer wall of tubular cells and collecting ducts earlier. A concentration gradient was thereby formed between the peritubular fluid and the tubular lumen, enabling transport of the injected radioisotope across the tubular wall (Fig. 1).

The circulatory differences in mammalian and avian kidney seemed to offer better conditions for testing the existence of transtubular transport. Renal blood supply in birds is mediated by both the renal artery and the vena portae renalis, which simultaneously drains the venous system of the pelvic leg on the same side (Sperber, 1948). Blood flow of the portal circulation omits the glomerules. Its branches enter close to the vasa efferentia of the glomerules, directly into the common peritubular capillary net. If a substance being studied is transported across the tubular wall, it appears in ureteral urine after injection into portal circulation much earlier than a substance excreted only by glomerular filtration, which would be brought to the glomerular membrane only after recirculation through the entire body (Fig. 2). Moreover, the participation of other factors is completely excluded (Vostál and Heller, 1963).

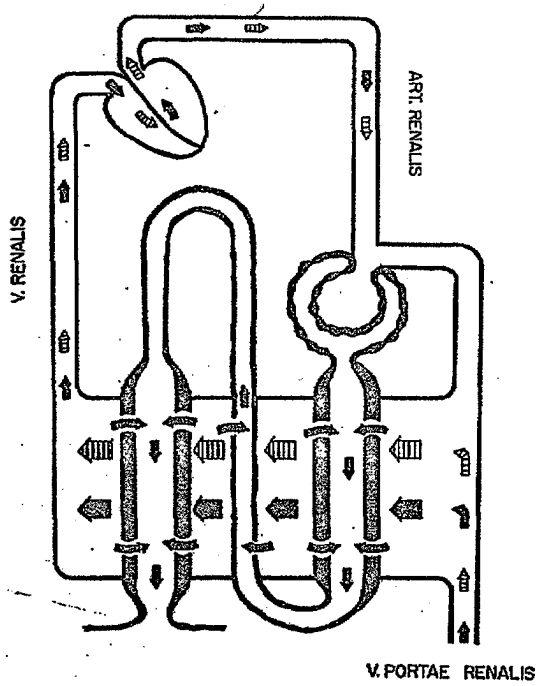

FIG. 2. Schematic drawing showing transtubular transport testing in the avian kidney. Tested substance (black arrows) is injected together with glomerular substance (striped arrows) into the renal portal system of chicken.

MATERIALS AND METHODS

Male chickens, *Gallus domesticus*, variety White Leghorn, weight 2–3 kg, were used as experimental animals. They were anesthetised by sodium pentobarbital (30 mg/kg i.p.) and ureters were cannulated from cloaca or after perineal incision. The outer diameter of the polyethylene catheters, always inserted at the same dis-

tance from the cloaca and tied in place, was such as to fit snugly into the ureters. To provide adequate urine flows intravenous injection of hypertonic 5% sodium chloride or 10% urea solution was started at the beginning of each experiment and supplemented by continual infusion as required during the course of experiment. Urine flow of 0.6–1.0 ml/kg/minute from each side was considered adequate. When the urine flow was adequate, the leg vein—a branch of vena ilica externa—on the side of the urine collection, was cannulated. After three control drops of urine directly from the cannula opening into small glass test tubes (arranged in an order similar to that used in stopflow technique) had been obtained, isotonic radioisotope solution in 0.9% NaCl containing inulin was rapidly injected and collection of individual urine drops continued. The volume of injected test solution was always 2 ml; the injection took 1–2 seconds for completion. The radioactivity of the injected solution was in all instances the same, i.e. 100 μCi; the amount of simultaneously injected inulin used as the reference glomerular substance was 100 mg. Experiments with individual isotopes were carried out in groups of 4–6 animals. The integrity of the portal circulation was tested in all animals after the experiment by intraportal injection of 0.2 mg/kg phenol red in saline and the functional state of portal circulation visually evaluated by observation of a considerable excess of red color excreted in the urine from the experimental side (Campbell, 1960). Exceptional animals whose portal circulation could not be clearly demonstrated to be functionally sound, were not included in the study. As the radioactivity on the control side was regularly delayed and appearing with the inulin, after the first experiments corrections for excretion from the control side were not carried out.

Radioactive lead $^{212}Pb^{++}$ (Thorium B), carrier-free, was produced by electrodeposition in a radioemanator containing radiothorium ($RdTh^{228}$, half-life 1.9 years). Electrodeposits were dissolved in 2 N nitric acid p.a., neutralized and suitably diluted by 0.9% sodium chloride solution. Copper $^{64}Cu^{++}$ (spec. activity 2.5 Ci/gm Cu) as well as freshly produced cadmium $^{115}Cd^{++}$ (spec. activity cca 0.1 Ci/gm Cd), both in the form of chloride salts, were supplied by Zentralinstitut für Kernforschung, Dresden, DDR. However, preliminary experiments showed that the carrier content was toxic for experimental animals, despite high specific activity of freshly produced radioisotope. Therefore, cadmium had to be injected as a mixture with cystein hydrochloride in 1:20 molar ratio (Vander, 1962). Manganese $^{52}Mn^{++}$ (carrier-free) and mercury $^{203}Hg^{++}$ (spec. activity 50–70 mCi/gm Hg), both also in the form of chloride salts, were products of the Institute of Nuclear Research, Prague. Cobalt $^{57}Co^{++}$ (carrier-free) in the form of cobaltous chloride and hexavalent molybdenum $^{99}Mo^{6-}$ (spec. activity 5 Ci/gm Mo) in the form of ammonium molybdenate as well as chromium $^{51}Cr^{6-}$ in the form of sodium chromate were obtained from Radiochemical Centre in Ammersham, England. Radioactivity of the individual drops of urine was measured in a well-type scintillation counter without further adjustments of the sample and, when necessary, corrections for radioisotope decay were made. Results were expressed in counts per minute per individual drop. Measurement time was selected after radioactivity sampling demonstrated significant differences from the background, within a $\pm$ 5% error. Urine drops, after proper dilution in the same

test tubes, were ther
method (Harrison, 1

Figure 3 demonst
of inulin and radioa
chicken and individ
and isotope. Quite si
radioisotopes of oth
available. These ex
radioactivity appea

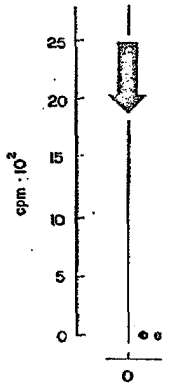

FIG. 3. Excretion
stantaneous injection
of a chicken at the mo
two figures represent t
separately from left ur
total volume of urine
appearance of detectab

which does not pas
graphic presentatio
ferent radioisotopes
were obtained by a
percents were calcul
and the first evidenc

In Figs. 4 and 5
isotopes of heavy
studied metal ions,
the ion, appear in
portal circulation
merely by glomeru
other ions (Vostál a

test tubes, were then analysed for inulin by a micromodification of diphenylamine method (Harrison, 1942) with alkali treatment.

RESULTS

Figure 3 demonstrates a typical result of one of experiments in which a mixture of inulin and radioactive lead $^{212}Pb^{++}$ was injected into the portal circulation of a chicken and individual drops of subsequently produced urine analysed for inulin and isotope. Quite similar results were obtained in other experiments with lead and radioisotopes of other heavy metals, wherever their gamma-emitting isotopes were available. These experiments demonstrated that after simultaneous injections radioactivity appears in urine much earlier than simultaneously injected inulin,

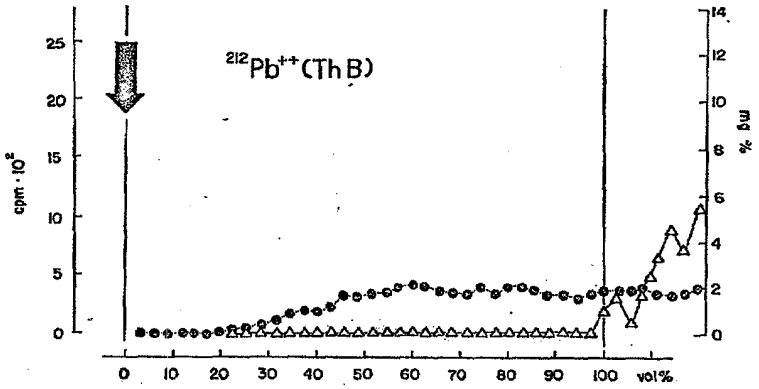

FIG. 3. Excretion pattern of radioactive lead isotope (●) and inulin (△) after an instantaneous injection of 100 μCi $^{212}Pb^{++}$ together with 100 mg of inulin into the left leg vein of a chicken at the moment indicated by an arrow. Different symbols in this and the following two figures represent the concentration of tested substances in single drops of urine collected separately from left ureter. Individual drops are on the abscissa expressed as a percentage of total volume of urine excreted by the kidney in the time interval between injection and first appearance of detectable traces of inulin.

which does not pass through the tubular wall in either of the two directions. For graphic presentation and comparison of individual experiments as well as different radioisotopes additional calculations were made. Although individual drops were obtained by a modified stopflow technique (Malvin *et al.*, 1958) urine volume percents were calculated from the volume of urine obtained between the injection and the first evidence of measurable inulin concentrations.

In Figs. 4 and 5 typical excretion patterns obtained with representative radioisotopes of heavy metals are shown. Both figures clearly demonstrate that all studied metal ions, independent of their valence or positive or negative charge of the ion, appear in the urine, after simultaneous injection with inulin into the portal circulation of chicken kidney, much earlier than a substance excreted merely by glomerular filtration. As in previous experiments with sodium and other ions (Vostál and Heller, 1963), the data demonstrate that the studied heavy

metals ions, as well as cadmium cysteinate hydrochloride, are transported across the tubular wall distal to the glomerulus, in the chicken kidney. Significant increase in radioactivity appeared at 32–62% of the urinary volume excreted during the interval between the injection and first traces of inulin in the urine. Although individual heavy metals differed in their excretion pattern, the recorded differences in level and extent of radioactivity excretion remained within limits of one

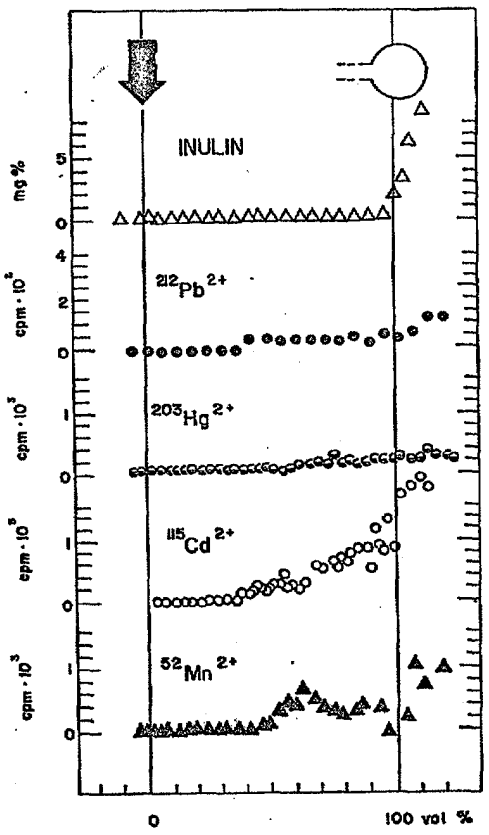

FIG. 4. Cumulative graph of typical excretion patterns of radioactive lead, mercury, cadmium (as cysteinate hydrochloride) and manganese after instantaneous injections with inulin into the renal portal system of chicken kidney at the time indicated by an arrow.

order. These differences may be primarily attributed to variable urine flow relative to different efficacies of scintillation equipment for energetically different radiation levels of various isotopes. Also, the possible effect of various degrees of binding on plasma and tissue components, as well as possible differences in diffusion coefficients should be considered in interpretation of the results. Current knowledge of the chicken kidney does not allow evaluation of the role of each

FIG. 5. Cum
denum and ch
system of chick

part of the n
Therefore, d
by possible

In view of
in the appe
differences i
the question
have for th
heavy meta
transcellular
as other es

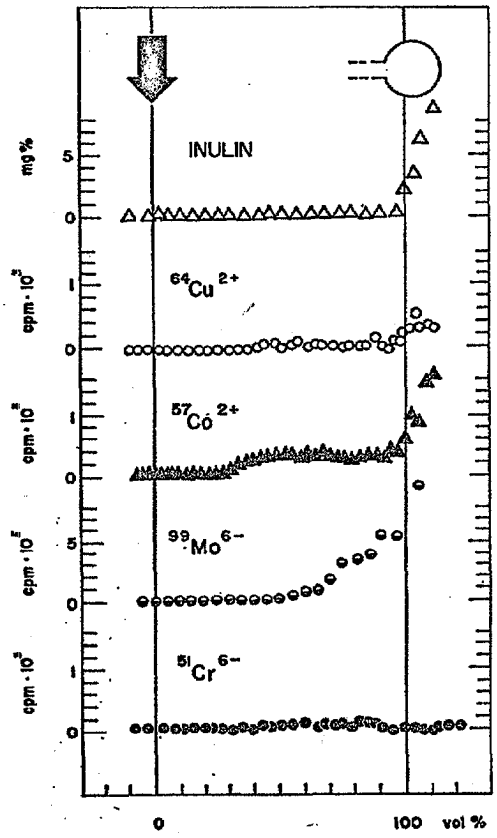

FIG. 5. Cumulative graph of typical excretion patterns of radioactive copper, cobalt, molybdenum and chromium ions after instantaneous injections with inulin into the renal portal system of chicken kidney at the time indicated by an arrow.

part of the nephron reached by intraportal injection in the individual experiments. Therefore, different excretion patterns cannot, for the time being, be explained by possible differences in excretory mechanisms for the several metals.

DISCUSSION

In view of the fact that Chinard (1955) has demonstrated that time differences in the appearance of injected metals and inulin in urine cannot be explained by differences in diffusibility of these substances in tubular fluid in nephron lumen, the question remains as to significance the proof of transtubular transport might have for the study of renal excretion of metals. It can be generally assumed that heavy metals should be, except for their increased ability to be bound onto intravascular and/or intracellular ligands, excreted by the same renal mechanism as other essential cations and anions. These mechanisms might, however, differ

considerably in individual cases, as shown by the well known differences in the excretory mechanism of chemically rather similar ions as e.g. sodium, lithium and potassium, or calcium, strontium and magnesium on the other hand (Smith, 1953). The participation of a tubular component, evident in the excretion of the above-mentioned essential cations, has been demonstrated for heavy metal ions by our results. Along with increased binding to plasma proteins—if we consider the fraction bound to blood corpuscles as not available for excretion—and therefore a decreased plasma diffusible fraction of heavy metals in blood, probably the importance of glomerular filtration in the mechanism of excretion of at least some representatives of the heavy metals group diminishes, and the higher binding ability of intracellular ligands of tubular cells may take precedence over the intravascular ligands. Rapid increase in the concentration of the metals studied, in peritubular capillary blood immediately after intraportal injection, permitted our demonstration of transtubular transport for all studied heavy metal ions, although the quantitative degree of this transport differed considerably for individual ions.

If the majority of all previously mentioned factors, probably responsible for differences found in excretion patterns, be neglected, one common factor of decisive importance for the existence of any transport in the living organism remains, i.e. the level of the diffusible fraction in blood. This diffusible fraction, consisting theoretically of ionized and unionized bound component, could be predicted for heavy metals on the basis of similar behavior of other ions in plasma. Its level, however, would probably remain under the level of our detectable limits (Berlin and Gibson, 1963; Vostál, 1963). It would depend not only on the metal-binding ability of various biological ligands as, e.g. amino-, carboxy-, and thiol-groups, where the sequence of metal affinity might differ considerably (Passow *et al.*, 1961), but would also be determined by the fact that some of these ligands are on nondiffusible macromolecular tissue or plasma proteins while others are on diffusible molecules, e.g. natural amino- and other organic acids. The level of the diffusible component would therefore probably be conditioned by many complex factors already in physiological homeostasis some of which might be affected by additional toxic effects of heavy metals, in which, for example, aminoacids might change considerably (Thoelen and Pletcher, 1953; Clarkson and Kench, 1956).

The diffusible fraction may also pass from the vessels into the extracellular fluid and consequently across the cellular membrane into the intracellular fluid; it could then be easily transported into the tubular cells. The presence of specific macromolecular nondiffusible ligands in renal tubular cells with known high affinity for a number of heavy metals, competes thereafter for entering metal ions within the diffusible fraction. The resulting decrease in metal concentration in the diffusible fraction encourages further transport of metal ions into the cell from the extracellular fluid, following the concentration gradient. Although the steady state between the concentrations of diffusible metal and metal bound to nondiffusible macromolecular ligands inside the cell is highly shifted in favor of the bound component, the diffusible fraction, although low, may come into contact with the cell membrane separating the tubular cell from the nephron lumen and, following the concentration gradient, penetrate into the tubular fluid (Fig. 6).

This is probably not the only mechanism underlying the observed transtubular transport of metal ions, since after intrarenal administration, considerable diffusion of free metal ion into the extracellular fluid may occur, reflecting the fact that binding to blood components is not yet in equilibrium. Consequent formation of a high concentration gradient in diffusible fractions considerably favors transport across the tubular cell. The existence of a diffusible component formed mainly by organic bound metal ions has also been suggested to explain metal reabsorption through the intestinal wall (Saltman, 1965) and could well be one of the many renal mechanisms by which the heavy metal ions are excreted from the body into the urine.

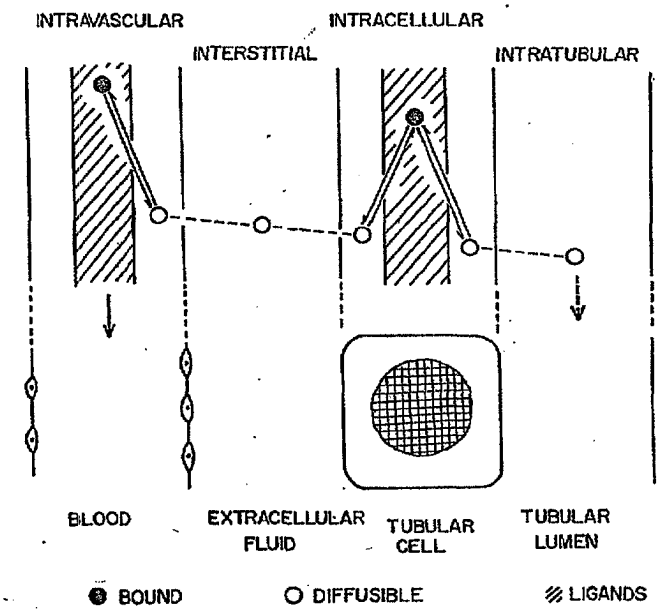

FIG. 6. Schematic drawing showing possible transport of heavy metals diffusible fraction (○) following the concentration gradient across the renal compartments into the tubular fluid.

It may be concluded therefore that we have demonstrated that all studied heavy metal ions, i.e. copper, cobalt, chromium, molybdenum, manganese, mercury, and lead as well as cadmium cysteinate hydrochloride are similar to sodium and other ions in being able to pass into the urine not only by glomerular filtration, but also by direct transport across the tubular wall. Whether or not and to what extent this mechanism participates in the normal excretion of these metals, as well as the possibility of participation of active secretory processes in this transtubular transport, still remain open questions. The approach here utilized seems useful for obtaining further information on the mechanisms of renal excretion of metals.

REFERENCES

BERGSTRAND, A., FRIBERG, L., MENDEL, L. AND ODEBLAD, E. (1959). The localization of subcutaneously administered radio-active mercury in the rat kidney. *J. Ultrastructure Research*, **3**, 234–240.

BERLIN, M. AND GIBSON, S. (1963). Renal uptake, retention and excretion of mercury. I. A study in the rabbit during infusion of mercuric chloride. *Arch. Environmental Health* **6**, 617–625.

CAMPBELL, D. E. S. (1960). Modification by bromcresolgreen or probenecid of the excretion and diuretic effect of three mercurial diuretics: Diurgin, Chlormerodrin and Mercumatilin. *Acta pharmacol. et toxicol.* **17**, 213–232.

CHINARD, F. P. (1955). Comparative renal excretions of glomerular substances following instantaneous injections into a renal artery. *Am. J. Physiol.* **201**, 795–798.

CHINARD, F. P., AND ENNS, T. (1955). Relative renal excretion patterns of sodium ion, chloride ion, urea, water and glomerular substances. *Am. J. Physiol.* **182**, 247–250.

CLARKSON, T. W., AND KENCH, J. E. (1956). Urinary excretion of amino acids by men absorbing heavy metals. *Biochem. J.* **62**, 361–372.

COLLINS, R. J., FROMM, P. O., AND COLLINGS, W. D. (1961). Chromium excretion in the dog. *Am. J. Physiol.* **201**, 795–798.

GAYER, J., GRAUL, E. H., AND HUNDESHAGEN, H. (1962). Die Lokalisierung des Transportes von Hg^{++}-Ionen in der Niere durch Stop-flow-Analyse. *Klin. Wochenschrift* **40**, 953–955.

HARRISON, H. E. (1942). *in* "Principles of Renal Physiology" by H. W. Smith, p. 210. Oxford University Press, New York, 1957.

HUBSH, J. B., LOVAAS, A., PICCIRILLI, A. AND PUTNAM, T. E. (1960). Urinary excretion of radium in dogs. *Am. J. Physiol.* **199**, 513–515.

MALVIN, E. L., WILDE, W. S. AND SULLIVAN, L. P. (1958). Localisation of nephron transport by stop-flow analysis. *Am. J. Physiol.* **194**, 135–140.

MAMBOURG, A. M., AND RAYNAUD, C. (1965). Etude a l'aide d'isotopes radioactifs, du mécanisme de l'excretion urinaire du mercure chez le lapin. *Rev. fran. études clin. et biol.* **10**, 414–418.

PASSOW, H., ROTHSTEIN, A. AND CLARKSON, T. W. (1961). The general pharmacology of the heavy metals. *Pharmacol. Rev.* **13**, 185–224.

RODIN, A. E., AND CROWSON, C. N. (1962). Mercury nephrotoxicity in the rat. 1. Factors influencing the localisation of the tubular lesions. *Am. J. Pathol.* **61**, 297–313.

SALTMAN, P. (1965). The role of chelation in iron metabolism. *J. Chem. Educ.* **42**, 682–687.

SIMMONDS, J. P. (1942). Renal pathological changes in hypertension and glomerulonephritis. Clinical interpretation. *J. Am. Med. Assoc.* **120**, 89–93.

SMITH, H. W. (1955). *In* "The kidney. Structure and function in health and disease," pp. 39–62. Oxford University Press, New York.

SPERBER, I. (1948). Investigations on the circulatory system of the avian kidney. *Zool. Bidrag, Uppsala* **27**, 429–448.

THOELEN, H., AND PLETCHER, A. (1953). Behaviour of amino acids and enzymes in heavy metal poisoning. III. Acute experimental mercuric poisoning. *Helv. Physiol. et Pharmacol. Acta* **11**, 64–66.

VANDER, A. J. (1962) Effect of cadmium on renal tubular sodium transport. *Am. J. Physiol.* **203**, 1–5.

VOSTÁL, J. (1963). Mechanisms of renal lead excretion. *Biochem. Pharmacol.* Conf. Issue 2, 207.

VOSTÁL, J., AND HELLER, J. (1963). Transtubular transport of solutes in the kidney. *Proceedings, 2nd Int. Congress of Nephrology, Excerpta Medica Internat. Congress Ser. No. 78, Amsterdam,* pp. 130–132.

WILLS, J. H. (1953). Characteristics of uranium poisoning. *In* "The Pharmacology and Toxicology of Uranium Compounds" (C. Voegtlin and H. C. Hodge, eds.) Vol. 1., pp. 237–280. McGraw-Hill, New York.