RENAL PHARMACOLOGY[1,2]

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Major recent advances in renal pharmacology include studies on the mode of action of hormones and other naturally occurring compounds on the kidney, the development of new diuretics and antibacterial drugs, and investigation of the toxic effect of widely used drugs.

Action of hormones on the kidney.—The mode of action of antidiuretic hormone has been extensively reviewed by Orloff & Handler (1). By analogy from studies utilizing amphibian skin and bladder (2, 3, 4), it is generally considered that the primary action of the hormone is an increase of permeability to water of the distal and collecting tubular epithelium. Three theories have been advanced to explain this action at the cellular level: (*a*) vasopressin stimulates the secretion of hyaluronidase by the tubular epithelial cells [Ginetzinsky (5)]; (*b*) there is interaction between a disulphide bridge in the octapeptide molecule of the hormone with free sulphydryl groups on the cell membrane [Fong et al. (6), Rasmussen et al. (7), Schwartz et al. (8)]; (*c*) vasopressin regulates the concentration of an intracellular compound, adenosine-3′,5′-monophosphate (cyclic 3′,5′-AMP), which is thought to be more directly responsible for the permeability changes [Orloff & Handler (9)].

The first theory was supported by Dicker & Eggleton (10) who confirmed that the excretion of hyaluronidase in water diuresis varied inversely with urine flow. This theory was criticized by Berlyne (11) who found no such correlation using a different method of estimation which he considered to be more accurate. This hypothesis is now considered unlikely, as it has been impossible to duplicate the action of vasopressin, whether *in vitro* [Leaf (4); Bently (12)] or *in vivo* [Rosenfeld, Hirata & Brest (13)], by the use of hyaluronidase in high concentration.

The second hypothesis is based on the reversible binding of tritiated vasopressin to kidney and bladder tissue and its release by cysteine which ruptures the postulated disulphide linkage between the hormone and its receptor. There is, as yet, no clear evidence that such a linkage would necessarily alter the permeability characteristics of the membrane.

[1] The survey of literature pertaining to this review was concluded in August, 1964.

[2] The following abbreviations are used: ADH (antidiuretic hormone); ADP (adenosine diphosphate); AMP (adenosine monophosphate); ATP (adenosine triphosphate); C_{H_2O} (free-water clearance); C_{urate} (creatinine clearance); DOPAmine (dihydroxy phenylalanine); DNA (deoxyribonucleic acid); PAH (*p*-aminohippuric acid); pKa (dissociation constant); RNA (ribonucleic acid); $T^c_{H_2O}$ (tubular reabsorption of osmotically-free water); and Tm_G (tubular maxima glucose).

The third and probably the most satisfactory hypothesis is that vasopressin catalyzes the action of the enzyme adenylcyclase which converts adenosine triphosphate (ATP) to cyclic 3′,5 -AMP [Davoren & Sutherland (14)]. The action of the latter nucleotide closely mimics that of vasopressin on the toad bladder [Orloff & Handler (9)]. Theophylline also has a similar action by inhibiting the inactivation of the nucleotide by conversion to 5-AMP, a reaction catalyzed by cyclic nucleotide phosphodiesterase. Further support is given by the observation [Brown et al. (15)] that vasopressin increases the production of cyclic 3′,5′-AMP in dog kidney particles *in vitro*. If this view is correct, however, it merely advances the problem by a single step, as the mechanism of the effect of cyclic 3′,5′-AMP on cellular permeability to water remains unexplained.

Important advances in knowledge of aldosterone action are reported by Edelman et al. (16) and Porter & Edelman (17). Their experimental results were derived from the toad bladder, but it is likely that the results apply to the mammalian kidney. They consider that aldosterone enters the cell nucleus where it stimulates deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) synthesis. The latter, in turn, enhances synthesis of enzymes concerned in supply of energy necessary for sodium transport. After preincubation of the bladder for 12 hr, aldosterone added to the medium enhances sodium transport after a latent period of 90 min; the effect then steadily increases over the next 4.5 hr. The increase is not dependent on the continued presence of aldosterone in the medium, as it persists if replaced by aldosterone-free solution. Use of tritium-labelled aldosterone showed that the binding was almost entirely in the cell nuclei. Prior incubation of the bladder with actinomycin, an inhibitor of nuclear RNA synthesis, and with panomycin, an inhibitor of ribosomal RNA protein synthesis, abolished the effect of aldosterone on sodium transport. These compounds did not, however, affect vasopressin stimulation of sodium transport, suggesting that their action was specific for aldosterone-stimulated nucleoprotein synthesis. Tritium-labelled uridine was shown to be incorporated within the nuclei at a greater rate in the aldosterone-treated tissue. It was also clearly shown that enhanced RNA synthesis preceded the rise in sodium transport [Porter, Bogoroch & Edelman (18)]. Participation of the tricarboxylic acid cycle [Edelman, Bogoroch & Porter (19)] was suggested by the necessity for the simultaneous presence of either glucose or pyruvate and by the demonstration that aldosterone increases the cellular content of high energy phosphate bonds. Oligomycin [Davis et al. (20)], which inhibits mitochondrial formation of phosphorylated intermediates in high-energy phosphate-bond synthesis, also prevented sodium transport. The transport parallelled cellular content of an unknown phosphorylated intermediate, produced from reduced pyridine nucleotide. This reacts with adenosine diphosphate (ADP) to form ATP, but studies of the effect of added ADP indicated that ATP itself was not the direct source of energy for ion transport.

Goldberg, McDonald & Zimmerman (21) reported that dihydroxy-

phenylethylamine (DOPAmine) caused a marked increase of urinary sodium in four patients with congestive heart failure. This action has not been reported after administration of other sympathomimetic amines. The results have been confirmed and extended in a larger number of patients and in normal subjects [McDonald et al. (22)]. Natriuresis was shown to be possibly due to an increase in glomerular filtration rate and in renal plasma flow. The cardiac output was increased and peripheral resistance was reduced, but the renal fraction of the cardiac output was unaffected.

Antibacterial agents.—Nalidixic acid [Lesher et al. (23)] is an antibacterial drug which is suitable for use in acute and chronic urinary infections at a dosage of 1 g per 6 hr. It has activity *in vitro* against most gram-negative urinary pathogens, especially *E. coli* and *Proteus* [Barlow (24)] an antibacterial spectrum comparable to nitrofurantoin. It is, however, less effective against *P. pyocyaneus*. Nalidixic acid is a naphthyridine derivative, 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-5-carboxylic acid and is chemically unrelated to any other established antibacterial agent. It is rapidly absorbed by mouth [McChesney et al. (25)] and is excreted partly unchanged; however, the greater portion is excreted either as a glucuronide or as the 7-hydroxymethyl metabolite, some of which is also conjugated. The excretion of both nalidixic acid and its 7-hydroxymethyl derivative resembles nitrofurantoin; it is pH dependent. Alkalinization of the urine increases the amount excreted in the free and active form from 13 to 30 percent of the administered dose. There is no accumulation in any particular tissue, and, therefore, the main antibacterial action is in the urine itself.

Side effects have been minor [Slade (26)]; the main complaints have been of rashes, nausea, or vertigo. Treatment of some patients continuously for over three months has not caused any significant toxicity. Renal failure is claimed not to contraindicate the use of the drug, but would presumably reduce urinary concentration of the unchanged drug and, therefore, limit its effectiveness. Strains rapidly develop resistance to nalidixic acid and also because of the high cost of the drug, it should not be regarded as routine in management of urinary infections. Possibly, its main use will be in the control of *Proteus* infection.

Roelsen (27) has reported a polyneuritis after nitrofurantoin therapy and was able to trace 33 cases. In the vast majority, polyneuritis only occurred in patients with impaired renal function and after prolonged therapy with the drug. Loughridge (28) found that the average plasma level of nitrofurantoin in patients receiving 300 mg daily was 1.8 μg per ml, if renal function was normal, but 5.1 to 6.5 μg per ml, if there was significant uraemia. The toxicity is possibly due to interference with the anaerobic formation of acetyl coenzyme A from pyruvate and coenzyme A [Paul et al. (29)]. Nitrofurantoin's main action as an antibacterial agent is by local concentration within urine.

Tubular transport mechanisms.—Berglund (30) has compared the effects of carinamide and probenecid on thiosulphate secretion by the renal tubules

of dogs. Probenecid has no such effect, but carinamide strongly inhibited transport. He postulated a competitive inhibition of transport: as in carinamide, the sulphone group is not directly bound to any unsaturated organic radicle; whereas, in probenecid, it is directly attached to a benzene ring.

Vander (31) reports on the effects of zinc, cadmium, and mercury on proximal tubular transport of PAH and glucose. Zinc reduced transport of both substances, but cadmium inhibited secretion of para-aminohippuric acid (PAH) alone, whereas mercury mainly reduced tubular maxima glucose (Tm_G).

Kalbfleisch et al. (32) have studied the effects of alcohol on magnesium reabsorption. Both in normals and alcoholics, ingestion of ethyl alcohol caused increased urinary output of magnesium and calcium and reduced excretion of potassium. The effect was maximal 60 to 80 min after the dose, lasted about 140 min, and was not due to changes in glomerular filtration rate and renal plasma flow. The increased urinary excretion of magnesium was claimed to account for the magnesium deficiency found in chronic alcoholics and cases of delirium tremens.

Diuretics.—The discovery of the diuretic and saluretic potency of several $\alpha\beta$-unsaturated ketone derivatives of aryloxyacetic acids is an important advance. The chemistry of these compounds has been reported by Schultz et al. (33), and the one which has been chosen as the most suitable for use in man is 2,3-dichloro-4(2-methylene butyroyl)-phenoxyacetic acid: $CH_2{:}C(C_2H_5)\cdot CO\cdot C_6H_2Cl_2\cdot O\cdot CH_2\cdot COOH$ or ethacrynic acid.

In dogs [Baer et al. (34, 35, 36)], a single intravenous injection of 1 mg per kg caused a rapid increase in output of sodium, potassium, chloride, and water which was maximal within 15 min and persisted at a lower level for 2 hr. The effect was about three to four times greater than that produced by the same dose of hydrochlorothiazide. Whereas hydrochlorothiazide depressed sodium reabsorption to only 95 percent of the filtered load, the corresponding figure for ethacrynic acid was as low as 85 percent. The Na:Cl ratio, after the latter diuretic, was only slightly less than unity, and the urine pH fell only slightly. Metabolic acidosis and alkalosis did not appreciably alter the effect of the drug. Preliminary reports of the effects of ethacrynic acid in man are encouraging [Melvin, Farrelly & North (37); Foltz (38); Cannon, Ames & Laragh (39)]. Excessive loss of water and electrolytes sometimes occurs, and severe hypokalaemic alkalosis may develop. The potency is roughly equivalent to that of mersalyl [Daley & Evans (40)], but the newer diuretic has the advantages of a more rapid action and being active in states of alkalosis as well as acidosis. Dollery, Parry & Young (41) have made an extensive clinical study of the use of the diuretic in hypertensive and edematous patients. It was found to be effective in some patients resistant to mercurials and thiazides and, in general, to be an effective diuretic and adjunct to anti-hypertensive drugs. The potency and speed of action proved dangerous in some patients who passed over 9 liters of urine after a 200 mg dose; one of the patients died suddenly the next day. Although the

potassium loss is less in normal subjects treated with ethacrynic acid than with thiazides, it may be greater in severely edematous patients. In patients with a large diuresis, there was an abrupt fall of urea and uric acid clearances and a marked contraction of the plasma volume. The most troublesome side effects were nausea and diarrhea. Ledingham (42) considers that the diuretic will be valuable in the treatment of severe pulmonary edema by intravenous injection, especially if there is anaemia requiring urgent blood transfusion. The drug can be mixed with the blood if so desired. Clinical activity was confirmed by Schröder, Sannerstedt & Werkö (43) in 16 edematous patients. Satisfactory diuresis occurred in all but two, and there was no significant increase of urinary potassium, although serum potassium was reduced. One patient developed a moderate thrombocytopenia during therapy.

Goldberg et al. (44) have studied the effects of ethacrynic acid on the urinary concentrating and diluting mechanism in normal human subjects and in cases of diabetes insipidus. In maximally hydrated subjects and in cases of diabetes insipidus, the drug caused a reduction in free-water clearance (C_{H_2O}), together with a reduction of glomerular filtration rate and an increased output of potassium and hydrogen ion. During hydropenia, tubular reabsorption of osmotically free water ($T^c_{H_2O}$) was considerably reduced. During the peak of diuresis, the urine was usually virtually isotonic with plasma. The results certainly suggest important effects on the countercurrent multiplier system in the loops of Henle and were interpreted as indicating an important action on the ascending limb of the loop, where sodium chloride is reabsorbed in excess of water. These results are confirmed by Earley & Friedler (45) who found that the renal concentrating ability was virtually abolished by the drug. In states of water diuresis, free-water clearance was reduced but not completely eliminated. The addition of chlorothiazide caused a further decrease of free-water clearance. They agree that the data suggest that the main locus of action is on sodium transport by the cells of the ascending limb of the loop of Henle.

Kleinfelder (46) reports studies on a new diuretic, fursemide, or 4-chloro--N-(3-furyl-methyl)-5-sulphamoylanthranilic acid. The drug had an unusual dose-response curve. Sodium excretion followed a steepening curve when graphed against the logarithm of the dose, but the increase in potassium output was linear. The curve suggested that fursemide would increase in activity above the dose range of 12.5 to 100 mg tested. The main action occurred during the first 4 hr, and although the drug was not superior to hydrochlorothiazide in normal subjects, it was almost twice as effective in patients with edema. The Na:K ratio was more favourable in the case of fursemide, with consequent reduced risk of potassium depletion. Similar results were obtained by Timmerman, Springman & Thomas (47), with the exception that no advantage in respect of the Na:K ratio was found.

Hutcheon, Mehta & Leonard (48) reported a satisfactory increase in sodium and chloride output in cases of congestive heart failure, but there was also a considerable rise in urinary potassium. Diuresis occurred soon after

administration of the drug and lasted 6 to 12 hr. There was, at first, an increase of osmolal clearance, followed by excretion of a more dilute urine. The results were interpreted as being due to a dual action of inhibition of sodium reabsorption in the proximal tubule and in the ascending limb of Henle's loop. There was no significant effect on plasma electrolyte concentration, and the diuretic response was significantly greater than that obtained by hydrochlorothiazide. Vorburger (49) considers that fursemide is effective in cases in which there is gross reduction of glomerular filtration rate, a situation in which many other diuretics are often completely impotent.

Schirmeister & Willmann (50) consider that fursemide will be particularly valuable for the treatment of pulmonary and cerebral edema. After intravenous injection of the drug, there was an increase of inulin, PAH, urea, and uric acid clearances not accounted for by the great increase in urinary volume. Increase in clearance (C_{urate}) was shown not to be due to reduction of tubular reabsorption, but rather to an increase in the amount filtered.

Muschaweck & Hajdú (51) found that fursemide at high dosage in dogs and cats had a considerable hypotensive effect. The blood sugar and plasma uric acid content were not changed by the drug, and there was no significant inhibition of carbonic anhydrase. No toxic effects were found after intravenous therapy for 2 wk. Suki, Rector & Seldin (52) give evidence that fursemide reduces sodium and water reabsorption both in the proximal tubule and the ascending limb of Henle's loop but has no effect on reabsorption in the distal and collecting tubules. In dogs, the drug increased the urine volume to 40 percent of the glomerular filtration rate; but free water clearance (C_{H_2O}) decreased, despite a rising osmolal clearance (C_{osm}). The latter effect was, however, secondary to antidiuretic hormone (ADH) release from shrinkage of the extracellular volume and disappeared when this volume was replenished by massive saline infusions. In hydropenic dogs given ADH and mannitol infusions, $T^c_{H_2O}$ increased, suggesting an action on the ascending limb of Henle's loop. Rise in K output could be explained entirely by increased distal exchange of K for Na in the distal nephron.

Several reports are available [Harvey (53); Barry et al. (54); Peters & Brunner (55); Berman, Önen & Chisholm (56); Teschan, Gagnon & Murphy (57); Eliahou (58); Dawson (59)] on the use of osmotic diuretics, particularly infusions of mannitol, to prevent the development of acute oliguric renal failure. The effect may come partly from a vasodilation of afferent glomerular arterioles and partly from the fact that intratubular urine flow, at any stated reduction of arterial blood pressure and glomerular filtration rate, is greater in conditions of osmotic diuresis. Thus, shock or hemorrhage must be both more profound and more prolonged before acute oliguric renal failure occurs.

Thurau (60) has suggested that a tubular-arteriolar feedback mechanism via the juxtaglomerular apparatus may also be of importance. A sustained urine flow in the macula densa segment of the nephron may be a major factor in preventing renal vasoconstriction. Stagnant urine in this

segment probably has a high sodium content which stimulates release of renin and consequent afferent arteriolar spasm.

Mannitol infusions prevented acute oliguric renal failure in anesthetized dogs and cats [Peters & Brunner (55)] in which the systolic blood pressure had been lowered to values below 50 mm Hg by acute hemorrhage, although there was considerable fall in the glomerular filtration rate. The evidence for the beneficial effects of mannitol diuresis is now sufficient for it to be used routinely during any operative procedure which is known to carry a high incidence of acute tubular necrosis. These procedures include major cardiac surgery, probably excluding mitral valvotomy and ligation of a patent ductus arteriosus, operations on either the thoracic or abdominal aorta, surgery for the relief of obstructive jaundice, major abdominal operations where it is possible that there will be a risk of postoperative peritonitis, and heroic excisions of primary carcinomata where the growth has spread to involve several adjacent organs.

Antidiuresis due to thiazide diuretics.—Many recent papers report on the mechanism of thiazide-induced antidiuresis in patients with diabetes insipidus. Some workers consider that reduction of the glomerular filtration rate is of major importance [Alexander & Gordon (61); Havard & Wood (62); Kovács, Dávid & László (63)], whilst others claim this is usually insignificant [Crawford, Kennedy & Hill (64); Reerink, Brongers & Schouten (65)]. Reduction of glomerular filtration rate may lead to an increase of urine osmolality, both in vasopressin-sensitive and nephrogenic diabetes insipidus. There may be a decrease of plasma sodium after thiazide therapy [Linke (66); Weber & Gautier (67); Robson & Lambie (68)] which tends to reduce thirst and consequent excessive water intake. In a study of seven patients, Skadhauge (69) reports there was a 28 to 39 percent reduction of urine volume with increase of urine osmolality from 7 to 128 percent. Plasma sodium, on an average, fell by 4 meq per liter, plasma chloride by 8 meq per liter and plasma osmolality 21 mOsm per kg. Significant reduction of the glomerular filtration rate occurred in only one patient. Thirst was reduced after one day, but the maximal effect on urine volume did not occur until therapy had been continued for 3 to 4 days.

Penicillamine in the treatment of cystinuria.—Eldjarn & Pihl (70) reported that mixed disulphides were, in general, more soluble that symmetrical disulphides, and the suggestion that D-penicillamine might be a useful thiol for the treatment of cystinuria was made. This was first tested by Crawhall, Scowen & Watts (71) who found that D-penicillamine greatly reduced cystine output in two cystinurics, with a corresponding rise of the more-soluble mixed disulphide of cysteine and penicillamine. There could conceivably be any of the following reactions: (*a*) $PeSH + Cy{\cdot}S{\cdot}S{\cdot}Cy \rightleftharpoons Pe{\cdot}S{\cdot}S{\cdot}Cy + Cy{\cdot}SH$, (*b*) $Pe{\cdot}S{\cdot}S{\cdot}Cy + Pe{\cdot}SH \rightleftharpoons Pe{\cdot}S{\cdot}S{\cdot}Pe + Cy{\cdot}SH$, or (*c*) $Cy{\cdot}S{\cdot}S{\cdot}Cy + Pe{\cdot}S{\cdot}S{\cdot}Pe \rightleftharpoons 2\ Pe{\cdot}S{\cdot}S{\cdot}Cy$, where Cy is the cysteine and Pe is the penicillamine radicle.

Under physiological conditions, only the first reaction seems to be

quantitatively important [Eldjarn & Hambraeus (72)]. Further, favourable reports of the use of D-penicillamine in cystinuria have been published [Lotz & Potts (73); Crawhall, Scowen & Watts (74)]. With 2 to 3 g D-penicillamine daily, cystine output in cystinurics was reduced by 80 to 100 percent. In normal subjects, penicillamine greatly increases the urinary output of cysteine radicals, chiefly as penicillamine-cysteine disulphide [Hartley & Walshe (75)]. Less favourable opinions have been given by Eldjarn & Hambraeus (72) who reported that the beneficial effect of formation of soluble mixed disulphide was neutralized by a simultaneous increase of free cystine output, with a corresponding negative sulphur balance. Studies on neutral sulphur output [Milne & de Rousse (76)] in cystinurics receiving penicillamine do not, however, support a large increase in urinary cystine excretion. Increase of urinary sulphur after penicillamine was roughly equal to the amount administered as the drug, about half being excreted as sulphate and half as "neutral sulphur," chiefly in the form of the soluble mixed disulphide.

The balance of evidence, therefore, supports the use of D-penicillamine in the management of cystinurics who have proved resistant to the simple high fluid regime of Dent & Senior (77). Since about 50 percent of D-penicillamine is metabolized to inorganic sulphate, a daily dosage of at least 2 g is necessary to combine with the large amount of cystine excreted in homozygous cases of cystinuria, averaging 1 g per day.

D-Penicillamine is much less toxic than the L form, but the latter may cause anorexia, weight loss, rashes, convulsions, and a reversible nephrotic syndrome [Scheinberg & Sternleib (78); Fellers & Shahidi (79); Adams et al. (80); Seven, Kliman & Peterson (81)]. Probably, both drugs owe their toxicity to inhibition of pyridoxal and pyridoxal-dependent enzyme systems. Both isomers are capable of blocking pyridoxal-5-phosphate by nonenzymatic combination to form a thiazolidine derivative [du Vigneaud, Kuchinskas & Horvath (82)].

Both isomers, therefore, inactivate the enzymatic reaction if incubated with pyridoxal-5-phosphate before combination with the apoenzyme has taken place. If, however, the coenzyme and apoenzyme are pre-incubated first, only L-penicillamine is inhibitory, showing that this isomer also inhibits the holoenzyme [Ueda, Akedo & Suda (83)]. Routine treatment of cystinuria with 2 g D-penicillamine daily will cause pyridoxal deficiency, as shown by abnormal output of xanthurenic acid after ingestion of L-tryptophan [Asatoor & Milne (84)] within one month; but the deficiency is easily correctable by supplements of pyridoxal hydrochloride.

Excretion of weak acids and bases.—Weiner & Mudge (85) have reviewed current knowledge of renal tubular mechanisms of excretion of organic acids and bases, including many important drugs. They give the evidence for the present view of a three-component system of excretion, including glomerular filtration, proximal tubular secretion, and distal tubular back diffusion which is pH dependent. Lipoid solubility of the unionized fraction of the weak base

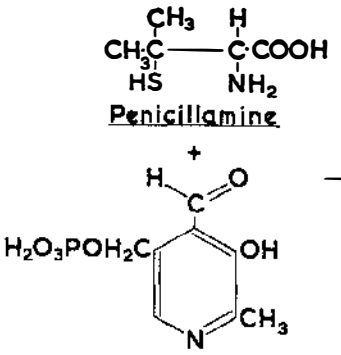

FIG. 1. Pyridoxal-5-phosphate.

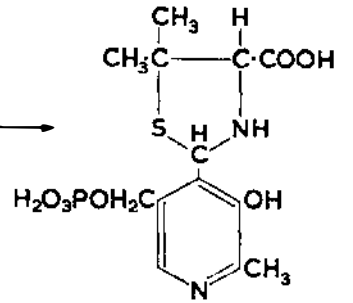

FIG. 2. Thiazolidine Derivative.

and acid and the dissociation constant (pKa) are the two major factors in determining whether the phenomenon of pH-dependent excretion will occur. The greater the lipoid solubility, the more is the chance of passive back diffusion of the unionized fraction, with consequent reduction of clearance below the renal plasma flow and often below the glomerular filtration rate.

The effect of pKa is described in relation to acids, but undoubtedly similar principles apply to weak bases. If the pKa of the acid is above 7.5, e.g., barbital, the clearance will not be affected by urinary pH changes. If between 3.0 and 7.5, e.g., phenobarbital, probenecid, and salicylate, the excretion will be much higher in alkaline urine; but usually the main rise in clearance occurs between the pH range of 6.0 to 8.0. In the case of stronger acids with pKa below 3.0, e.g., PAH and many sulphonic acids, the excretion may be theoretically pH dependent; but the effect is too low to be easily measurable and is, therefore, of no importance. In the case of stronger acids, the concentration of the diffusible unionized fraction is too low for significant back diffusion to occur. If, for instance, back diffusion was 0.5 percent in alkaline urine and 5 percent in highly acid urine, this would involve a tenfold difference in the amount of acid reabsorbed, but would only result in a small proportionate change from 99.5 percent to 95 percent in the amount excreted and in the clearance of the drug.

Important drugs which have recently been shown to be excreted in a pH-dependent manner are pethidine [Asatoor et al. (86)], amphetamine [Asatoor, Johnson & Milne (87)], and levorphanol [Braun, Hesse & Malorny (88)]. Milne (89) has claimed that pH-dependent excretion is important for four reasons: (*a*) it may be useful for enhancing urinary output of the drug in treatment of poisoning; (*b*) alteration of urinary pH may assist detection of the unchanged drug in urine in the diagnosis of addiction and attempted suicide or homicide; (*c*) study of the metabolism and excretion of a drug may give widely different results depending on whether the urine is highly acid or

highly alkaline; full knowledge is only obtainable if studies are made both in states of mild acidosis and mild alkalosis; and (*d*) the excretion of several naturally occurring indolic acids and bases is greatly influenced by changes of urinary pH [Milne et al. (90); Sandler & Spector (91)]; only if the pH of urine is controlled can metabolic studies, involving the output of these substances, give correct and reproducible results.

Immuno-suppressive therapy in renal transplantation.—Current interest in treatment of chronic renal failure by homotransplantation of kidneys, either from living donors or cadavers, has led to intensive research into the problem of prolongation of graft survival by immunosuppressive drugs. This is now more popular than use of total-body irradiation. Before 1959, rejection of grafts in dogs had usually occurred within 11 to 20 days. Calne (92, 93) and Zukoski, Lee & Hume (94, 95) recorded significantly increased survival by use of immunosuppressive chemotherapy. 6-Mercaptopurine and its imidazole derivative, azathioprene (Imuran), have been the most widely used agents [Calne & Murray (96); Calne (97); Calne, Alexandre & Murray (98)]. Alexandre et al. (99), in an extensive study, consider that a combination of azathioprene and azaserine proved the most successful both for prolongation of survival and for efficient functioning of the grafted kidney. Incipient rejection of the graft was best treated by actinomycin and cortisone and was effective in 50 percent of trials. In double transplants from unrelated donors, the results suggested that the unknown compatibility factors were of greater importance than the degree of the immunological response in the recipient. Zukoski, Callaway & Rhea (100) found that 6-methyl mercaptopurine considerably prolonged homograft survival in dogs; urethan plus 6-azauracil was moderately effective and cyclophosphamide and vinblastin sulphate were ineffective at tolerated dosage levels. Pierce & Varco (101) have shown that once tolerance is well established, immunosuppressive therapy may sometimes be discontinued. In one case, renal function remained satisfactory for 400 days after 6-mercaptopurine had been withdrawn.

In man, a few cases of long survival after renal homografts from living donors or cadavers have been reported [Merrill et al. (102); Hamburger et al. (103); Shackman, Dempster & Wrong (104); Calne et al. (105)]. Immunosuppressive therapy has proved more successful and gives rise to less serious complications than total-body irradiation. Results have been summarized in several recent reviews [Murray et al. (106); Merrill (107); Calne (108)]. Excluding transplants from monozygotic twins, eight cases are reported up to September 1963 to have survived over one year. Of these, three survived more than two years after the operation [Merrill (109)]. The eight survivors are the successful cases from a total of 216 transplantation operations, of which 148 were from living donors. It remains very dubious whether the benefit received by these patients can be balanced against the risk of disability produced in the donors by the hazards of life with a single kidney. This carries the risk of an increased incidence of acute renal failure from uretic obstruction and a more serious prognosis in traumatic incidents or

unilateral renal disease involving the remaining kidney. This ethical objection does not, of course, apply to the single long-term success from a graft taken from a cadaver. The incidence of success from cadaver transplants was, however, only 1 in 68, which compares unfavourably with the 7 in 148 success rate where living donors were used.

Immunosuppressive therapy in the control of renal homograft rejection must, therefore, be regarded at present as an important theoretical advance, but is still in the early experimental stage when concrete therapeutic benefit to human patients is considered.[3]

Nephrotoxicity from drugs and poisons.— Since Spühler & Zollinger (110) described 18 fatal cases of renal failure due to overdosage of phenacetin, there has been considerable investigation of the nephrotoxic effects of the drug. Most cases are reported from Switzerland, West Germany, Scandinavia, and Australasia where abuse of phenacetin is more common than in the United States, Canada, or England; but there are several descriptions of cases in the latter countries [Lakey (111); Rapoport, White & Ranking (112); Schreiner (113); Friend (114); Harvald (115); Reynolds & Edmondson (116); Harrow, Sloane & Liebman (117); Sanerkin & Weaver (118); Brown & Pell-Ilderton (119)]. The nephropathy is characterised by hyposthenuria, progressive azotemia with a normal or only slightly raised blood pressure, pyuria without gross proteinuria, and by increased liability to secondary pyelonephritis. Pathologically, there is prominent involvement of the interstitial tissue of the kidneys, with round-cell infiltration and secondary fibrosis. Interference with the medullary blood supply leads to severe papillary necrosis.

Phenacetin may be taken as analgesic for relief of headache or rheumatism; but, in many of the addicts in Switzerland and Scandinavia, it is taken because it, or the preparations which contain it, produces mild euphoria and is reputed to increase the power of concentration. Moeschlin (120) gives three reasons for believing that the phenacetin is a nephrotoxic agent: (*a*) improvement occurs if the drug is withdrawn before renal damage is too extreme; (*b*) there is a lack of relation of kidney damage to the condition for which the drug was taken; and (*c*) there is early pathological evidence of renal involvement before subjective symptoms have occurred.

Large amounts of phenacetin usually must be ingested before significant nephropathy occurs. Examples are given by Moolten & Smith (121) of 3.5 kg of the drug and by Meyler (122) of 7 and 13 kg in two patients. Reports of large series of patients have been made by Scandinavian workers. Lindeneg et al. (123) report 42 cases of phenacetin abuse of whom 39 were females. All

[3] Experience gained during 1964 has shown that this statistical analysis is reasonable when grafts are considered from parents, unrelated donors, and cadavers. The success rate, however, with transplants from siblings of patients is now very encouraging, being over 50 per cent on a two-year basis. The results might presumably be predicted from the closer antigenic compatibility of siblings as compared with all other possible donors (123a).

but 6 of these had obvious renal involvement, 18 had uremia, and 12 had renal papillary necrosis. Recognition of voided papillae during periods of hematuria was especially useful in diagnosis. Larsen & Møller (124) studied 205 patients taking large quantities of phenacetin and found reduced renal function in one third, compared to a control series of 9 percent in 337 patients not taking the drug. Kasanen & Salmi (125) found that of patients with renal disease in Finland, one third gave a history of phenacetin abuse compared to only one sixth in control patients without kidney disease. In individuals who were found to have taken over 5 kg of the drug, 85 percent had renal involvement. Bengtsson (126) reported on 75 cases of chronic nonobstructive pyelonephritis and 94 cases of renal papillary necrosis; a history of phenacetin consumption was obtained in 36 percent of the former and 79 percent of the latter. Secondary hypertension was much more frequent in the pure pyelonephritis cases. Renal function studies proved useful in differential diagnosis, as cases with renal papillary necrosis showed a disproportionate reduction of urinary concentrating and acidifying capacity as compared to fall of glomerular filtration rate.

Of 1350 consecutive necropsies at Sydney Hospital [Jacobs & Morris (127)], 3.7 percent of the cases had renal papillary necrosis. Almost all of these patients had taken phenacetin over a prolonged period, and in 60 percent large quantities had been consumed. Of 100 factory workers, Kasanen, Forsström & Salmi (128) report phenacetin abuse in 55 percent of the patients with chronic pyelonephritis compared to only 10 percent in controls without clinical evidence of renal damage. They concluded that ingestion of 1 g phenacetin daily for 5 to 10 yr is highly likely to cause kidney damage. Nordenfelt & Ringertz (129) in a survey of 1800 workers found that 130 habitually took phenacetin, and, of these, 29 percent had renal damage, whilst the incidence was 50 percent in those who had taken more than 6 kg. Microscopic examination of the kidneys was made in 23 patients; the usual finding was chronic interstitial nephritis and distal tubular damage, with papillary necrosis in 33 percent of the group. Tan et al. (161) report a series of 23 patients with severe renal failure and a history of prolonged excessive use of phenacetin-containing compounds.

These clinical and statistical studies are supported by experimental renal damage from analgesics in animals, although the results have not been completely consistent. Keller, Cottier & Reubi (130) found no pathological changes in the kidneys of rabbits given phenacetin and injected intravenously with cultures of *E. coli*. Miescher, Schnyder & Krech (131), however, found some interstitial infiltration in rabbit kidneys after treatment with phenacetin and injections of *E. coli*, but later they were unable to confirm their previous results [Miescher & Studer (132)]. Clausen (133) reported considerable interstitial inflammatory changes in rabbit kidneys with coliform infections after large quantities of phenacetin and acetylsalicylic acid had been given. The drugs alone caused dilatation of the convoluted and collecting tubules and, occasionally, a secondary fibrosis. More prolonged and

heavier dosage corresponding to the amounts recorded in man [Clausen (134)] caused a predominantly distal tubular damage with especially severe changes at the corticomedullary junction. There was dilatation of the tubular lumen with epithelial atrophy, fibrosis, and deposition of calcium-containing debris within the lumen. Abrahams, Rubenstein & Levin (135) reported papillary changes in rats given acetylsalicylic acid and phenacetin, but the lesions in animals are not identical with the papillary necrosis of human phenacetin addicts, possibly because there is less secondary bacterial infection.

The nephrotoxic effects of analgesics do not parallel their tendency to produce methemoglobulinaemia or intravascular hemolysis. Phenacetin, and to a lesser extent acetylsalicylic acid, can cause hemolysis in individuals with a deficiency of erythrocytic glucose-6-phosphate dehydrogenase [Houston & Barlow (136)]. In addition, the drug may cause acute hemolysis sufficient to precipitate acute oliguric renal failure [MacGibbon et al. (137)] by a sensitivity reaction from production of an abnormal plasma hemolysin. Normal erythrocytes, the hemolysin, and phenacetin or its chief metabolite N-acetyl-*p*-aminophenol must all be present for hemolysis to occur. A third hemolytic phenacetin or N-acetyl-*p*-aminophenol shorten the life span of the rabbit and mechanism is less acute, and requires the technique of determining the life span of labelled erythrocytes to demonstrate is presence. Large doses of either dog erythrocyte [Pletscher, Studer & Miescher (138)]. A similar effect in man is relatively mild in most addicts, but can become severe in renal insufficiency when the hemolytic action is potentiated by that directly due to the uraemia [Friis, Fogh & Nissen (139); Friis & Nissen (140)]. The hemolytic action in these individuals is severe enough to produce intracytoplasmic hemosiderin within renal tubule cells [Reynolds & Edmondson (116)]. It is, however, very doubtful whether this degree of renal hemosiderosis could lead to intersititial nephritis [Leonardi & Ruol (141)].

There is, therefore, little doubt that prolonged and large doses of phenacetin, and, to a lesser degree, other widely used analgesics, can cause interstitial nephritis and renal papillary necrosis and probably predispose to secondary bacterial infection and pyelonephritis, but the mechanism of their action remains unexplained.

Killen & Lance (142) have studied the nephrotoxicity of intravenous, radiological contrast media in the dog. The incidence and severity of azotaemia were greatest in animals receiving Urokon, Neo-Iopax, and Diodrast, whereas Miokon and Hypaque appeared relatively nontoxic. Morphological changes were confined to the renal tubular cells, where all grades of injury from cloudy swelling to complete cellular dissolution were seen. Proteinaceous casts and cellular debris were prominent within the tubular lumen.

Fink, Roenick & Wilson (143) have reviewed the nephrotoxicity of oral cholecystographic agents and state that 26 cases of acute oliguric renal failure have been reported in the last 5 years. Administration of these compounds to jaundiced dogs after ligation of the common bile duct produced

functional and morphological abnormalities of the kidneys. There were marked diffuse tubular degenerative changes, and formation of proteinaceous casts within the lumen. More severe lesions were produced by ipodate sodium and iopanoic acid than by bunamiodyl. Obstructive jaundice increases nephrotoxicity for two reasons: (*a*) there is increased excretion of the contrast medium in the urine, as none can be eliminated in the bile; and (*b*) severe jaundice can, itself, cause renal tubular degenerative changes [Allen (144)]. Additional renal damage may occur from fall of blood pressure directly due to the drugs [Köhler & Holsti (145); Funck-Brentano, Amiel & Méry (146); Blythe & Woods (147)], and from operative shock during attempts to relieve the biliary obstruction.

Of 20 patients developing acute oliguric renal failure, 14 had nonopacified gall bladders, one had faint opacification, and one developed the failure only after a high dose of the contrast medium had been given. The remaining four patients revealed normal concentration of the medium within the gall bladder. Acute renal damage is often due to the summation of action of various injurious agents, and this valuable study suggests that cholecystography is more dangerous in the severely jaundiced patient and that, if possible, operation should be postponed for several days after radiological investigations of the gall bladder and bile ducts.

Proximal tubular damage from ingestion of outdated tetracycline was first reported by Gross (148). A 38-year-old woman suddenly developed polyuria, polydipsia, glycosuria, aminoaciduria, hypercalcuria, a low plasma phosphate with hyperphosphaturia, a reduced plasma uric acid content, acidosis, and severe lethargy, the syndrome resembling the de Toni-Fanconi syndrome. A full recovery occurred after withdrawal of the drug. Frimpter et al. (149, 150) described three similar cases, and attributed the condition to formation of anhydrotetracycline and epianhydrotetracycline from exposure of the original tetracycline to air, moisture and heat over a considerable period. Other cases [Ehrlich & Stein (151); Rosenthal (152)] have been described in pediatric practice. The aminoaciduria is suggestive of nonspecific tubular damage and varies considerably from patient to patient. Another child [Sulkowski & Haserick (153)] developed a similar renal lesion, but in addition a photosensitive rash over the nose and cheeks; the syndrome superficially resembled disseminated lupus erythematosus. The tetracycline capsules contained a brownish gummy substance containing 3.5 percent anhydrotetracycline and 6.5 percent epi-anhydrotetracycline, with depression of the melting point from 212° C to 140° C. There was increased plasma α-aminonitrogen but no evidence of hepatotoxicity, and, consequently, this was attributed to the anti-anabolic effect of the antibiotic [Shils (154, 155)]. There will be less risk of this type of renal damage in the future, as tetracycline is now prepared with lactose rather than citric acid as excipient, a change which has been proved to lessen the chances of spontaneous chemical change.

Papper & Papper (156) have reviewed the effects of anesthetic drugs on

renal function. General anesthesia caused a fall in renal blood flow, glomerular filtration rate, and output of both water and electrolytes. The effects are usually due to renal and splanchnic vasoconstriction. Halothane [Mazze et al. (157)] has effects similar to other general anesthetic drugs. Meperidine and barbiturate [Papper et al. (158)] resemble morphine in producing oliguria, but, as there is no increase in urinary concentration, this is not ascribed to stimulation of ADH release but rather to renal vasoconstriction. Atropine in dogs [Solomon, Davis & Boone (159)] rather inconsistently produced a decrease in urinary volume, sometimes accompanied by a reduction of glomerular filtration rate.

Reidenberg et al. (160) reviewed acute renal failure due to nephrotoxins. Carbon tetrachloride, mercury, and ethylene glycol were found to cause the largest number of cases. A comprehensive list of other nephrotoxic agents with a useful bibliography is included in their report.

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