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Research Article

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No renal medullary gradient for salicylate was demonstrable during both hydropenic and hydrated states. In contrast, both free and conjugated APAP concentrations rose sharply in the inner medulla during hydropenia, reaching a mean maximal value at the papillary tip exceeding 10 times the cortical concentration ($P < 0.001$), a distribution similar to that of urea. Salicylate had no effect on the APAP gradient, but hydration markedly reduced both the APAP and urea gradients in the medulla. The data indicate that APAP probably shares the same renal mechanisms of transport and accumulation as urea and acetamide, and that papillary necrosis from excessive phenacetin may be related to high papillary concentration of APAP.

Renal Accumulation of Salicylate and Phenacetin: Possible Mechanisms in the Nephropathy of Analgesic Abuse

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ABSTRACT Since either aspirin or phenacetin might be causative in the nephropathy of analgesic abuse, studies were designed to examine the renal accumulation and distribution of the major metabolic products of these compounds, salicylate and *N*-acetyl-*p*-aminophenol (APAP) respectively, in dogs. Nineteen hydropenic animals were studied, of which seven were given phenacetin, nine received acetyl salicylic acid, two were given both aspirin and phenacetin, and one received APAP directly. Two of three hydrated animals were given phenacetin and one was given aspirin. During peak blood levels of salicylate and (or) APAP, the kidneys were rapidly removed, frozen, sliced from cortex to papillary tip, and analyzed for water, urea, APAP, and salicylate.

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INTRODUCTION

Since 1953 when Spühler and Zollinger (1) reported a series of cases in which chronic interstitial nephritis was associated with a high intake of certain analgesic compounds, many papers have appeared on the subject of the nephropathy of analgesic abuse. Renal failure is commonly associated with this condition, and pathologically, an almost uniform finding is necrosis of the renal papilla (2). Recently, Kincaid-Smith (3) has suggested that papillary necrosis is the primary factor in the pathogenesis of this disease.

Many questions remain, however, regarding the etiologic agents involved and the mechanisms of the renal tissue damage resulting from them. The clinical evidence for the entity, analgesic-induced nephropathy, while highly suggestive, is largely inferential, and the results of toxicity studies in animals have been both inconsistent and inconclusive (4–6). There are still conflicting opinions as to whether aspirin or phenacetin is the important offending agent. Gilman (7) has postulated that salicylate might diffuse back through renal tubular epithelial cells at medullary sites where tubular pH is low, favoring the diffusion of the undissociated and presumably freely dif-

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fusible form of salicylate. Hence, theoretically, a mechanism is possible which can lead to the medullary accumulation of salicylate. On the other hand, an inspection of the chemical structure of phenacetin and its major metabolic product, *N*-acetyl-*p*-aminophenol (APAP) (8), reveals a close resemblance to acetamide, an analogue of urea (see Fig. 1), which has been shown to concentrate preferentially in the renal papilla (9). The present study was therefore undertaken to determine whether salicylate or APAP, or both, might be distributed in the kidney to form an increasing concentration gradient from cortex to papillary tip. The rationale for this was based on the following two assumptions: (*a*) insofar as any component of analgesic mixtures might be nephrotoxic, the degree of injury it produces should be related to its tissue concentration, and (*b*) the site of maximum injury should correlate with the site of maximum concentration. Hence since the main site of injury in the nephropathy of analgesic abuse is the medulla, with papillary necrosis being the characteristic feature (3), it seemed reasonable to ask whether the main metabolic products of aspirin (salicylate) or of phenacetin (APAP) might tend to concentrate in these regions of the kidney.

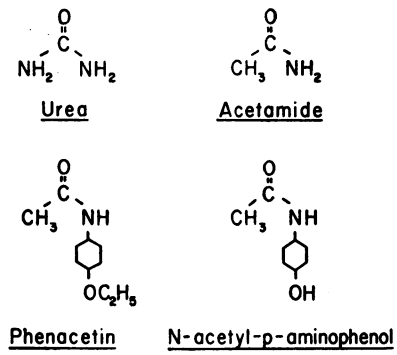

FIGURE 1 Comparison of the chemical formulas of urea, acetamide, phenacetin, and *N*-acetyl-*p*-aminophenol. See text.

METHODS

Mongrel female dogs weighing between 14 and 30 kg were used for all experiments. Hydropenia was induced by water deprivation for 18–24 hr. 5 units of pitressin tannate in oil was given 16 hr before each experiment. Control urine samples for use as blanks were obtained by direct bladder puncture. The analgesic agent being studied was given by mouth in gelatin capsules with up to 75 ml of milk or water to aid absorption. Nine dogs received phenacetin alone (300 mg/kg body weight). Of these, seven were hydropenic until removal of the kidney and two received an acute water load (30 ml/kg) as 5% glucose in water administered over a period of 30 min before the kidneys were removed during the ensuing diuresis. In these latter animals pitressin was omitted.

Nine dogs received aspirin alone in dosages of 170–330 mg/kg body weight. Eight of these were hydropenic and one received a water load as described above, before removal of the kidneys. Two additional hydropenic animals were given both aspirin (170 mg/kg) and phenacetin (300 mg/kg) simultaneously. One additional hydropenic animal was given APAP directly (150 mg/kg).

2–3 hr after drug administration, anesthesia was induced by intravenous sodium pentobarbital or sodium pentothal. A retention catheter was inserted and the bladder was emptied by the air washout technique. In the hydropenic animals, when urine osmolality exceeded 1400 mOsm/kg a final urine specimen was collected and blood samples were obtained. Both kidneys were then quickly removed after clamping the renal pedicles. The kidneys were immediately frozen in acetone and dry ice. In the hydrated animals the kidneys were similarly removed 1 hr after institution of the water load.

Two cross-sectional slices, ¼ inch thick, were cut from each frozen kidney by band saw. From each slice at least two series of tissue samples, each weighing from 75 to 500 mg, were cut at the levels of the cortex, the outer papilla (the outer portion of the "white medulla" adjacent to the outer red zone of the medulla), and the papillary tip. One series of samples from each kidney was used to determine tissue water content by drying in an oven at 103–105°C for 48 hr. Another series was used for analysis of salicylate and APAP. In six experiments with aspirin and three with phenacetin an additional series from each kidney was used for urea analysis by a modification of the Conway microdiffusion technique (10). Five hydropenic animals which were given no drug were used to obtain control levels of urea from cortex to papillary tip. These data were reported elsewhere as part of another study (11), but they are utilized here as controls because the experimental preparation and methods of analyses were identical with those of the present study.

Salicylate levels were determined on nitric acid digests of tissue and in blood and urine by the method of Trinder (12). Concentrations of free and conjugated APAP were determined in all plasma and urine samples of the phenacetin-loaded dogs by the extraction and spectrophotometric assay of Brodie and Axelrod (13). In two phenacetin-loaded dogs, one hydropenic and the other hydrated, independent determinations were made of free and conjugated APAP concentrations in kidney samples, also by the method of Brodie and Axelrod (13). Recovery studies were done by adding known amounts of salicylate and APAP to minced beef and dog kidney. Recovery values were consistently in the range of 93–97% for salicylate and 95–100% for APAP. Spurious

TABLE I

Summary of Urine, Plasma, and Renal Tissue Levels of Salicylate, Unconjugated N-Acetyl-p-Aminophenol (APAP), and Urea

Dog No.	State of hydration	Drug given		Salicylate concentrations (*mmoles/L*) and unconjugated APAP concentrations (*μmoles/L*)					Urine osmolality	Urine pH	Urea concentrations	
		Aspirin	Phenacetin	Plasma	Cortex	Outer papilla	Papillary tip	Urine			Cortex	Papillary tip
		mg/kg	*mg/kg*						*mOsm/kg*		*mmoles/liter*	*mmoles/liter*
Controls*	Hydropenic								1667 ± 58		36 ± 10	778 ± 206
S_1	Hydropenic	170	—	132	703	1164	829	—	—	—	—	—
S_2	Hydropenic	330	—	201	1438	1468	1397	—	1550	—	31	776
S_3	Hydropenic	170	—	109	564	455	612	—	1585	—	27	610
S_4	Hydropenic	170	—	132	876	533	521	511	1830	—	42	677
S_5	Hydropenic	170	—	117	553	535	620	—	2200	5.43	22	897
S_6	Hydropenic	170	—	125	646	496	496	482	1740	5.50	—	—
S_7	Hydropenic	170	—	163	760	814	986	774	2200	6.22	45	270
S_8	Hydropenic	170	—	132	668	657	727	877	2000	5.78	20	345
S_9	Hydrated	170	—	186	252	267	328	34	211	6.17	—	—
P_1	Hydropenic	—	100	29	30	86	193	1934	1486	5.49	15	789
P_2	Hydropenic	—	300	93	90	437	1590	2927	2238	7.15	27	838
P_3	Hydropenic	—	300	62	56	186	1079	2875	1570	7.05	—	—
P_4	Hydropenic	—	300	85	93	303	713	2650	1486	5.98	—	—
P_5	Hydropenic	—	300	21	36	89	212	782	2280	6.05	—	—
P_6	Hydrated	—	300	82	67	119	140	334	635	7.55	—	—
P_7	Hydrated	—	300	65	36	20	38	187	168	4.60	17	27
SP_1	Hydropenic	170		187	913	658	635	671	1400	6.00	—	—
			300	91	13	314	629	1735	—	—	—	—
SP_2	Hydropenic	170		132	882	518	549	627	2310	6.20	—	—
			300	55	30	144	338	2365	—	—	—	—

* Control data are means ± SD from 10 kidneys taken from five hydropenic animals and processed by methods identical with those used in the present series of experiments (11).

effects of the anesthetic agent on these analyses were ruled out in control animals similarly prepared but not given aspirin or phenacetin. Salicylate and APAP concentrations of zero were found in all kidney samples from these control animals.

Mean renal tissue concentrations were obtained for each dog by adding the measured concentrations at the same level in both left and right kidneys and dividing by two. Urine pH was measured by a pH meter (model G, Beckman Instruments, Inc., Fullerton, Calif.). Statistical analyses were performed by standard techniques (14).

RESULTS

Renal tissue concentrations for salicylate. A summary of the salicylate concentrations from all experiments in which aspirin was administered is given in Table I. Fig. 2 shows the renal concentration gradients for salicylate and for urea in one representative experiment during hydropenia (dog S_5). The results were consistent in all experiments. Despite the presence of a normal medullary urea gradient (see Table I for values obtained from control animals), no gradient was present for salicylate from cortex to papillary tip. As illustrated in Fig. 4 which summarizes the tissue gradients from all 11 experiments employing aspirin, the ratio of papillary salicylate concentration to cortical concentration was no different from one. While no gradient existed for salicylate, mean renal tissue concentrations at all tissue lev-

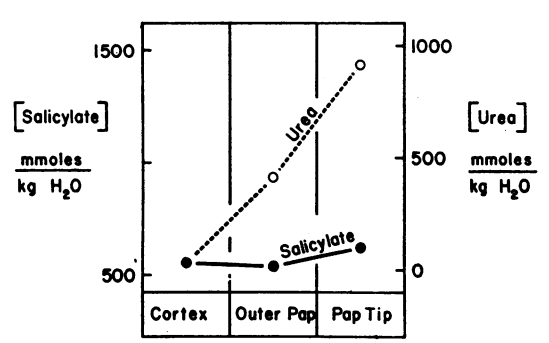

FIGURE 2 Tissue concentrations of urea and salicylate in dog S_8. Note the absence of a medullary gradient for salicylate in contrast to the typical urea gradient. See text.

els were approximately five times those of plasma in the hydropenic animals. Hydration in dog S_9 resulted in a reduction in tissue salicylate concentration at all levels to values only slightly above plasma (see Table I).

Renal tissue concentration for APAP during hydropenia. (See Table I for a summary of urine, plasma, and tissue concentrations from all APAP experiments.) In Fig. 3 are illustrated the tissue concentrations at cortex, outer papilla, and papillary tip for APAP and for urea in one representative experiment in dog P_2. In contrast to salicylate, a distinct medullary gradient for free APAP is apparent, resembling qualitatively the medullary urea gradient. In Fig. 4 are illustrated the mean values for the ratios of outer papilla/cortex and papillary/cortex free APAP concentrations from all nine experiments in which phenacetin was administered. The mean papillary tip/cortex APAP ratio exceeded 10 ($P < 0.001$). The mean papillary tip/cortex ratio for urea was somewhat greater than 20/1, and was similar to the normal urea medullary gradient obtained previously in this laboratory from control hydropenic dogs treated similarly (see Table I).

Since APAP can exist in both the free and conjugated forms in body fluids (8), conjugated APAP was measured in addition to free APAP in tissues, blood, and urine of several of the experimental animals to assess whether the free and conjugated forms were distributed in a similar manner qualitatively. As shown in Fig. 5 which illustrates the renal tissue APAP gradients from dog P_3 given phenacetin, the gradient for conjugated APAP was approximately 10/1, and the

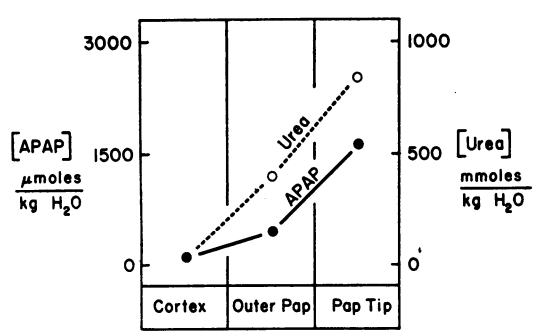

FIGURE 3 Renal tissue concentrations of APAP and urea in a representative experiment during hydropenia in dog P_3. Note the similarity of the medullary urea and APAP gradients. See text.

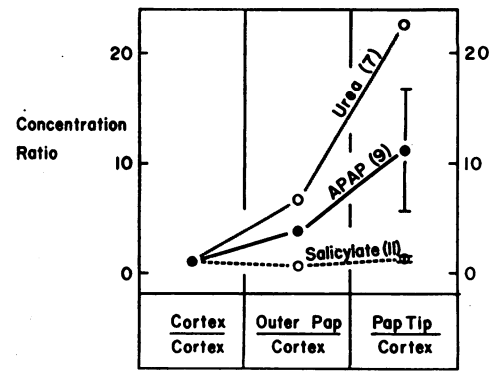

FIGURE 4 Mean tissue gradients for urea, APAP, and salicylate from nine animals who received APAP, eleven animals who received salicylate, and from seven hydropenic animals in which renal analyses for urea were performed. The data for papillary tip/cortex for APAP and salicylate represent means ± standard deviation. See text.

gradient for free APAP was 19/1. Hence our measure of free APAP in all of the phenacetin experiments summarized in Table I and Fig. 4 provides a reasonably good index of the renal distribution of conjugated and total APAP also. The renal tissue data obtained from hydrated animals, discussed below, support this conclusion.

One additional experiment was performed in which APAP itself was administered to a hydropenic animal instead of phenacetin, and the tissues were analyzed as in the phenacetin experiments. The data showed also the typical renal medullary APAP gradient with concentrations for APAP of 178 in the cortex, 340 at the outer papilla, and 800 μmoles/kg H_2O at the papillary tip.

Effects of simultaneous administration of aspirin and phenacetin on renal tissue gradients of salicylate and APAP. Because of the possibility that one agent may affect renal accumulation of the other, two hydropenic dogs (SP_1 and SP_2) were given both drugs together. The data from both experiments are contained in Table I, and the tissue gradients from dog SP_2 are illustrated in Fig. 6. It is apparent that, similar to the results from the studies involving the use of one drug alone, the characteristic medullary gradient for APAP was still present, whereas no gradient for salicylate was demonstrable.

Effects of hydration on the renal distribution of APAP. Fig. 7 summarizes the data on the in-

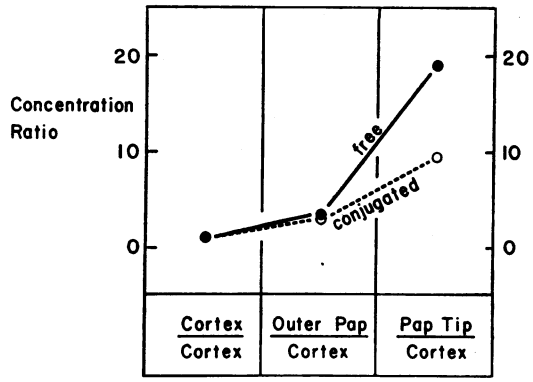

FIGURE 5 Renal tissue gradients for free and conjugated APAP in dog P_8 during hydropenia. Note the similarity in distribution of both the free and conjugated forms. See text.

trarenal distribution of both free and conjugated APAP in dog P_7, a water-loaded animal given phenacetin. The urine osmolality was 168 mOsm/kg at the time of removal and freezing of the kidneys. The striking feature of these data is the virtually total obliteration of the APAP medullary gradient which was present in the hydropenic animals. This was true for both the free and conjugated forms. This effect of hydration on the APAP gradient resembled the typical "medullary washout" of urea which also occurred in dog P_7. The cortical and papillary tissue urea concentrations of this animal were 17 and 27 mmoles/liter, respectively (Table I), compared to mean values from hydropenic animals of 36 and 788 mmoles/liter for cortex and papillary tip.

Effect of urinary pH on tissue distribution of

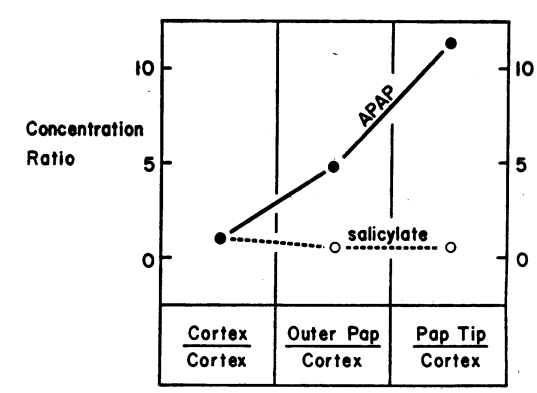

FIGURE 6 Tissue gradients for salicylate and APAP from dog SP_8 during hydropenia. The presence of salicylate did not influence the medullary distribution of APAP. See text.

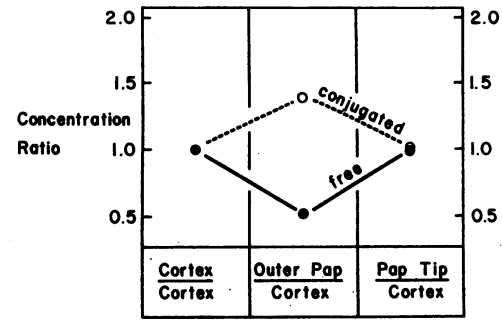

FIGURE 7 Effect of hydration on free and conjugated APAP in dog P_7 during water diuresis. Hydration totally obliterated the typical APAP gradients for both the free and conjugated forms of the compound. See text.

salicylate and APAP. Data on urinary pH at the time of removal of the kidneys for analysis are summarized in Table I. It is clear that variations in urinary pH from 5.43 to 6.22 in the salicylate experiments during hydropenia had no significant effects on the renal medullary distribution gradients. In APAP experiments during hydropenia, the highest urine pH values in two experiments were associated with the highest medullary gradients, although the significance of this observation is open to question.

DISCUSSION

Brodie and Axelrod (8) have shown that in both man and dog, within 2–4 hr after the administration of phenacetin, most of the drug has been de-ethylated to form APAP, a part of which is conjugated presumably with sulfate and glucuronate. Moreover, plasma and tissue levels of phenacetin during this period had declined to unmeasurable amounts. Hence, to the extent that tissue toxicity is related to phenacetin, it is most likely a function of the tissue concentration of APAP.

Our data demonstrate that a renal distribution gradient exists for APAP in hydropenic dogs given phenacetin alone, APAP alone, or phenacetin in combination with aspirin. It would appear therefore that the major metabolic product of this drug is a solute capable of traversing the renal tubule and accumulating in the interstitial and (or) cellular fluid of the medulla as do the structurally related compounds urea, methyl urea, and acetamide (9). It has been generally assumed that the intrarenal transport of urea is passive in na-

ture, i.e., movement takes place downhill according to its concentration gradient. Therefore, one might argue that APAP, a chemical analogue of urea, moves similarly across the collecting duct epithelium into the medullary interstitium. Accordingly, the development of an intramedullary concentration gradient for APAP may be explained by the behavior of the vasa recta functioning as countercurrent exchangers (15).

An alternative explanation is possible. Evidence has accumulated recently, suggesting the presence of an active transport system for urea in the collecting duct of the rat (9, 16–18). This concept has received strong support from studies in the dog (11) which have demonstrated an uphill gradient for urea between final urine and papillary tip when the medullary electrolyte gradient was experimentally abolished. This uphill urea gradient was reduced or obliterated by iodoacetate, an inhibitor of anaerobic glycolysis, and by acetamide, an analogue of urea. Hence, it is conceivable that APAP participates in this proposed active transport system, sharing the same carrier or transport sites as urea and acetamide. The relatively high urine/papilla ratio for APAP compared to urea might be due to a greater intrinsic capacity of this system for transport of urea than for APAP. Regardless of the mode of transport into the medullary interstitium, APAP would accumulate according to the principles of countercurrent exchange (14), with a gradient towards the papillary tip. Of course, the above described mechanisms for urea transport are not necessarily mutually exclusive. Urea may be reabsorbed both passively and via a carrier-mediated system. The relative importance of these two mechanisms in the physiology of the medullary urea concentrating mechanism remains to be determined.

In contrast to the data on APAP, no medullary gradient for salicylate was demonstrable during peak blood levels. The papillary tip concentration of this compound was no different from its concentration in the cortex, and hydration had no effect on the relative tissue distribution of the drug. The finding of a renal tissue salicylate concentration which was five times the concentration in plasma of dehydrated animals is somewhat at variance with previous reports. Smith, Gleason, Stoll, and Orgorzalek studied the distribution of salicylate in rats given sodium salicylate and found a concentration in renal tissue water to be approximately equal to that in plasma (19). Surprisingly, few additional data are available on the organ/blood concentration ratio of this common drug. In two patients who died of salicylate intoxication, extremely large variations were found, kidney/blood ratios being 8.0/15.0 in one patient, and 82.4/0.6 in the other (20). It should be noted that marked hydration of an animal receiving salicylate lowered the renal concentration of this drug at all tissue levels to values close to that of plasma. Different degrees of hydration, therefore, might explain some of the differences between Smith's data and our own.

The consistently high tissue/plasma ratios for salicylate found in our studies during hydropenia may be explained in two possible ways. The data are compatible with active reabsorption of salicylate throughout cortical, medullary, and papillary portions of the nephron. This explanation, however, does not account for the absence of an intramedullary concentration gradient similar to that obtained with APAP. If salicylate entered the medulla by reabsorption along the collecting duct, then the vasa recta would be expected to facilitate medullary accumulation of the compound with the highest concentration at the papillary tip. A better explanation is that the high tissue levels of salicylate in the kidney are due to binding with a major constituent of tissue such as protein. It has already been demonstrated that salicylate may bind with plasma protein (21). According to this concept, salicylate may enter the medulla via the blood supply, and transport from the tubular lumen of the collecting duct is not required to explain our data. The absence of an intrarenal concentration gradient for salicylate as well as the failure to relate urinary pH to tissue salicylate concentration does not support the concept proposed by Gilman (7) that a high papillary concentration of salicylate should occur because of favorable osmotic and pH conditions for back diffusion at this site. Salicylate failed to accumulate in the medulla according to a gradient despite the presence in the hydropenic animal of low urinary pH and normal medullary gradients for urea.

With regard to the mechanism of renal damage in the nephropathy of analgesic abuse, the present study does not directly answer the question of which drug is more injurious to the kidney. It

does, however, support the contention that phenacetin would more likely be the cause of the papillary necrosis since APAP accumulates in highest concentrations at the papillary tip after the administration of phenacetin. This interpretation supports the proposal of Kincaid-Smith (3) that the primary lesion in the pathogenesis of the nephropathy of analgesic abuse is papillary necrosis and consequent interstitial nephritis. According to this hypothesis, the more generalized renal involvement and the changes in the cortex are secondary to the primary alterations in the renal medulla.

If APAP behaves like urea physiologically, then the observed effects of hydration in reducing the intrarenal gradient for APAP and its absolute concentration at the papillary tip are predictable. This could be explained either by a passive washout of APAP and urea at high rates of tubular urine flow, as suggested by Ullrich and Jarausch (22), or by a decreased rate of collecting duct reabsorption because of the fall in tubular concentration of urea or APAP occurring with water diuresis (11). These effects of hydration in diminishing the renal gradient for APAP suggest that dehydration as well as the amount of analgesic compound consumed may have a bearing on the development of the renal lesions. It might offer a possible explanation for the failure to produce papillary necrosis in the majority of animals given large doses of phenacetin for long periods of time (4–6) if no effort were made to limit fluid intake. This contention is supported by the preliminary results of studies performed by Kincaid-Smith, Saker, McKenzie, and Muriden in the rat (23). These investigators were able to produce papillary necrosis and other lesions of the medullary interstitium more consistently in rats deprived of fluids overnight, compared to a control group of animals on an ad lib. water intake. Another implication of clinical importance is that adequate hydration may provide some protection against the devolpment of papillary necrosis in patients who consume large quantities of analgesic mixtures containing phenacetin.

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