(2) Wills, J. H., Kunkel, A. M., Brown, R. V., and Groblewski, G. E., *Science*, **125**, 743(1957).
(3) American Medical Association, United States Pharmacopeia Nomenclature Committee, *J. Am. Med. Assoc.*, **182**, 733(1962).
(4) Sundwall, A., *Biochem. Pharmacol.*, **5**, 225(1960).
(5) Jager, B. V., Stagg, G. N., Green, N., and Jager, L., *Bull. Johns Hopkins Hosp.*, **102**, 225(1958).
(6) Kondritzer, A. A., Ellin, R. I., and Edberg, L. J., *J. Pharm. Sci.*, **50**, 109(1961).
(7) May, J. R., Zvirblis, P., and Kondritzer, A. A., *ibid.*, **54**, 1508(1965).
(8) Poziomek, E. J., Hackley, B. E., Jr., and Steinberg, G. M., *J. Org. Chem.*, **23**, 714(1958).
(9) Swintosky, J. V., *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 395(1956).
(10) Wilbrandt, W., *Schweiz. Med. Woschr.*, **94**, 737(1964).
(11) Swintosky, J. V., *Nature*, **179**, 98(1957).

Keyphrases

Pralidoxime salts—absorption, elimination
Absorption, elimination rates—pralidoxime salts
Biologic half-life—pralidoxime salts
Pharmacological activity—pralidoxime salts

4-Acetamidophenyl 2,2,2-Trichloroethyl Carbonate

Particle Size Studies in Animals and Man

By LEWIS W. DITTERT*, H. JACK ADAMS†, FRED ALEXANDER, CLIFFORD W. CHONG, THEODORE ELLISON‡, and JOSEPH V. SWINTOSKY*

4-Acetamidophenyl 2,2,2-trichloroethyl carbonate (ATC) was prepared in coarse, regular, and fine particle sizes by sieving and grinding. The powders were found to dissolve in water at significantly different rates in a mechanically stirred system. When administered orally in suspensions to mice, the LD_{50}'s of the ATC powders were as follows: coarse—3340 mg./Kg.; regular—2461 mg./Kg.; and fine—1796 mg./Kg. The three ATC powders produced significantly different blood plasma concentrations of total acetaminophen in mice during the 0- to 2-hr. period after oral administration. In humans, the blood plasma concentration curves produced by oral administration of coarse and regular ATC were nearly identical. However, both had lower peaks and slower rates of decline than those produced by fine particle ATC or acetaminophen. Forty-eight-hour recovery of acetaminophen from human urine indicated that all the ATC powders were as efficiently absorbed as acetaminophen itself. It was concluded that ATC is a true prodrug of acetaminophen and that the acetaminophen blood plasma concentrations produced by orally administered ATC can be controlled to some degree by controlling its particle size.

ONE OF THE MAIN objectives of preparing prodrugs is to influence the dose–time–action profiles of drugs with known pharmacologic activities. The authors' studies with the prodrug carbonate esters of acetaminophen (1, 2) have been directed primarily toward prolonging its duration of action. This is done conveniently when the prodrug is much less soluble in water and has a slower dissolution rate in aqueous fluids than the parent drug. Under these circumstances, the appearance of the parent drug in the body is slowed by the slow dissolution of the prodrug in the gastrointestinal tract. Previous studies have indicated that the dissolution rates of the acetaminophen prodrugs, rather than their rates of hydrolysis, determine the rates at which acetaminophen is released to the tissues following their oral administration (2).

4-Acetamidophenyl 2,2,2-trichloroethyl carbonate (ATC) is much less soluble in water than acetaminophen (1). If the duration of action of ATC is indeed controlled by its dissolution rate in the gastrointestinal tract, then it would be expected that the particle size of ATC powder administered orally might have a pronounced influence on its dose–time–action profile. This paper describes the influence of particle size on oral toxicity of ATC in mice, on the blood plasma concentrations of total acetaminophen it produces in mice and in humans, and on the urinary excretion of acetaminophen after its oral administration to humans.

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EXPERIMENTAL

Preparation of ATC Powders of Various Particle Sizes—ATC was prepared in coarse, regular, and

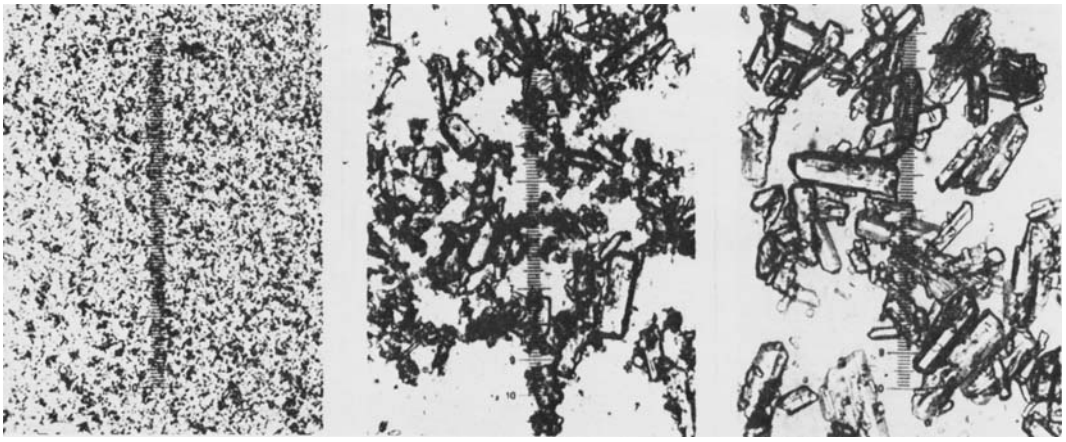


Fig. 1—Photomicrographs of the fine, regular, and coarse-particle ATC used in this study (scale = 6.03 μ/small division).

fine particle sizes by the following techniques: coarse—ATC particles which passed through a U. S. No. 140 mesh screen (hole size 150 μ) and remained on a U. S. No. 170 mesh screen (hole size 88 μ). Regular—ATC passed once through a U. S. No. 80 mesh screen in a Fitzpatrick model M comminuting mill (The W. J. Fitzpatrick Co., Chicago, Ill.) with hammers forward. Fine—ATC passed once through a fluid energy mill (Trost Jet Mill, Helme Products, Inc., Helmetta, N. J.).

Photomicrographs (125× magnification) of the powders are shown in Fig. 1. The volume surface mean diameters (d_{vs}) of the regular and fine powders were measured by air permeation using a Fisher subsieve sizer (Fisher Scientific Co.). After preparing and measuring the particle sizes of the powders, colloidal silicon dioxide[1] was added to comprise 2% by weight of the final powder to aid in dispersion.

Dissolution Rates of ATC Powders—The rate of dissolution of each of the ATC powders was determined by a method similar to that used by Parrott *et al.* (3). The apparatus consisted of a 2-L. three-neck round-bottom flask stirred by a glass impeller driven by a constant-speed electric motor (720 r.p.m.). The flask contained 1,500 ml. of 0.01% dioctyl sodium sulfosuccinate[2] solution which immediately wetted and dispersed the powders. One-hundred and fifty milligrams of the powders (about twice the amount necessary to make a saturated solution) was added through a powder funnel, and the appearance of ATC in solution was followed spectrophotometrically.

Oral Toxicity in Mice—Male Carworth Farms mice, weighing between 12 and 18 Gm., were randomly divided into groups of 10 and dosed orally with drug suspended in a 0.5% dispersion of gum tragacanth in water. Concentrations were calculated so that each animal received a dose volume of 20 ml./Kg. Control animals received 20 ml./Kg. of 0.5% tragacanth. After dosing, the animals were observed daily for 7 days; if no deaths occurred on the sixth and seventh days, the study was terminated. If deaths occurred during this 2-day period, the study was continued until no deaths occurred for two consecutive days. LD_{50}'s and potency ratios with 95% confidence limits were calculated by the Logit chi-square method of Berkson (4).

Blood Plasma Concentrations of Acetaminophen in Mice—Male Carworth Farms mice, weighing between 12 and 18 Gm., were randomly divided into groups of 10 and dosed orally with 1,000 mg./Kg. of drug suspended in a 0.5% dispersion of gum tragacanth in water. Concentrations were calculated so that each animal received a dose volume of 20 ml./Kg. At selected time intervals after dosing (0, 15, and 30 min., 1, 2, 4, and 6 hr.), heparinized blood was collected and pooled for each treatment group. The blood was centrifuged and the plasma analyzed for total acetaminophen (free + conjugated) by the method described below.

Protocols for Blood and Urine Studies in Humans—Twelve adult males were employed in a 6 × 6 crossover study with 6 subjects receiving 1 Gm. of coarse-particle ATC and 6 subjects receiving 1 Gm. of fine-particle ATC in hard gelatin capsules. Specimens of oxalated blood were collected at 0, 0.5, 1, 2, 3, 4, 7, 10, 12, and 14 hr. postdrug administration. Total urines were collected in polyethylene bottles at the following 8-hr. periods: 0, 0–8, 8–16, 16–24, 24–32, 32–40, and 40–48 hr. postdrug administration. (In this study, no changes in protocol were necessary.)

In a second study, 12 adult males (6 × 6 crossover) were given 1 Gm. of regular-particle ATC and 463 mg. of acetaminophen (molar equivalent of 1 Gm. of ATC) in hard gelatin capsules. Specimens of oxalated blood were collected at 0, 2, 4, 6, 8, 10, and 12 hr. postdrug, and total urine was collected in polyethylene bottles at 0, 2, 4, 6, 8, 10, 12, 14, 16, 24, 30, 36, and 48 hr. postdrug. (In this study, one subject was eliminated; and another, scheduled to receive acetaminophen during the second part of the crossover, was dropped.)

Analytical Method for Acetaminophen in Blood Plasma and Urine—The method employed for the determination of total acetaminophen (free + conjugated) in blood plasma and urine is similar to that used by Brodie and Axelrod (5). All samples were stored at −15° until assayed.

[1] Cab-O-Sil, Cabot Corp., Boston, Mass.
[2] Aerosol OT, American Cyanamid Co., Pearl River, N. Y.

One milliliter of the plasma or urine was transferred to a 12-ml. Pyrex graduated centrifuge tube containing 1 ml. of concentrated hydrochloric acid and diluted to 5.5 ml. with distilled water. The unstoppered tube was then placed in an autoclave at 15 lb. pressure for 1.5 hr. The tube was cooled to room temperature and the volume adjusted to 6 ml. A 5-ml. aliquot of the reaction mixture was transferred to a 40-ml. centrifuge tube containing 5 Gm. of dibasic potassium phosphate. The tube was shaken on a mechanical shaker for 5 min. (The pH of the final solution is between 7 and 8.) Twenty-five milliliters of isoamyl alcohol–ether solvent (prewashed with base, acid, and distilled water) was added, and the tube was shaken mechanically for 10 min. and then centrifuged for 10 min. to separate the phases. Twenty milliliters of the ether phase was transferred to a 40-ml. Pyrex glass-stoppered bottle containing 7 ml. of 0.01 *N* HCl, and the mixture was shaken mechanically for 5 min. and centrifuged for 5 min. Four milliliters of the aqueous phase was transferred to a colorimeter tube, and 1 ml. of a freshly prepared phenol solution (1% in water) was added and mixed. One milliliter of a freshly prepared sodium hypobromite solution (sufficient bromine water added to 1 *N* sodium carbonate solution to produce a slightly yellow color) was added, and the mixture was allowed to stand at room temperature for 20 min. The percent transmittance was read on a Coleman Junior spectrophotometer at 620 mμ against a blank consisting of 1 ml. of pretest plasma or urine treated in the same manner as the test specimens. The concentrations of acetaminophen in the specimens were determined by interpolation of a standard curve of transmittance *versus* concentration of *p*-aminophenol hydrochloride. Freshly prepared standard solutions of *p*-aminophenol hydrochloride were run through the analytical procedure along with the test and blank plasma and urine specimens.

RESULTS AND DISCUSSION

Photomicrographs of the coarse, regular, and fine ATC powders (Fig. 1) show that the coarse powder was of a relatively uniform particle size with nearly all particles falling in the 90- to 150-μ size range. The size range of this powder can be estimated from the photomicrograph in which each small division on the scale has a value of 6.03 μ. The fine powder also appears to be relatively uniform in particle size and was found by air permeation measurements to have a volume surface mean diameter (d_{vs}) of about 3.3 μ. The regular powder was not uniform in size and appears to be a mixture of relatively small particles (2–10 μ) and relatively large particles (80–100 μ). It was found to have a d_{vs} of about 22 μ. Dissolution rate experiments with the three powders showed that the solutions approached saturation at significantly different rates in the first 60 min. (see Fig. 2). Thus, significant differences in the onset and peak magnitude of the biological effects of ATC in the three particle sizes were anticipated.

Table I shows the LD_{50}'s of the three ATC powders when administered orally to mice. The LD_{50}'s and relative potencies were calculated by the Logit chi-square method of Berkson (4). The results show that both regular- and coarse-particle ATC were significantly less toxic than fine-particle ATC at the 95% confidence level. The toxicities of the regular and coarse ATC powders were not significantly different from each other at the 95% confidence level, but the actual LD_{50}'s strongly suggest that there were differences in the availabilities of these two powders for absorption in mice.

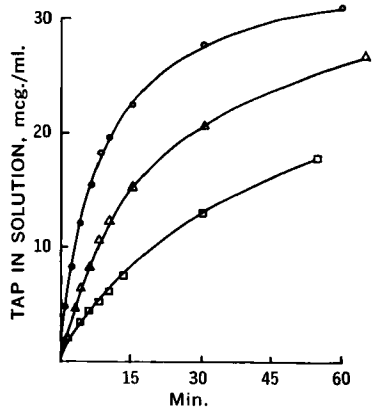


Fig. 2—Plots showing the relative rates of dissolution of fine (O-O), regular (Δ-Δ), and coarse (□-□) particle ATC powders in 0.01% dioctyl sodium sulfosuccinate solution in an apparatus similar to that used by Parrott et al. *(3). The plots illustrate different rates of* in vitro *dissolution and suggest that the powders would release ATC for absorption from the gastrointestinal tract at considerably different rates following oral administration.*

It was felt that a more sensitive test of availability, such as determination of drug plasma concentrations, might demonstrate even more unequivocally the differences in the availabilities of the three powder forms of ATC. Therefore, groups of mice were dosed orally with 1,000 mg./Kg. of the powders, and plasma concentrations of total (free + conjugated) acetaminophen were determined at various time intervals postdrug administration. The results in Fig. 3 show that, regardless of particle size, all three ATC powders produced peak plasma acetaminophen concentrations at about 1-hr. postdrug, suggesting that in each case absorption of ATC in the mouse was essentially ended after about

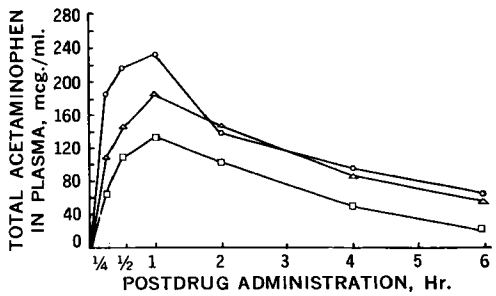


Fig. 3—Plots of plasma concentrations of total acetaminophen (free + conjugated) in mice following oral administration of 1,000 mg./Kg. of fine (O-O), regular (Δ-Δ), and coarse (□-□) particle ATC powders suspended in a 0.5% dispersion of gum tragacanth in water. The plots suggest that, in each case, the absorption of ATC was essentially ended after about 1 hr. The plasma concentrations in the 0- to 2-hr. period are dependent upon the particle sizes of the powders and, presumably, are controlled by their rates of dissolution

1 hr. However, Fig. 3 also shows that there were substantial differences in the plasma concentrations of acetaminophen produced by the powders during the first 2 hr. Thus, the fine-particle material gave rise to a substantially higher maximum plasma concentration during the first hour than the regular material; and the regular material gave rise to a substantially higher maximum plasma concentration than the coarse material.

Between 2 and 6 hr., the disappearance rates of acetaminophen from the plasma appeared to be identical for each of the three particle sizes of ATC. This supports the view that absorption of the administered doses was ended before 2 hr. The coarse-particle material gave the lowest peak and the lowest overall plasma concentrations of acetaminophen at all collection times, suggesting that the fraction of the dose absorbed was lowest from this form of ATC. The regular and fine-particle materials gave virtually superimposable plasma concentration curves between 2 and 6 hr. suggesting that the fractions of the doses absorbed were virtually identical from these two forms of ATC.

The higher plasma concentrations observed for the fine-particle ATC during the first hour suggests that absorption was fastest from this material. Also, the sudden drop in plasma concentration from Hour 1 to Hour 2 suggests that absorption-distribution equilibrium was not maintained during the first hour of absorption of fine-particle ATC. However, after absorption was ended, at about Hour 1, distribution equilibrium between the blood and other tissues was established by Hour 2; and the plasma concentrations observed thereafter were virtually the same as for the regular particle size ATC powder.

Figure 4 shows a plot of the LD_{50}'s of the three ATC powders (Table I) *versus* the peak plasma concentrations of total acetaminophen (log scale) at 1 hr. postdrug administration shown in Fig. 3. This plot shows that there was good correlation between the toxicities of the ATC powders and the peak plasma concentrations. The latter, in turn, appear to be related to the rate of absorption. In the mouse, the rate of absorption appeared to be a function of the particle size; therefore, there was excellent correlation between: (*a*) particle size and acute oral toxicity, (*b*) particle size and peak plasma concentration, and (*c*) peak plasma concentration and acute oral toxicity.

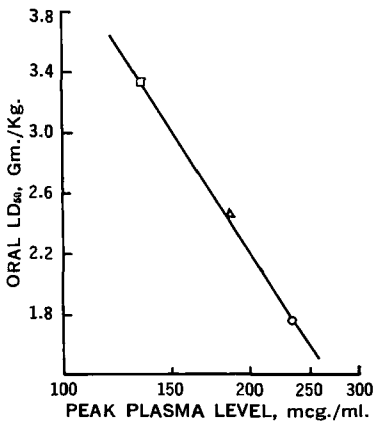


Fig. 4—Plot showing the correlation between the LD_{50}'s (Table I) and the peak plasma concentrations of total acetaminophen (log scale) at 1-hr. postdrug (Fig. 2) produced by oral administration of coarse (□), regular (Δ), and fine (○) particle ATC to mice.

TABLE I—ORAL LD_{50}'S AND POTENCY RATIOS WITH 95% CONFIDENCE LIMITS FOR COARSE, REGULAR, AND FINE-PARTICLE ATC POWDERS IN MICE[a]

LD_{50}	mg./Kg.
Coarse	3340 (1800–3710)
Regular	2461 (1820–2905)
Fine	1796 (1341–2079)
Relative Toxicities	
Coarse *vs.* regular	1.24 (0.93–1.59)[b]
Regular *vs.* fine	1.38 (1.12–1.73)[c]
Coarse *vs.* fine	1.71 (1.23–2.10)[c]

[a] Data calculated by the Logit chi-square method of Berkson (4). [b] Not significant at the 95% level. [c] Significant at the 95% level.

The results obtained with the three particle sizes of ATC in mice stirred interest to determine how particle size would affect the performance of ATC in humans. Two 6 × 6 crossover studies were carried out to compare (*a*) coarse-particle ATC with fine-particle ATC, and (*b*) regular-particle ATC with acetaminophen with respect to the plasma concentrations of acetaminophen produced in humans. Plots of the time profiles of plasma total acetaminophen in subjects administered 1 Gm. of coarse, regular, or fine-particle ATC or 436 mg. of acetaminophen (molar equivalent of 1 Gm. of ATC) orally in hard gelatin capsules are shown in Fig. 5.

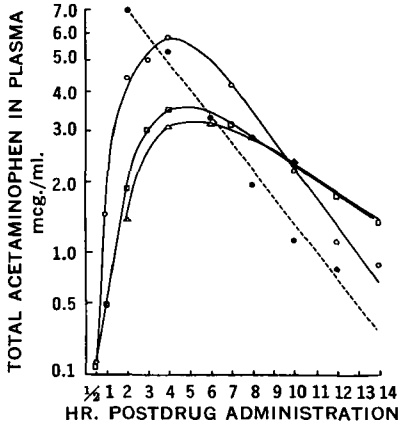


Fig. 5—Semilog plots of plasma concentrations of total acetaminophen (free + conjugated) in humans following oral administration of 1 Gm. of fine (○-○), regular (Δ-Δ), or coarse (□-□) ATC powders or 463 mg. of acetaminophen (●-●) (molar equivalent of 1 Gm. of ATC) in hard gelatin capsules. The study was carried out in two parts on 6 × 6 crossover plans. The acetaminophen and fine-particle ATC curves descend at rates corresponding to half-lives of about 3.4 and 3.2 hr., respectively, suggesting that no further absorption or conversion of ATC to acetaminophen took place after about 6 hr. following oral administration of fine-particle ATC. The regular and coarse-particle ATC curves descend at a rate corresponding to a half-life of about 6 hr. suggesting that absorption of ATC and conversion to acetaminophen was continuing well beyond 6 hr., perhaps as long as 14 hr., with these powders.

TABLE II—AVERAGE URINARY RECOVERY OF TOTAL (FREE + CONJUGATED) ACETAMINOPHEN FOLLOWING ORAL ADMINISTRATION OF COARSE, REGULAR, OR FINE-PARTICLE ATC OR ACETAMINOPHEN TO HUMANS (6 × 6 CROSSOVER)

Material Administered	Dose	Total Acetaminophen (mg./ml.) Collected per Postdrug Period (hr.) 0–8	8–16	16–24	24–32	32–40	40–48	Total	(Limits)
Acetaminophen	463 mg.	189.4 (40.9%)	70.7 (15.3%)	20.1 (4.3%)	←——7.8——→	←——(1.7%)——→		288.0 (62.2%)	(84.4–413.1) (18.2–89.3%)
Coarse ATC	1 Gm.	100.1 (21.6%)	142.3 (30.7%)	35.8 (7.7%)	23.9 (5.2%)	6.6 (1.4%)	2.2 (0.5%)	310.9 (67.1%)	(179.5–413.2) (38.8–89.2%)
Regular ATC	1 Gm.	112.3 (24.3%)	127.5 (27.6%)	41.7 (9.0%)	←——25.0——→	←——(5.4%)——→		306.5 (66.3%)	(207.4–432.1) (44.8–93.4%)
Fine ATC	1 Gm.	111.5 (24.1%)	134.6 (29.1%)	77.4 (16.7%)	10.1 (2.2%)	4.0 (0.9%)	1.9 (0.4%)	339.5 (73.3%)	(236.4–413.1) (51.1–89.2%)

The plasma concentration curves of regular and coarse-particle ATC were very similar, confirming the wide degree of overlap in the range of particle sizes in these two powders. In humans, where the gastrointestinal tract is much longer than in the mouse, proportionately smaller doses were given; and both the large and small particles in the regular-particle ATC powder were probably completely dissolved and absorbed. Since there was a relatively high proportion, on a weight basis, of coarse particles in regular-particle ATC powder (see Fig. 1), it was reasonable to expect that this powder would behave more like coarse-particle ATC in humans in which a large fraction of the administered dose is dissolved and absorbed.

Figure 5 shows that after peak drug concentrations were reached, the rates of plasma clearance with regular and coarse particle ATC were slower (half-life about 6 hr.) than that observed with acetaminophen (half-life about 3.4 hr.). Gibaldi and Schwartz (6) have shown that prolonged absorption of a prodrug of penicillin G gave a blood concentration plot of penicillin G with the appearance of prolonged excretion; that is, the half-life of the descending curve was much longer than that observed following administration of penicillin G itself. They pointed out that the apparent half-life from the "tail" of the plot was an artifact caused by absorption of the prodrug during the period in which the blood concentration curve was descending. Similarly, the slower apparent rate of clearance of acetaminophen from the blood of humans administered regular and coarse-particle ATC is probably due to prolonged or delayed absorption of the larger particles in these powders.

On the other hand, the plasma concentrations of acetaminophen produced by fine-particle ATC reached a higher, slightly earlier peak (4 hr.) than those produced by coarse or regular-particle ATC, and the rate of plasma clearance (half-life about 3.2 hr.) in the fine-particle case was the same as that observed for acetaminophen (half-life about 3.4 hr). This effect is probably due to "complete" absorption of fine-particle ATC within about 5 hr. postdrug and is indicative that ATC was completely converted to acetaminophen (and trichloroethanol) by about 5 hr. postdrug. Thus it appears that the low aqueous solubility of ATC would enable the formulator to lower the peak and extend the duration of acetaminophen (and presumably also trichloroethanol) blood levels by merely controlling the particle size of administered ATC powder.

The plasma concentration data obtained with mice suggested that coarse-particle ATC was not as completely absorbed as regular or fine-particle ATC, and it was of interest to know if this was also true in humans. Therefore, the urines of the subjects administered coarse, regular, or fine-particle ATC powder or a molar equivalent dose of acetaminophen were collected for 48 hr. following administration of the drug and analyzed for total acetaminophen content. The results shown in Table II suggest great variability in absorption efficiency from subject to subject with a given form of the drug. Also, the absorption efficiencies vary to the degree that the ranges overlap for each form of ATC and for acetaminophen. However, there is a trend toward greatest absorption efficiency for the fine form of ATC. Perhaps more importantly, these results confirm that ATC is a true prodrug of acetaminophen which releases molar equivalent amounts of acetaminophen to the tissues at a slower rate following oral administration than does acetaminophen itself. As a result, when a coarse powder form of ATC is administered to humans, acetaminophen blood levels show a profile which is suggestive of a slower, more prolonged release and absorption of this prodrug.

CONCLUSIONS

1. In mice, only a fraction of the dose of ATC appeared to be absorbed orally from the coarse powder form. The dissolution rate of the ATC powder controlled the blood plasma concentrations of acetaminophen (and presumably also trichloroethanol) during the first 2 hr. after oral dosing. The peak plasma concentration attained during this period was directly related to the LD_{50} of the powder.

2. In humans, coarse, regular, and fine-particle ATC powders appeared to be completely absorbed orally. The slow dissolution of the larger particles in the coarse and regular ATC powders caused the plasma concentrations of acetaminophen to reach lower peaks and descend at slower rates than those following oral dosing with acetaminophen or fine-particle ATC.

3. The low aqueous solubility of ATC would allow the formulator to control, to some extent, the blood levels and, presumably, the pharmacologic activity of ATC given orally. Thus, a prodrug might be employed to produce a sustained action of the parent drug in cases where the parent drug may not lend itself readily to formulation in common sustained-release dosage forms.

REFERENCES

(1) Swintosky, J. V., Caldwell, H. C., Chong, C. W., Dittert, L. W., and Irwin, G. M., *J. Pharm. Sci.*, **57**, 752 (1968).

(2) Dittert, L. W., Caldwell, H. C., Adams, H. J., Irwin, G. M., and Swintosky, J. V., *ibid.*, **57**, 774(1968).
(3) Parrott, E. L., Wurster, D. E., and Higuchi, T., *J. Am. Pharm. Assoc., Sci. Ed.*, **44**, 269(1955).
(4) Berkson, J., *J. Am. Statistical Assoc.*, **48**, 565(1953).
(5) Brodie, B. B., and Axelrod, J., *J. Pharmacol. Exptl. Therap.*, **94**, 22(1948).
(6) Gibaldi, M., and Schwartz, M. A., *Brit. J. Pharmacol. Chemotherap.*, **28**, 360(1966).

Keyphrases

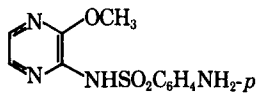
Prodrugs—acetaminophen carbonate esters
4-Acetamidophenyl 2,2,2-trichloroethyl carbonate
Particle size, effect—absorption, excretion, toxicity
LD_{50} values—acetaminophen carbonate esters
Plasma levels—acetaminophen
Urinary excretion—acetaminophen

Selective Acid-Catalyzed Hydrolyses of Methoxysulfanilamidodiazines

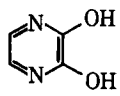
By VINCENT S. VENTURELLA*

The dilute acid hydrolysis of 3-methoxy-6-sulfanilamidopyridazine and several methoxysulfanilamidopyrimidines has been studied. Experiments show that in cases where an intermediate 2-pyrimidone is a possible postulation, further hydrolysis usually leads to the formation of sulfanilamide and the corresponding hydroxypyrimidine. A multistage route for the acidic degradation of 3-methoxy-6-sulfanilamidopyridazine, 2,4-dimethoxy-6-sulfanilamidopyrimidine, and 2-methylthio-4-methoxy-6-sulfanilamidopyrimidine is proposed.

RECENTLY, during a routine evaluation of the acidic degradation of the sulfanilamidopyrazine (I), it was found that the normally expected dilute acid cleavage to sulfanilic acid and 2-amino-3-methoxypyrazine did not occur (1), but that the products formed were sulfanilamide and 2,3-dihydroxypyrazine (II). Such a result



(I)



(II)

is in part unexpected, due to the fact that a 60% HBr solution is routinely used to effect the cleavage of difunctional methoxypyrazines (2), although there is a report (3) that 2,3-disulfanilamidopyrazine forms II under similar conditions. Since the result obtained with (I) may be due to the activation of the CH_3O position by the p-$H_2NC_6H_4SO_2NH$ group, it was desirable to test the mutual activation of these groups on the other substituent in similar sulfanilamidodiazines, such as those with the pyridazine and pyrimidine rings. The hydrolyses (except where noted) were carried out in refluxing 2 *N* HCl.

Hydrolysis of 3-methoxy-6-sulfanilamidopyridazine (III) (sulfamethoxypyridazine)[1] gave the expected sulfanilic acid, but activation of the ether position occurred forming 3-hydroxy-6-aminopyridazine. This result is surprising, even though Jacobs (4) states that the pyridazine ring is susceptible to substitution by nucleophilic reagents, because the present study has shown that a CH_3O group in the 3 position is not susceptible to nucleophilic displacement under the reaction conditions employed. In 2 *N* HCl solutions, neither 3-methoxy nor 3-methoxy-6-aminopyridazine (IV) formed more than a nominal amount of hydrolyzed material (3-hydroxypyridazine; 3-hydroxy-6-aminopyridazine, respectively) after several hours reflux followed by standing in solution for 24–72 hr. This seemingly anomalous result demands further study although a partial explanation can be made on the basis of an induced increase in the basicity of N^2 in the substituted pyridazine. The basicity increase is probably lacking or very weak in 3-methoxy- and 3-methoxy-6-aminopyridazine; a *point which*

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