4. The water content of the reaction mixture has little effect on the rate of reaction of the corticosteroid (II) with I within the range studied.

5. The hydrolysis rate of the ester (III → II) and subsequently the overall reaction rate appear to be proportional to the water content of the reaction mixture.

These kinetic results imply that the USP blue tetrazolium assay for these corticosteroid hemisuccinate esters has a high variability potential since it is a timed measurement (90 min). These results show that III does not react directly with I and that II must be formed. Therefore, the USP blue tetrazolium assay cannot differentiate between the corticosteroid (II) and its ester (III) and thus is not a stability-indicating assay of III. In fact, if hydrolysis of the ester of these corticosteroids occurred, the results might show an increase in corticosteroid since the reaction rate of the corticosteroid is about three times as fast as the hemiester in alcohol USP.

CONCLUSIONS

1. The reaction of corticosteroid esters (III) with I is sequential. The ester is hydrolyzed first, and the resulting corticosteroid (II) reacts with I. The hydrolysis step is the rate-determining step.

2. The USP blue tetrazolium assay is not stability indicating for the esters of these corticosteroids.

3. As the percent of water in the reaction mixture decreases, the time for complete reaction increases for the USP blue tetrazolium assay of C-21 hemisuccinate esters of corticosteroids.

REFERENCES

(1) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 622.

(2) S. Gorog and G. Szasz, "Analysis of Steroid Hormone Drugs," Elsevier, New York, N.Y., 1978, p. 331.

(3) A. S. Meyer and M. C. Lindberg, *Anal. Chem.*, **27**, 813 (1955).

(4) C. A. Johnson, R. King, and C. Vickers, *Analyst*, **85**, 714 (1960).

(5) D. E. Guttman, *J. Pharm. Sci.*, **55**, 919 (1966).

(6) R. E. Graham, E. R. Biehl, C. T. Kenner, G. H. Luttrell, and D. L. Middleton, *ibid.*, **64**, 226 (1975).

(7) J. W. Mauger, A. N. Paruta, and R. J. Gerraughty, *ibid.*, **58**, 574 (1969).

(8) E. Garrett, *ibid.*, **51**, 445 (1962).

(9) V. D. Gupta, *ibid.*, **67**, 299 (1978).

(10) M. D. Smith and D. Hoffman, *J. Chromatogr.*, **168**, 163 (1979).

(11) R. E. Graham and C. T. Kenner, *J. Pharm. Sci.*, **62**, 103 (1973).

(12) R. O. Rechnagel and M. Litteria, *J. Lab. Clin. Med.*, **45**, 818 (1955).

(13) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, pp. 242, 324.

(14) R. E. Graham, E. R. Biehl, and C. T. Kenner, *J. Pharm. Sci.*, **66**, 965 (1977).

(15) *Ibid.*, **65**, 1048 (1976).

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New Evaluation of Potential Methylmercury Scavengers

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Received November 5, 1979, from the **Department of Biochemistry and the* ‡*Department of Biophysics, Indiana University School of Medicine*, and the §*Veterans Administration Medical Center, Indianapolis, IN 46202.* Accepted for publication March 24, 1980.

Abstract □ A biological assay was developed to evaluate rapidly the relative efficacy of marketed and experimental mercurial scavengers. Rat liver mitochondrial protein (1.0 mg) was titrated against methylmercuric chloride to the inhibitory level of mitochondrial respiration. Respiration induced by adenosine 5′-diphosphate with succinate (plus rotenone) as the substrate was inhibited consistently by 20.7 ± 3.9 nmoles of methylmercury/mg of protein. Adenosine 5′-diphosphate-stimulated respiration (State 3) was restored with dimercaprol, penicillamine, and cysteine but not with serine. The antagonists glutathione, 3-mercaptopropionic acid, 2-mercaptoethanol, dithiothreitol, thioglucose, mercaptosuccinic acid, and thiosalicylic acid were effective. Glutathione was significantly superior to thiosalicylic acid and mercaptosuccinic acid. Sodium sulfide, thioacetamide, and ethylenediaminetetraacetic acid were completely inactive. Substitution of glutamate (plus malate) for succinate (plus rotenone) as the substrate did not alter the responses significantly. The rat liver mitochondrial assay provides preliminary information about the efficacy and toxicity of water-soluble thiols. Investigations utilizing encapsulated water- and lipid-soluble mercaptans are in progress.

Keyphrases □ Mercurial scavengers—thiol-containing compounds, evaluation of efficacy and toxicity using rat liver mitochondrial assay □ Mercaptans—evaluation of efficacy and toxicity as mercurial scavengers using rat liver mitochondrial assay

Agents currently used to alleviate clinical symptoms and to facilitate the rapid removal of methylmercury include chelators such as dimercaprol, penicillamine, and ethylenediaminetetraacetic acid (1–3). Unfortunately, the effectiveness of these antidotes is limited by their toxicity, chemical instability, and relatively short circulation time. Polythiol resins, thioacetylated steroids, mercaptosteroids, and lipid encapsulation of ethylenediaminetetraacetic acid have been proposed to improve on these mercurial scavengers (4–9).

BACKGROUND

Reports on the analysis of subcellular distribution of labeled mercury indicate significant accumulation of mercury by mitochondria (10). Furthermore, dose and time dependence of the mercurial in relation to the extent of damage incurred by isolated mitochondria has been documented (11–13). In addition, the technique for isolation of viable rat liver mitochondria is well known.

Early reports demonstrating reversal of mercurial inhibition by mercaptans dealt with isolated enzymes (14, 15). Thompson and Whittaker (14) showed significant protection of pigeon brain pyruvate oxidase activity against mercuric chloride inhibition by the addition of dimercaprol and glutathione. Barron and Kalnitsky (15) demonstrated 75% reactivation of mercuric chloride-inhibited succinoxidase by the addition of various dithiols. Sone *et al.* (11) recently studied the effects of methylmercuric chloride on mitochondrial respiration and found nearly complete restoration of succinate oxidation with thiol-containing reagents such as 2-mercaptoethanol, glutathione, cysteine, and dithiothreitol. The concentration required for reversal was not given. Less than 10% recovery of nicotinamide-adenine dinucleotide oxidation was restored by these reagents (11).

In summary, direct effects of mercurials on rat liver mitochondria have been demonstrated (10–13). Furthermore, reactivation of mercurial-inhibited enzymes by thiols has been shown (14, 15). In addition, relief of mercurial intoxication in mitochondria by thiols has been shown but has not been quantified (15). This report describes the use of mito-

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chondria in the evaluation of mercaptans as scavengers of methylmercuric chloride.

EXPERIMENTAL[1]

All mercaptans were dissolved in deionized, distilled water just prior to use. These reagents, as well as methylmercury, exhibit chemical instability and volatility. For example, a dilute solution of methylmercuric chloride loses 20% of its capacity to inhibit mitochondrial respiration after standing for 5 hr at room temperature.

Preparation of Mitochondria—Mitochondria were prepared by decapitation of rats (Sprague–Dawley strain) and rapid transfer of the liver to 0.25 *M* sucrose plus 1 m*M* ethylenediaminetetraacetic acid (pH 7.2) at 4°, followed by washing, homogenization, and centrifugation according to the method of Blair (16). The final pellet was resuspended in sucrose–ethylenediaminetetraacetic acid and assayed for total protein by the biuret method (17). The total amount of protein, the volume of material, and the final protein concentration were recorded for each experiment.

Measurement of Redox Reactions—The intactness of isolated mitochondria was determined by incubation of 1.0 mg of mitochondrial protein first with 15 m*M* glutamate plus 0.15 m*M* malate and then with 20 m*M* succinate plus rotenone (500 ng/ml) in a medium containing 250 m*M* sucrose, 5.0 m*M* monobasic sodium phosphate, and 3.0 m*M* $MgCl_2$ (Medium 1). State 3 respiration[2] was initiated by the addition of 1.5 μmoles of adenosine 5′-diphosphate and recorded on an oxygraph[3] equipped with a test platinum electrode and a 2.0-ml partially enclosed reaction vessel maintained at 30° by a circulating water bath. A respiratory control ratio of 5.0 for succinate (plus rotenone) as the substrate was used as a minimum standard. All mitochondria batches were subjected to control runs at the beginning of each experiment.

Inhibition of Mitochondrial Oxidative Metabolism—The minimum level of methylmercury required to inhibit State 3 respiration to the level of State 4 respiration[4] was determined by incubating 1.0 mg of rat liver mitochondrial protein with succinate (20 m*M*) plus rotenone (500 ng/ml) in Medium 1 to which an excess of adenosine 5′-diphosphate (3.0 μmoles) had been added. Approximately 1 min after the initiation of State 3 respiration, methylmercury was pipetted into the reaction vessel. Observations were terminated after several minutes of State 4 respiration or when the rate of State 3 respiration was indistinguishable from State 4 respiration. Titrations were conducted at the beginning of each experiment to evaluate interbatch variation.

The relative efficacy of the water-soluble thiols was examined using succinate plus rotenone as the substrate, adenosine 5′-diphosphate to initiate State 3 respiration, and a constant level of methylmercury in Medium 1. Thiols were titrated to a concentration sufficient to reinstate State 3 respiration. Values reported in Table I represent 50% recovery. These values were calculated by subtracting the State 4 rate from the recovery scavenger rate and dividing by the State 3 rate minus the State 4 rate of respiration, with subsequent matching of the scavenger concentration used to the appropriate percentage recovery. All agents were tested on several preparations of mitochondria.

RESULTS AND DISCUSSION

Inhibition of mitochondrial metabolism as a function of the amount of methylmercury added was reported previously (11, 12). No change in respiration was observed below 0.10 nmole of methylmercury/mg of rat liver mitochondrial protein. Above 11.0 nmoles of methylmercury, >91% inhibition of State 3 minus State 4 respiration was obtained.

Sone *et al.* (11) reported that dimercaprol reverses methylmercury-induced inhibition of rat liver mitochondrial metabolism. To quantify the proposed reversal, a series of experiments was conducted as described. Figure 1 displays actual tracings obtained when appropriate incubations were carried out. Addition of rat liver mitochondria to a reaction medium containing the substrate initiated State 2 respiration[5]. When adenosine 5′-diphosphate was added, State 3 respiration began. Without further additions, State 3 respiration terminated to give State 4 respiration (curve A). If 1.0 μmole of dimercaprol/mg of rat liver mitochondrial protein was mixed with actively respiring mitochondria, no change was observed (curve B). Introduction of a predetermined level of methylmercury slowed the State 3 rate to a pseudo-State 4 rate. Finally, a sufficient quantity of dimercaprol added to the medium immediately relieved mercurial inhibition (curves C–E). With levels of >50 nmoles of dimercaprol/mg of rat liver mitochondrial protein, >60% recovery routinely was obtained.

Table I—Titration of Methylmercury Scavengers [a]

Mercaptan	With Methylmercury: 50% Recovery Concentration, nmoles/mg of rat liver mitochondria	Ratio of Mercaptan to Methylmercury	Without Methylmercury: Control Run Concentration, μmoles/mg of rat liver mitochondria	Effect on State 3 Respiration
Glutathione	14.5[b]	0.54	0.70	None
Thiosalicylic acid	30.4	1.15	2.40	Inhibition
3-Mercaptopropionic acid	41.5	1.76	0.40	None
2-Mercaptoethanol	58.9	2.85	2.40	None
Dithiothreitol	65.1	2.48	4.50	None
Mercaptosuccinic acid	69.2	2.80	2.52	Inhibition
Thioglucose	126[b]	4.81	2.20	None
Sodium sulfide	—	—	1.17	Inhibition
Thioacetamide	—	—	33.2	None

[a] Results are given for the assay of mitochondrial incubations with mercaptans in the presence and absence of methylmercury. Titrations of mercaptans were carried out according to procedures outlined for penicillamine and dimercaprol. Results are reported as 50% recovery levels because of the greater sensitivity in this region of the titration curve to small changes in the concentration of the scavenger. Control runs were conducted in the absence of methylmercury using higher levels of the scavenger than required to produce recovery. Qualitative effects on State 3 respiration are included in the last column. [b] $p < 0.05$ (standard t test); individual 50% recovery concentrations were compared to the mean of all 50% recovery levels.

Since penicillamine has been an alternative therapy for acute mercurial intoxication (18), the efficacy of this compound was compared to dimercaprol with the mitochondrial assay procedure. Cysteine was included as a test reagent because of its close structural similarity to penicillamine and its qualitative scavenger capacity (11, 14). Serine also was selected because it structurally resembles penicillamine but contains a hydroxyl group instead of a sulfhydryl group.

Results of several experiments are shown in Fig. 2. Titration with penicillamine and cysteine produced similar results, although a slightly higher scavenger capacity was demonstrated by penicillamine. The optimal concentration of penicillamine and cysteine required to elicit a given percentage recovery of respiration was significantly higher than the dimercaprol concentration required to produce the same response. Serine has no capacity to reverse mercurial inhibition, thereby substantiating the requirement for sulfhydryl groups on scavenger molecules.

Compounds such as 3-mercaptopropionic acid and glutathione were able to relieve mercuric chloride inhibition of isolated enzyme systems. The scavenger efficacy of these mercaptans, as well as others selected for their variation in carbon chain length, extent of ionization, and location of sulfhydryl groups, was examined (Table I). To verify the requirement of reduced thiols for activity against methylmercury, sodium sulfide and thioacetamide were tested. Because ethylenediaminetetraacetic acid has been used in the treatment of heavy metal poisoning, it was tested also.

Glutathione, a tripeptide containing one sulfhydryl group, exhibited the greatest efficiency in relieving 50% of the mercurial-induced inhibition. Thiosalicylic acid showed 50% recovery with an average of 30.4 nmoles/mg of rat liver mitochondrial protein, making it nearly as effective as glutathione, although it inhibited State 3 respiration during the control run. 3-Mercaptopropionic acid exhibited a slightly superior mercaptan

[1] Methylmercuric chloride was obtained from ICN K and K Laboratories. Sucrose, succinate, rotenone, glutamate, malate, ethylenediaminetetraacetic acid, adenosine 5′-diphosphate, 2-mercaptoethanol, thioglucose, thiosalicylic acid, glutathione, and dithiothreitol were purchased from Sigma Chemical Co. Dimercaprol, penicillamine, and serine were products of Aldrich Chemical Co. Cysteine and mercaptosuccinic acid were supplied by Eastman Organic Chemicals. 3-Mercaptopropionic acid was obtained from Evans Chemetics. Thioacetic acid and thioacetamide were products of Matheson, Coleman and Bell.

[2] Active respiration of mitochondria in the presence of excess added substrate and adenosine 5′-diphosphate.

[3] Gilson model M5P.

[4] Respiration of mitochondria after added adenosine 5′-diphosphate has been phosphorylated but in the presence of exogenous substrate.

[5] Mitochondrial respiration in the presence of added substrate and phosphate.

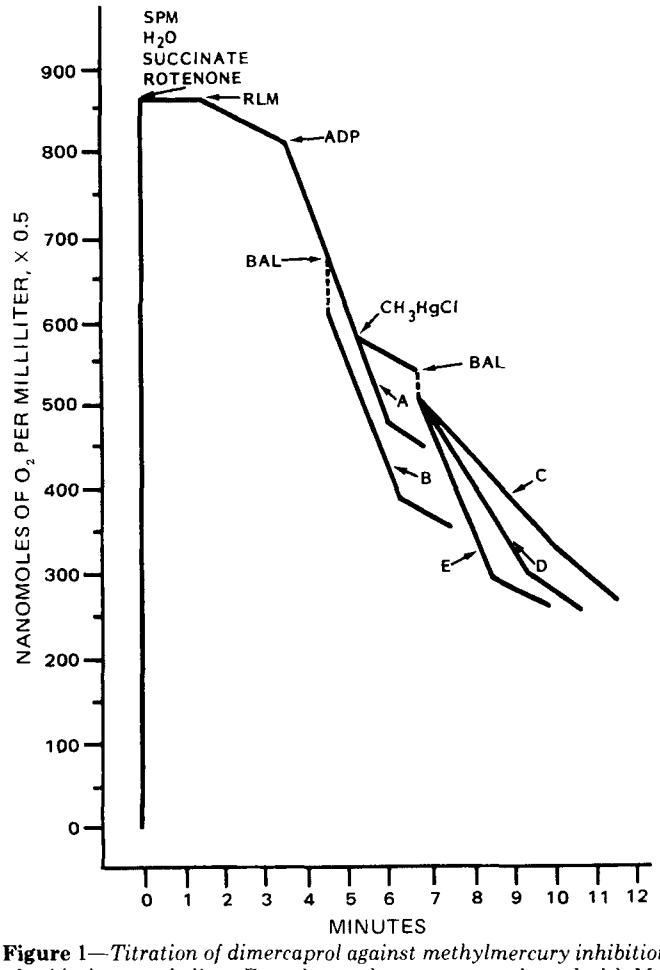

Figure 1—*Titration of dimercaprol against methylmercury inhibition of oxidative metabolism. Experimental runs were conducted with Medium 1 (20 mM succinate, 500 ng of rotenone/ml, 20 mg of mitochondria/ml, and 3.0 μmoles of adenosine 5′-diphosphate). Key: A, control run without methylmercury or dimercaprol; B, scavenger control run of 1.0 μmole of dimercaprol/mg of protein added to mitochondria after the initiation of State 3 respiration; and C–E, inhibitory effect of methylmercury (200 μg/ml) and the level of recovery using 0.05 (C), 0.10 (D), and 10 (E) μmoles of dimercaprol/mg of rat liver mitochondrial protein. Each run required ~10 min. SPM = 250 mM sucrose, 5.0 mM monobasic sodium phosphate, and 3.0 mM $MgCl_2$; RLM = rat liver mitochondria; ADP = adenosine 5′-diphosphate; and BAL = British anti-Lewisite.*

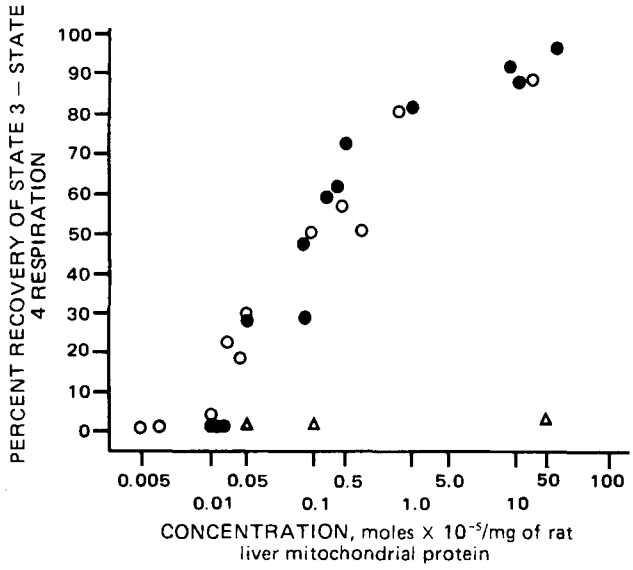

Figure 2—*Titration of penicillamine, cysteine, and serine against methylmercury. Mitochondria (20 mg/ml) were incubated in the presence of Medium 1 (20 mM succinate and 500 ng of rotenone/ml). State 3 respiration was initiated with excess adenosine 5′-diphosphate (3.0 μmoles). Inhibition of State 3 respiration to the level of State 4 respiration was obtained using 200 μg of methylmercury/ml. Aliquots of cysteine (○), penicillamine (●), and serine (△) were added to the reaction vessel, which was observed for reestablishment of State 3 respiration. The calculation of the percent recovery is described under* Experimental.

to methylmercury ratio to those of 2-mercaptoethanol and dithiothreitol, but the concentrations required were not statistically different. Mercaptosuccinic acid relieved 50% of the observed inhibition, although the scavenger capacity was reduced by a progressive increase of State 3 inhibition. Thioglucose was markedly less efficient as a mercurial scavenger. Sodium sulfate and thioacetamide proved to be ineffective scavengers and inhibitory at sufficient levels. Ethylenediaminetetraacetic acid was neither effective as a mercurial scavenger nor toxic at a concentration of 168 nmoles/mg of rat liver mitochondrial protein.

To compare the effect of an alternative substrate on mercurial scavenger efficacy, glutamate plus malate was substituted for succinate plus rotenone and several experiments were conducted as outlined. The test compounds were 3-mercaptopropionic acid, 2-mercaptoethanol, mercaptosuccinic acid, and thioacetamide. In each case, the assay responded as observed when succinate plus rotenone was used as the substrate. Furthermore, levels required for 50% recovery were statistically indistinguishable from those reported here. Thioacetamide was ineffective, as expected.

Dimercaprol proved to be a significantly superior methylmercury scavenger to penicillamine in the assay. The improved efficacy of dimercaprol compared to penicillamine also was observed in whole animal studies (8). Because dimercaprol has a relatively greater lipid solubility than penicillamine and contains an additional thiol group, the differences observed are reasonable. Nevertheless, doubling the number of sulfhydryl groups per molecule does not strictly increase its effectiveness by a factor of two. Thus, the potential of dimercaprol to bind heavy metals perhaps is antagonized by its characteristic tendency to form intermolecular disulfide bridges (18).

In this assay, glutathione was a significantly better mercurial scavenger than are the other mercaptans. Glutathione contains some ionized groups at physiological pH, so ease of transport through the inner mitochondrial membrane is not an applicable explanation. Furthermore, no damage to respiratory components was observed when glutathione was incubated with mitochondria (Table I). The explanation for the enhanced scavenger capacity of glutathione remains undetermined but is under investigation.

With the exception of glutathione and thioglucose, the mercaptans tested were not statistically different, indicating no minimal requirement for structural design beyond the availability of a free sulfhydryl group and water solubility. The inactivity of thioacetamide and sodium sulfide against methylmercury was predictable (14).

The assay is a new approach for preliminary testing of mercurial antidotes. Information is obtained not only about the efficiency of the mercurial scavenger but also about its behavior under control conditions. Although mercaptans such as thiosalicylic acid and mercaptosuccinic acid are effective scavengers, they also inhibit respiration in this system (Table I). The advantages of time and the number of animals involved make this assay attractive for first approximations of scavenger capacity. These studies are being extended to encapsulation of water-soluble mercaptans and lipid-soluble thiols.

REFERENCES

(1) L. Magos, *Br. J. Ind. Med.*, **25**, 152 (1968).

(2) W. T. Longcope, J. A. Leutscher, Jr., E. Calkins, D. Grob, S. W. Bush, and H. Eisenberg, *J. Clin. Invest.*, **25**, 557 (1964).

(3) H. Matsumoto, A. Suzuki, and C. Norita, *Life Sci.*, **6**, 2321 (1967).

(4) T. W. Clarkson, H. Small, and T. Norseth, *Arch. Environ. Health*, **26**, 173 (1973).

(5) L. Magos and T. W. Clarkson, *Chem.-Biol. Interact.*, **14**, 325 (1976).

(6) H. Selye, T. Mels, and S. Szabo, *Int. Urol. Nephrol.*, **2**, 287 (1970).

(7) V. Eybl, J. Sykora, M. Koutenska, J. Koutensky, and F. Mertl, *Arzneim.-Forsch.*, **23**, 867 (1973).

(8) L. K. Steinrauf, B. Cox, E. Foster, and R. T. Blickenstaff, *J. Pharm. Sci.*, **67**, 1739 (1978).
(9) V. E. Rahman, M. W. Rosenthal, and E. A. Cerny, *Science*, **180**, 300 (1973).
(10) T. Norseth and M. Brendeford, *Biochem. Pharmacol.*, **20**, 1101 (1971).
(11) N. Sone, M. K. Larsstuvold, and Y. Kagawa, *J. Biochem.*, **82**, 859 (1977).
(12) J. H. Southard and P. Nitisewojo, *Biochem. Biophys. Res. Commun.*, **52**, 921 (1973).
(13) J. H. Southard, P. Nitisewojo, and D. E. Green, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **33**, 2147 (1973).
(14) R. H. S. Thompson and V. P. Whittaker, *Biochem. J.*, **41**, 346 (1947).
(15) E. S. Barron and G. Kalnitsky, *ibid.*, **41**, 351 (1947).
(16) P. V. Blair, *Arch. Biochem. Biophys.*, **181**, 550 (1976).
(17) G. A. Gornall, C. J. Bardawill, and M. M. David, *J. Biol. Chem.*, **177**, 751 (1949).
(18) "The Pharmacological Basis of Therapeutics," 5th ed., L. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1975, pp. 944–958.

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Aspirin—A National Survey IV: *In Vitro* Dissolution of Aspirin Formulations

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Abstract □ The results of a national survey of the *in vitro* dissolution rates of aspirin tablets are presented. Dissolution profiles by both the proposed USP XX basket method and a paddle method are compared. The methods were used to analyze 59 tablet formulations representing 38 manufacturers. Each tablet was subjected to the dissolution procedure in 500 ml of pH 4.5 buffer solution, and an aliquot was sampled automatically and analyzed by an automated system. In 30 min, 22% of the samples tested using the basket method failed the proposed USP XX dissolution requirement. Seventy-five percent of the samples tested by the paddle method also failed the proposed dissolution requirement.

Keyphrases □ Aspirin—*in vitro* dissolution, comparison of basket and paddle methods □ Analgesics—*in vitro* dissolution of aspirin formulations □ Dissolution—aspirin formulations, *in vitro*, basket and paddle methods compared

A national survey (1) of aspirin tablet products was conducted at the National Center for Drug Analysis during the summer and fall of 1978. The purpose of the survey was to ascertain the quality of these products and the adequacy of the USP monograph (2). The USP presented a dissolution test in the *Pharmaceutical Forum* (3) that specifies use of a basket and requires 80% dissolution in 30 min.

During the survey, selected aspirin samples representing each manufacturer and dosage level were assayed for their dissolution rates *in vitro* using both a basket and a paddle and the dissolution medium (pH 4.5 buffer) and speed of rotation (50 rpm) specified in Ref. 3. The guidelines (4) for the dissolution testing were followed. The dissolution rate for each sample was determined by taking aliquots automatically (5) every 10 min over 60 min and analyzing the dissolution medium for aspirin by an automated procedure. The purpose of this experiment was to compare the dissolution characteristics of the selected marketed samples by the proposed USP XX dissolution test using the proposed basket method and the paddle method. The paddle method also was used because it has been a more discriminating test for differentiating drug products than the basket method in this laboratory.

EXPERIMENTAL

Apparatus—A dissolution apparatus[1] and a 76-liter aquarium tank with a constant-temperature water bath maintained at 37° were used.

For the sampling[2], an automatic analyzer with a sampler[3], three pumps[4], a manifold, and a timer[5] was used together with a cycle timer[6] for the sampling interval timer. Six sampling probes with filters[7] were required.

For the determinative step, an automatic analyzer with a sampler[3], pumps[8], a manifold, and a timer[5] was connected to a spectrophotometer[9] equipped with a quartz flowcell[10]. A 100-mv recorder[11] was connected to the spectrophotometer.

Reagents—ACS grade chloroform was washed with water and filtered through paper on the day of use. The pH 2.2 buffer solution was prepared by diluting 250.0 ml of 0.2 *M* KCl and 39.0 ml of 0.2 *M* HCl to 1 liter with water. The 0.2 *M* KCl was prepared by dissolving 14.911 g of potassium chloride in water and diluting it with water to 1 liter. The 0.2 *M* HCl was prepared by diluting 17.0 ml of concentrated hydrochloric acid to 1 liter with water. The pH 4.5 buffer solution was prepared by adding 2.99 g of sodium acetate to 1.66 ml of acetic acid and diluting to 1 liter with water. The buffer solution used for the dissolution medium was boiled to remove air bubbles.

Standard Preparation (324-mg Tablets)—Approximately 325 mg of USP reference standard aspirin was weighed accurately and dissolved in 5.0 ml of 95% ethanol. This solution was diluted to 1 liter with the pH 4.5 buffer solution. The standard was prepared fresh daily and was used immediately.

Sample Preparation (324-mg Tablets)—One tablet was placed in each dissolution vessel or basket. For 60 min, samples were withdrawn

[1] Model 72RL, Easi-Lift multiple-spindle dissolution drive, Hanson Research Corp., Northridge, CA 91324.
[2] C. E. Wells, T. W. Moore, and W. E. Juhl, unpublished data.
[3] AutoAnalyzer sampler II, 127-A000, Technicon Instruments Corp., Tarrytown, NY 10591.
[4] AutoAnalyzer proportioning pump I, 105-A200-01, Technicon Instruments Corp., Tarrytown, NY 10591.
[5] Flexopulse timer, model HG93A603, Eagle Signal Time Division, Gulf Western Industries, Davenport, IA 52803 (5).
[6] Three-cam, 10-min cycle timer, Westbrook Timer Sales, Westbrook, CT 06498.
[7] Filter tips, 20 μm, Centauer Chemical Co., Stamford, CT 06902.
[8] AutoAnalyzer proportioning pump III, 133-A014-04, Technicon Instruments Corp., Tarrytown, NY 10591.
[9] Model PM2DL, Carl Zeiss, Oberkochen, West Germany.
[10] Ten millimeters, 18 μl (886881) or 80 μl (886878), Beckman Instruments, Fullerton, CA 92634.
[11] Servo/Riter II, PS01W6A, Texas Instruments, Houston, TX 77001.

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