Influence of Organic Groups on the Polarographic Stability of Mercury Compounds

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Polarograms of 26 organic mercurials with different R structures were made. The half-wave potentials of these compounds correlate qualitatively with the polarizability of the R structure and the polarities of the mercury-chloride bond in the first wave and the carbon-mercury bond in the second. The polarographic reductive stability of the ring molecules below that of the chain compounds agrees with the greater electronegativity of the rings. Compounds which have found clinical usefulness as diuretics have similar polarographic properties. It is suggested that the electron density of the mercury may be the basis of the biological selectivity of these organic compounds.

IN SPITE OF their great affinity for sulfhydryl groups, organic mercurials show considerable differences in binding with protein. While these differences may be due in some instances to the configurations of the mercurials or to affinities for buffer ions or secondary groups of the protein surface, it seems also that their individual affinities for the sulfhydryl group itself must be a factor.

As an instance of this latter phenomenon it was found that phenylmercuric hydroxide (PMOH) differed from *p*-chloro-mercuribenzoate (PCMB) in its binding on egg albumin (1). Although both have similar configurations and both are known to combine readily with sulfhydryl, PMOH reacted with one SH under the same conditions that PCMB reacted with two. Moreover, under the same conditions, PMOH showed considerable non-sulfhydryl binding while PCMB possessed no affinity for secondary sites.

In this study the half-wave potentials of a series of organic mercury compounds of the type RHgX were determined in order to estimate the influence of a wide assortment of R structures and substituents on the ease of reduction of the mercury-anion bond. It is hoped that this information may suggest some correlation between the binding capacity of organic mercurials for proteins and the electronic influence of the organic moiety, possibly becoming a basis for the interpretation of their biological activity.

The compounds were studied in the supporting electrolyte, 0.1 *M* $NH_4Cl \cdot NH_4OH$, pH 7.4 being present mainly as the chloride species (2), except for 1,1′-oxalyldiiminobis (2-methoxy-trimethylenemercuri) bis-(2,5-dioxo-4-imidazolidineacetic acid, and 2,4-dioxo-3-imidazolidylmercuri-mercaptoacetic acid. In the former compound the mercury is bound to the nitrogen of the imidazolidine ring; in the latter compound the mercury is bound in a mercaptide.

None of these reductions is reversible; nevertheless, the waves are considered to be largely diffusion-controlled as judged by the normalcy of the polarograms at the low concentration levels employed (3) and the agreement between the depolarizing influence of the R groups and the polarographic stability.

METHODS AND MATERIALS

The mercurials studied are listed in Table I. Many were obtained as generous gifts from pharmaceutical houses[1] (PMOH) and phenylmercuric acetate (PMAC) were purchased from Berk and Co.; *p*-chloromercuribenzoate (PCMB) was obtained from Bios Laboratories, and *p*-tolylmercurichloride (PTMC), methylmercurichloride (MMC), and ethylmercurichloride (EMC) were purchased from Delta Chemical Works, Inc.

PMOH, PMAC, and PCMB were recrystallized before using. The other compounds were used as obtained. The purity of these compounds was judged to be sufficient for these studies from the data obtained with the samples.

Stock solutions of the mercurials were made at concentration level of $2 \times 10^{-4} M$. The mercurial was dissolved usually in water. In some cases (as with PCMB) it was necessary to dissolve it in a small amount of concentrated NH_4OH, neutralize with HCl, and dilute with supporting electrolyte solution to the desired concentration. Stock solutions were diluted with 0.2 *M* ($NH_4OH \cdot NH_4Cl$) before use, and the polarographic stability of the mercurial was observed at a concentration of $1 \times 10^{-4} M$. Possible decomposition of the solution was determined from a comparison of the polarograms of older solutions with those of a freshly prepared one. Where necessary, solutions were freshly prepared weekly.

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[1] Abbott Laboratories, Ciba Pharmaceutical Products, Inc., Lakeside Laboratories, Inc., Merck Sharp and Dohme Research Laboratories, Parke Davis and Co., The Squibb Institute, Sterling Winthrop Research Institute, The Upjohn Co., and Wyeth Laboratories, Inc.

The polarograms were obtained with an American optical recording electropolarizer model G-1 using a dropping mercury electrode. The drop time of the mercury electrode was 2.5 seconds per drop with a constant pressure of 16.5 cm. of mercury.

The temperature of the mercurial solutions was kept constant at 25° in a water bath. The solutions were deoxygenated by passing in oxygen-free nitrogen for 20 minutes. The solutions were allowed to equilibrate in the water bath for 10 minutes prior to the determinations.

RESULTS

Except for a few compounds—2-hydroxymercuri-3-methoxy-3-phenyl propanoic acid, N-(3-chloromercuri-2-piperidino-propyl) nicotinamide dihydrochloride, 1,1′-oxalyldiiminobis(2-methoxy-trimethylenemercuri)bis(2,5-dioxo-4-imidazolidineacetic acid), 2-acetomercuri-3-methoxy-butanoic acid, and 2,4-dioxo-3-imidazolidylmercuri-mercaptoacetic acid—which exhibited a single reductive wave, all of the mercurials listed in Table I were decomposed irreversibly in a two-step process. Benesch and Benesch (4) and later Wuggatzer and Cross (5) found that PCMB, phenylmercuric chloride (PMC), and phenylmercuric nitrate (PMN) are decomposed polarographically in two-electron steps, resulting in the formation of the organic mercurial radical in the first step and the reduction of this radical in the

TABLE I.—COMPOUNDS STUDIED POLAROGRAPHICALLY

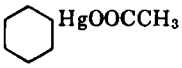
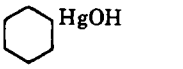
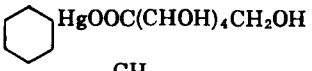
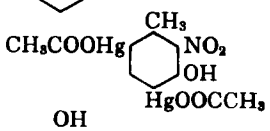
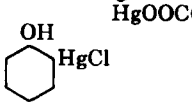
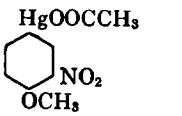
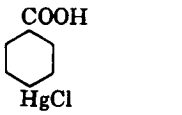
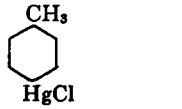
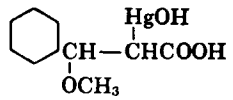
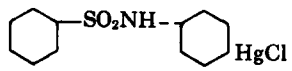
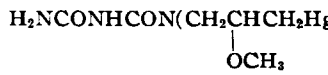
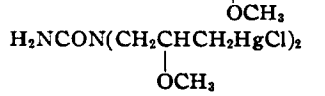
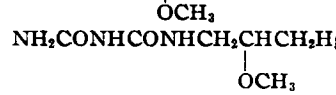
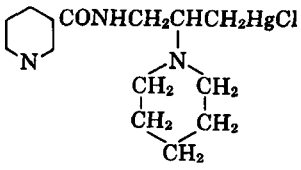
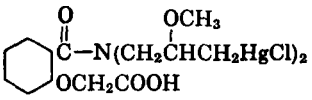
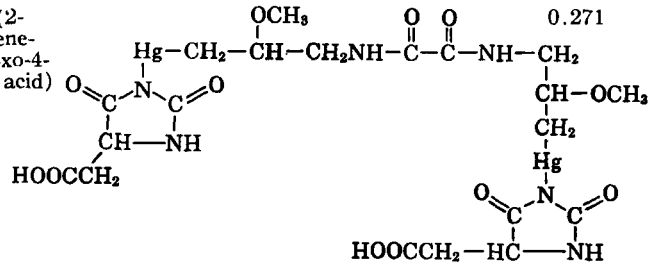
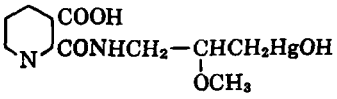
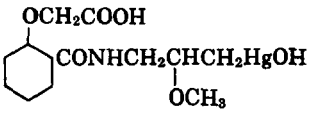
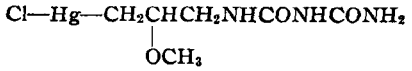
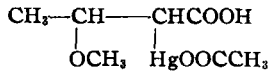
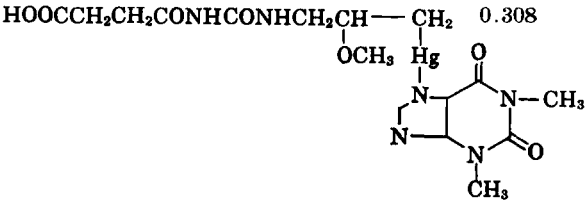
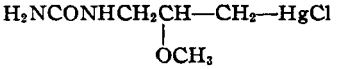
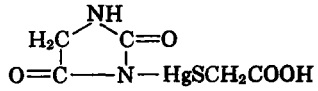
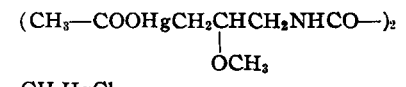
Compound Name	Formula	First Half-Wave Average, $-E_{1/2}$	Second Half-Wave Average, $-E_{1/2}$	Maxima Range, Volts
Acetomercuribenzene (phenylmercuric acetate, PMAC)		0.123	0.884	...
Hydroxymercuribenzene (phenylmercuric hydroxide, PMOH)		0.118	0.859	...
Glyconomercuribenzene		0.140	0.924	...
2-Nitro-3-hydroxy-4,6-diacetomercuritoluene		0.075	1.038	...
2-Chloromercuriphenol		0.131	0.838	...
1-Acetomercuri-3-nitro-4-methoxy-benzene		0.143	1.502	
4-Chloromercuribenzoic acid (*p*-chloromercuribenzoate, PCMB)		0.170	0.884	...
1-Methyl-4-chloromercuribenzene (*p*-tolylmercurichloride, PTMC)		0.178	0.923	...
2-Hydroxymercuri-3-methoxy-3-phenyl propanoic acid		0.196	...	...
1-Benzene sulfonamido-4-chloromercuribenzene		0.204	1.05	...
N,N-Bis(3-chloromercuri-2-methoxy-propyl)biuret		0.208	1.040	...
N,N-Bis(3-chloromercuri-2-methoxy-propyl) urea		0.216	0.958	...
N-(2-Methoxy-3-hydroxy-mercuri-propyl) biuret		0.235	0.864	...

TABLE I.—(*Continued*)

Compound Name	Formula	First Half-Wave Average, $-E_{1/2}$	Second Half-Wave Average, $-E_{1/2}$	Maxima Range, Volts
N-(3-Chloromercuri-2-piperidinopropyl) nicotinamide dihydrochloride		0.254	...	...
2-{N,N-Bis [3-(chloromercuri)-2-methoxypropyl] carbamyl-phenoxy} acetic acid		0.259	0.928	...
1,1′-Oxalyldiiminobis(2-methoxy-trimethylenemercuri)bis(2,5-dioxo-4-imidazolidineacetic acid)		0.271	0.900	...
2-(3-Hydroxymercuri-2-methoxy-propyl carbamyl) nicotinic acid		0.277	(1.02)	1.14 to 1.15
(Salyrganic acid) 2-(3-hydroxymercuri-2-methoxy-propyl) carbamyl-phenoxy acetic acid		0.279	(0.975)	1.06 to 1.08
1-[3-(Chloromercuri)-2-methoxy-propyl]biuret		0.283	(1.035)	1.15 to 1.17
2-Acetomercuri-3-methoxy-butanoic acid		0.285	...	...
Theophylline salt of N-(2-methoxy-3-hydroxy-mercuripropyl)-N′-succinyl urea[a]		0.308	0.882	...
N-(2-Methoxy-3-chloromercuripropyl) urea[b]		0.316	(0.975)	1.04 to 1.08
2,4-Dioxo-3-imidazolidyl-mercuri-mercaptoacetic acid		0.371	...	...
N,N′-Bis[3-(hydroxymercuri)-2-methoxy-propyl] oxamide, diacetate ester		0.372	(1.035)	1.09
Chloromercurimethane (methylmercurichloride, MMC)	CH_3HgCl	0.428	(1.30)	1.35 to 1.36
Chloromercuriethane (ethylmercurichloride, EMC)	CH_3CH_2HgCl	0.495	(1.39)	1.49 to 1.50

[a] Marketed as Mercurhydrin by Lakeside Laboratories. [b] Marketed as Neohydrin by Lakeside Laboratories.

second step to benzene and metallic mercury. This two-step reduction was also observed for a series of phenylmercuric amide and imide compounds (6).

It would be expected that the polarographic stability of PMOH, PMAC, and phenylmercuric glyconate should be identical because of the excess of chloride ion, and this is found to be true within ± 0.02 v. (see Table I). Taking this deviation as an allowable experimental error for all the determinations, it will be observed from the table that the R group of the mercurials has considerable influence on the half-wave potential. The phenyl ring compounds have the lowest and MMC and EMC the greatest negative $E_{1/2}$. Chain compounds with a β-methoxy group and biuret or urea in the chain have intermediate values. The entire range of voltage for the compounds varies from −0.127 v. for the phenyl compounds to −0.490 v. for EMC.

The direction of this change follows closely the depolarization effect of the R groups on the polarity of the mercury-chloride bond resulting in a change in the electron density of the mercury. Compounds in which mercury is attached directly to the benzene ring would be expected to have a higher polarity of the Hg-Cl bond, *i.e.*, a lower electron density on the mercury. The decomposition of these compounds at lower voltage agrees with the known polarizability of the benzene ring or its electronegativity to use Kharash's terminology (7).

The influence of substituents in the ring, on the other hand, seems to have relatively minor effects. The −E and −I effect of the *meta* nitro group is not shown, apparently being offset by the +E effect of the *p*-methoxy as in 1-acetomercuri-3-nitro-4-methoxy-benzene. Compound 2-nitro-3-hydroxy-4,6-diacetomercuritoluene having the same substituents in the ring has a lower decomposition potential. Possibly the electrophilic character of the two chloromercuri groups may be a factor. Compound 2-chloromercuriphenol shows no influence of an *ortho* phenolate ion. The influence of a *para* carboxyl ion in 4-chloromercuribenzoic acid (*p*-chloromercuribenzoate, PCMB) increases the half-wave potential significantly. The *para* methyl group in 1-methyl-4-chloromercuri-benzene (*p*-tolyl-mercurichloride, PTMC) and a substituted sulfonamide group in 1-benzene sulfonamide-4-chloromercuribenzene show considerable polarographic stabilizing influence.

Among the chain compounds the greater polarographic stability of chloromercurimethane (methylmercurichloride, MMC) and chloromercuriethane (ethylmercurichloride, EMC) suggests a large depolarization of the mercury-chloride bond. The influence of a β-methoxy and the other groups on the chain is shown by the lower decomposition potentials of the remaining compounds. Assuming a standard variation of ±0.02 v. compounds N-(3-chloromercuri-2-piperidinopropyl) nicotinamide dihydrochloride to 2-acetomercuri-3-methoxy-butanoic acid of Table I have the same decomposition voltage, −0.270 ± 0.02 v. Compound N-(3-chloromercuri-2-piperidinopropyl) nicotinamide dihydrochloride has a mercury attached to an aromatic pyridine ring with the substituted piperidine ring in the β-position to the mercury. The chain compounds of this intermediate group are all β-methoxy chain compounds which may account for their similarity of decomposition potential.

The decomposition potential, −0.373 v., of 2,4-dioxo-3-imidazolidylmercuri-mercaptoacetic acid is considerably higher than that of the group, −0.270 ± 0.02 v. This high value is obtained because the decomposition potential is that of a mercaptide in which the polarity of the Hg-S bond is less than the Hg-Cl bond (8).

Compound 2-acetomercuri-3-methoxy-butanoic acid is somewhat more stable than the group of methoxy compounds. Its decomposition potential is −0.285 v. It is interesting that the analog of this compound, 2-hydroxymercuri-3-methoxy-3-phenyl propanoic acid, in which the β-methyl is replaced with phenyl, is lower than the group −0.198 v. In both cases there is a carboxyl group bound to the same carbon as the Hg. The higher decomposition potential of 2-acetomercuri-3-methoxy-butanoic acid follows a depolarization effect of the carboxyl ion but a higher electronegativity of the phenyl ethyl group in 2-hydroxymercuri-3-methoxy-3-phenyl propanoic acid may also be a factor in lowering the potential.

Table I gives the decomposition potentials of the second wave for the aromatic ring compounds. Presumably at this potential the phenyl mercury radical is decomposed to benzene or a substituted benzene and mercury. Again using the results obtained with acetomercuribenzene (phenylmercuric acetate, PMAC), hydroxymercuribenzene (phenylmercuric hydroxide, PMOH), and glyconomercuribenzene as an estimate of the variations expected, the range of decomposition voltage obtained is −0.859 v. to −0.924 v. or −0.891 ± 0.03 v. It will be observed that only three of the compounds, 2-nitro-3-hydroxy-4,6-diacetomercuritoluene, 1-acetomercuri-3-nitro-4-methoxy-benzene, and 1-benzene sulfonamido-4-chloromercuribenzene, fall outside this range indicating that influences of substituents such as OH, COOH, or CH_3 are slight. From an electronegativity point of view, a small influence would be expected since the polarity of the mercury-carbon bond is less than that of the mercury-chloride bond. Compounds 2-nitro-3-hydroxy-4,6-diacetomercuritoluene and 1-acetomercuri-3-nitro-4-methoxy-benzene contain a nitro group in the ring and show unusually high polarographic stability. This strange effect is still in agreement with the idea of polarizability of groups when it is remembered that the nitro group is reduced under the conditions of these experiments to hydroxylamine in a four-electron step. This wave was observed at approximately −0.4 v. The +E effect of this reduced group would result in a decreased polarity of the carbon-mercury bond. This is in agreement with the greater negative half-wave potential of the compound.

A similar relationship was observed in a pH study of *o*-chloromercuriphenol. With increased pH there was an increased polarographic stability of the compound in a general correlation with the +E activity of the phenolate ion (see Table II). In this study a measure of the decomposition potential of the first wave gave anomalous results possibly due to bonding of the mercury to the *ortho* phenolate group. No significant change in the potentials of the first wave was observed over the entire pH range (5.07 to 10.23).

A similar relation between depolarization effects

TABLE II.—EFFECT OF pH ON SECOND HALF-WAVE OF *ortho*-CHLOROMERCURIPHENOL

pH	$-IIE_{1/2}$
5.07	0.608
5.98	0.763
6.70	0.776
6.70	0.840
7.73	0.796
8.71	0.884
10.23	0.946
10.23	0.888

and half-wave potentials was also observed in a study of the second decomposition of the chain compounds. The results of this study are given in Table I. Several of the chain compounds, 2-(3-hydroxymercuri-2-methoxy-propyl carbamyl) nicotinic acid; (salyrganic acid) 2-(3-hydroxymercuri-2-methoxypropyl) carbamyl-phenoxy acetic acid; 1-[3-(chloromercuri)-2-methoxy-propyl] biuret; N-(2-methoxy-3-chloromercuripropyl) urea; N,N'-bis-[3-(hydroxymercuri)-2-methoxypropyl] oxamide, diacetate ester; chloromercurimethane (methylmercurichloride, MMC), and chloromercuriethane(ethylmercurichloride, EMC), exhibited maxima with sloping plateaus at the second reduction. This made the estimation of their half-wave potentials difficult and inaccurate. The positions of the maxima are reported along with the approximate $E_{1/2}$. Undoubtedly, these abnormalities are due to adsorption and reactivity of the radicals but the position of the maximal wave is of some interest in terms of the polarization effects of the R groups. With PMOH as standard with a decomposition voltage of -0.891 ± 0.03 v., it will be observed that chain compounds N-(2-methoxy-3-hydroxymercuri-propyl) biuret; 2-{N,N-bis[3-(chloromercuri)-2-methoxy-propyl] carbamyl-phenoxy} acetic acid, and the theophylline salt of N-(2 - methoxy - 3 - hydroxy - mercuripropyl) - N'-succinyl urea are equally stable polarographically. These are β-methoxy chain compounds and the theophylline salt of N-(2-methoxy-3-hydroxy-mercuripropyl)-N'-succinyl urea has, in addition, an added theophylline ring. Compounds chloromercurimethane (methylmercurichloride, MMC) and chloromercuriethane (ethylmercurichloride, EMC) appear again as the most stable, correlating the +I effect of the methyl and ethyl chains, respectively. Compounds N,N-bis(3-chloromercuri-2-methoxy-propyl) biuret; N,N-bis(3-chloromercuri-2-methoxy-propyl) urea; 2-(3-hydroxymercuri-2-methoxy-propyl carbamyl) nicotinic acid; (salyrganic acid) 2-(3-hydroxymercuri-2-methoxy-propyl) carbamylphenoxy acetic acid; 1-[3-(chloromercuri)-2-methoxy-propyl] biruet; N-(2-methoxy-3-chloromercuripropyl) urea, and N,N'-bis[3-(hydroxymercuri)-2-methoxy-propyl] oxamide, diacetate ester are methoxy chain compounds and have an average decomposition potential of -0.983 ± 0.06 v.

As was stated before, 2-hydroxymercuri-3-methoxy-3-phenyl propanoic acid; N-(3-chloromercuri-2-piperidinopropyl) nicotinamide dihydrochloride; 1,1' - oxalyldiiminobis(2 - methoxy - trimethylenemercuri)bis(2,5-dioxo-imidazolidineacetic acid); 2-acetomercuri-3-methoxy-butanoic acid, and 2,4-dioxo-3-imidazolidylmercuri-mercaptoacetic acid appeared anomalous in that they exhibited a single reductive wave. Presumably this reduction may involve the addition of two electrons. An electronic interpretation would require that the two mercury bonds have the same polarity. This cannot be answered with the present data available, but it is interesting that in 2,4-dioxo-3-imidazolidylmercuri-mercaptoacetic acid the mercury is bonded to sulfur and nitrogen and in 1,1'-oxalyldiiminobis-(2-methoxy-trimethylenemercuri)bis(2,5-dioxo-4-imidazolidineacetic acid), to nitrogen and carbon. Possibly, proximate groups may also influence the polarity of the bonds.

DISCUSSION

The relation of the depolarizing influence of the R group structure with the polarographic stability of the compounds at the first wave is consistent in general with a mercury-chloride bond polarity of Hg^{d+}–Cl^{d-}. Those groups attracting electrons decrease the electron density on the mercury and those donating electrons decrease the polarity. Since the polarographic influence of the R group in the second reduction is in the same direction, an electronic interpretation would suggest that the polarity of the carbon-mercury radical bond is C^{d+}–Hg^{d-}. Radical formation does not occur apparently with all the compounds studied. In these cases there may be similar polarities in the two mercury bonds, as for instance in a compound in which the mercury is bonded to nitrogen and sulfur. This situation can be represented as N^{d-}–Hg^{d+}–S^{d-}.

At present, some organic mercurial compounds of the type

$$R—CH_2—\underset{\displaystyle OY}{\underset{|}{CH}}—CH_2—HgX$$

have found clinical usefulness as diuretics. While the X and Y groups have a small influence on activity, it is evident that the nature of the R group is fundamental (9). Compounds of the type R—Hg—X in which R is simply an aromatic ring or an aliphatic chain are not useful because of toxicity. It is interesting that the ring compounds of this type have low polarographic stability (see Table I) and those where R is a simple alkyl have large negative half-wave potentials. Among the intermediate compounds, polarographically speaking, are those which have found some clinical usefulness.

At present, it is impossible to correlate more closely toxicity or diuretic activity with half-wave potentials but this study suggests at least a possible basis for biological selectivity of the mercury compounds. From the demonstrated correlation between polarographic stability and polarizability of groups, it can be concluded that the R, X, and Y groups exert effects on the polarity of the mercury bonds and on the electron density around the mercury atom.

According to Boyer (2) the ease of dissociation of the mercury compound is apparently not of prime importance in biological activity. It seems from the work of Weiner and Müller (10) and others that the intact molecule is involved, at least in diuretic activity. If this is true, then possibly the electron density on the mercury plays the determining role in biological activity. This view is suggested by the rough correlation observed in the present work between polarographic stabilities and clinical usefulness

and by the fact that compounds of different polarographic stabilities exhibited correspondingly different inhibitory activities on trypsin proteolysis (work in progress).

This view of mercury activity proposes a mechanism by which organic groups may produce selectivity by controlling the electron density on the mercury moiety.

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A New Oral Gelatinized Sustained-Release Dosage Form

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A new sustained-release micropellet was prepared which takes advantage of the relationship of gelatin solubility to the hardness of the gelatin caused by formalin treatment. This new gelatin micropellet could be produced more easily than the coated micropellet employed in the usual sustained-release dosage forms.

THE SUSTAINED-RELEASE principle has universal application in the field of practical pharmacy. It is generally understood that gelatin is digested in the human gastrointestinal tract and that the rate of hydrolysis of the gelatin can be varied by hardening. This suggested that if medication was dissolved or suspended in a gelating sol and the gelatin treated with formalin-isopropanol, the rate of hydrolysis in the gastrointestinal tract would decrease. In this study, the relationship of the hydrolysis to the grade of gelatin hardening was applied to produce a sustained-release gelatin micropellet.

As described in the *Experimental* section, the technique utilized to produce the gelatin sustained-release micropellet is easier than the more complicated and painstaking process required to produce the conventional coated micropellet. The gelatin micropellet (containing medication) had a diameter of 0.3 to 0.5 mm. To obtain the desired sustained-release effect, the gelatin micropellet was stored in 10% formalin-isopropanol at 2–5° for varying periods of time. From the results of the *in vitro* dissolution test, the treated gelatin micropellet was found to prolong protease hydrolysis.

Urinary excretion, blood concentration, and biological kinetic theory data have been employed to evaluate oral sustained-release dosage forms and such studies have been reported recently in the literature (1–5). We used chemical determinations of the amount of medication in the blood or urine to examine the delay in absorption and urinary excretion of the medication imbedded in the treated gelatin micropellets. As gelatin can be digested in the gastrointestinal tracts of carnivorous and omnivorous animals, five dogs and three humans were used to evaluate the *in vivo* sustained-release of sulfanilamide (SA) and riboflavin (RF) through blood concentration determinations.

EXPERIMENTAL

Preparation of Dosage.—Table I shows the physical properties of gelatin and mineral oil used in the production of the micropellets.

An apparatus to produce the micropellets also is shown in Fig. 1. The apparatus consists of a stainless steel vessel, stirring wing, and motor. Two-hundred grams of water was added to 60 Gm. of gelatin. After the gelatin swelled 40 Gm. of SA powder (less than 50 μ in diameter) was added.

TABLE I.—PHYSICAL PROPERTIES OF GELATIN AND MINERAL OIL

	Viscosity, cps.	pH	M. p., ° C.	Sol-gel Transformation ° C.
Gelatin	23	6.70	30.5	25.7
		Sp. Gr.		Viscosity, cps.
Mineral Oil		0.853 (15.5° C.)		13.0 (35° C.) 22.0 (20° C.)

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