the corresponding control group of animals is noted. This finding is in agreement with that reported by Jacobson *et al.* for spleen-shielded mice exposed to 1025 r(7). By histological technics these workers have shown that bone marrow recovery appears to be complete in mice by the 8th day post-irradiation. Since, however, first evidence of a reversal of peripheral leucocyte and reticulocyte depression apparently occurs on the 6th day following irradiation(7), it would appear that body weight recovery of the spleen-homogenate-treated mice in the present investigation occurs only after the appearance of bone marrow recovery.

Multiple injections of spleen homogenates were not employed in the present investigation. It seems reasonable to suppose, however, that such treatment might confer greater protection upon irradiated mice than do single injections, especially at higher X-ray dose levels.

The present demonstration of the post-irradiation protection afforded by injectable spleen homogenates in irradiated mice provides a research tool of promise for investigation of the nature of the protective spleen factor. Studies along these lines are now in progress.

Summary and conclusions. 1. The mortality of adult LAf_1 mice, exposed to whole-body X-irradiation in doses of 650, 700 and 800 r, was either prevented or reduced markedly following a single intraperitoneal injection of spleen homogenate administered either one hour or as long as 45 hours after radiation exposure. The equivalent of 1 to 2 spleens was injected into each irradiated mouse. The protective factor was present in homogenates from spleens of young mice (1 to 3 weeks old), and to a smaller degree in homogenates of spleens from adult mice. 2. The post-irradiation protection afforded by spleen homogenate injection was reflected in minimized weight losses observed in the spleen-homogenate-treated mice as compared with the control animals. The data suggest a possible quantitative relationship between the amount of spleen substance injected and the degree of protection obtained.

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Human Assay of Three New Mercurial Diuretic Agents: A Promising Preparation for Oral Use.* (19541)

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A method for the bioassay of diuretic agents in ambulant patients with congestive failure

* This investigation was supported (in part) by a research grant from the National Heart Institute of the National Institutes of Health, U. S. P. H. S., and by the Diuretic Fund of Department of Pharmacology, Cornell University Medical College.

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was developed in our clinics(1). This method was employed in the present study. The plan provides for the comparison of the Unknown with the Solution of Mercuhydrin Sodium (meralluride sodium) by intramuscular injection, which we have adopted as the "Standard." The measure of response is the loss of body weight 24 hours after the dose. From dose-response curves for the Unknown and the Standard, the potency of the Unknown is determined. The assay is so designed as to supply data suitable for statistical analysis, and the precision of the difference in potency between the Unknown and the Standard is expressed in terms of confidence limits. This method has provided an efficient tool for the screening of new diuretic agents and for the investigation of their relative efficacy by various routes of administration.

In the present study, this method was used in the screening of 3 organic mercurial compounds which displayed diuretic action in laboratory animals(2-4). In addition to the diuretic activity, observations for possible toxicity were made: gastrointestinal effects; local irritant action; a complete blood count before the first dose and within 2 weeks after the last dose; similarly for blood urea nitrogen; urine examined during the action of each of the large doses, as well as before and within 2 weeks after the study. The results form the subject of this report.

Preparations. There were 4 preparations. The Standard was the injectable solution of Mercuhydrin sodium of commerce as supplied in sterile multiple-dose vials of 10 cc each. Of this solution, each cc is stated(5) to contain 123 mg of meralluride sodium and 9.4 mg of free theophylline. Two of the new agents were thiol compounds. Preparation "E" (3-carboxymethylmercaptomercuri-2-methoxy propylurea) was supplied in tablets containing 20 mg for oral administration. Preparation "F" (3(α-carboxyethylmercaptomercuri)-2-methoxy propylurea) was supplied in a sterile vial containing 439 mg of powder which we dissolved in 10 cc of water. This was used for intramuscular injections which were made within the hour. Of this solution, each cc represented 43.9 mg of the compound. This material was also supplied in tablets for oral administration, each containing 21.75 mg. Preparation "D" (3-chloromercuri-2-methoxy propylurea) represents meralluride from which the succinyl group was removed from one end and the theophylline from the other. It was supplied for intramuscular injection in the form of sterile ampules containing 2 cc of solution, each cc of which is stated to contain 23.85 mg of the compound. It was also supplied in the form of tablets for oral administration, each tablet stated to contain 18.35 mg of the drug. The tablets were always given at one time, in single doses of from 1 to 9 tablets. The comparisons as shown in the charts were made in terms of molecular weight, but since a molecule of each compound contains 1 atom of mercury, the values for relative potency as expressed in millimols of the compound or in milligrams of mercury are identical. It may be mentioned parenthetically that the significance of the common expression of relative potency of organic mercurials in terms of mercury content is open to question.

Results. None of the compounds in these assays produced changes in the blood count, blood urea nitrogen, or urine. They all caused gastrointestinal symptoms by oral administration and the incidence of these in relation to dosage and diuretic effectiveness is described below. When the interpretation of the weight loss was complicated by vomiting or watery diarrhea, the values in such cases were excluded from the calculation of potency.

An attempt to assay preparation "E" proved unsuccessful. Thirty-three patients received a total of 67 oral doses ranging from 20 to 100 mg. In the group of 21 patients who received the 60 mg dose, 48% developed in a period of hours, one or more of the following gastrointestinal symptoms: nausea, abdominal cramps, diarrhea, and in one case bloody stools. The diuretic effect was negligible, average weight loss of 0.12 lb 24 hours after the dose. With the 100 mg dose in 11 patients, 64% developed the foregoing symptoms, in one instance also vomiting. Again, the diuretic response was negligible, average weight loss of 0.18 lb in 24 hours. The possibility of delayed diuretic response was examined in a group of 10 patients (12 doses)

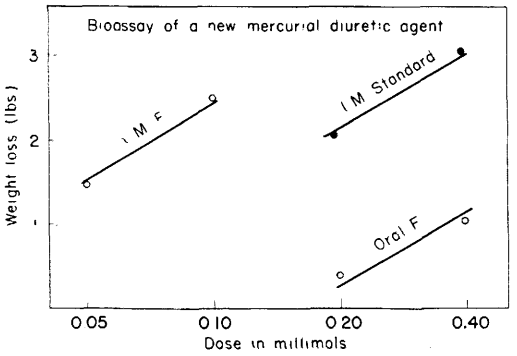

FIG. 1. Comparison of 3(α-carboxyethylmercaptomercuri)-2-methoxy propylurea by oral and intramuscular administration with the Standard, intramuscular mercuhydrin.

who were weighed 24 hours and again 48 hours after doses of 60, 80, and 100 mg. No delayed action was in evidence; their average weight loss in 24 hours was 0.35 lb, and in 48 hours the average weight returned to a level above that before the dose. "E" was manifestly unsuitable as an oral diuretic.

Fig. 1 shows the potency of preparation "F" by the intramuscular and by the oral route, determined in one assay. The 3 curves represent the same 37 patients, and each is based on 74 doses. The intramuscular doses were 0.5 cc (21.95 mg) and 1.0 cc (43.9 mg). The doses of the Standard (meralluride by intramuscular injection) were 1 cc (123 mg) and 2 cc (246 mg). It may be seen that by the intramuscular route, "F" has 2.5 times the diuretic potency of the Standard in terms of millimols and approximately 3.5 times in terms of milligrams of the compounds.

In the case of "F" by the oral route, patients received single doses ranging from 21.75 to 174 mg. Fig. 1 shows that "F" by the oral route has 9.2% of its potency by the intramuscular route, or conversely it takes approximately 11 times as much "F" by the oral as by the intramuscular route to produce the same diuretic effect. If it may be assumed, as has been shown in the case of other mercurial agents(6,7) that by the intramuscular route absorption is nearly complete, there is indication that only about 9% of a dose is absorbed from the gastrointestinal tract (Table I). The largest oral doses, 174 mg, which gave rise to gastrointestinal symptoms of the type listed above after one-fourth of the number of such doses given, yielded a small diuretic effect equivalent to that resulting from less than 0.5 cc (61.5 mg) of the Standard (meralluride by intramuscular injection). As in the case of preparation "E", there was no evidence of delayed diuretic response; 21 patients who received the 174 mg dose showed an average weight loss of 0.8 lb in 24 hours and in 48 hours a return to an average of 0.45 lb below the control weight. Preparation "F" did not appear to be especially promising as a diuretic agent for oral administration.

Fig. 2 shows the results of a preliminary assay of preparation "D" by oral administration. There were 3 dose levels, 73.4, 110.1, and 165.15 mg. The oral administration shows a potency of 70.7% of the Standard in terms of millimols and 122% in terms of milligrams of the compounds. At the level of dosage, 110.1 mg (6 tablets), a fairly good diuretic effect was obtained, equivalent to the effect of 1.1 cc of mercuhydrin in this group of patients. This dose produced gastrointestinal symptoms after 30% of the number of such doses given. These results may be compared to those of preparation "F" (see above) with which a dose causing an approximately similar incidence of local toxicity produced a diuretic effect half as much. As in the case of the other two compounds, preparation "D" also failed to show any diuretic action de-

TABLE I. Potency and Precision of the Assays.*

Route	Potency† expressed as % of: Mercuhydrin i.m. as standard	Particular unknown i.m. as standard	Confidence limits at P = .05
3 (α-carboxyethylmercaptomercuri)-2-methoxy propylurea			
Intramusc.	252.2		142.9 & 364.4
Oral	23.3		7.1 " 39.8
"		9.2	3.8 " 14.3
3-chloromercuri-2-methoxy propylurea			
Intramusc.	249.7		187.5 " 321
Oral‡	71.0		57.6 " 87.4
"		28.5	21.6 " 36.7

* Calculations according to C. I. Bliss, Statistical Methods in Vitamin Research, Academic Press, New York, 1951.

† Molecular terms.

‡ Combination of two assays.

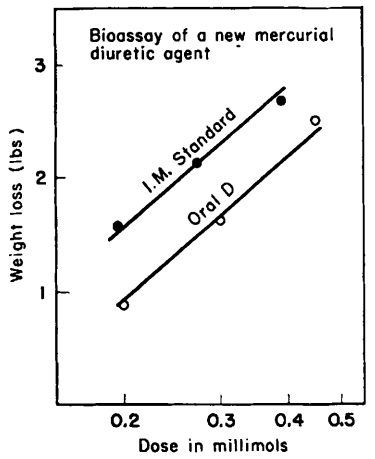

FIG. 2. Comparison of 3-chloromercuri-2-methoxy propylurea by oral administration with the Standard, intramuscular mercuhydrin.

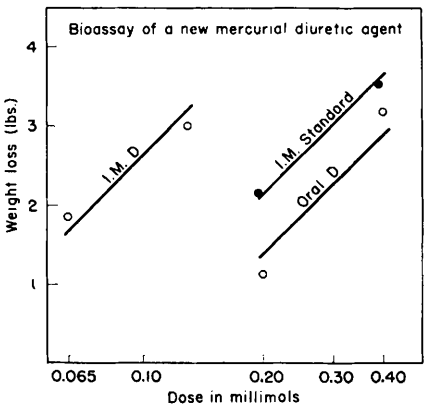

FIG. 3. Comparison of 3-chloromercuri-2-methoxy propylurea by oral and intramuscular administration with the Standard, intramuscular mercuhydrin.

veloping after the 24-hour period; 23 patients who received 33 doses of from 55.05 to 165.15 mg by the oral route showed an average weight loss of 2.2 lb at the 24-hour weighing and an average gain of 0.3 lb above this at the 48-hour weighing. Preparation "D" clearly presented more favorable properties than either "E" or "F". Since the assay shown in Fig. 2 was made with only 15 patients, a more definitive assay was carried out. The results are shown in Fig. 3. The 2-dose method was used. The same 27 patients are represented in each of the three curves, and each is based on 54 doses. The potency of preparation "D" by the oral route in this larger assay confirms the value established in the preliminary assay, 71.2% of that of the Standard (by intramuscular injection). Preparation "D" by the oral route has 28.5% its potency by the intramuscular route, and again, on the basis of fairly complete absorption from the intramuscular injection, this value would indicate the extent of its absorption from the gastrointestinal tract.

Preparation "D" is a much more potent material than meralluride as shown in the comparison in which both were given by intramuscular injection. The curve represents "D" in doses of 23.85 mg and 47.7 mg respectively. It may be seen that preparation "D" has 2.5 times the potency of the Standard in terms of millimols and 4.3 times in terms of milligrams of the compounds (Table I).

It may be mentioned that, while the first two preparations which were thiol compounds were relatively painless by intramuscular injection, preparation "D" caused considerable local discomfort and pain in the muscle area of injection.

The data on gastrointestinal symptoms produced by preparation "D" have been assembled in Table II. The general pattern of the response of patients to the local irritant action in the gastrointestinal tract was substantially similar for all three compounds. There are marked variations in susceptibility to the irritant action in the gastrointestinal tract in the same patient at different times, and in different patients. A small dose sometimes produced these unpleasant symptoms in a particular patient who at other times tolerated well more than 4 times as much. As may be seen in Table II, about one-half of the patients tolerated without gastrointestinal symptoms a dose which was more than 4 times as large as the one which in others produced gastrointestinal irritation. Nevertheless, there is a general dosage-response trend with respect to this effect: When expressed in terms of patients, the incidence of gastrointestinal reactions rose from 18 to 64% as the dose was gradually increased through a 4-fold range; when expressed in terms of doses, an essentially similar trend was in evidence.

Comment and Conclusions. Three organic mercurial preparations possessing diuretic ac-

TABLE II. Gastrointestinal Irritation after Oral Administration of 3-chloromercuri-2-methoxy propylurea.

	Doses (mg)					
	36.7	55.05	73.4	110.1	146.8	165.15
No. of patients who received dose	14	3	38	15	32	16
% of patients who developed symptoms*	18	100	25	37	64	53
No. of doses administered	15	3	55	20	36	21
Types of gastrointestinal toxicity						
Nausea	1		3		5	
Abdominal cramps		1	3	2	3	4
Vomiting	2	1	1		9	1
Diarrhea	1	1	4	4	5	5
% of doses causing gastrointestinal toxicity	27	100	20	30	61	48

* Where a patient received a particular dose twice, but developed gastrointestinal symptoms after only one of them, it was counted as ½.

tivity were assayed by the oral route against the Standard, mercuhydrin solution given intramuscularly, in patients with congestive failure. Of the three materials, the 3-chloromercuri-2-methoxy propylurea proved to be the most effective, producing with oral doses a diuretic response equivalent to results obtained by the conventional doses of intramuscular mercuhydrin. The diuretic potency of this compound when given orally is somewhat more than one-fourth of its potency by intramuscular injection, and by the latter route 4.3 times (in milligrams) as potent as intramuscular mercuhydrin. We are not aware of any mercurial diuretic with such a favorable ratio of intramuscular to oral potency, namely 4:1. In the case of the thiol compound we tested in this study, the ratio was 11.1. In another study(7) in which mercuhydrin was tested by the intramuscular and oral routes, this ratio was 24:1. There still remains the problem of gastrointestinal irritation. By the method employed in the bioassay, it was necessary to give the total dose at one time, in the case of the larger doses as many as 9 tablets. This, therefore, subjected the local irritant action to a rigorous test. As the results stand, it appears that approximately one-half of the population with congestive heart failure might be able to tolerate by the oral route doses of this compound which produce highly effective diuretic responses. This is as far as the investigation in clinical pharmacology has carried the problem. It is now necessary to establish the most satisfactory dosage plans for the use of this material by the oral route. If approximately one-half of the population can tolerate as many as 9 tablets given at one time without gastrointestinal distress, it may well turn out that by dividing this amount into several fractions taken at intervals during the day, satisfactory diuretic effects may be obtained with less interference from gastrointestinal symptoms. The protracted use of the material over periods of weeks and months may disclose other problems which are not revealed by the single dose bioassay method. It remains for clinical trials to decide these matters.

The authors are indebted to Mrs. Rita Simpson and Miss Susan Otto for technical assistance.

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Received April 29, 1952. P.S.E.B.M., 1952, v80.