Chromatography Studies of the Excretion Products after Meralluride Administration in Normal Subjects and Cardiac Patients

By John H. Moyer, M.D., Richard A. Seibert, Ph.D. and Carroll A. Handley, Ph.D.

Separation of the urinary excretory products of the organomercurial diuretic, meralluride (Mercuhydrin) has been possible using adsorption chromatography. Practically all of the mercury is excreted in a form closely resembling the administered meralluride. Most of the excretion occurs within the first 6 hours in both cardiac patients and normal subjects. The amount of mercury excreted as degradation products is insufficient to produce diuresis. Therefore, it is concluded that the diuretic action of meralluride depends on the specific organic mercurial molecule.

One concept for the diuretic action of organomercurial compounds is that they release inorganic mercury in the body. The inorganic mercury is thought to inhibit the renal tubular enzymes which are responsible for sodium and chloride reabsorption. Although inorganic mercury compounds, such as bichloride of mercury, do produce diuresis, they are less potent than organomercurial diuretics. The fact that all organomercurial compounds do not have diuretic action suggests that a specific organomercurial configuration is necessary. Furthermore, differences in the potencies of the various organomercurials indicate that their diuretic potency is not related to their capacity to release inorganic mercury but, instead, is inherent in the organomercurial molecule. Kessler and Pitts1 have shown that the mercury in the organomercurials is rapidly cleared from the blood and eliminated, whereas the nondiuretic organomercurials are slowly excreted. More direct evidence was presented by Weiner and Müller2 when they failed to demonstrate, by polarography, inorganic mercury in the urine during mercurial diuresis. These investigators found that mersalyl (Salyrgan) was mainly excreted as a mersalyl cysteine complex. They calculated that less than 12.5 per cent of the injected mercury was excreted in a significantly altered form.

In the current investigation meralluride (Mercuhydrin), a sodium salt of a carboxylic acid, was found to be selectively adsorbed by passing it through a chromatography column of acid aluminum oxide, an anionotropic adsorbent. Inorganic mercury and noncarboxylic mercury compounds were not adsorbed. Therefore, using meralluride as the organomercurial diuretic, the form and rate of mercury excreted in the urine were studied in dogs3 (fig. 1). The current report presents similar observations made on normal subjects and patients with heart failure.

Method

The adsorbent material for the chromatography column was selected after extensive trials of the known anionotropic adsorbents. Acid aluminum oxide was found to adsorb meralluride quantitatively when the latter was passed through the column. The column was prepared by packing about 5 Gm. of acid aluminum oxide into a 1.2 x 18 cm. glass column. Glass wool was used to retain the adsorbent. The solutions, containing the mercurial compounds to be separated, were passed through the column with the aid of slight suction. Twenty milliliters of distilled water were passed through the column to quantitatively remove the nonadsorbed fraction (fraction B) of mercury (fig. 2). To elute the adsorbed mercury compound, 10 ml. of 5 per cent sodium carbonate followed by 25 ml. of distilled water were passed through the column (fraction E). These two fractions, as well as a sample of the untreated urine (unchromatographed urine), were analyzed for mercury content according to the method of Laug and Nelson.4 The sum of the mercury in the two fractions should approximate closely the total mercury in the urine (unchromatographed urine). In order to check the accuracy of the method, samples containing known amounts of inorganic mercury and meralluride were passed
CHROMATOGRAPHY STUDIES OF MERALLURIDE EXCRETION

494 CHROMATOGRAPHY STUDIES OF MERALLURIDE EXCRETION

Mercury From Degradation Products of Meralluride

Mercury

Meralluride

0 1 2 3 4 5 6 7

Hours

Mercury Excretion Rate Over 6 Hour Period

Ordinate, per cent of meralluride recovered in urine.


through the column. It was observed that each fraction could be separated quantitatively.

Two groups of individuals who received meralluride were studied. The first group was composed of normal subjects without evidence of heart failure or renal disease. The meralluride was administered intravenously, intramuscularly, and subcutaneously in 2 ml. doses which are equivalent to 78 mg. of meralluride Hg. There were 6 subjects in this group. The urine was collected in 6 hour fractions for a total of 24 hours following the administration of the meralluride. All 6 of the subjects were given meralluride intravenously and intramuscularly, but only 4 were given the drug subcutaneously.

The second group of individuals who received meralluride consisted of 9 patients with cardiac failure who were responsive to diuretics. The etiology of the heart failure in all of the patients was arteriosclerotic heart disease. Five patients received the meralluride intravenously and 6 received it intramuscularly.

Prior to administration of the meralluride, both to the noncardiac and to the cardiac patients, two 6 hour control specimens of urine were collected. The volume of urine during these control periods as well as the excretory rates of sodium, potassium, and mercury (if any) were measured.

After the meralluride was administered (2 ml. = 78 mg. meralluride Hg), the urine was collected at 6 hour intervals for the next 24 hours. Each specimen was passed through the previously described chromatography column and the fractions were analyzed for mercury. Urinary sodium and potassium excretion rates were determined. The serum electrolytes (sodium, potassium and chloride) were found to be within normal limits in all of the patients studied.

RESULTS

Noncardiac subjects rapidly excreted the drug predominantly in a form adsorbed by the column. About 68 per cent was excreted in the adsorbable form (meralluride mercury) in the first 6 hours when given by the intravenous route and 63 per cent and 64 per cent after the intramuscular and subcutaneous routes respectively. By the end of 24 hours, 85 per cent had been excreted when the drug was given intravenously and 81 per cent and 82 per cent when the drug was given intramuscularly or subcutaneously (fig. 2). Of the mercury recovered, less than 3 per cent was in the degraded (non-meralluride Hg) form following administration by any route. Although the amounts are too small to be significantly different, it appears that the major part of the degraded form was also excreted during the first 6 hours.

Figure 3 compares the sodium and potassium excretion rates in total milliequivalents with the mercury excretion in milligrams during successive 6 hour periods following administration of meralluride. Since the different routes of injection show such similar patterns of excretion, only the intravenous route is presented graphically. During the first 6 hours 53 mg. of mercury was excreted in the adsorbable form (meralluride Hg). During the same period, 168 mEq. of sodium were excreted as compared to only 44 mEq. during the 6 hour period immediately preceding the administration of meralluride. Thus, the period of maximum sodium, water and mercury excretion coincided, and the curves for sodium and mercury elimination paralleled each other throughout the 24 hours. By the end of 18 hours, the sodium excretion fell below the control values and remained there for at least the next 6 hours. Potassium excretion was not changed significantly throughout the 24 hour period. Mercury in the degraded meralluride fraction (fraction B) comprised an insignificant amount, being less than 1 mg. in any one period which is insufficient to produce diuresis.

In the cardiac patients responding to meralluride, and in the noncardiac subjects, mercury was excreted in a qualitatively similar
Moyer, Seibert and Handley 495

Meralluride Given: Intravenously, Intramuscularly, Subcutaneously

Degradation Products 6, 12, 18 Hours After Meralluride Given

HOURS AFTER MERRALLURIDE GIVEN

Fig. 2 Top. Total meralluride mercury recovered in the urine compared to nonmeralluride mercury (degradation products) excretion rate in normal subjects (mean values) comparing three routes of administration.

Fig. 3 Bottom. Comparison of the excretion rates of meralluride mercury and its degradation products of meralluride with sodium (A) and potassium excretion (C) during successive 6 hour periods. Maximum increase in sodium excretion simultaneous with maximum excretion of meralluride mercury (B). Degradation products (D) of meralluride in urine, insignificant.

Manner. Here again, the maximum period of meralluride mercury excretion occurred during the first 6 hours following drug administration but the amount ranged from 29 mg. (37 per cent) to 45 mg. (58 per cent) after intravenous injection and from 13 (17 per cent) to 42 mg. (54 per cent) after intramuscular injection. Excretion rate following intravenous administration was approximately two times greater during the second 6 hour period in the cardiac patients than it was in the noncardiac subjects. After 24 hours, the total mercury excreted, which ranged from 80 to 99 per cent of the injected mercury, was about equal in the normal subjects and in the responsive cardiac patients. As in the normals, an insig-

Fig. 4 Top. Total mercury excretion in a patient with heart failure comparing intravenous and intramuscular administration. Excretion rate less rapid initially following intramuscular route but the total excretion about the same. Excretion of degradation products less than 5 per cent of the excreted mercury.

Fig. 5 Bottom. Excretion rates of sodium and potassium compared to those of meralluride during successive 6 hour periods following the administration of 2 ml. (78 mg. Hg) of meralluride to patients in heart failure. Maximum excretion rate of meralluride mercury coincided with that of sodium following both routes of administration.

cant amount (less than 3 per cent) was excreted as degradation products (fig. 4). In 48 hours or less, the excretion of mercury was complete in all patients.

In figure 5 the sodium excretion rate in total milliequivalents when meralluride was given to the cardiac patients is summarized. When given intravenously there was a rather marked rise in sodium excretion which remained high for 18 hours but the maximum was during the first 6 hours. After intramuscular injection, there was a marked rise in sodium output also and again the maximum rate was during the first 6 hours. There was no significant effect on potassium excretion. The mercury excretion paralleled sodium excretion just as it did in the normal subjects.
Discussion

It appears from the data shown here that meralluride passes through the body, exerts its effect on the renal tubules, and appears in the urine in a form quite similar to the injected drug. By utilizing the acid adsorbent chromatography column, we have been able to separate the urine mercury into two fractions. Mercury in the degraded meralluride fraction comprised an insignificant amount, being less than 1 mg, which is insufficient to produce diuresis in any one period. The exact nature of the two fractions is unknown. Evidence from paper chromatography as well as from polarographic studies by others, indicates that even the degraded form is still an organic radical. After 6 hours about 60 to 70 per cent of the administered mercury is excreted following either the intramuscular or the intravenous route of drug administration in normal subjects. In the cardiac patients, mercury was excreted in a manner qualitatively similar to that of the normal subjects. There appeared to be a moderate delay in the cardiac patients following intramuscular administration; this was probably due to delayed adsorption in patients with an abnormal circulation and a slight increase in tissue fluid.

Burch and his associates, using radioactive mercury, found that in normal subjects excretion is almost complete in 24 hours, but takes longer in cardiac patients. He states that this could lead to cumulative toxic effects. In our series we found negligible amounts in the urine 48 hours after injection, and total mercury recovery was essentially complete during this time interval. Studies on a large number of patients receiving mercurial therapy for prolonged periods failed to show any indication of accumulation of mercury.

Summary

A method for quantitatively separating the excretory products of meralluride has been described. It has been demonstrated by this method that practically all the mercury is excreted in a form closely resembling the administered drug. The method involves the use of adsorption chromatography which separates the excretory products into two fractions. One fraction, which represents by far the larger amount, is strongly adsorbed by the column. The other fraction is readily washed through the column by distilled water. The majority of the mercury is excreted within the first 6 hours in both cardiac and normal patients as well as in laboratory animals. The total amount of mercury excreted in 24 hours was similar in noncardiac and cardiac patients and was 80 to 90 per cent of that injected. In all subjects the mercury in the degradation products was of insufficient quantity to produce the diuresis exhibited. Therefore, it seems evident that inorganic mercury could not produce the diuresis. Instead, it seems quite likely that the diuretic action depends on the organic mercurial molecule.

Summario in Interlingua

Es describite un metodo pro le separation quantitativa del productos excretori de merallurido. Le metodo ha demonstrate que practicamente omne le mercurio es excernite in un forma multo simile al droga administrate. Le metodo se basa super le uso de chromatographia absorptional que separa le productos excretori in duo fractiones. Un de iste fractiones, le qual representa per multo le plus grande quantitate, es fortemente absorbite per le columna. Le altere fraction es prestemente lavate a transverso le columna per aqua distillate. Le plus grande portion del mercurio es excernite intra le prime sex horas tanto in subjectos normal e cardiac como etiam in animales de laboratorio. Le excretion total de mercurio intra 24 horas eseva simile in subjectos non-cardiac e in patientes cardiac. Ilo amontava a 80 a 90 pro cento del quantitate injicite. In omne le subjectos studiate le mercurio in le productos de degradation eseva de quantitates insufficiente pro producer le diuresi que eseva presente. Ergo, il pare evidente que mercurio inorganic non poteva producer le diuresi. Il es plus tosto satis probable que le action diuretic depende del organic molecule mercurial.
REFERENCES


Chromatography Studies of the Excretion Products after Meralluride Administration in Normal Subjects and Cardiac Patients

JOHN H. MOYER, RICHARD A. SEIBERT and CARROLL A. HANDLEY

Circ Res. 1957;5:493-497
doi: 10.1161/01.RES.5.5.493

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/5/5/493