# THE COMBINATION OF MERCURIAL DIURETICS WITH DIMERCAPROL (2:3-DIMERCAPTOPROPANOL); THE EFFECT ON DIURETIC ACTIVITY AND TOXICITY

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NOTE ON THE PREPARATION OF THE COMPOUNDS OF MERCURIAL DIURETICS WITH 2:3-DIMERCAPTOPROPANOL (DIMERCAPROL)

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Received July 16, 1952

RECORDS of the diuretic action of mercury compounds go back as far as the sixteenth century. Paracelsus reported the use of mercurous chloride as a diuretic and there are many references to the combined use of digitalis and mercurous chloride, but because of the occurrence of untoward toxic effects, the use of inorganic mercury compounds tended to fall into disrepute. The accidental discovery of the diuretic properties of novarsarol (a double salt of sodium mercurichlorophenyl oxyacetate with diethylbarbituric acid) introduced as an antisiphylitic agent, led to an extensive search for less toxic organic mercurials. As a result, a number of organic derivatives of mercury have been introduced as diuretics and are used in cases of œdema due to heart, kidney and liver disease, although, because of the risk of toxic effects on the cardio-vascular system, preliminary tests of the susceptibility of the patient and caution in their use are recommended. Also, because of their selective action on the kidney, these compounds cannot be used in cases of advanced chronic nephritis and acute renal disease. One of the organic mercurials introduced recently in the United States by Lehman is thiomerin, the disodium salt of $N(\gamma$-carboxymethylmercaptomercuri-$\beta$-methoxy)-propyl-camphoramic acid. This compound differs in that the organic mercurial has been combined with a monothiol derivative and it has been shown in animal experiments to be considerably less toxic to the heart than the commonly used mercurial diuretics (Lehman[1]). Clinical trials showed it to be well tolerated, producing much less local irritation at the injection site than other organic mercury compounds and to be well absorbed from subcutaneous injection, the diuretic response after administration in this way being similar to that produced by equivalent doses of other mercurial diuretics given intravenously. The nephrotoxicity of the mercury is not, however, eliminated and the use of thiomerin is still contra-indicated in cases of advanced chronic nephritis and acute renal disease.

In view of these results with the combination of an organic mercurial with a monothiol, it was thought to be of interest to study similar products

of the combination with a dithiol derivative, such as dimercaprol to see if the reduction in toxicity is maintained or even further reduced, and also whether some reduction of the renal toxicity could be achieved.

Two such compounds have been prepared by Mr. Sharp of the Wellcome Laboratories of Tropical Medicine. They are the product of combination of two molecules of mersalyl and one molecule of dimercaprol, which has been given the name "balmersal" and the disodium salt of 2:3-bis-(3'-camphoramido-2'-methoxypropylmercurimercapto)-propan-1-ol or "balmercamph," the product of dimercaprol and the organic mercurial used in thiomerin.

In spite of a large amount of work on the subject, evidence on how the mercury compounds produce their diuretic effect is conflicting. The two fundamentally different points of view, one, that the diuretic effect is primarily extra-renal, the mercury producing a dilution of the blood which acts as the stimulus for diuresis (Jendrassik,[2,3] Saxl and Heilig[4,5]), and two, that the effect is a direct one due to the action of the mercurial on the kidney (Govaerts,[6] Bryan, Evans, Fulton and Stead,[7] Gremels[8]) have still to be reconciled, and studies of the behaviour of the mercurials with dimercaprol have been made in the hope of contributing to this problem.

## Experimental

*Diuretic activity in rats.* A qualitative comparison of the diuretic activity of the compounds was made in rats. Cross-over tests were carried out on groups of 4 rats, each weighing about 200 g., the animals being selected so that the total weights of the groups were as nearly equal as possible. The animals were starved overnight and moderate hydration with 5 ml./100 g. of warm water preceded treatment. The compounds were given by intramuscular injection in doses equivalent to 8 mg. of Hg./kg., balmersal and balmercamph being given as solutions in distilled water and mersalyl and thiomerin as suitable dilutions of the commercially prepared injections with distilled water. A control group of animals for each test group was injected with a similar volume of saline and after 1 week's rest the control and test groups were reversed. The volume of urine excreted every 15 minutes from the appearance of the first drop was noted for the first 6 hours and also the total volume excreted in 24 hours. The animals were kept at a temperature of 18° to 22° C. to minimise as far as possible variations in renal flow due to temperature. Figure 1, giving the mean results of 4 such cross-over tests, shows that combination with dimercaprol does not affect the diuretic activity of mersalyl, balmersal being at least as active as mersalyl at this dose. Although there was some increase in urinary excretion in the first 6 hours in most of the groups, particularly from 4 to 6 hours, these differences were variable and the significant increase constituting the real diuretic effect occurred between 6 and 24 hours. These results are similar to those of Dicker[9] and Lipschitz, Hadidian and Kerpscar,[10] who reported a diuretic effect in rats about 10 hours after intramuscular injection of the mercurial compounds. Under the conditions of the test neither thiomerin nor balmercamph show definite diuretic activity.

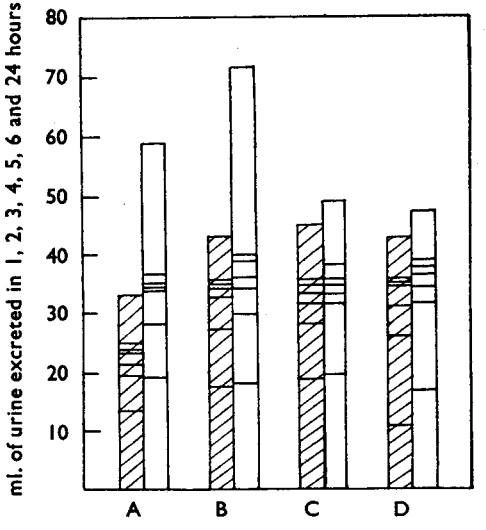


FIG. 1. Comparison of the diuretic activity of mersalyl, balmersal, thiomerin and balmercamph in rats. The results are the mean of 4 cross-over test with each compound. Cross hatch, control; open, test period.

Dose:—Equivalent of 8 mg. of Hg/kg. by intramuscular injection.

A. Mersalyl. B. Balmersal. C. Thiomerin. D. Balmercamph.

The dose range over which mercury compounds show diuretic activity in rats is a narrow one and in order to determine the effect of combination with dimercaprol on this range and to obtain a more quantitative comparison of the activity of the compounds, a modification of the method of bioassay of diuretics described by Lipschitz *et al*[10] was employed. Groups of 8 rats, of approximately equal weight were used, and hydrated with 2·5 ml./100 g. of saline solution instead of water. The volume of urine excreted in 24 hours after treatment was noted and the excretion expressed as a percentage of the liquid administered. The excretion was compared with that produced by a standard dose of urea. The dose of 25 millimols/kg., was selected since it produced an approximately similar diuretic effect. The "diuretic activity" was calculated as the difference between the logs of the excretion and the urea excretion. The curve relating the log dose to diuretic activity is shown in Figure 2. Diuretic activity increases with increasing dosage of mersalyl to a maximum at the dose corresponding to 8 mg. of Hg./kg. above which it rapidly decreases. With the dose 32 mg. of Hg./kg. complete oliguria occurred in some cases and all the animals treated with this dose died within a few days. The diuretic activity of balmersal is similar to that of mersalyl up to a dose equivalent to 8 mg. of Hg./kg. but the maximum activity occurs at 16 mg. of Hg./kg. and there is still activity at 32 mg. of Hg./kg. None of the animals treated with the latter dose died. The curve for thiomerin is similar, though the activity is lower than that shown by the other two compounds, the maximum activity shown by the dose corresponding to 16 mg. of Hg./kg. being less than the maximum for both mersalyl and balmersal. Diuretic

activity disappears at the dose corresponding to 32 mg. of Hg./kg. This dose was also toxic, all the animals dying in 3 days. No significant diuretic activity was obtained with balmercamph.

*Diuretic activity in dogs.* The activity of mercurials is potentiated by certain other drugs, particularly acidifying salts such as ammonium chloride (Keith, Barrier and Whelan[11]). The effect of the combination of the mercurials with dimercaprol on this property was studied in dogs.

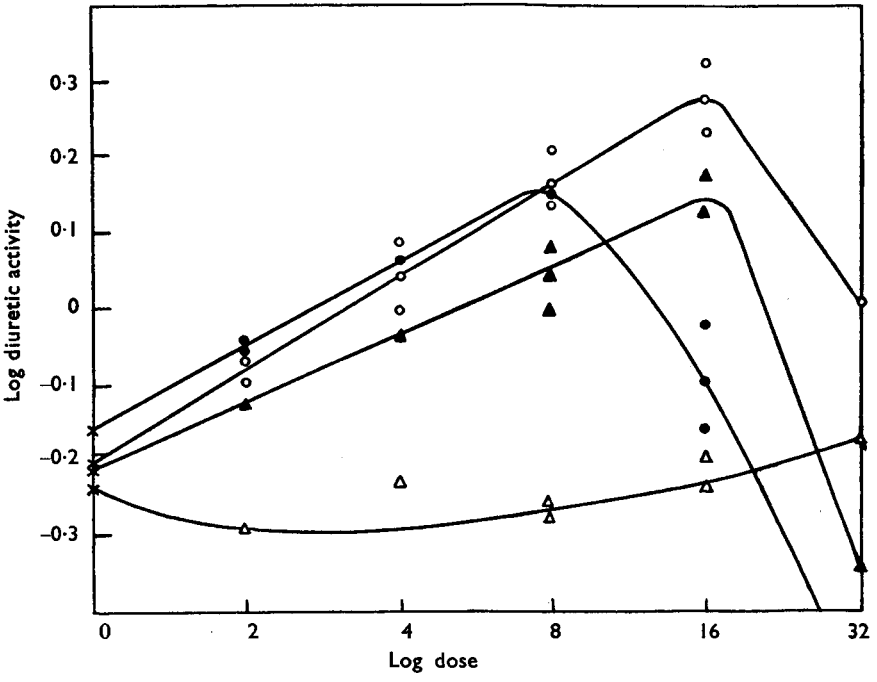


FIG. 2. Dose—response curves for diuretic activity of the 4 compounds in rats. The diuretic activity is measured as the difference between the logs of the excretion and the excretion after the standard dose of urea. × controls, ●—● mersalyl, ○——○ balmersal, ▲——▲ thiomerin, △——△ balmercamph.

The mercurial diuretics have been shown to be active in normal (Schloss[12]) and sometimes even in dehydrated dogs (Roby and Pfeiffer[13]). To ensure standard conditions the animals were deprived of food and water for 18 hours and then hydrated immediately before treatment with approximately 50 ml./kg. of milk and water. Bitches weighing approximately 15 kg. were used and they were catheterised immediately before, and at 2, 4, 6, and 24 hours after hydration, the volume of urine obtained at each period being noted. The excretion was expressed as a percentage of the volume of liquid administered. This constituted a control excretion period for each dog. After 2 days rest, the experiment was repeated, an intramuscular injection of the mercurial compound in a dose equivalent to 1·2 mg. of Hg./kg. being given immediately after hydration. After a further 3 days rest, the control and test periods were repeated, ammonium

chloride, 100 mg./kg., being given orally at the same time as the milk and water and also during the 2 days rest between the two periods. The results are given in Figures 3 and 4 and the diuretic effect calculated as the difference in percentage excretion between the test and the control period is given in Table I. A definite diuretic response was obtained with mersalyl, the onset of diuresis occurring between 2 and 4 hours and still

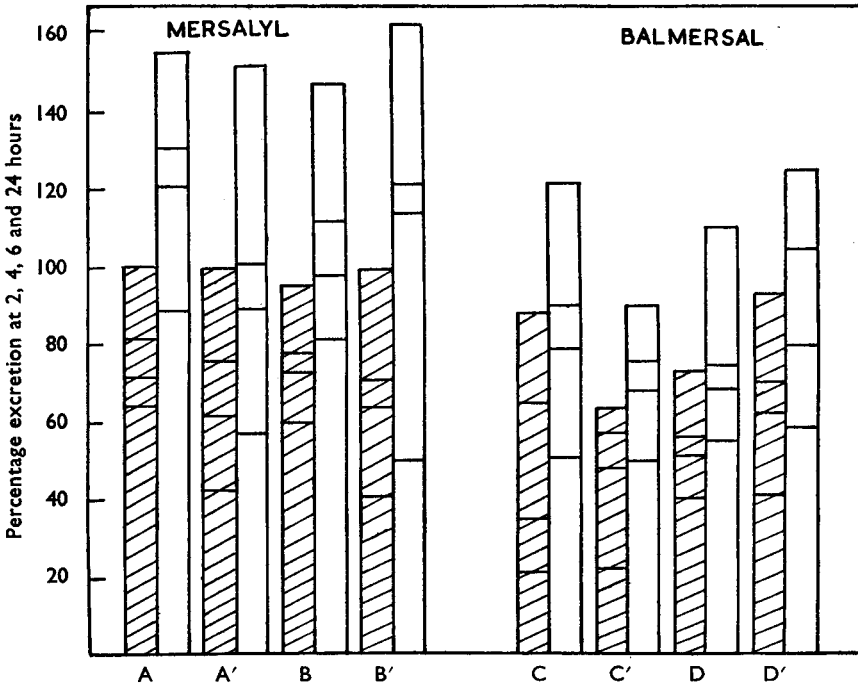


FIG. 3. Diuretic effect of mersalyl and balmersal in dogs, with a dose equivalent to 1·2 mg. of Hg./kg. given alone and after premedication with ammonium chloride. The excretion measured as the percentage of the liquid administered, is shown at 2, 4, 6 and 24 hours. Cross hatch, control; open, test period.

A. Dog 16. Mersalyl alone.
A′. ,, Mersalyl and ammonium chloride.
B. Dog. 14. Mersalyl alone.
B′. ,, Mersalyl and ammonium chloride.
C. Dog 16. Balmersal alone.
C′. ,, Balmersal and ammonium chloride.
D. Dog 30. Balmersal alone.
D′. ,, Balmersal and ammonium chloride.

being well marked in the 6 to 24 hour period. An overall increased effect was obtained when treatment was accompanied by premedication with ammonium chloride. The diuretic response with balmersal was similar although somewhat smaller than that obtained with mersalyl. The effect does not appear to be potentiated by simultaneous treatment with ammonium chloride. In one case (dog 43), a definite diuretic response was obtained with thiomerin, similar in magnitude to that obtained with an equivalent dose of mersalyl and the effect was increased to about the

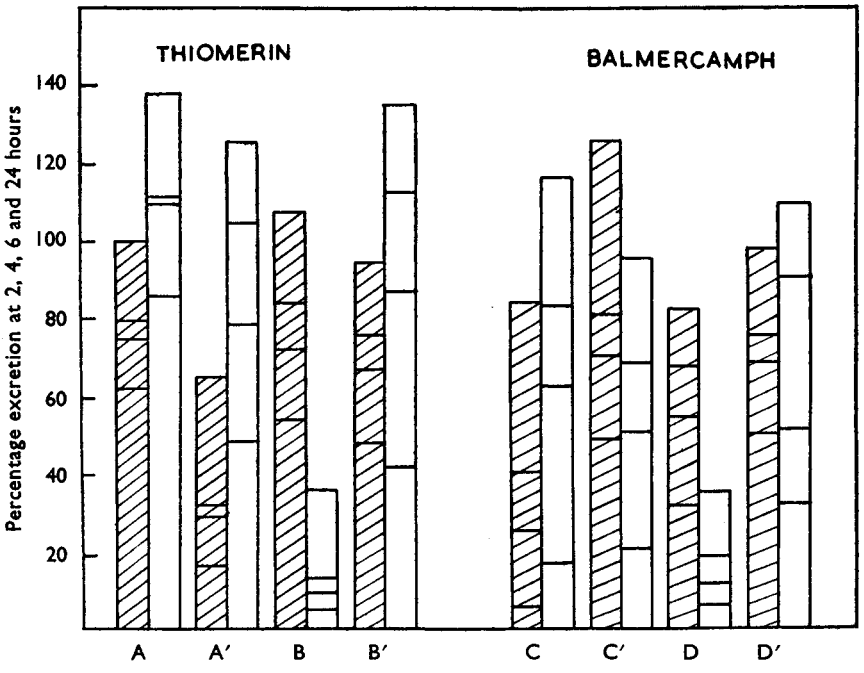


FIG. 4. Diuretic effect of thiomerin and balmercamph in dogs with a dose equivalent to 1·2 mg. of Hg./kg. given alone and after premedication with ammonium chloride. The percentage excretion at 2, 4, 6 and 24 hours is shown. Cross hatch, control; open, test period.

A. Dog 43. Thiomerin alone.
A′. ,, Thiomerin and ammonium chloride.
B. Dog 45. Thiomerin alone.
B′. ,, Thiomerin and ammonium chloride.
C. Dog 14. Balmercamph alone.
C′. ,, Balmercamph and ammonium chloride.
D. Dog 45. Balmercamph alone.
D′. ,, Balmercamph and ammonium chloride.

TABLE I

DIURETIC EFFECT IN DOGS AFTER A DOSE CORRESPONDING TO 1·2 MG. OF HG/KG. BY INTRAMUSCULAR INJECTION

| Compound | Dog number | Diuretic effect expressed as difference in percentage excretion for control and test period | |
|---|---|---|---|
| | | Drug alone | Drug + ammonium chloride |
| Mersalyl .. .. | 16 | 49 | 66 |
| | 14 | 36 | 68 |
| Balmersal .. .. | 16 | 34 | 26 |
| | 30 | 37 | 32 |
| Thiomerin .. | 43 | 41 | 60 |
| | 45 | − 71 | 40 |
| Balmercamph .. | 14 | 32 | − 29 |
| | 45 | − 46 | 12 |

same extent by previous and simultaneous treatment with ammonium chloride. In dog 45, a reduced urinary excretion was shown when the animal was first treated with the drug, similar to the effect obtained in rats treated with high doses of the compound, although the dose employed in

the test lies within the therapeutic range. The response when the test was repeated with ammonium chloride was normal. The slight increase in urinary excretion in dog 14 is probably not significant and the results with balmercamph confirm those obtained in rats, that the diuretic effect of the organic mercurial is lost by combination with dimercaprol.

*Effect on electrolyte excretion.* In addition to their diuretic effect, organic mercurials have a marked chloruretic effect (Kourilsky, Corre, Delcambre and Scordel,[14] Keith *et al.*[11]) and inhibit sodium tubular reabsorption causing as a result an increased sodium excretion (Farah,

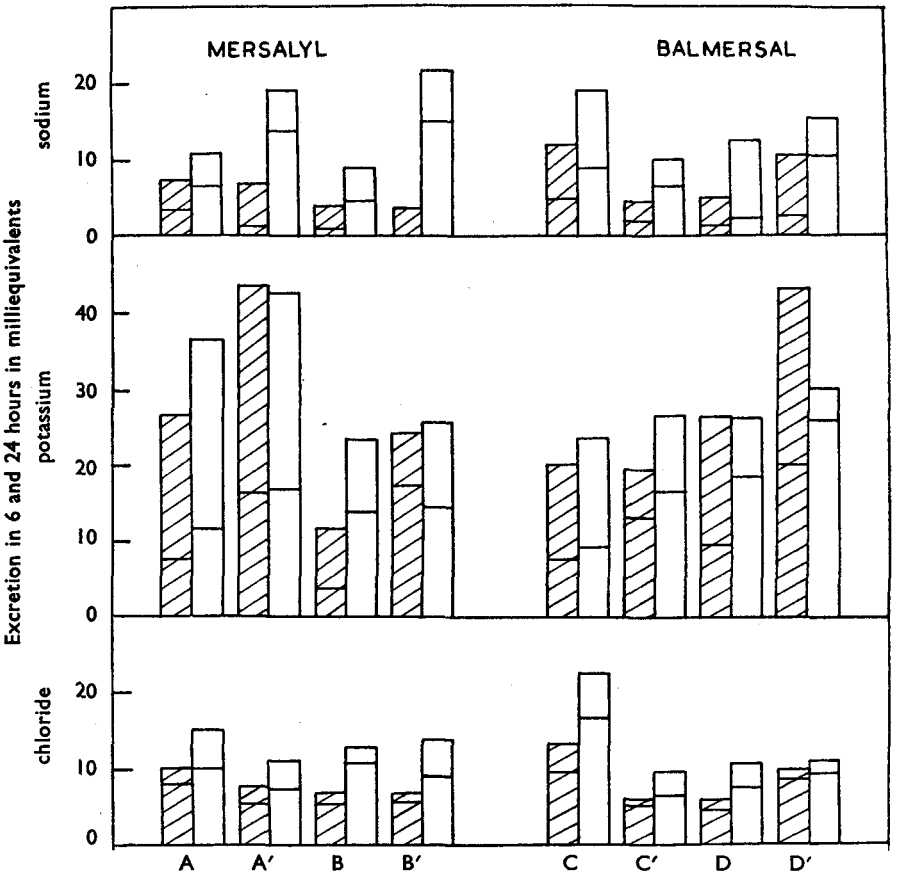


FIG. 5. Electrolyte excretion in dogs after mersalyl and balmersal, equivalent to 1·2 mg. of Hg./kg. intramuscularly, alone and with ammonium chloride. The total weight excreted, expressed as milliequivalents of Na, K and NaCl in 6 and 24 hours is shown. Cross hatch, control; open, test period.

A. Dog 16. Mersalyl alone.
A′. „ Mersalyl and ammonium chloride.
B. Dog 14. Mersalyl alone.
B′. ● „ Mersalyl and ammonium chloride.
C. Dog 16. Balmersal alone.
C′. „ Balmersal and ammonium chloride.
D. Dog 30. Balmersal alone.
D′. „ Balmersal and ammonium chloride.

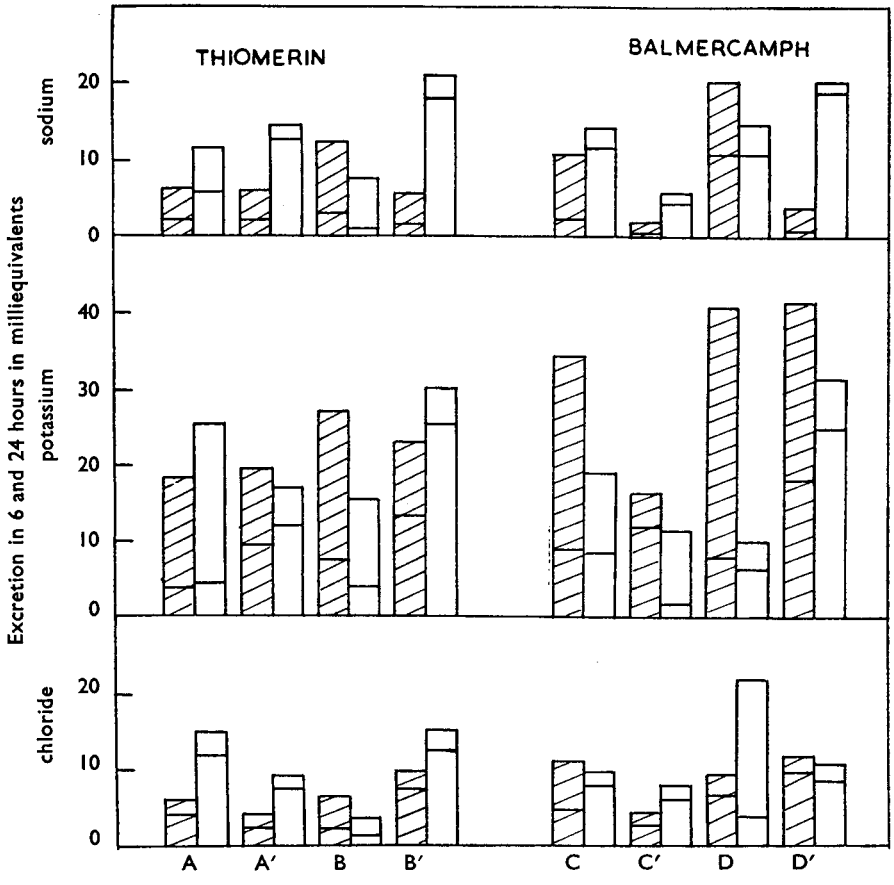


FIG. 6. Electrolyte excretion in dogs after thiomerin and balmercamph, equivalent to 1·2 mg. of Hg./kg. intramuscularly, alone and with ammonium chloride. The total weight excreted expressed as milliequivalents of Na, K and NaCl in 6 and 4 hours is shown. Cross hatch, control; open, test period.

A. Dog 43. Thiomerin alone.
A′. ,, Thiomerin and ammonium chloride.
B. Dog 45. Thiomerin alone.
B′. ,, Thiomerin and ammonium chloride.
C. Dog 14. Balmercamph alone.
C′. ,, Balmercamph and ammonium chloride.
D. Dog 45. Balmercamph alone.
D′. ,, Balmercamph and ammonium chloride.

Cobbey and Mook[15]). Blumgart, Gilligan, Levy, Brown and Volk[16] in their studies on the action of mercurial diuretics, also reported increased potassium and magnesium excretion but little or no effect on the excretion of phosphates and sulphates. Comparison of the effect of the compounds on electrolyte excretion is made in Figures 5 and 6. Balmersal, mersalyl and thiomerin all produce an increase in chloride and sodium excretion, the effect being similar whether the drug is given alone or with ammonium chloride. The effect on the potassium excretion is variable but there is no indication of a general tendency for the potassium excretion to increase.

Similarly no general increase of electrolyte excretion was found with balmercamph. Thus the variation in electrolyte excretion runs roughly parallel with the diuretic effect, the greatest increase being found where the diuretic effect is greatest.

*Toxicity.* In the course of the experiments on rats with varying doses of the compounds, indications were obtained that combination with dimercaprol had considerably reduced the acute toxicity of the mercurial derivatives. With the dose corresponding to 32 mg. of Hg./kg. all of the animals treated with mersalyl or thiomerin died while all those treated with balmersal and balmercamph survived. Two dogs were given balmersal in a dose equivalent to 4 mg. of Hg./kg. 3 times weekly for 6 weeks with very little general toxic effect, while a similar dose of mersalyl has been reported to cause acute nephrosis terminating in death within 2 weeks (Minatoya and Hoppe[17]). These results were confirmed quantitatively by determination of the LD50 intravenously in mice (Table II). In balmersal, the toxicity has been reduced to approximately one fifth that of mersalyl and that of balmercamph to about one sixth that of thiomerin.

TABLE II

ACUTE INTRAVENOUS TOXICITIES IN MICE

| Compound | LD50 mg./kg. |
|---|---|
| Mersalyl | 99 |
| Balmersal | 475 |
| Thiomerin | 79 |
| Balmercamph | 512 |

*Renal toxicity.* After absorption of mercury compounds, the mercury tends to become more concentrated in certain organs, particularly the kidney. Single large doses of mercury or smaller doses acting over a period result in necrotic changes in the tubular epithelium of the kidney (Burmeister and McNally[18]). The comparative renal toxicity after a single large dose and repeated smaller doses of the compounds was studied in rats, by determination of the amount of proteinuria produced and by histological examination of the kidneys at varying periods of time after treatment. The resultant proteinuria in groups of 4 rats after a single injection of the compounds equivalent to 20 mg. of Hg./kg. and after two 5-day periods of treatment with a dose equivalent to 4 mg. of Hg./kg. once daily is shown in Table III. A single high dose of thiomerin caused a very marked proteinuria, a 15- to 20-fold increase in the normal protein excretion being produced in the first 4 days. The protein excretion then gradually decreased and had returned to normal in about 14 days. No significant increase in protein excretion was observed with balmercamph. Except on the first day, only a 4- to 5-fold increase in protein excretion was obtained with balmersal and approximately normal values from the twelfth day onwards. The dose for mersalyl was beyond the diuretic range and almost complete anuria resulted. Although insufficient urine was obtained for quantitative analysis, qualitative tests indicated that a very high protein concentration was present. The results with repeated smaller doses are similar, a 2- to 3-fold increase was obtained with thiomerin, the effect being somewhat less with balmersal and greater with mersalyl. Protein excretion returned to normal when treatment was stopped. No significant increase in proteinuria was obtained with balmercamph.

TABLE III

PROTEIN EXCRETION (mg./100 g. rat) IN RATS AFTER TREATMENT WITH MERCURIAL DIURETICS BY INTRAMUSCULAR INJECTION

| Day | Single dose equivalent to 20 mg. of Hg/kg. | | | | 4 mg. of Hg/kg. for 2 × 5 day periods | | | | Control |
|---|---|---|---|---|---|---|---|---|---|
| | Mersalyl | Balmersal | Thiomerin | Balmercamph | Mersalyl | Balmersal | Thiomerin | Balmercamph | |
| − 3 to 0 (mean) | 1·09 | 0·71 | 0·92 | 1·45 | 0·80 | 1·20 | 1·30 | 1·12 | 1·18 |
| 1 | Anuria | 7·23 | 15·67 | 4·1 | 2·87 | 1·84 | — | 1·11 | 0·75 |
| 2 | ,, | 3·34 | 22·0 | 0·94 | 4·88 | 2·4 | 2·7 | 0·88 | 0·45 |
| 3 | ,, | 2·34 | 27·75 | 1·22 | 6·3 | 1·84 | 3·3 | 0·6 | 1·73 |
| 4 | All dead | 4·68 | 15·28 | 0·61 | 1·13 | 1·52 | 1·15 | 1·06 | 0·93 |
| 5 | — | 3·06 | 5·12 | 0·52 | 2·63 | 0·61 | 2·03 | 2·71 | 0·95 |
| 6 | — | 2·0 | 5·65 | 0·89 | 4·32 | 1·72 | 2·03 | 0·99 | 1·1 |
| 7 | — | 2·0 | 5·6 | 0·88 | — | 1·85 | — | 1·08 | 0·86 |
| 8 | — | 3·3 | 5·43 | 1·26 | 6·49 | 1·29 | 1·1 | 0·85 | 0·34 |
| 9 | — | Insufficient | 2·19 | 2·11 | — | 1·72 | 1·47 | 1·03 | 0·72 |
| 10 | — | ,, | 3·13 | 1·22 | 2·11 | 0·97 | 1·31 | 0·81 | 0·71 |
| 11 | — | 1·73 | 4·11 | 0·92 | — | 0·74 | 2·5 | 0·67 | 0·66 |
| 12 | — | Insufficient | 2·35 | 1·08 | 1·91 | — | 1·13 | 0·57 | 0·37 |
| 13 | — | 1·19 | Insufficient | 0·51 | 1·15 | 0·92 | 0·62 | 0·57 | 0·78 |
| 14 | — | — | — | — | — | — | — | — | — |
| 15 | — | Insufficient | 2·69 | 0·62 | 0·81 | 1·12 | 1·2 | 0·25 | 0·98 |
| 16 | — | ,, | 1·49 | 1·10 | 0·87 | 1·78 | 1·73 | 0·77 | 0·96 |
| 17 | — | ,, | Insufficient | 2·75 | 1·0 | 0·74 | 0·93 | 0·36 | 0·99 |
| 18 | — | ,, | ,, | 0·75 | 2·8 | 0·98 | 1·62 | 1·49 | 0·39 |
| 19 | — | 0·48 | 0·25 | 0·40 | 1·65 | 0·56 | 1·28 | 0·55 | 0·68 |
| 20 | — | 0·42 | Insufficient | 0·56 | 0·65 | 0·53 | 1·71 | 1·26 | 0·79 |
| 21 | — | 0·19 | 0·69 | 0·81 | 0·66 | 0·68 | 1·29 | 1·31 | 0·47 |
| 22 | — | 0·97 | 1·03 | 0·41 | 1·66 | 1·45 | 1·56 | 0·59 | 0·65 |
| 23 | — | 0·48 | 0·46 | 0·26 | 0·28 | 2·07 | 1·64 | 0·67 | 1·03 |
| 24 | — | — | — | — | 1·48 | 1·75 | 1·86 | 1·44 | 2·13 |

The progressive effect of the renal lesions was followed histologically and confirmed the observations made from the protein excretion. The kidneys were examined 24 hours, 1 week and 4 weeks after the single injection, 24 hours after the 5 and 10 repeated injections, and 1 and 4 weeks after the 10 injections. 24 hours after the single injection equivalent to 20 mg. of Hg./kg., severe acute nephrosis with damage to the cortical tubules and production of hyaline and granular casts, was seen in the animals given mersalyl, balmersal and thiomerin. There was some localised severe damage with balmercamph, but nothing like that produced with the other three compounds. The animals dying on the fifth day after treatment with mersalyl still showed similar severe damage. After 1 week the kidneys from the animal receiving thiomerin still showed generalised dilation of the cortical tubules with many granular and hyaline casts. There was a similar moderate generalised dilatation in the case of balmersal but the damage was less severe. In the case of balmercamph, no gross changes were observed. After 4 weeks, recovery was complete in all cases and the kidneys appeared normal, except for occasional patchy plasma cell infiltration and a slight generalised œdema.

5 daily injections of mersalyl, equivalent to 1 mg. of Hg./kg., produced acute cortical degeneration with large irregular nuclei in the damaged tubules. Similar areas of disintegration of the cells of the convoluted tubules forming granular cases were found with thiomerin. The damage

with balmersal was somewhat similar but less severe, fewer than 10 per cent. of the tubules being affected. 5 injections of balmercamph appeared to have no effect on the kidney, but after a further 5, occasional single cortical tubules showed a swelling of the epithelium and pyknosis of the nuclei. 1 week after the end of treatment, granular casts were still seen in the convoluted tubules of the animal treated with mersalyl and disintegration of the cells and nuclei from those treated with thiomerin but

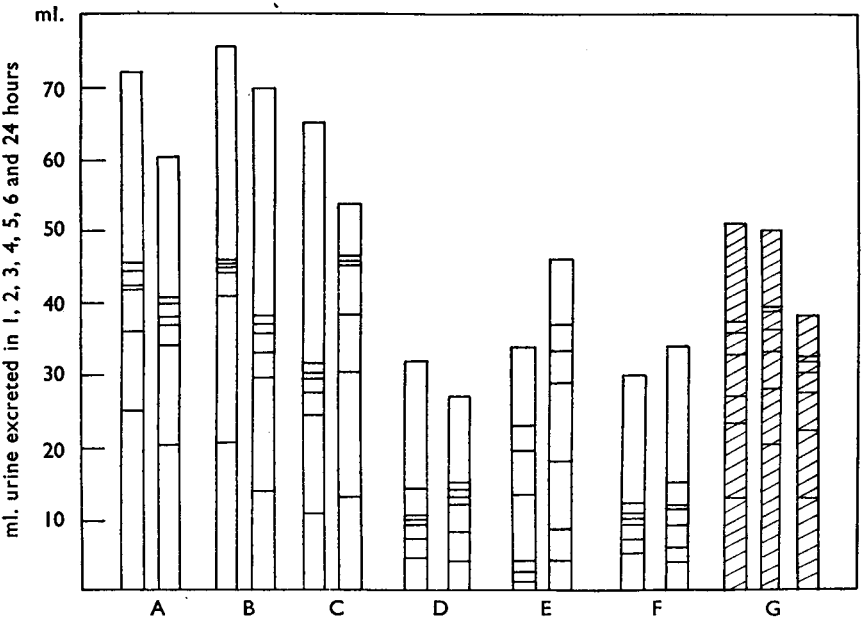


FIG. 7. Diuretic effect of mersalyl, 8 mg. of Hg./kg. administered intramuscularly with 1, 2, 5, 10 and 50 times the molecular equivalent of dimercaprol.

A. Mersalyl.
B. Mersalyl and dimercaprol 1 : 1.
C. „ „ 1 : 2.
D. Mersalyl and dimercaprol 1 : 5.
E. „ „ 1 : 10.
F. „ „ 1 : 50.
G. Controls.

those given balmersal and balmercamph were more or less normal. After 4 weeks, recovery was complete and the kidneys were normal in all cases.

*Local toxic action.* Mercury exerts a local toxic effect at the site of contact with the tissues, and the local irritant effect of mercurial diuretics when given intramuscularly or subcutaneously is well known. One of the advantages of thiomerin is that it shows a reduced local toxic effect and appears to be satisfactorily absorbed from subcutaneous tissue. The relative local irritant action of the compounds was compared in rats. The sites after 3 consecutive daily subcutaneous injections into a shaved area of approximately 1 sq. in. was examined. Balmersal and balmercamph showed an irritant effect similar to that of mersalyl; with all 3 compounds it was more marked than with thiomerin.

*Mechanism of diuretic action.* Simultaneous administration of mercurial diuretic and dimercaprol has been reported to eliminate the diuretic effect (Farah and Maresh[19]). In view of the well maintained diuretic activity of balmersal the effect of simultaneous administration of mersalyl and dimercaprol in the test conditions described, was examined. Mersalyl, equivalent to 8 mg. of Hg./kg., was given by intramuscular injection simultaneously with doses of dimercaprol corresponding to 1, 2, 5, 10 and 50 times the molecular equivalent. The results of the qualitative comparison are given in Figure 7. Mersalyl and dimercaprol when given in equivalent amounts showed a diuretic effect similar to that of mersalyl itself. This effect was abolished completely when 10 times the equivalent of dimercaprol was given and intermediate results were obtained in the other molecular proportion. The time of diuresis was also similar, the bulk of the increased urinary excretion occuring between 6 and 24 hours. Quantitative measurement of the diuretic action by comparison with urea, confirmed these results (Fig. 8).

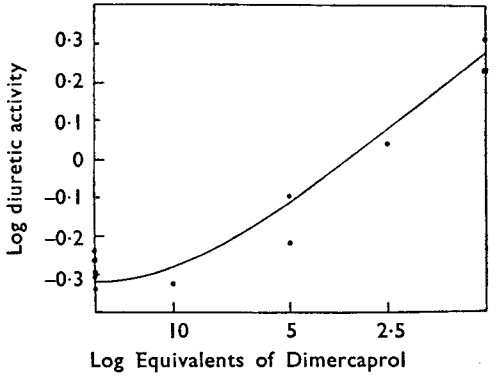


FIG. 8. Dose-response curve for diuretic action of mersalyl, 8 mg. of Hg./kg., in rats, administered with varying molecular proportions of dimercaprol.

It has been suggested that the diuretic effect of the mercurials may be due to inactivation of one or more enzyme systems. Hatta[20] showed that mercuric chloride inhibited many proteolytic enzymes and that the inhibition could be reversed by substances such as potassium sulphide which precipitated the mercury, and studies with the radio-active metal have confirmed that protein binding of the mercury occurs in the body. The findings of Fawaz and Fawaz[21] that mersalyl in therapeutic doses has no effect on the succinic oxidase activity of cortical homogenates, indicate that mercurials may react with proteins other than through the ·SH groupings. The results with dimercaprol suggest that when only equivalent amounts are administered, the mercury reacts preferentially with the protein, producing the usual diuretic effect, but when increasing amounts are given, a mass action effect is obtained and the dimercaprol binds the mercury more firmly than do the tissues. Attempts to find evidence for an extra-renal action of the compounds by determination of the effect on the concentrations of electrolytes in the blood gave inconclusive results. Measurements of the concentration of sodium potassium and chloride ions showed no significant alteration after injection of any of the compounds.

## Discussion and Conclusions

From the standpoint of practical value in replacing present mercurial diuretics with less toxic drugs, the compounds with dimercaprol do not appear to provide the complete answer. Because of the greatly reduced acute toxicity and some modification of the renal toxicity with maintenance of an equivalent diuretic effect, balmersal may be of some value. The effects on cardiovascular responses have still to be determined. From the theoretical point of view, the experiments on renal toxicity lend some support to the theory that the mercurials owe their diuretic activity at least in part to a mild tubular irritation which inhibits tubular reabsorption. In the case of balmercamph where combination with dimercaprol has practically eliminated renal toxicity, little or no diuretic effect is observed, while with balmersal where some nephrotoxic action is still maintained, diuretic activity is still present.

The equivalence of the diuretic effect of mersalyl and balmersal suggests that the reaction with mersalyl and dimercaprol is a reversible one and that balmersal is slowly decomposed in the body leaving the mercury free to exert its diuretic effect. The results with mersalyl and varying equivalents of dimercaprol confirm this, since the diuretic effect is gradually abolished when increasing amounts of dimercaprol are administered. These preliminary results also suggest that mercury may react with proteins other than through the SH-groupings but further work with varying doses of mercury and dimercaprol and observations on other enzyme systems and specific inhibitors is necessary to confirm this.

## Summary

1. The two compounds formed by combination of 1 molecule of the dithiol, 2:3-dimercaptopropanol (dimercaprol) with 2 molecules of the organic mercury compounds, mersalyl and $\gamma$-hydroxymercuri-$\beta$-methoxypropylcamphoramide (mercurophyllin U.S.P.) are called respectively balmersal and balmercamph.

2. The diuretic effect of these compounds is compared in rats and dogs with that of mersalyl and thiomerin, a monothiol derivative of mercurophylline and thioglycollic acid. The diuretic activity of balmersal is at least equal to that of mersalyl, but the introduction of the second thiol group in balmercamph abolishes the diuretic effect.

3. The effect of the compounds on electrolyte excretion is studied in dogs.

4. Measurements of the intravenous LD50 in rats, show that the acute toxicity is considerably reduced by combination of the organic mercurials with dimercaprol. Some reduction of the renal toxicity also occurs.

5. Administration of increasing equivalents of dimercaprol to rats reduces the diuretic activity of mersalyl, suggesting that the combination of mersalyl and dimercaprol *in vivo* is a reversible reaction.

6. The significance of these results in the interpretation of the mechanism of the diuretic action of organic mercury derivatives is discussed.

I wish to thank Mr. Sharp of the Wellcome Laboratories of Tropical Medicine for his co-operation and the preparation of the compounds with dimercaprol, Dr. David Trevan for the preparation and examination of the histological preparations of the kidneys and Miss A. Facey for valuable technical assistance.

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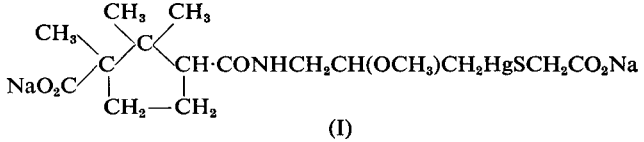
## A NOTE ON THE PREPARATION OF COMPOUNDS OF MERCURIAL DIURETICS WITH 2:3-DIMERCAPTOPROPANOL (DIMERCAPROL)

BY T. M. SHARP

LEHMAN[1] has shown that the acute toxicity of mercurial diuretics is greatly reduced by combining the mercury with monothiols such as thioglycollic acid. One of these is now in clinical use in the United States of America under the names thiomerin and mercaptomerin.[1] It was thought that a similar or greater reduction in toxicity might be attained by combining



(I)

known mercurial diuretics with dimercaprol, 2:3-dimercaptopropanol, which is a well established antidote for heavy metal poisoning. It is now shown that 2 mols. of mersalyl will combine readily in alkaline solution

(II)