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SECTION MEETINGS

OHIO VALLEY	
Children's Hospital, Cincinnati	October 23, 1948
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17058. A Comparative Study of the Local Toxic Action of Mercurial Diuretics.

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The mercurial diuretics have today a secure place in the management of the edematous patient because of their effectiveness and dependability. However they give rise, on occasion, to various side effects of which irritation at the site of injection and an acute toxic action on the heart are the most significant. This latter action may sometimes be fatal[1-3] and in an attempt to eliminate it a new type of mercurial has recently been made available under the name Thiomerin.* This drug is identical with Mercuzanthin (Mercurophylline, USP XIII) except that it contains sodium mercapto acetate in chemical combination with the mercury instead of theophylline. In animal experiments it failed to produce the typical ventricular tachycardia and fibrillation which occurs after the other diuretics.[4] Thiomerin also appears to be less toxic to tissue and has been proposed for clinical use by subcutaneous injection. It therefore seemed desirable to make a detailed comparison of the mercurial diuretics with respect to the reaction which they produce at the site of injection. Thiomerin, Mercuzanthin, and Mercuhydrin were used in this study.

Subcutaneous Injection in Mice. It has

1 DeGraff, A. C., and Nadler, J. E., *J. Am. Med. Assn.*, 1942, **119**, 1006.

2 Ben-Asher, Solomon, *Ann. Int. Med.*, 1946, **25**, 711.

3 Volini, Italo, Levitt, R. O., and Martin, Richard, *J. Am. Med. Assn.*, 1945, **128**, 12.

* Thiomerin has also been identified as MT6.

4 Lehman, R. A., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 428.

previously been shown in rabbits, that addition of theophylline to a parent mercurial such as Salyrgan or Mercuzan, protects the skin from the severe necrosis following intradermal injection.[5] However, this technic was not found to give a sufficiently sensitive or consistent response for the study of mercurials of a less irritant character. The albino mouse was selected as a more suitable animal since, unlike the rabbit, the skin over the abdomen is thin enough so that necrosis cannot escape detection, and large enough groups of animals can be used to evaluate the effect of individual variation.

Three groups of 5 mice in the weight range of 20 to 30 g were selected at random and the hair carefully clipped over the abdomen. The first group received Thiomerin, the second Mercuzanthin and the third Mercuhydrin all by subcutaneous injection in a dose of 0.03 cc of undiluted solution as supplied for clinical use (40 mg of mercury per cc). A one quarter cc tuberculin syringe with a 27 gauge hypodermic needle was used with the bevel up. All the animals were injected on the same day. Approximately 24 hours after the injection the mice were examined for evidences of toxicity. They were then anesthetized with a barbiturate and photographed in color. These photographs appear as Fig. 1.

It will be observed that in the case of Mercuzanthin the destruction of tissue has progressed in all animals to the point of skin breakdown over a substantial area of the abdomen. The very dark discoloration accompanying some of the lesions suggests the presence of extravasated blood. In every case there is a white ring surrounding the area of necrosis which is presumably due to inflammatory exudate.

The response to Mercuhydrin is of similar character but somewhat less severe, more superficial, and involves a smaller area of skin. Nevertheless perforation of the skin will be observed in the first animal on the left in Fig. 1. Extravasation of blood appears not to have occurred in this group.

None of the animals injected with Thiomerin shows pathology which can be detected on gross examination.

Intramuscular Injection in Rats. Because of these marked differences in local toxicity, studies were undertaken to compare the histopathology occurring after injection of Thiomerin, Mercuzanthin and Mercuhydrin. For this purpose the tibialis anterior muscle of the rat was selected as a more homogeneous tissue than the skin for the precise administration of a measured volume of drug. Each rat was etherized very lightly, the hair shaved from the leg and 0.03 cc of undiluted drug was injected into the belly of the muscle using a one-quarter cc syringe and 27 gauge needle. Twenty of the rats received saline control injections into the corresponding muscle of the opposite leg. The rats were killed by a blow on the head after a period of 4, 24, or 96 hours. The skin was stripped away and the injected muscle gently freed from adherent muscle bundles and dissected free. Muscles were fixed in Bouin's solution, embedded in paraffin and cut at 10 μ. Each muscle was sectioned serially at intervals of 500 μ. The sections were examined by the pathologist without foreknowledge of the treatments given and the pathology was scored as "none", "slight", "moderate", "marked", or "very marked" in the following categories: "disruption of muscle integrity", "cellular infiltration", and "edema". The results are given in the Table.

Disruption of Muscle Integrity. It appears from the Table that after 4 or 24 hours the 3 drugs show definite disruption of tissue in distinction to the saline control. After 96 hours the disruption is less than at 4 or 24 hours in the case of Mercuzanthin and Mercuhydrin while Thiomerin injected muscles now show no disruption and cannot be distinguished from the controls.

Cellular Infiltration. After 4 hours all of the drugs as well as saline have produced a slight cellular response as indicated in the Table. Twenty-four hours after injection the response to saline has not increased while that to the three mercurials has increased a great deal. After 96 hours the infiltration has disappeared in the case of Thiomerin and

[5] DeGraff, A. C., Batterman, R. C., and Lehman, R. A., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 373.

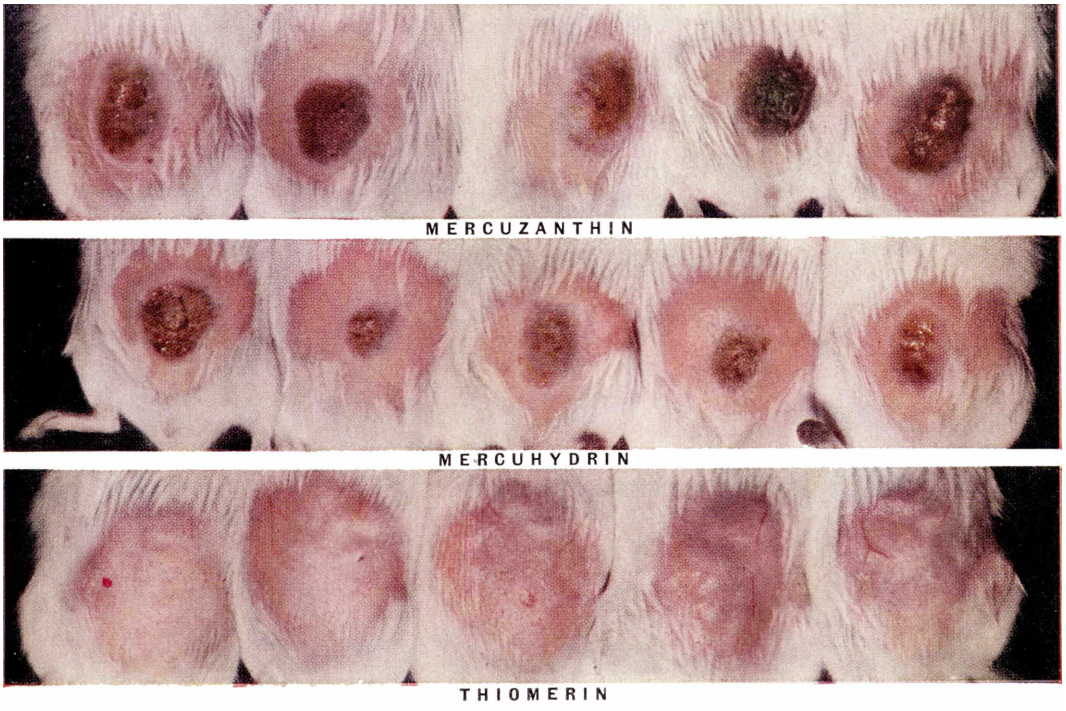

FIG. 1

Photographs of mice 24 hours after the subcutaneous injection of 0.03 cc. of a mercurial diuretic at a concentration of approximately 40 mgm of mercury per cc. All of the mice in a row received the same treatment. *Top Row*: Mercuzanthin (Mercurophylline USP XIII); *Middle Row*: Mercuhydrin (Meralluride); *Bottom Row*: Thiomerin.

TABLE I.
Scores for Examination of Muscle Sections After Injection of Mercurial Diuretics.*

Drug	4 hr			24 hr			96 hr		
	DI	CI	ED	DI	CI	ED	DI	CI	ED
Saline	0	+	0	0	0	0	0	0	0
	0	+	+	0	+	0	0	0	0
	0	+	+	0	+	0	0	0	0
	0	+	+	0	+	0	0	+	0
	+		+	0	+	0	++	+++	+
				0	+	+			
				0	+	+			
				0	+	+			
				+	+	+			
				+	++	+			
Thiomerin	+	+	+	+	++	+	0	0	0
	+	+	++	+	++	++	0	0	0
	+	+	++	++	++	++	0	+	0
	++	++	++	++	++	++	0	+	0
	++	++	++	++	++	++	0	+	+
				++	+++	++			
				++	+++	++			
				++	+++	++			
				++	+++	++			
					+++	+++			
Mercuzanthin	+	+	+	++	++	+	0	++	+
	+	+	+	++	+++	++	+	++	+
	++	+	+	+++	+++	++	++	++	+
	++	+	++	+++	++++	++	++	++	++
				+++	++++	+++	++	+++	++
Mercuhydrin	+	+	+	+	++	+	+	++	+
	++	+	+	++	++	++	++	+++	+
	++	+	++	++	++	++	++	+++	+
	+++	+	++	+++	+++	++	++	+++	+
	+++		++	+++	+++	++			

* 0 none; + slight; ++ moderate; +++ marked; ++++ very marked.
DI, disruption of muscle integrity; CI, cellular infiltration; ED, edema.

saline but is still present in the case of Mercuzanthin and Mercuhydrin.

Edema. Four hours after injection edema is present in every instance although saline shows less than the 3 drugs. The edema persists for at least 24 hours. After 96 hours it has disappeared from the saline and Thiomerin injected muscles but is still present in the others. It should be pointed out in this connection that absorption of these drugs is substantially complete one hour after injection.[6]

The character of the response is illustrated in Fig. 2, 3, and 4. Fig. 2 is typical of the response 24 hours after injection of any one of the 3 mercurials and is characterized by the infiltration of polymorphs, lymphocytes and the appearance of edema fluid. The response to saline was similar but quantitatively less extensive. Fig. 3 illustrates the appearance of muscle 96 hours after injection of Mercuzanthin or Mercuhydrin and shows not only the persistence of the inflammatory exudate suggesting continued irritation but also the development of a reparative response as manifested by newly-formed connective tissue. Fig. 4 shows essentially normal muscle which was consistently observed 96 hours after injection of Thiomerin or saline.

Discussion. It has long been taken for granted that mercury compounds in which at least one valence bond bears an ionizable group are highly irritant to tissues. Thus Salyrgan with the configuration $RHg\overset{+}{\underset{-}{}}OH$ is severely and rapidly necrotizing.[5] The introduction of Mercuzanthin provided a compound of structure $RHg—N<$ in which the irritant action of the mercury has been considerably reduced by combination with theo-

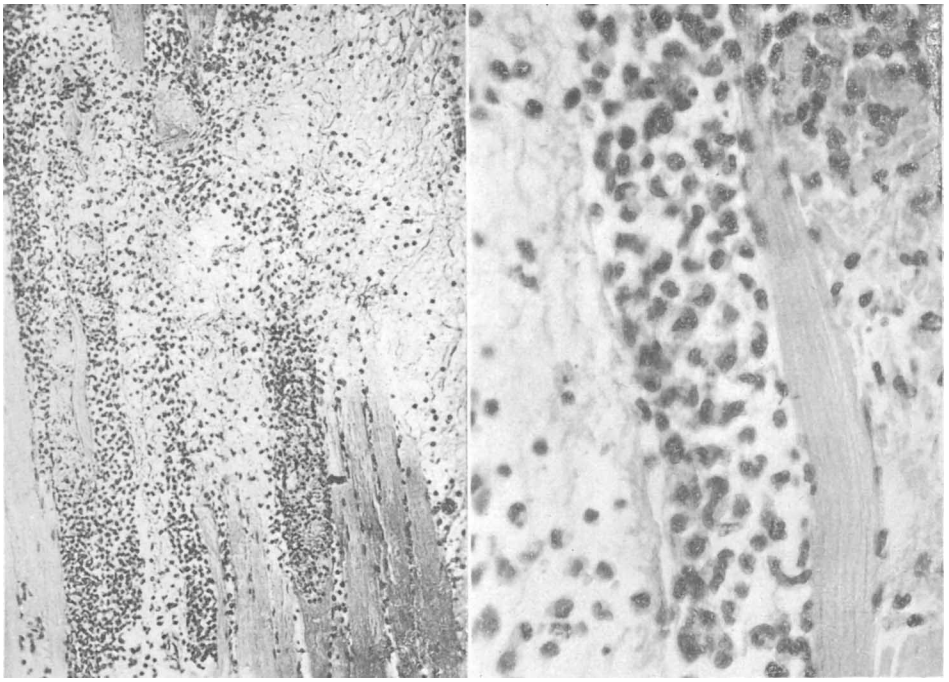

FIG. 2.
Section of rat muscle illustrating the response to Mercuzanthin, Mercuhydrin or Thiomerin 24 hr after inj. Left—×100; right—×450.

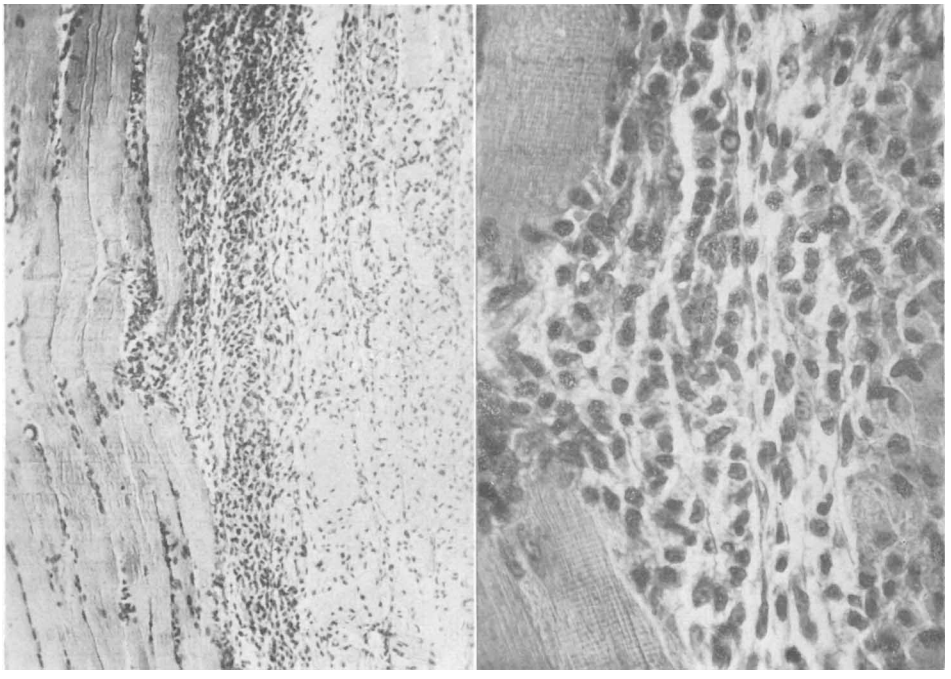

FIG. 3.
Section of rat muscle illustrating the response to Mercuzanthin or Mercuhydrin 96 hr after inj. Left—×100; right—×450.

FIG. 4.
Section of rat muscle 96 hr after inj. of Thiomerin or saline showing essentially normal tissue. ×100.

phylline. Nitrogen compounds of similar structure (such as succinimide) can be substituted for theophylline with comparable effect.[6,7] Salyrgan-theophylline and Mercuhydrin are theophylline-bearing drugs of this class. In Thiomerin the second valence bond of the mercury has been bound as a mercaptide to give a substance of the formula RHg–S– and this modification has been shown above to result in a drug of still less toxicity. In a previous report it was indicated that the addition of a sulfhydryl compound to a conventional diuretic will cause precipitation of the theophylline indicating that the Hg–S bond is chemically more stable than the Hg–N.[4] Hence the results here reported would be consistent with the conclusion that local irritant action in the mercurial diuretic series is proportional to the reactivity of the mercury which they contain. A logical extension of this series would be the compound RHg—C <– which might be expected to show even less local irritant action than Thiomerin. However, such a mercurial would also be expected not to have therapeutic activity.

It is beyond the scope of this report to discuss the effect of the Hg–S bond on diuretic activity. Clinical studies[8–10] seem to indicate that Thiomerin is at least as effective as the other mercurials in this respect.

It may be mentioned in passing that the sensitivity of mice to the subcutaneous injection of mercurials was found to be greater in the summer than in the winter months. All of the injections for the experiment which is illustrated in Fig. 1 were accordingly given on the same day (September) so that the comparison would be valid. In this connection the work of DiPalma and coworkers[11] on seasonal variations in the sensitivity of the skin to the production of reactive hyperemia is of interest.

Summary and Conclusions. Three mercurial diuretics, Thiomerin, Mercuzanthin and Mercuhydrin have been compared with respect to the tissue reaction which they produce. Thiomerin was tolerated by mice on subcutaneous injection without exhibiting gross pathology while Mercuzanthin and Mercuhydrin gave

6 Lehman, R. A., and Dater, Arnold, *J. Pharm. Exp. Therap.*, 1938, **63**, 443.

7 Hunt, W. H., Walter, L. A., and Fosbinder, R. J., *J. Am. Pharm. Assn.*, Sci. ed., 1942, **31**, 278.

8 Herrmann, George R., Chriss, John W., Hejmancik, Milton R., and Sims, Paul M., *Texas State J. Med.*, 1949, **45**, 79.

9 Grossman, J., Weston, R. E., Edelman, I. S., and Leiter, L., in press.

10 Batterman, Robert C., Unterman, David, and DeGraff, Arthur C., *J. Am. Med. Assn.*, in press.

11 DiPalma, J. R., Reynolds, S. M. R., and Foster, F. I., *Am. Heart J.*, 1942, **23**, 377.

rise to necrosis of the skin under the same conditions. After intramuscular injection in rats all three drugs gave an early inflammatory response characterized by the appearance of a polymorphonuclear exudate. In the case of Thiomerin this exudate was entirely resorbed without evidence of residual damage. After injection of Mercuzanthin, or Mercuhydrin, however, the irreversible nature of the response was indicated by marked fibroblastic proliferation.

These results would seem to furnish an adequate experimental basis for the clinical use of Thiomerin by subcutaneous injection.

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17059. Observations of Nitrite-Induced Postural Syncope in Patients with Mental Disease.

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Earlier work[1] discussed the vasomotor system in schizophrenia; it was concluded that no structural vascular defect exists and that schizophrenic patients often exhibit acral vasoconstriction. Investigation of the reactivity of postural vasoconstrictor mechanisms has also been made. Nitrite dilates vessels by relaxing smooth muscle. but does not prevent the action of nervous impulses on this muscle; accordingly, the degree to which the vasoconstriction overcomes the effect of nitrite affords data on the reactivity of postural vasoconstrictor mechanisms.

Material and methods. Twenty-four patients were studied. Their diagnoses varied (Table I). Four (Table II), all schizophrenics were studied only after lobotomy. Twenty-four normal subjects were also observed. After reassurance the subjects came to the laboratory fasting or 3 or 4 hours after a last meal. Patients were not permitted to smoke before or during experiments. Each subject was given 3 grains of sodium nitrite by mouth and allowed to rest for 30 minutes. He then lay on a board which had no protuberances except for a bicycle seat at right angles to it; this the patient straddled. After control observations were made, the board was tilted 60 degrees leaving the patient head-up with legs hanging. Systolic blood pressure was measured every few minutes by palpation. Auscultatory measurements were not made since they may be markedly in error with changing vasomotor tone; an arterial cannula was considered undesirable for measuring arterial pressure in this study. If syncope did not occur within forty minutes after tilting the patient, the observations were ended and were repeated no sooner than 2 days later, using 7 grains of sodium nitrite.

Observations. Subjects were classified as follows:

Group I. Syncope during tilting after 3 grains of sodium nitrite, *i.e.*, no increase in vasomotor reactivity (Fig. 1).

Group II. Syncope during tilting after 7 grains but not after 3 grains of sodium nitrite, *i.e.*, possible increase in vasomotor reactivity (Fig. 2).

Group III. Fall in blood pressure during tilting but no syncope after 7 grains of sodium nitrite, *i.e.*, definite increase in vasomotor reactivity (Fig. 3).

Group IV. Rise in blood pressure during tilting after 7 grains of sodium nitrite, *i.e.*, marked increase in vasomotor reactivity (Fig. 4).

Twenty-two normal subjects were in Group I and 2 in Group II. Seven of the 11 nonlobotomized schizophrenic patients exhibited

[1] Altschule, M. D., and Sulzbach, W. M., *Arch. Neurol. and Psychiat.*, 1949, **61**, 44.