STUDIES ON THE PERMEABILITY OF THE SKIN TO MERCURY*

By EDWIN P. LAUG

From the Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.

THE QUESTION of permeability of the skin should hold particular interest for the cosmetic chemist. This is so because the usual older criteria of acceptability of skin preparations, namely good physical properties, may no longer be adequate in characterizing an ointment or lotion. We should like to know, for example, whether ammoniated mercury applied as a surface bleach penetrates the skin, to what extent propylene glycol, or glycerol functions as a percutaneous vehicle, etc.

I realize that the use of mercurials in cosmetic chemistry probably has a very limited interest. Therefore, in presenting the problem of mercury penetration in considerable detail, I wish to emphasize that the individual findings are important only to the extent that they help to illustrate the principles and techniques involved. My chief interest, therefore, is to demonstrate a new method of approach in evaluating the properties of ointments, and I hope that these studies may stimulate further work in this direction.

The work on the skin permeability of mercury which is to be reported here is the outgrowth of studies made during the war under Office of Scientific Research and Development contracts. The object of the study was to reinvestigate the use of mercurial ointments for venereal prophylaxis of skin and mucous membranes, and to develop more efficient preparations. This involved the screening of a considerable number of ointments containing mercury in the form of calomel. For a calomel ointment to be acceptable, it had to possess (1) good mechanical properties, such as stability, spreadability, resistance to drying, etc.; (2) a minimum of irritating properties; (3) ability to penetrate the skin in amounts therapeutically effective both locally and systemically. The last of these three criteria was the one upon which we concentrated our attention.

That the skin is permeable to mercury has been well established, and its therapeutic usefulness adequately investigated by Cole and his

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associates (1, 2, 3). Proof of the cutaneous penetration of mercury has rested chiefly upon the finding of mercury in the urine, although systemic symptoms of mercurialism have also been frequently noted. While clinical studies on humans can be carried on with reasonable care to prevent contamination of excreta with mercury, this is not so easy with animals. Furthermore, in the screening of a large number of ointments, a rapid means for quantitative differentiation was very necessary. In this respect, the urinary excretion method left much to be desired and, in fact, could be regarded as hardly sufficiently quantitative.

Because of the fact that the new methods which we have adapted for the assay of mercurial ointments have applicability for other heavy metals, as well as the routine testing of numerous cosmetic preparations, it seems advisable to describe the procedures somewhat in detail.

A. Methods for Cutaneous Application

Obviously stringent precautions are necessary in determining cutaneous permeability in order to avoid serious errors. There must be assurance that the substance under study enters the body *via* the skin and only *via* this route. All oral contact through licking, etc., must, therefore, be avoided. In addition, if examination of excreta is to be made, ways must be found to prevent contamination by a flaking or running off of the test substance from the skin into the collection trays.

Rabbits used in our study were immobilized in a special head-hip stock (4). Hair from the back and flanks was removed by clipper. Shaving or chemical methods of depiliation were avoided because of the possibility of injury to the skin. An area of 4 × 6 in. [155 $(cm.)^2$ which is 8 per cent of the total body surface of a 2.5-kg. rabbit] was laid off saddle fashion from the back around to each flank. To avoid creeping of ointment the area was limited by strips of tape. An excess of ointment (4 gm.) was inucted with a glass rod for 3 minutes. Without removing the excess ointment the inunction site was then covered with rubberized cloth and the animal kept in the stock for 24 hours.

Because of greater availability and ease of handling, the rat proved to be more desirable as a test animal than the rabbit. But since it was not feasible to confine rats in individual stocks like rabbits, a technique for wrapping the animals was developed which proved rapid and effective. The animals were lightly etherized and the hair removed around the trunk between shoulders and hips. An area of $1\frac{1}{2}$ × 3 in. [29 $(cm.)^2$ which is 8 per cent of the total body surface of a 250-gm. rat] was laid off on the back in a manner similar to that for the rabbit. An excess of ointment (0.4 gm.) was inuncted with glass rod for 3 minutes. After inunction, and without removal of the excess oint-

ment still remaining on the skin, the animal was wrapped in a piece of celluloid sheeting formed into a cylinder fitted snugly around the trunk. The edges of the cylinder were cemented to the skin at the shoulders and hips so as to prevent slipping of the shield or possible seepage of the ointment. The procedure is illustrated in Fig. 1. As additional protection, the celluloid

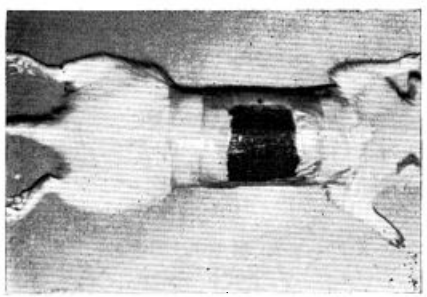

Figure 1.—For studies on the permeability of the skin to mercury

shield was covered with a layer of adhesive tape applied in such a way as to overlap the edges of the shield by about $^3/_4$ in. Thus "trussed," the animals were placed in individual cages with access to food and water. Experience with several hundred rats so prepared has shown that the celluloid shield proved to be sufficiently rigid to prevent the animal from getting in oral contact with any part of its trunk, yet did not interfere with walking. Although the animals occasionally struggled, the celluloid-to-skin seals were effective in confining the ointment beneath the shield.

B. Methods for Detecting Cutaneous Penetration of Mercury

The method for detecting and quantitating the cutaneous penetration of mercury depends on an entirely new principle. This method substitutes storage of mercury for excretion of mercury as a measure of how much has penetrated the skin. Of all the organs of the body, the kidney has the greatest capacity for storing mercury. This is shown very clearly in Table 1. Here it can be seen that the concentration of mercury in the kidney, following 24- and 48-hour exposures to calomel ointment, is of an entirely different order of magnitude than in other tissues. Convenient also for purposes of assay is the fact that the concentration of mercury in the control kidney is so low as to be of no quantitative significance. At any time following exposure to mercury, the amount in the kidney may be assumed to be a balance between three dynamic factors: (1) the rate of entrance of mercury, (2) the rate of excretion of mercury, and (3) the rate of storage of mercury. If the rate of excretion is slow (experiments have proved this) and the rate of storage rapid (intravenously injected mercury finds its way into the kidney in maximum amount with $1^1/_2$ hours), then it becomes possible to use the mercury content of the kidney as a measure of how much has penetrated the skin. Obviously this measure is only a relative one, but is chiefly useful in

TABLE 1—DISTRIBUTION OF MERCURY IN THE TISSUES OF THE RAT FOLLOWING CUTANEOUS APPLICATION OF A 30 PER CENT CALOMEL OINTMENT

Micrograms Mercury per Gram Wet Tissue											
Kidney	Liver	Blood	Muscle	Lung	Heart	Testes	Brain	Spleen	Stomach and Contents	Small Intestine and Contents	Bone
					Control*						
0.24	0.06	..	0.07	0.12	..	..	0.03	0	..	..	0.10
					24-Hour Exposure*						
27.2	0.84	0.64	0.16	1.23	0.61	0.10	0.13	2.38	0.21	0.47	0.22
					48-Hour Exposure*						
38.6	1.12	0.99	0.10	1.69	0.98	0.30	0.24	1.60	0.18	0.58	0.30

* Average of six animals on each treatment.

comparing the penetrating abilities of efficient types of mercurial ointments. This method is so sensitive that if as little as 9 micrograms enter the body of a 250-gm. rat, 25 to 50 per cent thereof can be detected in the kidneys in 24 hours.

The procedure which we have used for assaying mercurial ointments may be summed up as follows: A clipped area on the back is inuncted for 3 minutes with a mercury ointment, covered with rubber sheeting or celluloid, and contact of the ointment with the skin maintained for 24 hours. At the end of this time, the animal is sacrificed by exsanguination under ether anesthetic, and the kidneys removed for analysis of mercury (5). All results are expressed in micrograms of mercury per gram of wet tissue. Owing to the natural variability in test animals, 6 to 8 are used for an assay in order to be able to recognize significant differences between inunction procedures or ointments.

Before discussing the findings of chief interest, namely, the effects of various types of ointment bases and mercury compounds on the cutaneous penetration of mercury, a number of factors relating especially to the test animals and the methods of application must be considered briefly.

1. *Effect of Covering the Inunction Site*

According to Rothman (6), covering the skin results in interferences with the normal escape of moisture.

Moisture thus accumulated may produce softening and maceration of the stratum corneum, a condition which may be favorable to the retention of substances in close contact with the skin, and possibly also to penetration. While in our assays the inuncted areas of skin were always kept covered to avoid contamination errors, it seemed desirable to test whether this technique affected skin penetration. The results are shown in Table 2. It is clear that covering the inuncted area increases the absorption of mercury from every one of the six different ointments tested.

TABLE 2—EFFECT OF COVERING THE INUNCTED SKIN AREA ON THE CUTANEOUS PENETRATION OF MERCURY IN THE RABBIT

Storage of mercury in the kidney is used as a measure of penetration

Calomel Ointment	Micrograms Mercury per Gram Wet Kidney: Covered Skin*	Uncovered Skin*
1	26	7
2	45	13
3	17	8
4	20	9
5	6	4
6	22	5
Av.	23	8

* Average of two animals on each treatment.

2. *Location of the site of Inunction*

Generally speaking the abdominal skin in the rat and rabbit is considered thinner than the back skin. A comparison of the penetration of mercury at these two sites was, therefore, made and is shown in Table 3. It is to be noted that the site of inunction does not significantly affect absorption of mercury.

3. *Size of the Inunction Site*

In spite of the best precautions, seepage of the ointments sometimes occurred so that in effect a larger area of skin came into contact with the ointment than originally planned. In Table 4 are shown the results of deliberately halving the inunction areas which were routinely 8 per cent of the total body surface. The amount of mercury stored in the kidney is not halved but reduced by roughly one-third. From these data it can be inferred that small changes in exposure area cannot seriously influence the outcome of the results.

4. *Effect of Weight, Sex, and Litter*

The studies were generally conducted on groups of rats homogeneous with respect to age, weight, and sex, but heterogeneous with respect to litter. As examples of three possible variants, litter, sex, and weight were studied. It may be presumed that these variants could operate to produce differences between skin as characterized by such factors as texture, thickness, subcutaneous fat, number of hair follicles, etc., or differences between organs, such as the ability of the kidney to excrete or store mercury. It was found, however, that the cutaneous penetration of mercury, as judged by the level in the kidneys, is not significantly affected by the above factors.

TABLE 3—EFFECT OF THE LOCATION OF THE INUNCTION SITE ON THE CUTANEOUS PENETRATION OF MERCURY IN THE RABBIT

Storage of mercury in the kidney is used as a measure of penetration

Calomel Ointment	Micrograms Mercury per Gram Wet Kidney	
	Back Skin	Belly Skin
1	26	35
2	45	38
3	17	27
4	20	18
5	6	7
6	22	26
Av.	23	25

The results are averages of two animals on each treatment.

TABLE 4—EFFECT OF SIZE OF INUNCTION AREA ON THE CUTANEOUS PENETRATION OF MERCURY IN THE RAT

Storage of mercury in the kidney is used as a measure of penetration

Calomel Ointment	Micrograms Mercury per Gram Wet Kidney	
	Standard Area 29 (Cm.)^2	Half of Standard Area 14.5 (Cm.)^2.
C	5.3	3.6
D	29	23

The results are averages of six animals on each treatment.

5. *Species Difference*

Provided the same percentage of total body area is treated with an excess of ointment, no significant difference between the skin permeability of the rat and rabbit can be demonstrated by measuring the storage of mercury in the kidney. This was tested with two ointments of widely different penetrating capacities. The results are shown in Table 5.

6. *Removal of Excess Ointment*

Excess ointment was allowed to remain in contact with the skin routinely. The effect of removal of the excess by mechanical means causes quite a marked decrease in the absorption of mercury. This is shown in Table 6. These results are in consonance with the chemical experience of Cole and coworkers (7) who have reported that there is less penetration of mercury after the so-called "clean" inunction.

7. *Skin Conditioning*

(*a*) *Washing.* Ordinarily the openings of the skin, such as the hair shafts and sebaceous ducts which are regarded as the avenues of entrance for mercury, may be partially occluded by the presence therein of cell detritus and oily secretions. Cleansing the skin with soap and water would be expected to remove some of this "plugging" material without the more vigorous effects which follow the application of lipoid solvents such as alcohol or ether. However, a comparative study of washed and unwashed skins in two groups of rats showed definitely that treatment of the skin with soap and water, prior to inunction, had no effect on cutaneous penetration of mercury as measured by storage of the metal in the kidney even though the cleaning operation was seen to remove a visible yellowish scale of dander-like material.

(*b*) *Pretreatment With Lard.* "Softening" of the skin by thoroughly inuncting lard into the site 24 hours before application of a mercury ointment had a tendency

TABLE 5—EFFECT OF ANIMAL SPECIES ON THE CUTANEOUS PENETRATION OF MERCURY

Storage of mercury in the kidney is used as a measure of penetration through 8 per cent of the total body area

Calomel Ointment	Micrograms Mercury per Gram Wet Kidney	
	Rat	Rabbit
C	5.3	4.5
D	29	25

The results are averages of six animals of each species on each ointment.

TABLE 6—EFFECT OF REMOVAL OF EXCESS OINTMENT REMAINING ON THE SKIN OF THE RAT AFTER 2 MINUTES' INUNCTION

Storage of mercury in the kidney is used as a measure of penetration

Micrograms Mercury per Gram Kidney	
Excess Removed After 2 Min.	Excess Remained 24 Hr.
22	41
20	29
4	22
10	31
15	18
21	23
Av. 15	27

to increase the permeability of the skin for mercury.

All of the ointments presented to us for evaluation contained 30 per cent calomel, which was suspended in vehicles representative of three generally recognized classes, namely, fat, water-in-oil, and oil-in-water. It soon became apparent, however, after a number of assays had been made, that while it was easily possible to demonstrate marked differences in penetration of mercury from different types of vehicles, it was not easy to explain these differences. This was so because the ordinary multicomponent ointment presented such a formidable array of possible variables affecting penetration of mercury that the task of unravelling these would become hopeless. It must be admitted that the complete picture of absorption of mercury, or for that matter, of any substance, must not only take into account the individual effect of each component of an ointment but also the likelihood of interaction between components.

8. Single Component Vehicles

In attempting to arrive at a partial answer we have considered the following components of ointments and used them as separate vehicles for calomel: water, petrolatum, mineral oil, anhydrous lanolin, hydrous lanolin, corn oil, lard, pro-

TABLE 7—THE CUTANEOUS PENETRATION OF MERCURY WHEN APPLIED AS A 30 PER CENT SUSPENSION OF CALOMEL IN A NUMBER OF SINGLE COMPONENT VEHICLES

The measure of penetration is the storage of mercury in the kidneys of the rat

Micrograms Mercury per Gram Wet Kidney									
Control	Water	Petrolatum	Mineral Oil	Anhydrous Lanolin	Hydrous Lanolin	Corn Oil	Lard	Propylene Glycol	Oleic Acid
Series A, Females*									
0.07	4.4	6.0	5.6	6.0	5.9	13	11	19	26
Series B, Males*									
0.17	4.1	5.6	5.2	5.6	...	11	10	16	..

* Average of six animals on each treatment.

pylene glycol, and oleic acid. The result of this study is shown in Table 7. It is at once apparent that the vehicle exerts a profound influence on absorption. One of the most striking facts, however, is that petrolatum, mineral oil, and lanolin, which have found such extensive use in many ointment bases, support a penetration of mercury which is quite low, in fact, not markedly greater than when the calomel is merely applied as a simple paste with water. It is at present not permissible to extend the interpretation of these surprising findings to other penetrants besides mercury, but it is, nevertheless, very tempting. Such results suggest very strongly that a reinvestigation of some of our commonly accepted vehicles should be undertaken.

9. *Double Component Vehicles*

An attempt was next made to determine what effect two-component vehicles would have on the penetration of mercury. Four of these were constructed from the single substances investigated under Table 7. Each vehicle consisted of equal parts of the two components. The results are shown in Table 8 with values for the separate components added from Table 7 in order to facilitate comparison. It would appear that when a substance which supports good penetration of mercury is mixed with one which supports poor penetration, the properties of the combination resemble the former rather than the latter, as shown with vehicles A, C, and D. On the other hand, vehicle B gives penetration of mercury which is indistinguishable from that of either component. Notably, neither component separately gives good penetration.

10. *Effect of Lard and Petrolatum*

It had been suspected for some time that the presence of petrolatum in large proportions in many vehicles was not conductive to good penetration of mercury. To con-

TABLE 8—THE CUTANEOUS PENETRATION OF MERCURY WHEN APPLIED AS A 30 PER CENT SUSPENSION OF CALOMEL IN DOUBLE COMPONENT VEHICLES

The measure of penetration is the storage of mercury in the kidneys of the rat

	Double Component Vehicle	Kidney*, Micrograms Mercury per Gram Wet Weight	Single Component Vehicle	Kidney, Micrograms Mercury per Gram Wet Weight
A	50% Lard 50% Anhydrous lanolin	11	Lard Anhydrous lanolin	10 5.8
B	50% Mineral oil 50% Anhydrous lanolin	5.9	Mineral oil	5.4
C	50% Corn oil 50% Petrolatum	10	Corn oil Petrolatum	12 5.8
D	50% Propylene glycol 50% Petrolatum	14	Propylene glycol	17

* Averages of six animals.

firm this, four different mercurials, ammoniated mercury, metallic mercury, yellow oxide of mercury, and calomel were suspended, respectively, in each of two double component bases consisting of equal parts of lard and lanolin and equal parts of petrolatum and lanolin. These vehicles were selected for study because they frequently form the main base for a number of commonly used ointments. The concentration of mercury was 25 per cent in all cases. In Table 9 is shown ammoniated mercury, while of little or no significance in vehicle E, is highly significant in vehicle A. Presumably this marked effect of the vehicle may be attributed to some interaction with the compound, as has been suggested by Moncorps (*8*) who observed that the stability of ammoniated mercury was somewhat uncertain. It is, therefore, not unlikely that the reactivity of a compound with its vehicle may be one of the causes of differences between compounds.

TABLE 9—COMPARISON BETWEEN TWO TYPES OF VEHICLES CONSISTING OF LARD AND PETROLATUM WITH LANOLIN AS COMMON CONSTITUENT ON THE PENETRATION OF FOUR DIFFERENT MERCURIALS

The concentration of mercury in all ointments is 25 per cent. The measure of penetration is the storage of mercury in the kidneys of the rat

	Vehicle	Micrograms Mercury per Gram Wet Kidney			
		Calomel	Ammoniated Mercury	Metallic Mercury	Yellow Oxide Mercury
E	50% Petrolatum 50% Lanolin	4.2	5.5	7.9	11
A	50% Lard 50% Lanolin	8.8	19	14	23

The results are averages of six animals on each treatment.

the marked superiority of the lard-component vehicle over the petrolatum-component vehicle. The increase in penetration of mercury from calomel, metallic and yellow oxide is roughly twofold, but in the case of the ammoniated mercury the increase is nearly fourfold. Besides the profound difference in penetration of mercury, which can be attributed directly to the influence of the vehicle, it must be emphasized that the compound of mercury also has an effect on penetration, yellow oxide giving the highest and calomel the lowest. It is to be noted that the difference between calomel and

11. Effect of Wetting Agents

Numerous observations have indicated that the presence of wetting agents might be very important to the penetration of substances through the skin. In order to study the effect of wetting agents on mercury penetration, two factors were considered: (1) type of wetting agent, (2) type of vehicle. For the first experiment a well-known aryl alkyl polyether alcohol (Triton NE) was mixed in 2 per cent concentration in three different bases, representative of (A) a pure animal fat, (B) a pure mineral base, (C) an oil-

TABLE 10—CUTANEOUS PENETRATION OF MERCURY AS AFFECTED BY THE ADDITION OF TRITON NE TO THREE DIFFERENT TYPES OF VEHICLES

The measure of penetration is the storage of mercury in the kidneys of the rat

Micrograms Mercury per Gram of Wet Kidney					
Ointment A		Ointment B		Ointment C	
Without Wetting Agent	With 2% Wetting Agent	Without Wetting Agent	With 2% Wetting Agent	Without Wetting Agent	With 2% Wetting Agent
9.1	13	5.7	7.0	6.7	13
Significant $p = 0.03$		Difference Between Groups Not Significant $p > 0.05$		Significant $p = 0.02$	

Composition of Vehicle A		Composition of Vehicle B		Composition of Vehicle C	
Anhydrous lanolin	50%	White petrolatum	100%	Propylene	65.1%
d-Isoascorbic acid palmitate	0.1%			Cetyl alcohol	3.7%
Lard	47.8%			Stearic acid	4.0%
Beeswax	2.1%			Glycerol monostearate	17.0%
				KOH	0.18%
				Borax	0.02%
				Water	10.0%

The results are averages of eight animals on each treatment.

in-water emulsion type, the latter being chosen because its properties might be expected to be most modified by the presence of a wetting agent. The results are given in Table 10. There was no effect on B, the vehicle which contained no water. In the case of vehicles A and C, marked increase of mercury penetration was brought about by the wetting agent. The effect of the wetting agent on C is perhaps to be expected but on A more difficult to interpret because, like B, the vehicle contained no water.

With the indication that in certain bases, Triton NE enhances the penetration of mercury, it seemed of interest to determine whether differences between wetting agents could be demonstrated. The effect of 2 per cent concentration of Triton NE, Aerosol OT (sodium dioctyl sulfosuccinate) and Duponal C (sodium lauryl sulfate) in base A was studied. No differences in cutaneous penetration of mercury were found. Whether this would hold for the oil-in-water base or certain other types remains at present undetermined. The most that can be said for the tests with wetting agents is that the degree of increased penetration of mercury is disappointingly small as compared with that observed by Duemling (9).

12. Effect of Stiffening of the Vehicle

Many of the ointments submitted for assay were rather stiff and did not spread easily. It was noted that this characteristic seemed to

cause a lower penetration of mercury. To test the effect of the stiffening, an inert diluent, talc, was added in 15 per cent concentration to an oil-in-water base of the following composition:

Propylene glycol	25%
Starch glycerite	34%
Stearic acid	4%
Glyceryl monostearate	2%
Spermaceti	2%
Water	32%

Penetration of mercury from the above base, with and without 15 per cent talc filler, was compared. The result is shown in Table 11. Here it can be seen that the addition of an inert agent, with resultant stiffening action, reduces the penetration of mercury. In addition to these observations the problem had another more practical aspect: Ointments were designed which contained in addition to 30 per cent calomel, also 15 per cent sulfathiazole, the latter being added in powder form as a diluent of the base. All of the bases containing the sulfathiazole were stiffer and somewhat less spreadable. Upon examination of 6 different ointments representing oil-in-water, animal fat, and petrolatum base, it was clearly shown that the presence of sulfathiazole reduced the penetration of mercury by about 33 per cent. In no case was there evidence of chemical action between the mercury and the sulfur.

13. State of Subdivision of Calomel

Distinction was made between two different calomel powders on the basis of particle size. (a) Ordinary fine milled: range of size 10 to 100 micra. (b) Micronized: range of size 1 to 10 micra. It seemed desirable to determine what effect particle size would have on the penetration of mercury. Four different types of vehicles were used, oil-in-water, water-in-oil, 100 per cent petrolatum, and 100 per cent propylene glycol. In selecting these vehicles cognizance was taken of the possibility that the effect of particle size on penetration might be related to solubility of the finely divided calomel. Hence, two of the vehicles contained water and propylene glycol. Table 12 shows the results. It is clear that better penetration is obtained with micronized calomel but the effect is not favored by any particular vehicle. Possibly the better penetration of mercury from the ointment containing micronized calomel may result from the greater facility with which these small particles can be forced into the skin appendages.

TABLE 11—EFFECT OF A STIFFENING AGENT (TALC) ON THE CUTANEOUS PENETRATION OF MERCURY THROUGH THE SKIN OF INDIVIDUAL RATS

The measure of penetration is the storage of mercury in the kidney

Micrograms Mercury per Gram of Wet Tissue	
Calomel Ointment +15% Talc	Calomel Ointment Without Talc
13	23
14	18
17	16
13	13
19	24
17	22
Av. 15	20

14. Concentration of Mercury

Since the application of mercurial ointments was always designed to leave an excess on the skin, the question arose whether reduction in concentration of the mercurial might be affected without seriously reducing its cutaneous penetration. The possibility of conserving mercury by this means was obvious. Four different mercurials were examined, of which three, the metallic 50 per cent and 10 per cent, the ammoniated 4 per cent, and the yellow oxide 0.93 per cent are official U.S.P. preparations. Adjustments were made to give a large range of concentration of mercury. The results are given in Table 13. With the exception of the yellow oxide, where a 25-fold increase in mercury produced a significant rise in cutaneous penetration, it can be seen that changes in concentration of mercury produce relatively little change in mercury penetration. It should be emphasized that these results can only be interpreted strictly from short 24-hour exposures. When the skin exposure schedule is extended up to 4 days these higher concentrations of mercury do lead to greater penetration of mercury. This is in consonance with clinical experience of Cole, *et al.* (1).

TABLE 12—EFFECT OF PARTICLE SIZE OF CALOMEL ON THE CUTANEOUS PENETRATION OF MERCURY

The measure of penetration is the storage of mercury in kidney tissue of the rabbit and the rat

Micrograms Mercury per Gram Wet Kidney		
	Range of Size of Calomel Particles	
Type of Vehicle	10–1000 Micra	1–10 Micra
100% petrolatum*	16	26
Oil-in-water*	18	35
Water-in-oil*	11	32
100% propylene glycol†	15	28
Av.	15	30
Increase in penetration		100%

* Average of two rabbits on each treatment.

† Average of six rats on each treatment.

TABLE 13—EFFECT OF MERCURY CONCENTRATION IN THE CUTANEOUS PENETRATION OF MERCURY

The measure of penetration is the storage of mercury in the kidney tissue of the rat

Calomel		Metallic Mercury			Ammoniated Mercury		Yellow Oxide Mercury	
Per Cent Metallic Mercury								
25	8.3	50	25	10	25	4	25	0.93
Micrograms Mercury per Gram of Wet Kidney								
7.9	6.8	12	13	10.5	12	11	13	7.0
Vehicle		Vehicle			Vehicle		Vehicle	
Petrolatum 100%		Oleate of mercury		4%	Anhydrous lanolin	5.3%	Anhydrous lanolin	1.0%
		Lanolin		60%	White petrolatum	94.7%	Yellow petrolatum	99.0%
		White wax		10%				
		White petrolatum		26%				

The results are averages of six animals on each treatment.

15. Effect of Aging

Generally speaking, reassay of ointments after standing for months gave check results. In ointments containing lard, however, a measurable increase in penetration of mercury was observed. The work of Menschel (10) indicates that interaction between mercury and the lard base may take place with formation of mercuric oleate. According to our results, mercuric oleate gives by far the best penetration. In Table 14 are shown two assays of a lard containing calomel ointment, with an interval of 16 months intervening. It can be seen that after the prolonged contact between calomel and lard, significantly greater penetration of mercury occurred than from the freshly prepared ointment. By contrast no change occurred in the petrolatum vehicle.

TABLE 14—EFFECT OF AGING ON THE PENETRATION OF MERCURY FROM TWO CALOMEL OINTMENTS

The measure of penetration is the storage of mercury in the kidneys of the rat

	Micrograms Mercury per Gram of Wet Kidney	
	Assayed Immediately	Assayed After 16 Months
30% Calomel 70% Benzolinated lard	11	17
30% Calomel 70% Petrolatum	5.6	5.5

The results are the averages of six animals on each treatment.

SUMMARY

A new method for determining penetration of mercury through the skin has been worked out. This method depends on the principle that the quantity of mercury which accumulates in the kidney during a 24-hour cutaneous exposure to mercury is a measure of the penetration of this metal through the skin. The results of a study of a number of factors concerned in cutaneous penetration of mercury are as follows:

1. Covering the site of the skin inunction increases penetration nearly fourfold.
2. Location of the inunction site appears to have no effect on penetration.
3. Penetration through the skin of the rat and rabbit is approximately equal.
4. "Clean inunction" reduces penetration.
5. Skin conditioning has no marked effect on penetration.
6. Adjustments of the size of the exposure area are not critical for penetration.
7. Sex, litter, and weight have no significant influence on penetration.
8. The vehicle has the greatest influence on penetration, the compound of mercury less, and the concentration of mercury least.
9. Reduction in the particle size of calomel increases penetration.
10. "Stiffening" of an ointment tends to reduce penetration.
11. The effectiveness of wetting agents in increasing penetration is dependent on the type of vehicle.
12. There is evidence to show that the aging of certain calomel ointments, notably those containing lard, increases penetration.

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