STUDIES ON THE RETENTION OF HEXACHLOROPHENE (G-11) IN HUMAN SKIN

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Received for publication June 19, 1948

Recent studies (Traub, Newhall, and Fuller, 1944, 1945; Seastone, 1947; Gump, 1945) on the compound hexachlorophene (G-11) (2,2'dihydroxy 3,5,6,3',5',6'hexachloro-diphenyl-methane)[1] have indicated a prolonged bacteriostatic effect on human skin. In order to explain this protracted effect, studies were conducted to determine whether hexachlorophene could be recovered from skin for several days following its application and whether the skin lipoids play any part in the retention of the material. As a control, the residual bacteriostatic effectiveness of zephiran chloride (dimethylbenzylalkylammonium chloride) was examined.

MATERIALS AND METHODS

One per cent hexachlorophene (G-11) liquid soap was prepared as follows: Ten grams of the monopotassium or monosodium salt of hexachlorophene[2] were dissolved in 50 ml of hot 95 per cent ethyl alcohol. This alcoholic solution was added with thorough mixing to 1 liter of soap solution prepared by dissolving 200 grams of clear potash soap in 800 ml of hot distilled water.

The method of determining numbers of skin organisms was essentially that of Price (1938) as modified by Pohle and Stuart (1940). The individual to be tested wet his hands to a point approximately 1 inch above the wrist joint in a liter of sterile distilled water in a sterile basin; to this wet skin area Ivory soap was applied for 25 seconds and the hands were thoroughly massaged with the lather for 75 seconds. The hands were then rinsed for 25 seconds in the liter of sterile water, and, after stirring, 1.0- and 0.1-ml samples were plated. Counts were made after incubation at 37 C for 48 hours and reported as numbers of organisms per liter of wash water. To inhibit the hexachlorophene carried over in the wash water 1 per cent sterile sheep serum was added to the agar plates. The individuals used in the tests consisted in part of laboratory personnel, the remainder being students, with men and women about equally represented. Most of the counts obtained before treatment ranged from 1 to 10 million organisms per liter of basin water.

In determining the residual hexachlorophene in the skin, the application of the agent was carried out as follows: 3 to 5 ml of the liquid soap were applied to the hands after wetting the skin with tap water; the resulting lather was massaged into the skin for 3 minutes, rinsed in tap water, and the application repeated in the same manner, giving a total contact time of 6 minutes. After a final tap

[1] W. S. Gump, U. S. Patent no. 2,250,480.

[2] Obtained from Givaudan-Delawanna, Inc., Delawanna, New Jersey.

water rinse, the hands were dried and the residual hexachlorophene was extracted from the skin of both thumbs by inserting each thumb in turn into the same 60-ml centrifuge tube containing 15 ml of ether. The tube was inverted for 1 minute over each thumb, with Penrose tubing connecting the thumb and the tube to prevent leakage. The ether was then distilled off with a water-cooled condenser. Ten ml of broth were added to the residue, and the mixture was sterilized by autoclaving at 15 pounds pressure for 15 minutes. This redissolved ether extract was diluted serially with sterile broth, and 5.0 ml were inoculated with 0.1 ml of a 24-hour broth culture of *Staphylococcus albus*, standardized turbidimetrically. These tubes were then incubated at 37 C and read at 24-, 48-, and 72-hour intervals. Control dilutions in broth showed that the autoclave temperature had no effect on the activity of hexachlorophene.

A series of controls in which the thumbs of 50 individuals were extracted with ether prior to contact with hexachlorophene failed to show any inhibitory effect

TABLE 1

Amount of extractable hexachlorophene at varying time intervals following three consecutive six-minute daily washes

TIME AFTER HEXACHLOROPHENE*	INHIBITORY DILUTION OF EXTRACT	AVERAGE CALCULATED AMOUNT OF HEXACHLOROPHENE	AVERAGE AMOUNT OF HEXACHLOROPHENE PER SQUARE INCH OF SKIN
		μg	μg
Immediately	1/1,000 dil.	100	8.0
24 hours	1/50 dil.	5	0.4
48 hours	1/10 dil.	1	0.08
72 hours	No inhibition	None	—
96 hours	No inhibition	None	—

* Sum of 10 individuals per test.

in the undiluted extracts. The amount of hexachlorophene in 5 ml of broth necessary to inhibit growth for 48 hours at 37 C of the standard inoculum of *Staphylococcus albus* was found to be 0.1 μg (0.0001 mg). The highest inhibitory dilution of skin extract was assumed to contain this amount of hexachlorophene. The total average skin area of both thumbs was assumed to be 12 square inches. Chemical methods or the use of a spectrophotometer in quantitatively determining the amounts of hexachlorophene present in various solvents was found to be inadequate for detecting the small amounts encountered in this experimental work.

EXPERIMENTAL RESULTS

The amount of hexachlorophene deposited on the skin by three consecutive daily 6-minute washes with the agent in liquid soap was determined immediately afterward, and at 1-, 2-, 3-, and 4-day intervals following the last contact. Table 1 shows that approximately 8.0 μg of hexachlorophene were recovered from each square inch of skin immediately after the third application. Twenty-four hours after the third application the amount recovered was 0.4 μg per square

inch, and after 48 hours 0.08 μg was found. Residual hexachlorophene was not detectable 72 or 96 hours after the last contact with the agent.

The amount of hexachlorophene recovered when 2 per cent hexachlorophene bar soap[3] was substituted for the liquid soap tested above was determined. The same three consecutive daily 6-minute washes were employed. In six individuals the average amount recovered immediately after the last application was 0.8 μg per square inch of skin, or only one-tenth the amount recovered after a 1 per cent liquid soap.

A parallel series of tests with 1 per cent zephiran chloride was planned in order to determine its persistence in skin as compared to 1 per cent hexachlorophene. However, since no prolonged bacteriostatic effect was noted, further study of this material was abandoned. Table 2 presents the results indicating the absence of any residual effect on the skin flora 24 hours after three daily 6-minute appli-

TABLE 2

Comparison of residual effects of zephiran chloride and 1 per cent hexachlorophene following three consecutive six-minute daily washes

SUBJECTS	ORIGINAL COUNT	24 HOURS AFTER ZEPHIRAN CHLORIDE	SUBJECTS	ORIGINAL COUNT	24 HOURS AFTER HEXACHLOROPHENE	SUBJECTS	ORIGINAL COUNT	48 HOURS AFTER HEXACHLOROPHENE
A	14,100,000	7,380,000	F	2,830,000	230,000	K	23,200,000	441,000
B	4,810,000	3,480,000	G	9,560,000	430,000	L	7,740,000	231,000
C	5,230,000	3,490,000	H	3,180,000	90,000	M	14,000,000	185,000
D	240,000	280,000	I	3,360,000	120,000	N	6,950,000	620,000
E	1,250,000	770,000	J	2,220,000	109,000	O	5,130,000	840,000

Figures represent the number of organisms per liter of wash water.

cations of zephiran chloride. The hexachlorophene effect is still evident 48 hours after application.

In view of the fat solubility of hexachlorophene noted by Gump (1945), an attempt was made to determine whether its residual bacteriostatic effect was due to solution and retention in the natural fats and oils of the skin. If this were true, the extraction of the skin lipoids before treatment with hexachlorophene should reduce the residual bacteriostatic effect. However, the opposite effect was obtained. The normal resident bacterial counts of six individuals were determined prior to the start of the experiment. The hands were then immersed for 2 minutes in equal parts of acetone and ether, and this extraction was repeated in order to ensure as complete removal of fats and oils as possible, after which the hands were soaped with 1 per cent hexachlorophene soap solution for 3 minutes and rinsed, and the procedure was repeated, giving a total contact time of 6 minutes. Twenty-four and 72 hours following this single hexachlorophene wash, skin counts were taken.

A control group of 6 individuals was tested after an ether-acetone wash without

[3] The solid soap was furnished through the courtesy of Johnson and Johnson, New Brunswick, New Jersey.

hexachlorophene; another group of 6 persons was studied after a single hexachlorophene 6-minute wash without ether-acetone pretreatment. Table 3 shows the greater and more prolonged effect of hexachlorophene on those persons whose skin had been defatted before application of the agent. The residual material recovered from the ether-acetone mixture showed no inhibitory effect upon hexachlorophene *in vitro*.

TABLE 3

Comparison of residual effect of hexachlorophene on defatted and normal skin

SUBJECTS	TREATMENT	BEFORE TREATMENT	24 HOURS AFTER WASH	72 HOURS AFTER WASH
A	Defatted with ether-acetone. 6-minute hexachlorophene wash	3,620,000	10,000	740,000
B		1,710,000	90,000	2,540,000
C		220,000	200,000	180,000
D		11,940,000	20,000	150,000
E		2,920,000	100,000	640,000
F		2,710,000	40,000	200,000
Average		3,840,000	76,600	762,000
G	Defatted with ether-acetone. No hexachlorophene wash	1,830,000	230,000	1,970,000
H		2,440,000	1,420,000	1,550,000
I		220,000	140,000	92,000
J		4,020,000	4,720,000	3,760,000
K		2,290,000	1,130,000	2,650,000
L		3,590,000	610,000	1,190,000
Average		2,400,000	1,370,000	1,868,000
M	6-minute hexachlorophene wash. No ether-acetone treatment	8,940,000	1,750,000	1,930,000
N		750,000	20,000	1,140,000
O		2,380,000	210,000	1,160,000
P		11,230,000	4,630,000	3,150,000
Q		7,340,000	1,170,000	2,220,000
R		4,330,000	123,000	120,000
Average*		5,830,000	1,300,000	1,620,000

The figures represent the number of organisms per liter of wash water.

* The counts shown above are higher than those in table 2 because only a single hexachlorophene application was used.

In a previous study (Seastone, 1947), it was noted that the bacteriostatic potency of hexachlorophene was depressed about 100-fold by the addition of 1 per cent serum to the broth or agar used for testing. A brief experiment was conducted to determine whether albumin or globulin is responsible for this effect. Sterile purified egg albumin was diluted in sterile water to a concentration comparable with that of normal serum albumin (4.8 per cent). One-tenth-ml amounts of this solution were added to 9.8-ml amounts of sterile broth containing

varied concentrations of hexachlorophene. These were inoculated with 0.1 ml of the standarized 24-hour broth suspension of *Staphylococcus albus*, incubated at 37 C, and read at 24-, 48-, and 72-hour intervals. Purified human globulin was similarly diluted to a concentration comparable with that of normal serum (1.9 per cent) and the above-described procedure was repeated. Controls on the albumin, globulin, whole serum, and hexachlorophene broth were included. It appears from table 4 that both globulin and albumin show the hexachlorophene-inhibiting effect of whole serum.

TABLE 4

Depressing effect of serum components on bacteriostatic action of hexachlorophene

Hexachlorophene.....	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Control.....	−	−	−	+	+
1% serum.....	−	+	+	+	+
0.0483 % albumin.....	−	+	+	+	+
0.0189% globulin.....	−	+	+	+	+

\+ = growth of standard inoculum of *Staphylococcus albus* in 72 hours.
− = no growth in 72 hours.

DISCUSSION

The prolonged bacteriostatic effect of hexachlorophene, as might be expected, is explained by retention in the skin of detectable amounts of the material during the time interval in which its effect on the skin flora is apparent. When a solid soap vehicle was substituted for a liquid, the amount of retained hexachlorophene was reduced about tenfold. This is of interest in view of a similar observation (Seastone and Erickson, 1948) based on skin counts, in which it was shown that the solid soap vehicle is also less effective in reducing the resident bacterial skin flora. The most obvious explanation for this difference lies in the fact that a much larger amount of the agent comes into contact with the skin when it is dissolved in a liquid soap vehicle.

The unexpected enhancement of bacteriostasis following defatting of the skin may indicate that a larger reservoir becomes available for the retention of hexachlorophene following this treatment. It implies that the residual hexachlorophene activity is not due to solution and retention in the lipoids of the skin.

One finding of incidental interest, not reported elsewhere in this paper, is the rare occurrence of unusually high skin counts in individuals using hexachlorophene. This has been seen only twice in our experience, the counts being several million per liter of wash water. In both cases the skin flora proved to be entirely gram-negative bacilli of the coliform group and in both cases these high counts were transitory since tests after a few days' continued use of the compound showed characteristic low counts. The phenomenon could not be induced again in these individuals, indicating that a permanent resistant flora was not established.

SUMMARY

Hexachlorophene (G-11) has been recovered from skin 2 days after three consecutive daily 6-minute applications of 1 per cent solution in liquid soap.

Approximately ten times less hexachlorophene was recovered from skin following the use of a 2 per cent preparation in solid soap.

One per cent aqueous zephiran chloride exhibited no prolonged residual bacteriostatic effect on the skin.

Preliminary treatment of skin with acetone and ether increased and prolonged the bacteriostatic effect of hexachlorophene in the skin.

The effect of whole serum in reducing the bacteriostatic effect of hexachlorophene could be duplicated by egg albumin or serum globulin.

REFERENCES

GUMP, W. S. 1945 Development of a germicidal soap. Soap San. Chem., **21**, March, 36; April, 50.

POHLE, W. D., AND STUART, L. S. 1940 The germicidal action of cleansing agents. J. Infectious Diseases, **67**, 275–281.

PRICE, P. B. 1938 New studies in surgical bacteriology and surgical technique with special reference to disinfection of skin. J. Am. Med. Assoc., **111**, 1993–1996.

SEASTONE, C. V. 1947 Observations on the use of G-11 in the surgical scrub. Surg. Gynecol. Obstet., **84**, 355–360.

SEASTONE, C. V., AND ERICKSON, T. C. 1948 A comparison of solid and liquid soap as vehicles for G-11 (hexachlorophene) in the surgical wash. *In press.*

TRAUB, E. F., NEWHALL, C. A., AND FULLER, R. J. 1944 Studies on the value of a new compound used in soap to reduce the bacterial flora of the human skin. Surg. Gynecol. Obstet., **79**, 205–216.

TRAUB, E. F., NEWHALL, C. A., AND FULLER, R. J. 1945 New cutaneous bactericidal agent used in soap. Arch. Dermatol. Syphilis, **52**, 385–388.