Percutaneous Toxicity of Thioglycolate Mixtures in Rabbits

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Abstract □ The percutaneous absorption of pH 9.3, 0.60 *N* ammonium thioglycolate was ascertained in rabbits. Applications to a clipped skin site were made daily for 20 days, and the mortality rate was followed over 3 weeks. Surfactants, mainly anionic in nature, were incorporated into the mixtures at 0.5% activity, and the LD_{50} or the daily dosage causing 50% mortality was determined. The lowest toxicity was displayed by mixtures with sodium oleate, sodium stearate, and alkyl aryl sulfonate (VII) (LD_{50}: 4.1–4.4 ml/kg); intermediate activity was found with the sodium salt of alkyl aryl polyether sulfonate (III), a protein condensation product with oleyl chloride (IV), and sodium *N*-methyl-*N*-"coconut oil acid" taurate (VI) (LD_{50}: 3.4–3.7 ml/kg); and the highest activity was found with technical lauryl sulfate (V), the nonionic aromatic polyglycol ether condensate (I), and the cationic alkyldimethylbenzylammonium chloride (II) (LD_{50}: 2.3–2.8 ml/kg). The surfactant-free solution was relatively innocuous at 6.5 ml/kg/day. The LD_{50} fell with increased levels of wetting agents. The pH 9.3 sodium thioglycolate lotion with 4.0% III lowered the LD_{50} compared to the corresponding ammonium thioglycolate mixture. The percutaneous toxicity decreased when treatment was interrupted for several daily periods. Some insight into the penetration of 0.60 *N* thioglycolate through the skin was afforded by administration of the ^{35}S-compound at 1.0 ml/kg. The application of a pH 8.6 ammonium thioglycolate mixture with 4.0% III at 1.0 ml/kg over 20 days increased urinary ^{35}S-excretion; the activity fell during the last half of the tests. Although the tracer experiments showed some correlation with the general qualitative findings for several of the "cold" lotions, discrepancies were noted in relation to the LD_{50} values.

Keyphrases □ Thioglycolates—percutaneous toxicity, effect of surfactants, percutaneous penetration, rabbits □ Cosmetics—thioglycolates, percutaneous toxicity, effect of surfactants, percutaneous penetration, rabbits □ Burns, chemical—thioglycolates, percutaneous toxicity, effect of surfactants, percutaneous penetration, rabbits

The evaluation of the percutaneous irritancy of surfactants and other agents is a constant concern to workers in the cosmetic field. Extrapolation of animal skin patch tests to humans poses many problems. Among the earlier approaches toward surfactant screening (1) was an acute test involving the administration of large amounts of product to the clipped rabbit trunk over which a rubber sleeve was fitted. In subacute tests, lower dosages were applied to the clipped skin daily for 20 or 90 days. An occasional reversal in the results between humans and the animal species was noted. Since then, numerous reports have described patch testing procedures in humans and animals with cosmetic formulations and wetting agents and have presented pertinent statistical designs (2, 3) and physicochemical considerations in the absorption (4, 5) and anatomical changes (6). Middleton (7) noted that surfactants reduced the water binding with the stratum corneum in humid atmospheres. Good correlation between animal and human patch tests was found with highly active irritants and nonirritants, but poor agreement was found with materials of mild and moderate activity (8).

Although thioglycolates, especially the ammonium salt, have been used for many years in the permanent waving of hair, quantitative percutaneous toxicity data are sparse. Draize *et al.* (9) advanced preliminary subacute toxicity findings for rabbits administered a commercial 7% ammonium thioglycolate lotion daily at 0.5–4.0 ml/kg. In a later report (10), a 25% sodium thioglycolate solution containing a tracer amount of ^{35}S-thiol and without a wetting agent was applied once to the clipped skin of several rabbits at specified levels. Urinary specimens were withdrawn by catheter hourly over 4–5 hr, and the ^{35}S-activity was ascertained. Although significant penetration occurred, the amount absorbed per unit time showed little relation to the applied dosage. Other percutaneous tracer studies have been carried out *in vitro* with ^{14}C-labeled steroids (11), *in vitro* and *in vivo* with ^{35}S-dimethyl sulfoxide, (12), and *in vitro* with ^{3}H- and ^{14}C-labeled compounds employing human and hairless mouse skin (13).

In the current investigation, rabbits were subjected to 20-day percutaneous tests with pH 9.3, 0.60 *N* thioglycolate with ammonium hydroxide, among other formulations. Emphasis was on the changes in toxicity elicited by the type and level of wetting agent. The LD_{50}, expressed as the daily thioglycolate dosage, was deduced for these formulations by the usual statistical practice. For several mixtures, ^{35}S-thioglycolate was introduced and the urinary radioactivity was determined for single and repeated daily application to the skin site.

EXPERIMENTAL

Test Materials—Thioglycolic acid of high purity was distilled under vacuum. For the mixtures, ammonium hydroxide was added to the acid solution to yield 0.60 *N* ammonium thioglycolate with a final pH of 9.3, corresponding to 0.50–0.53 *N* free ammonia. The commercial wetting agents[1] were incorporated generally at 0.5% activity. With one product (L-7), the acid was treated with ammonium carbonate solution to a final concentration of 0.80 *M* and the pH was increased from 8.2 to 8.6 with ammonium hydroxide. Another mixture (L-19) was adjusted to pH 9.3 with sodium hydroxide.

For the product containing 0.5% active sodium oleate (L-3), the addition of the soap caused creaming on standing. With sodium stearate, the solution of the soap in hot water was cooled, and on addition to ammonium thioglycolate, some precipitation occurred in the resulting system (L-4). Preliminary tests also were instituted with a lotion (L-20) containing 0.20 *N*-oxidized salt, diammonium dithiodiglycolate of pH 9.30 with ammonium hydroxide (reducing agent concentration 0.050 *N*; detergent II at 4.0% activity).

Rabbits of either sex were purchased from one Illinois farm and housed individually. They were administered a stock pelleted feed and water *ad libitum*. The animals were observed for 2 weeks before use, at which time they weighed 2.3-3.0 kg. With few exceptions, the minimum number employed per dosage was 11.

General Animal Treatment Procedure—A right lateral site comprising about 15% of the body surface was clipped closely with electric clippers 3 days prior to treatment with the mixtures. Animals displaying nicks or cuts were excluded. A volume of mixture based on body weight was delivered to the skin site with a glass syringe with intermittent in-

[1] In the tables and text, the commercial wetting agents or surfactants are: I, Neutronyx 600 (aromatic polyglycol ether condensate; Onyx Oil & Chemical); II, BTC (alkyldimethylbenzylammonium chloride; Onyx Oil & Chemical); III, Triton X-200 (sodium salt of alkyl aryl polyether sulfonate; Rohm & Haas); IV, Maypon 4C (protein condensation product with oleyl chloride; Maywood Chemical Works); V, Duponal WA (technical lauryl sulfate; du Pont); VI, Igepon TC-42 (sodium *N*-methyl-*N*-"coconut oil acid" taurate; Antara Chemicals, Division of General Aniline); and VII, Nacconal NRSF (alkyl aryl sulfonate; National Aniline, Division of Allied Chemical & Dye). The respective thioglycolate lotions are designated L-1 *et seq.*

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Table I—Percutaneous Toxicity Data for Thioglycolate Mixtures [a] (20-day Tests)

Mixture	Ammonium Thioglycolate, *N* (pH)	Wetting Agent, Activity Percent, Type	Dosage Range Explored, mg/ml	Number of Animals	Mean Number of Applications prior to Death	Slope of Probit–Log Dose Curve	LD_{50}, ml/kg/day (mg of Thioglycolic Acid/kg/day) [b]
L-1	0.608 (9.32)	None	6.5	12	12[c]	—	>>6.5 (>>365)
L-2	0.613 (9.32)	I, 0.5, nonionic	2.0–2.75	36	8	4.24	2.61 ± 0.33 (147 ± 18.6)
L-3	0.593 (9.34)	Sodium oleate, 0.5, anionic	3.75–4.5	41	9	15.38	4.08 ± 0.10 (223 ± 5.5)
L-4	0.603 (9.34)	Sodium stearate, 0.5, anionic	3.75–4.5	42	13	26.02	4.24 ± 0.06 (236 ± 3.3)
L-5	0.610 (9.34)	II, 0.5, cationic	2.0–3.0	50	7	13.33	2.34 ± 0.06 (132 ± 3.4)
L-15	0.606 (9.34)	II, 4.0	0.95–1.5	32	8	7.21	1.43 ± 0.11 (79.7 ± 6.1)
L-21	0.600 (9.34)	II, 10.0	0.70–1.0	33	8	7.72	0.906 ± 0.066 (50.0 ± 3.6)
L-6	0.610 (9.34)	III, 0.5, anionic	3.0–4.0	37	11	5.22	3.50 ± 0.25 (197 ± 14.0)
L-7	0.600 (8.60)	III, 1.0	3.0–3.75	58	9	12.54	3.42 ± 0.12 (188 ± 5.5)
L-12	0.609 (9.29)	III, 1.0	3.0	11	12[d]	—	Four dead; 36.4% mortality
L-14	0.597 (9.32)	III, 4.0	1.75–2.5	33	10	4.81	2.11 ± 0.25 (116 ± 3.7)
L-19	0.600 (9.31 with sodium hydroxide)	III, 4.0	2.0–2.5	35	8	7.94	1.69 ± 0.11 (93.3 ± 6.1)
L-8	0.610 (9.35)	IV, 0.5, anionic	2.0–4.0	36	12	6.70	3.69 ± 0.21 (207 ± 11.8)
L-8A	0.600 (9.35)	IV, 0.15	4.0	20	19	—	Four dead; 20.0% mortality
L-9	0.610 (9.35)	V, 0.5, anionic	2.5–3.0	35	11	15.99	2.75 ± 0.07 (155 ± 3.9)
			3.0	11	9	—	Eight dead; 72.7% mortality
			4.0[e]	9	9	—	Three dead; 33.3% mortality
L-11	0.606 (9.32)	VI, 0.5, anionic	3.0–4.0	36	11	10.00	3.44 ± 0.14 (192 ± 8.9)
			3.0	11	13	—	Four dead; 36.4% mortality
L-11A	0.600 (9.35)	VI, 0.125	3.0	12	(20)	—	One dead; 8.3% mortality

[a] The pH of each mixture was brought to 9.3 with ammonium hydroxide except for L-7, which contained ammonium carbonate, and L-19, which contained sodium hydroxide. [b] The LD_{50} applies to the daily dosage causing death in 50% of the animals treated for 20 days and observed for 3 weeks. The dosage, in milligrams, is based on thioglycolic acid. The standard error of the mean appears after each ± sign. [c] The lone death occurred after 12 applications. [d] As with L-12, the mortality was 36.4% with L-6 at 3.0 ml/kg (11 animals). [e] Applied on 4 consecutive days followed by a rest period of 3 days (20 applications; duration of 32 days). Two rabbits died following application 12; the third one died after the fourth application.

unction with the side of the syringe. The procedure was well standardized and performed by one individual. Body weight was determined daily, and the dosage was adjusted accordingly.

The tests were conducted daily for 20 consecutive days, and the animals were observed for 3 weeks after the last application. Tissues from animals succumbing before the conclusion of the tests and from those sacrificed after the observation interval were examined grossly and microscopically. Except as otherwise stated, at least three dosages were investigated per mixture; the protocol allowed testing of up to three formulations at a given time. If necessary, the animals were restrained by hand.

The percutaneous LD_{50} was determined by converting the percentage mortalities to the probits and plotting the probits *versus* log dosages according to literature methods (14,15).

^{35}S-Thioglycolate Studies—^{35}S-Labeled thiol was introduced at a definite level into the test mixture and then inuncted into the skin site as described. Single and repeated daily applications were made; with one series, four skin sites were clipped and the mixture was applied to a different area each day over 4 days. The animals were housed in circular steel cages, with the floors covered with hardware cloth. The total sulfur content of each 24-hr urine sample was ascertained by oxidation to the sulfate with the Folin reagent, precipitation with barium chloride, and counting the tared barium sulfate (16).

RESULTS

Table I presents percutaneous toxicity data for 0.60 *N* thioglycolate brought to pH 9.3 with ammonium hydroxide and containing one nonionic wetting agent (I), the cationic agent (II), and the anionic detergents sodium oleate, sodium stearate, and III–VI, each at 0.5% activity, as well as II at 4.0 and 10.0% and III at 1.0, 2.0, and 4.0%. Agent III (4.0%) also was employed in the study of pH and sodium salt effects in 20-day skin tests. From at least three dosages per formulation, the LD_{50} values were ascertained. These values, together with the slope, *b*, of the probit–log dosage curves, are shown in the table. The effect of lower levels of IV (0.15%) and VI (0.125%) is also demonstrated for single dosages. A typical probit–log dosage curve appears in Fig. 1.

The LD_{50} refers to the daily level of a formulation killing 50% of the animals. The frequency of deaths varied over the 20-day testing period. The average number of applications preceding death is based on all dosages per mixture. The LD_{50} also is expressed in terms of milligrams of acid.

Mixtures Containing Surfactants at 0.5% Activity—Table I can be divided into three groups in order of increasing percutaneous toxicity.

Group A (LD_{50}: 4.1–4.4 ml/kg; L-3 and L-4)—With few exceptions, the respective sites treated daily with L-3 and L-4 were unirritated except for mild peripheral involvement which cleared before the last application. Several rabbits displayed prominent hair regrowth and "dry" skin conditions during the last half of the tests. Body weight losses were significant only in those animals that succumbed. The survivors recovered very rapidly over the 3-week observation period.

As with most of the test formulations, the mixtures were more easily absorbed following the first two or three applications, and some resistance was encountered during the last third of the tests. Death followed depression in two animals administered L-3 (3.75 and 4.0 ml/kg); convulsive activity ensued in two on L-4 (4.0 and 4.5 ml/kg), which also died. The average number of applications for all dosages employed prior to death was nine for L-3 and 13 for L-4.

Preliminary screening of percutaneous toxicity of a similar mixture containing the anionic agent, VII, at 0.5% activity indicated a 4.4-ml/kg LD_{50}, or 240 mg/kg in terms of the acid. Deaths occurred after an average of 12 applications. Extensive convulsive activity was noted in two animals, one receiving 3.0 ml/kg and the other receiving 4.0 ml/kg and succumbing 6 days after Treatment 20.

Group B (LD_{50}: 3.4–3.7 ml/kg; L-6, L-8, and L-11)—The mixtures containing III, IV, and VI produced similar LD_{50} values, and the skin sites

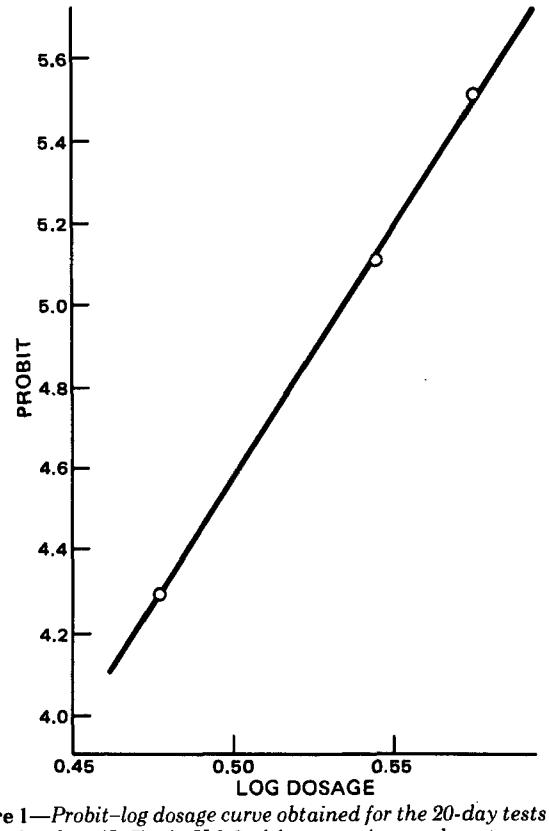

Figure 1—*Probit–log dosage curve obtained for the 20-day tests of 0.60* N *thioglycolate (L-7) of pH 8.6 with ammonium carbonate, ammonia, and 1.0% surfactant III.*

resembled those of Group A or were somewhat more irritated. Convulsive activity was displayed by four animals on L-6 (3.0 and 4.0 ml/kg; dead after four to nine applications), two on L-8 (3.0 ml/kg), and one on L-11 (3.5 ml/kg). One animal on L-8 at 3.0 ml/kg underwent severe convulsions after Treatment 20 but recovered during the 3-week observation period.

Group C (LD_{50}: 2.3–2.8 ml/kg; L-2, L-5, and L-9)—Greater toxicity was observed with the three mixtures containing I (L-2), III (L-5), and V(L-9). The skin sites with L-2 and L-9 were moderate in activity, especially at the peripheries. In marked contrast, L-5 provoked extreme skin alterations; intense inflammation arose after only one or two applications. This inflammation was followed by widespread irritation and necrosis so that inunction into the marginal sites was necessary with several rabbits due to the leathery consistency of the initially treated areas. Such animals screamed and struggled invariably during the first half of the tests, and body weight losses were excessive. A typical skin condition is illustrated in Fig. 2. Central nervous system involvement was apparent with two animals each on L-2 (3.0 and 3.5 ml/kg), L-5 (2.5 ml/kg), and L-9 (2.5 ml/kg).

Percutaneous Toxicity of Mixtures with Wetting Agents—Ammonium thioglycolate (L-1; detergent absent) was essentially without percutaneous effect (20-day tests) (Table I). Only one of 12 animals succumbed. Except for dry skin, involvement was minimal and hair regrowth was prominent during the second half of the tests. With the addition of a wetting agent, greater amounts of mixture were absorbed. Differences were apparent, depending on the detergent. Further studies were implemented to alter the levels of several wetting agents.

The LD_{50} of the thioglycolate formulation markedly decreased with increased II, the values being 2.34 ± 0.06, 1.43 ± 0.11, and 0.906 ± 0.066 ml/kg for mixtures containing 0.5, 4.0, and 10.0%, respectively (Fig. 3). Based on the testing of one group at a single dosage, III, the lotion with 1.0% (L-12), was similar in activity to the 0.5% mixture (L-6). The LD_{50} of L-7 with the wetting agent at 1.0% activity and pH 8.6 with ammonium carbonate at 0.80 *M* was similar to the L-6 LD_{50} (3.42 ± 0.11 ml/kg; for L-6, 3.50 ± 0.25 ml/kg). A much greater change was noted with the 0.60 *N* thioglycolate containing ammonium hydroxide at 0.60 *N* when the activity of III was raised to 4.0% (L-14; LD_{50}: 2.11 ± 0.25 ml/kg). A comparison of L-14 with the corresponding mixture of pH 9.31 with sodium hydroxide (L-19) showed the latter to be more toxic (LD_{50}: 1.69 ± 0.11 ml/kg or 93.3 ± 6.1 mg/kg based on the acid).

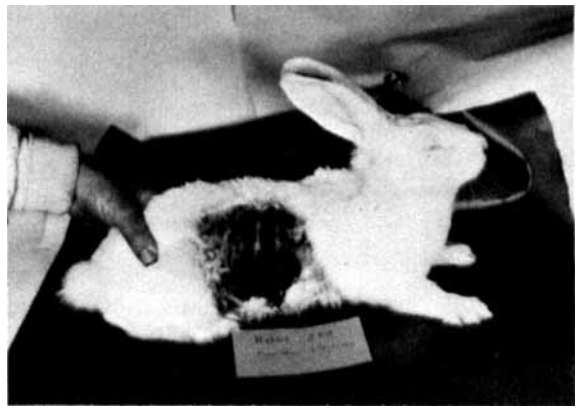

Figure 2—*Skin site of an animal after five daily applications of 0.60* N *ammonium thioglycolate containing 0.5% II (L-5) at 2.0 ml/kg/day.*

The effect of even lower detergent activities was screened with two of the anionic agents incorporated into pH 9.3, 0.60 *N* thioglycolate. With IV at 0.15% (L-8A), the mortality at 4.0 ml/kg was only 20% while the LD_{50} of L-8 was 3.7 ml/kg. The mean numbers of applications preceding death were 12 and 19 for L-8 and L-8A, respectively. Surfactant VI at 0.125% activity was responsible for one death of 12 animals at 3.0 ml/kg (L-11A) as compared to a mortality of 36.4% with L-11 at the same daily dosage.

Compound II at 4.0% activity also was incorporated into a mixture containing the oxidized salt, diammonium dithiodiglycolate at 2.0 *N* and adjusted to pH 9.30. The percutaneous application of L-20 at 3.5 ml/kg daily to five animals led to 100% mortality after an average of three treatments.

Relation of Percutaneous Treatment Frequency to Toxicity—Early runs with several mixtures showed that when 1 or more days, such as weekend periods, were missed in the percutaneous treatment schedule, toxicity decreased. The data are illustrated in Table I for L-9, which was applied for 4 days followed by a 3-day rest period. The se-

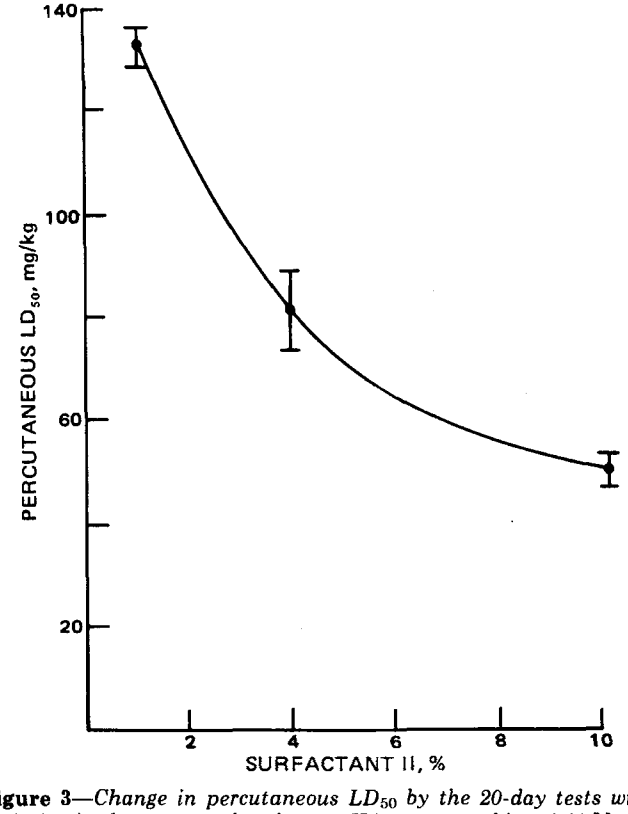

Figure 3—*Change in percutaneous LD_{50} by the 20-day tests with variation in the content of surfactant II incorporated into 0.60* N *ammonium thioglycolate (L-5, L-15, and L-21). The values are in milligrams of thioglycolic acid.*

Table II—Total Urinary ^{35}S Excreted over Periods up to 72 hr following a Single Percutaneous Application of Thioglycolate Mixtures at 1.0 ml/kg [a]

Product	Wetting Agent, Activity Percent	Percentage ^{35}S Excreted ± *SEM* at 24 hr	48 hr	72 hr
L-1	None	16.22 ± 0.55 (19)	4.29 ± 0.38 (14)	2.17 ± 0.30 (8)
L-1 [b]	—	17.23 ± 1.76 (3)	4.53 ± 1.11 (3)	1.73 ± 0.15 (3)
L-5	II, 0.5	20.85 ± 1.18 (8)	5.90 ± 1.12 (4)	1.98 ± 0.18 (4)
L-15	II, 4.0	22.10 ± 0.94 (7)	4.98 ± 0.34 (4)	2.00 ± 0.13 (4)
L-3	Sodium oleate, 0.5	7.72 ± 1.07 (5)	2.80 ± 0.34 (5)	1.07 ± 0.35 (5)
L-7 [c]	III, 1.0	18.12 ± 0.60 (17)	6.66 ± 0.82 (6)	1.90 ± 0.40 (4)
L-7 [b]	—	17.87 ± 1.09 (6)	5.28 ± 1.21 (4)	2.04 ± 0.45 (4)
L-14	III, 4.0	15.60 ± 1.45 (5)	4.40 ± 0.25 (4)	1.83 ± 0.10 (4)
L-19	III, 4.0 (with sodium hydroxide)	16.07 ± 0.71 (7)	4.50 ± 0.46 (5)	1.84 ± 0.22 (5)

[a] Composition of mixtures is given in Table I; pH was 9.3 with ammonium hydroxide except for L-7 (pH 8.6 with ammonium carbonate) and L-19 (pH 9.3 with sodium hydroxide). The decreases in 24-hr mean values for L-1, L-14, L-7, and L-19 compared to those for L-5 and L-15 were statistically significant at $p < 0.01$, except that for L-7 at 1.0 mg/kg *versus* L-5 (Fisher t value, 2.31; $p < 0.05$). The L-3 24-hr mean was markedly lower than any of the other averages, and the difference in L-5 and L-15 values was not significant. Number of animals is given in parentheses. [b] Dosage of 2.0 ml/kg. [c] For one animal, the ^{35}S of the treated skin site at 72 hr was 0.22%.

quence was repeated until 20 applications had been made. Three of the nine animals (33.3%) treated at 4.0 ml/kg succumbed, compared to a 72.7% mortality with a lower dose (3.0 ml/kg) applied daily on 20 consecutive days. The respective skin conditions were much milder in the series administered 4.0 ml/kg.

Anatomical Findings for Tissues from Thioglycolate-Treated Rabbits—Aside from the skin conditions, the gross and microscopic examination of tissues revealed little extreme alteration except for occasional lung congestion and, in a few cases, possible kidney cortical hemorrhage.

^{35}S-Thioglycolate Studies—Thioglycolate mixtures containing up to 0.40 μCi ^{35}S (most commonly 0.10–0.20 μCi) were applied to the skin site as in the "cold" series, and urine was collected over 24 hr following treatment and analyzed in duplicate.

Single Application—Urinary ^{35}S-levels 24, 48, and 72 hr following a single percutaneous thioglycolate mixture application at 1.0 ml/kg are shown in Table II. Based on the mean 24-hr values, the II-containing lotions L-5 and L-15 were responsible for percentages (20.85 ± 1.18 and 22.10 ± 0.94, respectively) that exceeded those for L-1, L-3, L-7, L-14, and L-19 as well as for L-1 or L-7 at 2.0 ml/kg. Mixture L-3 was responsible for the lowest excretion of ^{35}S at 24 hr (7.72 ± 1.07); at 48 and 72 hr, the values were 2.8 and 1.1%, compared to the respective means of 5.0 and 2.0% for the other mixtures. The residual ^{35}S-level was generally far below 1% at the 96th hr.

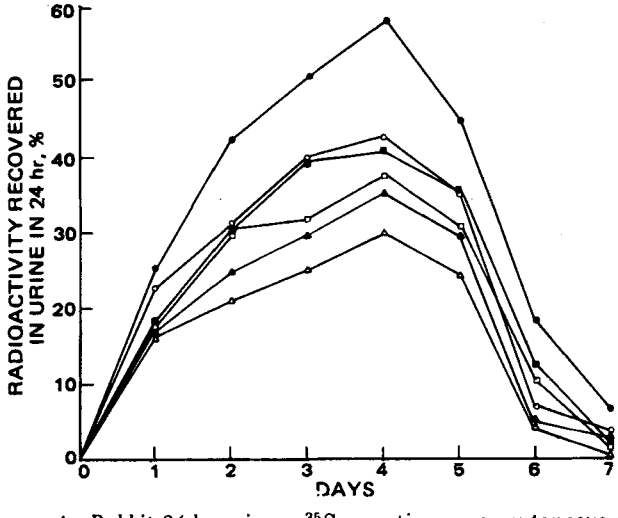

Figure 4—*Rabbit 24-hr urinary ^{35}S-excretion on percutaneous administration of the following mixtures concurrently in one series, each at 1.0 ml/kg/day and containing 0.1–0.2 μCi of labeled thioglycolate per dose for 4 days. Key: △, L-1, without wetting agent; ▲, L-19, with 4.0% III; □, L-7, with 1.0% III; ■, L-14, with 4.0% III; ○, L-5, with 0.5% II; and ●, L-15, with 4.0% II. The products comprised 0.60 N ammonium thioglycolate of pH 9.3 except for the sodium salt of the same pH (L-19) and the ammonium carbonate-containing system (L-7; pH 8.6). The additional points depict the residual ^{35}S-activity.*

Repeated Daily Applications—With more than one application of an agent at 1.0 ml/kg, further ^{35}S-distribution patterns could be discerned. Figure 4 presents graphical data for one series of rabbits inuncted daily for 4 days, the residual activity being included for 3 additional days. Mixture L-15 elicited the highest excretion of ^{35}S; L-5 and L-14 showed progressively lower activity, followed by L-7, L-19, and L-1. Both L-15 and L-1 present extremes in percutaneous efficacy with the remaining products being intermediate. This general pattern was observed with several other rabbits. Variations also were apparent in residual activity and were not dependent strictly on the applied isotope level.

A further illustration of the multiple-treatment schedule and of variations in ^{35}S-activity is presented in Fig. 5, where L-1 and L-7 were employed daily at 1.0 ml/kg for 5 days at levels of 0.203 and 0.043 μCi, respectively. The residual output of tracer was ascertained for an additional 4–5 days. With L-1, the overall urinary specific activity was 1.36×10^5 cpm and accounted for 37.6% of the isotopic dosage. With L-7, the corresponding figures were 3.65×10^4 cpm and 47.7%. The action of L-1 is shown in Fig. 6 for nine consecutive daily applications at 1.0 ml/kg. Treatment was not continued due to the development of anuria. Concurrently, a second animal was administered L-7 at the same dosage over 20 days. With this animal, the ^{35}S increased from the start but fell after the ninth application, reaching a low by the 13th day and then rising to a small plateau that persisted to almost the end of the tests. The residual activity thereafter was rather small.

Although many of the tests were conducted with thioglycolate systems at 1.0 ml/kg, much higher daily volumes of agent with incorporated ^{35}S also were investigated. No clear correlation could be drawn between dosage and absorption based on isotopic excretion. A few rabbits were anuric. With L-7 at 3.0 ml/kg applied daily for 5 days, the urinary ^{35}S-excretion amounted to 14.9, 30.7, 30.1, 28.7, and 27.1%, respectively, for Days 1–5. With two additional animals under the same conditions, the peak values at the 5th day were 18.0 and 16.4%. With a rabbit receiving L-19 at 2.0 ml/kg/day, the initial 24-hr value was 18.5%; the excretion levels fell progressively to 4.7% at the seventh application, and the animal succumbed 1 day later.

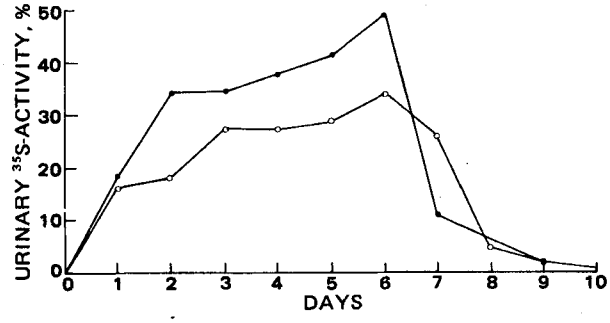

Figure 5—*Urinary ^{35}S-activity following percutaneous application of 0.60 N ammonium thioglycolate mixtures without wetting agent (L-1) (○) and with 1.0% III (L-7) (●) daily for 5 days. The ^{35}S-levels were 0.203 and 0.043 μCi/day with L-1 and L-7, respectively. The residual ^{35}S up to Days 9–10 was 13.8 and 13.7% of the total dosages applied based on urinary specific activities of 5.00×10^4 and 1.05×10^4 cpm for L-1 and L-7, respectively.*

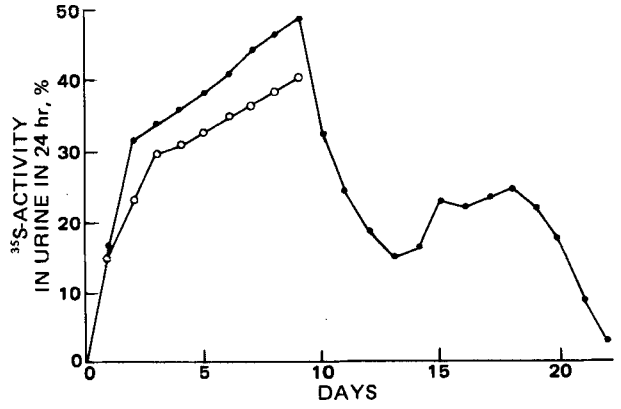

Figure 6—*Urinary ^{35}S-elimination with nine daily applications of the surfactant-free L-1 (○) and with 20 applications of L-7, containing 1.0% III (●). Since the rabbit treated with L-1 became anuric after the ninth treatment, further work was discontinued. The daily ^{35}S-dosage was 0.20 μCi.*

Repeated Application at Multiple Skin Sites—Percutaneous tests were instituted with L-5 and L-15 applied to rabbits with four clipped skin sites, a fresh one being inuncted with 1.0 ml/kg/day for 4 days. Histograms presenting the ^{35}S-excretion in such animals as well as in those with the single clipped skin site are shown in Fig. 7. Aside from little difference in each pair after the first treatment, animals with the multiple skin sites displayed higher isotopic elimination and the skin areas were less irritated. In another experiment employing L-15 (1.0 ml/kg) and multiple skin sites, the values were 18.2, 35.1, 38.3, and 41.3% at Days 1–4. With L-1 under comparable conditions, the urinary ^{35}S-levels were 12.4, 13.8, 17.1 and 15.8%, respectively, and were lower than the values from rabbits with a single clipped skin site.

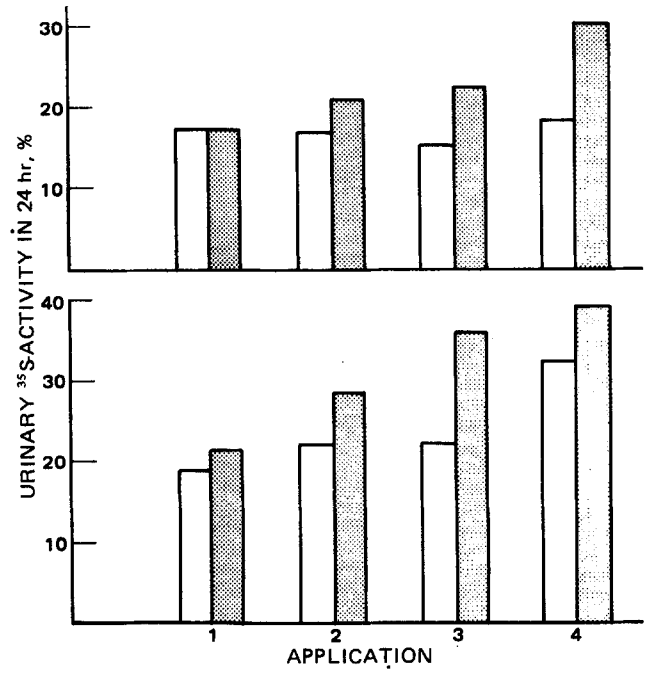

Figure 7—*Urinary ^{35}S-changes accompanying percutaneous 0.60 N ammonium thioglycolate containing II at 0.5% (L-5) (top) and at 4.0% (L-15) (bottom series) daily for 4 consecutive days. The solution was delivered either to the same clipped skin site (open areas) or to a different one of four sites each day (shaded areas). The daily ^{35}S-dosage was 0.20 μCi.*

DISCUSSION

The wetting agents were anionic except for the nonionic I and the cationic II. Wide differences in toxicity could be observed between the anionic agents compared on the basis of 0.5% activity by the 20-day tests. Thus, sodium oleate and stearate and, possibly, VII were responsible for the lowest toxicity of 0.60 *N* ammonium thioglycolate; III, IV, and VI were intermediate; V shared the highest toxicity with the nonionic and cationic surfactants. The solution without wetting agent, L-1, produced minimal skin alterations, and the LD_{50} was far in excess of 6.5 ml/kg.

The toxicity of thioglycolate with increasing surfactant percentage also was studied with II and III. The former was responsible for more extreme skin conditions, and the increase in percutaneous toxicity plateaued at ~10.0% (Fig. 3). Surfactant III caused little change in percutaneous activity at 0.5 and 1.0%. With 4.0%, the toxicity mounted but was below that elicited by the lotion with 4.0% II. The percutaneous toxicity of the pH 9.3 sodium salt solution exceeded that of the ammonium salt with 4.0% III as the wetting agent in each. As expected, a decrease in surfactant content, as illustrated by 0.15% IV (L-8A; Table I), led to a lowered percutaneous toxicity. Hair regrowth in the respective skin sites with several lotions was indicative of lower product irritancy; the hair growth cycle was minimally affected.

The derivation of percutaneous LD_{50} values in the present experiments is novel and in contrast to the usual parenteral toxicity. Repeated applications as in the 20-day testing schedule are mandatory for such data. A 2.0-ml/kg dose is the consumer level in hair waving as referred to the volume of product used and the consumer body weight. In addition, this method can shed light on percutaneous toxicity in regard to operators who are in almost daily contact with such lotions. In this report, the LD_{50} deduced for the various mixtures refers to mortality over 20 days of treatment followed by an observation interval of 3 weeks. Since the deaths resulted after an average of seven to 11 applications, an overall lethal dosage might be approximated. However, as noted from the ^{35}S-experiments, the percutaneous absorption was greatly reduced during the second half of the tests, at which time the respective skin sites underwent clearing and became more resistant to further inunctions.

An LD_{50} of 4.0 ml/kg would imply an overall dosage of 80 ml/kg or in excess of 4.4 g/kg based on thioglycolic acid for the survivors. Considering the large amount of mixture that is not absorbed due to poor wetting qualities coupled with losses as a result of evaporation, oxidation, and mechanical reasons, most of this dosage is not realized in the percutaneous testing. The large losses notwithstanding, many variables were controlled, so the resulting LD_{50} values do reflect the outstanding differences among the surfactants selected.

In another thioglycolate study, investigators (9) applied a 7% commercial ammonium thioglycolate lotion of undisclosed brand and wetting agent to rabbits for 90 days. The mortality at daily levels of 2.0 and 4.0 ml/kg was 11.8 and 61.1%, respectively. Such values might be in the range deduced for mixtures of similar thiol content in the current experiments and with VII or the sodium soaps at 0.5% activity.

^{35}S-Thioglycolate was incorporated into the test fluids to follow percutaneous absorption, emphasis being directed to levels of 1.0 ml/kg applied once or on repeated days. At single dosage, the ^{35}S-elimination by the II-containing mixtures L-5 and L-15 was in the same range but was significantly higher than the fluids containing III and sodium oleate as well as L-1 (Table II). In fact, the surfactant-free solution was as effective as the mixtures containing III and even exceeded the effectiveness of L-3 with sodium oleate in activity by the single-dosage criterion. At 2.0 ml/kg, L-1 and L-7 caused ^{35}S-excretion similar to that of the lower dosage, about one-fifth of the applied activity being eliminated over 72 hr.

In a previous study (10), single applications of a surfactant-free sodium thioglycolate solution to rabbits produced an average 29% excretion for five animals at 330 mg/kg as determined hourly for 4–5 hr after treatment and 7, 11, and 24% for three animals at 660 mg/kg. The last group succumbed in 24 hr. The extent of thiol absorbed was not closely related to the total applied. Compared to the current findings, the ^{35}S-activity with 330 mg/kg was somewhat higher than the 24-hr value for the sodium salt system containing 4.0% III (L-19), and the amount of salt inuncted was less (68 mg/kg).

The single-application series employing ^{35}S-labels presents discrepancies as already discussed in relation to the data of Table I. Inconsistencies persisted when repeated application of ^{35}S-thiol mixtures was instituted for 4 or 5 days, a period when skin irritation was usually prominent. As expected, tracer excretion was highest with L-15 and lowest with L-1, with the remaining products being intermediate (Fig. 4). The relative position of the latter may bear some qualitative relationship to the respective LD_{50} values, but the number of animals was too small for statistical testing. The sodium thioglycolate lotion (L-19) appeared to elicit a lower tracer excretion than the ammonium salt (L-14), both based on 4.0% III, but L-19 displayed a greater percutaneous toxicity (Table I). In contrast to the single-treatment series, ^{35}S-cumulation occurred, and it was difficult to correct for the prior residual activity with

each ensuing treatment. With either L-1 or L-7, each applied at 1.0 ml/kg daily for 5 days, the activity determined over 4–5 days after the final treatment was ~14%; the overall ^{35}S-excretion was 38 and 48% for L-1 and L-7, respectively (Fig. 5). A correlation might exist with the LD_{50} findings, a difference of 10% in isotopic elimination possibly being significant. At any rate, these discrepancies as well as those cited by others (10) bear out that percutaneous toxicological studies are fraught with difficulties.

^{35}S-Activity in one rabbit receiving L-7 at 1.0 ml/kg/day for 20 days reached a maximum at the 9th day and then fell rapidly. Based on cumulative activity, the peak may have been reached before the ninth treatment in this instance as well as with L-1 (Fig. 6). The question might be raised as to whether such tests should be continued daily for ≥20 days. Although the percutaneous penetration was apparently less during the last half of the tests, the absorption rallied over the final five to six inunctions, and a new peak or plateau was established. In addition, the seven to 11 applications preceding death were an average. Several animals malingered and succumbed toward the end of the treatment period and even into the 1st week of the observation interval.

To circumvent the extreme inflammation and necrosis at the skin sites with such products as L-5 and L-15, multiple areas were treated. A new site was involved for 4 days at 1.0 ml/kg/day. Greater absorption based on ^{35}S-excretion was noted for the second treatment compared to the single-site series. The present approach is well suited for extreme skin irritants and can yield further quantitative data in addition to those involving a single area that is repeatedly insulted. With L-1, the multiple-site method led to lower tracer excretion. Presumably, only after several applications at a given site with its ensuing irritancy, as small as it might be, was the percutaneous penetration heightened. This behavior also was apparent with L-3, a mixture that exceeded L-1 in toxicity (Table I) but that ranged lower than the latter in ^{35}S-activity elimination after a single application.

The most prominent form of sulfur excreted by the rabbit on tracer administration is neutral sulfur, followed by inorganic sulfur and ethereal sulfate (10, 16). Since oxidation is often cited as a source of thioglycolate activity loss, the disulfide, a prominent metabolite, also was screened. A diammonium dithiodiglycolate at 2.0 *N* of pH 9.3 with 4.0% II caused death after a few daily applications at 3.5 ml/kg or at 1150 mg/kg based on the acid. The thiol content as determined by titration was low (0.050 *N*). These exploratory runs justify further study of the disulfide as such and with ^{35}S-labeling.

The relative classification of the mixtures according to LD_{50} values and as spot-checked by ^{35}S-thioglycolate may have application to the human experience, but care must be taken in extrapolating such data. Along with the subacute methods, additional tests for cosmeceuticals should include acute toxicity coupled with primary irritancy evaluation employing mucous membranes and instillation of agents into the animal eye (1, 17). Thioglycolate is a potent chemical agent, which is readily metabolized on parenteral and percutaneous administration. It affects the nervous system and blood sugar (9, 18), *in vitro* tissue metabolism (19, 20), and sulfhydryl-dependent enzyme systems (21, 22).

Another approach may be useful in studying percutaneous thioglycolate absorption. Several vehicles might be suggested that are rapidly absorbed through the skin such as dimethyl sulfoxide, which also facilitates the penetration of other agents (23). Stoughton (12) demonstrated the rapid percutaneous absorption of the ^{35}S-agent *in vivo* and *in vitro*. However, by the *in vivo* approach, much sulfoxide is metabolized in other areas of the body; little, if any, is excreted in the urine. In percutaneous experiments with thioglycolate–dimethyl sulfoxide mixtures employing such species as the rabbit, the overall urinary sulfur output might reflect primarily the activity or metabolism of the thioglycolate.

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