

VEHICLE ROLE OF PETROLATUM

Absorption Studies with Metallic Test Compounds in Guinea Pigs

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Abstract. An *in vivo* isotope technique (disappearance measurements) was used to compare, in the guinea pig, the percutaneous absorption of sodium chromate (Na_2CrO_4), cobaltous and mercuric chlorides mixed with petrolatum and distilled water. In 6 of the 8 concentrations studied, absorption was higher from distilled water, but only in one mercury concentration was the difference statistically significant. Absolute absorption increased with increasing concentration of the test substances, and with increasing volume of the test substance-petrolatum mixture, respectively. From the point of view of absorption there was no advantage in first dissolving Na_2CrO_4 in water before mixing with petrolatum. The greater reliability in patch testing obtained with test substances in petrolatum compared with distilled water seems to be due to the sealing capacity of petrolatum (among other factors hydration and prevention of evaporation) and not to any absorption-promoting property. A comparison between different ways of administering Na_2CrO_4 showed, however, that iontophoresis and the use of sodium lauryl sulphate as vehicle or pretreatment agent gave the largest absorption.

Patch tests showed that petrolatum was preferable to water as a vehicle when using *non-occlusive* adhesive tapes (5). Chromium in petrolatum gave a lower threshold of sensitivity than in distilled water, with an *occlusive* test method (6). Consequently, it is conceivable that petrolatum, besides its sealing capacity (5), which prevents evaporation of the test substances and facilitates hydration, may also possess properties promoting penetration and absorption of the type for example, described (4) for dimethyl sulfoxide (penetrant carrier). Such properties would warrant a more general use of petrolatum in, among other things, patch testing. In order to elucidate this problem, chromium, cobalt and mercury compounds were mixed with petrolatum and distilled

water respectively and administered to guinea pigs, after which the percutaneous absorption rate was determined by a method previously described (disappearance measurements). In this connection an occluded skin depot (9) was used, so that it was unnecessary to take the evaporation factor into consideration.

MATERIAL AND METHODS

Experimental animals. White guinea pigs of both sexes were used in about the same proportions; weight 300-500 g.

Disappearance measurements. Details were given in a previous study (9). The radioactivity above a topical deposit of the isotope-labelled penetrant on the skin was continuously recorded for at least 5 hours by a scintillation detector. The decrease in activity is an indication of absorption and is expressed mathematically in terms of a disappearance constant ($k \text{ min}^{-1}$).

Test substances. *Sodium chromate* ($\text{Na}_2\text{CrO}_4 \cdot 4\text{H}_2\text{O}$), analytical reagent (Mallinckrodt Chemical Works, USA). *Cobaltous chloride* (CoCl_2), analytical reagent (British Drug Houses, England). *Mercuric chloride* (HgCl_2), analytical reagent (E. Merck AG, Germany).

Isotopes. ^{51}Cr (half-life 27.8 days), ^{60}Co (half-life 71 days), and ^{203}Hg (half-life 47 days) were obtained from The Radiochemical Centre, Amersham, England.

Vehicles. Petrolatum (Vaselinum, *Pharmacognomia Svecica* 1967) and distilled water. The mixtures with petrolatum were delivered with crystals less than 0.3 mm of the test substances (Fig. 1), whereafter the respective isotopes were added. In order to investigate whether higher absorption is obtained if the crystals are first dissolved in water before mixing with petrolatum, 0.40% Na_2CrO_4 was prepared also from a 50% water solution of the salt (Fig. 2).

Concentrations. The lowest concentration of the test substances was near that usually applied to man in patch testing. Na_2CrO_4 : 0.40, 1.87, and 5.59%. CoCl_2 : 2.00, and 5.69%. HgCl_2 : 0.14, 2.17, and 6.49%.

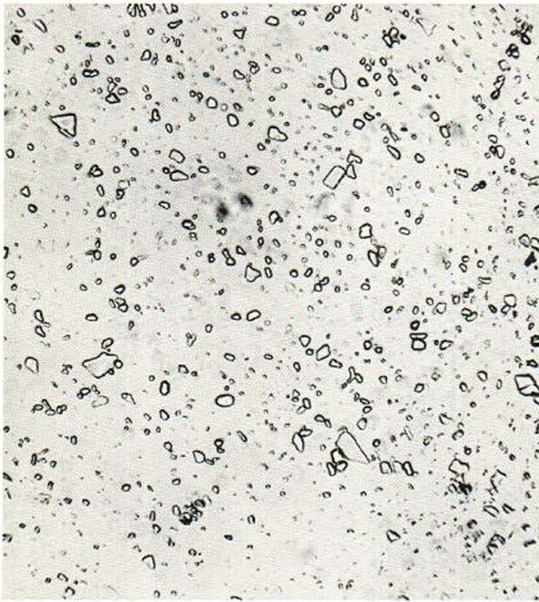


Fig. 1. 0.40% Na_2CrO_4 -petrolatum mixture with crystals less than 0.3 mm ($\times 100$).

Volume of mixture applied. Of Na_2CrO_4 in petrolatum 0.25, 0.50 and 1.00 ml were applied; of CoCl_2 and HgCl_2 0.50 and 1.00 ml. Of the water solutions only 1.00 ml was applied, as smaller volumes did not cover the exposure area in the skin depot (9).

Statistical analysis. χ -square tests and analysis of vari-

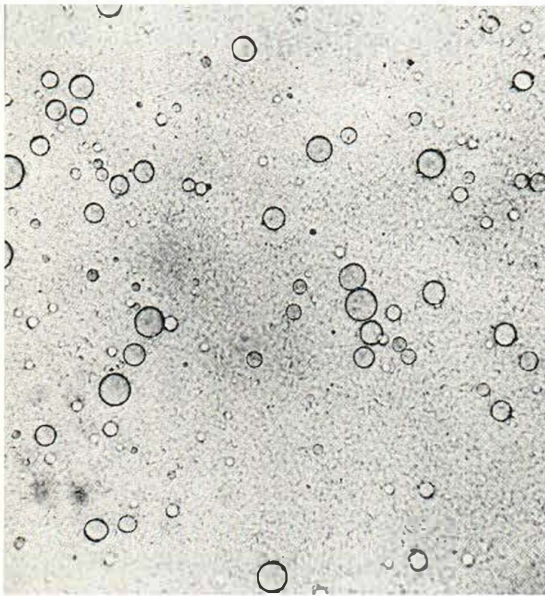


Fig. 2. 0.40% Na_2CrO_4 -petrolatum mixture, prepared from a 50% aqueous solution of Na_2CrO_4 ($\times 100$).

ance were carried out in order to estimate the effect of varying the vehicle. In some series the absorption rate was reduced to such an extent that the disappearance technique (9) was not sufficiently sensitive to permit a quantitative determination of the absorption (i.e. the disappearance constant was less than $3.4 \cdot 10^{-5} \text{ min}^{-1}$). In this case, the χ -square test was made, where the cell frequency, below and above respectively this sensitivity limit of the disappearance technique, was given in a fourfold table. However, this method of analysis sometimes (8) gave results which were misleading. To the rest of the experiments, where all values of the disappearance constant were greater than $3.4 \cdot 10^{-5} \text{ min}^{-1}$, the analysis of variance was applied.

RESULTS

Tables I-III and Fig. 3 show *relative* ($k \text{ min}^{-1}$) and *absolute* ($\mu\text{g cm}^{-2} \text{ h}^{-1}$) absorption from mixtures of Na_2CrO_4 , CoCl_2 , and HgCl_2 with petrolatum and distilled water. As previously pointed out (9), in some series the disappearance technique was not sufficiently sensitive to permit quantitative determinations of the absorption. For results below the stated sensitivity limit ($3.4 \cdot 10^{-5} \text{ min}^{-1}$), arbitrary values may be used (e.g. corresponding to 0 and $3.4 \cdot 10^{-5}$) for calculating the mean values (9-11). In the series, shown in the tables, where this method of calculation was applied, both values for the mean have been given and are put in parentheses. In the series where all the values were higher than the stated sensitivity limit, the mean and the standard error were calculated on the basis of the individual observations.

Petrolatum compared with distilled water as vehicle

Chromium (series, 1, 4, 6, 9, 10, 13, Table I, Fig. 3). In the three concentrations higher absorption was obtained from distilled water. At the concentration 5.59%, this tendency was most evident ($0.01 < P < 0.05$).

Cobalt (series, 14, 16-18, Table II). At the concentration 2.00%, absorption was slightly higher from distilled water ($0.3 < P < 0.5$). At the concentration 5.69% absorption was the same ($0.7 < P < 0.8$).

Mercury (series 19, 21-25, Table III). At the concentrations 0.14 and 2.17%, absorption was higher from distilled water, and for 2.17% the difference was statistically significant ($P < 0.001$). At the concentration 6.49%, ab-

Table I. Absorption of chromium from petrolatum and distilled water

Series no.	Na ₂ CrO ₄ conc. %	Mixed with	Volume of mixture applied, ml	Total no. of experiments	$k \cdot 10^5 \text{ min}^{-1}$				Mean absorption	
					<3.4 No.	3.4-6.6 No.	6.7-10.1 No.	10.2- No.	Relative ($k \cdot 10^5 \text{ min}^{-1}$)	Absolute ($\mu\text{g Cr cm}^{-2} \text{ h}^{-1}$)
1	0.40	Distilled water	1.00	10	—	4	4	2	8.8 ± 1.4 ^b	1.5
2	0.40	Petrolatum	0.25	10	3	2	4	1	(5.4-6.4) ^c	(0.2-0.3)
3	0.40	Petrolatum	0.50	10	—	3	6	1	7.4 ± 0.7 ^b	0.6
4	0.40	Petrolatum	1.00	10	—	6	—	4	7.4 ± 1.2 ^b	1.3
5	0.40	Petrolatum	1.00 ^a	10	2	3	4	1	(5.9-6.6) ^c	(1.0-1.1)
6	1.87	Distilled water	1.00	12	—	6	4	2	6.9 ± 0.6 ^b	5.6
7	1.87	Petrolatum	0.25	10	4	1	5	—	(4.8-6.2) ^c	(1.0-1.2)
8	1.87	Petrolatum	0.50	10	6	1	2	1	(3.4-5.4) ^c	(1.4-2.2)
9	1.87	Petrolatum	1.00	10	1	7	1	1	(5.4-5.8) ^c	(4.3-4.7)
10	5.59	Distilled water	1.00	10	—	5	2	3	7.6 ± 0.7 ^b	18.3
11	5.59	Petrolatum	0.25	10	6	4	—	—	(1.5-3.6) ^c	(0.9-2.2)
12	5.59	Petrolatum	0.50	10	4	4	1	1	(3.9-5.2) ^c	(4.7-6.3)
13	5.59	Petrolatum	1.00	10	3	4	1	2	(5.1-6.1) ^c	(12.3-14.7)

^a Prepared from a 50% aqueous solution of Na₂CrO₄.

^b Standard error.

^c See text.

sorption was higher from petrolatum ($0.01 < P < 0.02$).

Absolute absorption

Expressed as $\mu\text{g Cr, Co, and Hg cm}^{-2} \text{ h}^{-1}$, it increased with increasing chromium (Table I, Fig. 3), cobalt (Table II), and mercury (Table III) concentration applied.

Effect of volume of test substance-petrolatum mixture applied

On increasing the volume of the applied mixture from 0.25 to 0.50 and 1.00 ml the absolute ab-

sorption of chromium increased for all the concentrations (Table I, Fig. 3). The same results were obtained for cobalt and mercury when the volume was increased from 0.50 to 1.00 ml (Tables II, III).

Comparison between different methods of preparing Na₂CrO₄-petrolatum mixture

Absorption from a mixture of Na₂CrO₄ in crystalline form (series 4, Fig. 1) was somewhat higher ($0.10 < P < 0.20$) compared with a mixture prepared from a 50% aqueous solution of the salt (series 5, Fig. 2).

Table II. Absorption of cobalt from petrolatum and distilled water

Series no.	CoCl ₂ conc. %	Mixed with	Volume of mixture applied, ml	Total no. of experiments	$k \cdot 10^5 \text{ min}^{-1}$				Mean absorption	
					<3.4 No.	3.4-6.6 No.	6.7-10.1 No.	10.2- No.	Relative ($k \cdot 10^5 \text{ min}^{-1}$)	Absolute ($\mu\text{g Co cm}^{-2} \text{ h}^{-1}$)
14	2.00	Distilled water	1.00	10	6	3	—	1	(3.1-5.2) ^a	(3.0-5.0)
15	2.00	Petrolatum	0.50	10	8	2	—	—	(1.3-4.0) ^a	(0.6-1.9)
16	2.00	Petrolatum	1.00	10	8	2	—	—	(1.1-3.8) ^a	(1.1-3.7)
17	5.69	Distilled water	1.00	10	4	5	1	—	(3.3-4.7) ^a	(9.0-12.8)
18	5.69	Petrolatum	1.00	10	6	2	1	1	(3.2-5.3) ^a	(8.7-14.5)

^a See text.

Table III. Absorption of mercury from petrolatum and distilled water

Series no.	HgCl conc. %	Mixed with	Volume of mixture applied, ml	Total no. of experiments	$k \cdot 10^5 \text{ min}^{-1}$					Mean absorption	
					No.	No.	No.	No.	No.	Relative ($k \cdot 10^5 \text{ min}^{-1}$)	Absolute ($\mu\text{g Hg cm}^{-2} \text{ h}^{-1}$)
19	0.14	Distilled water	1.00	10	2	—	7	1	—	(7.5–8.2) ^a	(1.5–1.6)
20	0.14	Petrolatum	0.50	10	3	4	3	—	—	(4.8–5.8) ^a	(0.5–0.6)
21	0.14	Petrolatum	1.00	10	2	3	3	2	—	(6.5–7.2) ^a	(1.3–1.4)
22	2.17	Distilled water	1.00	20	—	3	6	5	6	10.8 ± 0.8^b	33.4
23	2.17	Petrolatum	1.00	10	5	1	2	1	1	(5.1–6.8) ^a	(15.8–21.1)
24	6.49	Distilled water	1.00	15	13	—	1	1	—	(1.3–4.2) ^a	(12.1–39.0)
25	6.49	Petrolatum	1.00	10	4	2	3	1	—	(4.8–6.2) ^a	(44.6–57.6)

^a See text.^b Standard error.

DISCUSSION

A review of the literature (1–3, 7, 15) shows that there is no generally accepted definition of a vehicle. Most authors consider that a vehicle is therapeutically inactive and exerts its effect by carrying the active ingredient. By definition there does not appear to be a clear distinction between a vehicle and an adjuvant.

When comparing absorption from the vehicles petrolatum and distilled water it was found that

this was higher from distilled water in 6 of the 8 concentrations studied ($3/3$ chromium, $1/2$ cobalt and $2/3$ mercury concentrations). The differences were not large, however, and only in one mercury concentration was it statistically significant. In the remaining cobalt concentration the absorption was the same, and in the remaining mercury concentration higher absorption was obtained from petrolatum.

In a previous investigation (6) the threshold of sensitivity was determined for hypersensitive patients, for chromium in an alkaline buffer (pH 12.0), in petrolatum and in distilled water (pH 4.1–5.2), and parallel with this the percutaneous absorption rate was determined in guinea pigs. "Excellent" agreement between the test results for man and the experimental findings for guinea pigs was obtained: chromium in the alkaline buffer (chromate) gave the lowest threshold of sensitivity and showed simultaneously the highest absorption; next came chromium in petrolatum, and finally in distilled water (bichromate). These results do not contradict the present which were obtained with chromate in distilled water (pH 8.0–8.4). Corresponding determinations of the threshold of sensitivity in patients hypersensitive to cobalt, nickel and mercury (14) showed small differences between petrolatum and distilled water; and the results seem to be connected, among other factors, with the simultaneous occurrence of hypersensitivity to other metal compounds. Thus, it

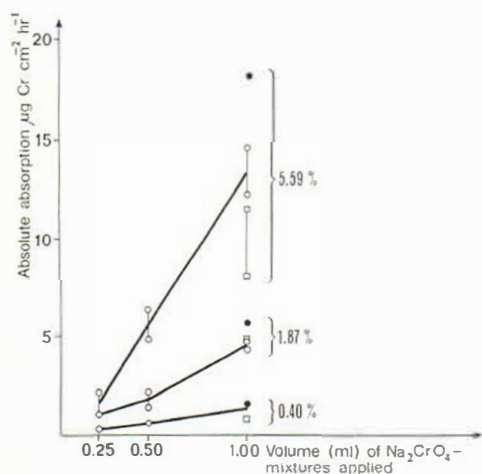


Fig. 3. Absolute absorption of chromium from mixtures of Na_2CrO_4 (0.40, 1.87, and 5.59%) with petrolatum and distilled water. \circ — \circ , mixed with petrolatum; \bullet , mixed with distilled water (pH 8.0–8.4); \square — \square , mixed with distilled water + HCl (pH 2.9–3.0).

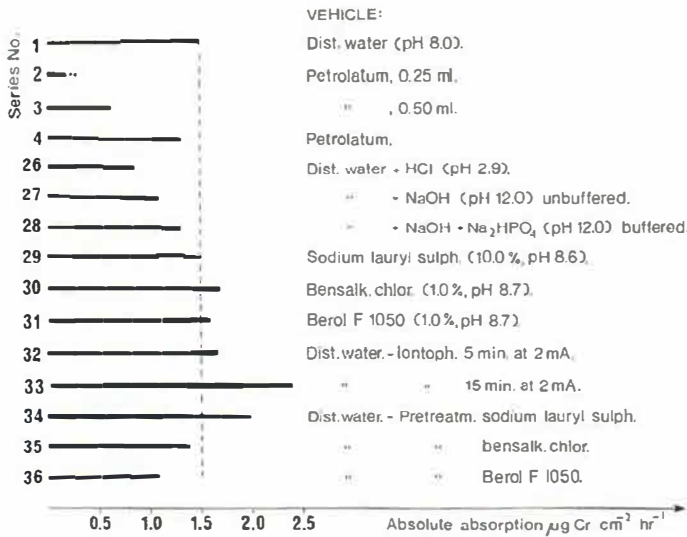


Fig. 4. Absolute absorption from 0.40% Na₂CrO₄ in different vehicles, after iontophoresis and pretreatment. 1.00 ml of the respective mixtures was applied, if not otherwise stated.

was not possible to demonstrate any absorption-promoting property of petrolatum compared with distilled water, but the greater reliability of petrolatum in patch testing is due to its sealing capacity (5).

As is evident from Tables I-III and Fig. 3 absolute absorption increased with the increasing volume of test substance-petrolatum mixture. Consequently, from a practical point of view it is desirable to apply the respective test ointments in sufficiently large amounts in patch testing.

With regard to absorption, there is no apparent advantage in dissolving the Na₂CrO₄ crystals in water before mixing with petrolatum.

Fig. 4 shows a comparison between chromium absorption from Na₂CrO₄-petrolatum mixtures, from a buffered and an unbuffered solution with varying initial pH (11), from mixtures with detergents (10), after pretreatment of the exposure area with these detergents (10), and after iontophoresis (12) (concentration 0.40% Na₂CrO₄). The largest absorption was obtained after iontophoresis for 15 min at 2 mA (series 33), then after pretreatment with sodium lauryl sulphate (series 34). When comparing with detergents as vehicles (series 29-31), absorption was lower from petrolatum. A similar comparison for 5.59% Na₂CrO₄ showed that the detergents (10), but also an alkaline buffer (11) gave higher chromium absorption than from petrolatum.

An analogous comparison for HgCl₂ showed that dimethyl sulfoxide both as vehicle and as

pretreatment agent (13), alkyl aryl sulphate and soap (8) gave higher mercury absorption than from petrolatum.

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