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PERCUTANEOUS ABSORPTION OF SODIUM CHROMATE (^{51}Cr), COBALTOUS (^{58}Co), AND MERCURIC (^{203}Hg) CHLORIDES THROUGH EXCISED HUMAN AND GUINEA PIG SKIN

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In previous studies (13, 20–22) an isotope-technique, the so-called disappearance measurements, was used to determine the percutaneous absorption rate of 10 metal compounds in living guinea pigs. In order to clarify the essential aspect, whether the absorption rate is identical *in vivo* and *in vitro* it was investigated in regard to sodium chromate, cobaltous and mercuric chlorides, using excised guinea pig skin, and the same technique and by analysing the bathing solution on the dermal side. Comparative studies were carried out also with excised human abdominal and mammary skin so as to extrapolate, if possible, results previously obtained in guinea pigs to conditions in man.

Materials and Methods

The diffusion chambers (1, 3, 12) were made of perspex. The skin was fixed between two rings that had a central hole with a diameter of 20 mm, i. e. an area of 3.1 cm^2, which was the same as *in vivo* (20). Four pegs in the upper ring protruded through the holes cut in the stretched skin and the corresponding holes in the lower ring (fig. 1). The lower part of the chamber, the recipient, had a volume of 3.8 cm^3, and contained the thermometer and apertures for inflow and outflow of the bathing solution. The pegs in the ring were placed in the corresponding holes in the lower part of the chamber, after which its upper part, consisting of a plate with a central opening of 20 mm, was put on top. The skin was clamped together with four brass screws in the corners of the chamber.

Heat: In order to enable investigations to be made at different temperatures the diffusion chamber was put on a lead plate heated by a hot-plate (fig. 1). The desired temperature was controlled by a thermometer situated in the recipient immediately below the dermis.

Bathing solution: Distilled water or 0.9 per cent sodium chloride solution was pumped through the recipient by a Sigmamotor pump,[1] by means of which different flow rates could be applied.

Disappearance measurements: A detailed description of the method used for disappearance measurements was given in a previous study (20). The radioactivity, above a skin deposit of an isotope-labelled compound in solution, was measured by a scintillation detector[2] connected with a high

[1] T-8, Sigmamotor, Inc, Middleport, N. Y., U.S.A.
[2] Philips PW 4111.

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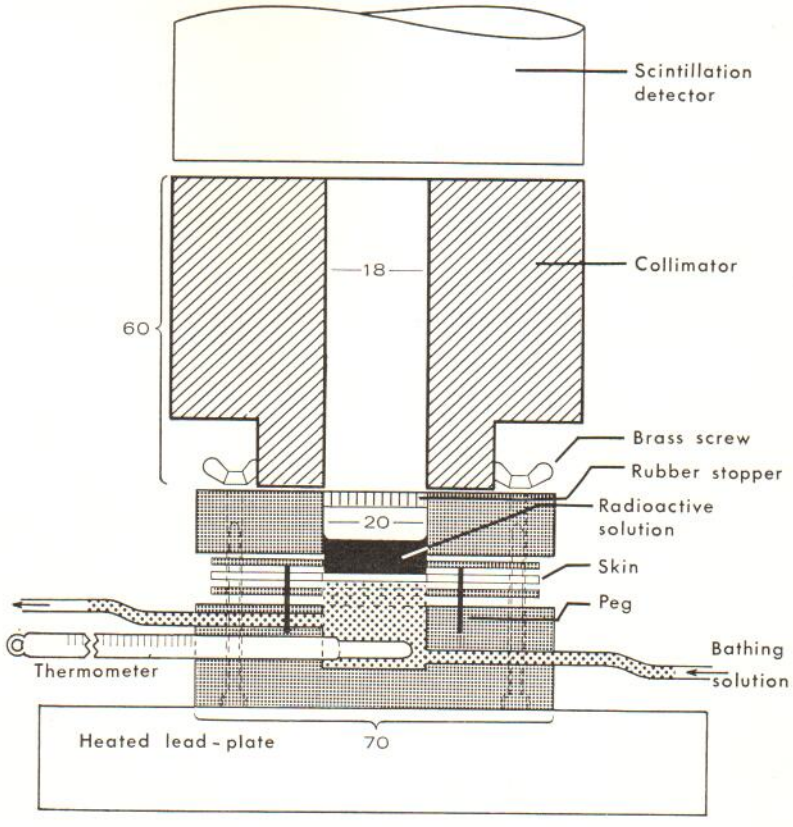

Fig. 1. Schematic representation of the diffusion chamber used for making disappearance experiments on excised skin. In order to show the individual parts, the chamber is not clamped together. Unit of measurement: millimeter.

voltage unit/preamplifier,[3] and a ratemeter.[4] The time constant of the ratemeter was set at 100 seconds and the oscillations were directly transferred to a potentiometer recorder.[5] The counting rate decreased continuously as a function of time, indicating disappearance from the surface. The observed decrease in activity was expressed mathematically in terms of a disappearance constant (k min^{-1}) (20), which is an expression of relative absorption. In order to obtain the same geometry as that applied in the *in vivo* experiments (20), a collimator with the same channel length (60 mm) and same diameter (18 mm) was used (fig. 1). The diffusion chamber and the scintillation detector were surrounded by lead shields so as to reduce background counting rate.

Radioanalysis of bathing solution: Samples of the bathing solution (4.0 ml) or mercuric sulphide precipitate (see below) were put in plastic tubes and measured in a scintillation detector[6] provided with a well-type crystal,[7] and electronic counter.[8]

Excised guinea pig skin was obtained from animals weighing 325–400 grams. They were killed by a blow on the head. The back skin was clipped (20) and then removed. The histological examination showed that the skin contained a thin subcutaneous layer of fat. The isotope solution was applied within 15 minutes after the animal was killed.

[3] Philips PW 4022.
[4] Philips PW 4042.
[5] Philips PR 4060 m/02.
[6] Philips PW 4119.
[7] Philips PW 4118/01.
[8] Philips PW 4032.

Excised human abdominal skin was obtained from the Department of Pathology, Karolinska Sjukhuset. It consisted of an unselected material of autopsies of patients who had suffered from the most common diseases, i. e. cardiovascular conditions and malignant tumours. The mean age of the 50 subjects was 62 ± 2 (s. e.) years. After death the bodies were washed with soap and water and then stored at + 4° C until autopsy was performed. The skin was taken from an area on the mid-line between the xiphoid process and the navel. The isotope solution was applied, on an average, 42 ± 3 (s. e.) hours after death.

Excised human mammary skin was obtained from patients operated at the Department of Plastic Surgery, Karolinska sjukhuset. In 10 women, mean age 38 ± 2 (s. e.) years, who had undergone a reduction mammaplasty according to Strömbeck's technique (14), the skin was excised from the lower part of the breasts. Before the operation the skin was washed with an 0.1 per cent bensalkonium solution. A certain amount of time was required for transport, preparation, and determination of background counting rate, but all experiments with fresh mammary skin could be started within 4 (mean 2.5) hours after the skin had been removed from the patients. Two adjacent pieces of skin were put each in its own diffusion chamber; one for immediate use, the other was stored for 48 hours at + 4° C in order to investigate the influence of storage on the absorption rate.

Compounds:[9] Sodium chromate (Na_2CrO_4), analytical reagent.[10] ^{51}Cr, half-life 27.8 days.[11]

Cobaltous chloride ($CoCl_2$), analytical reagent.[12] ^{58}Co, half-life 71 days.[11]

Mercuric chloride ($HgCl_2$), analytical reagent.[13] ^{203}Hg, half-life 47 days.[11]

Sodium chloride (NaCl), analytical reagent.[13] ^{22}Na, half-life 2.6 years.[11]

The concentrations were the same as those used in previous *in vivo* studies (13, 22), and correspond to those applied in patch tests (6). The pH[14] of the freshly prepared aqueous solutions of the compounds were determined.

Experimental procedure: The subcutaneous tissues of the human skin were removed by blunt dissection. After the human and the guinea pig skin were fixed between the perspex rings, their intactness was controlled by measuring the electrical conductivity at 1 volt (electrode diameter: 18 mm) according to Blank & Finesinger (5). Specimens, the conductivity of which exceeded 5 microamperes, were not used. The assembled diffusion chamber was put on the (heated) lead plate (fig. 1); the plastic tube was then attached through which the bathing solution was pumped. After the background counting rate had been determined, 1.0 ml (20) of the respective isotope solution was applied to the epidermis. In order to prevent evaporation outside the chamber, a rubber stopper was put above the deposit. During the 48-hour experiments, samples of the bathing solution were taken for analysis (see below).

Methodological Studies

In order to investigate the effect of variations in the type of bathing solution, flow rate, and temperature, *disappearance measurements* were made on excised guinea pig skin, and 0.080 M $HgCl_2$ solution was used as the test substance. In order to ascertain the effect on absorption of the clamping of the skin in the diffusion chamber, disappearance measurements were made with 0.239 M NaCl (21) ap-

[9] In the text and tables these are given as salts, despite their presence in the solutions as various complex compounds.
[10] Mallinckrodt Chemical Works, USA.
[11] Radiochemical Centre, Amersham, Buckinghamshire, England.
[12] British Drug House, LTD, England.
[13] E. Merck, AG, Germany.
[14] pH-meter 22, Copenhagen.

Table 1. *Disappearance constants ($k \cdot 10^5$ min^{-1}) in 10 experiments with 0.080 M $HgCl_2$-solution, applied on excised guinea pig skin. Bathing solution: 0.9 % NaCl. Temperature 34 ± 1° C*

Experiment	1	2	3	4	5	6	7	8	9	10	Mean ± s.e.
Flow rate ml/hr	17.9	17.7	17.5	16.5	16.5	17.2	17.3	15.8	14.6	14.8	16.6 ± 0.4 ml/hr
Electrical conductivity at 1 volt (microamperes) Electrode diameter: 18 mm	0.5	2.5	3.0	1.5	3.5	3.0	1.5	5.0	1.5	1.0	2.3 microamp.
Disappearance constant (k) calculated for intervals of:											
0—5 hours	5.8	8.9	6.4	8.4	10.7	13.4	9.9	2.8	7.8	2.7	7.7 ± 1.1
0—12 "	5.7	7.4	4.5	5.3	7.2	9.4	8.2	3.9	6.4	2.9	6.1 ± 0.6
12—24 "	1.0	1.6	1.4	4.0	3.3	2.7	2.9	4.1	1.9	0.6	2.4 ± 0.4
24—36 "	1.0	1.6	0	2.4	0.7	1.8	1.0	0	1.9	2.2	1.3 ± 0.3
36—48 "	3.1	0	1.4	3.4	0.8	0.9	0	0	1.0	0	1.1 ± 0.4
0—24 "	3.3	4.5	2.9	4.7	5.3	6.0	5.5	4.0	4.1	1.7	4.2 ± 0.4
24—48 "	2.0	0.3	0.6	2.9	0.8	1.3	0.5	0	1.5	0.6	1.1 ± 0.3
0—48 "	2.7	2.4	1.8	3.8	3.0	3.7	3.0	1.7	2.8	1.2	2.6 ± 0.3

plied to excised guinea pig skin (0.2 μA/1 volt), for 7 days; and with distilled water as bathing solution.

Results: In the left columns of tables 1, 2, 4, 5 the time intervals are given on which the disappearance constant (20) was calculated. Table 1 shows in detail the results of a series of 10 absorption experiments. The disappearance constant was highest during the first 12 hours. On comparing the four 12-hour periods a tendency to decrease was observed subsequently.

Table 2 gives the mean value and the standard error for the six series, each of which consisted of 10 absorption experiments where the type of bathing solution, the flow rate, and the temperature varied. On comparing distilled water with 0.9 per cent NaCl solution as bathing solutions at 24° C with a flow rate of 15 ml/hr, and also 0.9 per cent NaCl solution with heparinized blood (taken from the same animal that supplied the skin) at 34 C° and stationary bathing solution, no differences in absorption occurred. During the first 5 hours the disappearance constant increased with increasing flow rate, whereas for the other periods no differences were observed. Absorption was greater at 34° C than at 24° C; this was most evident during the first 24-hour period.

In the experiment with 0.239 M NaCl, the disappearance constant, calculated for the first 5 hours, was $3.5 \cdot 10^{-5}$ min^{-1}, and for the 24-hour intervals (0–24, 24–48, etc.): 1.8 – 1.1 – 0 – 0.3 – 0.7 – 0.1 – 2.0 · 10^{-5} min^{-1}.

Radioanalysis of bathing solution: 1. Investigation of 4.0-ml-samples taken directly during the disappearance measurements. 2. In the bathing solution [collected when disappearance measurements were made with 0.080 M $HgCl_2$ (table 1) during a 48-hour period (700–860 ml)] mercuric sulphide was precipitated with sodium sulphide in an acid solution in the presence of cadium chloride. The precipitate was filtered and put in a plastic tube.

3. Application of 0.233 mC ^{58}Co (1.58 μg Co) and 1.375 mC ^{203}Hg (1.95 mg Hg) respectively, with stationary bathing solution. The diffusion chambers were rinsed, at various intervals, 5–15 times; on each occasion 4.0 ml of an 0.9 per cent NaCl solution were used.

4. Application of $HgCl_2$ solutions (about 4 mg) with high activity (about 1.5–2.0 mC), and with continuously flowing bathing solution. Mercuric sulphide was precipitated according to procedure 2 above and analysed.

Table 2. *Means and standard errors of disappearance constants ($k \cdot 10^5$ min^{-1}) in 10 experiments in each series with 0.080 M $HgCl_2$-solution, applied on excised guinea pig skin Bathing solution, flow rate and temperature were varied*

Bathing solution	Distilled water	0.9% NaCl	0.9% NaCl	0.9% NaCl	0.9% NaCl	Heparinized blood
Flow rate ml/hr	15	15	0	16.6±0.4	167±14.3	0
Temperature °C in the recipient	24±1	24±1	34±1	34±1	34±1	34±1
Electrical conductivity at 1 volt (microamperes). Range Electrode diameter: 18 mm	2.0—5.0	0.5—5.0	1.0—3.0	0.5—5.0	0.5—3.0	0.7—4.0
Disappearance constant (k) calculated for intervals of:						
0—5 hours	5.2±1.0	5.4±0.8	5.7±0.8	7.7±1.1	9.5±1.1	5.7±0.8
0—12 "	3.5±0.8	3.5±0.4	4.1±0.5	6.1±0.6	5.1±0.6	4.3±0.6
12—24 "	1.3±0.6	1.1±0.2	3.0±0.8	2.4±0.4	2.1±0.5	1.3±0.4
24—36 "	1.5±0.5	0.9±0.2	2.2±0.4	1.3±0.3	2.2±0.3	1.6±0.6
36—48 "	1.2±0.4	1.4±0.5	1.6±0.4	1.1±0.4	1.6±0.4	1.8±0.6
0—24 "	2.4±0.6	2.3±0.2	3.7±0.5	4.2±0.4	3.7±0.4	2.8±0.4
24—48 "	1.3±0.4	1.1±0.3	1.8±0.4	1.1±0.3	1.9±0.3	1.7±0.5
0—48 "	1.8±0.5	1.7±0.2	2.8±0.4	2.6±0.3	2.8±0.3	2.2±0.5

Results: 1. No activity.

2. 0–310 counts per minute were found.

3. The first 4.0-ml-sample invariably contained the largest amount of ^{58}Co and ^{203}Hg respectively. Absorption increased gradually with time: on rinsing 44 hours after application (previous rinsing 24 hours earlier), the first 4.0-ml-sample contained 0.1 ng[15] of cobalt and 6 ng of mercury respectively; and after 8 days, 44 ng of cobalt and 64 ng of mercury respectively.

4. Absorption increased gradually with time, and, compared with experiment 3, larger amounts of ^{203}Hg were recovered (table 3). Despite successive filtering, small amounts (about 1 ng of mercury) were still found when measuring 4.0-ml-samples of the filtrate.

Percutaneous Absorption of Na_2CrO_4 (0.034 M), $CoCl_2$ (0.085 M), and $HgCl_2$ (0.005, 0.080, 0.239 M) Through Excised Human Skin (stored abdominal; fresh, and stored mammary skin) and Guinea Pig Skin (fresh back skin).

Results: Table 4 gives the mean values and the standard errors of the disappearance constants (k) for the 8 periods of time in the 12 series, each consisting of 10 absorption experiments. The disappearance constants were throughout highest during the first 12-hour period, after which they decreased; but when k was compared for the following three periods (12–24, 24–36 and 36–48 hours) in each series, no distinct tendency could be observed. When the three compounds were compared k was lowest for Na_2CrO_4 during the first 12-hour period for both human and guinea pig skin; the other periods did not show any distinct tendency. When the three $HgCl_2$ concentrations were compared, the disappearance constant was highest for the 0.005 M solution during the first 12-hour period. For the other time intervals the highest values were obtained for fresh guinea pig skin with an 0.080 M solution, and for stored human abdominal skin with an 0.239 M solution.

Table 5 gives the ratios between the means of the disappearance constants for each period of time when comparing the different types of skin. For example, during the period 0–5 hours, the disappearance constant for 0.034 M Na_2CrO_4 solution was on an average $5.7 \cdot 10^{-5}$ min^{-1} for freshly excised guinea pig skin, and 1.6 ·

[15] nanogram.

Table 3. *Recovered amount of mercury in continuously-flowing bathing solutions after application of 1.0 ml of $^{203}HgCl_2$ solutions, with high activities (about 1.5–2.0 mC), on excised guinea pig skin*

mC applied mg Hg " electrical conductivity μA/1 volt	1.996 3.906 1.0	1.951 4.297 2.5	1.590 3.906 0.8	1.578 4.297 1.0	Calculated absorption from 0.005M $HgCl_2$ (1 mgHg) (Table 4)
Hours:	μg Hg	μg Hg	μg Hg	μg Hg	μg Hg
0—5	0	0	—	—	39.90
0—12	—	—	0.002	0.003	51.84
5—24	0.026	0.023	—	—	13.68
12—24	—	—	0.030	0.006	3.60
24—36	—	—	0.050	0.008	0.72
36—48	—	—	0.062	0.034	1.44
24—48	0.184	0.269	—	—	4.32
48—72	1.197	0.466	0.098	0.058	—
72—96	1.570	1.050	0.143	0.049	—
96—120	1.431	1.343	0.296	0.073	—
120—144	1.764	1.777	0.494	0.082	—
144—168	2.163	1.244	0.662	0.115	—

10^{-5} min^{-1} for stored human abdominal skin; a ratio of 3.6. For the 8 periods of time where these types of skin were compared with regard to the five concentrations, the highest ratio was obtained with Na_2CrO_4 (range 3.0–8.0, mean 5.3), and the lowest with 0.239 M $HgCl_2$ solution (range 0.5–2.7, mean 1.6). Calculated for all the 40 ratios the mean for freshly excised guinea pig skin/stored human abdominal skin was 3.1. For the concentration 0.080 M $HgCl_2$ the mean ratio between fresh guinea pig skin/fresh human mammary skin was 1.8 (range 0.9–3.4); between stored/fresh human mammary skin 1.1 (range 0.9–1.6); and between stored human mammary skin/stored human abdominal skin 1.7 (range 1.3–2.2).

Discussion

There are many advantages in using *in vitro* methods for studying percutaneous absorption. *First,* they enable investigations to be made directly on human skin, whereby sensitive isotope methods can be employed without radiation hazards. *Second* by using adjacent pieces of skin from the same animal or human being individual differences can be practically eliminated. *Third,* for low absorption rates, exposure time can be considerably prolonged. The need for caution in applying *in vitro* results to *in vivo* conditions was emphasized i. a. by Lindsey (10) and Sulzberger (16). Ainsworth (1) showed, however, that for rat, rabbit, and pig skin, and using tributyl phosphate as the test substance, there was good agreement in respect of absorption rates when comparing *in vivo* and *in vitro* techniques. It was shown that for water (7), Ca-ions (15), and tributyl phosphate (1) the absorption rate remained unchanged when using fresh or stored skin. Treherne (1, 18) found that freshly resected whole skin consumed oxygen at a rate which was practically unchanged several hours later.

A number of *in vitro* techniques for the study of penetration and absorption have been published (1–4, 8, 9, 12, 15). In these the determination of the absorption rate was based on chemical, gas-chromatografic or isotopic analysis of the dermis or of the bathing solution on the dermal side. In order to facilitate comparison with previous results (13, 22) obtained *in vivo,* the same isotope-technique (disappearance measurements) (20) was applied and the bathing solution was analysed as a supplement. Earlier, the same method was employed by Fredriksson (9) for the determination of the absorption rate of parathion.

In the *in vivo* experiments the disappearance curve showed wide fluctuations;

Table 4. *Means and standard errors of disappearance constants ($k \cdot 10^5$ min^{-1}) in 10 experiments in each series with aqueous solutions of Na_2CrO_4, $CoCl_2$ and $HgCl_2$, applied on excised human skin and guinea pig skin*

Temperature: 34 ± 1° C. Electrical conductivity at 1 volt: 0.1–5.0 μA. Mean flow rate of bathing solution (0.9 % NaCl): 14–33 ml/hr

Substance, Conc.	Na_2CrO_4 0.034 M		$CoCl_2$ 0.085 M		$HgCl_2$ 0.005 M		$HgCl_2$ 0.080 M				$HgCl_2$ 0.239 M	
Type of Skin	Fresh guinea pig	Stored human abdominal	Fresh guinea pig	Stored human abdominal	Fresh guinea pig	Stored human abdominal	Fresh guinea pig	Stored human abdominal	Fresh human mammary	Stored human mammary	Fresh guinea pig	Stored human abdominal
Disappearance constant (k) calculated for intervals of:												
0—5 hours	5.7±0.5	1.6±0.4	6.4±0.6	2.3±0.5	13.3±1.4	4.6±0.7	7.7±1.1	2.3±0.5	4.9±0.6	4.7±0.8	8.7±0.6	3.2±0.5
0—12 "	3.9±0.3	0.9±0.2	4.0±0.4	1.6±0.3	7.2±0.6	2.2±0.4	6.1±0.6	1.7±0.3	3.3±0.2	3.0±0.4	5.7±0.3	2.1±0.3
12—24 "	1.4±0.6	0.2±0.1	1.2±0.3	0.2±0.1	0.5±0.2	0.2±0.1	2.4±0.4	0.5±0.2	0.7±0.2	1.1±0.3	1.1±0.3	1.1±0.3
24—36 "	0.9±0.2	0.3±0.2	0.2±0.1	0.1±0.1	0.1±0.1	0.1±0.1	1.3±0.3	0.8±0.2	0.9±0.3	1.0±0.3	1.1±0.2	0.7±0.2
36—48 "	0.8±0.2	0.1±0.1	0.5±0.2	0.2±0.1	0.2±0.1	0.2±0.1	1.1±0.4	0.8±0.3	1.2±0.4	1.1±0.4	0.7±0.3	1.3±0.4
0—24 "	2.4±0.2	0.5±0.1	2.6±0.3	0.8±0.1	3.8±0.4	1.1±0.2	4.2±0.4	1.1±0.2	2.0±0.1	2.0±0.3	3.3±0.2	1.6±0.2
24—48 "	0.8±0.2	0.2±0.1	0.2±0.1	0.1±0.1	0.3±0.1	0.1±0.0	1.1±0.3	0.7±0.2	0.9±0.3	1.0±0.3	0.9±0.2	1.0±0.3
0—48 "	1.6±0.2	0.2±0.1	1.3±0.2	0.4±0.1	1.8±0.1	0.5±0.1	2.6±0.3	0.9±0.1	1.4±0.2	1.5±0.2	2.1±0.2	1.3±0.2

Table 5. *Ratios obtained on comparison between disappearance constants for various periods of time and types of skin*

	Stored human abdominal skin / Fresh guinea pig skin						$HgCl_2$ 0.080 M		
	Na_2CrO_4 0.034 M	$CoCl_2$ 0.085 M	$HgCl_2$ 0.005 M	$HgCl_2$ 0.080 M	$HgCl_2$ 0.239 M	Mean	Fresh guinea pig skin / Fresh human mammary skin	Stored human mammary skin / Fresh human mammary skin	Stored human mammary skin / Stored human abdominal skin
Calculated for intervals of:									
0—5 hours	3.6	2.8	2.9	3.4	2.7	3.1	1.6	1.0	2.0
0—12 "	4.3	2.5	3.3	3.6	2.7	3.3	1.9	0.9	1.8
12—24 "	7.0	6.0	2.5	4.8	1.0	4.3	3.4	1.6	2.2
24—36 "	3.0	2.0	1.0	1.6	1.6	1.8	1.4	1.1	1.3
36—48 "	8.0	2.5	1.0	1.4	0.5	2.7	0.9	0.9	1.4
0—24 "	4.8	3.3	3.5	3.8	2.1	3.5	2.1	1.0	1.8
24—48 "	4.0	2.0	3.0	1.6	0.9	2.3	1.2	1.1	1.4
0—48 "	8.0	3.3	3.6	2.9	1.6	3.9	1.9	1.1	1.7
Mean	5.3	3.0	2.6	2.9	1.6	3.1	1.8	1.1	1.7

and in certain cases the slope was so slight that the possibility of subjectivity (20) could not be disregarded when indicating values on the curve for calculating the disappearance constant. On the other hand, for the *in vitro* experiments the fluctuations were less pronounced, probably owing to the constant temperature and flow rate and the absence of the animals' respiratory movements and convulsions. Despite the minimal slope of the disappearance curve in many cases, there was not the same difficulty in indicating values. For comparing the absorption rate through different types of skin, the period of 0–5 hours was chosen, i. e. the same period as in the *in vivo* experiments (13, 20–22), and, moreover, 0–12, 12–24, 24–36, 36–48, 0–24, 24–48, and 0–48 hours.

There are some differences in the choice of temperature, flow rate, and type of bathing solution in the diffusion chambers when comparing the methods used by various authors (1–4, 8, 9, 12, 15, 18). As is evident from the methodological studies with 0.080 M $HgCl_2$ on excised guinea pig skin (table 2), an increase in temperature of 10 degrees centigrade caused the absorption rate to increase during the first 24-hour period; whereas variations in the type of bathing solution (distilled water, 0.9 per cent NaCl solution or heparinized blood) did not have any influence. One explanation of the fact that the absorption rate was found to decrease when the four 12-hour periods were compared, is probably the protein-precipitating properties of certain metal compounds. Consequently, $HgCl_2$ is not an ideal test substance for methodological studies since protein precipitation may have masked effects due to changes in temperature, flow rate and type of bathing solution.

In order to avoid leakage a certain amount of pressure has to be used in clamping the skin in the diffusion chambers. How this pressure affects the absorption rate is not known, but a risk of barrier damage exists as pointed out by Fredriksson (9). The absorption rate *in vivo* for 0.239 M NaCl solution through stripped skin was found to be about 5 times greater than that through normal skin (21). Since the experiment carried out *in vitro* showed a constant low absorption rate during a period of 6 days, this indicates that the skin was not manifestly damaged when it was clamped in the diffusion chamber. The rise in absorption rate on the 7th day points to bacterial decomposition or lysis of the skin. Furthermore, the human skin had been "pretreated" with soap or bensalkonium solution which could also injure the barriers. In the present investigation the intactness of the skin was checked by measuring its electrical conductivity (5). Another source of error may have been that contact with perspex seems to affect

the composition of the $HgCl_2$ solution; it was observed that the pH of a 1.0 ml 0.080 M solution had increased by 0.11 units after 48 hours (19). In order to investigate whether $HgCl_2$ is adsorbed by perspex, a rectangular piece (surface 2.0 cm^2) was put in a plastic tube, after which 1.0 ml of a $^{203}HgCl_2$ solution, with the same activity as in the disappearance measurements, was added. After 48 hours the piece of perspex was removed from the isotope solution and then rinsed with running tap water for different periods of time. After 5 minutes' rinsing, 0.18 per cent remained, and after 60 minutes 0.09 per cent of the activity that was originally applied.

That the skin had been stored did not have any effect; with 0.080 M $HgCl_2$ solution as the test substance there were no differences in the absorption rate through fresh and stored (48 hours at $+4°$ C) mammary skin. On the other hand, absorption was greater through stored human mammary skin than through stored human abdominal skin. Similar variations in the absorption rate through human skin from different parts of the body have been described earlier (15).

Analyses of 4.0-ml portions of the bathing solution from ordinary disappearance experiments did not show any activity. After precipitation of mercuric sulphide in the bathing solution from 48-hour experiments, small amounts of ^{203}Hg were recovered. However, it was not possible to get a quantitative idea of the absorption. These results were expected in view of the low activity applied (about 7 μC), the large amount of carrier and the high degree of dilution. Only when the activity had been substantially increased (about 1.5–2.0 mC) was there a definite absorption (table 3). There was a discrepancy, however, between the absorption obtained in this way, and the one calculated on the basis of the disappearance measurements with 0.005 M $HgCl_2$ (table 4). Absorption was greater in the latter case. The applied method of precipitating mercuric sulphide was incomplete, as some activity could be shown to be present in the filtrate; moreover, a certain volatilization of the mercury may have occurred (11, 17) although this cannot account entirely for the difference. As is shown in table 3 there is a latent period before ^{203}Hg can be recovered from the bathing solution. Absorption increased gradually with time and a steady value was not obtained within 7 days. *In vivo,* the penetrating molecules meet the blood at the papillary capillaries; whereas *in vitro,* they have to pass through the whole skin until they reach the bathing solution. Fredriksson found in his experiments with parathion (9) that larger amounts were present in the recipient if heparinized blood was used instead of saline as a bathing solution. His interpretation was that parathion was retained in the corium. Since good agreement was obtained with various methods using tributyl phosphate (1), the observations made in the present investigation indicate that $HgCl_2$ reacts with, and is stored in the skin, and diffuses only gradually into the bathing solution. This hypothesis is supported also by stripping experiments (20), where tape with attached hair and cells, as well as the remaining skin were investigated in the well[7]-scintillation detector.[6]

In table 6 the *in vivo* (13, 22) and *in vitro* absorption rates are compared for the first 5-hour period after application. For three of the concentrations (0.085 M $CoCl_2$, 0.005 M and 0.239 M $HgCl_2$) the disappearance technique was not sufficiently sensitive *in vivo* to permit a quantitative determination of the absorption. The mean values were therefore estimated by a method previously mentioned (20). As the same degree of accuracy cannot be obtained by the latter method, the *in vivo/in vitro* rates ratio for guinea pigs has, in these cases, been placed in brackets. The ratios varied between 0.1 and 1.4. For the chisquare tests, the cell frequency was given in a fourfold table, respectively below and above the stated sensitivity limit of the disappearance technique ($3.4 \cdot 10^{-5}$ min^{-1}) (20). These tests showed that in the highest $HgCl_2$ concentration (0.239 M) the lower absorption rate *in vivo* was statistic-

Table 6. *Comparison of absorption rates, in vivo and in vitro, of aqueous solutions of Na_2CrO_4, $CoCl_2$ and $HgCl_2$ during a 5-hour period*

$k \cdot 10^5$ min^{-1}					< 3.4	3.4-6.6	6.7-10.1	10.2-13.5	13.6-17.0	17.1-	Mean		Ratios		
%/5 hours					< 1.0	1.0-1.9	2.0-2.9	3.0-3.9	4.0-4.9	5.0-			guinea pig: in vivo / in vitro		in vitro: guinea pig / human
Compound	Conc. M	pH	Type of skin	Total No.	No.	No.	No.	No.	No.	No.	$k \cdot 10^5$ min^{-1}	mμM* cm^{-2} hr^{-1}		Chisquare (c) Analysis of variance (v)	
Na_2Cro_4	0.034	8.7	guinea pig, in vivo	10	—	7	3	—	—	—	6.4	42			
,,	,,	,,	,, , in vitro	10	—	7	3	—	—	—	5.7	38	1.1	P > 0.2 (v)	
,,	,,	,,	human, abdominal, ,,	10	9	1	—	—	—	—	1.6	11			3.6
$CoCl_2$	0.085	5.6	guinea pig, in vivo	10	6	3	—	—	1	—	(3.1–5.2**)	(51–86)			
,,	,,	,,	,, , in vitro	10	1	3	6	—	—	—	6.4	105	(0.5-0.8)	0.01 < P < 0.02 (c)	
,,	,,	,,	human, abdominal, ,,	10	8	2	—	—	—	—	2.3	38			2.8
$HgCl_2$	0.005	4.8	guinea pig, in vivo	10	2	—	7	1	—	—	(7.5–8.2**)	(7–8)			
,,	,,	,,	,, , in vitro	10	—	—	3	2	3	2	13.3	13	(0.6)	0.10 < P < 0.20 (c)	
,,	,,	,,	human, abdominal, ,,	10	3	5	2	—	—	—	4.6	4			2.9
,,	0.080	4.0	guinea pig, in vivo	20	—	3	6	5	5	1	10.8	167			
,,	,,	,,	,, , in vitro	10	2	2	4	2	—	—	7.7	119	1.4	0.02 < P < 0.05 (c)	
,,	,,	,,	human, abdominal, ,,	10	8	2	—	—	—	—	2.3	36			3.4
,,	,,	,,	human, mammary fresh, ,,	10	4	5	1	—	—	—	4.9	76			1.6
,,	,,	,,	,, , ,, stored 2 days ,,	10	4	4	2	—	—	—	4.7	73			1.6
,,	0.239	3.3	guinea pig, in vivo	15	13	—	1	1	—	—	(1.3–4.2**)	(60–194)			
,,	,,	,,	,, , in vitro	10	—	2	6	2	—	—	8.7	402	(0.1-0.5)	P < 0.001 (c)	
,,	,,	,,	human, abdominal, ,,	10	8	1	1	—	—	—	3.2	148			2.7

* Calculated on the metal component of the compound.

** Reference 20.

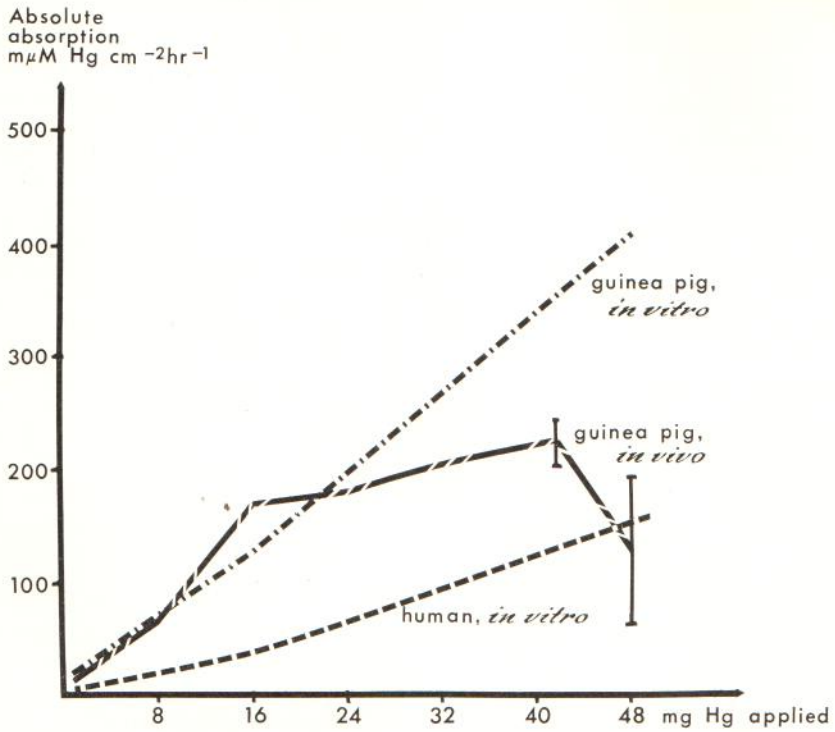

Fig. 2. The mean absolute absorption of mercuric chloride through guinea pig skin *in vivo* and *in vitro*; and through human abdominal skin *in vitro*; correlated to the applied amounts.

ally significant ($P < 0.001$). For the remaining four concentrations the ratios were, in two cases, greater than 1 (0.034 M Na_2CrO_4, 0.080 M $HgCl_2$), and, in two cases, less than 1 (0.085 M $CoCl_2$, 0.005 M $HgCl_2$). With the exception of the highest $HgCl_2$ concentration, there was, thus, good agreement between the *in vivo* and the *in vitro* absorption rates through guinea pig skin during the first 5-hour period.

Calculated absolutely as mμM (Cr, Co, Hg) cm^{-2} hr^{-1}, for the first 5-hour period the lowest values were obtained for Na_2CrO_4, and the highest for 0.239 M $HgCl_2$. On comparing the three $HgCl_2$ concentrations (fig. 2), it was found that absolute absorption *in vitro* increased with increasing concentration for both guinea pig and human skin, which was in contrast to the conditions, *in vivo*, for guinea pigs, where a "plateau value" was obtained (13).

SUMMARY

The same isotope-technique (disappearance measurements) applied in previous *in vivo* studies was used in the present investigation of the percutaneous absorption rate during 48 hours of aqueous solutions of 0.034 M sodium chromate (Na_2CrO_4), 0.085 M cobaltous chloride ($CoCl_2$), and 0.005, 0.080 and 0.239 M mercuric chloride ($HgCl_2$). Excised human (abdominal, mammary) and guinea pig whole skin were studied. The skin was clamped in a perspex diffusion chamber where temperature, flow rate and bathing solution could be varied. As a supplementary study the bathing solution was also analysed; absorption was found to be lower than when disappearance measurements were used. Possible explanations of this discrepancy are given.

1. When comparing absorption through guinea pig skin during the first five hours, the *in vivo/in vitro* rates ratio varied in the five given concentrations between 0.1 and 1.4. In the highest $HgCl_2$ concentration (0.239 M), an observed lower absorption rate *in vivo* was statistically significant.

2. During the first five hours absolute absorption through human and guinea pig skin *in vitro* increased with increasing con-

centration of $HgCl_2$. This was different from the *in vivo* observations where a "plateau value" was obtained.

3. The mean absorption rate for the five given concentrations was 3.1 times greater through freshly excised guinea pig skin compared to stored, human abdominal skin.

4. The mean absorption rate of 0.080 M $HgCl_2$ was 1.8 times greater through freshly excised guinea pig skin when compared to freshly excised human mammary skin; 1.7 times greater through stored excised human mammary skin than stored human abdominal skin and 1.1 times greater through stored mammary skin than freshly excised mammary skin.

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