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THE PERCUTANEOUS ABSORPTION OF SODIUM CHROMATE (^{51}CR) IN THE GUINEA PIG

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We have previously studied [Friberg *et al.* (3)], [Wahlberg and Skog (8)] the percutaneous absorption of some mercury compounds and attempted to indicate factors that change the absorption through the skin. We used an *in vivo* isotope technique, the so-called "disappearance measurements". In the present investigation we have employed the same method to ascertain the percutaneous absorption of sodium chromate and its distribution in the body after such absorption.

Material and Methods

Disappearance mesurements. A detailed description of the experimental method used in the present study was given in an earlier report (3). By "disappearance measurements" the activity over a skin depot containing the isotope-labelled compound in solution, was registered by a scintillation detector. The counting rate decreased continuously as a function of time indicating that absorption through the skin has taken place. The compound applied was subsequently found to be present in the blood, kidneys, urine, the lymph glands etc. The decrease in activity was expressed mathematically in terms of a "disappearance constant", which made it possible to compare various compounds, concentrations and methods of pretreatment.

In the previous report (3) the skin depot was located on the experimental animal's belly. We have shown (8), however, that there is no positive difference in the percutaneous absorption between back and belly skin, when concentrations of mercury chloride from 1 to 48 mg Hg/ml are used. In this investigation the depot was placed on the skin of the experimental animal's back.

Isotope: Sodium chromate labelled with ^{51}Cr (half-life, 27.8 days) was obtained from the Radiochemical Centre, Amersham, Buckinghamshire, England.

Sodium chromate: Analytical agent.[1]

Chromium concentrations: These were selected in such a way as to enable us to make direct comparisons with our earlier absorption investigations using mercuric chloride, methyl mercury dicyandiamide and potassium mercuric iodide (3, 8); the concentrations varied between 0.00048 M and 4.870 M. This highest chromium concentration is near to the maximum solubility of sodium chromate in water at room temperature. Only aqueous solutions of sodium chromate were

[1] Mallinckrodt Chemical Works.

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used. [With regard to the chemistry of sodium chromate see Udy (6).] At least 10 absorption experiments were made in connection with each concentration.

Histological examination: In order to determine the irritant effect of the sodium chromate on the skin, biopsies were performed after 5 hours' exposure, i. e. after the conclusion of the disappearance experiment. The preparations were fixed in formalin and stained with hematoxylin eosin.

pH: The results of pH determinations (pH meter 22, Copenhagen) of the sodium chromate solutions used, is shown in table 1.

Organ analyses: A scintillation detector[2] with a well-type crystal[3] was employed for the organ analyses; and each organ was counted at least 20 minutes. The organs investigated were: skin from the exposure area, blood, heart, lungs, spleen, liver, kidneys, bones, lymph nodes from the axillae and ingues. They were directly transferred to plastic tubes, and the activity was given as counts per minute per gram wet tissue. In addition to the organ analyses after the disappearance-experiments, distribution was also studied, especially for 3 concentrations: 0.00048, 0.261 and 4.870 M, where the amounts of carrier were 86.207 %, 99.970 % and 99.998 %. In these experiments a higher activity (about 0.2 mC) was used than that in the disappearance-experiments. The organs were investigated after exposition for 5 and 19 hours.

Results

Disappearance measurements: Table 1 shows the disappearance constant and the disappearance percentage per 5-hour period for 9 different chromium concentrations between 0.00048 M and 4.870 M. For the 2 lowest and the 3 highest concentrations there is only slight absorption, i. e. calculated relatively, less than 1.0 % for 5 hours. For these concentrations the disappearance technique

Table 1. *Disappearance constant (k) and disappearance percentage during 5 hours for Na_2CrO_4 for chromium concentrations between 0.000 48 and 4.870 M.*

%/5 hours			<1.0	1.0–1.9	2.0–2.9	3.0–3.9	4.0–4.9	5.0–5.9	Mean k. 10^5 min.$^{-1}$	Mean %/5 hours	Mean rate of absorption mμM/cm² and hour
k. 10^5 min. $^{-1}$			<3.4	3.4–6.6	6.7–10.1	10.2–13.5	13.6–17.0	17.1–20.5			
Cr M	pH	No.	No.	No.	No.	No.	No.	No.			
0.000 48	7.0	10	8	1	1	—	—	—	<3.4	<1.0	—
0.005	7.9	10	7	2	1	—	—	—	<3.4	<1.0	—
0.017	8.2	10	—	4	4	—	2	—	8.8	2.6	30
0.080	8.2	12	—	6	4	2	—	—	6.9	2.1	105
0.261	8.4	10	—	—	2	3	2	3	13.7	4.0	690
0.398	8.4	10	—	3	3	2	2	—	9.4	2.8	725
0.753	8.4	10	8	1	—	1	—	—	<3.4	<1.0	—
1.689	8.5	10	7	2	1	—	—	—	<3.4	<1.0	—
4.870	8.5	10	6	4	—	—	—	—	<3.4	<1.0	—

[2] Philips PW 4119.
[3] Philips PW 4118/01.

is not sufficiently sensitive to permit a quantitative determination of the amount of absorption; but that an actual absorption has taken place is shown by the organ analyses (see below), where chromium can be shown to be present, i. a. in the blood, kidneys, urine and regional lymph glands. The table shows that the relative chromium absorption is slight for low chromium concentrations, then rises with increasing concentrations and reaches a maximal percentage for concentration 0.261 M, when, on an average, 4.0 % is absorbed in 5 hours. With further increase in chromium concentration the relative absorption falls to less than 1.0 per cent.

Organ analyses

After the disappearance experiments the organ contents were low when calculated as counts per minute per gram wet tissue, owing to the degree of absorption, the high carrier amount, the short exposure time and the comparatively low activity (1—3 μC). ^{51}Cr could be shown to be present in the blood, kidneys, urine and lymph glands. In certain cases it was also found in small quantities in the liver, lungs, heart, spleen and bone tissue.

After the absorption experiments with about 0.2 mC, ^{51}Cr can be shown to be present in all the above-mentioned organs, and in higher amounts than after the disappearance experiments. As in the latter experiments, the blood, kidneys, urine and lymph glands occupy a special position, containing relatively much larger amounts than did the other organs investigated. The highest contents in the organs were obtained with 0.261 M solution. In all cases more ^{51}Cr was obtained in the organs after 19 hours' exposure than after 5 hours.

Histological examination

Microscopic changes of any considerable extent were observed, on the whole, only in the highest concentrations. These consisted in the epidermis of acanthosis, intra- and extracellular edema. Moreover, here and there vesicles were noted localized immediately under the horny layer filled with epidermal cells and leukocytes. In the dermis a moderate infiltration of mononuclear cells and leukocytes was observed.

Discussion

That chromium compounds can be absorbed via damaged skin and thus cause death in man was described i. a. by Urban (7) and Major (4). These compounds are absorbed also through normal skin according to Schwartz and Spier (5). Their experiments — where absorption was determined by organ analyses — showed that this varied between 0.015 and 0.07 % after 18 $^1/_2$—19 $^1/_2$ hours exposure to 0.25—2.5 mg % Cr. The method employed by us showed a considerably higher chromium absorption per 5-hour period with a maximum of 4.0 % of the amount applied, and absorption varied with the concentrations of the solutions employed. Thus with low concentrations of chromium (0.00048—0.005 M) chromium absorption was less over a 5-hour period than 1.0 % of the

amount of chromium applied. With higher concentrations of chromium the relative absorption increased gradually up to a maximal percentage (4.0 %) around 0.261 M, whereas a further increase in the chromium concentration did not heighten absorption, but caused it to recede gradually to less than 1.0 %, for a 5-hour period, for concentrations of 0.753 M and over.

The appreciably higher chromium absorption which we found when using the disappearance technique, as compared with that reported by Schwartz and Spier (5), is probably due to the fact that we employed quite different concentrations and that organ analysis does not afford such a good conception of the magnitude of absorption. They did not state the chromium concentrations used in each individual absorption experiment, and, in the only comparable concentration (0.00048 M) the disappearance technique is not sensitive enough, so that absorption could only be said to be less than 1.0 % per 5-hour period. When carrying out organ analyses we found only small amounts of ^{51}Cr, which were insufficient for making a quantitative estimation of the absorption. Contrary to our procedure, Schwartz and Spier used carrierfree solutions (50—250 μC) and applied longer exposure times. This is probably the explanation of the higher organ contents of ^{51}Cr that they obtained.

Schwartz and Spier point out that absorption is probably considerably greater than the organ analyses show. In experiments of this kind the percentage of recovery is, in general, low. Against the results obtained respecting the rate of absorption, in Schwartz and Spier's experiments and in ours with the disappearance technique it may be objected that the use of a closed depot increases absorption. This increase does, however, probably not depend on the maceration of the skin (3), but is due to the fact that a higher concentration gradient is obtained when using a closed depot, which prevents evaporation.

When determining chromium absorption by the disappearance technique, relatively low activity is employed, about 1—3 μC, whereas the amount of carrier is very high in order to make it possible to study such high chromium concentrations as 4.870 M. Since the maximum percentage of absorption is 4.0, this means that the quantities of ^{51}Cr found in the respective organs only permit qualitative estimations, i. e. that absorption has taken place. It is hardly possible to acquire any quantitative conception of the size of absorption. But as a complement to determinations of the absorption by the disappearance technique, organ analyses are of value.

When this work was planned we intended to investigate especially the distribution of ^{51}Cr-labelled sodium chromate (0.2 mC) for 3 different concentrations: 0.00048, 0.261 and 4.870 M, and for varying exposure times, but, unfortunately, carrierfree solutions were not available. When the amount of carrier contained in the concentration 4.870 M is as large as 99.998 %, organ analyses are, naturally, of less value than when carrierfree solutions can be used. Like Schwartz and Spier (5) we found that ^{51}Cr, after percutaneous application, could be shown to be present in the lymph glands, blood, spleen, liver, kidneys, urine, faeces, heart, lungs, and bones. As was expected, absorption was greater after 19 hours exposure than after 5 hours. The highest organ contents were obtained with 0.261 M solution. This agreed well with the results of the disappearance experiments.

As in our earlier investigations of the absorption of mercuric chloride and

methyl mercury dicyandiamide (3) we found that absorption of sodium chromate, expressed as a percentage of the applied amount, increased gradually with higher chromium concentrations up to a maximal percentage of absorption. For the two mercury compounds this was obtained with a 0.080 M solution and for sodium chromate with a 0.261 M solution. When the concentration of chromium was still further increased, the percentage of absorption was diminished; and with high concentrations (from 0.753 M onwards) it fell to less than 1.0 % of the applied amount per 5-hour period.

The simplest explanation of this fact is probably the protein-precipitating properties of these metal compounds, with increasing concentration. In an earlier investigation (8), however, we pretreated the skin with irritant concentrations of mercuric chloride, in order to obtain an optimal protein precipitation, but this had no definite effect on the rate of absorption. Similar results were reported by Blank and Gould (1), who reduced the protein-binding of surfactants in different ways, but found that there was no increase in penetration. They concluded from this that protein-binding does not present a major obstacle to the penetration.

Provided the percentage of absorption is known for a 5-hour period as well as the concentration of the chromium solution applied and the size of the exposure area, the absolute quantity of the chromium absorbed can be calculated. Certain objections can be raised against such a method of calculation (3), but our main object is to compare the absorption of different compounds. Calculated in absolute figures, as μg mercury or chromium absorbed per hour and cm^2 exposure area, we found as regards mercuric chloride (8) that absorption increased gradually with increasing concentrations up to a plateau of about 30—50 μg mercury for a concentration of 0.080 M. When the mercury concentrations were increased to 0.239 m no appreciable increase above this plateau value took place. The same condition appears to prevail with regard to sodium chromate for which the plateau appears to be around 30—40 μg chromium per hour and cm^2 exposure area. Unfortunately, the absolute chromium absorption cannot be calculated for all concentrations from 0.753 M onwards. As was previously mentioned the percentage of absorption is then less than 1.0 per 5-hour period. If instead, absorption is given as mμM mercury and chromium per hour and cm^2 exposure area, the plateau for mercuric chloride is around 150—300, and that for sodium chromate around 690—725, (i. e. compared on a molar basis, chromium is absorbed 2—3 times more rapidly than mercury). By way of comparison it may be mentioned that Fredriksson (2), when using the same method (disappearance measurements) reported the absorption of parathion to be 240—260 mμM and of sarin to be 7740 mμM per hour and cm^2 exposure area in the cat.

SUMMARY

The percutaneous absorption of sodium chromate in guinea pigs was studied during periods of 5 hours at 9 different concentrations: 0.000 48 M — 4.870 M, by means of an *in vivo* isotope technique, the so-called "disappearance measurements" and by organ analysis.

1. The *relative* absorption increased with increasing chromium concentration

to a maximal procentual absorption (4.0 %) with a 0.261 M solution. When the concentration was still further increased the absorption decreased to less than 1.0 % with 0.753 M and over. The *absolute* absorption also increased with increasing chromium concentration up to a plateau-value of approximately 690—725 mμ M per hour and cm^2 exposure area. A further increase in the chromium concentration above 0.398 M did not increase absorption above this plateau-value.

2. Organ analyses showed that after percutaneous absorption the highest content of ^{51}Cr, calculated in counts per minute per gram wet tissue, was observed in the lymph nodes, and that smaller amounts were found in the kidneys, urine, blood, liver and spleen.

RÉSUMÉ

L'absorption percutanée du chromate de sodium a été étudiée chez le cobaye pendant une durée de 5 heures avec des solutions de 9 concentrations différentes entre 0,000 48 M — 4,870 M, au moyen d'une technique utilisant un isotope *in vivo*, la soi-disant « mesure de disparition » ainsi que par des analyses d'organes.

1. L'absorption *relative* croît avec l'augmentation de la concentration en chrome jusqu'à un pourcentage d'absorption maximum (4,0 %) avec une solution de 0,261 M. En augmentant encore la concentration, l'absorption diminue jusqu'à moins de 1,0 % avec des concentrations de 0,753 M et plus. L'absorption *absolute* croît aussi avec la concentration en chrome, jusqu'à un plateau situé approximativement à 690—725 mμ M par heure et par cm^2 de surface de peau exposée. Une nouvelle augmentation de la concentration en chrome, supérieure à 0,398 M ne provoque pas une augmentation de l'absorption supérieure à ce plateau.

2. Les analyses d'organes ont montré qu'après l'absorption percutanée la plus grande partie de Cr^{51}, calculée en grammes de tissu frais et par minute, se trouve dans les ganglions lymphatiques, et que les plus petites quantités ont été trouvées dans les reins, l'urine, le sang, le foie et la rate.

ZUSAMMENFASSUNG

Es wurde die percutane Aufnahme von Natriumchromat bei Meerschweinchen über eine Dauer von 5 Stunden untersucht. 9 verschiedene Konzentrationen zwischen 0.000 48 M und 4,870 M wurden appliziert. Die Resorption wurde mit einer in vivo-Isotopentechnik, der sog. „Verlust-Messung" sowie durch Organanalyse bestimmt.

1. Die *relative* Resorption stieg mit steigender Chrom-Konzentration auf eine maximale prozentuale Resorption von 4 %, die mit einer 0,261 M-Lösung erreicht wurde. Bei weiterer Erhöhung der Konzentration fiel die Resorptionsrate wieder ab, bei Verwendung von 0,753 M und höher konzentrierten Lösungen auf weniger als 1 %. Die *absolute* Resorption stieg ebenfalls mit ansteigender Konzentration bis zu einem Maximum von 690— 725 mμ M pro Stunde und cm^2 Expositionsfläche. Weitere Erhöhung der Chrom-Konzentration auf über 0,398 M bewirkte keine weitere Steigerung.

2. Die Organanalysen zeigten, dass nach percutaner Resorption der höchste Cr^{51}-Gehalt auf Grund der Zählung pro Minute und Gramm Feuchtgewicht in den Lymphknoten festzustellen war. Geringere Mengen wurden in Nieren, Urin, Blut, Leber und Milz gefunden.

RESUMEN

Se estudió la absorción percutánea del cromato sódico en cobayas en períodos de 5 horas a concentraciones diferentes: 0,000 48 M — 4,870 M, por medio de una técnica de isótopos «in vivo» llamada «medidas de desaparición» (disappearance measurements) y por análisis orgánico.

1. La absorción *relativa* aumenta con la concentración del cromo hasta una absorción procentual máxima (4.0 %) con una solución de 0.261 M. Cuando la concentración es todavía mayor la absorción desciende a menos del 1,0 % con 0.753 M y más. La absorción *absoluta* también aumenta con la concentración del cromo hasta un nivel de aproximadamente 690—725 mμ M por hora y por cm^2 de área de exposición. Un aumento mayor de la concentración del cromo por encima de 0.398 M no eleva la absorción por encima de este nivel.

2. Los análisis orgánicos muestran que después de la absorción percutánea el contenido más elevado de ^{51}Cr, calculado en valores por minuto por gramo de tejido humedecido, se encontró en ganglios linfáticos, y los valores mínimos en riñón, orina, sangre hígado y bazo.

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