lines appeared on examination of the products when precipitated basic cupric carbonate was subjected to 18000 bars and 550°C in the 'simple squeezer'.

Eventually precipitated basic cupric carbonate was introduced into a low-pressure hydrothermal bomb and subjected to 500 bars total pressure (CO_2 partial pressure = 450 bars, H_2O partial pressure = 50 bars) at 180°C for 36 h. The bulk of the products was well-crystallized malachite, but a small yield of the rhombohedral substance was also obtained.

The yield was better than before, and the diffraction lines were reasonably strong. It proved to be quite simple to subtract the known lines of malachite from the pattern. In only one case was it necessary to estimate the intensity of a line from the earlier patterns.

The X-ray powder diffraction pattern at 25°C was obtained by means of a Norelco high angle recording diffractometer, using CuK_α radiation ($\lambda = 1{\cdot}5418$ Å) and a Ni filter. The results are given in the Table.

It was found that the presumed $CuCO_3$ crystallizes in the rhombohedral system. Systematic extinctions appeared to be the same as for calcite, and it is consequently assumed that the space group is also the same, *viz.* $R\,\bar{3}c$.

The hexagonal unit-cell dimensions at 25°C, obtained by least-squares, are:

$$a_0 = 4{\cdot}796 \pm {\cdot}005 \text{ Å}$$
$$c_0 = 15{\cdot}48 \pm {\cdot}01 \text{ Å},$$

yielding an axial ratio

$$c_0/a_0 = 3{\cdot}227.$$

The dimensions of the corresponding rhombohedral unit-cell are:

$$a_{rh} = 5{\cdot}856 \text{ Å}$$
$$\alpha = 48° 11'.$$

These dimensions are well within the range covered by the other rhombohedral carbonates. While it cannot be stated with absolute certainty that the present compound is actually $CuCO_3$, the X-ray evidence is strongly in favour of this being the case.

The stability of this compound is evidently dependent not only on the CO_2-pressure and the temperature, but also on the partial H_2O-pressure. It would seem that a CO_2-pressure which is rather high in comparison with the H_2O-pressure is necessary for its stability, and this might explain why it is not found in nature.

Assuming a rhombohedral unit-cell containing 2 molecules, the calculated density of $CuCO_3$ is 3·99 g/cm³ at 25°C. This value is not dependent on the conditions of formation, as seems to be the case for $NiCO_3$[4].

Powder data

$d_{obs.}$ in Å	$d_{calc.}$ in Å	$hk \cdot l$	$100I/I_0$
3·654	3·659	01·2	35
2·819	2·831	10·4	100
2·390	2·398	11·0	30
2·161	2·174	11·3	30
1·758	1·757	11·6	25
1·538	1·539	12·2	30
1·451	1·451	10·10	20

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Zusammenfassung

In Anwesenheit von Malachit wurde kristallines $CuCO_3$ durch Behandlung des basischen Karbonates unter 450 bar CO_2-Druck und bei 180°C gewonnen. Die rhomboedrische Einheitszelle (Raumgruppe $R\bar{3}c$) besitzt die folgenden Dimensionen:

$$a_{rh} = 5{,}856 \text{ Å}; \alpha = 48° 11'.$$

[4] J. GOLDSMITH, oral communication.

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Nucleic Acid Content in Mouse Epidermis Separated from Dermis by Different Methods

In various biochemical investigations of the skin, it is essential to obtain data separately for the epidermis and the dermis. This is especially important when pathological processes (e. g. the carcinogenesis) are being studied. In such cases, the interpretation of the data is difficult and often impossible if they refer to the whole skin. That is why different methods for separation of the epidermis from the dermis have been proposed: physical procedures–high vacuum[1]; splitting of the epidermis by means of a sharp razor or dermatoms[2,3]; scraping off the epidermis with a sharp safety-razor[4] or with a blunt instrument–in the last case after a preliminary loosening of the junction between the epidermis and the corium by means of tight stretching of the skin[5,6], or by heating the skin[7]; chemical reagents: unsaturated organic compounds[8,9]; acids[7,10,11]; alkali[7,12]; neutral salts[10,11]; enzymes: pepsin[13], trypsin[14–17], collagenase, esterase[15].

All methods mentioned above have different advantages and shortcomings, pointed out in the literature cited. It is most probable that for every kind of investigation a different suitable method should be used. The great importance of NA studies in connection with such pathological processes in skin as regeneration, carcinogenesis etc., makes it most desirable to specify which of the suggested methods would be applicable for nucleic acid estimation in epidermis.

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Experimental methods. All investigations were carried out on 3-month old Agnes-Bluhm white mice. The hair of the skin was plucked off with a mixture of wax and colophony.

1. *Removal of the epidermis.* (a) *Scraping method.* The incised depilated skin is stretched fur side up and the epidermis scraped off with sharp safety razor. (b) *Heat method*[7]. The skin is placed with corium downwards on a metal plate and heated 2 min at 50° ± 0·5°C. The epidermis is then removed by means of a blunt scalpel. (c) *Citric acid method.* This procedure, elaborated by us, leads to a spontaneous separation of the epidermis as a whole layer. The sacrificed and depilated animals are injected subcutaneously in the back region with 20 ml of ice cold 2% citric acid, the needle being introduced through the tail in order to avoid the flow out of the solution. The injected mice are kept in the refrigerator at 1–2°C for 2 h and then the epidermis is separated from the dermis as an intact layer. The epidermis separated by this method manifests a considerable O_2-consumption. (d) *Trypsin method*[15]. The incised and depilated skin is incubated at 37°C for 1 h in 0·5% solution of trypsin in phosphate buffer with pH 7·8. The epidermis is then scraped off with a blunt scalpel.

2. *Nucleic acid estimation.* The separated epidermis is immediately placed in ice-cold absolute alcohol. After changing the alcohol, the material is treated twice with ether and dried at 37°C overnight. The estimation of NA was then carried out spectrophotometrically according to our two-wave-length method[18], which eliminates the ultraviolet absorbing contaminants. The results are expressed in mg of phosphorus per 100 g of dried delipidated tissue.

3. *Histological and histochemical controls.* In order to estimate morphologically the degree of separation of the epidermis, as well as the modifying action of the different procedures on the cytochemically detected NA, the separated epidermis and dermis were fixed 40 min in the Helly fixative and paraffin sections were stained with Unna's methylgreen-pyronine.

Results and discussion. The highest values for the NA content are obtained (Table) when the epidermis is removed by scraping or by the heat procedure, both methods giving the same values: 193 mg% RNA-P and 270 mg% DNA-P, the quotient RNA/DNA varying between 0·70 and 0·75. The NA content of epidermis separated by means of citric acid is considerably decreased – 25% for RNA and 33% for DNA, which increases slightly the quotient RNA/DNA to 0·84. Lowest values are obtained by the trypsin method, the RNA content being 31% and the DNA content 43% lower in comparison with the data from the first two methods.

The lower values obtained by the last two methods can be explained by the incomplete separation of the epidermis cells and by the partial extraction of cellular components including NA. As our histological observations have shown, the scraping removes completely the epidermis cells, the quantity of dermis being negligible. The using of a blunt instrument by the heat procedure removes all epidermal cells too, but yields pure epidermis without any traces of the corium. The citric acid separates an intact layer of epidermis, but here and there groups of basal epidermal cells rich in NA remain on the dermis. The most incomplete method is the separation of epidermis with trypsin, a considerable amount of basal cells remaining with the dermis.

The cytochemical detection of NA reveals no visible extraction of NA by all four methods used. Additional biochemical investigations have shown, however, that in citric acid solution a significant amount of substances including NA can be extracted.

Separation method	Replicates	RNA-P[a]	DNA-P[a]	RNA/DNA
Scraping	7	196 ± 8	262 ± 11	0·75 ± 0·03
Heat	14	191 ± 4	275 ± 7	0·70 ± 0·02
Citric acid	6	148 ± 4	178 ± 11	0·84 ± 0·03
Trypsin	7	134 ± 7	153 ± 6	0·88 ± 0·06

[a] Expressed as mg of phosphorus per 100 g of dry delipidated epidermis.

Compared with some literature data[19], our values for RNA and DNA in epidermis removed by the heat procedure are high, which indicates that other chemical methods lead to loss of NA too. The higher quotient RNA/DNA (1·44) obtained by the authors cited is due to the contaminants interfering with NA estimation when the classical Schmidt-Thannhauser or Schneider procedures are used[18].

It is obvious from these data that, for NA estimation, only the scraping method especially combined with the heat procedure are suitable for isolation of the epidermis. Separation by chemical reagents or enzymes may influence considerably the NA content of the epidermis.

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Résumé

Des études comparatives ont été effectuées sur le contenu en AN de l'épiderme de souris blanches séparé du derme par quatre méthodes différentes. Ce n'est que la méthode mécanique (scraping method) et la méthode à chaud de Baumberger *et al.*[7], qui se sont montrées convenables pour les déterminations quantitatives des AN.

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Demonstration of Aliinase in a Protein Preparation from Onion

The recent identification of the major sulfur-containing volatiles of macerated onion as alkyl disulfides[1] plus the observation by Vilkki[2] that ammonia and pyruvate are released from an amino acid isolated from onion in the presence of onion juice suggests the presence in onions of an enzyme similar to the garlic alliinase of Stoll and Seebeck[3] capable of hydrolyzing S-alkyl-L-cysteine sulfoxides to the corresponding alkyl esters of alkyl thiosulfinic acids, ammonia, and pyruvate. Recently Virtanen and Matikkala[4] have isolated from onion the sulfoxides of both S-methyl and S-propyl cysteine.

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