*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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Although garlic alliinase was first prepared 11 years ago by STOLL and SEEBECK, no description of its isolation from the related species, onion, has previously been reported. The present paper forwards evidence to support the occurrence of such an enzyme in a soluble protein fraction of onions which was prepared as an acetone powder from an 0·75-saturated ammoniumsulfate-precipitated fraction obtained from the supernate of the homogenate of peeled chopped onions. The homogenate was prepared by blending for 30 s 250 g of tissue with 150 ml 0·3 *M* sucrose in 0·2 *M* phosphate buffer, pH 6·8, at 4°C.

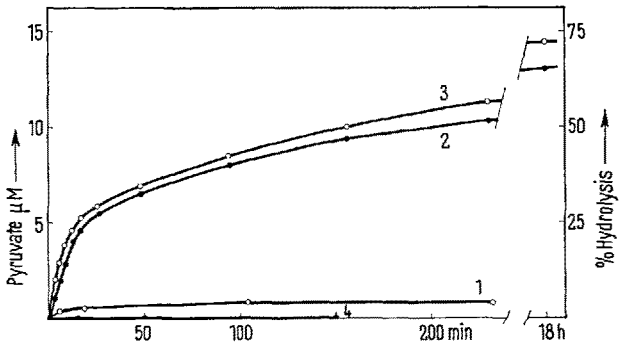
The course of enzymatic production of pyruvate from a synthetic preparation of (±)-S-propyl-L-cysteine sulfoxide is shown in the Figure. At pH 8·4 in the presence of pyrophosphate buffer and pyridoxal phosphate (conditions for optimal activity), the reaction proceeds rapidly for the first 10 min and 20% hydrolysis to pyruvate. The reaction then proceeds at a slower rate to about 70% in 18 h. The Michaelis constant for this substrate was found to be equal to $5 \times 10^{-3}$ *M*. This biphasic mode of the course of the reaction is probably due to the observed difference in the rates of hydrolysis between the (+) and (−) forms of the substrate. The (+) form of S-propyl-L-cysteine is also hydrolyzed faster than (+)-S-methyl and (±)-S-allyl cysteine sulfoxide. Neither the corresponding S-alkyl-L-cysteines nor cycloalliin[5] are hydrolyzed. At pH 4·9, the reaction proceeds slowly and terminates at about 4% hydrolysis, thus illustrating the instability and low activity of this enzyme at this pH.

The reaction mixture ('Experimental' 9·1 ml) at pH 8·4 and 37°C in 0·07 *M* pyrophosphate contained 200 μ*M* of amino acid (see Fig.), 5 μ*M* of pyridoxal phosphate, and 18·1 mg of dialyzed enzyme protein nitrogen in a closed Thunberg tube containing 1·4 ml of 1 *N* HCl in the side arm. The reaction was terminated by addition of the acid in the side arm, centrifuged and stored at −26°C. 'Blank' was identical with 'Experimental' except that amino acid added after addition of HCl. Amino acid was determined by the methods of KORNBERG and PATEY[6] and ROSEN[7], pyruvate by the method of KACHMAR and BOYER[8]; ammonia by the method of BROWN *et al.*[9]; thiosulfinate by the method of CARSON and WONG[10], using a pure sample (infrared) of the propyl ester of propylthiolsulfinate for standardization. Benzene extract of enzyme digest was used for analysis after removal of benzene by distillation *in vacuo*. (Pyruvic acid was identified by the infrared spectrum of its 2,4-dinitrophenylhydrazone.)

These results (see Table) demonstrate that both ammonia and pyruvate, produced as a result of enzyme action, correspond within experimental error to the decrease in the amount of amino acid. Thiosulfinate as measured by the method of CARSON and WONG[10] appeared in amounts less than half of those expected. This indicates losses due to instability during the reaction (this method does not detect thiosulfonates), during the preparation of the sample for analysis and/or the production of other sulfur-containing substances from the presumed primary product of reaction, propyl thiosulfenic acid[3].

The enzyme thus appears to be similar to garlic alliinase in its specificity towards S-alkyl-L-cysteine sulfoxides, its lability[3], and in its requirement for pyridoxal phosphate for maximal activity[11]. It differs from the garlic enzyme in its pH optimum and its optimal action on (+)-S-propyl-L-cysteine sulfoxide. The garlic enzyme has been reported to act optimally on (+)-S-allyl-L-cysteine sulfoxide, over a broad pH range extending from pH 5 to pH 8[3]. The lack of action on S-substituted cystine derivatives differentiates it sharply from the C-S lyase of *Albizzia lophanta*[12].

We wish to thank G. F. BAILEY and E. GONG of this laboratory for the infrared determinations.




Production of pyruvate from S-propyl-L-cysteine sulfoxide by a protein preparation from onions. The enzyme reaction, carried out in 1 ml at 37°C, contained the following: curve 1, 20 μ*M* of (±)-S-propyl-L-cysteine sulfoxide ($\alpha_D = -6{\cdot}9$, $c = 1$) and 1·55 mg of enzyme protein nitrogen at pH 4·9; curve 2, as in curve 1 + 70 μ*M* pyrophosphate buffer (final pH = 8·4); curve 3, as in curve 4 + 0·05 μ*M* of pyridoxal phosphate; curve 4, as in curve 3, except boiled enzyme used. Pyruvate determined according to method of KACHMAR and BOYER[8]

Stoichiometry of Onion Alliinase Action

| Substance | Blank μ*M* | Experim. μ*M* | Change μ*M* |
|---|---|---|---|
| Amino Acid | 200 | 126 | −74 |
| Pyruvic Acid | 2 | 75 | +73 |
| Ammonia | 6 | 74 | +68 |
| Thiosulfinate | 0 | 34 | +34 |

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*Zusammenfassung*

Eine proteinhaltige Darstellung aus Zwiebeln enthält ein Enzym, welches stöchiometrisch S-Alkyl-L-cysteinsulfoxyde in Brenztraubensäure und Ammoniak spaltet. Daneben entsteht, nicht-stöchiometrisch, ein benzollöslicher, lauchartig riechender, mit N-Äthylmaleinimid reagierender Stoff, wahrscheinlich das Alkylthiosulfinsäurealkylester. Das Enzym wirkt optimal auf (+)-S-Propyl-L-cysteinsulfoxyd bei pH 8,4 in Gegenwart von Pyridoxalphosphat.

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