

13. GORDON, J.; ROSE, B. and SEHON, A. H.: Detection of 'non-precipitating' antibodies in sera of individuals allergic to ragweed pollen by an *in vitro* method. *J. exp. Med.* 108: 37 (1958).
14. VOSTI, K. L. and RANTZ, L. A.: Measurement of type and non-type specific group A hemolytic streptococcal antibody with an hemagglutination technique. *J. Immunol.* 92: 185 (1964).
15. RAFFEL, S.: *Immunity*; 2nd Ed., p. 369 (Appleton Century-Crofts, New York, N. Y. 1961).
16. PETERSON, R. D. A.; WICKLAND, P. E. and GOOD, R. A.: Endotoxin activity of a house dust extract. *J. Allergy* 35: 134 (1964).
17. THOMAS, L.: The Physiological disturbances produced by endotoxin. *Ann. Rev. Physiol.* 16: 467 (1954).
18. ISHIZAKA, K.: Gamma globulin and molecular mechanisms in hypersensitivity reactions. *Progress in Allergy*, Vol. 7, p. 32 (Karger, Basel/New York 1963).
19. Unpublished observations.
20. MUNOZ, J.: Effect of bacteria and bacterial products on antibody response. *Advances in Immunology*, Vol. 4, p. 397 (Academic Press, New York, N. Y. 1964).

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## Studies on the Atopic Allergen in Ripe Tomato Fruits

### II. Further Chemical Characterization of the Purified Allergen

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#### *Introduction*

In a previous paper (10) the isolation and purification of the atopic allergen in ripe tomato fruits has been described. The active fraction was shown to be a polydisperse mixture of closely related protein-carbohydrate complexes. Physicochemically, the allergen closely resembled many other purified inhalant allergens, while its sedimentation constant likewise proved to be of the order of magnitude expected for atopic allergens.

Current studies on the ultraviolet and fluorescence spectra of inhalant allergens have shown these antigens to be characterized by the incorporation of 1-deoxy-2-ketose sugars and their derivatives, linked to the  $\epsilon$ -amino group of lysine residues through carbon atom C-1 (2, 4, 5).

In the present paper evidence will be presented for the incorporation of similar N-glycosidic 1-amino-1-deoxy-2-ketose groups within the molecular structure of the purified tomato allergen; it will also be shown that this allergenic glycoprotein is produced artificially by non-enzymatic browning (Maillard) reactions between proteins and reducing sugars during the ripening process.

#### *Materials and Methods*

The tomato fractions D, F and G used in this study were prepared as described before (10); fraction D represents the crude allergenic fraction obtained by  $(\text{NH}_4)_2\text{SO}_4$ -precipitation of the dialyzed extract of ripe tomatoes, F the globulin fraction and G the purified tomato allergen fraction. Fractions F and G were both obtained by chromatography of fraction D on DEAE-Sephadex.

## Spectroscopic Measurements

Ultraviolet absorption spectra were determined of 0.05% solutions in 0.01 M phosphate buffer at pH 7 and room temperature, against the same buffer as a blank; a Zeiss spectrophotometer, type PMQ II, was employed (1 cm quartz cells).

Fluorescence emission spectra were established of 0.1% solutions under the same conditions as above, using the Zeiss spectrophotometer equipped with a special fluorescence attachment; excitation energy in this instrument is provided by a low-pressure mercury arc, from which a beam of monochromatic incident light of 365 nm wavelength is isolated by means of a filter inserted between the energy source and the cuvettes. The beam of fluorescent secondary light is withdrawn perpendicular to the exciting beam and is subsequently dispersed in a monochromator.

## Analytical Methods

Methods of assay for protein nitrogen, hexoses and amino acid composition have already been given (10). Free  $\epsilon$ -amino groups ('available' lysine content) of the fractions were determined by a micro-modification of the method of CARPENTER (11); values obtained were not corrected for losses of the 2,4-dinitrophenyl- $\epsilon$ -N-lysine complex during acid hydrolysis.

The method of KEENEY and BASSETTE (14) was used for the qualitative assay of browning intermediates in the isolated fractions: after digestion in 0.1 N oxalic acid at 100°C for 2 h, the methylfurfural yield was determined by spectrophotometric measurement of the 2-thiobarbituric acid reaction product at 443 nm. The  $E_{443}$ -values were corrected for absorption of the allergen at the same wavelength.

## Digestion Studies

Proteolytic enzyme digestions were carried out with crystalline trypsin ( $2 \times$  cryst., NBC) and  $\alpha$ -chymotrypsin (Boehringer) at pH 8 and 37°C under a constant stream of nitrogen in a pH-state attached to a Radiometer TTT<sub>1</sub> titrator equipped with a glass-electrode and a magnetic valve; 0.01 M NaOH was added from a micro-burette, with automatic pH-control, to a solution containing 25 mg of allergen and 100  $\mu$ g of enzyme in 25 ml 0.01 M  $\text{CaCl}_2$ .

## Results

## (a) Optical Studies

The ultraviolet absorption spectrum of the active fraction from tomatoes is significantly different from that of many other (glyco)-proteins (6), indicating additional chromophores to be incorporated in the molecular structure besides the u. v. absorbing aromatic side chains of tyrosine, phenylalanine and tryptophan. The characteristic differences with normal protein spectra become at once apparent if a comparison is made between the absorption spectra of fractions F and G; these spectra have been given in Fig. 1.

Subtraction of the rather normal absorption spectrum of fraction F from that of fraction G reveals prominent peaks in the difference curve at 250 nm, 305 nm and at 325 nm, while marked absorption still occurs at wavelengths over 325 nm (Fig. 2).

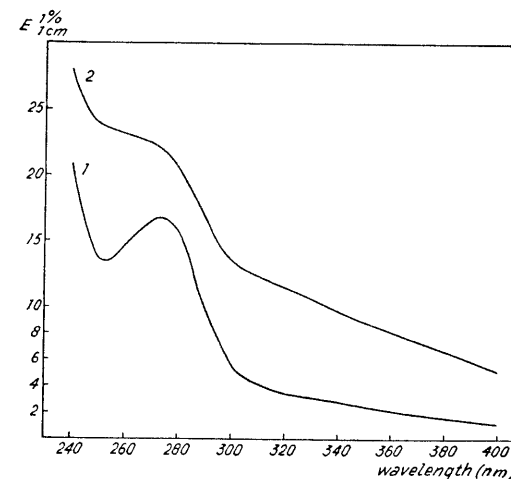


Fig. 1. Ultraviolet absorption spectrum of fraction F (1) and fraction G (2) in phosphate buffer (0.01 M, pH 7).

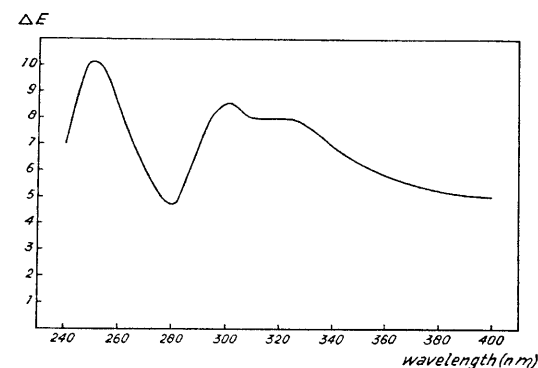


Fig. 2. Difference spectrum, obtained by subtracting curve 1 in Fig. 1 from 2.

Similar characteristic maxima have been observed in the difference spectra constructed for purified inhalant allergens. Analysis of the u. v. absorption spectra of a large number of these allergens (5), together with extensive investigations of synthetic model compounds (6, 7) and of model systems containing crystalline proteins (or simple amines) and reducing sugars have indicated that

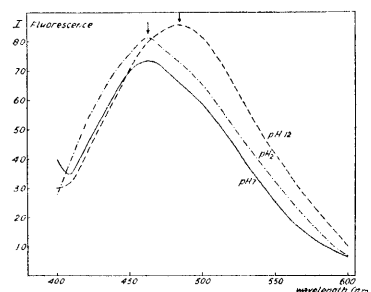


Fig. 3. Fluorescence spectra of purified tomato allergen at pH 2 (0.01 N HCl), pH 7 (0.01 M phosphate buffer) and pH 12 (0.01 N NaOH) at 20°C. Excitation: 365 nm. Fluorescence intensity in arbitrary units.

(1) the increase of absorption at 250 nm is largely the result of denaturation of the protein moiety of the atopen molecules;

(2) the maximum at 305 nm is specifically attributable to the 1,2-enol of a N-substituted 1-amino-1-deoxy-2-ketose residue;

(3) absorption at 325 nm and above is due to degradation products of the above mentioned ketose (2,3-enediol and -anions, comp. ref. 5), the incorporation of the 1,2-enol and 2,3-enediol tautomers and of their anions accounting for the brown colours of the allergens in solution.

Besides these characteristic features of u.v. absorption, the atopic allergen from ripe tomatoes has also been found to emit blue fluorescence upon irradiation with ultraviolet light of 365 nm wavelength.

In Fig. 3 the fluorescence spectra have been represented for the allergen at different pH-values. Fluorescence emission proved to be pH-dependent, the intensity being higher at pH 2 and pH 12 than at neutral pH, while the maximum of fluorescence shifted from about 460 nm at pH 7 to about 485 nm at pH 12.

It has already been demonstrated in a separate study on the fluorescence spectra of a large number of purified atopic allergens and of deoxyribulose-proteins that, in acid and neutral medium, the maximum of fluorescence emission at about 445 nm is to be attributed to the 1,2-enolic tautomer of the N-substituted ribulose in these compounds; under alkaline conditions the 1,2-enol is converted into (a stabilized form of) the 2,3-enediol tautomer, fluor-

Table I  
Analytical data.

| Fraction | Protein N % | Hexose % | Uronic acid % | Lysine content %* lysine %** | Avail. lysine %** | E <sub>443</sub> nm*** |
|----------|-------------|----------|---------------|------------------------------|-------------------|------------------------|
| D        | 8.5         | 22.5     | 7.5           | N. d.                        | 2.6               | 0.36                   |
| F        | 10.2        | 12.6     | 1.5           | 3.6                          | 3.0               | 0.12                   |
| G        | 8.5         | 21.0     | 8.0           | 3.5                          | 1.6               | 0.60                   |

\* After HCl hydrolysis.

\*\* Assayed according to CARPENTER (11).

\*\*\* Assayed according to KEENEY and BASSETTE (14); values given for 5 mg allergen per 5 ml of the T.B.A.-furfural reaction product.

escing at about 490 nm (2, 3). In the tomato allergen, as isolated from the ripe fruits, both the 1,2- and 2,3-enolic tautomers occur, causing the ultimate fluorescence maximum to lie at about 460 nm.

#### (b) Chemical Analysis

Additional evidence for the incorporation of N-substituted sugar residues in the molecular structure of the purified tomato atopen was obtained by chemical analysis. The 1-amino-1-deoxy-2-ketoses and other browning intermediates are known to be easily converted in oxalic acid medium into furfural compounds (14). These relatively unstable sugar residues could indeed be demonstrated in the skin-reactive tomato fraction G; the absorption at 443 nm of the thiobarbituric acid-furfural reaction product proved to be higher for the active fraction G than for fractions F and D (compare Table I).

Moreover, the 'available' lysine content, as determined by the method of CARPENTER (11), was found to be much lower than the amount of lysine found in the allergen after total acid hydrolysis with HCl; the method of Carpenter measures the number of free  $\epsilon$ -amino groups of lysine residues, i. e. the  $\epsilon$ -amino groups available for reaction with dinitrofluorobenzene in the native allergen. Of the  $\epsilon$ -amino groups in the allergenic fraction G, 55% was in fact found to be blocked by covalent substitution; a much lower degree of blockade was found for the compounds in fraction F.

In accordance with these findings the active fraction from tomatoes was observed to be relatively resistant to the action of the proteolytic enzyme trypsin. Trypsin has a narrowly restricted specificity, limiting its action to the rupture of bonds linking the car-

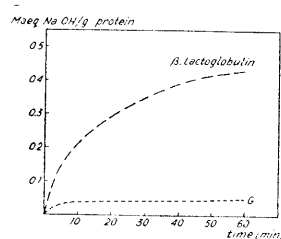


Fig. 4. Trypsin digestion curves of purified tomato allergen (G) and  $\beta$ -lactoglobulin. Conditions: see Materials and Methods section.

boxyl group of a basic amino acid (lysine, arginine) to adjacent amino acids in the peptide chain.

In Fig. 4 the digestion curve has been given of fraction G treated with trypsin, in comparison to the curve obtained with  $\beta$ -lactoglobulin (after slight denaturation at pH 8.5). Of the protein  $\beta$ -lactoglobulin, with its lysine content about 3 times higher than found in fraction G, about 8 times more peptide bonds are split during the period of incubation than of fraction G; of fraction F about twice the number of peptide bonds is hydrolyzed under the action of trypsin. It should be emphasized at this point that the treatment of the allergen with 8 M urea prior to digestion, or with 2-mercapto-ethanol and iodoacetic acid did not influence the results.

In similar experiments a remarkable resistance was also noted of the allergen fraction against the action of  $\alpha$ -chymotrypsin; this might be due to masking of the aromatic side chains by the carbohydrate moiety of the allergen. In contrast, the allergen proved to be susceptible to the action of pepsin.

#### (c) Origin of the Allergen

Comparable fractions were isolated from different batches of tomatoes under strictly identical conditions (4°C, pH 4–8 etc.), i. e. the water-soluble non-dialyzable material and the compounds precipitable with ammonium sulphate. Three batches were investigated, viz: orangegreen (unripe) tomatoes, fresh red-ripe tomatoes and ripe tomatoes stored for 14 days at room temperature; significant data have been collected in Table II.

Three atopic patients with a positive skin reaction to tomato extract were injected intracutaneously with the products obtained.

Table II  
Comparative data on extracts obtained from different batches of tomatoes.

| Material                                        | Yield**<br>(in mg/kg tomatoes) | E <sub>1cm</sub> <sup>1%</sup> 305nm | I. fluor<br>460 nm*** | Skin-reactivity<br>U/mg |
|-------------------------------------------------|--------------------------------|--------------------------------------|-----------------------|-------------------------|
| Green-orange tomatoes                           | 100–150                        | 4.60                                 | 6                     | < 1000                  |
| Red-ripe                                        | 100–150                        | 9.80                                 | 11                    | 2500                    |
| Red-ripe stored 14 days at room temperature     | 100–150                        | 12.0                                 | 12                    | 2700                    |
| Freezedried tomato juice stored 1 year at 20°C* | 320                            | 18.2                                 | 40                    | not done                |

\* 10–20% H<sub>2</sub>O.

\*\* of high-molecular weight material prepared by the procedure applied for fraction D (cf. section Materials and Methods).

\*\*\* of 0.10% solutions in 0.1 M phosphate buffer pH 7; intensity I in arbitrary units.

It was found that the skin-reactive potency of the fractions parallels the degree of browning; with increasing degree of maturation of the fruits the skin-reactivity, together with u. v. absorption at 305 nm and with the intensity of fluorescence emission, were observed to increase.

These experiments therefore demonstrate that the active tomato allergen is being artificially produced during the process of ripening and during storage of the fruits. Also, browning reactions seem to proceed in tomato extract concentrates, as evident from the data in Table II, fourth line.

#### (d) Inactivation Studies

As pointed out before (10) the allergen is remarkably stable at acid and neutral pH, and resists the action of many protein denaturing agents. However, in alkaline solution its stability proved to be far less pronounced. Upon incubation at pH 12 and room temperature the brown colour of the allergen solution rapidly faded, the solution becoming colloidal (not due to bacterial contamination), while sometimes precipitation occurred of denatured protein material. These changes could be followed with the aid of ultraviolet and fluorescence spectroscopy.

Fluorescence intensity was observed to decrease rapidly upon incubation of solutions of fraction G at pH 12, the reduction of

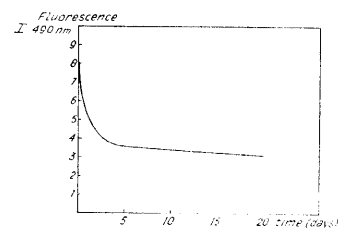


Fig. 5. Fluorescence-time curve. Tomato allergen incubated at pH 12 (0.01 M NaOH) and 20°C. Fluorescence intensity at 490 nm in arbitrary units.

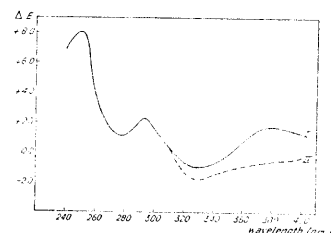


Fig. 6. Difference spectra, obtained by subtracting the ultraviolet absorption curves of fraction G at pH 7 from these at pH 12. I without incubation, II after incubation at 20°C during 24 h.

fluorescence emission amounting to about 75% in two days (Fig. 5); the phenomenon proved to be irreversible.

Similarly, the difference curve of u. v. absorption (spectrum of the allergen solution at pH 12 minus that at pH 7) was subject to marked alteration (Fig. 6).

The difference curve constructed for these spectra before incubation reveals a minimum at 325 nm and a maximum at 375 nm; this would indicate the conversion of the 1,2-enolic tautomer of the N-glycosidic residue into the (anion of) the 2,3-enediol isomer, as discussed in separate papers (1, 2). The 2,3-enediol form, like other reductones of a related structure, is unstable in alkaline medium and is liable to undergo further rearrangement, polymerization reactions, or  $\beta$ -elimination, all of which would ultimately lead to a denatured and unstable glycoprotein. These degradation reactions could actually be seen in operation during incubation: the maximum

in the difference curve at 375 nm declines and finally disappears completely, while the absorption differences between the uv-spectra at pH 12 and pH 7 in the whole range of wavelengths over 300 nm gradually diminish and become negative.

These findings supplement experiments done with other atopic allergens and are in confirmation of the mechanism proposed for their inactivation (1). Moreover, they afford an appropriate explanation for the marked loss of skin-reactivity observed during chromatography on DEAE-Sephadex, especially if the allergen is eluted at low flow rates and at room temperature: the allergen occurs in fact adsorbed onto the surface of the alkaline anion-exchange resin, where the effective alkalinity is considerably higher than in the effluent volume, and is therefore liable to undergo similar degradation reactions during adsorption as described above.

Because of the reductone nature of the lysyl-sugar determinant some preliminary experiments were finally conducted on the influence of oxidation upon the activity of the allergen fraction. To this end  $K_3Fe(CN)_6$  in acid medium was chosen as an oxidizing agent.

In a typical experiment 10 mg of fraction G was dissolved in 4 ml of a 0.1 M phthalic acid buffer (pH 5.6), 1 ml of a solution of 1%  $K_3Fe(CN)_6$  in water was added and the mixture was kept at 70°C for 40 min (compare ref. 12). The solution was then dialyzed against a phosphate buffer 0.01 M, pH 6.0, containing 0.01 M sodium EDTA, followed by dialysis against several changes of distilled water; after lyophilization a grey-white powder was obtained ( $G_{ox}$ ).

In Fig. 7 the fluorescence spectra have been given of the allergen before and after oxidation: a fall in effective fluorescence ( $I_{460}-I_{410}$ ) of about 75% may in fact be observed. In Fig. 8 the curve has been drawn obtained by plotting the difference between the u. v. absorption spectrum of the allergen before oxidation and after oxidation. The minor peak in this curve at 280 nm (often only a mere shoulder) is attributable to the oxidation of aromatic amino acid residues, while the maxima at 325 and 350 nm correspond to the removal by oxidation of the above mentioned enolic tautomers of the N-glycosidic lysyl-sugar residues in the molecular structure. Although oxidation of other groups in the allergen structure cannot be entirely excluded, it seems unlikely that changes other than at the site of the 1-amino-1-deoxy-2-ketoses will have occurred to any great extent because reductones of this and of a related structure

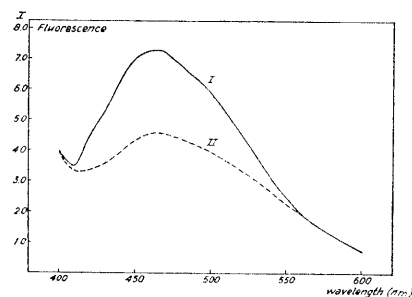


Fig. 7. Fluorescence spectra of fractions G (I) and  $G_{ox}$  (II) at 20°C and pH 7.

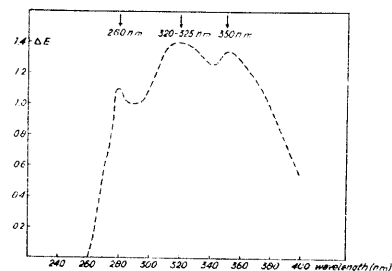


Fig. 8. Ultraviolet absorption difference curve, obtained by subtracting the spectrum of  $G_{ox}$  from that of G.

are very easily oxidized, and because of the relatively mild conditions employed. It may therefore be surmised that, in the present experiments, oxidation has led to specific fall in the number of N-glycosidic determinants.

Skin-reactivity of the oxidized allergen fraction was assayed in three atopic patients with a positive intracutaneous (immediate) reaction to a tomato extract. It follows from Table III that the specific reactivity of the active fraction is in fact considerably diminished after treatment with  $K_3Fe(CN)_6$ . Consequently, the integrity of the structural areas involving the N-glycosidic protein-sugar linkages apparently is essential for imparting skin-reactivity to the allergenic glycoprotein in atopic individuals hypersensitive to tomato fruit. A similar result has been described for the human dandruff allergen (1).

Table III  
Skin-reactivity of fractions G and  $G_{ox}$ .

| Patient | G* | $G_{ox}$ * |
|---------|----|------------|
| 1       | ++ | —          |
| 2       | +  | —          |
| 3       | ++ | ±?         |

\* 2  $\mu$ g injected intracutaneously

### Discussion

Marked biochemical alterations have been reported to occur during the ripening process of tomatoes: from the green to the red ripe stage the total amount of reducing sugars increases to about 4%, the total amount of free acids increases, together with the nitrogen content of the soluble material, while the softening process is thought to be due to conversion of the insoluble propectins of the middle lamellae to water-soluble pectins, which are subject to further cleavage by the enzyme pectinesterase (13, 17, 18). Moreover, non-enzymatic internal browning of tomato fruit is a wellknown phenomenon (15, 16). Hence, it is not surprising that it has been possible to isolate from ripe tomatoes a polydisperse mixture of high-molecular weight and chemically closely related glycoproteins incorporating sugar bound N-glycosidically to the  $\epsilon$ -amino group of lysine residues; such protein-sugar linkages are known to be generated during non-enzymatic browning reactions between free amino groups and reducing sugars. The remarkable point of the present study, however, is that this particular fraction specifically exerts biological activity in atopic subjects hypersensitive to tomato fruit.

The experimental results described in this paper clearly demonstrate a close correlation between skin-reactivity and the degree of browning, i. e. the number of (lysyl)-1-amino-1-deoxy-2-ketose residues in the allergen preparation. Interestingly, a similar relationship has recently been found to exist between sugar bound N-glycosidically to the milk protein  $\beta$ -lactoglobulin and skin-reactivity during the study of cow's milk hypersensitivity in atopic patients (8, 9).

In combination with previous findings on the structure of inhalant allergens the present data therefore strongly implicate the

structural areas involving the N-glycosidic protein-sugar linkage to be the characteristic *allergenic* determinants in the molecules of atopic allergens, including those isolated from foods.

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### Summary

Purified tomato allergen has been subjected to several analytical procedures and both the ultraviolet absorption spectra and the fluorescence emission curves have been studied. Spectroscopic analysis indicates the incorporation of N-glycosidic 1-amino-1-deoxy-2-ketose groups in the allergen molecules.

Moreover, the 'available' lysine content of the atopic allergen is much lower than the total amount of lysine present in the digest after HCl hydrolysis and in the active fraction browning intermediates may be demonstrated. The allergen is relative resistant to the action of trypsin and chymotrypsin.

The tomato allergen is unstable in alkaline medium, the skin-reactive potency decreases markedly by mild oxidation with  $K_3Fe(CN)_6$  at pH 5.6 and the allergen has been found to be artificially produced by non-enzymatic browning reactions during the ripening and the storage of tomato fruits.

It is suggested structural centres involving the N-glycosidic protein-sugar linkage to be the characteristic allergenic determinants in the atopic allergen molecules.

### References

1. BERRENS, L.: A proposed mechanism for the inactivation of atopic allergens in dilute solution. *Immunochemistry* (in press).
2. BERRENS, L.: Fluorescence of atopic allergens. *Immunochemistry* (in press).
3. BERRENS, L.: Fluorescent intermediates in the Maillard reaction. *Rec. Trav. Chim.* **85**: 1117-1128 (1966).
4. BERRENS, L.: Studies on the human dandruff allergen. IV. Immunochemical analysis of a purified allergen preparation. *Dermatologica*, Basel (in press).
5. BERRENS, L. and BLEUMINK, E.: Spectroscopic evidence for N-glycosidic linkages in atopic allergens. *Int. Arch. Allergy* **28**: 150-170 (1965).
6. BERRENS, L. and BLEUMINK, E.: The influence of sugars on the u. v. absorption spectrum of proteins. *Rec. Trav. Chim.* **85**: 59-69 (1966).

7. BERRENS, L. and BLEUMINK, E.: Ultraviolet absorption of N-glycosidic (lysine-sugar) linkages. *B. B. A.* **115**: 504-507 (1966).
8. BLEUMINK, E. and BERRENS, L.: Synthetic approaches to the biological activity of  $\beta$ -Lactoglobulin in human allergy to cow's milk. *Nature, Lond.* **212**: 541-543 (1966).
9. BLEUMINK, E.; BERRENS, L. and YOUNG, E.: The atopic allergen in cow's milk. 6th European Congr. Allergology, September 1965, Stockholm.
10. BLEUMINK, E.; BERRENS, L. and YOUNG, E.: Studies on the atopic allergen in ripe tomato fruits. I. Isolation and identification of the allergen. *Int. Arch. Allergy* **30**: 132-145 (1966).
11. CARPENTER, K. J.: The estimation of the available lysine in animal-protein foods. *Biochem. J.* **77**: 604-610 (1960).
12. CHOI, R. P.; KONCUS, A. F.; CHERREY, G. and REMALEY, R. J.: Determination of protein reducing value of milk as an indication of the presence of non-fat dry milk solids. *J. Milk Food Techn.* **16**: 241-246 (1953).
13. DALAL, K. B.; SALUNKHI, D. K.; BOL, A. A. and OBSON, L. E.: Certain physiological and biochemical changes in the developing tomato fruit. *J. Food Sci.* **30**: 504-508 (1965).
14. KEENEY, M. and BASSETTE, R.: Detection of intermediate compounds in the early stages of browning reaction in milk products. *J. Dairy Sci.* **42**: 945-960 (1959).
15. RICH, S.: Fertilizers influence the incidence of tomato internal browning in the field. *Phytopat.* **48**: 448-454 (1958).
16. TOMPKINS, D. R.: Factors related to internal browning of tomato fruits. Thesis 1964.
17. WINSOR, G. W.; DAVIES, J. N. and MASSEY, D. M.: Composition of tomato fruit. III. Juices from whole fruit and locules at different stages of ripeness. *J. Sci. Food Agric.* **13**: 108-111 (1962).
18. WINSOR, G. W.; DAVIES, J. N. and MASSEY, D. M.: Composition of tomato fruit. IV. Changes in some constituents of the fruit walls during ripening. *J. Sci. Food Agric.* **13**: 141-145 (1962).

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