to LAURENT[8] the main component belongs to carbonic anhydrase B. By use of immunoelectrophoretic analyses, with interrupted groove, it was demonstrated that there was a complete reaction of identity between the $3S\gamma_1$-globulin and the carbonic anhydrase B (Figure 2).

Immunofluorescent technique using a rabbit anti-$3S\gamma_1$-globulin has revealed that labelling was distributed in the whole cytoplasma of all tubular cells, the glomerule being faintly fluorescent (Figure 3). A $3S\gamma_1$-antiserum of which the antibodies were precipitated by a pure $3S\gamma_1$-preparation did not show any further fluorescence.

As we know that carbonic anhydrase is commonly distributed in the kidney, the above data suggest an identity between this enzyme and the $3S\gamma_1$-globulin. Therefore, we may assume that on the basis of similarities on chemical structure, activity and immunological behaviour, the plasma $3S\gamma$-globulin and the carbonic anhydrase B are a unique component[9,10].

Résumé. La $3S\gamma_1$-globuline, précédemment isolée du plasma humain normal, a fait l'objet d'une comparaison immunologique avec l'anhydrase carbonique B isolée à partir d'érythrocytes. L'immunoélectrophorèse en gélose et l'immunofluorescence analysée sur le tissu rénal ont montré l'identité immunologique de ces 2 protéines.

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Detection and Identification of Pyridosine, a Second Lysine Derivative Obtained Upon Acid Hydrolysis of Heated Milk

Recently, a new basic amino acid, found by ERBERSDOBLER[1] in an acid hydrolysate of overheated milk, has been identified by HEYNS[2] and FINOT[3] as ε-N-(2-furoylmethyl)-L-lysine or 'furosine'[3]. This compound X can also be obtained by acid hydrolysis of ε-N-(1-deoxy-2-ketose)-L-lysine (derivative of glucose (I)[3,4]; derivative of lactose (II)[3]).

A second lysine derivative, compound Y, formed simultaneously with furosine during acid hydrolysis of I and II, has now been detected and identified in hydrolysates of overheated milk. This compound cannot be detected by applying the standard conditions of chromatography (SPACKMAN[5]), since it is eluted far after phenylalanine from the 'neutral' column, and well before lysine from the 'basic' column. Its migration is extremely sensitive to changes in pH; the peak can, however, be detected under special conditions (Figures 1 and 2).

Compound Y was isolated by chromatography on Dowex 50 (form H^+) as a peak eluted after furosine[3], and precipitated with methyl ethyl ketone from a methanolic solution of the dried chromatographic fraction. Its structure was determined by UV, IR, NMR and mass spectrometry (operating conditions as previously described[3]) and confirmed by synthesis.

The mass spectrum of the trifluoroacetyl-methyl ester derivative (molecular peak at m/e 364: $C_{15}H_{19}N_2O_5F_3^+$) shows that compound Y possesses the carbon skeleton of lysine and glucose from which it derives and that only 1 nitrogen can be trifluoroacetylated; the presence of ions at m/e 180, 166, 152, 138, and 124, obtained by elimination of the fragments $(CH_2)_n$–CH–(NH–CO–CF_3)–CO_2–CH_3 (n = 0, 1, 2, 3, 4) reveals that the α-nitrogen has been trifluoroacetylated; the last 2 fragments (n = 3, 4) are formed mostly by rearrangement of 1 hydrogen (m/e = 139, 125) or 2 hydrogens (m/e = 126). The great stability of the ion at m/e 125 indicates that the ε-nitrogen belongs to a ring ($C_6H_6NO_2^+$). The ring hypothesis is consistent with the IR bands at 1620 (s), 1550 (s), and 870 (m) cm^{-1} (γ-pyridone), and is confirmed by the UV-spectrum (maxima: 277 nm in neutral solution, 300 nm in basic solution, 246 and 275 nm in acidic solution) typical of the 3-hydroxy-4-pyridone structure. The UV-spectrum is similar to that of a natural compound, β-(1,4-dihydro-3-hydroxy-4-oxo-1-pyridyl)-alanine, namely mimosine or leucenol (maxima: 280 nm in neutral solution, 305 nm in basic solution, and 272 with shoulder at 250 nm in acidic solution) isolated by RENZ[6] and synthesized by ADAMS[7] and SPENCER[8].

The remaining substituents of the ring are given by the NMR-spectrum (Figure 3): singlets at δ (ppm) 8.16 and 7.20 (2 non coupled hydrogens), and at 2.65 (methyl). The methyl group must be either in position 5 or 6; the more probable position 6 has been confirmed by synthesis. The latter has been performed using the technique of HEYNS[9], by condensing α-N-formyl-L-lysine with the corresponding γ-pyrone, allomaltol, prepared according to BROWN[10]; the reference product, namely ε-(1, 4-dihydro-6-methyl-3-hydroxy-4-oxo-1-pyridyl)-L-lysine (V), has the same spectroscopic behaviour as compound Y. The trivial name pyridosine is proposed for this new amino acid.

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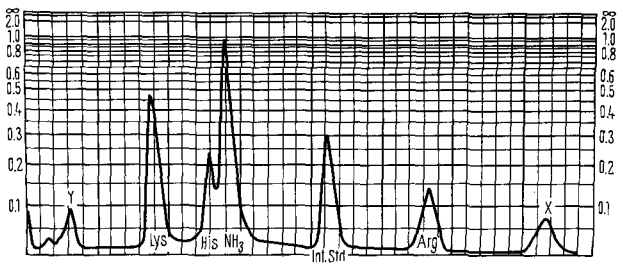


Fig. 1. Basic amino acid chromatography of a scorched roller dried milk after 6 *N* HCl hydrolysis. X, furosine; Y, second hydrolysis product of inactivated lysine; internal standard (Int Std), 2-amino-3-guanidino-propionic acid. Conditions: Resin PA-35 BECKMAN; column height, 9.5 cm; diam. 0.9 cm; buffer 0.38 *N* pH 4.26: 16 ml; then buffer 0.35 *N* pH 5.28; speed: 50 ml/h.

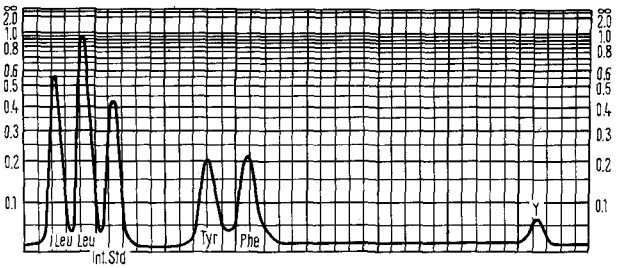


Fig. 2. Neutral amino acid chromatography of a scorched roller dried milk after 6 *N* HCl hydrolysis. Y, second hydrolysis product of inactivated lysine. Conditions: Resin PA-35 BECKMAN; column height, 50 cm; diam. 0.9 cm; buffer 0.2 *N* pH 4.25 until the complete elution of the internal standard peak (norleucine); then buffer 0.35 *N* pH 5.28; speed: 50 ml/h.

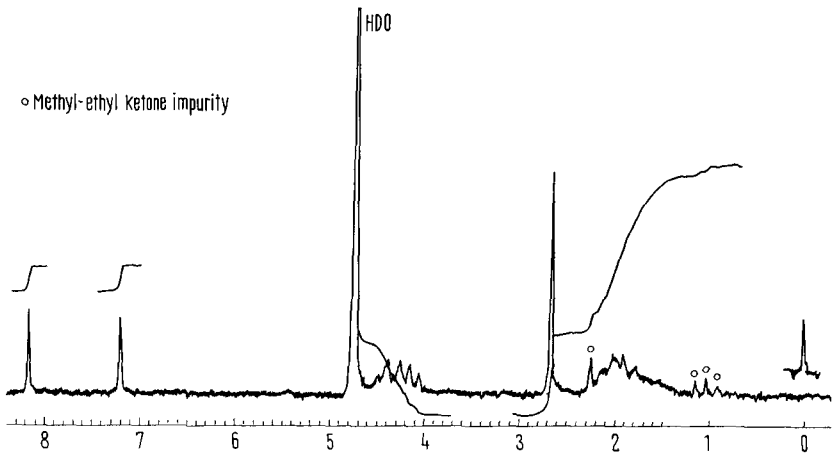
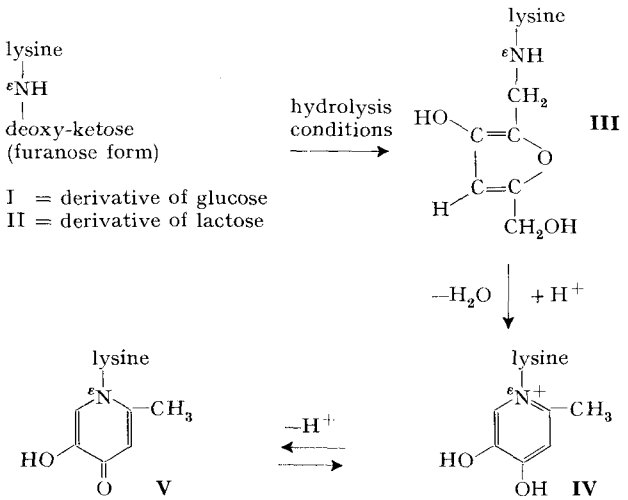


Fig. 3. NMR-spectrum of compound Y in D_2O. Internal standard: sodium 3-(trimethylsilyl)-propansulfonate (Merck, Darmstadt).

This isomer of furosine probably derives from the β-furanose form of I and II, through III, which in acidic solution can easily yield IV (ELMING[11])



The equilibrium between IV (aromatic cation) and V (neutral molecule) explains the delayed migration of compound Y on polystyrene sulfonated resins used with acidic buffers, in spite of its neutral behaviour on paper electrophoresis at pH 3.9 according to BISERTE[12].

In acid hydrolysates of treated milk, the determination of the 2 products obtained from inactivated lysine (furosine and pyridosine) could be a quick checking of the loss in available lysine provoked by the Maillard reaction. A systematic investigation of the hydrolysates of lysine inactivated by reducing sugars, might then enlarge the field of application of this technique to any foodstuff[13].

Résumé. Un deuxième dérivé de la lysine inactivée, l'ε-(1, 4-dihydro-6-methyl-3-hydroxy-4-oxo-1-pyridyl)-L-lysine, a été trouvé dans les hydrolysats de laits surchauffés; il se forme à partir de la deoxy-D-lactulose-L-lysine et peut être mis en évidence dans des conditions particulières de chromatographie. Sa structure a été déterminée par spectrométrie de masse, UV, IR et RMN; le nom de pyridosine est proposé pour ce nouvel acide aminé.

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