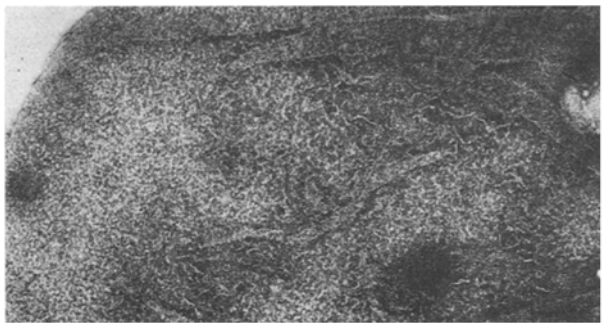

Fig. 1. Control bovine erythrocyte membrane, negatively stained with phosphotungstic acid. ×15,000.

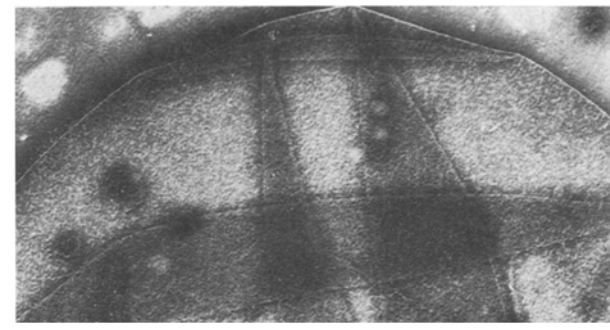

Fig. 2. Bovine erythrocyte membrane, negatively stained with phosphotungstic acid. Blood irradiated for 300 min with UV-rays. ×15,000.

Results and discussion. The detailed fine structure of erythrocyte membrane is very similar in the case of pig, bovine and human erythrocytes. The results obtained for non-irradiated membranes are in agreement with the results of DANON and PERK[3].

The membranes have a fine granular structure, the rather thick folds are localised mainly in peripheric areas of the object. After UV-irradiation, erythrocyte membranes are always thinner, the surface structure is less granular and the folds appear chiefly in the central areas. These changes may be related to changes of membrane permeability, following UV-irradiation.

The fine structure of erythrocyte membrane, although similar, is not identical for the species investigated. It seems that the size of the granules may be correlated to ATP level and osmotic propreties of the cell[5,6,14].

The results obtained by the negative staining method seem to confirm the differences in the ultrastructure of membranes of the different animals. We observed several conal-like and thread-like structures, and also pit structures, observed by HARRIS and AGUTTER[18]. After UV-irradiation, the number of thread-like structures is much smaller; sometimes they disappear completely.

Our results suggest that erythrocyte membranes, subjected to UV-irradiation, are less granular, smoother, thinner and take the appearance of old erythrocytes or erythrocytes with enzymatic defects, e.g. with the defficiency of glucose-6-phosphate dehydrogenase (G-6-PD)[19] or erythrocytes with decreased metabolic activity[12,13,20,21]. The suggestion is only preliminary one and needs further investigation.

Résumé. Nous avons étudié l'effet des rayons UV sur l'ultrastructure des globules rouges des mammifères. L'examen électronoscopique montre qu'après ce rayonnement la membrane des globules rouges devient moins granulaire et moins épaisse. La répartition de ses plis ressemble à celle que présentent les globules plus vieux ou atteints de défauts enzymatiques (p. ex. le manque de G-6-PD).

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Evidence that Pepsitensin is Angiotensin I

FERNANDEZ et al.[1] obtained a highly purified pepsitensin[2] from ox plasma incubated with pepsin at pH 6.0, and identified it as the decapeptide angiotensin I. More recently, however, HOCHSTRASSER et al.[3] isolated a pepsitensin from denatured renin-substrate treated with pepsin at pH 3.0 and concluded that it was the undecapeptide angiotensin I-yl-leucine. These results would suggest that pepsin might act at a different peptide bond in plasma renin-substrate, according to the pH of incubation: either the Leu^{10}-Leu^{11} or the Leu^{11}-Val^{12} bond would be broken at pH 6 of pH 3, respectively. In order to verify this hypothesis we have identified the products of the peptic proteolysis of synthetic tetradecapeptide renin-substrate by pepsin, at both pH 6 and pH 3.

Materials. The tetradecapeptide renin-substrate was a product of Schwarz/Mann, Orangeburg, New York. It had

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isoleucine in position 5 of the peptide chain, and so did the other peptides used in this study, which were synthesized by the solid phase method[4,5]. All the peptides were purified by counter-current distribution and characterized by their partition coefficient (K) in n-butanol:acetic acid: water (4:1:5). Their purity was demonstrated by the obtention of a single component on high voltage paper electrophoresis with pyridine acetate buffer, pH 4.9, and by thin layer chromatography on silica gel (TLC) with the following solvent systems: A) n-butanol:acetic acid:water (4:1:1); B) n-butanol:pyridine:water (30:20:6:24); C) propanol:water (2:1); D) ethyl acetate: pyridine:acetic acid:water (3:2:1:1). Amino acid analyses were on a Beckman model 120C amino acid analyzer.

Angiotensin I-yl-leucine had $K = 0.13$ and the following Rf values: A, 0.27; B, 0.56; C, 0.57; D, 0.33. The amino acid molar ratios were: Asp, 1.05; Arg, 0.98; Val, 1.04; Tyr, 0.94; Ile, 0.93; His, 1.96; Pro, 1.01; Phe, 0.97; Leu, 2.07.

Angiotensin I Had $K = 0.21$ and the following Rf values: A, 0.20; C, 0.52; D, 0.24. The amino acid analysis showed the following molar ratio; Asp, 1.09; Arg, 0.99; Val, 1.01; Tyr, 0.93; Ile, 0.91; His, 2.06; Pro, 1.10; Phe, 0.96; Leu, 1.05.

Leu-Val-Tyr-Ser had $K = 0.70$ and the following Rf values: A, 0.52; B, 0.53; C, 0.67; D, 0.69. The amino acid molar ratios were: Leu, 1.02; Val, 1.04; Tyr, 0.95; Ser, 0.97.

Val-Tyr-Ser had $K = 0.31$ and the following Rf values: A, 0.40; B, 0.41; C. 0.55; D, 0.51. The amino acid molar ratios were: Val, 1.04; Tyr, 0.91; Ser, 1.04.

Methods. A solution of renin-substrate in water (1 mg/ml) was adjusted to pH 3.0 or 6.0 by addition of 0.1 N HCl or 0.1 N NaOH, and equilibrated at 37°. The incubation was initiated by addition of 2 µg/ml (at pH 3) or 4 µg/ml (at pH 6) of 3×-crystallized pepsin (Sigma). Aliquots of 0.2 ml were removed at various times, boiled for 3 min, and submitted to TLC with solvent system D and to paper electrophoresis at 1000 volts for 90 min, in 2 M acetic acid (pH 2.4). After 24 h the remaining solution was boiled for 3 min, freeze-dried and submitted to preparative paper electrophoresis in the same conditions as for the previous aliquots. The separated compounds were eluted with water, the eluates were evaporated to dryness, hydrolyzed for 72 h with 6 N HCl at 110° under N_2, and submitted to amino acid analysis.

Results and discussion. The main products of peptic proteolysis of renin-substrate, at either pH studied, were identified as angiotensin I and Leu-Tyr-Ser by TLC and paper electrophoresis. In neither pH was it possible to detect the appearance of angiotensin I-yl-leucine, Val-Tyr-Ser or angiotensin II in the incubation mixtures.

The solution remaining after 24 h of peptic hydrolysis at pH 6.0 was submitted to preparative paper electrophoresis and the two Pauly-positive fractions were eluted, hydrolyzed and analyzed for their amino acid composition. The results, shown in the Table, confirm that the main products were angiotensin I and Leu-Val-Tyr-Ser, although molar ratios indicate in fraction I the presence of other peptides, including some intact substrate. Since all of the other possible fragments of peptic hydrolysis of the tetradecapeptide would have negligible biological activity (with the exception of angiotensin II), it may be concluded that pepsitensin, whether produced at pH 3 or pH 6, is identical with angiotensin I. This agrees with the results obtained with native renin substrate[1], and it seems probable that the same would happen also in the case of the denatured substrate[6].

Amino acid analyses of the 2 fractions from the hydrolysate of renin substrate by pepsin at pH 3.0

Amino acid	Fraction I		Fraction II	
	Expected	Found	Expected	Found
Asp	1	1.28	0	0
Arg	1	1.11	0	0
Val	1	0.81	1	1.00
Tyr	1	0.87	1	1.03
Ile	1	0.70	0	0
His	2	1.87	0	0
Pro	1	1.00	0	0
Phe	1	1.22	0	0
Leu	1	1.46	1	0.95
Ser	0	0.29	1	1.45

Résumé. L'angiotensine I et le Leu-Val-Tyr-Ser sont les principaux produits des hydrolyses peptiques du tétradécapeptide substrat de la rénine, réalisés à pH 3 et à pH 6. Ces résultats indiquent qu'il n'y a qu'une seule pepsitensine laquelle est identique à l'angiotensine I.

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Basal Forebrain Heating and Osmotic Reactivity of the Thirst Mechanism in Dogs

There is controversial data regarding the coupling between the hypothalamic temperature and feeding as well as drinking behavior. Andersson and Larsson[1] found that heating of the preoptic anterior hypothalamus (PO/AH) region in goats increased drinking and suppressed food intake. On the other hand Spector et al.[2] and Hamilton and Ciaccia[3] reported that the same procedure in rats produced increase of food consumption and a tendency to reduce water intake. The present study was performed on conscious dogs in order to check whether local heating of the basal forebrain influences water intake and changes the osmotic reactivity of the thirst mechanism.

Material and methods. Experiments were carried out on 5 male mongrel dogs. Each of them was chronically implanted with 4 thermodes and 2 copper constantan thermocouples. The heater of the thermode consisted of a miniature carbon resistor[4] placed at the end of a 0.8 mm