However, caudal to the obex, there appeared to be more stained cells in the dorsal half of the nucleus where the 'cell nests', which were described by Keller and Hand[7], are located. In this region, many of the stained cells appeared to cluster into small groups (fig. 3) whereas elsewhere in the nucleus, they tended to lie singly.

The stained cells varied in shape. Most were round or oval and a few were fusiform (figs 2, 3). The round profiles were of 2 types: one type was medium-sized (15–18 µm in diameter) and the other type was smaller (8–12 µm). The oval profiles were larger and measured 22–26 µm in their long diameter. The fusiform cells measured 25–28 µm in their long diameter.

Discussion. The present study has demonstrated histochemically that in the cuneate nucleus of the cat, glutamic acid dehydrogenase, an enzyme which is involved in the degradation of glutamic acid to oxoglutaric acid, is located both in the neuropil and in several types of neuronal perikarya. In the neuropil, staining was slightly more intense in the dorsal part of the nucleus where primary afferent fibers of dorsal root origin have been shown to terminate predominantly[7], than in the ventral part. This would correlate well with the findings of pharmacological experiments in which a high content of glutamate has been found not only in the dorsal roots and dorsal root ganglia, but also in the dorsal column nuclei[2]. Furthermore, the glutamate content in the cuneate nucleus of the rat has also been shown to increase when the dorsal column fibers were stimulated[8]. In contrast, Sims et al.[9], in their histochemical study of succinic semialdehyde dehydrogenase in the rat brainstem, observed heavy staining for this enzyme, which destroys GABA, in the cuneate neuropil, but no staining at all of the cuneate neurons.

The identity of the stained neuronal perikarya observed in the present study is not known. Of particular interest is the observation of Galindo et al.[4] that hair, touch and proprioceptive cells in the cuneate nucleus were excited by iontophoretically-administered glutamate, the hair cells being the most responsive. It is not possible at this stage to correlate the latter observations with the present findings. However, several anatomical studies using horseradish peroxidase tracing techniques have led to the identification of the gracilo-thalamic projection cells in the cat[10] and the cuneothalamic projection cells in the rat[11]. In the latter study, Tan and Lieberman[11] were able to distinguish 2 classes of cuneothalamic projection cells in the rat; these cells were mostly medium-size round or oval cells which were within the range of diameters of the stained neurons of the same shape and size in the present study.

In the cat gracile nucleus, Berkley[10] has identified the neurons which projected to the inferior olivary nucleus as small, round cells of less than 10 µm diameter. The possibility that some of the stained neurons observed in the present study might be cuneothalamic and inferior olivary projection neurons will have to be verified.

The results of pharmacological experiments suggest that the medial lemniscal fibers are non-cholinergic and that the excitatory neurotransmitter substance is likely to be an amino-acid[12], and since the thalamus has been shown to have a high content of glutamate[13], and since the thalamic relay cells in the dorsal column nuclei are excited by iontophoretically-applied glutamate[14–18], it might be suspected that glutamate might be the excitatory transmitter substance in the lemniscal terminals in the somatosensory thalamus. Experiments are now underway to study the effect of an electrolytic lesion of the nucleus ventralis posterolateralis of the contralateral thalamus on the glutamic dehydrogenase activity in cuneate neurons of the cat. In addition, the effect on enzyme activity following rhizotomy of C5–C8 dorsal roots are also being studied.

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Functional properties of lyophilized hemoglobin in the presence of amino acids after 13 months of conservation

P. Labrude, C. Chum, F. Pelgrims and C. Vigneron

Centre régional de Transfusion sanguine et d'Hématologie, F-54500 Vandoeuvre les Nancy, and Faculté des Sciences pharmaceutiques et biologiques, F-54001 Nancy Cedex (France), 9 February 1981

Summary. Four lyophilisates of hemoglobin, each protected by an amino acid salt, were conserved for 13 months. The determinations carried out (oxyhemoglobin, methemoglobin, p50, Hill's number, and visible spectrum) demonstrated that the hemoglobin had retained its functional properties.

Oxyhemoglobin is very unstable in solution, even at low temperatures[1]. One would think that this molecule's conservation would be improved after lyophilization. However, 50% of the hemoglobin is oxidized to methemoglobin[2,3] during freeze-drying. This alteration can be prevented by the previous admixture of a number of very different substances: carbohydrates (glucose, ...), amine buffers and macromolecules[4]. However, recent evidence has shown that

Chemical and physiological values in hemoglobin-amino-acid salt lyophilisates after 13 months of storage at +4 °C.

	Saline balanced hemoglobin solution	Lyophilisate* without protective compound	Lyophilisate* with a protective compound	L arginine/ L aspartate	L lysine/ L aspartate	L arginine/ L glutamate	L lysine/ L glutamate
Colour	Red	Brown	Red	Red	Red	Red	Red
Dissolution	-	≫ 5 min	< 5 min	< 5 min	< 5 min	< 5 min	< 5 min
Appearance	Clear red	Turbid brown	Clear red	Clear red	Clear red	Clear red	Clear red
Spectrum $\frac{\text{OD 578 nm}}{\text{OD 560 nm}}$	1.50-1.70	1.30-1.46	1.50-1.70	1.62	1.63	1.67	1.63
pH	7.0±0.10	7.10±0.10	7.0±0.10	6.88	6.94	7.02	6.88
Hb O_2 (%)	> 97	< 60	> 95	94	93.5	94	92.5
MetHb (%)	< 3	> 40	< 5	4.2	3.2	4.7	5.1
p50 (kPa)	3.50-4.40	1.70-1.92	3.30-4.40	3.12	3.45	3.19	3.79
Hill number	2.6-2.9	1.65-1.85	2.5-2.7	2.62	2.73	2.63	2.37

*These lyophilisates are analyzed immediately after dessiccation.

these lyoprotectors fail to protect the hemoprotein from auto-oxidation after prolonged conservation, resulting in up to 40% methemoglobin after 12 months at +5 °C according to Bonderman et al.[5] or at +20 °C according to Pristoupil et al.[6]. Recently, we have demonstrated the effectiveness of various amino acids and the salts of 4 of them: L aspartates of L arginine and L lysine, and L glutamates of L arginine or L lysine[7]. In 17 trials using these amino acid salts, less than 5% methemoglobin was present after lyophilization. 4 samples were conserved at +4 °C, in the dark, under air. The results of analyses carried out after 13 months of conservation are presented.

Methods. The solution of hemoglobin was prepared from blood which had passed its maximum conservation date. The red blood cells were separated and washed twice with 0.1 M NaCl and were hemolyzed with demineralized water. The hemolysate was centrifuged 2 times at 25,000×g for 30 min and the stromas decanted. The hemoglobin solution was dialyzed for 15 h at +4 °C against demineralized water, and finally centrifuged to separate the last stromas.

The samples containing 5×10^{-6} M tetrameric hemoglobin and 2.5×10^{-3} M amino acid salt were lyophilized under the following conditions: freezing at −40 °C, primary drying to −10 °C in 16 h, and secondary drying to +5 °C in 8 h (Kreel experimental apparatus, Nancy, France). The samples were immediately sealed and conserved.

After 13 months, the contents of the vials were examined (to check the colour) then reconstituted using 5 ml of 0.15 M Sörensen phosphate buffer solution at pH 7.4 and immediately analyzed. The following determinations were made; dissolution time, appearance, visible spectrum (Beckman DBGT spectrophotometer), pH, oxyhemoglobin content (Hemoximeter OSM 2 Radiometer), methemoglobin content[8], dissociation curve at 37 °C and PCO_2 5.32 kPa (Hem-O-Scan Aminco) with determination of the p50 and Hill's number, and cellulose acetate electrophoresis (Cellogel Sebia) at pH 8.6.

Results and discussion. The results are represented in the table, except for those of the electrophoreses, which were normal and revealed strictly characteristic migration spots. The values were extremely homogeneous, with no salt demonstrating more or less hemoglobin protective capacity than the others. Methemoglobin content was very low; much lower than we obtained after storage using other protective molecules under identical conditions; α D glucose: 35% MetHb after 12 months; Tris: 80% MetHb after 18 months, and other reported results[5,6].

The superiority of amino acid salts, for example arginine aspartate, as hemoglobin protectors can be demonstrated by comparing this salt with glucose, which is most often referred to in published reports. This is illustrated by comparing curves of methemoglobin content (logarithm of percentage) as a function of the protector's concentration (in moles). The linear equations obtained are:

$y=-8.28x+1.71$ for glucose ($p<0.001$),

$y=-13.2x+1.54$ for arginine aspartate ($p<0.001$).

We are preparing a more exhaustive comparative study of currently proposed protectors[4].

In conclusion, these amino acid salts are very effective in maintaining hemoglobin integrity not only during freeze-drying but also after conservation at +4 °C. It would seem logical to distinguish 2 classes of lyoprotectors: those whose effectiveness is limited to the drying process and several weeks after, and those allowing prolonged conservation.

Various studies having shown that it is possible to freeze-dry and then conserve hemoglobin in a satisfactory functional state, future research should be orientated towards understanding the mode of action of these various molecules. The excellent results we obtained, which as for as we know are unparalleled in current reports, should prove valuable in perfecting oxygen carriers and standards for hematological use, and in the lyophilization of fragile proteins, where analogous protectors could be used.

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