endotoxin-like substance in the bacterial genera appeared minimal. The chromatography analysis[8] did, however, reveal a large percentage of branched-chain fatty acids in *F. multivorum* that were not present in *P. paucimobilis* and may account for the difference in activity. The presence of endotoxin-like activity also may partially explain the virulence of these organisms during infection in man. This evidence suggests that additional studies, including the pyrogenic assay and Shwartzman reaction, may be helpful in further defining the endotoxicity of these organisms.


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## Aging of the erythrocyte. XV. Isoosmotic lysis times


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*Summary.* Isoosmotic lysis times of various density (age) fractions of human, pig and ox erythrocytes in glycerol and ammonium chloride solutions decreased with increasing cell density.


The question of age-releated changes in erythrocyte osmotic properties seems to be of importance since simple tests based on osmotic fragility are employed in creening for red cell membrane disorders[2-5] and in some cases the observed alterations in values of appropriate parameters might be due to changes in the mean age of erythrocyte populations studied. Data concerning this point are contradictory: some authors found increased osmotic fragility of in vivo aged erythrocytes of serveral mammalian species[6-9] but others did not observe any significant correlation between osmotic fragility and cell age[10-12]. In those studies various techniques of red cell fractionation according to age were employed and the cells were of different species origin. This report describes isoosmotic lysis of erythrocytes from 3 mammalian species, separated according to age, by the same method.

*Material and methods.* Human, pig and ox blood was taken into citrate. Erythrocytes were separated according to density (and age)[13] by the method of Murphy[14].

Isoosmotic lysis times ($ILT_{50}$) were determined by introducing 50 µl of appropriately diluted erythrocyte suspension in phosphate-buffered saline to 1.5 ml of 300 mM glycerol or 163 mM $NH_4Cl$ in a spectrophotometric cuvette and monitoring changes in optical density at 640 nm (initial value: 1). Control samples incubated in saline did not show changes in optical density greater than 0.01 during the incubation period. The time needed for a decrease in optical density to 0.5 was defined as $ILT_{50}$ in the appropriate solution. The lysis was carried out at ambient temperature (20 ± 1 °C).

*Results and discussion.* Successful separation of erythrocytes from all species according to density (age) was demonstrated, among other tests, by an increase in the mean cell hemoglobin concentration in successive red cell fractions. The average ratio of this parameter in 17% bottom cells and in 17% top cells was 1.07 for ox erythrocytes, 1.11 for pig red cells and 1.18 for human cells. These values may reflect interspecies differences in separation efficiency of in the rate of age-related changes in the red cell hemoglobin concentration.

Absolute $ITL_{50}$-values averaged from all density fractions are given in table 1. They coincided with values obtained for non-fractionated erythrocyte samples, therefore there was no discernible effect of the separating centrifugation on the parameters studied. Glycerol lysis time was not determined for human erythrocytes since rapid lysis in this version of the test[2,3], precluded accurate measurements. Changes in $ILT_{50}$ accompanying increasing cell density shown in table 2, demonstrate a significant acceleration of lysis in both sulutions for red cells of all species examined.

Table 1. $ILT_{50}$ (sec) of erythrocytes from various species in glycerol and $NH_4Cl$ solutions

| Species | Glycerol Mean | SD | n | $NH_4Cl$ Mean | SD | n |
|---|---|---|---|---|---|---|
| Human | | | | 155 | 31 | 5 |
| Pig | 450 | 96 | 5 | 111 | 35 | 6 |
| Ox | 830 | 264 | 5 | 156 | 61 | 6 |

Table 2. Relative $ILT_{50}$-values of different density fractions of erythrocytes (fraction 1, lightest cells; fraction 6, densest cells; mean ± SD, n = 5-6 (different individuals)

| Fraction No. | Glycerol Pig | Ox | $NH_4Cl$ Human | Pig | Ox |
|---|---|---|---|---|---|
| 1 | 100 | 100 | 100 | 100 | 100 |
| 2 | 86 ± 8* | 93 ± 9 | 78 ± 12* | 95 ± 4 | 87 ± 10 |
| 3 | 79 ± 14* | 82 ± 10* | 77 ± 13* | 91 ± 9 | 74 ± 12* |
| 4 | 73 ± 17* | 81 ± 12* | 70 ± 19* | 79 ± 8** | 67 ± 10** |
| 5 | 51 ± 9** | 74 ± 10** | 65 ± 16** | 78 ± 10* | 64 ± 12** |
| 6 | 47 ± 10** | 74 ± 6** | 65 ± 19* | 78 ± 13* | 67 ± 14** |

Statistical significance of differences with respect to fraction 1: * $p < 0.05$, ** $p < 0.01$ (one-tailed Student's t-test).

This is in contrast with the lack of considerable alterations in osmotic fragility of bovine erythrocytes in hypotonic NaCl solutions[10]. This is understandable, since $ILT_{50}$ may depend on several parameters including permeability of the cell membrane for a given solute, relative critical hemolytic volume of a cell and mechanical properties of the membrane. As a result, red cells of various ages can differ in the time at which hemolysis occurs[15], though showing no difference in the final fraction of cells lysed in a hypotonic solution. Therefore, although fractionation of bovine erythrocytes according to age was impossible using graded osmotic hemolysis in hypotonic NaCl solutions[10], isoosmotic lysis stopped after various time intervals might provide a general basis for such a procedure. It was observed that the fraction of 1% of bovine erythrocytes most resistant to glycerol lysis had about 2-fold higher activity of a cell-age dependent enzyme, glucose-6-phosphate dehydrogenase, than the whole red cell population, in agreement with this prediction.

1 I am indebted to Prof. W. Leyko for many stimulating discussions.
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## Chromosomal homology in southern Akodon[1]

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*Summary.* Differential staining (G and C) of southern South American *Akodon* are presented. *A. olivaceus, A. longipilis* and *A. sanborni* all have the same karyotype (2n = 52, NF = 58). A virtually identical band sequence is observed. This situation is interpreted using the canalization model of chromosomal evolution which stresses an optimum karyotype for each adaptive zone. Despite the high degree of conservation of the chromosome structures, the specific status of these species is supported by maintenance of distinctness when they occur in areas of sympatry.

The genus *Akodon* (Rodentia, Cricetidae) is the largest among akodontine sigmodontine rodents and is one of the most confusing and complex taxa of South American rodents. It currently contains 7 subgenera that perhaps deserve the status of full genera[3]. Karyotypically *Akodon* is characterized by chromosome multiformity (2n = 14–52)[3–6] with a wide array of intraspecific and intrapopulational polymorphisms. Such extensive chromosomal diversity indicates that chromosome rearrangements must have occurred quite frequently during the evolution and diversification of the genus. Nevertheless, when bands are analyzed, this variation is shown to be more illusory than real and hence, consistent stability of banding patterns seems to be a rather common situation in the genus.

This paper presents for the first time the banded karyotypes (G and C) of *Akodon (Akodon) olivaceus olivaceus, A. (Akodon) olivaceus brachiotis, A. (Abrothrix) longipilis* and *A. (Abrothrix) sanborni.*

Live trapped animals were collected from the following places in Chile: *A. o. olivaceus* (2 males and 2 females) from Santiago and La Serena, *A. o. brachiotis* (3 males and 4 females) from Valdivia, *A. longipilis* (3 males and 3 females) from Valdivia and Osorno Mountain, *A. sanborni* (1 male and 4 females) from Osorno Mountain.

Voucher specimens in accordance with Osgood[7] were deposited in the Collection of Mammals, Institute of Ecology and Evolution, U. Austral of Chile (IEEUACH). Mitotic plates were obtained by the standard air dried technique described elsewhere[8]. G-bands were obtained by using the trypsin method of Seabright[9] and C-bands as described by Schnedl[10]. Induction of the C-banding pattern was difficult, probably because of the minimal amount of heterochromatin present. A total of 190 representative spreads were photographed and 50 were selected for interspecific comparisons.

The diploid complements of these species, characterized by the same diploid numbers and overall karyotypic morphology, present 52 chromosomes (NF = 58) with 22 pairs of acrocentric and 3 pairs of submetacentric chromosomes (pairs 15, 22 and 25). Sex chromosomes are formed by a medium sized subtelocentric X and a small acrocentric Y. 90 specimens from 14 localities were previously karyotyped by standard technique and no evidence of intra or interpopulation variation in chromosome complement was observed. Their banded karyotypes show a virtually identical G-banding pattern in both autosomes and sex chromosomes, indicating a high degree of homology (fig. 1). The same remarkable correspondence is observed in the C-banding patterns which are characterized by low amounts of centromeric heterochromatin (fig. 2).

*A. olivaceus, A. longipilis* and *A. sanborni* together with *A. lanosus, A. xanthorhinus* and *A. markhami* occupy the southern-most peripheral distribution of the genus[3]. In contrast with vole mice from the Central Andean region (presumed geographical area of differentiation), these southern species are characterized by chromosome stability and high diploid number[3]. More recently, the same karyotype and chromosome stability described here, has been found in *A. xanthorhinus*[11].

Homology in banding patterns among recognized species is not an unusual phenomenon in mammals. It has been demonstrated in Primates[12], Cetacea[13], Pinnipedia[14], Carnivora[15], Chiroptera[16] and also in rodents as *Neotoma*[17], *Tylomys*[18], *Rattus*[19], and *Mus*[20]. Bianchi et al.[21] have described G-banding homology in Argentinian *A. obscurus* (2n = 34), *A. molinae* (2n = 42, 43, 44) and *A. azarae*