the synthesis of this type of hepatic GAG is unmeasurably low, but its quantity gradually increases in liver fibrosis as a consequence of catabolic inertness and extreme reduced turnover rate. Although the existence of keratan sulfate in liver has not been proved so far[3], our data suggest that normal and diseased liver is capable of synthesizing this special type of GAG.

During chronic liver injury the synthesis of specific GAG increased disproportionately (figure 2, table). However, the degree of stimulation was much lower than demonstrated previously by the in vivo incorporation of $(^{35}SO_4)^{2-}$ [20]. The factors controlling the formation of GAG in chronically injured liver are not known. The changing population of cell types during fibrogenesis[21] in particular the increased number of mast cells[22] and the ability of the hepatocyte to synthesize collagen[23] necessitate cell-type differentiated studies of GAG synthesis in liver. Furthermore, it remains to be established whether the augmentation of GAG synthesis is a feature of parenchymal regeneration during chronic injury rather than a disease specific phenomenon[10].

20 K. Becker, Z. ges. exp. Med. *144*, 222 (1967).
21 F. Hutterer, E. Rubin, E. J. Singer and H. Popper, Cancer Res. *21*, 206 (1961).
22 K. Murata, M. Okudaira and K. Akashido, Acta derm.-vener., Stockh., suppl. *73*, 157 (1973).
23 K. Sakakibara, M. Saito, M. Umeda, K. Enaka and Y. Tsukada, Nature *262*, 316 (1976).

Mn^{2+} electron spin resonance studies on ATP phosphoribosyltransferase from *E. coli*[1]

A. R. Tebar, A. Ballesteros and J. Soria
Instituto de Catálisis y Petroleoquímica, C. S. I. C., Serrano, 119 Madrid-6 (Spain), 11 February 1977

Summary. ESR binding studies of Mn^{2+} with each of the substrates and products suggests that substrate bridge complexes are formed in the reaction. This prediction is confirmed, comparing Mn^{2+} + ADP and Mn^{2+} + ADP + enzyme spectra.

ATP phosphoribosyltransferase (EC. 2.2.2.17) is the first enzyme of the histidine biosynthetic pathway, and it is allosterically inhibited by the end product, histidine[2]. It catalyses the reversible reaction of ATP and phosphoribosylpyrophosphate (PRibPP) to yield phosphoribosyl-ATP (PRibATP) and pyrophosphate[2]. The reaction requires Mg^{2+}[2], but as in most processes with participation of nucleotides, Mn^{2+} can substitute for Mg^{2+}[3]. Studies in our laboratory with the E. coli enzyme have dealt with conformational changes and association-dissociation processes effected by substrates and other ligands. We have reported on fluorescence data[4], association-dissociation[5], steady state kinetics[6] and nitroxide spin labelling of the enzymes[7]. In this paper, an ESR study has been carried out, in which use is made of the Mn^{2+} cation to obtain information on the environment of the metal during the catalytic reaction.

ESR parameters of aqueous Mn^{2+} solutions*

Ligand (mM)	$\frac{\Sigma H_i}{6}$ (G)	Relative intensity $\frac{\Sigma Y_i}{6}$	$\frac{\Sigma a_i}{6}$ (G)
–	29	346	96
ADP (0.3)	35	63	97
ADP (0.15) Enzyme (9 mg/ml)	40	54	97
ADP (0.15) Enzyme (9 mg/ml) Histidine (1)	38	68	94
ADP (0.3) PRibPP (0.3)	–	< 5	–
ATP (0.3)	52	21	97
ATP (0.15) PRibPP (0.15) Enzyme (9 mg/ml)	42	62	97

* The $MnCl_2$ concentration was 0.1 mM: in spectra in the presence of enzyme it was 0.05 mM. At these levels, the relative intensity of the signal is a function of the manganous ion concentration. ΔH_i is the peak-to-peak width of the first derivative ESR signal, Y_i is the peak height, a_i is the hyperfine splitting constant.

Materials and methods. ATP phosphoribosyltransferase was purified basically according to the method of Parsons and Koshland[8], as previously described[5], including a Sephadex G-200 step. Protein was determined by the method of Lowry et al.[9], with insulin standards. PRibPP (from Sigma) was used without further purification. PRibATP was prepared according to Klungsoyr and Kryvi[10], including a Sephadex G-10 final step; the purity was tested with ATP phosphoribosyltransferase, using a value of 3100 M^{-1} cm^{-1} for the extinction coefficient of PRibATP at pH 8.0 and 290 nm[11].

ESR measurements were carried out in a JEOL JM-PE-3 spectrometer, working at 23 °C and at X band (9.53 GHz), using a modulation amplitude of 4 G and microwave power of 6 mW. The solutions were contained in a quartz cylindrical cell, standard for the JEOL equipment. The solvent was in all cases 50 mM Tris-HCl buffer, pH 8.0. Analytical grade manganous chloride was obtained from Merck.

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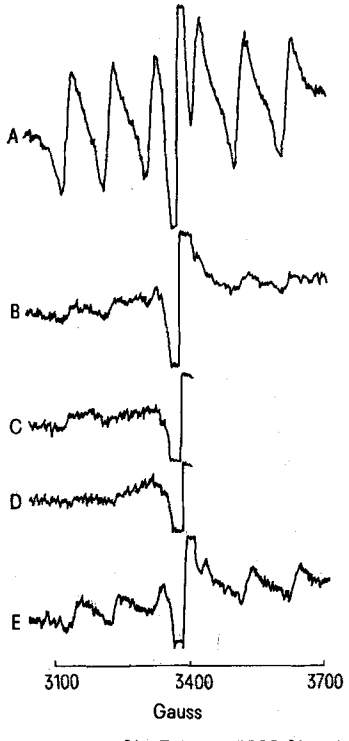
2 B. N. Ames, R. G. Martin and B. J. Garry, J. biol. Chem. *226*, 2019 (1961).
3 R. M. Bell and D. E. Koshland, Jr, Bioorg. Chem. 1, 409 (1971).
4 A. R. Tébar, V. M. Fernández, R. Martin Del Rio and A. Ballesteros, FEBS Lett. *50*, 239 (1975).
5 A. R. Tébar, V. M. Fernández, R. Martin Del Rio and A. Ballesteros, Experientia *29*, 1477 (1973).
6 A. R. Tébar and A. Ballesteros, Mol. cell. Biochem. *11*, 131 (1976).
7 A. R. Tébar, A. Ballesteros and J. Soria, Experientia *33*, 1014 (1977).
8 S. M. Parsons and D. E. Koshland, Jr, J. biol. Chem. *249*, 4104 (1974).
9 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).
10 L. Klungsöyr and H. Kryvi, Biochim. biophys. Acta *227*, 327 (1971).
11 D. E. W. Smith and B. N. Ames, J. biol. Chem. *240*, 3056 (1965).

Results. The aqueous Mn^{2+} spectrum consists of a signal with 6 hyperfine components that showns a value of $g=2.008$ (figure, A) and can be assigned to the cation Mn^{2+} surrounded by 6 molecules of water. Following the conditions of Reed et al. (using nucleotide in considerable excess)[12], it is possible to suppress most of the free Mn^{2+}. The actual ESR observed spectra will represent the contributions from signals due to free Mn^{2+} [i.e., $Mn^{2+}(H_2O)_6$], to manganese affected by dipolar interactions and to manganese-nucleotide complex, although generally the bound metal does not contribute significantly to the signal when free metal is present[13]. In fact, Cohn and Townsend failed to detect the Mn^{2+} spectrum of any of the complexes studied[14].

First, we have determined the contribution that each component makes to the binding of Mn^{2+}. The figure, B–E, shows the spectra of Mn^{2+} in the presence of each of the substates and products of the reaction. It can be observed that PRibATP binds to the metal more loosely than PRibPP, ATP and pyrophosphate do. Since we are working in a range of Mn^{2+} concentrations in which the intensity of the hyperfine structure is proportional to the concentration of free Mn^{2+}, the concentration of free manganese in each case can be calculated from the relative intensity of the spectra in the figure. In this way and assuming 1:1 binding, values of 17 μM, 13 μM and 8 μM for the dissociation constants (K_D) of PRibPP, ATP and PP_i, respectively, to the Mn^{2+} are obtained. The K_D of Mn^{2+} and PRibATP appears to be of the order of 40 μM. The K_D of ADP (spectrum not shown, see the table) is 53 μM. The parameters of the manganese signal in several different conditions are presented in the table. In the presence of 0.3 mM ADP, the intensity of the signal is reduced by a factor of 5 and the linewidth of the hyperfine components is enlarged. (In control experiments done in the absence of Mn^{2+}, no ESR signal was obtained.) When enzyme (9 mg/ml or 134 μM per dimer of 67,000 daltons[10]) is present in the Mn^{2+}+ADP solution, the lines become broader and slightly smaller. The presence of 1 mM histidine produces a narrower and larger signal and smaller hyperfine constant, indicating a change in the dielectric constant of the medium surrounding the cations. In the presence of ADP plus one of the substrates of the enzyme (PRibPP, 0.3 mM), the signal practically disappears. In the spectrum of a solution of 0.3 mM ATP, the hyperfine components become broader and their intensity drops by a factor of 3 in relation to the ADP solution. When testing a mixture containing 0.05 mM Mn^{2+}, 0.15 mM PRibPP, 0.15 mM ATP and 9 mg/ml enzyme, one would expect that, following the tendency observed in lines 5 and 6 of the table, the hyperfine structure would disappear in the presence of PRibPP and ATP. However, when enzyme is also present in great excess, not only does the signal not disappear but its intensity increases and the linewidth diminishes. This fact can be explained by considering that the solution components react to form PRibATP and PP_i. Part of the Mn^{2+} is released probably bound to the PRibATP just formed, whose affinity constant for Mn^{2+} is appreciably lower than those of the other ligands of the enzyme.



ESR spectra. *A* 0.05 mM Mn^{2+}; *B* 0.05 mM Mn^{2+}, 0.15 mM PRibPP; *C* 0.05 mM Mn^{2+}, 0.15 mM ATP; *D* 0.05 mM Mn^{2+}, 0.15 mM PP_i; *E* 0.05 mM Mn^{2+}, 0.15 mM PRibATP. The sharp signal in the middle of the spectra corresponds to DPPH (α,α'-diphenyl-β-picrylhydrazyl), used as a standard to determine the g values and compare signals intensity.

Discussion. The determination of dissociation constants for the nucleotides and their metal complexes by electron spin resonance have been exploited by Cohn and coworkers[14,15]. The accuracy of the values so obtained may prove to compare favorably[15] with the accuracy of the more classical techniques of equilibrium dialysis and sedimentation gradient. Here, the values determined for the MnATP and MnADP complexes are of the same order as those previously obtained under similar conditions[16]. According to Mildvan[17], substrates which participate in substrate bridge complexes (E-S-M) are generally nucleoside di- and triphosphates which have a high affinity ($K_D < 1$ mM) for the metal ion. In our case (compounds from 2 to 4 phosphate groups), the K_D values obtained (see above) are well below 1 mM, which suggest that the reaction mechanism is one involving substrate bridge complexes.

The dissociation constants for the binary 1:1 Mg^{2+} complexes of the same compounds (e.e., PRibPP, ATP, PP_i and PRibATP) have recently been measured by Morton and Parsons[18], seeking information on the optimal Mg^{2+} requirements of ATP phosphoribosyltransferase of S. typhimurium. It is somewhat surprising that the values of K_D for the Mn^{2+} complexes are 10–15 times lower than those of the Mg^{2+} complexes[18].

When ADP is introduced into the manganese solution, the loss of intensity of the hyperfine structure would logically be due to the presence of ADP in the manganese first coordination sphere. The broadening of the lines indicates an increment of the dipolar interactions, which

12 G. H. Reed, H. Diefenbach and M. Cohn, J. biol. Chem. *247*, 3066 (1972).
13 R. A. Dwek, Nuclear Magnetic Resonance in Biochemistry, p. 254. Clarendon Press, Oxford 1973.
14 M. Cohn and J. Townsend, Nature *173*, 1090 (1954).
15 W. J. O'Sullivan and M. Cohn, J. biol. Chem. *241*, 3104 (1966).
16 R. M. C. Dawson, D. C. Elliot, W. H. Elliot and K. M. Jones (Ed.), Data for biochemical Research, 2nd ed. Oxford University Press, New York 1969.
17 A. S. Mildvan, in: The Enzymes, 3rd ed., vol. 2, p. 445. Ed. P. D. Boyer. Academic Press, New York 1970.
18 D. P. Morton and S. M. Parsons, Archs Biochem. Biophys. *175*, 677 (1976).

could be interpreted as a slight segregation of the free Mn^{2+} in pockets, raising the metal concentration in these pockets. The presence of the enzyme itself in the Mn^{2+}+ ADP solution has the same effect on the signal, enlarging the linewidth but reducing its intensity. A similar interpretation is therefore considered: the complexing reduces the amount of free Mn^{2+} and the segregation enhances the metal concentration in certain regions of the aqueous solution. Since the K_S of ADP for ATP phosphoribosyltransferase is 175 μM (A. Ballesteros, unpublished), this means that under the present experimental conditions nearly 60% of the MnADP complex is bound to the enzyme. Our results (cf. lines 2 and 3 in the table) do not show significant direct interactions of either MnADP or Mn^{2+} with the enzyme. This indicates that the metal does not interact with the protein, at least not with high affinity. Hence, in the ternary complex (enzyme, nucleotide, metal), the nucleotide must act as a bridge[17]. When histidine is added to the system, it does not affect the spectrum appreciably, as would be expected for a ligand which binds at a molecule separated from the metal by the nucleotide.

Karyotypes of shrews of the genera *Cryptotis* and *Blarina* (Mammalia: Soricidae)[1]

H. H. Genoways, J. C. Patton III and J. R. Choate

Carnegie Museum of Natural History, 4400 Forbes Avenue, Pittsburgh (Pennsylvania 15213, USA); Department of Zoology, University of Georgia, Athens (Georgia 30602, USA); and Museum of the High Plains, Fort Hays Kansas State College, Hays (Kansas 67601, USA), 25 March 1977

Summary. Cryptotis parva has a diploid number of 52 and a fundamental number of 50. Blarina brevicauda in Nebraska and Pennsylvania has a diploid number of 49 or 50 and a fundamental number of 48. Blarina carolinensis in Nebraska and Kansas has a diploid number of 52 and a fundamental number of 62. The X-chromosome in all 3 species is a large metacentric chromosome. The Y-chromosome is a small acrocentric in Blarina, whereas in Cryptotis it is a small subtelocentric.

Information has been published (for example, Baker and Hsu[2]; Fedyk and Ivanitskaya[3]; Meylan[4–9]; Meylan and Hausser[10,11]; Hausser et al.[12] and papers cited therein) on various aspects of the karyology of shrews (family Soricidae), but few data are available for the North American genera Cryptotis and Blarina. The genus Cryptotis is represented in the United States by only one species, C. parva (the least shrew), which occurs throughout much of the eastern half of this country as well as in mesic and montane habitats in Mexico and Central America (distribution and habitats summarized by Whitaker[13]). The relationships of this species to other members of the genus in Latin America recently have been reviewed (Choate[14]), and the taxonomy of the species in the United States probably contains few problems. This is not true, however, for the genus Blarina (short-tailed shrews), the distribution of which includes only the eastern half of the United States and adjacent regions of Canada (Hall and Kelson[15]). Prior to 1972, the genus Blarina generally was assumed to consist of only one species, B. brevicauda; a second species, B. telmalestes, had been described from the Dismal Swamp of coastal Virginia and North Carolina (Paul[16]), but was of doubtful taxonomic status (Choate[17]). Then, in 1972, Genoways and Choate[18] presented data indicating that in Nebraska a large, northern subspecies (B. b. brevicauda) and a smaller, southern subspecies (B. b. carolinensis) were behaving as good biological species. Subsequently, most authors have treated these taxa as a distinct species (B. brevicauda and B. carolinensis, respectively). Later, based on their study of fossils of Blarina, Graham and Semken[19] recognized a third Recent species (B. kirtlandi) in the genus. We continue to recognize only 2 species of Blarina in this paper.

Certainly, much additional systematic work is needed on North American shrews, especially Blarina. To aid in these studies, we present below the karyotypic data on these shrews that we have amassed over the past several years. The only previously published information for these shrews pertained to B. brevicauda talpoides (Meylan[6,8]) and B. b. kirtlandi (Lee and Zimmerman[20]). All karyotypic preparations were made according to methods described by Baker[21].

Cryptotis parva (figure 1). The diploid number for the least shrew is 52 and the fundamental number without the sex-chromosomes is 50. The autosomes, which are all acrocentric, range in size from one large pair to several minute pairs. The X-chromosome is a large metacentric and the Y-chromosome is a small subtelocentric.

Blarina brevicauda (figure 2). Specimens of B. b. brevicauda from Nebraska have a diploid number of either 49 or 50 and a fundamental number of 48. The polymorphism in diploid number is the result of a Robertsonian fission/fusion between a pair of large acrocentric autosomes and a pair of small acrocentric autosomes. Specimens with a diploid number of 48, resulting from fusion of both members of these pairs, were not represented in our material. The X-chromosome is a large metacentric and the Y-chromosome is a small acrocentric.

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2 R. J. Baker and T. C. Hsu, SWest. Nat. *14*, 488 (1970).
3 S. Fedyk and E. Y. Ivanitskaya, Acta Theriol. *17*, 475 (1972).
4 A. Meylan, Revue suisse Zool. *72*, 636 (1965).
5 A. Meylan, Revue suisse Zool. *73*, 548 (1966).
6 A. Meylan, Can. J. Zool. *45*, 1119 (1967).
7 A. Meylan, Revue suisse Zool. *74*, 685 (1967).
8 A. Meylan, Revue suisse Zool. *75*, 691 (1968).
9 A. Meylan, Revue suisse Zool. *78*, 603 (1971).
10 A. Meylan and J. Hausser, Z. Säugetierk. *38*, 143 (1973).
11 A. Meylan and J. Hausser, Revue suisse Zool. *81*, 701 (1974).
12 J. Hausser, J.-D. Graf and A. Meylan, Bull. Soc. vaud. Sci. nat. *72*, 241 (1975).
13 J. O. Whitaker, Jr, Mammal. Spec. *43*, 4 (1974).
14 J. R. Choate, Univ. Kansas Publ., Mus. Nat. Hist. *19*, 195 (1970).
15 E. R. Hall and K. R. Kelson, The mammals of North America, vol. 1, p. 53. Ronald Press 1959.
16 J. R. Paul, J. Mammal. *46*, 496 (1965).
17 J. R. Choate, J. Mammal. *53*, 116 (1972).
18 H. H. Genoways and J. R. Choate, Syst. Zool. *21*, 106 (1972).
19 R. W. Graham and H. A. Semken, J. Mammal. *57*, 433 (1976).
20 M. R. Lee and E. G. Zimmerman, J. Mammal. *50*, 333 (1969).