The ATP (Figure 3A) also associates the enzyme, although not so highly and surely not so specifically as histidine. This effect, contrary to that of the PRPP, is hard to explain if ATP binds only at the catalytic center; therefore another site(s) (allosteric) for ATP would be required, as KLUNGSOYR and KRYVI[17] have postulated. This finding is also in agreement with the KRYVI and KLUNGSOYR kinetic data[18] referring to ATP inhibition at high concentrations.

In Figure 3B it is clearly displayed that 2-thiazolalanine (also a feedback inhibitor), associates the protein not so efficiently as histidine does; this fact agrees with our thiazolalanine studies in vivo, in which the wild type is more resistant to this inhibitor than other *E. coli* K12 strains and *S. typhimurium* LT 2.

In the elution of a denatured sample (lyophilized and stored for one month at −15 °C) with only 5% of the initial activity (experiment not shown), nearly all the protein is excluded, and there are small amounts of hexamer and tetramer that could be responsible for the residual activity elicited by dissociation by the PRPP. It is conceivable that reversible tetramer and hexamer formation are intermediate steps towards irreversible high order polymerization.

Kinetic data of derepression of the histidine operon (unpublished experiments) point towards a positive control for the first enzyme. We are working on a biosynthetic model of regulation, of the JACOB-MONOD type, in which the association-dissociation processes reported in this paper play a central role[20].

Resumen. Se han realizado estudios de asociación-disociación por filtración en gel con el primer enzima de la biosíntesis de histidina en *Escherichia coli*, en presencia de sustratos y ligandos. Se observa una interconversión reversible entre las formas dímero, tetrámero y exámero, y una agregación irreversible de orden superior.

A. R. TÉBAR, V. M. FERNÁNDEZ,
R. MARTINDELRÍO and A. O. BALLESTEROS

Departamento de Catálisis, Consejo Superior de Investigaciones Cientificas, Serrano 119, Madrid 6 (Spain), 17 May 1973.

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RNase Activities in Blood Serum of Several Vertebrates

The presence of Ribonuclease in the serum of several animals has been reported[1–5]. Problems of like the origin and biological function of these serum enzymes were approached by some authors, but they remain controversial. Origin of serum RNase has been accepted to be pancreatic[6], but no direct evidence was ever reported. On the contrary, the hypothesis that the pancreas is the exclusive source of serum RNase was not sustained by pancreatectomy experiments[7].

Whatever the uncertainties in this field, this protein proves to be very useful for philogenetic studies, as recently emphasised by BARNARD[8,9] who demonstrated the existence of three well-defined classes among the vertebrates as concerns RNase content of the pancreas. As similarities between pancreatic and seric RNase have been proved to exist in different animal species[5], a comparative study of serum RNase activities in several vertebrates has been undertaken in the present investigation.

Representative species of the 3 groups classified by BARNARD were chosen for our purpose. Among those with very high pancreatic RNase activities – 200 to 1,200 μg/g of pancreatic tissue (group A) – we have studied ox, hamster, goat, rat, sheep and guinea-pig. Belonging to the group with a very low content of pancreatic nucleasic activity – 0 to 20 μg per g (group C) – we studied cat, pigeon, rabbit, dog and human sera. Other species included in this study, horse, pig and chicken, belong to the intermediary group, the group B, in BARNARD's classification.

Materials and methods. Blood samples from ox, sheep, goat, pig and horse were obtained from the slaughter-

Total serum protein in vertebrates

	Total protein[a] (mg/ml serum)
Ox (*Bos taurus*)	74.4
Hamster (*Mesocricetus auratus*)	63.7
Goat (*Capra hircus*)	80.5
Rat (*Rattus norvegicus*)	71.4
Sheep (*Ovis aries*)	61.4
Guinea-pig (*Cavia porcellus*)	50.0
Horse (*Equus caballus*)	72.8
Pig (*Sus scrofa*)	81.1
Chicken (*Gallus domesticus*)	43.1
Cat (*Felis catus*)	72.2
Pigeon (*Columba livia*)	27.6
Rabbit (*Oryctolagus cuniculus*)	60.7
Dog (*Canis familiaris*)	60.7
Mouse (*Mus musculus*)	69.9
Human	86.0

[a] Mean values of at least 3 different samples.

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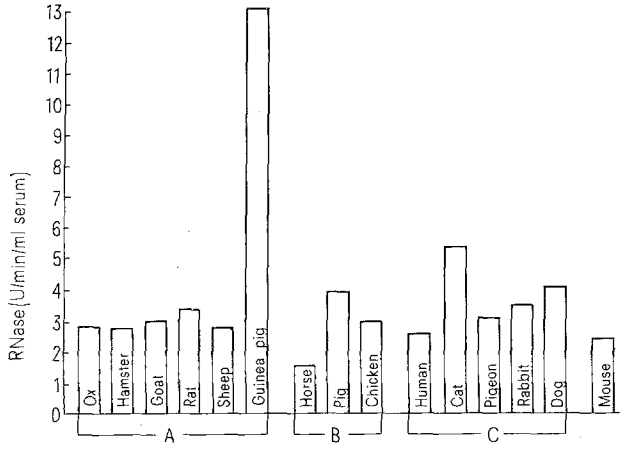
house. Blood samples from all the other animals were collected by decapitation. Wistar rats, Charles Rivers mice and Syrian hamsters, from strains maintained by random breeding in our laboratory animal house, were studied. Dogs, cats and guinea-pigs were submitted to an adaptation period of at least 4 weeks before bleeding. Chicken, pigeons and rabbits were commercially purchased and utilized without any previous care.

Human serum collected from the forearm veins was also analysed for RNase activity. All the blood samples were collected from individuals submitted to a fasting period varying between 12 and 24 h. Clear sera were obtained after blood coagulation at room temperature and centrifuged at 3000 rpm for 30 min. Total protein was determined by the method of LOWRY[10] and the results found are °listed in the Table.

Alkaline RNase activity was assayed by pipetting 20 µl of serum into a mixture of 0.25 ml of 0.25 *M Tris*-HCl buffer pH 7.45, 0.1 ml of 20 m*M* EDTA and 0.08 ml of 75 m*M* NaCl. 0.25 ml of 1.2% RNA was added and digestion was allowed to proceed for 20 min at 37 °C. The reaction was stopped and undigested RNA precipitated by the addition in the cold of 0.25 ml of 0.75% uranyl-acetate in 25% perchloric acid. The assays were run in duplicate as well as blank tests. After standing in an ice bath for 30 min, the tubes were centrifuged at 0 °C for 30 min at 12,000 *g*. Clear supernatant aliquots of 0.2 ml were diluted with 5 ml of distilled water and absorbance determined at 260 nm. 1 unit of RNase activity corresponds to an increase in the absolute absorption value of 1000, within the range of linearity[11].



Serum RNase activities – mean values of at least 3 different blood samples – of 15 vertebrate species. A, B and C correspond to the 3 groups classified by BARNARD[9].

Results and discussion. RNase activities found in the sera of the 15 vertebrate species mentioned above are represented in the Figure. These results show that serum RNase activities in the different vertebrates do not differ in the same striking way as pancreatic RNase and do not even allow one to group them into distinct classes.

A first conclusion to be drawn is that the activity of circulating RNase is not directly dependent on the pancreatic content of this enzyme. All the species belonging to group A in BARNARD's classification have serum RNase activities which are not distinguishable from those found in individuals of group B and C. Exception is made for the guinea-pig, which presents a high content of pancreatic RNase and also exhibits a high serum RNase activity, being in this respect the outstanding species among those included in the present study. On the other hand, the cat, belonging to the group with very low pancreatic RNase content, revealed a moderately higher level of serum enzyme compared to the other species studied.

These facts may be interpreted in 2 possible ways, either the origin of serum RNase is not related to the pancreatic enzyme, or a strong regulatory mechanism exists limiting the circulating levels of these enzymes. Such a treshold regulation would very probably be connected with the biological function of the blood serum nucleases in vertebrates[12].

Résumé. Les activités de la RNase ont été déterminées dans le sérum sanguin de 15 espèces de Vertebrés appartenant aux trois groupes définis par BARNARD. Les résultats obtenus montrent que les activités de la RNase n'offrent pas de différences (exception faite pour le cobaye), ce qui exclut l'existence d'une relation directe, simple entre RNase pancréatique et sérique. On relève l'interêt phylogénétique de ces protéines et de l'origine de la RNase sérique.

M. C. LECHNER and M. C. DUQUE MAGALHÃES

Laboratório de Farmacologia, Centro de Biologia, Instituto Gulbenkian de Ciência, Oeiras, (Portugal), 21 May 1973.

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Nitrate Reductase Activity in Developing Wheat Ears

A review of the literature shows that accumulation of protein in the cereal grains involves translocation of nitrogen from the vegetative parts, particularly the leaves. Some reports of uptake of nitrogen by the plant during the grain development phase are available[1–3]. Involvement of nitrate reductase (NR) and protease enzymes in the synthesis and mobilization of organic nitrogen to the grains has been suggested by the studies of HAGEMAN et al.[4], RAO and CROY[5] and NAIR and ABROL[6]. 'High' protein in the grains has been attributed to enhanced levels of translocation of amino acids from the leaves[7,8].

That the developing wheat ears are the seats of sulphate reduction and synthesis of sulphur amino acids was shown by the studies of GRAHAM and MORTON[9]. However, to the author's knowledge, no report where in the occurrence of nitrate reducing system vis-a-vis synthesis of amino acids in the developing ears is available. In this communication, experimental evidence is presented which suggests that NR activity in the developing wheat ears viz. grains and glumes, is of significance as a contributing factor towards the synthesis of amino acids.

Materials and methods. Wheat var. *Kalyansona* was grown in the fields of our research institute at 4 different