

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<sup>10</sup> and the results found are listed in the Table.

Alkaline RNase activity was assayed by pipetting 20  $\mu$ l of serum into a mixture of 0.25 ml of 0.25 M Tris-HCl buffer pH 7.45, 0.1 ml of 20 mM EDTA and 0.08 ml of 75 mM 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<sup>11</sup>.

**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.

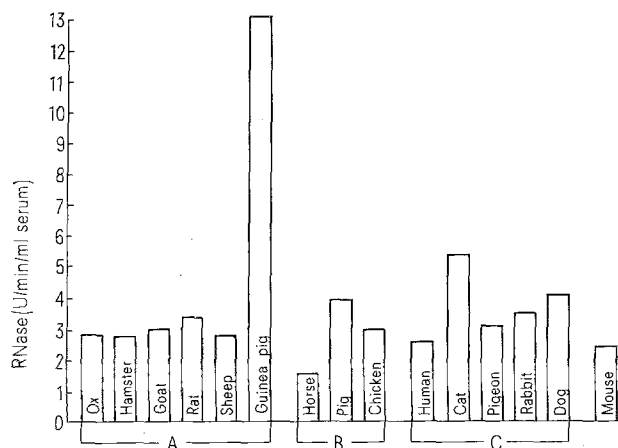
A first conclusion to be drawn is that the activity of circulating RNase is not directly dependent on the pancreatic content of this enzyme. All the species belonging to group A in BARNARD's classification have serum RNase activities which are not distinguishable from those found in individuals of group 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 threshold regulation would very probably be connected with the biological function of the blood serum nucleases in vertebrates<sup>12</sup>.

**Résumé.** Les activités de la RNase ont été déterminées dans le sérum sanguin de 15 espèces de Vertébrés 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'intérêt phylogénétique de ces protéines et de l'origine de la RNase sérique.

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21 May 1973.



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<sup>9</sup>.

<sup>10</sup> O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* 193, 265 (1951).

<sup>11</sup> T. UCHIDA and F. EGAMI, in *Progress in Nucleic Acid Research*, (Ed. G. L. CANTONI and R. DAVIES; Harper, New York 1966), p. 4.

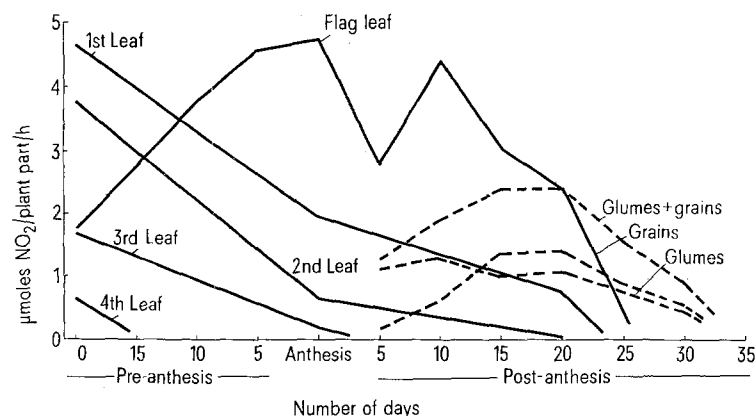
<sup>12</sup> The technical assistance of Mrs. A. ALMEIDA is gratefully acknowledged.

## 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<sup>1-3</sup>. 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.<sup>4</sup>, RAO and CROY<sup>5</sup> and NAIR and ABROL<sup>6</sup>. 'High' protein in the grains has been attributed to enhanced levels of translocation of amino acids from the leaves<sup>7,8</sup>.

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<sup>9</sup>. 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



NR activity in leaves and developing ears (grains and glumes), expressed as  $\mu\text{mole NO}_2^-$  released on total plant part basis.

nitrogen fertility levels viz.  $N_0$ ,  $N_{60}$ ,  $N_{120}$  and  $N_{180}$  (kg N/hect). Nitrogen was applied in the form of urea in 2 equal split doses, one at the time of sowing as basal dose and the other in 60-day-old plants. NR activity in the plants grown at  $N_{120}$  was determined by the in vivo method of KLEPPER et al.<sup>4</sup> For determination of NR in the grains, these were cut in half and incubated for 1 h. After incubation, the medium with the tissues was boiled in water-bath for effective removal of the nitrite.

**Results and discussion.** It was observed that NR activity ( $\mu\text{moles of NO}_2^-$  released per h) in the grains increased with age. Highest activity was between 15–20 days followed by a decrease. Little or no activity was detectable at 32–35 days.

On a per-gram fresh weight basis, decrease in NR activity in the 1st, 2nd, 3rd and 4th leaves (counting from top downwards) after anthesis was observed. NR activity was high in the flag leaf and was detectable up to 25–27 days. Activity in the grains and glumes was low and was detectable up to 30–32 days. However, it was

observed that the total leaf area available for NR activity decreased after anthesis, while that of ears viz. grains and glumes showed an increase. The approximate ratio of leaf to ear (fresh weights) was 1:1 at 10 days after anthesis in plants grown at 4 different levels. It shifted to 1:8 in the case of  $N_0$  and approximately 1:5 at all other fertility levels at 30 days stage (Table).

Thus calculation on  $\text{NO}_2^-$   $\mu\text{moles}$  released per plant part basis viz. leaves, ears (grains and glumes) show distinctly that within 10–32 days the ear contributes significantly to nitrate assimilation (Figure). The above findings are of interest from the viewpoint of elucidation of biochemical factors affecting protein accumulation in the wheat grains.

**Zusammenfassung.** Die Fruchtstände der Weizenpflanzen reduzieren während ihrer Entwicklung beachtliche Mengen von Nitratstickstoff.

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Fresh weight of ears and leaves at different post anthesis stages at 4 nitrogen fertility levels (var. Kalyansona)

Fertility levels	Plant part	No. of days after anthesis		
		10	20	30
Fresh weight (g) *				
N <sub>0</sub>	Ears	4.9	10.4	15.8
	Leaves	6.2	3.6	1.9
N <sub>60</sub>	Ears	9.8	22.4	23.7
	Leaves	10.6	9.0	5.5
N <sub>120</sub>	Ears	13.9	26.4	23.0
	Leaves	17.0	7.3	3.9
N <sub>180</sub>	Ears	20.3	21.4	28.6
	Leaves	25.9	10.1	5.3

\* Average of 5 plants.

<sup>1</sup> R. F. WILLIAMS, A. Rev. Pl. Physiol. 6, 25 (1955).

<sup>2</sup> R. H. M. LANGER, in *The Growth of Cereals and Grasses* (Ed. FL. L. MILTHORPE and J. D. IVINS; Butterworths, London 1966), p. 213.

<sup>3</sup> P. J. RANDALL, Proc. Symp. Biology of Storage Proteins Canberra (1972).

<sup>4</sup> L. KLEPPER, D. FLESHER and R. H. HAGEMAN, Pl. Physiol. 48, 580 (1971).

<sup>5</sup> S. C. RAO and L. I. CROY, Agric. Fd. Chem. 20, 1138 (1972).

<sup>6</sup> T. V. R. NAIR and Y. P. ABROL, Proc. 2nd Sabrao Congress, New Delhi (1973).

<sup>7</sup> L. J. CRUZ, C. G. CAGAMPANG and B. O. JULIANO, Pl. Physiol. 46, 743 (1970).

<sup>8</sup> J. A. JOHNSON and J. P. MATTERN, Summary Report of Research Findings, Agency for International Development, Department of State, Washington, D.C. (1972).

<sup>9</sup> J. S. D. GRAHAM and R. K. MORTON, Aust. J. biol. Sci. 16, 357 (1963).

## Zur Biosynthese von Biopterin in Säugetieren

Biopterin ist ein essentieller Kofaktor zur Biosynthese von Adrenalin und Serotonin im Organismus von Säugetieren<sup>1-3</sup>. Im menschlichen Harn wurden bisher Biopterin, Sepiapterin und Neopterin gefunden<sup>4-6</sup>. Jedoch ist die Herkunft dieser Substanzen bei Säugetieren noch nicht geklärt.

Zur Untersuchung dieser Fragestellung injizierten wir männlichen Ratten und Mäusen Ringer's Lösung mit <sup>14</sup>C-GTP (U) (jeweils 2  $\mu\text{Ci}$  in 0,2 ml Lösung) in die Schwanzvenen. Dem nach der Applikation eine Woche lang gesammelten Urin wurde nichtradioaktives Biopterin (jeweils 4  $\mu\text{Mol}$ ) hinzugefügt. Der Urin wurde mit