Fertilizer residue from labelled urea in a clay soil[1]

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Summary. The proportion of nitrogen obtained by wheat Pak-70 from N^{15} labelled urea applied at the rate of 120 kg N/ha was less than 35%. Leaching of nitrogen beyond the root zone was shown to be negligible. Most of the nitrogen was therefore left in the soil as residue. The increase in soil inorganic nitrogen values after the wheat harvest confirmed the presence of residual nitrogen.

The introduction of high yielding varieties of crops having high nutritional requirements, and the progressive use of fertilizer by farmers in Pakistan, has resulted in large inputs of fertilizer into the soil from year to year. A corresponding increase in soil nitrogen may occur in well-aerated fine-textured soils. This residual nitrogen, while beneficial in lessening the need for fresh fertilizer nitrogen[2], also represents a potential loss and health hazard if it leaches beyond the root zone and pollutes the ground water. The present experiment was aimed at studying the residual effect of labelled urea applied to wheat at optimum rates during the first growing season.

Materials and methods. The experiment was conducted at the Atomic Energy Agricultural Research Centre, Tandojam. Wheat Pak-70 *(Triticum aestivum)*, a semi-dwarf cultivar, was used. The treatments consisted of 0 and 112 kg N/ha, applied as urea labelled with 1% N^{15} atom excess. The labelled urea was applied to microplots of size 1.5×1.5 m situated in the centre of the main plots of size 8×8 m. Due to late availability of labelled fertilizer, the whole of the nitrogen was applied at the time of sowing. All of the experimental plots received a basal dose of P at the rate of 22 kg P/ha. 3 irrigations of 7.5 cm each in addition to the soaking dose were applied to the wheat during the growing season. Wheat plant samples were obtained from N^{15} microplots at harvest to determine fertilizer utilization by wheat. Percentage of N^{15} excess in plant tissue was determined by the Siebersdorf Laboratory of the International Atomic Energy Agency in Vienna. The texture of the experimental plots varied between clay and clay loam (clay content 30–55%). Total soluble salts ranged between 810 and 985 ppm, pH ranged between 7.7 and 8.3, and organic matter ranged between 0.6 and 1.2%. The rainfall during the growing season was negligible (1.3 mm).

Table 1. Fertilizer nitrogen derived by wheat from added urea, and the 'A'-values

Plot No.	N^{15} excess in added urea (%)	N^{15} excess in plant tissue[a] (%)	N derived from fertilizer (%)	A-value (kg N ha^{-1})
R1	1	0.365	36.5	208
R2	1	0.310	31.0	267
R3	1	0.300	30.0	280
R4	1	0.290	29.0	294

[a] Total plant tissue above ground. Roots and stubs were not sampled.

Table 2. Inorganic nitrogen in soil before sowing and after wheat harvest

Soil layer (cm)	Before sowing (kg N ha^{-1})		After harvest (kg N ha^{-1})		Change (kg N ha^{-1})	
	NH_4–N	NO_3–N	NH_4–N	NO_3–N	NH_4–N	NO_3–N
0–15	54	17	176	153	+122	+136
15–30	74	24	228	153	+154	+129
30–60	183	45	219	153	+ 36	+108
60–90	159	54	387	228	+228	+174
Total 0–90	470	140	1010	687	+540	+547

Each figure represents the average of 4 replications. kg N ha^{-1} for each layer was calculated as concentration (ppm) on a wet weight basis (as sampled) × bulk density of soil at the time of sampling (g cm^{-3}) × depth of soil layer (cm) × 10^{-1}.

Results and discussion. The proportion of nitrogen derived by the wheat from the added labelled urea and the 'A'-value data are given in table 1. The A-values were calculated according to Fried and Dean[3]. It is evident that only about one third of the applied fertilizer was used by the wheat. This low utilization was due to higher uptake of nitrogen by wheat from the soil N, as is demonstrated by the A-values. Average nitrogen utilization by wheat in Pakistan from different nitrogen sources was of the order of 50% or less[4,5].

The proportion of nitrogen not utilized by wheat may have been either left in the soil, leached beyond the root zone or lost from the soil-plant system in the gaseous form. The data in table 2, which reports the nitrate and ammonium values of the soil samples obtained before wheat sowing and after the harvest, show that a large amount of fertilizer nitrogen was left in the soil. This residue was due primarily to insufficient fertilizer utilization by the wheat owing to the shorter growing season (wheat was planted 5 weeks later than the normal sowing time of wheat for the region) and the late application of urea. There was high variability in the soil inorganic nitrogen measurements particularly among the samples obtained after the wheat harvest (average coefficient of variability for the samples obtained before sowing = 12%, and for those after harvest = 27%). Some of the nitrogen found at the lower depths in the profile may have mineralized from the native soil organic matter during the warmer part of the growing season. This nitrogen apparently remained unutilized by wheat near maturity and moved upwards with the soil solution under the influence of increasing evapotranspirative demand at the surface. The application of fertilizer usually stimulates the release of soil N by enhancing mineralization of organic soil nitrogen (priming effect)[6]. Ammonium fertilizers generally possess a greater stimulatory effect than nitrates.

The greater part of the nitrogen was concentrated in the top 30 cm. The lower values for nitrogen at 30–60 cm depth after wheat harvest were probably due to uptake by roots from this zone. At the end of the growing season the increase in the concentration of NO_3 was slightly greater than that of NH_4, which showed that nitrification was in progress even at the end of the growing season. High residual effects of applied nitrogen were also observed by other workers. Olsen et al.[7] and Muir et al.[8] related the large amounts of nitrogen found in the soil profile to heavy nitrogen fertilization of crops. Power et al.[9] found that the residual effect of four nitrogenous fertilizers persisted for 3 years following continuous fertilization for 4 years. With urea, gaseous losses to the extent of 50% were observed at the end of the residual period.

Nitrogen leaching. Leaching of nitrogen into the ground water was traced indirectly by following the movement of water in the soil profile. Soil moisture curves prepared before and after each irrigation (not shown here) showed that downward movement of water was not beyond 1 m of the soil under wheat. As the movement of highly mobile NO_3 is closely associated with water, it is credible that the same could be true for nitrogen. The plant cover further reduces the amount of nitrogen available for leaching due to utilization by the plants.

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Monophosphate, the only phosphoric ester of thiamin in the cerebro-spinal fluid

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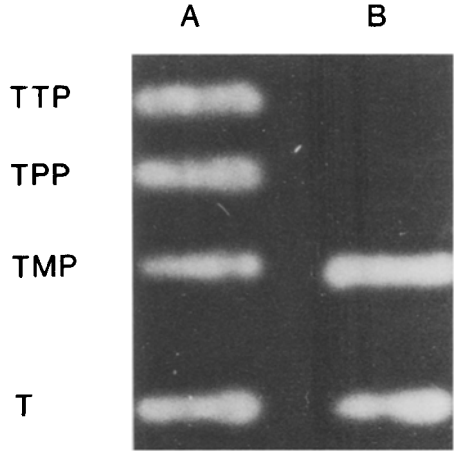
Summary. With a specific and sensitive electrophoretic-fluorometric method, thiamin was found in the cerebro-spinal fluid of different mammals both in free and phosphorylated form, monophosphate being the only thiamin phosphoric ester. In humans, its amount was about 60% of total thiamin. Alcoholism greatly lowered total thiamin content, affecting both thiamin forms.

In animal tissues thiamin is present in 4 forms: free (T), mono- (TMP), pyro- (TPP) and tri-phosphate (TTP), TPP being 80–90% of the whole thiamin[1,2]. However, in rat plasma only T and TMP were found[3]. Sinclair[4,5] was unable to find thiamin phosphates in normal human cerebro-spinal fluid (CSF). Recently, Spector[6] reported that in rabbit CSF thiamin is contained mostly in an unspecified 'phosphorylated' form and that both free and phosphorylated thiamin enter the brain as well as the CSF by a saturable transport mechanism. Our current interest on the physiological functions of the thiamin phosphates in the central nervous system[7] prompted us to investigate which thiamin phosphate is present in normal CSF of humans and other mammals, as a first approach to the study of its origin and physiological meaning. The results are briefly reported here.

Materials and methods. Human CSF was obtained by lumbar puncture, in the morning, after 10–14 h of starvation; fluids containing erythrocytes or increased proteins were discarded. From other mammals the CSF was obtained by puncture of the cisterna magna, after Pentothal anesthesia. CSF was stored in the cold until the moment of chemical analysis, usually 30 min after withdrawal. After centrifugation at $350 \times g$ for 10 min in order to eliminate possible leukocytes, the samples (1–3 ml) were deproteinized with trichloroacetic acid (8% final concentration) and centrifuged again in the cold at $20{,}000 \times g$ for 10 min. The supernatant was purified and analyzed for thiamin compounds, using the electrophoretic or the direct fluorometric method described by Patrini and Rindi[8]. A comparison of the two methods for total thiamin (sum of T and phosphorylated thiamin) determination in a series of 6 CSF samples gave the following mean results (μg of total thiamin/l $\pm$ SE): 23.74 ± 1.24 (electrophoretic method) and 23.96 ± 0.98 (direct fluorometric method). The differences between paired results were not statistically significant (sign-test[9]). This allowed the electrophoretic or direct fluorometric method to be interchangeably used for total thiamin estimation (table).

Results. First a set of experiments was devoted to investigating any possible hydrolysis of CSF thiamin phosphates before and during the electrophoretic procedure. Thus, a known amount of TMP (about 50 ng) and TPP (370–400 ng) was added to a 1-ml sample of a pool of human CSFs, which was analyzed after a week's storage in a deep freezer. Another sample of the same CSF pool, but without addition of thiamin compounds, was immediately analyzed. The recoveries of the added compounds were (means of 2 experiments): TMP, 102.1%; TPP, 97.2% and total thiamin, 97.8%. This indicated the good stability of the single thiamin phosphoric esters in the CSF even after long storage at a low temperature. In another experiment only TPP was added to a sample of CSF. This was stored for



Electrophoretic analysis[8] of: *A* Thiamin compounds in pure solution (thiamin (T), 116.3; thiamin-monophosphate (TMP), 83.2; thiamin-pyrophosphate (TPP), 89.9; thiamin-triphosphate (TTP), 88.2 ng); *B* 10 ml of a pool of human cerebro-spinal fluids, carried through the entire analytical procedure[8]: only T (91.3 ng; 42.82% of total T) and TMP (121.9 ng; 57.18% of total T) were found.