

## ROLE OF BORON IN NUCLEIC ACID METABOLISM OF GERMINATING WHEAT SEEDLINGS

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### ABSTRACT

Wheat seeds soaked for four hours in distilled water (control), 0.125%, 0.25% and 0.50% solutions of boric acid and borax were transferred to polythene dishes for germination. Treatments with 0.125% boric acid and borax were found to be optimum for accelerating seed germination process and seedling growth. Boron seems to help in mobilising nitrogenous hydrolysates from germinating seeds to young shoots. Both boric acid and borax increased ribonuclease activity in young shoots and isolated germinating seeds at all levels of concentration as compared to control. Ribonuclease, therefore, seems to be associated with the biosynthesis of RNA in actively growing tissues. There is also an increase in ribonucleotide concentration both in shoots and seeds with an increase in the concentrations of these compounds.

### INTRODUCTION

A CRITICAL survey of literature reveals that out of all the hypotheses put forward by different investigators to explain the role of boron in plant metabolism, the recent suggestions<sup>1-3</sup> about its role in nucleic acid metabolism deserve detailed studies. These suggestions seem to be quite plausible in view of the higher concentrations of nucleic acids and their intervention in vital activities of meristematic tissues coupled with the observations that boron is localised in meristematic regions of plants. Boron has also been ascribed to play an important role in protein metabolism.<sup>4,5</sup> Its deficiency often decreases the protein content of plant tissues. Our observations<sup>6</sup> have already indicated that boron participates in protein biosynthesis through its control on RNA content.

The present investigation was undertaken to understand the role of boron, both in the form of boric acid and borax, in nitrogen mobilisation and polyribonucleotide metabolism during seed germination and seedling growth. Our major aim was to interpret the effects of boron on the above phenomenon in the light of the alterations brought about, if any, in the levels of total nitrogen, RNA, ribonucleotides and ribonuclease activity.

### MATERIALS AND METHODS

Wheat seeds (*Triticum vulgare*) of Mexican variety (*Lerma rojo*) were soaked for four hours in distilled water (control), 0.125%, 0.250% and 0.50% solutions of boric acid and borax. Seedlings were raised in polythene dishes on a double layer of Whatman No. 44

filter-paper. Twenty healthy seeds were kept for germination in each dish and four such dishes were put under each treatment. Distilled water was applied to the germinating seeds at regular intervals of six hours. The experiment was conducted in a dark room at 26° C. Percentage germination and heights of the shoots in centimeters were recorded after every 24 hours interval.

Total nitrogen in young roots and shoots was separately determined in each sample by Microkjeldahl method.<sup>7</sup>

Plant tissues (germinating seeds and shoot tips) under similar treatments were pooled together for the preparation of acetone powder according to the method cited in Colowick and Kaplan.<sup>8</sup> RNA was estimated by the method of Schneider.<sup>9</sup> The changes in ribonuclease activity in the acetone powder were measured by a method described by Pardee and Kunkee.<sup>10</sup> Ribonucleotides were estimated by Ceriotti method.<sup>11</sup>

### RESULTS AND DISCUSSION

*Effect on Seed Germination.*—Our observations (Fig. 1) clearly showed that 0.125% boric acid treatment was optimum for bringing about hundred percent seed germination in 48 hours. Even though higher concentrations of boric acid and borax were found to delay the process, yet the control seeds required the maximum time (192 hours). At 0.125% and 0.25% concentrations boric acid was found to be more effective as compared to borax. Hastening of seed germination by boron has also been reported<sup>12</sup> in the case of pineapple, ash and yellow accacia.

**Effect on Nitrogen Mobilisation.**—Maximum promotive effect on shoot growth was shown

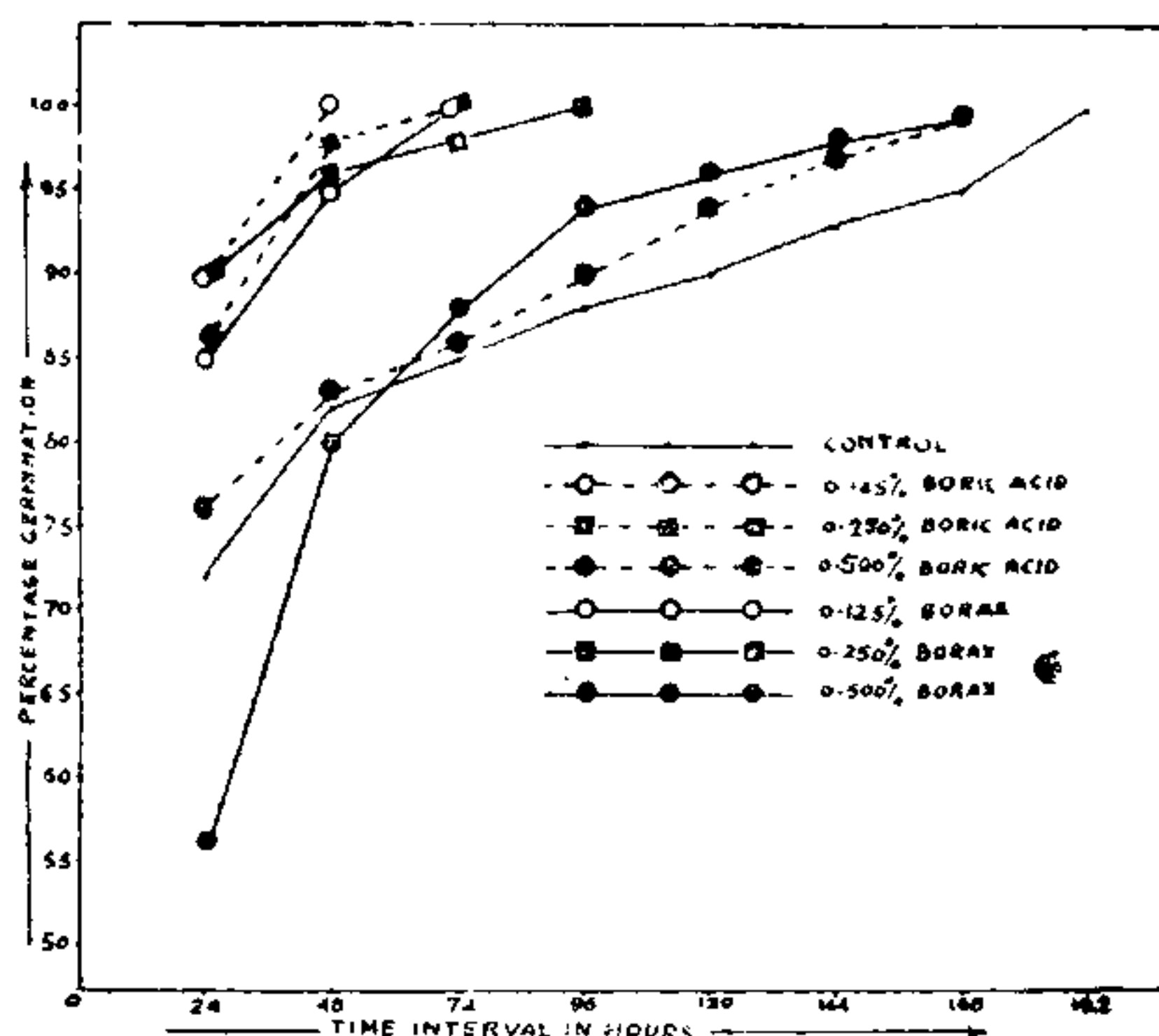


FIG. 1. Effect of various concentrations of boric acid and borax on percentage germination of wheat seeds.

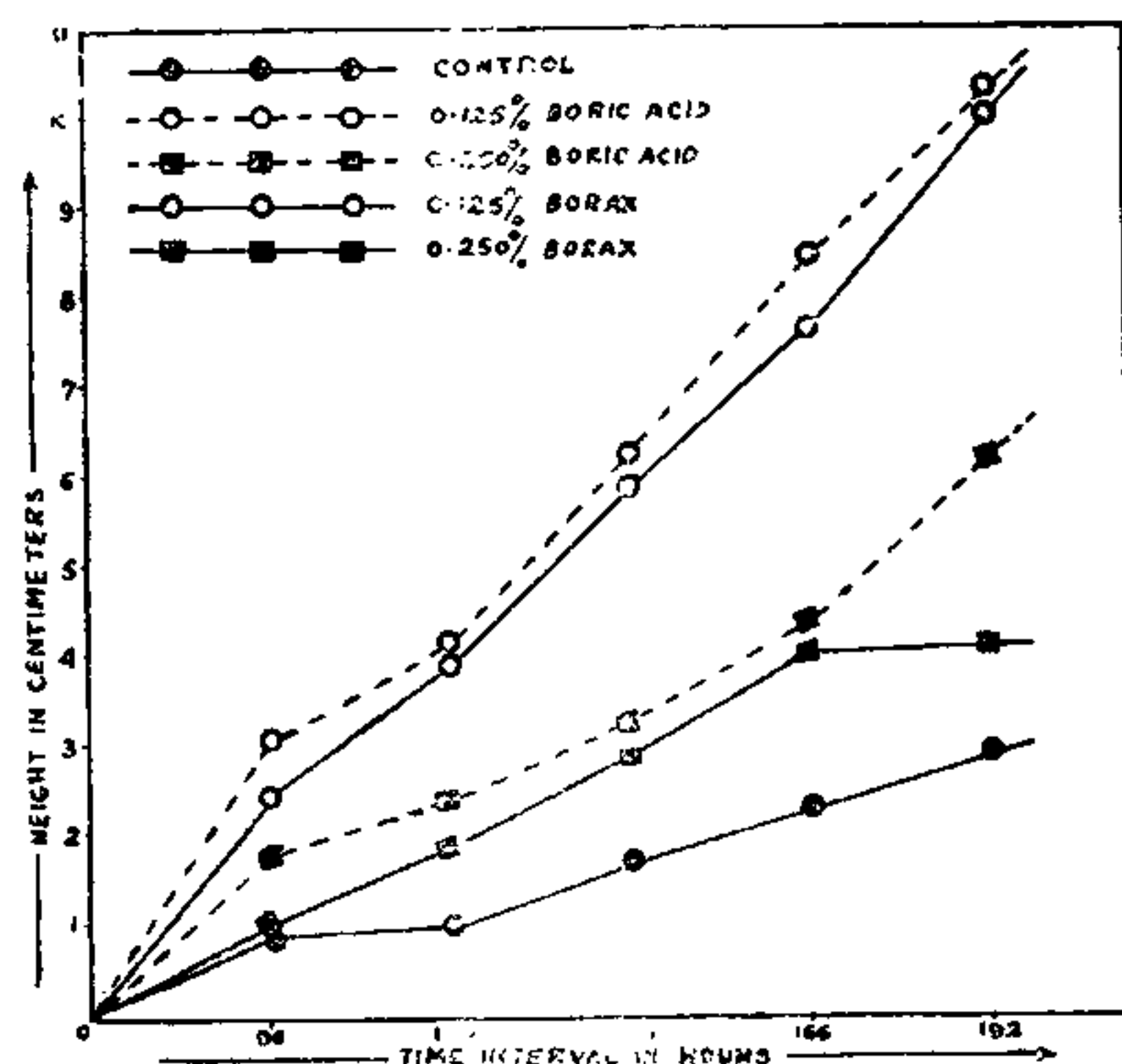


FIG. 2. Effect of various concentrations of boric acid and borax on the shoot growth of wheat seedlings. Each value represents a mean of 80 seedlings.

by 0.125% boric acid treatment (Fig 2). An explicit biochemical reasoning for this accelerated shoot growth due to boron treatments could be gathered by studying the nitrogen status of the young shoots for which the only source of nitrogen is the germinating seed. Our investigations (Table I) showed that there was a statistically highly significant increase in the total nitrogen content of shoots treated with 0.125% and 0.25% boric acid. With an increase in the concentrations of boric acid and borax, the rate of shoot growth was retarded and in the case of 0.5% treatments

there was a negligible shoot growth after seed germination. Borax treatments had a less pronounced effect on shoot growth and its nitrogen status as compared to boric acid. Control roots contained higher concentrations of total nitrogen as compared to those treated with boric acid and borax. Boron thus helps in hydrolysing the seed proteins and directing their mobilisation more towards shoots than to the roots.

**Effect on Nucleic Acid Metabolism.**—Protein synthesis is known to be turned off in all parts of the resting seed. One reason for this might be the unavailability of the mRNA which is considered to be activated during imbibition.<sup>13</sup> Our observations (Table II) also showed a significant rise in the RNA content of wheat seeds after imbibition.

TABLE I

Statistical evaluation of percentage nitrogen in germinating wheat seedlings treated with varying concentrations of boric acid and borax

	Treatments				
	Control	Boric acid		Borax	
	- B	0.125%	0.250%	0.125%	0.25%
Germinating shoots	0.828	1.064	0.989	0.914	0.839
S.D.	..	0.1258	0.0681	0.1826	0.1223
t value	..	3.253	4.090	0.759	0.536
Germinating roots	0.546	0.504	0.434	0.462	0.429
S.D.	..	0.0955	0.0643	0.0731	0.0714
t value	..	0.760	3.010	1.988	2.834

1. Each value represents a mean of six replications.

2. Significant at 5% level when  $t_{10}$  as compared to control  $\geq 2.23$ . Highly significant at 1% level when  $t_{10}$  as compared to control  $\geq 3.17$ .

Maximum ribonuclease activity was exhibited by 0.125% boric acid-treated wheat seeds. Both boric acid and borax were found to activate ribonuclease at all concentrations. Simultaneously, there was an increase in the RNA content of wheat seeds with increasing boron concentrations. At 0.125% concentration, both boric acid and borax were found to depolymerise RNA to the maximum, probably to be transported as nucleotides to meristematic tissues. Therefore, the seeds treated with 0.125% boric acid and borax were found to contain the minimum amount of RNA.

Studies in the shoot tips of the germinating seedlings (Table II) showed that both boric acid and borax enhanced the ribonuclease activity as compared to that in the control plants.

TABLE II

Changes in RNA, ribonuclease activity and ribonucleotides in germinating wheat seeds and shoot tips treated with varying concentrations of boric acid and borax

	Treatments										
	Seeds					Shoot tips					
	Control (-B)		Boric acid		Borax		Control (-B)		Boric acid		Borax
D.r.	Ger.	0.125%	0.25%	0.125%	0.25%	D.r.	Ger.	0.125%	0.25%	0.125%	0.25%
Ribonucleic acid ( $\mu\text{g./mg.}$ of acetone powder)	240.00	182.66	256.00	304.00	229.33	239.33	225.35	295.73	394.36	304.36	507.04
Unit RNase activity	0.00	5.00	20.00	15.00	17.00	12.00	25.00	30.00	38.00	30.00	34.00
Free Ribonucleotides ( $\mu\text{g./mg.}$ of acetone powder)	28.33	24.14	21.66	22.14	20.75	22.14	10.00	15.23	16.42	13.81	17.61

D.r.: Dormant, Ger.: Germinating.

The activity of this enzyme was found to increase with increasing concentrations of boron compounds. A simultaneous increase in the RNA content and ribonuclease activity with all the concentrations of these compounds was very intriguing. On the basis of these observations, the present authors support the suggestion put forward by Barker and Douglas<sup>14</sup> that the ribonuclease of the growing tissues is involved in the synthesis of RNA.

The values of ribonucleotides were found to increase with increasing concentrations of boric acid and borax in the case of young shoot tips. An increase in ribonuclease activity due to boron treatments could not be responsible for an increase in ribonucleotide concentration because of a simultaneous rise in RNA levels in shoot tips. Boron, therefore, seems to play some role in ribonucleotide biosynthesis.

A decline in ribonucleotide concentration in boron-treated seeds as compared to the control seeds showed that more ribonucleotides were probably transported from seeds to actively growing tissues for RNA synthesis.

#### CONCLUSION

Wheat seeds soaked in 0.125% boric acid for four hours take one-fourth time to reach 100% germination as compared to the control seeds. This treatment is also optimum for significantly mobilising nitrogenous hydrolysates from germinating seeds to young shoots as well as for promoting maximum shoot growth.

Boron treatments also increase ribonuclease activity which seems to help in the biosynthe-

sis of RNA in young shoots. An accumulation of ribonucleotides in boron-treated shoot tips shows that the element might be involved in ribonucleotide biosynthesis.

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1. Shkol'nik, M. Ya. and Maevskaya, A. N., *Fiziol. Rast.*, 1967, 9 (3), 270
2. Tima-hov, N. D., *Dokl. Akad. Nauk. SSSR* (Russ.), 1966, 169 (6), 1459.
3. Sherstnev, E. A., *Ibid.*, (Russ.), 1967, 175 (5), 1190
4. Vladimirov, E. N., *Uzb. Biol. Zh.* (Russ.), 1966, 10 (6), 18.
5. Enileev, Kh. Kh. and Andryushchenko, V. K., *Ibid.*, (Russ.), 1963, 7 (4), 23.
6. Sai'i, H. S., Dani, I. M., Allag, I. S. and Sareen, K., *Curr. Sci.*, 1969, 38 (15), 355.
7. A.O.A.C., 1965.
8. Colwick and Kaplan, *Methods in Enzymology*, 1955, 2, 63.
9. Glick, D., *Methods of Biochemical Analysis*, 1966, 14, 155.
10. Pardee, A. B. and Kunkel, J., *Biol. Chem.*, 1952, 199, 9.
11. Ceriotti, G., *Ibid.*, 1956, 214, 59.
12. Stepanov, V. I., *Nauka. Issledk. Ap. Nomenklat.* (Russ.), 1963, 45, 23.
13. Marcus, A. and Ferley, J., *Proc. Natl. Akad. Sci., U.S.A.*, 1964, 51, 1075
14. Barker, G. R. and Douglas, T., *Nature*, 1960, 188, 643.