PROTEIN SYNTHESIS DURING EARLY GERMINATION

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THE control of early developmental processes and subsequent growth is mainly dependent on the specific transcriptional and translational functions in the living organisms. Protein synthesis is considered to be the earliest molecular process in seed germination[1]. Regulation of the protein synthesising machinery is related to repression as well as derepression of the genome.

During germination, part of the proteins are hydrolysed to peptides and amino acids in the storage organs and translocated to the developing embryos. There seems to be a sequential appearance of various proteins in germinating seeds. There has been speculation on the time of synthesis of proteins during germination. Bhatia and Nilson[2] showed that proteins did not change until 48 hr in wheat seed germination; while Maller[3] observed changes in protein separation by 4 to 12 hr in onion. Hence it was aimed to study the process of protein synthesis in mungbean (*Phaseolus aureus* L.) and runner bean (*Phaseolus coccineus*) axes during early germination.

Materials and Methods

The mungbean seeds were purchased from the local market. The runner bean seeds were purchased from Supergran, Mechelen, Belgium. Uniform mungbean seeds according to size and weight were sterilized with 1% calcium hypochlorite for 2 min and washed in sterile water. The seeds were germinated in 0·01 M potassium dihydrogen phosphate pH 6·0 at 30° C in a metabolic shaker with 80 to 90 oscillations per minute. At the end of each incubation, which varied from 4 to 10 hr, the axes were separated manually, after arresting the incubation by plunging the seeds in cold water. They were immediately taken for analysis or stored at −15° C until needed.

Embryonic axes of runner bean were excised manually and stored at 4° C in a desiccator in dark until needed. The axes were germinated in dark at 30° C in a medium referred as GM (Germination Medium)[4] containing *tris*-HCl (pH 7·6), 0·005 M; KCl, 0·02 M; sucrose, 10 mg.ml^{-1} and chloramphenicol, 10 μg.ml^{-1}. The incubation was stopped by the addition of ice-cold water at the end of each interval. The axes were either used immediately or stored at −15° C until required.

Protein Synthesis in vivo.—Studies on initiation of protein synthesis were conducted with 10 uniform mungbean seeds/10 runner bean axes. The incubation medium contained 0·2 μc and 0·3 μc of ^{14}C-leucine-(U), (331 μc per μmole) for mungbean and runner bean respectively. The incubation was arrested by adding cold leucine (10^{-2} M). The axes were then homogenized in a buffer containing *tris*-HCl (pH 7·8), 0·01 M; $MgCl_2$, 0·01 M; and KCl, 0·02 M. The contents were centrifuged briefly and the volume made to 10 ml. Suitable aliquots were precipitated with trichloroacetic acid (TCA). The TCA-insoluble material was incubated at 80° C for 20 min, cooled and filtered on Whatman GF/C glass fibre filters. The precipitate was washed twice with 10% TCA and once with 70% ethanol. The filter was dried and counted with 5 ml of scintillation mixture (4 g PPO per litre of toluene) and radioactivity counted in a Packard (model 574) Liquid Scintillation Spectrometer. The results are expressed as cpm label incorporated into acid-insoluble material per 10 axes.

Separation of Protein on Polyacrylamide Gels.—The axes were homogenized in 3 or 5 ml buffer consisting of *tris*-HCl (pH 7·8), 0·01 M; magnesium acetate, 0·001 M; KCl, 0·005 M and mercaptoethanol, 0·0015 M respectively. The homogenate was centrifuged at 165,000 g for 180 min in a SW 50 rotor. The resulting supernatant was subjected to separation of proteins on polyacrylamide gels. Before applying the sample on the gel, the protein concentration in all the samples (different hours) was adjusted to be uniform by proper dilution. This solution was uniformly mixed with 40% sucrose and an aliquot was layered on the gel and electrophoresed.

The disc electrophoresis of Davis[5] was adopted using 7·5% acrylamide with *tris*-glycine buffer (pH 8·3). The electrophoresis was conducted with 6 to 8 mA per gel using bromophenol blue as tracker. After electrophoresis, the gels were stained in a solution of 1% amido black in 7% acetic acid and destained electrophoretically in 7% acetic acid after an hour. The coloured bands of proteins were traced using a Varicord densitometer.

Results

In mungbean axes, protein synthesis is initiated after the 4th hour. A little decrease is observed at the 7th hour, followed by a further increase towards the 10th hour (Fig. 1). Protein synthesis in excised embryonic axes of runner bean is found

to initiate even by the first hour of imbibition and increases at later stages. The incorporation of leucine is inhibited by cycloheximide (Table I).

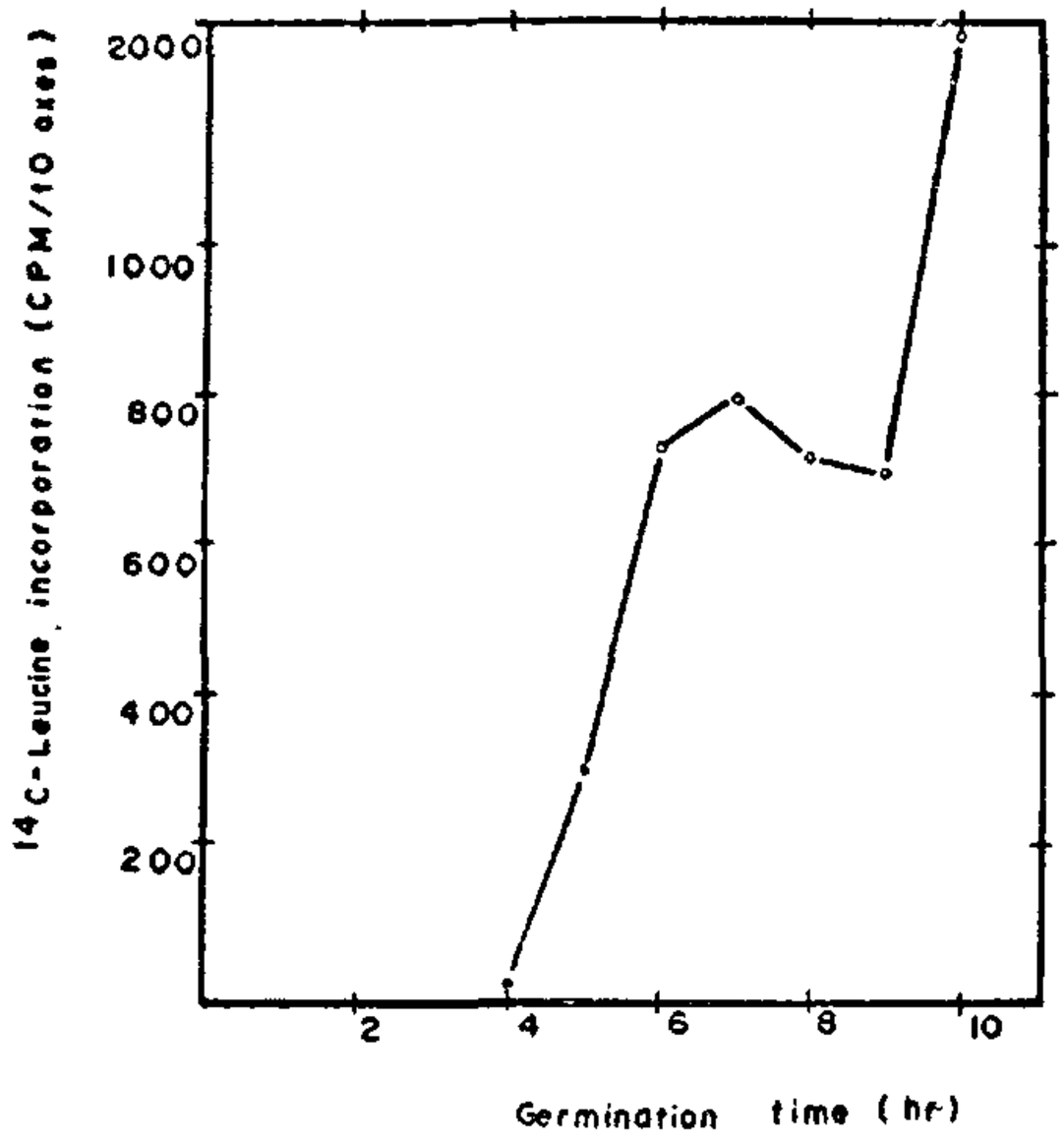

FIG. 1.

TABLE I

Effect of cycloheximide on protein synthesis in runner bean axes

Time (hr) From	normal	+Cyclo-heximide (1 mg/ml)
	(*cpm*/10 *axes*)	
0 to 1	543	23
5 to 6	6863	97
11 to 12	13705	85

(Fig. 2). There is a marked difference in the separation pattern of different protein bands during early germination hours in mungbean. Little change is observed in the pattern between 4 and 6 hr. By 8 and 10 hr periods, the number of bands increases. In 10 hr period, sharp tracings are obtained for almost all the bands (Fig. 2) and the newly formed bands become very conspicuous as compared to that found by 8 hr (indicated by arrows).

The supernatant fractions of runner bean obtained from ungerminated and 12 hr germinated embryonic

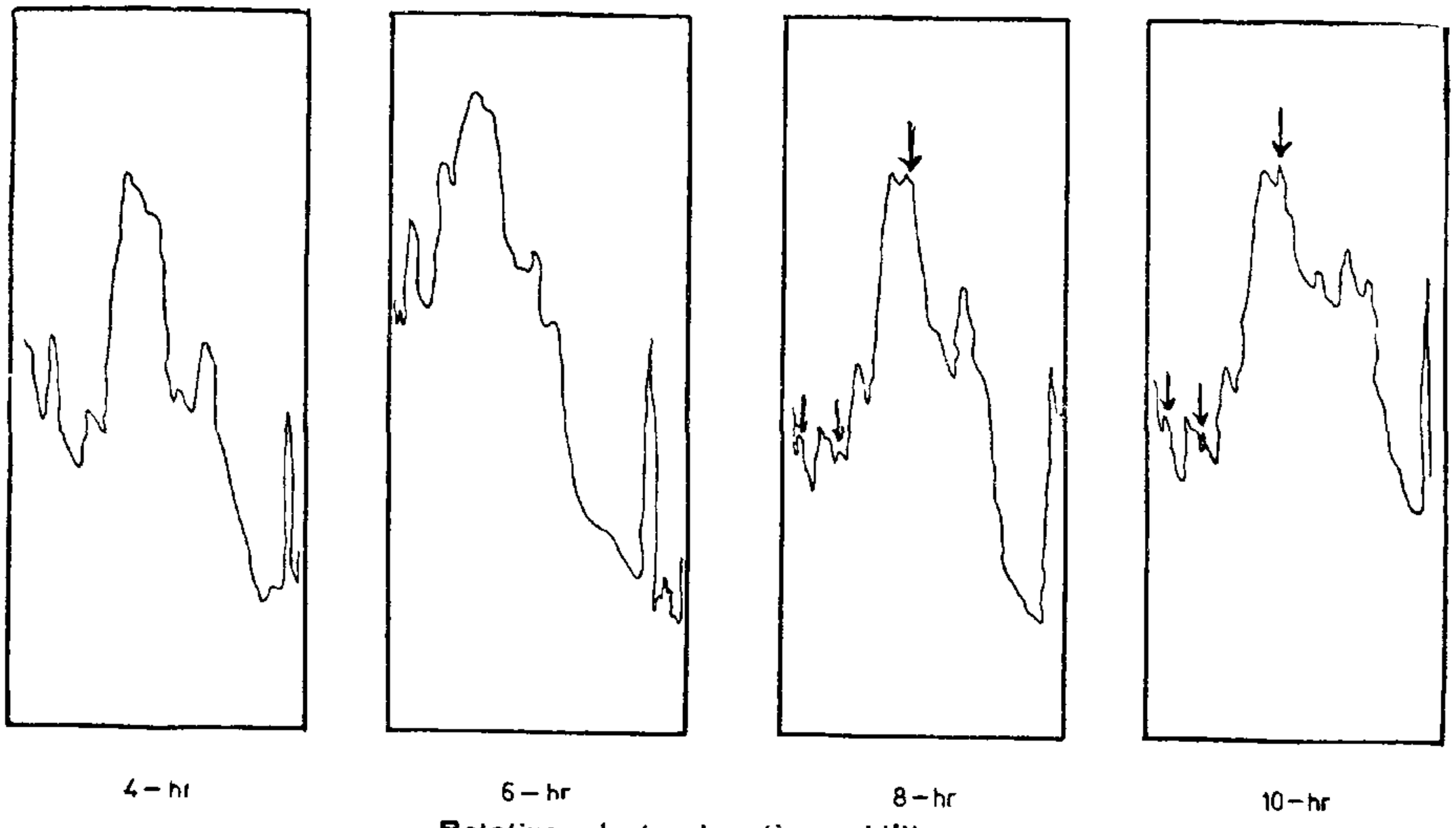

FIG. 2

The protein fraction from high-speed (165,000 g) supernatant when electrophoresed gave the changing pattern of proteins during germination and the results are indicated in the densitometer tracings

axes were also subjected to electrophoresis. The separation patterns (Fig. 3) are modified in germinated axes. The difference between dry and germinated axes is clear, as seen in the diagram.

Discussion

There is relative increase in the synthesis of protein in both mungbean and runner bean axes as proved by ^{14}C-leucine incorporation. The protein breakdown and resynthesis occur during early germination period; this is in agreement with the electrophoretic separation of proteins (Figs. 2 and 3). Protein synthesis is initiated after 4 hr in mungbean and even from the first hour in runner bean axes. Since the leucine incorporation is inhibited by cycloheximide, it is evident that *de novo* protein synthesis occurs even from the first hour of germination in runner bean. In barley seeds[6] also, protein synthesis starts even by the first hour of germination.

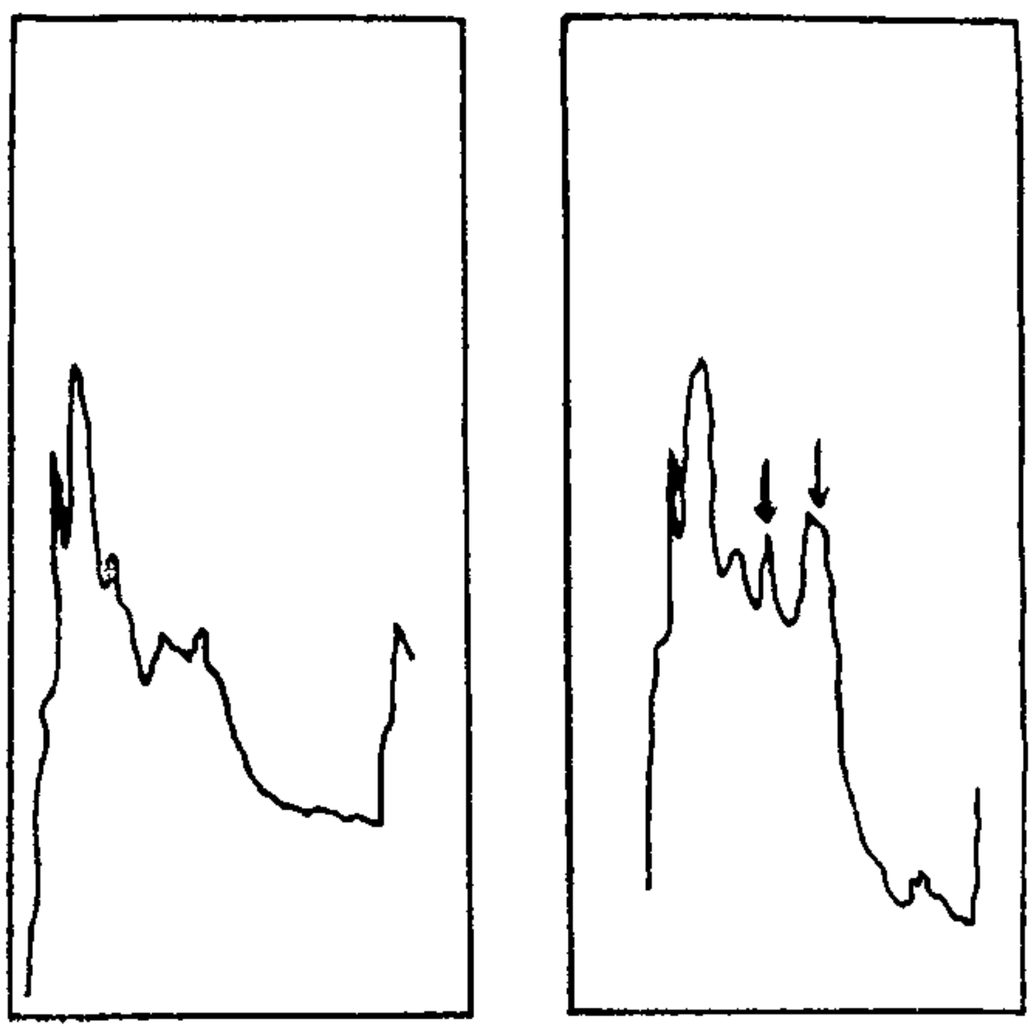

FIG. 3

The fact that protein synthesis is observed at very early stages of germination, points out that the synthesis may be directed by a preformed messenger as reported for cotton embryos[7]. It is also probable that imbibition triggers the protein synthesising machinery by the formation of polysomes which are required for protein synthesis[1]. Levinthal *et al.*[8] reported that synthesis of early proteins is dependent on conserved messenger in *Bacillus subtilis* spores.

The formation of polyribosomes and the consequent activation of protein synthesising capacity, immediately after imbibition during early developmental stages in wheat embryos[9], provide evidence for protein formation. Hence, it can normally be expected that during early germination, varied changes in proteins do take place. The results presented in Figs. 2 and 3 reveal that spectra of protein are altered during germination of embryonic axes. Since the axes are growing fast and metabolically very active, synthesis of proteins may occur as evidenced by the electrophoretograms. The formation of additional bands and modified bands may be due to new synthesis. It is also probable that the storage proteins are degraded and give rise to a different protein pattern. Further, proteins present in ungerminated axes may be transformed and lead to the formation of new bands. However, in contrast to the present results, Bhatia and Nilson[2] reported in wheat seeds, that no difference could be observed in protein separation patterns before 48 hr of germination. In *Phaseolus vulgaris* seeds[10], the number of bands of albumin disappears during early stages of germination and several new components are formed in later germination period.

It was observed that different classes of proteins synthesised could reflect the time of transcription of the corresponding portions of the genome. Though there appears to be a definite variation in protein separation patterns, identification of individual protein components is, at best, very uncertain. In addition, in the absence of the genetic data, the appearance and disappearance of particular bands cannot be ascribed to gene function which modifies secondarily in each tissue to meet specialized requirements for growth and development, as reported by Bhatia and Nilson[2].

Acknowledgement

The author thanks Prof. A. R. Carlier, Laboratory of Plant Biochemistry, Carnoy Institute, Louvain, Belgium, for providing laboratory facilities.

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