the dissociation of the –COOH group of the spore surface decreases. Below pH 2, which corresponds roughly to the isoelectric point of the basic and acidic groups of the spore surface[10], the electric charge is presumably reversed.

The exposure of spores to a low pH, even if for a short time, however, could produce changes of the spore surface other than changes of the electric behaviour. To test this possibility, an experiment was performed, the results of which are shown in Figure 2. Heat-shocked spores were divided into 3 aliquots. The first was incubated at pH 7.3 with pyruvate for 30 min, the second preincubated at pH 1.1 at 37°C for 5 min and than washed and incubated at pH 7 with pyruvate for 30 min; the third was incubated at pH 1.1 at 37°C with pyruvate for 5 min.

The results clearly demonstrate that the exposure for 5 min of spores at pH 1.1 does not produce any change in the permeability behaviour of spores to pyruvate at pH 7.3, and therefore it can be assumed that only reversible changes in the electric behaviour of the spore surface and the pyruvate molecule are responsible for the large keto-acid uptake at pH 1.1.

Discussion. The results reported in this paper strongly support the view that pyruvate does not induce germination under the usual experimental conditions of spore germination (neutral pH) because it cannot penetrate the cell in an amount sufficient to activate the dormant enzymatic systems ('substrate' hypothesis). The difference in germination-inducing capacities of alanine and pyruvate at neutral pH are not to be interpreted, therefore, as a consequence of inability of the spores to metabolize pyruvate, but of the impossibility of an actual contact between the enzymatic systems *inside the cell* and pyruvate *outside the cell.*

When pyruvate is allowed to come inside the cell through an artifice such as a short preincubation with the keto-acid at low pH, or by using alanine which easily penetrates and causes formation of pyruvate inside the cell, germination of the spore occurs.

Riassunto. È stata studiata la permeabilità delle spore batteriche al piruvato. Il piruvato penetra nelle spore soltanto a pH basso, quando la molecola del chetoacido ed i gruppi polari della superficie delle spore sono largamente indissociati. Le modificazioni della superficie, che si verificano nel corso della esposizione a pH acido, non hanno alcuna importanza nel fenomeno. Questi risultati sono messi in relazione con il fatto che il piruvato induce germinazione delle spore soltanto a pH basso e contribuiscono all'ipotesi che un agente germinante, per essere attivo, deve penetrare nella spora ed attivare i sistemi enzimatici quiescenti.

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The Distribution of Nutrients in the Seed Parts of Bengal Gram

In India, Bengal gram (*Cicer arietinum* L.) constitutes a major pulse crop and over 5 million tons are produced annually. Besides other nutrients, it serves as a good source of cheap protein. It is consumed in different forms, generally involving removal of certain component parts, the seed coat and embryo[1].

Investigations on the chemical composition of different anatomical parts of some cereal grains[2–7] have shown that the whole seed is quite an inhomogenous entity and that the various parts differ in respect of their chemical composition. Similar information in respect of Bengal gram, or as a matter of fact of any dicot seed, is not available except for the distribution of nitrogen in the seed parts of ripening pea (*Pisum sativum*)[8].

Seeds of Bengal gram, variety Pb7 were soaked, overnight, in glass distilled water just sufficient to be absorbed completely by the grains. The separation of the seeds into three components, the seed coat, cotyledons, and embryo, was carried out by hand. The components were then dried, weighed and powdered in a coffee grinder to pass a 36 mesh sieve. For chemical analysis, A.O.A.C.[9] methods were employed. The carbohydrate content was, however, obtained by difference.

Analysis for a few constituents of the cotyledons and the seed coat separated mechanically, without soaking the seeds, showed that soaking did not bring about any significant migration of the nutrients. The chemical composition of the different component parts is shown in the Table.

It may be seen from the Table that, although, the embryo is the richest part of the whole seed, it, being smallest

Chemical composition of the component parts of Bengal gram

Constituents	Seed coat	Embryo		Whole seed	
		Cotyledons	Rest of the embryo	Determined	Calculated [a]
Proportion (%)	14.55	84.21	1.22		
Protein (%) (N ×6.25)	2.86	25.00	36.99	21.06	21.92
Ether Extractives (%)	0.22	5.32	13.08	4.73	4.67
Ash (%)	2.82	2.56	5.14	2.74	2.63
Crude Fibre (%)	48.25	1.00	3.10	8.90	7.96
Carbohydrates (%) (by difference)	45.85	66.12	41.69	62.57	62.82
Phosphorus (mg/100 g)	23.0	290.0	744.0	272.0	256.6
Iron (mg/100 g)	7.5	5.1	11.0	5.6	5.5
Calcium (mg/100 g)	1000.0	70.0	110.0	200.0	205.8

[a] Calculated from the content of the different seed parts.

[1] Although botanically the two cotyledons in *Cicer* are part of the whole embryo, in the present paper, where ever embryo has been used it should be taken to mean the rest of the embryo, i.e. the whole embryo minus the cotyledons.

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in proportion, accounts for only an insignificant part of the total value of the seed. The seed coat, however, being fairly large in proportion is extremely low in most of the constituents, except calcium, and would, therefore, contribute very little to the nutritional value of the seed. On the other hand, the cotyledons, being the principal component and fairly well balanced in their chemical composition, account for almost the entire food value of the seed. Thus, in view of the above results, the seed coat and the embryo, if removed, during milling, would not appreciably lower the food value of Bengal gram.

Zusammenfassung. Die bengalische «Gram»-Pflanze (*Cicer arietinum* L.) ist in Samenschale, Cotyledonen und Restteile des Embryos zerlegt worden. In diesen Fragmenten wurde die Verteilung von Protein, Extraktstoffen, Asche, Faseranteilen sowie Calzium, Phosphor und Eisen näher untersucht. In den relativ massiven Samenschalen fand sich auffallend viel Calcium, während die übrigen untersuchten Stoffe in den andern Pflanzenteilen (mit Ausnahme vom Faseranteil) nur geringfügig vorhanden waren. Die Cotyledonen, die mehr als 85% dieses Getreides ausmachen, zeigen chemisch ausgeglichene Verhältnisse.

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Paper Chromatographic Study of the Role of Sulphur-Containing Amino Acids in the Process of Induction in the Chick Embryo

Brachet[1] propounded a hypothesis that -SH containing ribonucleoproteins are important in the phenomenon of inducation. Chloroacetophenone (CAP), which is a specific and irreversible inhibitor of the -SH groups (Beatty[2]) was found to produce malformations predominantly in the brain region and also cause a fall in the induction capacity of the Hensen's node as the concentration was increased (Lakshmi[3,4]). It was felt desirable to know which of the sulphur-containing amino acids were affected, and to what extent, by the said chemical. Therefore a chromatographic analysis was undertaken.

Materials and Methods. The control and experimental samples for the analysis were prepared as follows: Chick embryos at the primitive-streak stage, explanted *in vitro* by the technique of New[5], were treated with 0.0005*M* and 0.0015*M* CAP solutions and the corresponding control solutions for 15 min in the manner described in the earlier work (Lakshmi[3,4]). The organizer region (anterior one-third of the primitive-streak) of these control and experimental embryos was excised and suspended in acetone (Durrum, Block, and Zweig[6]). In a large number of such experiments (75 embryos/sample), sufficient protein material (dry weight: control 1.25 mg, experimental 2.3 mg for the first concentration and 1.5 mg and 2.85 mg in the control and experimental for the second) was obtained. The samples were evaporated and hydrolysed under vacuum by 1:1 by volume of 6*N* HCl and 90% formic acid at 110° C for 24 h (Durrum et al.[6]). The hydrolysates were evaporated, washed repeatedly in distilled water and dried *in vacuo*. The residues were dissolved separately in 500 µl of 10% isopropyl alcohol and spotted on Whatman No. 1 filter paper. Standard amino acids (B.D.H. and E. Mercks products) were also spotted for guidance. In each chromatogram 100 µl of the samples and 100 µl (100 µg) of the standard amino acids were applied. The spotted papers were run in Butanol: acetic acid: water (4:1:5) descending system (Alexander and Block[7]) till the solvent dripped off the paper, dried and developed with platinic iodide (Winegard et al.[8]), which is specific for sulphur-containing amino acids.

Experimental Results. In the chromatograms of both control and experimental samples, we could not detect cystine or cysteine, whereas methionine and glutathione were very clearly detected. It was noticed that the areas of the bleached portions pertaining to methionine and glutathione differed considerably between the controls and the experimentals. Therefore an estimation was done based on the method of Fisher et al.[9,10], according to which

$$A/\log a = B/\log b = K \text{ (constant)}$$

where A is the area of the standard spot, a the concentration of the standard aminoacid and B is the area of the amino acid whose concentration (b) is to be determined. Accordingly the areas of the control and experimental methionine and glutathione spots were accurately measured and the concentrations were calculated by applying the above formula. The control sample was found to contain 2.566 µg of methionine/1000 µg of the protein. The experimental sample contained 0.7907 µg of methionine/1000 µg of the protein. The amount of glutathione in the control sample was 15.98 µg/1000 µg of the protein whereas that in the experimental sample was 0.8283 µg/1000 µg of the protein. It would seem that both methionine and glutathione are affected by the lower concentration (0.0005*M*) of CAP. It is obvious, however, that glutathione is affected to a much larger extent than methionine. Methionine content of the lower concentration control sample and the higher concentration (0.0015*M* CAP) control sample did not seem to differ much. The difference between methionine content of the control and experimental samples was almost maintained even after the increase of the CAP concentration. Glutathione, though it could be detected in the control sample of the higher concentration, could not be detected at all in the experimental sample.

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