

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

ten wurde die Verteilung von Protein, Extraktstoffen, Asche, Faseranteilen sowie Calcium, 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

BRACHET¹ propounded a hypothesis that -SH containing ribonucleoproteins are important in the phenomenon of induction. Chloroacetophenone (CAP), which is a specific and irreversible inhibitor of the -SH groups (BEATTY²) 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 NEW⁵, were treated with 0.0005M and 0.0015M 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 ZWEIG⁶). 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 6N 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 BLOCK⁷) till the solvent dripped off the paper, dried and developed with platinum 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 amino acid 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 (0.0005M) 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 (0.0015M 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.

¹ J. BRACHET, *Chemical Embryology* (Interscience Publishers Inc. 1950).

² R. A. BEATTY, *Proc. Roy. Soc. B* 138, 575 (1951).

³ M. S. LAKSHMI, *J. Embryol. exp. Morph.* 10, 373 (1962).

⁴ M. S. LAKSHMI, *J. Embryol. exp. Morph.* 10, 383 (1962).

⁵ D. A. T. NEW, *J. Embryol. exp. Morph.* 3, 326 (1955).

⁶ E. L. DURRUM, R. J. BLOCK, and G. ZWEIG, *Paper Chromatography and Paper Electrophoresis* (Academic Press Inc., New York (1958)).

⁷ P. ALEXANDER and R. J. BLOCK, *Laboratory Manual of Analytical Methods of Protein Chemistry* (Pergamon Press, 1960).

⁸ H. M. WINEGARD, G. TOENNIES, and R. J. BLOCK, *Science* 108, 506 (1948).

⁹ R. B. FISHER, D. S. PARSON, and G. A. MORRISON, *Nature* 161, 764 (1948).

¹⁰ R. B. FISHER, D. S. PARSON, and R. HOLMES, *Nature* 164, 183 (1949).

The observation that glutathione, which contains -SH groups, is affected by CAP is of significance. It is conceivable that the reduction of glutathione observed might have been reflected in the fall of induction capacity of the Hensen's node on treatment with chloroacetophenone¹¹.

Zusammenfassung. Die Organisatorregion von Hühnchenembryonen wurde mit CAP behandelt und die Wirkung des CAP auf schwefelhaltige Aminosäuren papierchromatographisch untersucht. Die Analyse ergab, dass Glutathion stärker beeinflusst wurde als Methionin. Die Reduktion des Glutathiongehaltes könnte dem Abfall der Induktionskapazität des Hensenschen Knotens entspre-

chen, der durch die Behandlung mit CAP hervorgerufen wird.

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Secretion of Saliva in the Rabbit after Postganglionic Parasympathetic Denervation

One to three days after postganglionic parasympathetic denervation the parotid gland of the cat shows a secretory activity occurring in bursts and assumed to be due to a paroxysmal release of acetylcholine from the degenerating nerve-endings¹. A similar, although more continuous, 'degeneration secretion' has been observed in submaxillary and sublingual glands of cats^{2,3} and dogs⁴.

In the present experiments the effect of unilateral postganglionic parasympathetic denervation of the submaxillary and parotid glands was studied in rabbits. As in cats³ the chorda tympani was dissected along the submaxillary duct and cut as close to the gland as possible. The parotid gland was denervated by section of the auriculotemporal nerve, which contains its parasympathetic secretory fibres⁵. Morphine-urethane was used for the former, ether anaesthesia for the latter operation.

One to five days later acute experiments in urethane anaesthesia were carried out. The parotid ducts were exposed and cannulated; the submaxillary ducts were can-

nulated through the mouth. The cannulae used gave about 50 drops out of 1 ml of distilled water.

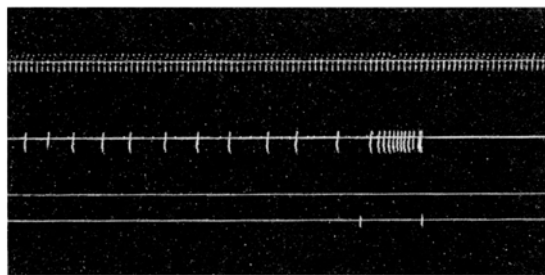
One to three days after section of the auriculotemporal nerve the parotid gland of the rabbit showed a 'degeneration secretion'; the flow was particularly marked on the first two days, when drops of saliva could fall with intervals of 3–5 min. The secretion appeared in paroxysms with intervals of 1–2 min. It was increased by eserine and abolished by Hoechst 9980 ($\alpha\alpha$ -diphenyl- γ -piperidino-butylamide) as shown in the Figure, and must therefore be assumed to be caused by acetylcholine.

Contrary to the parotid gland, the submaxillary gland of the rabbit is normally in a permanent state of spontaneous activity⁶. One to three days after the operation a 'degeneration secretion' could be observed in the submaxillary gland, superimposed on the slow spontaneous flow. During the first two days, when the flow was particularly pronounced, drops of saliva fell every 4–6 min from the denervated gland whereas the contralateral, normal gland secreted about one drop per h. The 'degeneration secretion' was much more regular in the submaxillary than in the parotid glands; only in some few rabbits there was a tendency to paroxysmal flow from the submaxillary glands. Eserine augmented the flow and Hoechst 9980 reduced it to the level seen in the normal gland.

Zusammenfassung. Eine Degenerationssekretion erscheint beim Kaninchen in den ersten drei Tagen nach postganglionär-parasympathischer Denervierung der Submaxillaris- und Parotisdrüsen.

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'Degeneration secretion' from left parotid gland of the rabbit. Tracings from above: time in min; drops of saliva from left denervated gland; right control gland; signal. Postganglionic, parasympathetic denervation of left gland 24 h before the experiment. At first signal 100 μ g eserine/kg, at second signal 100 μ g Hoechst 9980/kg was given intravenously.

¹ N. EMMELIN and B. C. R. STRÖMBLAD, *J. Physiol.* **143**, 506 (1958).

² N. EMMELIN, *J. Physiol.* **154**, 1-2P (1960).

³ N. EMMELIN, *J. Physiol.* **162**, 270 (1962).

⁴ D. A. COATS and N. EMMELIN, *Exper.* **18**, 177 (1962).

⁵ I. NORDENFELT and P. OHLIN, not published.

⁶ I. NORDENFELT and P. OHLIN, *Acta physiol. scand.* **41**, 12 (1957).

The 5-Hydroxytryptamine Content of the Brain and Some Other Organs of the Hedgehog (*Eri-naceus europaeus*) During Activity and Hibernation

HESS¹ compares hibernation with the state of sleep and considers both these conditions to be dependent upon an

autonomic central regulation. According to this opinion the functional balance in both these states is shifted towards a trophotropic predominance. SUOMALAINEN² considers that sympathetic hypofunction is essential to hiber-

¹ W. R. HESS, *Z. vgl. Physiol.* **26**, 529 (1939).

² P. SUOMALAINEN, *Biochem. Z.* **295**, 145 (1938).