chandran of the C.B.R.I. for the help rendered during the laboratory investigation. The junior author (N. G. K. N.) is indebted to the C.S.I.R. for financial assistance.

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A PAPER CHROMATOGRAPHIC ANALYSIS OF THE ENZYMATIC ACTIVITY *IN VITRO*

IV. Isolation, Identification and Estimation of Glutamic Acid as a Product of Liver Histidase Activity

HISTIDINE when reacted with histidase is believed to yield ammonia, formic acid and glutamic acid. However, Walker and Schmidt[6] suggested that the end product of this reaction was α-formamidino glutaric acid. Tauber[5] states that the end products could not be isolated. The present paper-chromatographic study, using the modified technique,[3] aims at isolation, identification and if possible estimation of these end products.

The enzyme histidase was prepared by grinding rat liver tissue with phosphate buffer of pH 8·2, and 1% l-histidine solution in glass-distilled water. The control and experimental tubes were prepared and incubated for 3 hours at 38° C. as in our previous study.[4]

Horizontal circular paper chromatography after the method of Giri and Rao[2] was done. The chromatogram was divided into six sectors (Fig. 1) and spots (0·005 ml.) were placed from control and experimental tubes. Sectors 1, 3 and 4 were spotted with 0·005 ml. from the experimental tube at 0, 2 and 3 hours of incubation periods respectively. The spot of sector 2 consisted of 0·005 ml. of glutamic acid of known strength (0·005 mg./0·01 ml.) besides the contents of control tube, the sectors 5 and 6 were spotted with identical quantities and strengths of glutamic acid and threonine, the two overlapping amino-acids.

The chromatogram thus prepared was run for 10-12 hours using *n*-butanol : acetic acid : water (4 : 1 : 5) as the solvent. After drying, the sectors 2 and 5 were partially cut away from the neighbouring sectors and stained with 0·5% ninhydrine in 95% acetone to serve as guide sectors for marking the position of glutamic acid bands on the untreated sectors. The corresponding unstained portions of sectors 1, 3, 4 and 6 were cut out and used as wicks for the separation of glutamic acid and other overlapping amino-acids, if any, by the modified technique (*loc. cit.*). The proximal parts of the sectors 1, 3 and 4 were then stained with Pauly's reagent, specific for histidine, to confirm the complete breakdown of histidine by 2 hours of incubation with the enzyme. The unstained cut-out bands were stitched to the one inch wide strip of chromatography paper which could be fastened to the frame even by cotton threads instead of using silver wire springs mentioned in the modified technique (*loc. cit.*). The sector 6 unstained band served as control wick for the strip chromatograms. These strip chromatograms were run with pyridine : water (8 : 2) as solvent for 8 hours and stained with ninhydrine. The glutamic acid band was clearly identified at 2-hour incubation as seen in Fig. 2. The similarly

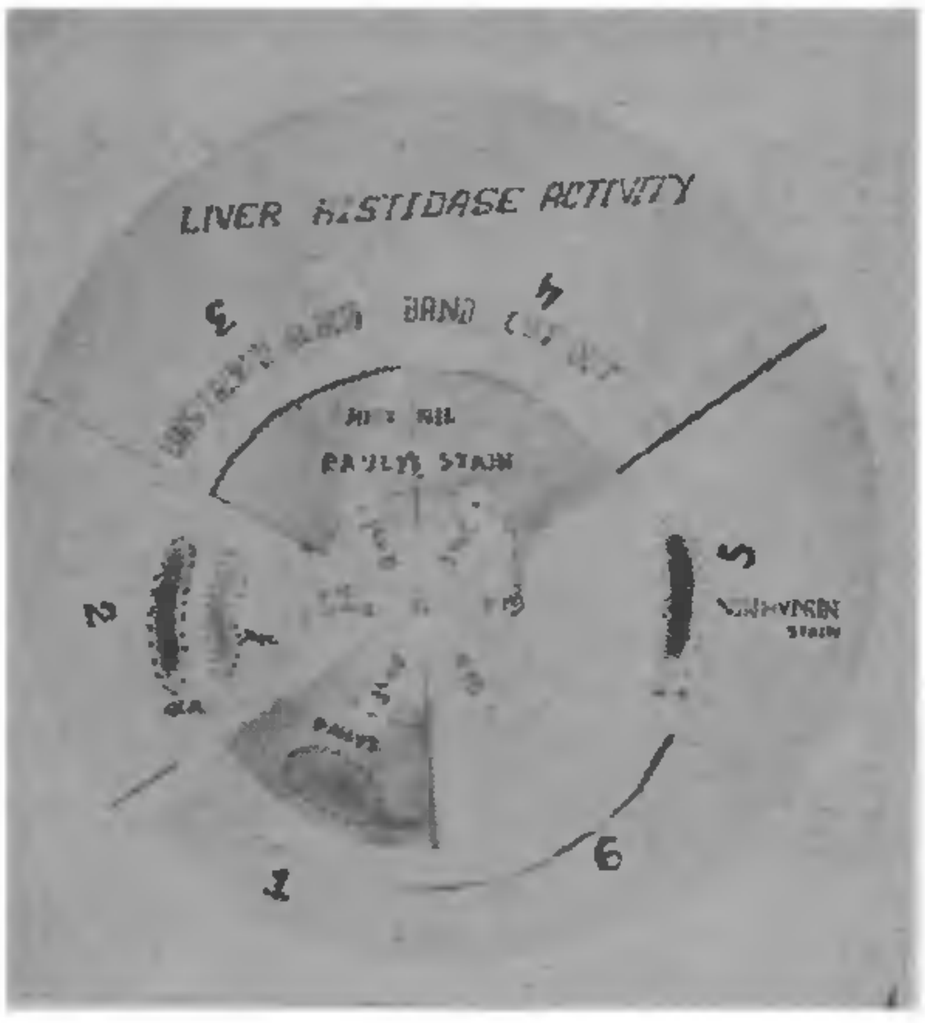

FIG. 1

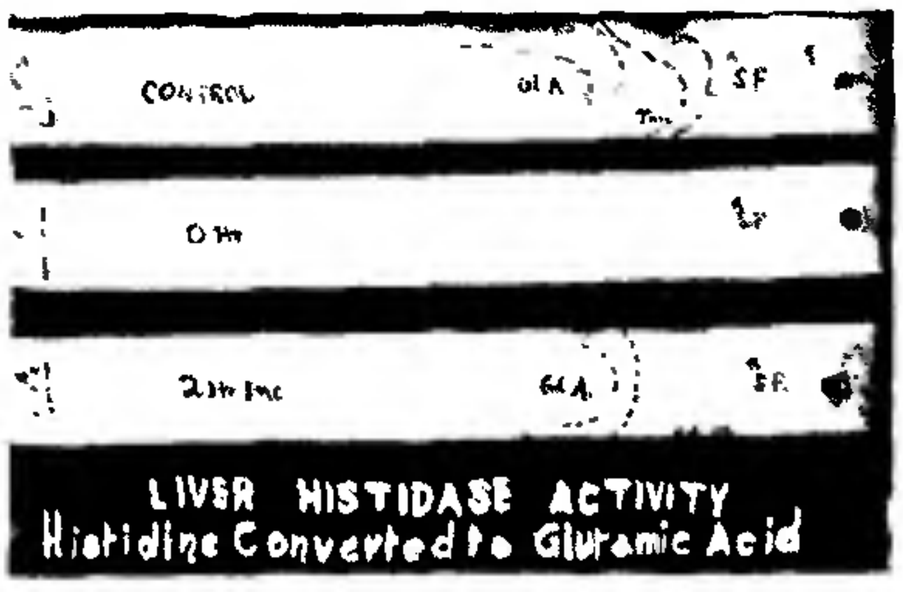

FIG. 2

developed and stained band of glutamic acid after 3 hours of incubation period from sector 4 was cut out and eluted in 4 ml. distilled water in a test-tube immersed in water-bath at 50° C. for 15 minutes. The colour densities of the control glutamic acid and the above strip were measured at 400 mμ wavelength on a Coleman Junior spectrophotometer.

Hence the only chromatographically identifiable band of breakdown products of histidine by histidase is that of the glutamic acid. On calculation it is seen that the glutamic acid obtained from histidine breakdown does not fully account for the mole-per-mole mathematical relationship expressed by Fruton.[1]

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G.R. Medical College, R. K. JAIN.
Gwalior, *March* 21, 1966. I. P. AGARWAL.

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PRESERVATION OF *GNETUM* MATERIAL IN NATURAL COLOUR

PLANT collectors always find it difficult to preserve *Gnetum* specimens in good condition. If they are preserved in the normal FAA solution experience shows that they turn dark soon after bottling and the preservative too gradually becomes a dark decoction.

However, I found the following technique to be sitisfactory for preservation of *Gnetum* material in almost natural colour. Freshly collected specimens of *Gnetum*—thin stems, leaves, cones—are first water-cleaned and then air-dried. The material is then kept in pure glycerine for about three to four days and then is bottled in castor oil after removing the extra glycerine by gently pressing the material between blotters. Material preserved in this way in January 1963 has not so far turned dark. This preserved material can be used for morphological as well as anatomical studies. Hand-cut sections are treated first with petroleum ether to get rid of the oil present. Then the usual technique of double staining with saffranin and light green is followed. It is to be noted that instead of aqueous stain, saffranin in 50% alcohol is to be used. Later steps remain the same.

For microtome sections, pieces of the material preserved by the above technique are kept in petroleum ether for a few hours to remove oil. Then the usual process for preparing paraffin blocks is followed.

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SELECTIVE CONTROL OF *LORANTHUS* ON TEAK

Loranthus spp., the parasitic weed on many horticultural plants, have been observed to be prolifically growing on the valuable timber yielding teak tree (*Tectona grandis*) in several forest ranges of Malabar. Parasitic growth of this weed reduces the timber yield of teak if not kills the tree ultimately. Since mechanical removal of this weed is laborious, time-consuming and uneconomical, preliminary experiments on the selective herbicidal control of *Loranthus* on teak were conducted. Chlorophenoxy, triazine and urea type of hormone herbicidal formulations were employed. However, the chemical 1 : 1-dimethyl 4 : 4-bipyridylium has been observed to be more efficacious and suitable. The critical time for the spray application of this chemical for the herbicidal destruction of *Loranthus* is observed to be early summer when the teak tree is devoid of its foliage. No residual toxicity of the chemical was noticed. It was not absorbed through non-chlorophyllous parts of tree and was washed off by the rain when it got inactivated on contact with soil.

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A CONTRIBUTION TO THE STOMATAL STUDY OF *MARSILEA MINUTA* LINN.

GUPTA[1] investigated the epidermal features of some American Marsileas, which proved to be of some diagnostic value. He has also investigated the epidermis of some Indian species of *Marsilea* including *M. minuta* Linn.[2]

During the course of an ecological and morphological study of *Marsilea minuta* Linn. growing locally, I also investigated the stomatal characters of its three life forms, *viz.*, (1) water form with floating leaves ; (2) water form with immersed leaves ; and (3) terrestrial form inhabiting drier habitat.