blue colour was read at 720 nm. The standard curves of glucose were prepared to estimate the cellulase activity.

A close correlation was obtained between the enzyme production and the aggressiveness of the isolates (table 1) as it has been previously reported in *R. solani*[4] and in *Verticillium*[5]. The agressive isolate R_5 has interestingly recorded a maximum activity on day 4 which followed a sudden decrease in the latter periods of incubation. Whereas the less aggressive isolate R_1 showed a slight increase in activity, only after prolonged period of incubation. That the increased production of cellulase only by the agressive isolate further substantitates the earlier work of Weinhold and Motta[6] in cotton, where it has been showed that the cell wall degrading enzymes are responsible for the host damage, even before the symptoms are apparent. A thorough investigation of isoenzymes present both in the culture filtrates and in host tissues infected with aggressive and less agressive isolates will throw more light on the nature of enzymes involved during early pathogenesis.

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CYTOKININ GLUCOSIDE ACTIVITY IN GUAVA (*PSIDIUM GUAJAVA* L.) SEEDS

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CYTOKININ glucosides have been suggested to be the storage forms and serve to regulate the levels of free forms of cytokinins in the plants[1]. The occurrence of zeatin glucoside in coconut milk[2] and glucosylated forms of zeatin riboside and the dihydrozeatin riboside has been reported as a metabolite of 8 [^{14}C]zeatin in the seedlings of *Phaseolus vulgaris*[3]. In an earlier paper[4] five different cytokinins were detected from immature seeds of guava. While three of them were identified as zeatin, zeatin riboside and zeatin nucleotide, a suggestion was put forward[4] that either of the rest one may be zeatin glucoside. The present paper reports the tentative identification of zeatin glucoside and zeatin riboside glucoside from the immature seeds of guava *Psidium guajava* L.

From about 2000 immature guava fruits (cv. Allahabad safeda) seeds were separated and homogenised twice with chilled 80% methanol. The combined methanolic extract was evaporated to aqueous *in vacuo* below 40°C and from it the fractions A and B were separated as described earlier[4]. Descending paper chromatography of fraction B (ammonia eluate of Zeo-karb column) was performed on Whatman No. 1 paper strip (45 × 10 cm) at 25–26°C using *n*-butanol: acetic acid: water (12:3:5 v/v) solvent

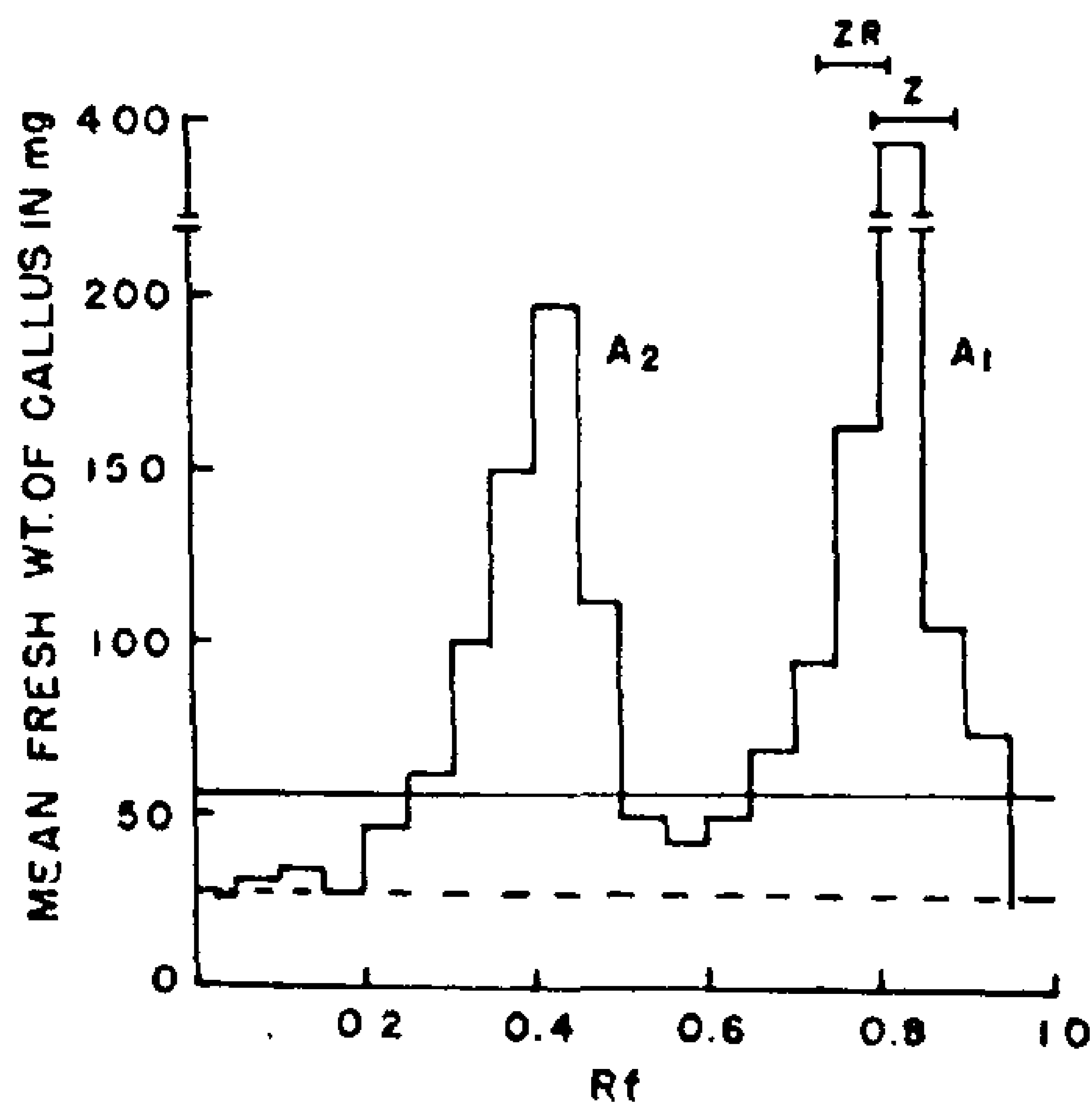

Figure 1. Cytokinin activity as measured by soybean callus test after paper chromatography in *n*-butanol: acetic acid: water solvent system of ammonia fraction obtained by Zeo-karb 225 column chromatography of guava seed extract. Quantity equivalent to 20 g fresh weight of tissue was chromatographed. The broken and solid horizontal lines represent control and least significant differences at 5% level respectively.

system. Sephadex LH-20 column chromatography[5] was carried out using a column (30 x 2 cm) and developing with 35% (v/v) ethanol at a flow rate of 20 ml/hr. Fifteen ml of each of the fractions were collected, dried and bioassayed by employing soybean callus test[6]. To convert the cytokinin glucosides to their respective free forms, β-glucosidase treatment was performed[2].

Preparative paper chromatography of fraction B showed two zones of activity at R_f 0.25–0.50 (A_2) and R_f 0.65–0.95 (A_1) (figure 1). Zeatin and zeatin riboside have already been identified[4] from factor A_1. After preparative paper chromatography, the factor A_2 was separately eluted with 50% ethanol, evaporated to dryness, taken up in 1.5 ml of 35% ethanol and fractionated through Sephadex LH-20 column. Following fractionation, two different zones of cytokinin activity could be evident, one eluting at fraction 4–5 and fraction 6–7 (figure 2). These displayed chromatographic properties indicative of zeatin riboside glucoside and zeatin glucoside[2]. Further evidence for the existence of these compounds were presented when treatment of factor A_2 with β-glucosidase (Sigma from almond, 1 mg enzyme/ml extract in 0.02 M Tris buffer, pH 5.2 for 22 hr at 37°C) prior to fractionation on Sephadex, resulted in the shift of activity from its original elution volume and subsequent detection of two peaks of activity which co-eluted with zeatin riboside and zeatin respectively (figure 3). $KMnO_4$ oxidation[7] in the presence of ethanol of an aliquot of the two peaks shows that about 15% of cytokinin activity still remains in the two fractions. This residual activity could be ascribed to the presence of dihydroderivatives of zeatin which, however, cannot be separated from zeatin by Sephadex LH-20 column chromatography using 35% ethanol as an eluant.

The glucoside of zeatin has been identified as a zeatin metabolite in *Lupin* seedling[8], and in *Phaseolus* leaf cuttings[9] it occurs as a glucoside of dihydrozeatin. The accumulation of cytokinin glucosides appear to be the characteristic feature of mature and ageing leaves of woody as well as herbaceous annuals[10]. These

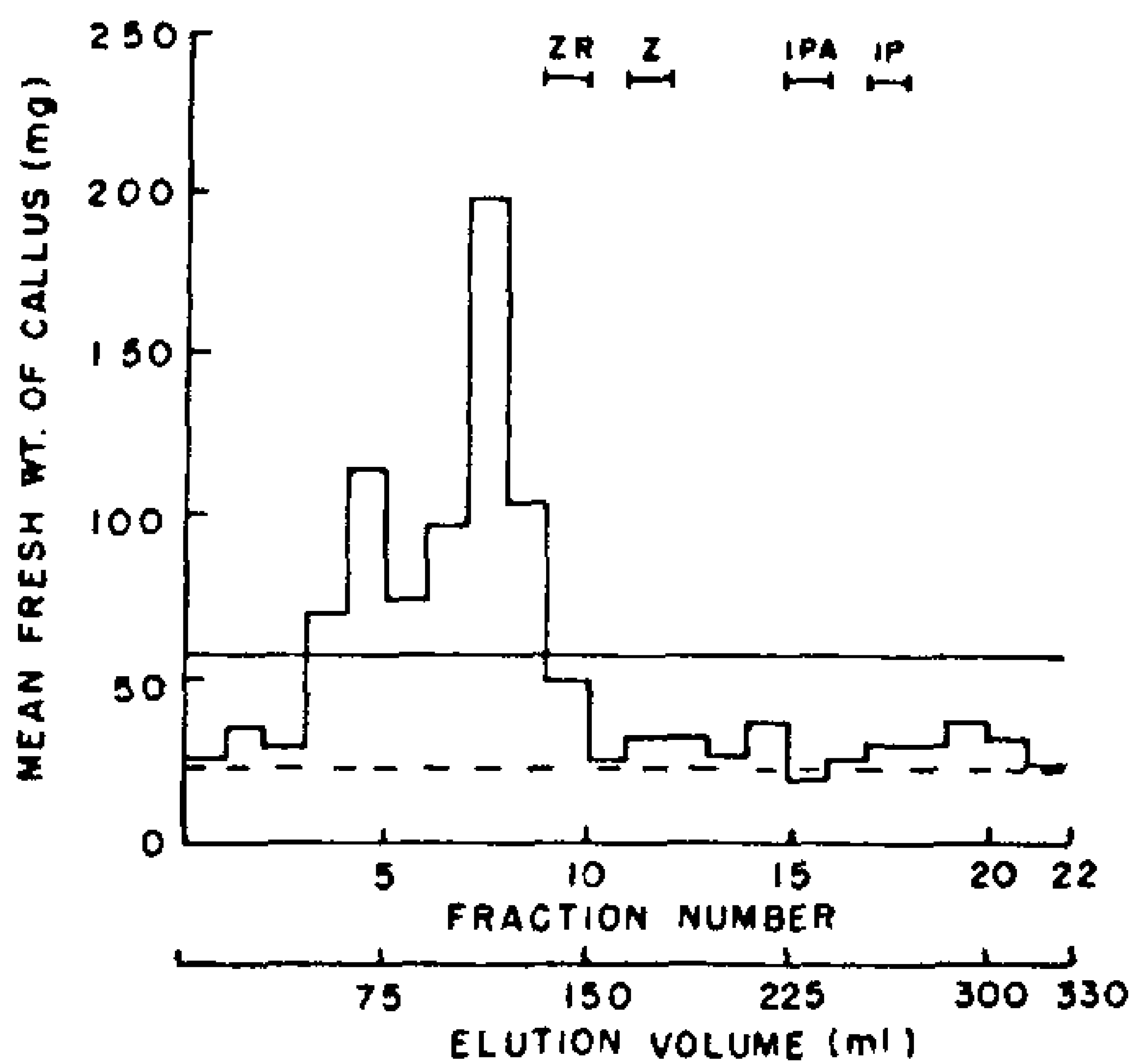

Figure 2. Separation of cytokinin activity by Sephadex LH-20 column chromatography of the active factor A_2 obtained as in figure 1 after preparative paper chromatography. Quantity equivalent to 10 g fresh weight of tissue was chromatographed on Sephadex LH-20 column eluted with 35% ethanol. The elution patterns of zeatin (Z), zeatin riboside (ZR), isopentyladenine (iP), isopentyladenosine (iPA) are shown by horizontal bars over the histogram. The broken and solid horizontal lines represent the same as in figure 1.

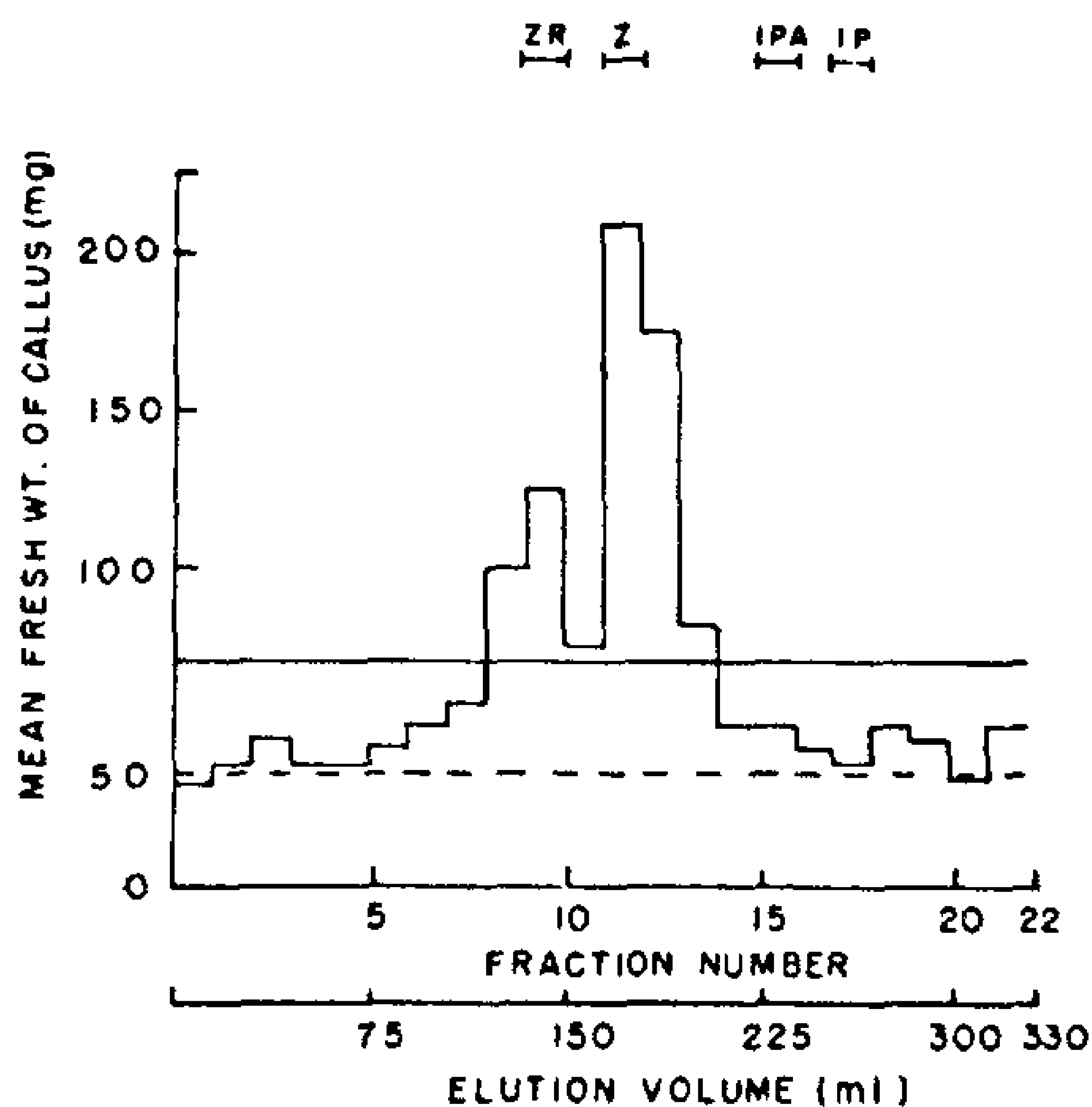

Figure 3. Cytokinin activity detected by soybean callus bioassay of factor A_2 obtained as in figure 1 after β-glucosidase treatment and subsequent fraction on Sephadex LH-20 column eluted with 35% ethanol. The elution patterns of zeatin (Z), zeatin riboside (ZR), isopentyladenine (iP) and isopentyladenosine (iPA) are shown by horizontal bars over the histogram. The broken and solid horizontal lines represent the same as in figure 1.

cytokinins undergo sequestration and fluctuate during the course of leaf/fruit growth. The accumulation of cytokinin glucoside in *Picea* leaves during growing season may be converted to free base forms of zeatin and zeatin riboside when active growth is resumed[11]. It is of interest to note that the unidentified cytokinins of guava fruit[12] undergo such phenomenon and thus it is tempting to investigate the seasonal changes in cytokinin glucosides during fruit growth in guava.

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LEVELS OF ACID AND ALKALINE PHOSPHATASES IN DIFFERENT ORGANS OF UNINFECTED AND INFECTED *CHANNA PUNCTATUS*, FOLLOWING STARVATION.

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THE fish survive starvation effects by readjustment of their metabolic machinery and utilization of internal source of energy (metabolites). Attempts have been made in the past to study the different metabolites[1-4] during the starvation of fish, but relatively little work has been done on the role of enzymes. The present study includes an evaluation of the levels of acid and alkaline phosphatases in the different organs of uninfected and infected *Channa punctatus* following starvation.

C. punctatus, (81) procured from local market during March–April, were maintained in the laboratory under starvation for 35 days, changing the tap water of aquaria daily.

The fish were sacrificed after 0, 7, 15, 21, 28 and 35 days of starvation and the liver, gill, kidney and spleen of the uninfected, and the parasitized fish (infected with *E. heterostomum*), were preserved at 0°C in 0.25 M sucrose solution; 5% homogenate of liver and gill and 2% homogenate of kidney and spleen were used. All homogenates were centrifuged at 3000 rpm for 10 min and the supernatants were used as enzyme source. Activities of acid and alkaline phosphatases were determined by standard methods[5]. The results were statistically analysed using t test.

The levels of acid and alkaline phosphatase activities in different organs of starved uninfected and infected *C. punctatus* have been given in figures 1 and 2.

In the uninfected fish, acid phosphatase activity increase in liver, kidney, gill and spleen following starvation; this increase was statistically significant in all stages of sacrifice in all the tissues except in liver and gill on the 15th day; the levels of significance are different in all the four organs. Alkaline phosphatase activity, on the other hand, is significantly higher in gill alone on the 15th, 21st and 28th days of starvation.

In infected fish acid phosphatase activity increased, following starvation; in liver, gill and kidney, this increase was found statistically significant in the liver (in all stages), kidney (in all stages except on 28th day) and gill (on the 15th and 21st days). Alkaline phos-