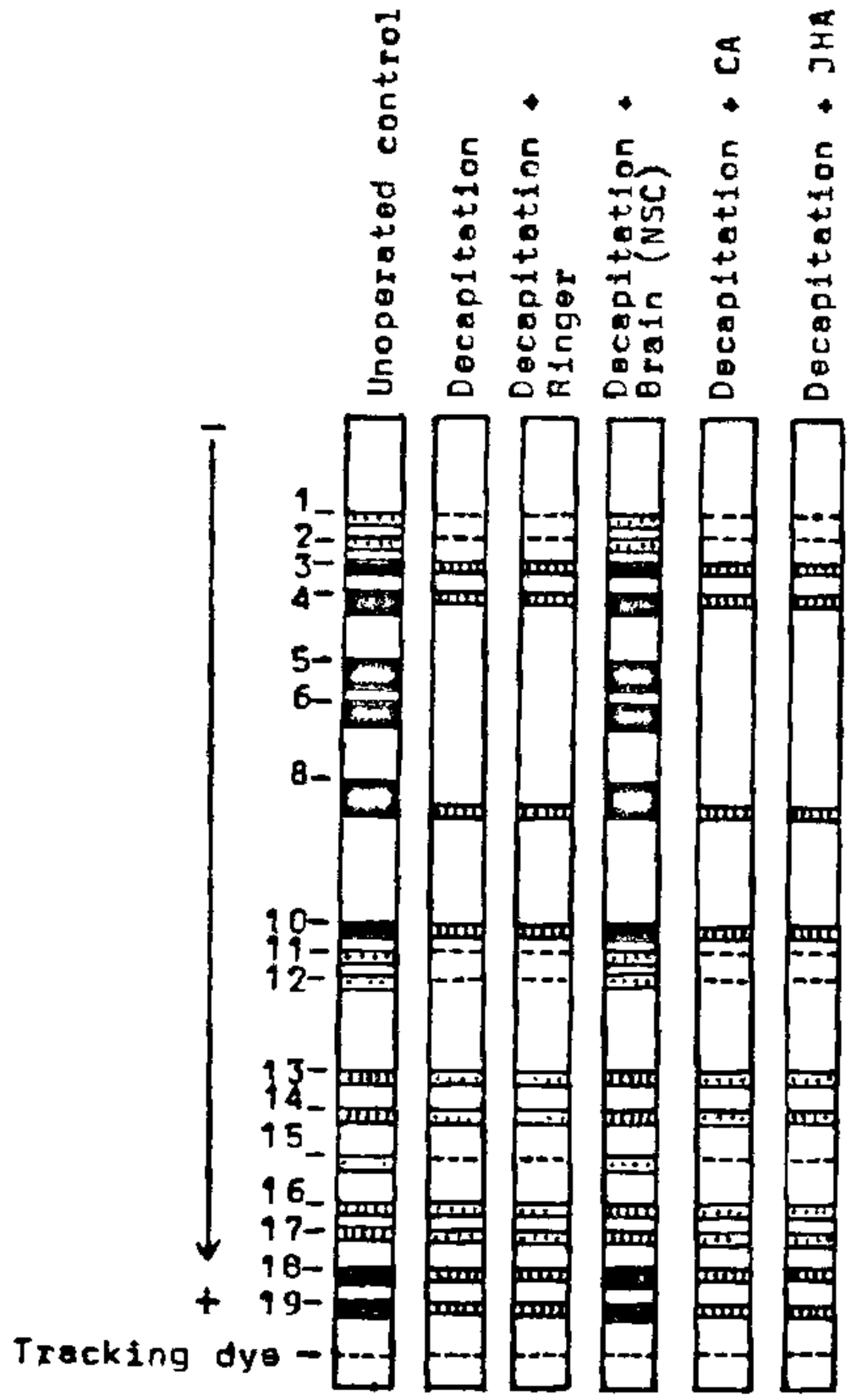

Figure 2. Effect of decapitation and CA and NSC-implantation on VG synthesis.

with NSC (figure 2). On the other hand, implantation of CA or treatment with JHA fails to produce any change in the haemolymph protein pattern. These results indicate that the tissue responsible for VG synthesis in the present insect is NSC and not the CA. Decapitated pupae without any treatment failed to produce VG in the haemolymph (table 1, figure 2). But when such pupae are implanted with NSC, they can synthesize the VG which subsequently appear in the haemolymph. During decapitation a part of CA can remain which could initiate VG synthesis. This possbility is however ruled out because decapitated pupae implanted with CA or treated with JHA failed to show the presence of VG in the haemolymph (figure 2).

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ENCYSTATION OF AXENIC *ENTAMOEBA HISTOLYTICA* AMOEBAE

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CLAIMS have been made regarding encysting axenically growing *Entamoeba histolytica* amoebae. Dobell's[1] encystation of *E. histolytica* growing in bacterial cultures also established the criteria for recognizing encystation if it has taken place. Dobell's description showed that four nucleated cysts of *E. histolytica* also should excyst to yield amoebae lying dormant within the cyst wall. Thus encystation should be followed by excystation to prove that the former had actually taken place and that four nucleated cysts had formed since only these are capable of excystation. A wide variety of factors are known to effect encystation of amoebae[2,3] but their application in encysting *E. histolytica* has not been reported. In the present study encystation of axenically growing *E.*

histolytica has been shown to have been triggered by β nicotinamide adenine dinucleotide (NAD) and thymidine and upon giving the cysts excysting conditions, excystation took place.

E. histolytica amoebae were axenically grown in Diamond[4] TPS1 medium as modified by Imam[5]. The encystation medium consists of autoclaved TPS1 Diamond medium containing 20 mg/100 ml NAD DPN + thymidine (both obtained from Himedia, Bombay) 50 mg/100 ml + 20% serum and 5% vitamin mixture and the pH was adjusted to 7.2. The amoebae (3–4 million) are incubated in this medium for 24 h at 33°C when the medium is again changed. The cysts were harvested after 48 h.

Encystation usually took 24 h to complete and the encystation medium was incubated with amoebae of *E. histolytica* for 24 h when the encystation medium was again changed. Thus usually after two changes of encystation medium, every 24 h, the cysts were harvested. The toughness of cyst wall was tested by incubating a sample of cyst in 1.5% HCl for 24 h. If this treatment leaves the cyst shape unaffected, then cysts are used for excystation in normal Diamond TPS1 as modified by Imam. This excysted the cysts and yielded a culture.

Encystation is usually above 80% of amoebae used for encystation. Excystation of cysts of *E. histolytica* has been usually 60–80% when the cysts had not previously been treated with HCl. After HCl treatment the excystment decreased from 15 to 20%.

The concentration of NAD was varied from 100 mg, 50 mg to 20 mg/100 ml. Whilst thymidine was kept constant at 50 mg/100 ml. When NAD was substituted with NADH or NADP, the percentage of encystation decreased and the cyst formed and did not appear to be of the same quality. However the cysts could stand 1.5–2% HCl treatment for 24 h without losing their shape and could excyst after HCl treatment up to 5–10%.

When uridine, guanosine, cytidine and adenosine (obtained from SISCO, Bombay) at 10 mg/100 ml concentration each were added together to TPS1 Diamond medium without RNA and autoclaved, later adding 20% serum and 5% vitamin mixture and distributing the medium in autoclaved screw capped tubes, it was possible to see cysts upon incubation of *E. histolytica* trophozoites for 96 h in this encystment medium.

When NAD was seitz filtered and then added to TPS1 medium, which is autoclaved for sterilization, encystation did not seem to have been effected.

Another set of encystment media was made in which NAD and thymidine were added in the usual concentrations to Diamond TPS1 medium in which the serum content was reduced to 10, 5, and 0%. These were incubated with trophozoites at 32° C. The best encystment results were obtained with encystment medium containing 5% and 0% serum.

When HCl treatment was not given to cysts the cysts were kept in a deep freezer of refrigerator for 14 days, after which period of time they are given excystment medium, it was observed they could excyst and amoebae and empty cysts walls were seen.

Incorporating the NAD DPN + thymidine in Boeck Dhroblav medium is being tried to see if these effectors can encyst *E. histolytica* in bacterial cultures.

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INTERACTION OF Ca^{2+} AND CALMODULIN ANTOGONIST, CPZ ON MITOCHONDRIAL Ca^{2+} ATPase ACTIVITY OF COWPEA LEAF DISCS DURING SENESCENCE

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ACTIVE Ca^{2+} extrusion from a variety of cells is mediated by a Ca^{2+} + Mg^{2+} ATPase. Membrane-bound ion-stimulated ATPase are involved in ion transport of higher plants[1–4]. The Ca^{2+} + Mg^{2+} ATPase requires mediation of calmodulin[5,6] and is known to be inhibited by various phenothiazines and related compounds[7,8]. These compounds interact with calmodulin in Ca^{2+}-dependent manner and do not compete for the Ca^{2+} binding sites of calmodulin[9]. Ca^{2+} stimulation of ATPase was reported by Kawasaki *et al*[10]. The influence of phenothiazines on Ca^{2+} ATPase in animal systems is well-known but studies on Ca^{2+} ATPase in plant tissue as influenced by phenothiazine drugs are scanty. The present study is aimed at understanding the influence of Ca^{2+} and CaM antogonist, chlorpromazine (CPZ), on the