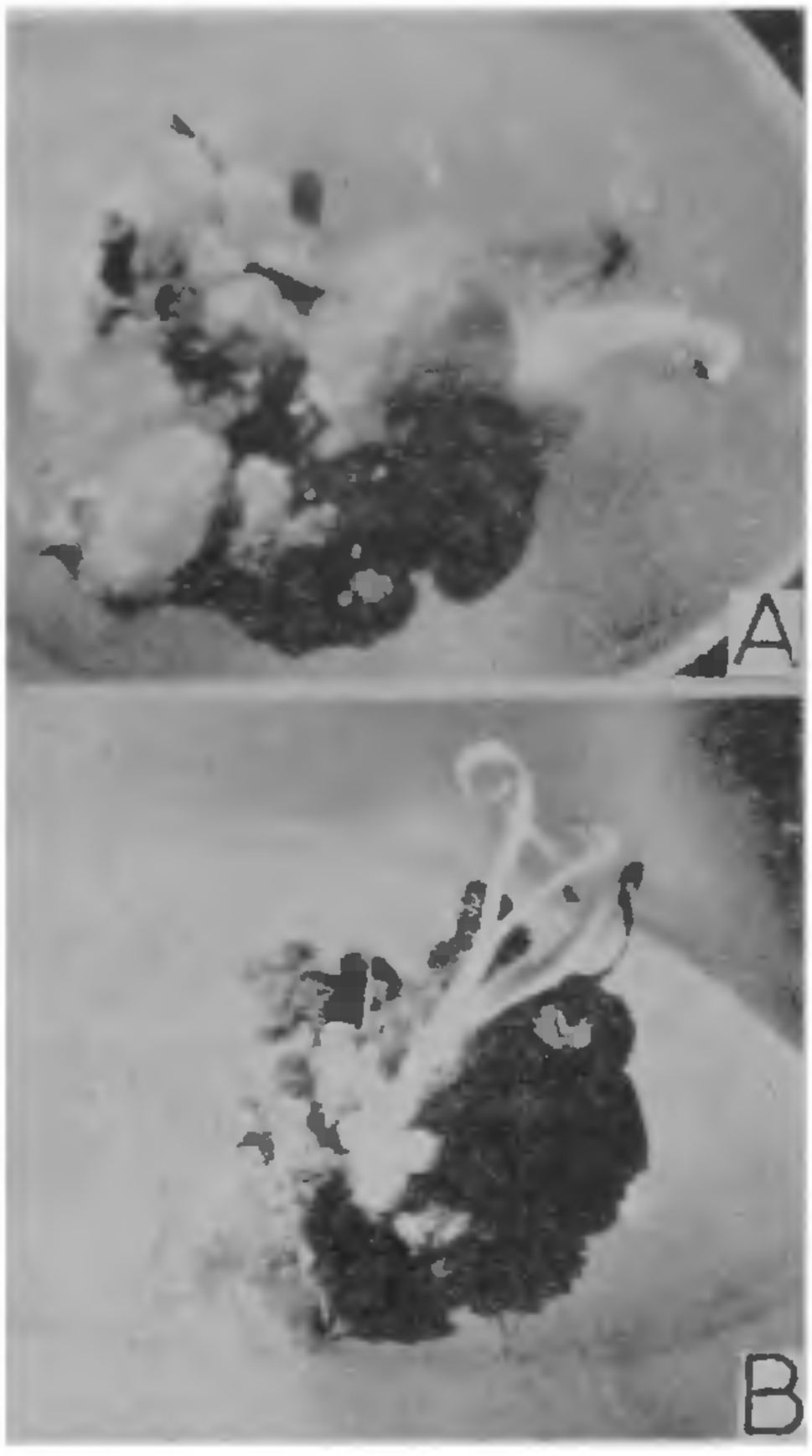

FIG. 1 A–B. A. Callus showing root formation on medium MS + organics and 1 mg/l of NAA. B. Plantlet redifferentiated from the callus showing both roots and shoot.

medium but without auxin and incubated in the light, occasionally redifferentiated to form both roots and shoots (Fig. 1 B), and subsequently the whole plants. Thus it can be concluded that, in *C. glaucus* auxin is one of the most significant key regulators for callus formation and redifferentiation but relatively unaffected by kinetin. Carter *et al.*[1], also reported that oat cultures showed de-differentiation to form large callus at higher concentrations of auxins (225 μmoles/l of 2,4-D and 5700 μmoles/l of IAA) and redifferentiation into large number of shoots and few roots when the callus was transferred to auxinfree medium. Such an auxin-controlled differentiation is also reported in rice, wheat, rye, millets (cited by Yamada[5]) and *Pennisetum mezianum* (unpublished). *C. glaucus*, however, differs from the above cereals in that, redifferentiation is possible only with the fresh callus because the potential for differentiation in *C. glaucus* declined after a few subcultures and could not be enhanced by various auxins and cytokinins supplemented to the medium.

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ALCOHOL DEHYDROGENASE POLYMORPHISM IN *DROSOPHILA ANANASSAE*

Introduction

ALCOHOL dehydrogenase (ADH) is polymorphic[1,2]. It is genetically specified[2]. Variation of ADH activity with different substrates has been studied in *Drosophila melanogaster*[3–6]. The present work reports on the ADH isoenzymes in the adult flies of *Drosophila ananassae* with alteration of the substrates.

Material and Methods

Young adults (3–4 days old) of two inbred geographic strains of *D. ananassae*, namely a_6 and a_{10} were individually homogenized in a glass homogenizer tube (5 ml) containing 0·1 ml of 0·05 M tris-phosphate buffer, pH 9·0 and centrifuged at 14,000 rpm for 10 minutes at 0° C. The supernatant was loaded on the gel. Electrophoresis was performed at 20–24° C for $2\frac{1}{2}$ hours at 25 volts/cm length of the 5% acrylamide gel in 0 05 M tris-phosphate buffer, pH 9·0. ADH activity was recognised by reduced tetrazolium deposition incubated at 37° C for $1\frac{1}{2}$ hours in a solution containing 15 ml 0·05 M tris-phosphate buffer, pH 9·0, 1·5 ml water, 3·8 mg NBT, 1·8 mg PMS, 8·0 mg NAD^+ and 0·2 ml substrate for each gel. We used ethanol, methanol, butanol, *n*-propanol, 2-propanol, octanol, amyl alcohol, allyl alcohol, benzyl alcohol and cyclohexanone as substrates. The scanning of the gels were made using microdensitometer IFO–451 (USSR) by Prof. L. I. Korochkin,

USSR Academy of Sciences, Novosibirsk. NAD^+, NBT, PMS and tris were obtained from Sigma Chemical Company (U.S.A.).

Results

Adult a_6 and a_{10} shows five forms of ADH activity with different substrates. They are represented as ADH_{1-5}. Their relative distances (in mm) with respect to the marker dye are respectively 18, 13, 8, 5 and 3.

Visual estimates as well as densitometric analyses of the stained gels (Figs. 1 and 2) show that adults of a_6 strain produce one isoenzymic form in reaction with ethanol, amyl alcohol, cyclohexanone, allyl alcohol and octanol, two isoenzymes with *n*-propanol 2-propanol and methanol and three isoenzymes with butanol. But in adults of a_{10} strain, while ethanol, benzyl alcohol, octanol, cyclohexanone, methanol and 2-propanol exhibit one isoenzyme of ADH, butanol, amyl alcohol allyl alcohol and *n*-propanol show activity of two isoenzymic forms. Benzyl alcohol could not show any activity of ADH in adults of a_6 strain.

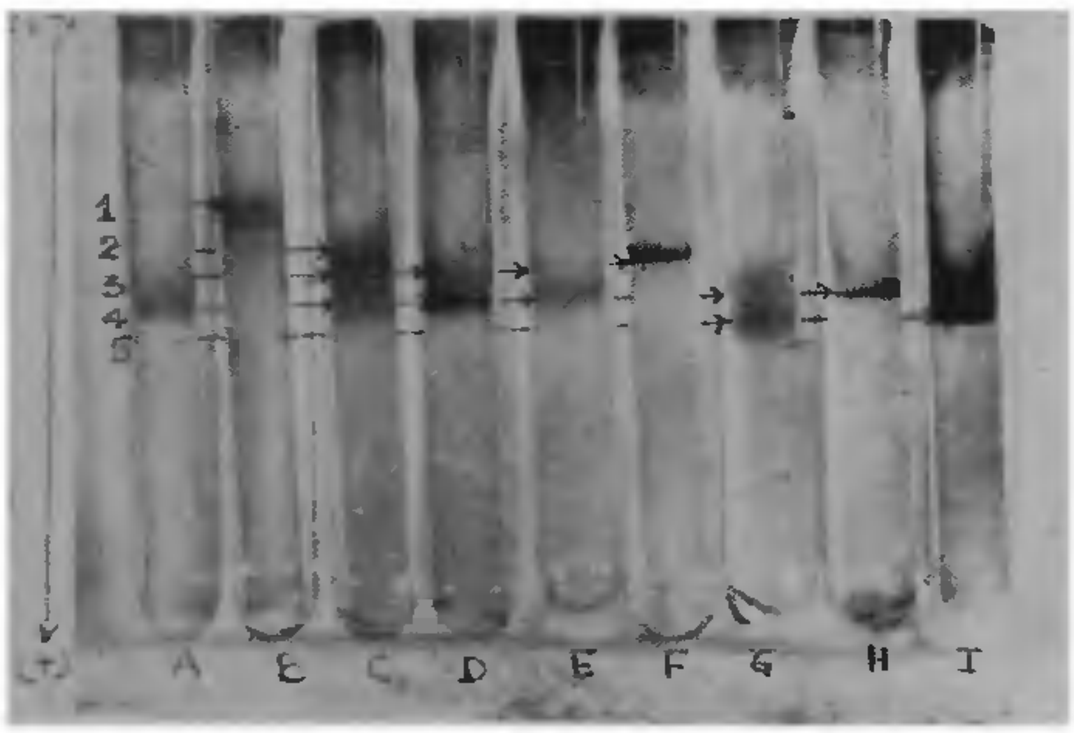

FIG. 1. Substrate specificity of ADH isoenzymes in adult *D. ananassae*. A: amyl alcohol, a_6; B: allyl alcohol, a_6; C: butanol, a_6; D: *n*-propanol, a_6; E: butanol, a_{10}; F: ethanol, a_{10}; G: amyl alcohol, a_{10}; H: octanol, a_{10}; I: cyclohexanone, n_{10}.

FIG. 2. Densitograms of alcohol dehydrogenase isoenzymes in adult of *D. ananassae* with butanol as a substrate. A : a_{10} strain; B : a_6 strain.

Discussion

It is interesting to see that the number of ADH isoenzymic forms expressed on the gel varies with different substrates. On single individual assay procedure, butanol shows the activity of three forms of ADH isoenzymes in a_6 but only two forms in a_{10}. Two isoenzymes are exhibited in a_6 adults in presence of methanol and 2-propanol but only one form of ADH in a_{10} adult. Furthermore, allyl alcohol and amyl alcohol show the presence of one ADH isoenzyme in the adults of a_6, but two in a_{10} adults. These differences in relation with appearance of ADH isoenzymes in the adults of two different geographic strains of *D. ananassae* give the proof of biochemical differences between the two strains, which is in accordance with our earlier report[7].

Ursprung and Leone[3] and Jacobson *et al.*[5] reported that methanol is unreactive with ADH in *D. melanogaster*. Our result differs from them in that ADH isoenzymes, in *D. ananassae*, are as good reactive with methanol as with other substrates, we used. We have obtained one isoenzymic form in adult a_{10} and two isoenzymic forms in adult a_6.

While several ADH isoenzymes are reactive with many substrates, other isoenzymes are specific for one or two substrates. Such overlap in substrate specificity was observed in xanthine dehydrogenase and aldehyde oxidase[8]. Differences in the isoenzyme pattern of the same developmental stage of the organism when stained with different substrates, indicates the existence of isoenzymes which are preferentially reactive with a particular substrate and may thus possibly perform different physiological functions. At present, we are not sure about the functional significance of each of the ADH isoenzymes. Further work is in progress.

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