HISTOCHEMISTRY

Tissue Dilution Artefact; a Re-interpretation of Variations in Levels of Succinic Dehydrogenase during Chemical Carcinogenesis

RECENT biochemical studies on succinic dehydrogenase in livers of rats fed with the liver carcinogen *p*-dimethylaminoazobenzene (DAB) for periods of up to 150 days have confirmed the well-established phenomenon that the level of this enzyme generally falls as feeding progresses (Table 1). Samples of tissue from each liver lobe were taken for histological and histochemical investigations, and the remainder was homogenized. Succinic dehydrogenase was estimated to within an accuracy of ± 10 per cent by the anaerobic methylene blue technique, basing the Q_{MB} values on dry weight. For histochemical purposes the tissue was frozen[1], stored for not longer than a week, and cut at − 20° C. in a refrigerated microtome. Succinic dehydrogenase was detected histochemically by incubating frozen sections in a neotetrazolium–succinate buffer system[2]; in addition, the periodic acid–Schiff method[3] was used to stain glycogen, while phospholipid was stained by the acid hæmatin method[4].

The histochemical results indicated that the succinic dehydrogenase activity of the liver cells was high in liver from rats fed on a complete diet or on a low-protein methionine-supplemented diet[5] with or without DAB, and that in the course of feeding this activity per cell did not alter detectably. In contrast, normal bile duct epithelium had a low activity, while connective tissue gave no apparent reaction ; as the period of feeding increased, the proportion of these less or non-reactive components became greater, and the gradual fall in Q_{MB} was qualitatively matched by a reduction in the proportion of liver cells, which histochemically still reacted in a manner comparable with normal liver cells. Sections have also been stained with hæmatoxylin and eosin, while the periodic acid–Schiff and acid hæmatin stains have provided useful information in distinguishing cell types. The latter, in particular, differentiates the weakly staining cells of bile duct and connective tissue in contrast to the strong reaction of liver cells.

When rats were allowed to remain on diet for 200 days, the Q_{MB} values, even of tissue samples selected from the same lobe, showed considerable variation. High Q_{MB} values, about 75–90 per cent of control values, were given by areas containing a high proportion of liver cells, though this proportion was not as high as in normal liver. Low values of Q_{MB}, 20–50 per cent of control values, were obtained from areas of liver composed principally of bile duct cells and fibrous tissue.

Daoust and Cantero[6], who studied the livers of Wistar rats, showed that as the period of feeding the carcinogen DAB was extended, so the relative proportion of liver cells decreased, while the proportions of connective tissue and bile duct cells increased. In these studies, rats of the August strain have been used, but the histology of these livers was qualitatively similar to that reported by Daoust and Cantero. Thus the proportion of liver cells containing a high level of succinic dehydrogenase fell, while the amount of much less active bile duct cells and inactive connective tissue increased ; the overall fall in succinic dehydrogenase was due, not to any change, malignant or otherwise, in any particular cell type, but rather to an alteration in the relative proportions of cell types. It is suggested that the term 'tissue dilution artefact' be used to describe this phenomenon.

Table 1. VARIATIONS IN VALUES OF Q_{MB} IN THE RIGHT ANTERIOR LOBES OF MALE RATS (AUGUST STRAIN) FED WITH THE LIVER CARCINOGEN DAB ON A LOW PROTEIN DIET

Period of feeding	Q_{MB} in right lobe (DAB)	Q_{MB} in right lobe (control)	DAB Q_{MB} as percentage of control values
No DAB		25 ± 4	
30 days	23	25	92
60 days	20	24	83
90 days	13	22	59
120 days	15	29	52
150 days	10	22	45

It is claimed then that this artefact may occur generally in biochemical studies of a tissue containing two or more cell types which may vary in proportion during the course of the experiment. A notable example of such investigations is that on livers of animals treated with liver carcinogens. In this kind of work the onus lies with the experimenter to demonstrate that biochemical comparisons reflect a true alteration in cell metabolism, and are not due to a change in the proportion of cell types of different metabolic activities. Rigid histochemical and histological checks become of the utmost importance in interpreting such biochemical results if invalid conclusions based solely on such data are to be avoided.

We are grateful to the British Empire Cancer Campaign for supporting this work.

G. R. N. JONES
L. BITENSKY
J. CHAYEN
G. J. CUNNINGHAM

Department of Pathology,
Royal College of Surgeons,
Lincoln's Inn Fields,
London, W.C.2.

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PHYSIOLOGY

Sub-fractionation of Human Serum Leucine Aminopeptidase

OUR earlier starch-gel electrophoretic zymograms[1], obtained with 1-leucyl-β-naphthylamide (LBNA) substrate, revealed a single leucine aminopeptidase (LAP) zone for human serum. The substitution of alanyl-β-naphthylamide (ABNA) substrate now consistently reveals, within the diffuse trail behind the primary zone, an additional less-intense zone (Fig. 1*a*), detectable also for some sera by LBNA. Occasional sera give three or more zones; and one chronic pancreatitis serum has given a remarkable zymogram with five zones which, when observed through a suitable blue filter (Dupont No. 9 plastic 'Varigam' filter), can be distinguished as eight zones (Fig. 1*b*).

Whereas some zymograms are distinctive because of this multiplicity of zones, others are distinctive