large tumour nodes occurred which, according to their histological structure, may be divided into hepatomas and cholangiomas. In the tumours with cholangioma structure, a positive reaction for succinic dehydrogenase activity is observed in accordance with the results of PEARSON et al., obtained by a different method[3]. Formazan crystals are detected in the outgrown ducts, succinic dehydrogenase activity being increased with the number of layers of the epithelium of their walls as well as with increasing of cellular cytoplasm. In the large cystic formations, no positive reaction is observed (Figure 3) with the exception of some rare cases, when the epithelium starts growing and destroying the structure of the cysts and the adjacent tissues. Differing from cholangiomas, solid tumours of the hepatoma type show a negative succinic dehydrogenase reaction (Figure 4). In single cases (3 animals), we observed solid tumours with nuclear histogenesis, which showed a positive succinic dehydrogenase reaction – less intensive than that of normal liver parenchyma. Probably these tumours represent a transition to a hepatocarcinoma, which later on may display a negative succinic dehydrogenase reaction. The possibility exists too that these tumours are cholangiocarcinomas in a stage of dedifferentiation and parallel loss of succinic dehydrogenase activity.

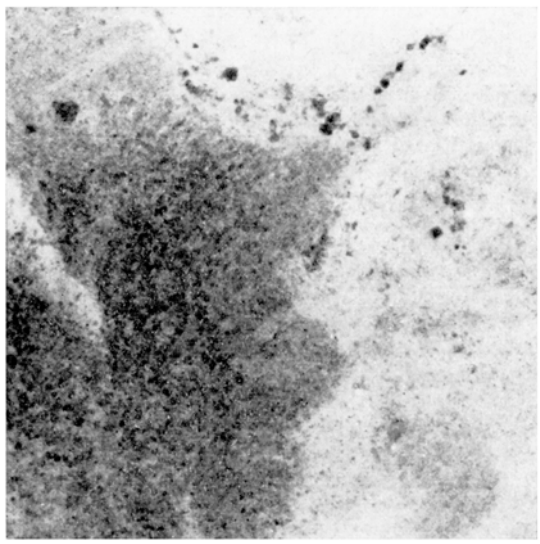


Fig. 4. Solid tumour node of the hepatoma type, no succinic dehydrogenase activity. Positive reaction in the adjacent liver parenchyma. ×100.

The results of our experiments show that, in the course of azo-dye induced rat liver cancerogenesis, the succinic dehydrogenase activity in parenchyma and bile duct cells follow a definite pattern. In typical hepatoma, the activity is markedly lower than in normal parenchyma cells and in hyperplastic parenchyma cell nodules. The negative reaction in normal bile duct cells becomes positive in cholangiofibrosis and cholangiomas with a possible subsequent decrease in more dedifferentiated cholangiocarcinomas.

The 'tissue dilution artefact' as commented on by JONES et al.[8] should be taken into account in the evaluation of biochemically obtained data, but our histochemical results confirm the biochemically obtained results, showing quantitative changes in liver succinic dehydrogenase activity in azo-dye cancerogenesis.

Résumé. L'auteur a étudié histochimiquement l'activité de la succine déhydrogénase lors de la cancérogénèse expérimentale du foie des rats induite par le 3'-méthyl-4-diméthylaminoazobenzène. On a observé une augmentation de l'activité enzymatique dans les stades précancéreux (cholangiofibrose et hypertrophie parenchymateuse) suivie d'une diminution et disparition de cette activité dans les tumeurs développés.

D. C. HADJIOLOV

Department of Pathology, Oncological Research Institute, Sofia (Bulgaria), December 27, 1963.

[8] G. R. N. JONES, L. BITENSKY, J. CHAYEN, and G. F. CUNNINGHAM, Nature *191*, 1203 (1961).

Progressive Localization of Neutral-Red Positive Region with Morphogenesis in *Limnaea*

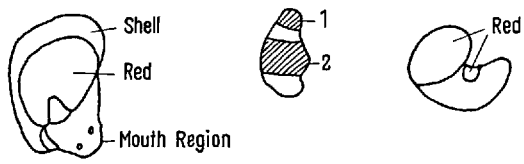
The classical method of vital staining in experimental embryology was given a sharp impetus by REVERBERI et al. REVERBERI[1] had, for example, pointed out a fruitful method of detecting the localization of mitochondria in Ascidian eggs by means of vital staining with Janus green. This and many other attempts to follow the reorientation and redistribution of tangible substances, which go hand in hand with chemodifferentiation, have been ably summed up by REVERBERI[2]. It is obvious that the changing pattern of localization of mitochondria (which houses the many important enzymes) and other substances, following fertilization, closely reflects the changing substratum which culminates into chemodifferentiation.

We have therefore followed this method, which REVERBERI et al. have so fruitfully employed in the case of marine molluscs and ascidia, in our work on fresh water mollusca, stained with Janus green and the supra vital stain, neutral red. As neutral red in very dilute solution has absolutely no toxic effect on *Limnaea* embryos (in fact, they can be kept in neutral red for 48 h and still hatch out into living snails), it is a particularly convenient agent for this sort of work. The *Limnaea* embryos are, however, far from being ideal material for study, namely, glass-clear embryos with little or no colouring matter. Actually, the fresh water molluscs, including *Limnaea*, have a regiment of natural colouring substances, as COMFORT[3] has pointed out, some U-V fluorescing components of which are manufactured, as we have found[4], before hatching. Nevertheless, the visible natural colour in the embryos is not too intense to mask the brilliant hue due to neutral red. Thus a worthwhile study can be carried out until just after hatching, when the outer shell begins to thicken.

[1] G. REVERBERI, Exper. *12*, 55 (1956).
[2] G. REVERBERI, in *Advances in Morphogenesis* (Academic Press 1961).
[3] A. COMFORT, Biol. Rev. *26*, 285 (1951).
[4] R. L. BRAHMACHARY and A. BHATTACHARYA, unpublished data.

Observations were carried out by putting the embryos with a few drops of water on groove slides. The general contour was clearly visible with a magnification of 60×. After this, more detailed observations could be carried out under a magnification of 240×. The embryos constantly move about giving one the impression that 'they are swimming about within their own eggs'. This makes it difficult to get photographs or even to draw pictures, but it also gives us the opportunity of observing it from different sides and angles.

A series of such observations reveal a remarkable trend of localization of the neutral red in course of morphogenesis. About eight days before hatching, almost the whole embryo is intensely red. At about the time of hatching, in snails which had been stained 144 h earlier, the colour was localized at the site of liver, digestive tube etc. It seems the coloured region lies in the posterior 1/3rd or 1/4th part of the body (see Figure). However, in this region, clear, faint, pink patches of presumably natural colour were also seen when embryos before the hatching stage were stained with Janus green alone. 30 h after hatching, the batch treated with neutral red alone, also showed two blackish band-like regions in the red posterior. Nevertheless, the misinterpretation due to these interferences cannot be very great, because the stain due to neutral red is very bright.



In order to be sure about this progressive localization, near-hatching embryos were kept overnight in neutral red. In spite of this, only the posterior (about 1/3rd part of the body) region was coloured intensely red. Again, a young embryo at the stage of 'eight days before hatching' was kept overnight in neutral red (see Figure). The whole body became red or pink but the most intense parts are shaded. As the region 2 contains the bulk of the body, this may well be intensely red but 1 (the posterior tip) is more intense than the neighbouring region. Two days later, the coloured region was seen to have contracted. It now spanned the region from the posterior up to almost the border of the eye. Near this border region, there were some points of concentration, but they were much less intense than the posterior region. The total coloured region was less than in the near-hatching embryos mentioned above. Four days before hatching, the colour receded to the posterior region only. At this stage, it was again kept in neutral red overnight, till it became uniformly coloured. A few hours later, on the third day before hatching, the colour was seen to have been sharply localized in the posterior half (see Figure). In the next two days there was progressive localization till the snail hatched. (The distortion and rearrangement in the staining pattern due to centrifuging has been studied but the data are as yet too scanty.)

We thus detect a progressive differentiation in the form of a progressive loss of 'neutral-red positive' reaction of regions other than the liver, digestive tube etc. The best-known differentiation of this type is EBERT's[5] finding that in chick embryos the capacity of synthesizing myosin is progressively restricted to the cardiac region alone.

Now, there are indications that neutral red may stain acid and alkaline phosphatase[6], and lysosomes, presumably because they are rich in the above substances. MINGANTI[7] had shown several years ago that phosphatase in *Limnaea* is synthesized only after the trochophore stage. It is possible that later on a progressive restriction sets in.

Further, EVOLA-MALTESE[8] detected a steady rise in and a certain degree of localization of alkaline phosphatase in sea urchin embryos and MINGANTI[7] detected a similar rise in *Limnaea* embryos, suggesting a 'functional differentiation of the digestive tract'. However, our results indicate that in *Limnaea*, after a certain stage, the progressive loss of neutral-red positive reaction is more striking than the 'functional differentiation'.

Our work will continue with Gomori stain and centrifuging experiments, as soon as a new batch of material is available[9].

Résumé. Les embryons de *Limnaea* ont été colorés au neutral rouge suivant la méthode de Janus Verte, signalée par REVERBERI pour les mitochondries. A un jeune stade (Veliger), l'embryon entier est taché par neutral rouge avec une coloration intense du conduit alimentaire; à un stade plus avancé, la tache se localise dans la partie postérieure et, au stade de l'eclosion à peu près, seuls l'estomac et la région hépatique présentent la tache.

R. L. BRAHMACHARY and A. BHATTACHARYA

Research and Training School and Gerontology Unit, Indian Statistical Institute, Calcutta (India),
December 28, 1962.

[5] J. D. EBERT, in *The Cell*, vol. 1 (Academic Press, 1959).
[6] A. B. NOVIKOFF, in *The Cell*, vol. 2 (Academic Press, 1961).
[7] A. MINGANTI, Riv. Biol. *42*, 295 (1950).
[8] C. EVOLA-MALTESE, Acta embryol. morphol. Exp. *1*, 99 (1957).
[9] *Note added in proof:* In subsequent observations, in a few cases, some stain was found in the frontal (foot) region. But the progressive loss of stain in the anterior region of the alimentary system was confirmed in *all cases*.

Relation Between Blockade of H^3-Noradrenaline Uptake and Pharmacological Actions Produced by Phenothiazine Derivatives

It has been shown that chlorpromazine decreases uptake of H^3-noradrenaline by the heart, spleen and adrenal glands when given before the labelled compound. Chlorpromazine does not cause release of H^3-noradrenaline bound in these tissues[1,2]. The phenothiazine derivative chlorpromazine has a variety of pharmacological actions, including antihistaminic, adrenolytic and sedative effects. The present study was carried out to see whether any of these actions might be related to the ability of phenothiazines to affect the uptake of H^3-noradrenaline. Four phenothiazine derivatives, each of which lacked certain actions were tested for their ability to block uptake of H^3-noradrenaline into rat hearts.

[1] G. HERTTING, J. AXELROD, and L. G. WHITBY, J. Pharmacol. exp. Therap. *134*, 146 (1961).
[2] J. AXELROD, G. HERTTING, and L. POTTER, Nature *194*, 297 (1962).