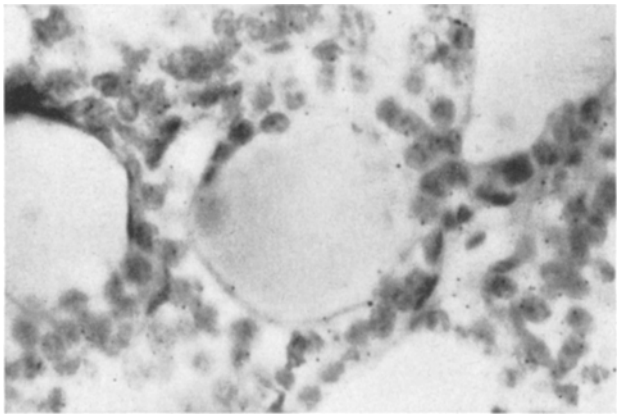

Fig. 1. Ordinary autoradiograph of rat thyroid. Scattered radioactivity derived from ^{3}H-retinyl acetate is seen associated with follicular cells and stroma. Stained with hematoxylin and eosin. ×1000.

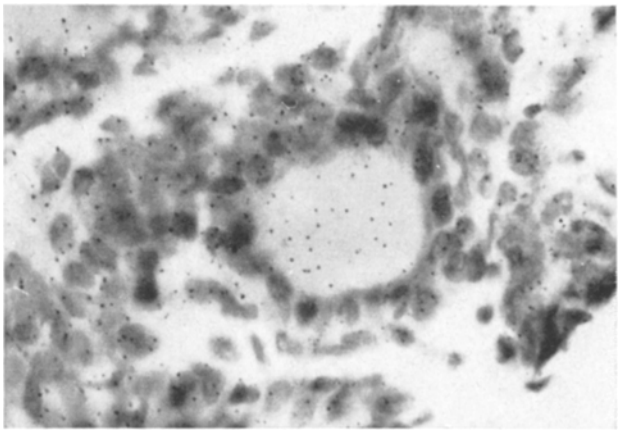

Fig. 2. Soluble-compound autoradiograph of rat thyroid. A higher concentration of radioactivity derived from ^{3}H-retinyl acetate is seen over follicular cells and stroma. Note particularly, concentrations of radioactivity over the colloid. Stained with hematoxylin and eosin. ×1000.

status of the animal can influence the size, weight and/or histological condition of the thyroid[3–10]. For example, JUNGHERR et al.[7] reported that thyroid hyperplasia occurred in bull calves as a result of vitamin A deficiency. In contrast, CARPENTER and SAMPSON[8] found that hypervitaminosis A produced thyroid follicles which were reduced in size, irregular in shape and contained only small amounts of colloid. Despite the apparent relationship, attempts to demonstrate the presence of vitamin A in the thyroid were not successfull. POPPER[11] and POPPER and GREENBERG[12] were unable to detect vitamin A fluorescence in rat thyroid using fluorescence microscopy techniques. The present study is the first to show that radioactivity derived from ^{3}H-retinyl acetate may be autoradiographically located in the follicular cells and colloid of rat thyroid. Furthermore, it appears that the colloid radioactivity is in a relatively soluble form, since it is seen only in freshly frozen tissue and is lost upon fixation and washing of the tissue, while at least a portion of the radioactivity associated with the follicular cells seems to be bound to the cells.

It is unfortunate that the results of the present study can do no more than locate radioactivity derived from injected vitamin A and not tell us more about the nature of the substance(s) it represents. It may be that the radioactivity does represent very small amounts of the vitamin A molecule itself, but it is equally as likely that the radioactivity represents a metabolic derivative of the vitamin or only a fragment of the original molecule or even a totally unrelated substance to which the ^{3}H isotope has been attached or has been exchanged for unlabeled hydrogen. In any event, the results of the present experiment warrant further investigation[13].

Résumé. Des études autoradiographiques démontrent la présence de radioactivité dans les cellules folliculaires et dans la colloïde de la thyroïde du rat à la suite de l'injection intrapéritonéale de l'acétate rétinyl-^{3}H (acétate de la vitamine A). La radioactivité de la colloïde se manifeste seulement dans le tissu congelé vivant; elle disparaît lors de la préparation histologique ordinaire. Par contre, une partie de cette radioactivité reste liée aux cellules folliculaires, même après la préparation histologique habituelle.

B. S. SHERMAN

Department of Anatomy,
State University of New York,
Downstate Medical Center,
Brooklyn (New York 11203, USA), 8 May 1970.

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Specific Stages of Cellular Response to Homeostatic Control During Diethylnitrosamine-Induced Liver Carcinogenesis

After application of a carcinogen, a latency period usually precedes the appearance of morphologically recognizable tumors. Experimental observations indicate that this preneoplastic period is a non-uniform interval which can be subdivided into specific stages by analyzing the stepwise cellular disorientation from organ-specific homeostatic control mechanisms.

Experiments were performed to obtain insight into the growth-behaviour of preneoplastic cells by the combined application of autoradiography and enzyme histo-

chemistry to serial sections of the liver during the preneoplastic period. The model chosen was the diethylnitrosamine-induced hepatoma of the rat. By feeding 5 mg diethylnitrosamine (DEN) per kg daily in the drinking water, hepatocellular carcinomas can be produced within 140–160 days[1]. Long before the appearance of hepatomas, characteristic changes of the enzymatic pattern of the liver are seen. About the 40th day of feeding the carcinogen, deficiencies of glucose-6-phosphatase and adenosine triphosphatase activity appear in circumscribed groups of hepatocytes[2,3]. Since, in fully developed hepatocellular carcinomas, glucose-6-phosphatase activity is, as a rule, much diminished[4–6], it can be assumed that the cells of the deficient areas are precursors of hepatoma cells. This reasoning by analogy was substantially confirmed by observation of further development. These early changes of parenchymal liver cells during DEN feeding facilitate precise analysis of the growth-behaviour of prospective tumor cells during the first stages of carcinogenesis. Mitosis is extremely rare in these areas[7]. Nothing is known as yet about the control of proliferation and its dependence on homeostatic regulation.

One clear-cut example of the influence of homeostatic regulation is the regeneration of the liver after partial hepatectomy[8]. Within a very short time the loss of parenchyma is compensated by excessive proliferation of the remaining liver tissue[9,10]. This fact suggested a study of such influences as might act on proliferation in enzyme-deficient areas in the partially resected liver of DEN-fed animals. Reports on the influence of partial hepatectomy on tumor growth deal with later stages of carcinogenesis[11–15].

Every 10 days from the start of DEN feeding until the 130th day, 2 rats were injected with tritiated thymidine. The substance was given i.p. 7 times at intervals of 6 h, the dose per injection being 100 µC (thymidine-6-H^3, specific activity 5 C/m*M*, Radiochemical Centre Amersham). Like continuous infusion of the label, this leads to a complete labelling of all cells in DNA synthesis during the experimental period. Concurrently with DEN-fed non-hepatectomized rats, 3 DEN-fed rats every 10 days after start of feeding were partially hepatectomized[16], and injections of tritiated thymidine were given to them at intervals of 6 h 7 times between 20 and 56 h after partial hepatectomy. All animals were sacrificed 4 h after the last injection. Serial sections of the liver (5 µm) were prepared at −20 °C in a cryostat. Subsequent sections were used for the determination of glucose-6-phosphatase and adenosine triphosphatase[17], and for the autoradiogram (stripping film Kodak AR 10). By this method, changes in enzyme activity may be topographically correlated with changes in DNA synthesis activity with great accuracy.

With untreated controls the labelling index of hepatocytes was 0.48% after 7 repeated injections of thymidine. Partially hepatectomized control animals, not treated with DEN, showed a labelling index of liver parenchymal cells of 92%, a value agreeing with the results of continuous infusion of tritiated thymidine[18].

As early as 10 days after the start of DEN feeding, characteristic changes are seen in the growth fraction after partial hepatectomy. In congruent serial sections, the activities of glucose-6-phosphatase and adenosine

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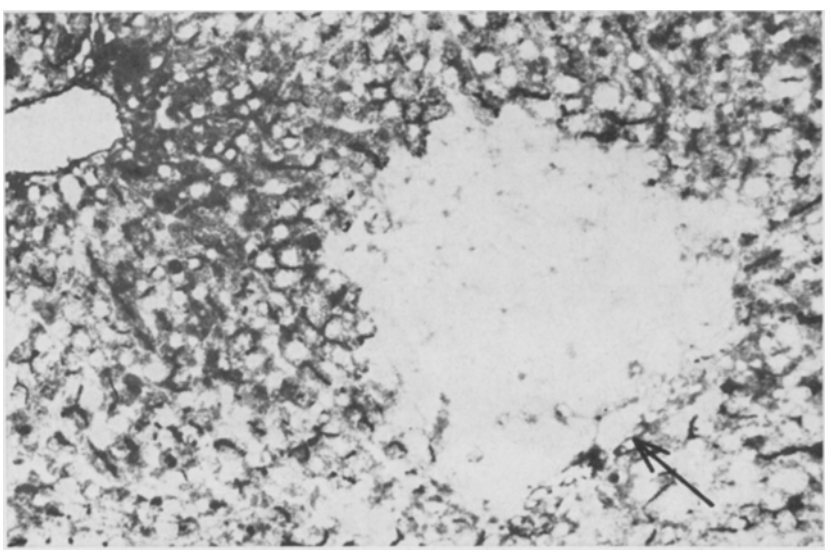

Fig. 1. Group of adenosine triphosphatase deficient hepatocytes adjacent to the central vein (arrow) of the liver lobule after feeding diethylnitrosamine.

triphosphatase are most distinct around the portal field, but fall off towards the lobular center. In the autoradiogram the cells synthetizing DNA are restricted to the areas of highest enzymatic activity. Within a broad fringe around the central vein, DNA synthetizing parenchymal liver cells are completely absent[19]. This inhibition of enzymatic activity and DNA synthesis is reversible. When feeding with the carcinogen is stopped, the labelling index rises.

After about 40 days of feeding DEN, the sharply circumscribed areas of complete enzyme deficiency become prominent (Figure 1). Until about the 90th day after the start of DEN feeding, the tritiated thymidine labelling index in these areas of enzyme deficiency is not significantly higher than in the surrounding parenchyma. Hence the loss of enzyme activity as shown by histochemistry does not immediately coincide with the beginning of proliferation of these cells (Figure 2).

Surprisingly enough, the cells regain, simultaneously with the loss of enzyme activity, their ability to respond to homeostatic regulation, although they are largely situated in lobular areas no longer responding to a growth stimulus during DEN feeding. After partial hepatectomy, however, nearly all cells of the enzyme-deficient areas are labelled (Figure 3). The growth fraction is about 90%, equalling almost the growth fraction of liver cells in the vicinity of the portal field. Those liver

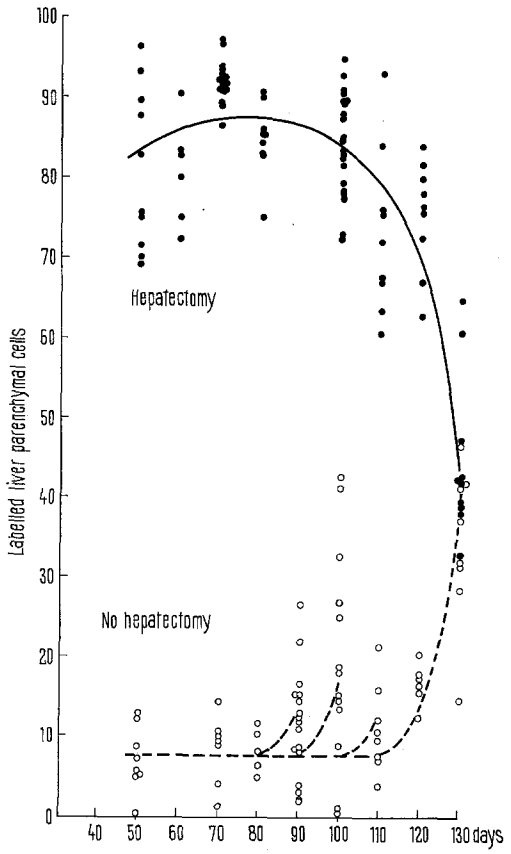

Fig. 2. Thymidine-H^3 labelling index (percent labelled liver parenchymal cells) of glucose-6-phosphatase and adenosine triphosphatase deficient cell groups at different intervals after start of feeding DEN (abscissa). Tritiated thymidine injected 7 times at intervals of 6 h. Animals sacrificed 4 h after last injection of thymidine. Closed circles: labelling indices of animals partially hepatectomized 60 h before sacrifice. Open circles: Labelling indices without partial hepatectomy.

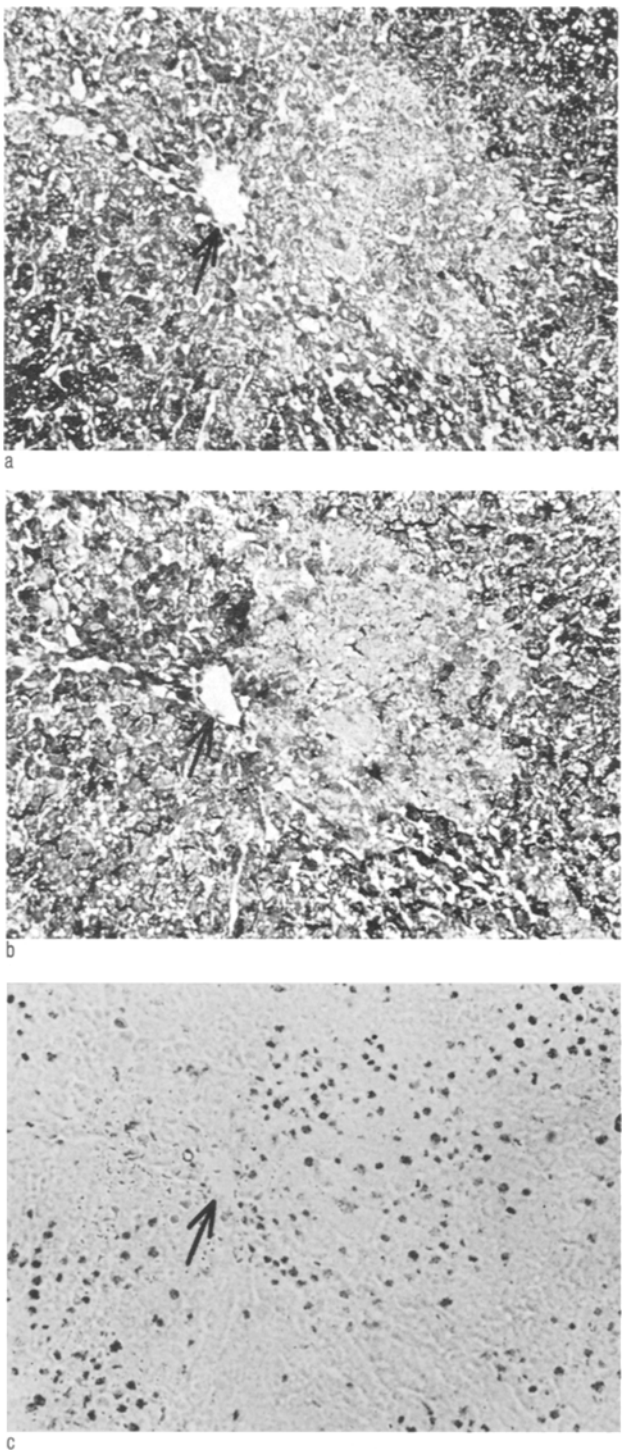

Fig. 3. Cryostat serial sections of rat liver 60 days after start of feeding 5 mg DEN/kg/day and 60 h after partial hepatectomy. Animals injected 7 times at intervals of 6 h with tritiated thymidine prior to sacrifice. In congruent areas adjacent to the central vein (arrows) cells deficient in glucose-6-phosphatase (a) and adenosine triphosphatase (b) show in the autoradiogram (c) of the subsequent section a high tritiated thymidine labelling index after partia hepatectomy. Figures a and b slightly counterstained with hemalaun.

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cells which may be regarded as prospective tumor cells show, until the 90th day, but little proliferation, not exceeding that of the residual parenchyma, if there is no special growth stimulus. Yet if a growth stimulus is provided by partial hepatectomy, their proliferation becomes extraordinary (Figure 2).

The phase of inducible proliferation in the enzyme-deficient liver cell groups is followed by another characteristic period: Endogenous proliferation starts gradually in some enzyme-deficient areas even without an additional growth stimulus, while other areas of deficiency lag behind in this development. In some enzyme-deficient cell groups the labelling index rises above 40% the 100th to 120th day after start of DEN feeding, whereas it is only about 5% in other deficient areas. From the 120th to 130th day onwards the labelling indices rise more steeply (Figure 2).

Simultaneously with the rise of endogenous proliferation, the inducibility of DNA synthesis after partial hepatectomy is lost. On the 130th day, the labelling index of enzyme deficient areas, even after partial hepatectomy, is not significantly higher than without preceding liver resection. These findings suggest that in the course of carcinogenesis liver parenchymal cells become capable of endogenous proliferation in the same degree as they lose their dependence on higher, organ specific, homeostatic control mechanisms.

Transformation of a prospective tumor cell into an autonomous hepatoma cell would, in view of these findings, be completed only after a prolonged period of latency, when the cell has passed through the following phases: 1. Period of weakening of the activities of glucose-6-phosphatase and adenosine triphosphatase activity of acinocentral hepatocytes coincidently with loss of ability to proliferate even after organ specific stimulation of growth. 2. Period of complete loss of the activities of glucose-6-phosphatase and adenosine triphosphatase within circumscribed acinocentrally localized groups of hepatocytes, coincidently with return of ability to proliferate, but for almost all hepatocytes of the enzyme-deficient cell groups only after specific stimulation by partial hepatectomy. 3. Period of autonomous proliferation of these enzyme-deficient cells without an additional growth stimulus, accompanied by a loss of response to organ specific homeostatic control.

Zusammenfassung. Die Latenzperiode bis zum Auftreten Diaethylnitrosamin-induzierter Hepatome ist in 3 spezifische Perioden zu unterteilen: Hemmung der Leberzellproliferation; Enzymverlust mit gleichzeitiger Induzierbarkeit der Proliferation durch partielle Hepatektomie; autonome Proliferation mit Verlust der Stimulierbarkeit durch partielle Hepatektomie.

H. Rabes[20], R. Hartenstein and P. Scholze

Pathologisches Institut der Universität, Thalkirchnerstrasse 36, D-8 München (Deutschland), 2 July 1970.

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Variation between Species in the Innervation of Intra-Testicular Blood Vessels

The innervation of the testicle in mammals is sparse, and considerable differences have been reported in its extent and distribution in a number of common laboratory species[1–7]. In particular, the occurrence of nerves to intra-testicular blood vessels and their pattern of distribution appears to be very variable[1,6,7], despite physiological evidence for the existence of nervous pathways which can affect the testicular circulation[8].

The intra-testicular sympathetic innervation of rats (Wistar strain), rabbits (Flemish Giants), guinea-pigs, cats and dogs has been examined by fluorescence microscopy. Testicles were obtained from 5 mature animals of each species after they had been anaesthetized with ether or Halothane, and transverse and longitudinal sections were prepared by the method of Falck and Owman[9] for the demonstration of catecholamine-containing nerve fibres.

Green-fluorescent, sometimes beaded nerve fibres were seen to form a moderately dense plexus around the main

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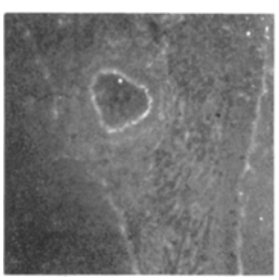

Fig. 1. Rat. Lack of fluorescent, sympathetic nerve plexus around major intratesticular artery. ×100.

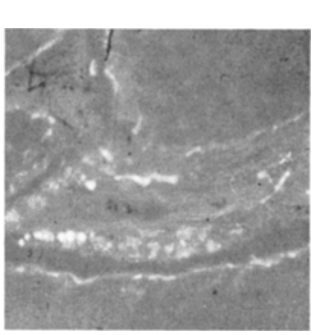

Fig. 2. Rabbit. Fluorescent nerve fibres forming a plexus around an artery deep in the testis. ×100.