It has been claimed that patients with neoplasms have an increased requirement for vitamin B_{12}. Carcinoma cells, like normal cells, need B_{12} and the infiltrative growth of the carcinomas could possibly increase the demand for B_{12}[4]. Following injection of vitamin B^{12} increased growth has been reported in some sarcomas[5].

4-Iodophenylalanine. This amino acid has earlier been found to localize almost selectively in the exocrine pancreas of normal mice[6]. The substance appeared to be transported across the pancreatic cell membrane similarly to an 'ordinary' amino acid but not to be accepted in the protein synthesis and therefore rapidly returned to the blood. In the rest of the body the distribution was very even, almost all tissues having lower concentration than the blood.

In the present investigation, a concentration of 4-iodophenylalanine estimated to approximately 3 times that of blood was seen in the actively growing parts of the Ehrlich ascites cell tumour (Figure 2). The lymphatic leukemia showed somewhat higher concentration than the blood, while the soft fibroblastic osteosarcoma did not take up the drug.

Unlike vitamin B_{12} 4-iodophenylalanine did not show an uptake which correlated with the growth rate of the tumour, since the soft fibroblastic osteosarcoma grew faster than the Ehrlich ascites cell tumour but did not concentrate the drug. Rather, it would seem that the Ehrlich ascites cells and to a less extent the lymphatic leukemic cells have a membrane transport system which can accept 4-iodophenylalanine, which is not the case for the cells of the soft fibroblastic osteosarcoma or the cells of most normal tissues. This may be of interest with regard to the possibility of slightly modifying naturally occurring compounds, such as amino acids, sugars, nucleosides, vitamins etc., to obtain selective localization in tumours. Another non natural amino acid which has been found to accumulate in certain tumours without being incorporated into proteins is 1-aminocyclopentane carboxylic acid[7].

One practical consequence from our findings might be the use of gamma-emitting vitamin B_{12} or 4-iodophenylalanine for clinical localization of tumours. However, these compounds do not accumulate equally in all tumours and a comprehensive mapping of their uptake in different neoplasms in various species must be done before their possible use as tumour diagnostic agents can be appraised[8].

Zusammenfassung. Die Verteilung von radioaktivem Vitamin B_{12} und 4-Jodphenylalanin in tumortragenden Mäusen wurde ganztierautoradiographisch untersucht. Die grösste Aufnahme von B_{12} wurde im weichen fibroblastischen Osteosarkom gefunden, während 4-Jodphenylalanin in einem Ehrlich-Ascites-Tumor aufgenommen wurde.

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Hepatic Carcinogenesis Threshold and Biphasic Mitochondrial Swelling Response in the Guinea-Pig During Diethylnitrosamine Administration[1]

The refractoriness of the guinea-pig to the carcinogenic action of amino azo dyes and aromatic amines, and the resistance of this species to carcinogenic agents in general, is well known. It was reported previously[2] that feeding of the potent hepatocarcinogen, 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB), to guinea-pigs, even at the high level of 0.12%, does not affect the pattern of swelling of isolated liver mitochondria. Although this is in striking contrast with the observations on rats[2-5], it is not actually unexpected in view of the refractoriness of the guinea-pig to azo dye carcinogenesis and the well-established correlation between the onset of carcinogenesis and alterations of mitochondrial swelling[2,3,5] in the rat. The report by Argus and Hoch-Ligeti[6] on the potent hepatocarcinogenic activity of diethylnitrosamine (DEN) in the guinea-pig eliminated the notion that this species is endowed with specific cancer resistance. Hence, this finding prompted an investigation of the possible effect of DEN administration on the swelling response of hepatic mitochondria of the guinea-pig and the correlation, if any, between the swelling response and the threshold of DEN-induced hepatic tumorigenesis.

Materials and methods. Random-bred male guinea-pigs (smooth-haired variety), housed singly in a cage, were used. The weight range at the beginning of the experiments was 170–260 g. The animals were maintained on Purina rabbit chow ad libitum, and vitamin C was supplied during DEN administration by depositing on the back of the tongue, 3 times weekly, 100 mg ascorbic acid in solution in 1 ml water; in the dose-response study, after the respective periods of DEN administration the vitamin C dosing was substituted by an abundant daily

[1] Supported by Research Grants Nos. CA-05431 and CA-05793 from the National Cancer Institute, United States Public Health Service. The expert technical assistance of Mrs. Shirley B. Bemis with the mitotic indexes is gratefully acknowledged.
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supply of fresh greens. Diethylnitrosamine was administered in the drinking water at the level of 0.042 ml/l, made up fresh daily. The average daily intake was 1.2 mg DEN/guinea-pig. The dose-response experiments were carried out as previously[3] in rats with an azo dye carcinogen. In the present study the groups contained 23–25 guinea-pigs each, DEN was administered for periods from 4–24 weeks, and all animals were sacrificed a total of 12 months after the beginning of the experiment[7]. The isolation of liver mitochondria (in 0.44 M sucrose plus 0.001 M ethylenediaminetetraacetate) and the measurement of mitochondrial swelling[2–4], and the determination of the mitotic index[8] on tissue samples taken in the swelling studies were carried out as previously.

Results and discussion. The dose-response of DEN-induced hepatic tumorigenesis in the guinea-pig is shown in Figure 1. No tumors appeared in the groups which received the carcinogen at this dose level for 4 or 8 weeks; however, there was a 21% tumor incidence in the guinea-pigs which received DEN for 12 weeks. The curve indicates that the onset of tumorigenesis is between 8 and 10 weeks. The minimum effective tumor dose (TD_5) and the 50% tumor dose (TD_{50}) of DEN in guinea-pigs under the present experimental conditions are 86 mg

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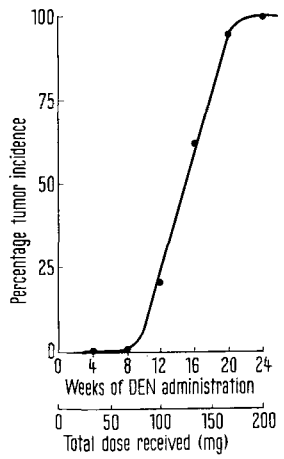

Fig. 1. Hepatic tumor incidence in 6 groups of guinea-pigs (23–25 animals each) as a function of the time of administration of diethylnitrosamine (DEN) at the average daily oral dose of 1.2 mg. The initial weight and diet of the animals, and the mode of DEN administration, were as in footnote[b] of the Table. In all groups the final tumor incidence was determined after 12 months.

Effect of diethylnitrosamine administration on the hepatic mitotic index of guinea-pigs[a]

Weeks of diethylnitrosamine administration[b]	Total number of cells counted	Mitotic index[c]
0	731	3.1
1	849	4.9
2	805	5.5
3	642	4.2
4	794	4.4
5	813	6.3
6	814	6.4
8	825	7.5
10	885	8.5
13	996	9.6
16	888	8.8
20	678	7.8

[a] The tissues were prepared and the numbers of cells in mitosis were counted as previously described[8]. [b] The guinea-pigs weighed 170–260 g at the beginning of administration. They were maintained on Purina rabbit chow diet supplemented with 100 mg ascorbic acid administered orally 3 times weekly. Diethylnitrosamine was administered in the drinking water; the average daily intake was 1.2 mg/animal. [c] The mitotic index represents the percent of the total nuclei counted which were in mitosis. For each interval of administration the mitotic indexes of 5 individual guinea-pigs were averaged.

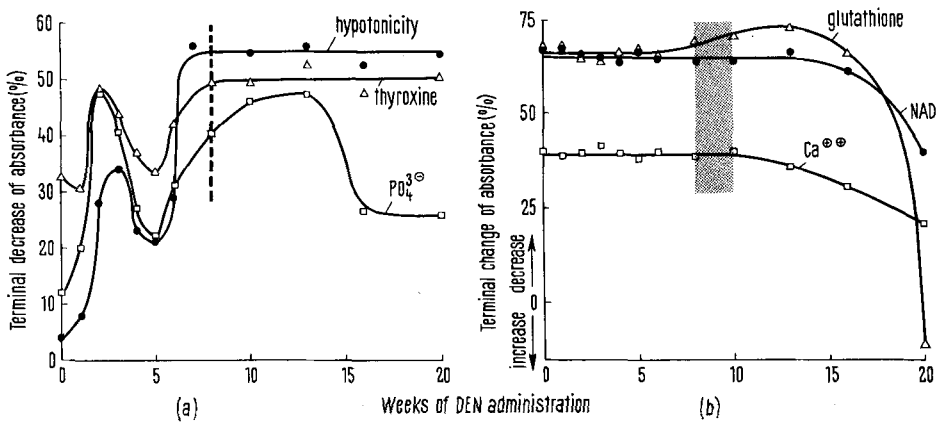

Fig. 2. Swelling of guinea-pig liver mitochondria as a function of the time of administration of diethylnitrosamine (DEN) at the average daily oral dose of 1.2 mg. The initial weight and diet of the animals, and the mode of DEN administration, were as in footnote[b] of the Table. The terminal absorbance change was calculated from the absorbance at 40 min (at 520 nm; 23–25 °C) relative to the zero time value. When swelling was induced by thyroxine ($1\times10^{-5}M$), phosphate ($4\times10^{-4}M$), glutathione ($5\times10^{-3}M$), NAD ($5\times10^{-3}M$) and Ca^{++} ($5\times10^{-3}M$) the assay was carried out in 0.30 M sucrose; when swelling was induced by hypotonicity the assay medium was 0.17 M sucrose without inducer agents. Both sucrose media were buffered with 0.02 M *Tris* hydroxymethyl aminomethane (pH 7.4). Each point in the curves represents the mean values of 5 experiments.

and 122 mg, respectively, as evaluated from the probit plot of the dose-response.

From the very onset of administration DEN brings about an increase in the number of mitoses in the liver. The Table shows that the mitotic index gradually increases during administration and reaches the highest level at 13 weeks, which is beyond the tumorigenesis threshold. Since the animal experiments were already terminated at the time when the mitotic indexes were read, no tissue sample was taken beyond 20 weeks of administration. Thus, further experiments will be necessary to ascertain whether the 13-week mitotic index represents the beginning of a plateau or a true maximum (as suggested by the Table) beyond which the mitotic index of liver tissue gradually decreases. The here observed gradual increase of the hepatic mitotic index in guinea-pigs during DEN-carcinogenesis is in agreement with the findings in rats[8].

Unlike 3'-Me-DAB[2] (inactive in the guinea-pig), DEN which is highly carcinogenic toward the liver of this species also brings about drastic changes in the swelling of liver mitochondria (Figures 2a and 2b). However, the DEN-elicited alterations of mitochondrial swelling response in the guinea-pig are much more complex than the simple monophasic change observed with 3'-Me-DAB in the rat[3,5]. With DEN in the guinea-pig the patterns elicited by the swelling-inducer agents studied here can be grouped in 2 classes: (A) those showing an early sharp maximum at 2 or 3 weeks followed by a decrease of swelling and, again, a subsequent appreciable rise terminating at 8–10 weeks (Figure 2a); and (B) those showing no change of swelling, or a gradual very small increase up to 11–13 weeks, at which time a marked decrease of swelling begins. While the exact biochemical significance of the 2 types of swelling responses is not understood at the present time, the changes (observed with inducers of class A) terminating at 8-10 weeks and the changes (observed with inducers of class B) beginning at 11–13 weeks, appear to mark the conclusion and onset, respectively, of 2 consecutive processes of mitochondrial alterations both required to bring about the neoplastic state. In fact, the time of transition between the 2 processes – at 8–10 weeks (shaded region in Figure 2b) – exactly coincides with the minimum time of DEN administration necessary to induce liver tumors in the guinea-pig (Figure 1).

Résumé. La corrélation préalablement établie entre l'incidence de tumeurs hépatiques induits par le 3'-méthyl-4-diméthylaminoazobenzène et le gonflement de mitochondries dans le rat a été étendue à la cancérogenèse induite par la diéthylnitrosamine dans le cobaye.

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Über die Chemodifferenzierung der Stammganglien der Ratte

Die Stammganglien sind ontogenetisch verschiedener Herkunft. Claustrum, Striatum und Nucleus amygdalae entwickeln sich aus der Anlage des Telencephalon, die Nuclei subthalamicus und endopeduncularis aus der des Diencephalon. Über die Abstammung des Pallidum bestehen verschiedene Ansichten. Für das menschliche Gehirn vertreten Grünthal[1,2] die Meinung, das Pallidum gehöre entwicklungsgeschichtlich zum Telencephalon, Spatz[3], Richter[4] u. a. zum Diencephalon. Für das tierische Gehirn besteht eine vergleichbare Diskussion[4]. Unsere Untersuchung greift diese Fragestellung für die Ratte auf, wobei ausser färberisch-lichtmikroskopischen vor allem fermenthistochemische Methoden verwendet werden. Ferner soll geprüft werden, ob ähnlich wie bei der Morphodifferenzierung die Chemodifferenzierung der genannten Strukturen in einer bestimmten Richtung fortschreiten und in welcher Entwicklungsperiode die Stammganglien ihre endgültige Ausgestaltung erfahren. Untersucht wurden 108 Ratten zwischen dem 18. Embryonaltag und dem 45. Lebenstag sowie 11 erwachsene Tiere.

Saure Phosphatase, Thiaminpyrophosphatase, Monoaminooxidase, unspezifische Esterase, Cholinesterase, Bernsteinsäuredehydrogenase, Glucose-6-Phosphatdehydrogenase, Lactatdehydrogenase und NADH zeigen in den dienzephalen Strukturen etwa um den 20. Embryonaltag eine erste starke Aktivitätszunahme. Eine vergleichbare Steigerung erfolgt in den telenzephalen Teilen der Stammganglien erst 2–3 Tage später. Ähnliche zeitliche Unterschiede bestehen auch hinsichtlich der Morphodifferenzierung. Stets verhält sich das Pallidum wie ein dienzephaler Gehirnabschnitt. – Saure Phosphatase und Thiaminpyrophosphatase nehmen unter den genannten Fermenten eine Sonderstellung ein. Ihre Aktivität, die ausschliesslich an das Zytoplasma der Nervenzellen gebunden ist, bleibt auch bei fortschreitender Entwicklung in den dienzephalen Arealen höher als in den telenzephalen. Auch diesbezüglich erweist sich das Pallidum als dienzephales Areal: einschliesslich der erwachsenen Tiere ist die Aktivität für saure Phosphatase und Thiaminpyrophosphatase im Pallidum höher als in Claustrum, Striatum und Nucleus amygdalae. Bei den anderen Fermenten (unspezifische Esterase, Cholinesterase, Succinodehydrogenase, Glucose-6-Phosphatdehydrogenase, Lactatdehydrogenase, NADH) gleichen sich die Aktivitäten in den di- und telenzephalen Strukturen zwischen dem 3. und 10. Lebenstag einander an, wenn auch für die verschiedenen Fermente zu unterschiedlicher Zeit. Nach dem 10. Lebenstag steigt die Aktivität dieser Fermente

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