is the result of either a process of sudden cavitation of the inner cell mass or a folding process involving the lateral edges of the embryonic disc[14]. Fluid has been noted in the amniotic sacs of some blighted human ova, and fluid is also present in very early human pregnancies before organ differentiation has progressed very far[15]. It is possible that the actual production and metabolism of amniotic fluid varies from one species to another, and given the variety of differences in mammalian extra-embryonic membranes, it would be difficult to extrapolate data from one species to another. However, the fundamental nature of the evolution of this membrane suggests some common denominator of function and purpose in all of the amniotes. From the data in our experiments, however, it is clear that in the hamster, the fetal urine plays little or no role in the maintenance of amniotic fluid volume[16].

Zusammenfassung. Experimentelle Prüfung beim Goldhamster, *Mesocricetus auratus*, ob Nieren- oder Gehirnmissbildungen einen Einfluss auf die Quantität der Amnionflüssigkeit ausüben, was nur für die Gehirnmissbildungen bejaht werden konnte.

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Ultrastructural Changes in Rat Liver Cells After Intramuscular Implantation of a Walker Tumor

Rats bearing Walker tumors exhibit an increase in liver weight with a decrease in hepatic drug-metabolizing enzyme activity and cytochrome content[1–3]. Experiments in this Institute have shown that, 3–4 weeks after i.m. implantation of this tumor, there is a slight diminution of P-450 content, ethylmorphine demethylation and aniline *p*-hydroxylation as well as an increase of microsomal proteins in the liver cells[4]. Administration of Walker tumor extract produces similar biochemical changes, indicating that a 'toxohormone' is released from the tumor tissue which affects the liver and leads to reduced activity in the hepatic microsomal system[5]. The ultrastructural equivalent of this hepatic microsomal hypofunction was studied in the present work.

Walker tumors were implanted i.m. into the hind limb of 16 female ARS/Sprague-Dawley rats (100 g). The livers of these animals and of 8 controls were examined with an electron microscope 1, 2, 3 or 4 weeks later, using standard techniques (fixation in osmium, dehydration in graded ethanol, embedding in Epon resin).

As compared with the controls (Figure 1), the tumor-bearing rats displayed progressive dilatation and ballooning of the rough-surfaced endoplasmic reticulum (RER) in hepatocytes (Figure 2). The granular membranes were irregularly-shaped and were broken up into smaller units, from the external surfaces of which the ribosomes had gradually detached themselves. These changes were conspicuous although they varied considerably among the animals of the same group and in the different areas of the same liver. The alterations were generally mild 2 weeks after implantation, became pronounced after 3–4 weeks, and were usually observed along with progressive smooth-surfaced endoplasmic reticulum (SER) hypertrophy (Figure 3).

In previous studies[6–8], with other types of tumors, mitochondrial abnormalities and lysosomal accumulation were the principal changes found in the hepatocytes. In the present work, however, conspicuous endoplasmic reticulum alterations were detected.

RER injury might represent the ultrastructural equivalent of hepatic microsomal hypofunction in rats bearing Walker tumors. SER proliferation, which is generally equated with enhanced drug detoxication by liver microsomes[9–11], is reminiscent of the hypoactive, hypertrophic

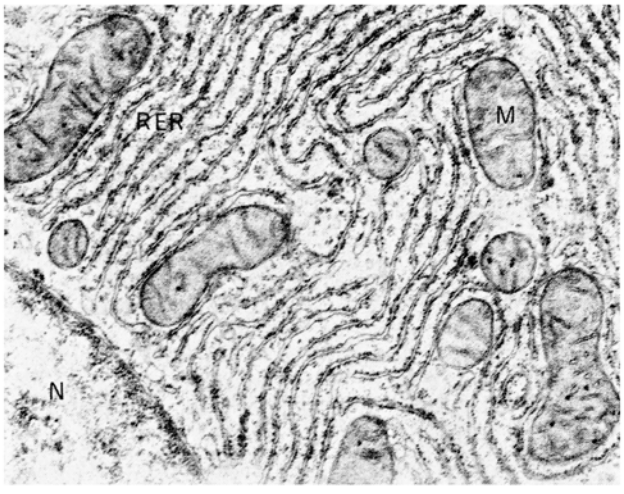

Fig. 1. Portion of a hepatocyte in control rat showing characteristic features. M, mitochondrion; RER, rough-surfaced endoplasmic reticulum; N, nucleus. ×14,133.

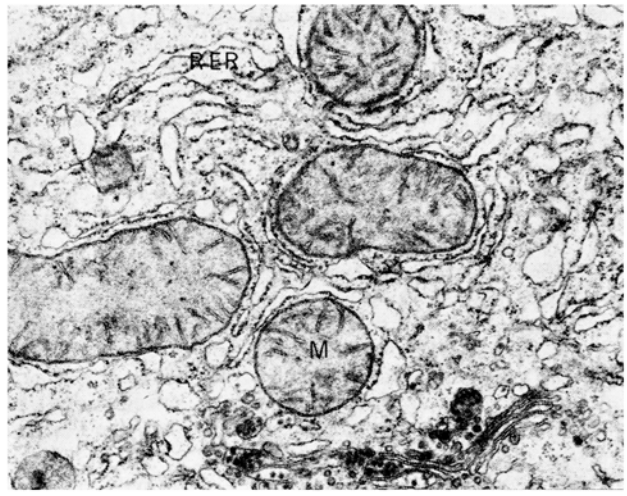

Fig. 2. RER dilatation, disorganization, disruption and degranulation in a rat hepatocyte 3 weeks after Walker tumor implantation. ×13,733.

endoplasmic reticulum noted by HUTTERER et al.[12] in the liver cells of rats given dieldrin or 3'-methyl-4-dimethylaminoazobenzene. Prolonged treatment with these compounds reduced the initial increase of enzyme activity, without affecting SER hypertrophy. Considering the biochemical data[1-3], SER accumulation in Walker tumor-bearing rats seems to represent a diminished functional capacity of the hypertrophic endoplasmic reticulum. However, further work is needed to establish whether these changes are due to a specific 'toxohormone' secreted by the tumor cells or to some nonspecific toxicity (e.g., protein breakdown products released from the tumor tissue, etc.).

Patients with advanced cancer might exhibit different nonspecific manifestations, including anorexia, weight loss, cachexia, increased susceptibility to infection, abnormal responses to drugs and various endocrine alterations, irrespective of the localization of the tumor. Obviously, not all of these changes are related to hepatic microsomes. However, some of them could result from faulty intermediary drug or hormone metabolism. It remains to be seen whether the structure and function of the endoplasmic reticulum membranes are likewise impaired in these cases[13].

Résumé. L'implantation i.m. de la tumeur de Walker chez les rats provoque une dilatation, une désorganisation et une dégranulation progressives du réticulum endoplasmique granuleux des hépatocytes. Ces changements, qui s'accompagnent en même temps d'une prolifération du réticulum endoplasmique lisse, représenteraient les manifestations ultrastructurales d'une insuffisance microsomale démontrée biochimiquement dans le foie des rats affectés par la tumeur de Walker.

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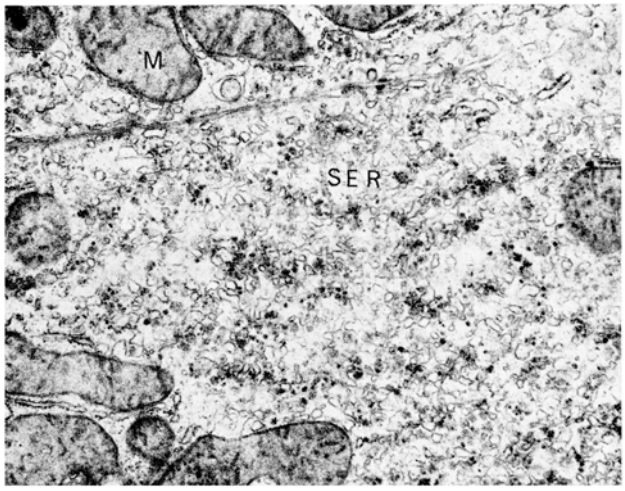

Fig. 3. SER accumulation in a rat hepatocyte 4 weeks after Walker tumor implantation. SER, smooth-surfaced endoplasmic reticulum. ×10,666.

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Interpretation of the Methylene Blue Reduction Test of Human Plasma and the Possible Cancer Protecting Effect of Selenium

Selenium has recently been implicated as a possible cancer protecting agent in humans by SHAMBERGER and FROST[1]. These authors found an inverse relation between the whole blood selenium concentrations from blood bank samples in 20 US cities and the human cancer mortality. They also report data which suggest a lower cancer mortality in areas of Canada where 'selenium indicator plants' are found than in areas where such plants are not found. In the present communication we present data from our laboratory which appear to support this hypothesis, and which explain earlier work on a presumed plasma cancer test based on the measurement of the methylene blue reduction time.

SAVIGNAC et al.[2] and BLACK[3] reported an abnormality in the methylene blue reduction time (MBRT) of plasma of cancer patients. A typical testing procedure consisted in the measurement of the time required for complete decolorization of a sample of plasma to which a known amount of methylene blue solution was added when the mixture was heated under anaerobic conditions on a steam bath[2]. The test was considered positive if the MBRT exceeded the average value of plasma of normal subjects. In systematic studies BLACK[3] reported the plasma of 75% of hospitalized cancer patients to give positive test results. Patients with nonmalignant neoplasia and other diseases gave overwhelmingly normal MBRT values. Subsequent studies[4] indicated practical limitations of the test for cancer diagnosis in that only approximately 50% of cancer cases gave positive test results. Moreover, in other groups of patients with pathological states the percentage of false positives ranged from 7 to 34%. The origin of the abnormal behavior of cancer patient plasma was originally considered to be dependent on the concentration of free sulfhydryl groups