Moreover, since G-6-Pase is now known to have multiple enzymatic activities[9,10], cells bearing the enzyme, under appropriate physiological stimuli, may also be able to generate G-6-P and Pi[11]. On this basis one might suggest that the G-6-P splitting enzyme activity in Kupffer cells plays a complex role in the various physiological functions of this cell type. It is of interest to note that a G-6-P hydrolyzing enzyme activity is also present in a spectrum of different cell types in virtually all of the lymphoid organs[8]. However, as cautioned in previous publications[1] although leukocytes and Kupffer cells are capable of hydrolyzing G-6-P and also demonstrate an identical organellar distribution of reaction product similar to that of liver cells, further studies are required to demonstrate whether the G-6-Pase like activity in RES cells is biochemically similar to liver G-6-Pase, particularly with respect to their kinetics and multifunctional specifities[12].

Zusammenfassung. Die Aktivitätsverteilung eines Glukose-6-Phosphat hydrolysierenden Enzyms wurde in der Leber 24 Stunden alter Ratten elektronenmikroskopisch untersucht. Die Verteilung der Enzymaktivität in den Kupffer-Zellen schien mit der in den Hepatozyten gefundenen identisch zu sein.

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Vitamin E Deficiency and Chemical Carcinogenesis

There are several reports in the literature on the carcinogenic and co-carcinogenic effect of heated fat[1,2]. SUGAI et al.[2] were the first to demonstrate that a polymeric fraction of heated fat accelerates the induction of tumors in rats given a low level (5 mg/100 g of the diet) of N-2-fluorenylacetamide (FAA). It was not clear whether the synergistic effect of heated fat was directly related to the presence of oxidative polymers in the diet or whether these compounds caused in vivo peroxidation of tissue unsaturated lipids which resulted in a more favorable environment for the neoplastic transformation. In an attempt to answer this question, we have superimposed vitamin E deficiency on animals receiving a low level of FAA. No long term experiments have been previously reported to determine whether a diet deficient in vitamin E and high in polyunsaturated fatty acids would accelerate or retard the induction of tumors in rats given a low level of carcinogen.

Materials and methods. 4 groups of 18 male rats each (Holtzman, 60 g) were fed a semisynthetic diet containing casein 20%, cerelose 66.1%, salt mixture 3.5%, choline chloride 0.3%, fat 10% and all the necessary vitamins. Groups 1 and 2 received 10% corn oil without and with 0.005% FAA respectively. Groups 3 and 4 received 10% stripped corn oil (Distillation Products Industries, Rochester, New York) without and with 0.005% FAA respectively. The animals were given feed and water ad libitum and their weights were recorded periodically. The deficiency of vitamin E in the blood of animals from groups 3 and 4 were confirmed by performing the dialuric acid hemolysis test[3]. Microquantities of blood were withdrawn periodically from the tail vein and used for obtaining the serum lipoprotein patterns[4]. The tissues of the animals were fixed in formalin and paraffin sections were stained with hematoxylineosin.

Results and discussion. The rats on diet containing stripped corn oil and FAA grew poorly as compared to controls, and 5 rats died within the first year. After 14 months on the diet, many rats of this group became quite emaciated and 3 more rats died. Therefore, 6 rats were sacrificed at this time and 4 remaining rats were sacrificed along with the animals in the other groups during the 18th month of the experiment.

Almost all the rats from group 4 (10 out of 13 which were histologically examined) developed liver tumors of the adenomatous type. No ear duct tumors or other tumors were observed in rats from this group. Rats from group 3, which were fed the same diet, but without the carcinogen, did not develop any tumors and excluded the possibility of malignant transformation due to stripped corn oil alone for this long time period (18 months). The serum lipoprotein patterns indicated an increase in high density lipoproteins of animals given the carcinogen (groups 2 and 4 vs groups 1 and 3 respectively) and are consistent with similar results reported before[5].

A low level of 0.005% FAA was chosen on the basis of the observations of SUGAI et al.[2]. However, in the present experiment (and confirmed by a subsequent experiment) this level of FAA was sufficient to induce tumors of liver and other tissues in rats given fresh corn oil (14 out of 14 from group 2). Thus, this observation precludes us from concluding that stripped corn oil (or vitamin E deficiency) acts synergistically in conjunction with 0.005% FAA to induce neoplasia. It is, however, possible that a still lower level than 0.005% FAA may demonstrate this effect.

A lower level of the carcinogen would mean a more prolonged period of experimentation, and, in the case of vitamin E deficient animals, it is impractical.

Since vitamin E and intracellular antioxidants are reported to be enriched in tumor tissues as compared to normal tissue[6,7], some retardation of tumor induction or tumor growth might be expected in acute vitamin E deficiency. Since the growth of the animals in group 4

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was poor, it was difficult to ascribe the decreased size of liver tumors observed in several rats from this group to vitamin E deficiency alone. Surprisingly, SWICK and BAUMANN[6] have reported that dietary vitamin E decreased the incidence of hepatomas when large amounts of the vitamin were fed after administration of 3'-methyl-4'dimethylamino-azobenzene.

MILLER et al.[8] used a diet containing 0.06% *p*-dimethylaminoazobenzene and low in vitamin E and have concluded that vitamin E does not exert any effect on the carcinogenicity of *p*-dimethylaminoazobenzene. Since they used relatively larger rats (initial wt, 180 g vs 60 g used in the present experiment), a lower level of fat (5% vs 10%), a much shorter total period of experimentation (6 months vs 18 months), and did not establish the vitamin E deficiency status of their rats, their conclusion may perhaps be questioned. However, the present results, obtained with a different carcinogen (FAA) under more controlled conditions are essentially in agreement with their data. Thus, it can be stated that vitamin E deficiency, under the present experimental conditions, does not accelerate the induction or growth of tumors by FAA in rats[9].

Zusammenfassung. Männliche Ratten erhielten während mehr als 14 Monaten eine an Vitamin E arme, jedoch an ungesättigten Fettsäuren reiche Diät. Vitamin E-Mangel (jeweils mit und ohne niedrigem N-2-Fluorenylacetamid-Gehalt) hemmte das Tumorwachstum nicht und führte zu keiner Zunahme der Karzinogenese, was durch Kontrollen bestätigt wurde.

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Fibrinolytic Activity of Rat Uterine Fluid

We wish to report some data elucidating the fibrinolytic system in rat uterine fluid because secretions from the rat uterus liquefy fibrin clots[1,2].

White rats (Sprague-Dawley strain) were anesthetized with ether and their uterine horns ligated at the cervix. 5–8 weeks later blood was collected in citrate by heart puncture under ether anesthesia. The uterus was isolated and the uterine fluid collected and centrifuged to remove cellular material. The uterine tissue was rinsed in water, blotted with filter paper and stored at −20 °C in small, tightly stoppered vials. Samples were assayed within 2 weeks. Fibrinolytic activity was determined by the fibrin plate method[3] using plasminogen-rich bovine fibrinogen. Plasminogen activator in tissues was extracted with *2 M* potassium thiocyanate[4]. Determinations are based on assays of serial dilutions in triplicates. Tests for protease activity were made on fibrin plates heated for 45 min at 85 °C to destroy plasminogen.

Rat uterine fluid was fibrinolytically active with extreme variations between individual samples confirming observations on mouse uterine fluid[5]. Lack of activity on heated fibrin indicated absence of protease, suggesting the active agent is a plasminogen activator. Lyzed zones obtained from samples equally diluted with barbital buffer were: 567, 644, 751, 1476 and 2054 mm² (diameter products). Converted into units of a standard tissue activator preparation simultaneously assayed (32 U/ml), concentrations of activator in the uterine fluid ranged from 70–2200 U/ml. A red-tinged sample produced slight activity on heated fibrin suggesting contamination with plasminogen originating in the blood. Solutions of uterine fluid were adjusted to different pH values and kept for 90 min at 37 °C. After neutralization the remaining activity was determined. The solutions were most stable around neutral reaction. Losses were encountered at alkaline (pH 9.2) or acid reaction (pH 5.0 and 4.0) and became larger at higher acidity (pH 3.0 and 2.2). There was still 25% of the original concentration after 90 min at pH 3.0. These results indicate that the uterine fluid contains a stable as well as an acid-labile plasminogen activator.

Uterine fluid (0.2 ml) was also collected from a normal rat in estrus and its activity assayed in serial dilutions. The undiluted activity of 203 mm² was considerably lower than in the weakest of the samples collected after ligation.

Rat plasma was inactive on regular or heated fibrin plates. Euglobulins, isoelectrically precipitated at pH 5.9 and redissolved to the original volume, were fibrinolytically active on regular (e.g. 139 mm²) as well as heated fibrin plates (70 mm²), confirming similar, previous determinations[6]. Others, determining euglobulin clot lysis times, reported marked[7,8] or very low[9] fibrinolytic activity in rat plasma, a discrepancy still unresolved. Addition of streptokinase did not enhance the activities. Addition of urokinase increased lysis on heated fibrin demonstrating presence of unconverted plasminogen. The activity in plasma is much lower than in the uterine fluid excluding the circulating blood as a possible source.

Assays of activator concentrations in the uterine tissue of rat produced curves in the double-logarithmic graph with slopes lower than the standard preparation suggesting contamination with inhibitors and allowing

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