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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approximate determinations only. Concentrations in ligated uteri (e.g. 96, 288, 576 U/g) and in controls (120, 192, 240, 335, 480 U/g) showed wide individual variations. For unknown reasons, these values are considerably higher than those reported in a previous study[10]. Following acid precipitation, the activity was lower than in the crude extract. Attempts to remove an inhibitor from the crude extract by adsorption to glass were negative.

Occasion was taken to determine the plasminogen activator concentration in the ovaries of 4 rats. Again, the individual results varied (66, 120, 360, 1120 U/g) and showed decreased slopes of the dilution curves.

Endometrial fibrinolytic activity of the rat is known to be localized at vascular structures[11] or at the surface epithelium[12], the activity of the latter being influenced by gonadal hormones[13]. The fibrinolytic activity appearing in the uterine cavity during desquamation of the endometrial surface epithelium is probably the source of the activity accumulating in the fluid in the ligated uterus. The observed wide variations in individual results could be caused by differences in individual response or in stage of hormonal cycle. This would also apply to the plasminogen activator in the ovaries which was observed in the hog to increase in concentration during pregnancy[14]. The complete suppression[5] of fibrinolytic activity in mouse uterine fluid by progesterone, and partial suppression by estrogen would seem to support the proposed origin. This would also explain why suppression of activity in the uterine fluid coincides with endometrial glandular activity[15] because endometrial glandular epithelium is fibrinolytically inactive[11-13, 16].

Zusammenfassung. Nach Abbindung des Rattenuterus wird bei relativ grosser individueller Streubreite ein fibrinolytisch hochaktives Sekret produziert. Die Aktivität wird durch einen in verschiedenen Konzentrationen vorhandenen Plasminogenaktivator, der vermutlich im Oberflächenepithel des Endometriums gebildet wird, verursacht.

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Disruption of Lecithin Spherulites During Hydrolysis by Phospholipase A: A Study by Electron Microscopy

The structure of lecithin spherulites (liposomes) has been studied extensively with the electron microscope[1]. Aqueous dispersions of lecithin stained with phosphotungstate show alternating electron-opaque and electron-lucent lamellae which represent respectively the hydrophilic and hydrophobic parts of the molecular aggregate. The lamellar structure of lecithin may be modified by at least two procedures. BANGHAM and HORNE[2] showed that lysolecithin when mixed with lecithin caused the progressive breakdown of the lamellar structure by release of particles 70–80 Å in diameter from the surface of the spherulite. GREEN et al.[3] demonstrated that β-hydroxybutyrate dehydrogenase (a phospholipid-requiring enzyme) caused the loss of internal structure of the liposome. The size of the liposome, however, was not changed. During a study of some biochemical properties of a phospholipase A from the venom of the Australian black snake (*Pseudechis porphyriacus*)[4], a change in morphology of the substrate lecithin during hydrolysis was noted. The observations form the basis of this report.

Lecithin was prepared from egg yolks and its purity was confirmed by thin layer chromatography; the ester: phosphorus ratio was 2.0. Venom of the Australian black snake was used as a source of phospholipase A (phosphatide acyl-hydrolase, E.C.3.1.1.4.). Phospholipase A is the major component of the venom, as shown by disc gel electrophoresis. Lecithin was ultrasonically dispersed at a concentration of 3 µmoles/ml in a solution of 0.22 *M* NaCl, 10 m*M* $CaCl_2$, and 1 m*M* EDTA. The pH was adjusted to 7.3 immediately before the experiment. The venom was used at a concentration of 0.16 mg/ml. The reaction was carried out at 25 °C with efficient stirring. At 15 min intervals duplicate 0.2 ml samples were taken for titration with methanolic KOH as described previously[4]. Samples for electron microscopy were taken before the addition of the enzyme and at several intervals (30 sec to 110 min) during the incubation. The samples were mixed with an equal volume of 2% sodium phosphotungstate (pH 7.4), dried on parlodion and carbon coated grids, and examined in an Hitachi HU-11C microscope at instrument magnifications of 30,000–50,000.

The reaction was followed for 110 min by titration. In that time 2.4 µmoles/ml of fatty acid were produced; 80% of the substrate was hydrolyzed. The effect of the venom on the lamellar structure of lecithin is shown in the electron micrographs. Figure 1 shows the appearance of a lecithin spherulite before the addition of the venom. After 2 min of incubation (< 1% hydrolysis) spherulites with intact concentric lamellae were rarely seen; instead, discontinuous stacks of phospholipid leaflets were encountered (Figure 2). The appearance of the leaflets suggested that they had split off a larger aggregate of leaflets. In Figure 3 is seen a small spherulite observed after 110 min

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