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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of incubation (80% hydrolysis). Some leaflets jut out from its surface. The adjacent leaflets apparently were cleaved off by exposure to the venom. No attempt was made to correlate the extent of hydrolysis with the electron microscopic appearance of the spherulites. It was apparent, however, that intact spherulites rarely were seen in the hydrolysate.

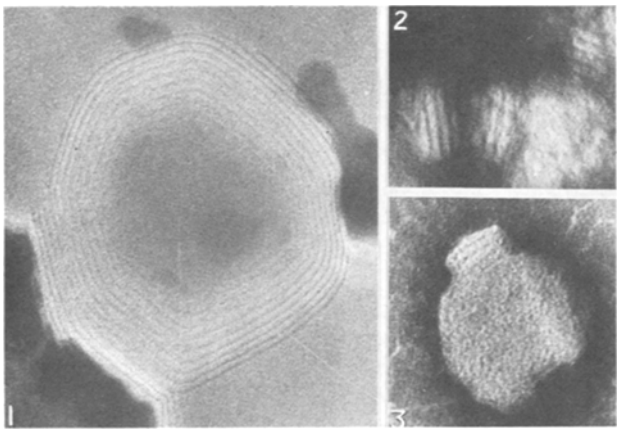

Fig. 1. Lecithin spherulite before exposure to phospholipase A. × 250,000.

Fig. 2. Stacks of bimolecular leaflets from a lecithin solution which had been hydrolyzed for 2 min. × 250,000.

Fig. 3. Lecithin spherulite after hydrolysis for 110 min. × 250,000.

It was expected that phospholipase A would disrupt the bilaminar aggregates of lecithin through the in situ generation of lysolecithin as based on the observations of BANGHAM and HORNE[2]. The results of the present study are consistent with their observations and further indicate that hydrolysis of lecithin by phospholipase A, under some conditions, is accompanied by cleavage of stacks of lamellae from the spherulite.

Zusammenfassung. Durch Phospholipase A des Giftes von *Pseudechis porphyriacus* spalten sich während der Lecithin-Hydrolyse Lamellenstapel von der Oberfläche der Phospholipidspheruliten ab.

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The Mechanism of Blood Vessel Permeability Derangement Under the Influence of Histamine, Serotonin and Bradykinin

Electron microscopic studies have shown[1] that under the influence of inflammatory agents or permeability factors (histamine, serotonin etc.) partial separation of endothelial cells occurs, thus increasing vessel permeability. To explain this phenomenon it was supposed that endothelial cells have contractile structure that contracts under the influence of permeability factors[2,3]. The shortlasting increase of vessel permeability under the conditions described[4] is in favour of this view. If permeability increase is the result of active contractility of cell structure, it must be an energy-requiring process. To verify this point of view, the effects of histamine, bradykinin and serotonin on vessels were studied, tissue respiration being suppressed by cyanide.

In the first series of experiments on rabbits, guinea-pigs and Wistar rats, solutions of NaCN (0.1 ml) (10^{-2} to $10^{-4}M$) in 0.1 *M Tris* HCl-buffer (pH 7.4) were introduced i.c. into shaved parts of the flanks. Then 0.1 ml solutions of bradykinin (0.5 µg)[5], histamine (10 µg) or serotonin (0.5 µg) were introduced into the same parts of skin with different intervals.

In control experiments the mediators were introduced into the parts of skin with *Tris* HCl-buffer previously injected. The injection of NaCN solutions served as an additional control. 20 tests were made on each rabbit, 10 on each guinea-pig and 8 on each rat. Immediately after the s.c. injections, Evans blue solution (20 mg/kg) was administered i.v. to test vessel permeability.

In all cases of control experiments, the mediators increased skin vessel permeability (the diameter of blueing exceeds 9 mm). Cyanide did not cause the derangement of permeability and circulation (the measurements of skin temperature). In the experiments on rabbits, $10^{-2}M$ NaCN fully and $10^{-3}M$ NaCN solutions partially inhibited the responses of vessels to histamine and bradykinin. The most pronounced inhibition occured 10–15 min after cyanide injections (Table).

In guinea-pigs and rats the effects[1] of cyanide were also significant, though somewhat less pronounced than in rabbits. $10^{-2}M$ NaCN solution significantly inhibited the influence of serotonin on rat skin vessels. Analyzing the data described above it is necessary to take into account that the true cyanide concentration in skin was lower than that administered, due to cyanide transfer into circulation.

In the second series the influence of cyanide on the development of vessel permeability derangement was studied on rat mesentery. NaCN (10^{-2}–$10^{-3}M$) solutions in *Tris* HCl-buffer in a volume of 0.1 ml were dropped on the part of the mesentery. In 3 min Indian ink (0.08 ml/100 g) was introduced i.v. and in 2 more min 0.1 ml of bradykinin (1 µg), histamine (5 µg) or serotonin (20 µg) was applied to the parts of mesentery affected by cyanide. In control experiments, mediators were used

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