studied. Cholesterol and phospholipids of serum, liver and aorta were determined daily during 7 days following injection of hormone, to evaluate time response of birds to this estrogen. Early and significant rise of serum and liver cholesterol and phospholipids was observed. Increase of aortic lipids was slower to appear, but significant changes in these parameters were also noted.

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Non-Enzymatic Lysis of Fibrin *s* by Bile.* (25934)

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The fibrin plate was devised by Astrup and Mullertz(1) to serve as a simple substrate for detecting fibrinolytic activity. Lassen(2) demonstrated that heating the fibrin to 86°C for 30 minutes destroyed adsorbed plasminogen. Lysis of unheated but not heated fibrin is interpreted to indicate the presence of plasminogen activators whereas lysis of both types of plate indicates that a proteolytic enzyme is present. Using fibrin plates, Albrechtsen *et al.*(3) found plasminogen activator in many body fluids. We have been unable to find a prior study of fibrinolytic activity in bile, although Albrechtsen(4) and Todd(5) investigated plasminogen activator in body tissues and reported its absence in liver. Consequently, bile was tested on the fibrin plate and appeared to contain a heat-resistant activator. Further investigation, however, showed that the observed lysis was not due to plasminogen activation, but rather was due to simple solution of fibrin *s*(6), formed in the absence of calcium. Fibrin *i*, formed in presence of calcium, was not dissolved by bile, but was susceptible to true plasminogen activators.

Materials and methods. Human or canine bile was either tested within 2 hours of collection or frozen at −20°C for not longer than 2 weeks. Na dehydrocholate (Decholin, Endo), Na taurocholate, (Delta Chemical Works) and Na glycocholate, (Delta and Mallinckrodt Chemical Works) were used as supplied by the manufacturers. *Fibrin plates* (1.2): 0.4 g of bovine fibrinogen (Fraction I, Armour Laboratories) was dissolved in 100 ml of 0.25 M borate-saline buffer, pH 7.4(7), and filtered through Whatman #1 filter paper with suction. Eight ml was pipetted into 100 mm Kimax flat bottomed Petri dishes and clotted with 0.1 ml thrombin (Upjohn, Inc., 50 u/ml in 0.9% saline). Clots were allowed

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TABLE I. Fibrinolysis by Bile.

	Unheated plate	Heated plate
T-tube W	+++*	++
A	+	0
EW	++	0
Surgical specimens		
Cholecystitis RC	+	0
RR	+	0
Dog bile		
Meningitis	+++	0
Total body irradiation	+++	0
Human autopsy		
No gall bladder disease EB	++	0
LG	++	0
Fresh voided urine	+++	0

* + = 10-100 mm² of lysis
++ = 100-200
+++ = 200-300
++++ = 300-400
+++++ = >400

to stand for 10-15 minutes before testing. One half of the plates were heated at 90°C for 20 minutes. Samples were tested by delivering one drop from a 0.1 ml pipette onto unheated and heated plates. Plates were incubated at 37°C for 24 hours. Fibrinolytic activity was recorded as the product of 2 perpendicular diameters of the clear lysis zone. Standard deviation was ± 7.5%. Freshly voided urine was employed as a known plasminogen activator. *Proteolytic assays.* The caseinolytic technic of Norman(8) was utilized without modification.

Results. Bile was tested for activators on heated and unheated fibrin plates (Table I). These results suggested the presence of plasminogen activator in bile. Bile and urine were tested after heating at 60°, 80° and 100°C for one hour. The lytic agent in bile was heat stable, whereas urokinase, a known activator, was destroyed by heating.

To discover what substance in bile was responsible for lysis of unheated fibrin, 1% solutions of several bile salts, Na taurocholate, Na glycocholate, and Na dehydrocholate (Decholin), were tested and found to lyse unheated but not heated fibrin. Lysis occurred readily at 22°C and 37°C but not at 2°C, and the area of lysis depended on concentration. The lytic property of bile salts was also stable at 100°C. As these substances are "biologic detergents," Alconox—a synthetic detergent composed of a mixture of hydrocarbon sulfonates, fatty alcohol sulfates, and complex phosphates—was tested and was a potent lytic agent similar to bile salts.

Bile salts and Alconox uniformly failed to activate proteolytic activity of plasminogen when tested by the caseinolytic technic. Bile salts did not cause delay of clotting of whole blood or produce subsequent clot lysis; Alconox, however, caused hemolysis and an infinite clotting time.

Urea dissolves fibrin *s*, formed in absence of calcium or fibrin stabilizing factor. Upon dialysis to remove urea the clot reforms(9, 10). Thirty per cent urea produced lysis on our plates, indicating presence of fibrin *s*. Heated fibrin *s* plates were not lysed by urea.

Plates prepared with 0.01 M $CaCl_2$ were subjected to 30% urea, bile, and bile salts (Table II). Addition of calcium prevented non-enzymatic lysis of fibrin but did not interfere with plasminogen activators. T-tube bile "W" was contaminated with bacteria and proteolysis was probably due to microbial growth.

Mullertz has reported that fibrin plates formed in the presence of citrate are more sensitive to enzymes than plates free of citrate(11). Because Armour fibrinogen contains 40 to 50% sodium citrate it became important to demonstrate whether the effects noted also occurred with citrate-free fibrin. Citrate was removed from a fibrinogen solution by dialysis for 24 hours against borate buffer with a change of buffer at 12 hours. Plates formed with citrate-free fibrinogen

TABLE II. Fibrinolysis with and without Calcium.

	No calcium	0.01 Ca	Heated
5 M urea	++	0	0
T-tube A	+	0	0
" W	+++	+++	+++
" EW	+	0	0
GB bile EB	+	0	0
10% desiccated ox bile in H_2O	+++	0	0
1% glycocholate	++	0	0
1% taurocholate	++	0	0
1% Alconox	+++	0	0
Urine	+++	+++	0

were sensitive to bile salts, detergent and urea and the effect was blocked by 0.01 M Ca.

Dialysis of lysed clots to remove the bile salt or detergent caused the clot to reform, demonstrating that the fibrin had been dissolved and not digested. Six ml of fibrinogen was clotted and then dissolved with 10 ml of 10% (W/V) bile salts or Alconox. The solutions were dialyzed at 2°C against 0.2 M phosphate buffer, pH 7.4, for 16 hours. At least partial recovery of the clot was evident in all cases, and both citrated and non-citrated clots could be recovered by this method.

Discussion. Olesen reported that bile salts activated fibrinolytic activity in guinea pig serum subjected to precipitation at pH 5.4 in presence of these salts(12). This appears to be a different phenomenon than we are reporting, because the bile salts required the presence of something in serum for fibrinolytic activity. The fibrin plates used by Olesen were not sensitive to direct application of bile salts although they did not have added calcium. This may represent an unexplained difference in fibrin prepared by a different method. Reconstitution of fibrin clots after dissolution by bile salts and detergent seems to be unequivocaal evidence that fibrin *s* was not digested enzymatically, but was dissolved. as it may be by a number of reagents: urea, lithium chloride, lithium bromide, sodium bromide, sodium iodide, guanidine hydrochloride, histamine dihydrochloride, and monochloroacetic acid(13). Whether the above reagents, bile salts and detergents all dissolve fibrin *s* by the same mechanism is undetermined. Only bile salts appear in physiological fluids in sufficient concentration to be confused with plasminogen activators in fibrin plate testing.

Fibrin *i*, formed in presence of calcium and fibrin stabilizing factor (FSF), is a copolymer of fibrin and FSF and is resistant to solubilizing reagents(14). It is suggested, therefore, that fibrin *i* be used in tests for enzymatic fibrinolysis to obviate artefacts such as that seen with bile. Bovine Fraction I (Armour) contains FSF so that addition of calcium during clotting gives fibrin *i*(13).

Summary. Bile salts and detergents cause "lysis" of fibrin *s* by a simple solution of fibrin. Fibrin *i* is not dissolved by bile salts and detergents. Fibrin *i* should be used in fibrinolytic assays for plasmin or plasmin activators.

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