nancy than concomitantly administered estrone and 17α-hydroxyprogesterone acetate.

Summary. Progestins found capable of maintaining pregnancy in rats castrated on the 8th day after insemination included progesterone, 17α-hydroxyprogesterone acetate, 17α-ethyl-19-nortestosterone, desoxycorticosterone acetate, 9α-bromo-11-ketoprogesterone and 6α-methyl-17α-hydroxyprogesterone acetate. The most potent compound in this respect was 6α-methyl-17α-hydroxyprogesterone acetate. Compounds which failed to maintain pregnancy include 17α-ethinyltestosterone, 17α-ethinyl-19-nortestosterone, 17α-ethinyl-17-hydroxy-5(10)-estren-3-one, and 17α-methyltestosterone. With the exception of 17α-hydroxyprogesterone acetate, all compounds which maintained pregnancy when administered concomitantly with estrone also maintained pregnancy when administered alone. Higher doses were required, however, for comparable results. Fair correlation between reported potency in the McPhail or Clauberg rabbit endometrium assays and pregnancy maintenance potency existed only for 6α-methyl-17α-hydroxyprogesterone acetate, 9α-bromo-11-ketoprogesterone and desoxycorticosterone acetate.

ADDENDUM: In experiments recently performed in this laboratory, it was found that pregnancy was maintained in 25/26 castrate rats fed 400 μg or more of 6α-methyl-17α-hydroxyprogesterone acetate/day. Pregnancy was not maintained in any of 10 castrate rats fed less than this amount/day.

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Fibrinolysis. I. Fibrinolytic Activity of Extracts from non-Pathogenic fungi.* (24398)

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Several proteolytic enzymes have been obtained from fungi. In particular, a crystalline protease was isolated by Crewther and Lennox (1) from a strain of *Aspergillus oryzae*. In an investigation to identify alternative sources of fibrinolytic agents, the additional observation was made that extracts of some non-pathogenic fungi are able to lyse purified fibrin and plasma clots of human and bovine source.

Materials and methods. Cultures of 160 fungi were obtained from many Institutions. In this article they are recognized by letters and numbers. The letter was arbitrarily assigned according to source, as shown below. The number which follows was either arbitrar-

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ily assigned or represented that supplied by parent Institution: (A) Department of Botany, Harvard University; (B) U. S. Army, Natick, Mass.; (C) Department of Mycology, Harvard Medical School; (D) Department of Microbiology, and of Botany, University of Mass., Amherst, Mass.; (E) Microbiology Institute, N.I.H., Bethesda, Md.; (F) Department of Microbiology, Duke University School of Medicine, Durham, N. C.; (G) Department of Zoology, University of Mich,. Lansing. Species of the following genuses were considered in the survey: Absidia, Alternaria, Aspergillus, Blastomyces, Candida, Chaetonium, Circinella, Conidiabolus, Cryptococcus, Cunninghamella, Epiderophyton, Epiocum, Geotrichum, Glicoladium, Hellicostylum, Histoplasma, Hormodendrum, Microsporum, Monosporum, Mucor, Mycophyton, Myrothecium, Neurospora, Nocardia, Penicillium, Phialophora, Phycomyces, Piptocephalis, Pleurotus, Pyrenochaeta, Rhizopus, Riptocephalus, Sardaria, Scopulariopis, Sporotrichum, Syncephalastrum, Thamnidium, Trichoderma, Trichophyton, Zygorhyncus. The cultures, received in solid culture media, were transferred to a Sabouraud's dextrose medium (containing Neopeptone, 10 g; Bacto-dextrose, 40 g; Bactor-agar, 15 g and water to 1000 ml). Liquid media were then prepared (containing sucrose 7.2 g; dextrose, 3.6 g; $MgSO_4$ crystals, 1.23 g; KH_2PO_4, 13.69 g; KNO_3 2 g; and H_2O to 1,000 ml). Aliquots of 100 ml were transferred to Erlenmeyer's flasks of 250 ml capacity. A sample of culture, approximately 1 cm in diameter, was transferred to each flask. Cultures were incubated at room temperature (25° C) and grew luxuriously in all cases. After specific time, the content of flasks was filtered on Whatman No. 2 paper under sterile conditions. To one volume of clear filtrate, occasionally stained with pigments produced by the culture, were added 2 volumes of 95% alcohol pre-cooled at –20°C. A mixture was immediately transferred to deep freeze at –20°C. A whitish precipitate formed almost immediately which separated slowly to the bottom. After overnight storage at –20°C, tubes were centrifuged at 2000 rpm/15′/4°C and the supernatant was discarded. The precipitate which contained traces of nitrogen, sugars and a large amount of salts was resuspended in saline 1/10 volume of the original filtrate, by agitation. To test the effect of extracts from fungi on the overall clotting process a system was prepared containing 0.2 ml of human platelet-poor plasma and 0.2 ml of serial dilutions of the fungal extracts. The mixture was then recalcified by adding 0.2 ml of 0.025 M Ca C12. To test the clot lysing activity of the extracts, 0.2 ml of fresh human or bovine platelet-poor plasma (which would not show retraction 4 hours after clotting) were added to 0.2 ml of saline solution and 0.2 ml of the undiluted extract. The mixture was clotted by addition of 0.2 ml of thrombin solution containing 100 NIH units/ml. After verifying that clots had formed normally, they were observed for lysis after 12 hours of incubation in water bath at 37°C. The direct fibrinolytic activity of the extracts was demonstrated by the use of the Astrup-Müllertz plates. Commercially available bovine fibrinogen (Armour) and highly purified human fibrino-

TABLE I. Fungi Producing "Lysing" Extracts.*

No.	Fungus	Range of activity					
		Human clot	Bovine clot	Human fibrin	Bovine fibrin	Casein	Egg albumin
B-82i	*Aspergillus oryzae*	+++	±	++	+	++	++
B-1273	" "	+++	+	++	+	+	—
B-4m	*Aspergillus flavus*	+	+	+	++	+++	+++
D-101	*Absidia coerulea*	±	±	±	+	+	—
A-10	*Aspergillus niduleus* (inactive extract for comparison)	—	—	—	—	—	—

* Extracts were prepared after 6 days of culture of the fungus in liquid media. The range of activity shows considerable variation of lytic activity on various media. Note also some interesting dissociation between ability to digest fibrin and to digest casein and egg albumin.

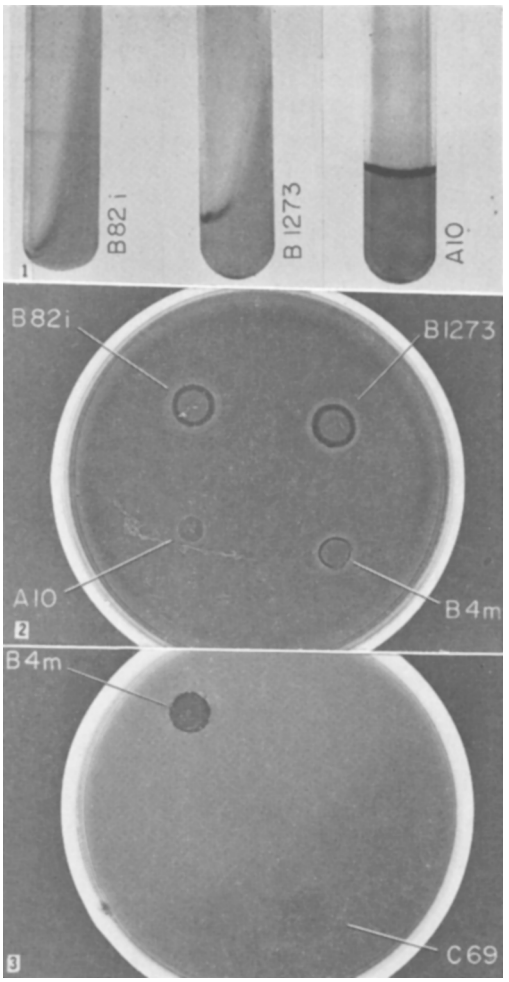

FIG. 1. Lysis of human plasma clots by extracts of fungi. B-82i (active), B-1273 (active), A-10 (inactive). Lysis of plasma clot was recorded after 12 hours of incubation.

FIG. 2. Lysis of heated human fibrin plates by extracts of fungi: active (B82i, B1273); active (B4m); inactive (A10).

FIG. 3. Lysis of bovine heated fibrin plates by extracts of fungi and clarifying effect of other extracts. The left half of plate shows lytic effects (B4m). The clarifying effect is shown on right side of plate (C69) for comparison.

gen were used. Human fibrinogen was prepared by the method of Cohn and purified by the method of Laki(2). The fibrin plates were prepared with the following methods: 1 ml of bovine thrombin containing 100 NIH units/ml were rapidly added to 10 ml of a 1% solution of bovine or human fibrinogen. After quick mixing, the contents of the tube were poured into a Petri dish. The plates were then heated at 85°C for 5 minutes. Three-hundredths ml of the extracts were finally deposited over the plates with a pipette. After incubation at 37°C for 12 hours, the degree of lysis was judged by measuring area of digestion of the fibrin plate. Digestion of casein and of egg albumin was studied by standard technics(3).

Results. Extracts from 4 non-pathogenic fungi were found to lyse human or bovine plasma clots or to destroy plates of human and bovine fibrin (Table I). Table also indicates that extracts from fungus B-1273 showed good activity against human plasma clot and human fibrin, but poor activity against bovine fibrin and casein, none against egg albumin. One ml of extract from fungus B-1273, in fact, had the equivalent lytic activity of 8 γ of trypsin on casein and of at least 250 γ on human fibrin. Experiments including several inactive fungi did not indicate any correlation between clot or fibrin lysing activity and lactic acid production or content in carbohydrates and nitrogen of the extracts. These data, however, are of little value because of the extreme impurity of the material studied. There was only minimal acceleration of clotting time by very low dilutions of extracts from fungi showing lytic activity. At higher concentration, the extracts showed anticoagulant effect, an activity shared by some non-lytic fungi. Extracts from other fungi, which did not lyse plasma clots or fibrin plates, showed a different property. They included a Scopulariopis (C-64), a Trichoderma (C-69) and a *Rhyzopus nigricans* (D-94). Their addition to the plate was followed by considerable clarification of the medium, as the extract apparently "spread" across the plate. Interpretation of this phenomenon is under investigation. Addition of serum to the extract inhibited both lysis and "clarification". Figs. 1, 2 and 3 give a visual demonstration of the phenomena observed.

Although 2 of the 4 "lytic" fungi belonged to the genus *Aspergillus* species *oryzae,* not all strains of the species showed lytic activity. Among 6 strains of *Aspergillus oryzae* studied, 2 were active (B-82i and B-1273) and 4 inactive (B-22b; B-871; B-6735; D-146). It was also noted that optimum activity was

TABLE II. Relationship of Life Time of Culture to Lytic Activity of Extracts of Fungi.*

No.	Fungus	Size of area of lysis (diameter), mm Days of culture 3	6	9	12
B-4m	*Aspergillus flavus*	3	7	3	4
B-1273	" *oryzae*	7	10.5	8	4

* Lytic activity on heated bovine fibrin plate.

reached at one definite point during growth which corresponded to initial formation of spores. It tended to fall rapidly after that (Table II).

Summary. A study of properties of extracts from cultures of 160 non-pathogenic fungi has shown that 4 cultures produce non protein materials able to lyse plasma clots and fibrin, both human and bovine source; at least one shows some specificity against human plasma clots.

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Modifications of "George Wright" Canine Tracheal Divider.* (24399)

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The "George Wright" tracheal divider(1), long used in canine bilateral bronchospirometry, provides access to valuable pulmonary information. Two modifications, necessitated in our pulmonary studies, have made the instrument more useful without altering its basic principle.

Modification No. 1. Anesthetized dogs were placed on dog boards for measurement of bilateral oxygen uptakes (VO_2) and tidal volumes (V_t) by oxygen-filled, Benedict-Roth recording spirometers, using the "Wright" brass-tubed tracheal divider. Under these conditions VO_2 and V_t of the right lung are larger(Rahn 2). However, with the original brass-tubed divider, V_t of the right lung and VO_2 of the left lung were larger.

Study of the regional anatomy of the dog's carina explained these discrepancies. The right pulmonary artery in a supine dog lies directly over the tracheal carinal bifurcation. Because of random differences in neck length and placement of the dog's head below the plane of the carina, a fulcrum point in the neck often was inadvertently established whereby the brass-tubed divider applied leverage upward, partially blocking the right pulmonary artery. Blood flow then shifted to the left lung by this resistance, and left lung VO_2 exceeded right lung VO_2.

To correct this condition, equivalent-diametered polyethylene tubes were substituted on the divider (Fig. 1). These tubes were stiff enough so placement of the divider was not handicapped, yet they were flexible enough to prevent inadvertent establishment of a leverage fulcrum. The VO_2 and V_t of the right lung always were larger than those of the left lung when measured with this modified divider. Right:left % VO_2 and % V_t of these dogs showed close agreement with right:left % weight.

Modification No. 2. Following excision of the 3 lower right lung lobes, leaving only the right apical lobe and complete left lung, the right apical lobe's size and weight increased about 150%. The remaining right apical lobe's expansion alters the angle of tracheal

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