incubation with vinegar containing graded amounts of antibiotics. The major effects were not seen after 2 weeks. The specific action is not known; the stimulus was noted by counting the total population at different periods. Further work is under way using this system to study the effects of antibiotics in adaptation to heat stress.

It is a pleasure to acknowledge with thanks the gift of staphylomycin from its discoverer, Dr. P. de Somers, Professor of Virology, Univ. of Louvain, and oxytetracycline (terramycin chloride) from Pfizer, S. A. Brussels.

1. Luckey, T. D., *Antibiotics, Their Chemistry and Non-Medical Uses,* H. D. Goldberg, Ed., D. Van Nostrand, Princeton, N. J., 1959, p174.

2. Moore, P. R., Luckey, T. D., Everson, G. A., McCoy, E., Elvehjem, C. A., Hart, E. B., *J. Biol. Chem.,* 1946, v175, 437.

3. Luckey, T. D., *Recent Progress in Microbiology,* Almquist and Wiksell, Stockholm, 1959, p340.

4. ———, *Nature,* 1963, v198, 263.

Received November 29, 1962. P.S.E.B.M., 1963, v113.

Effect of Podophyllin (P) and Podophyllotoxine (PT) on the Rat Litter *in utero*. (28296)

J. B. THIERSCH*

Department of Pharmacology, University of Washington, Seattle

In toxicity studies of Podophyllin (P) and Podophyllotoxine (PT)(5), it was shown that the LD_{50} for rats was approximately 15 mg/kg, i.p. Both compounds arrest cell mitosis in metaphase(2,4) and are used in treatment of condyloma accuminata. As the compounds resemble in their action on mitosis the effects of colchicine(2,4), their possible effect on the rat embryo was of interest. Didcock, Jackson and Robson(3) reported intra-amniotic and intraplacental injection of rat fetuses with PT on the 17th day of gestation (g.d.). The drug was lethal to the fetuses in doses of 20 mg by the intra-amniotic route and had an LD_{50} for the fetuses of 100 mg by intraplacental administration. Wiesner and Yudkin(7) destroyed 3 successive litters in Swiss mice in the same mothers with subcutaneous doses of 0.25 mg of PT, given at least 3 days after fertilization.

Controls. Thirty-six control rats, treated with a placebo, produced 98.3% normal fetuses, each 5 g in weight and 4.6 cm in length, and 1.7 $\pm$ 2.4% of all fetuses were resorbed, indicating that more than 4.1% resorption of fetuses is abnormal.

* This work was carried out with the aid of grants from the Population Council, Inc., and USPHS.

Procedure. Both compounds were given to groups of pregnant rats, prepared as described in detail previously(6). Both compounds were given dissolved in distilled water, intraperitoneally. Female rats were paired with males. On findings of massive vaginal sperm, the females were isolated, weighed and injected with P or PT. The animals were sacrificed on the day before littering. The uteri were removed in toto; their contents, fetuses, placentas, implantation sites and sites of fetal resorption, recorded. Surviving fetuses were fixed, cleared and stained with Alizerin. The maternal internal organs were microscopically studied, as were the sites of fetal resorption, and surviving placentas, ovaries and breast tissues.

Results with Podophyllotoxine (Table I). A 2 mg/kg dose, given on the 7th and 8th g.d. (that is, after implantation), led to the loss of 17.5% of all fetuses at term, and stunting of 5.7% of the survivors. Twenty-one percent of the placentas of the resorbed fetuses were found at term together with other live fetuses in the same uterus. Only one animal had its entire litter destroyed in this group and here 13 placental remnants, each 0.3 cm in diameter, were found at term *in utero*. The surviving fetuses were normal in size and weight

TABLE I. Podophyllotoxine in Pregnant Rats.

Dose i.p., mg/kg	Day of dose post coitum	No. rats	Total implant.	Fetuses, total implantation (%)				% mothers with entire litters resorbed	Litter wt	Fetus	
				Normal	Stunted	Dead	Compl. resorb.	Surviving plac. only			Wt	Lgth
2	7 & 8	21	183	66.8	5.7	2.2	15.3	21.4	4	39.9	5.3	4.1
5	7 & 8	10	102	8.7	32.3	—	59	86.5	33.3	17.7	2.8	3.1
5	11 & 12	18	177	5.1	—	—	94.9	100	89	27.4	5.5	4.1
5	15 & 16	6	60	—	41.6	56.8	1.6	100	33.3	9.5	2.4	2.2
.5	6 to 16	22	207	79.3	1.4	4.3	15	85	4.5	37.2	4.9	3.9
Placebo	7 & 8	36	332	98.3	—	—	1.7 ± 2.4	—	—	49	5.0	4.6

but for 5.7% which weighed 2.9 g each and measured 3.1 cm in length. Increasing the dosage of PT to 5 mg/kg i.p., the percentage of resorbed fetuses rose to 59%, and 33% of the litters were totally destroyed. Thirty-two percent of the surviving fetuses were stunted at term, varying from 1.5 to 3.1 cm in size and 1.0 to 1.5 g in weight. The same dosage administered on the 11th and 12th g.d. permitted the survival of only 5% of the fetuses and destroyed 16 out of 18 litters completely (or 89%). The few survivors were found to be normal in size and weight. All placentas survived the fetuses and were found at sacrifice. On the 15th and 16th g.d., the 5 mg/kg doses induced death in 56% of the fetuses, with stunting of all survivors. Thirty-three percent of all litters were completely destroyed. All placentas of the dead fetuses were found at sacrifice. The dead fetuses were attached to them as greenish-brown lumps, measuring 0.5 to 1 cm in length. A group of mother rats treated with 0.5 mg/kg doses from the 6th to the 16th g.d. showed good weight gain. Eighty percent of all fetuses survived; of these, only a rare one was stunted. Gross malformations were not observed in the fetuses at term, and only one litter was completely destroyed by the compound.

Results with podophyllin (Table II). The 5 mg/kg doses given on the 11th and 12th g.d. induced 90% fetal resorption and 63% total litter destruction, with stunting of all survivors. Eighty-one percent of the placentas of the resorbed fetuses were found at term. The 10 mg/kg doses led to total litter destruction when given on g.d. 11 and 12, but permitted the survival of one litter when administered on g.d. 15 and 16. The same dosage given on the 18th and 19th g.d. permitted the survival of only 4 fetuses in a group of 15 mother rats and destroyed 80% of all litters. All survivors were stunted and underweight. In both groups, the dead and necrotic fetuses were not delivered spontaneously but were found *in situ* at sacrifice *in utero*. Complications from extensive intra-uterine hemorrhages occurred in rats treated the 18th and 19th g.d. with loss of 3 mothers. The older

TABLE II. Podophyllin in Pregnant Rats.

Dose i.p., mg/kg	Day of dose post coitum	No. rats	Total implant.	Fetuses, total implantation (%)					% mothers with entire litters resorbed	Litter wt	Fetus	
				Normal	Stunted	Dead	Compl. resorb.	Surviving plac. only			Wt	Lgth
5	11 & 12	11	109	—	9.9	—	90.1	81.5	63.6	7.1	2.6	2.9
10	11 & 12	11	105	—	—	100	—	100	100	—	—	—
20	11 & 12	12	114	—	—	100	—	100	100	—	—	—
10	15 & 16	14	120	5.8	—	94.2	—	100	93	28	4.0	4.0
10	18 & 19	15	113	—	3.5	96.5	—	100	80	3.4	2.6	2.9
1	7 to 21	13	114	19.1	5.7	9.6	65.6	33	46.2	17.7	4.4	3.8
1	11 to 21	13	127	19.4	1.6	4.0	75	56	38.4	15.7	4.6	3.8
.5	6 to 16	23	220	77.5	0.4	4.1	18	80	8.7	45.8	5.3	4.1

fetuses apparently died within 6 to 12 hours following drug administration, with general edema, ascites and anasarca, similar to those observed following desacetyl-thio- and methylcolchicine(6). Three groups of rats were treated with smaller doses over longer periods of gestation. One group received 0.5 mg/kg from the 6th to 16th g.d.; another group received 1 mg/kg from the 11th to the 21st g.d., and a third group received the same doses from the 7th to 21st g.d. In all 3 groups, an effect on the litters was noted. In each group, several animals lost their entire litters. The 0.5 mg/kg doses permitted the survival of 78% of all fetuses. The 1 mg/kg doses resulted in 75% and 79% loss of fetuses. In spite of these findings, no gross malformations of the surviving fetuses were observed.

Microscopically, livers, spleens, pancreas, kidneys, thyroids, mesenteric lymph nodes, intestinal tracts of the mother animals were found to be natural in all groups treated with P and PT. However, the sternal marrow of animals treated with P on the 18th and 19th g.d. showed a mild depression of total cellularity of all nuclear elements.

Surviving placentas. The placentas surviving resorbed, dead or necrotic fetuses of rats treated on the 15th and 16th or 18th and 19th g.d. corresponded to normal structures found at the gestation age of the days of treatment. In several organs, central hemorrhages and necrosis were seen macro- and microscopically. In the groups of rats treated on the 11th and 12th g.d. great variation in placental structures was noted in the surviving remnants. Several placentas appeared to be normal organs, corresponding to the 12th g.d. Others showed varying states of intraplacental hemorrhage and marginal necrosis. Often a network of stroma filled with blood and varying numbers of relatively well-preserved giant cells in the periphery was noted. Placental remnants of rats treated on the 7th and 8th g.d. showed frequently advanced atrophy, necrosis and involution. The fetal capillary placenta was always absent. In all cases of placental remnants *in utero,* even in the absence of a single live fetus, mammary secretion in the mothers was noted.

Discussion. The present findings confirm previous observations of Wiesner and Yudkin (7), and of Didcock and Jackson(3) of the toxicity of P and PT to the fetus *in utero*. An explanation of the action might be found in the work of Baker, Miller, Davison and Smith (1), who reported inhibition of respiration of rat organ homogenates, especially those of the thymus, lymph nodes and spleen. In the present experiments, the absences of gross malformations was striking, even in fetuses from mothers treated from the 7th to the 21st g.d. This indicates that processes vital for the fetuses are affected by P or PT, terminating life. This is in contrast to other compounds which affect less important biochemical systems in the fetus, permitting the embryos to survive but inducing malformations.

Summary. The single LD_{50} for the adult rat was, for P or PT, approximately 15 mg/kg i.p. Toxicity for the rat fetus was tested in groups of pregnant rats injected with varying doses and at different gestation days. The compounds were found to be more toxic to the fetuses than to the mothers, with a peak of fetus sensitivity around midterm (11th and 12th g.d.). Doses of 5 mg/kg i.p., administered on the 11th and 12th g.d. destroyed more than 90% of all fetuses. Doses of 0.5 mg/kg or 1 mg/kg i.p., given daily over longer periods of gestation, led to a loss of 18% to 75% of all fetuses. General stunting, but no gross malformation, was observed in surviving fetuses.

1. Baker-Miller, Z., Davison, C., Smith, P. K., *J. Exp. Med.*, 1949, v90, 525.

2. Cornman, I., *Biol. Bull.*, 1947, v93, 192, 214.

3. Didcock, K., Jackson, D., Robson, J. M., *Brit. J. Pharmacol.*, 1956, v2, 437.

4. King, L. S., Sullivan, M., *Science*, 1946, v104, 244.

5. Philips, F. S., Chenoweth, M. B., Hunt, C. C., *Fed. Proc.*, 1948, v7, 249.

6. Thiersch, J. B., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 479.

7. Wiesner, B. P., Yudkin, J., *Nature*, 1955, v176, 249.

Received March 8, 1963. P.S.E.B.M., 1963, v113.

Lipoprotein Lipase Activity in the Dog Pancreas and Pancreatic Juice.* (28297)

JACQUES I. KESSLER,† MARTIN FINKEL, DAVID A. DREILING AND HENRY D. JANOWITZ

Division of Gastroenterology and Nutrition Laboratory, Department of Medicine and Pancreatic Research Laboratory, Department of Surgery, The Mount Sinai Hospital, New York City

Although the role of circulating lipoprotein lipase (LPL) "clearing factor" in the normal physiology of lipemia-clearing is subject to much controversy, there is increasing evidence that a variety of pathologic hyperlipemic states are associated with either a deficiency or an inhibitor of LPL(1-4). The hyperlipemia of acute pancreatitis in man and experimental animals(5) is well known; however no satisfactory explanation for this phenomenon has been suggested. Recently we have obtained evidence for inhibition of LPL by the plasma of rabbits with experimental acute pancreatitis, in which an elevation of triglycerides was also observed(6). In addition, Meng and Goldfarb(7) have shown that the intact pancreas is necessary for normal mobilization of LPL after heparin injection, and LeVeen, Giordone and Zinberg(8) have suggested that the pancreas may be one of the sources of LPL. Thus a disturbance in LPL metabolism might account for the hyperlipemia seen in pancreatitis. In an effort to clarify such a possible relationship between the lipid aberration of pancreatitis and LPL, canine pancreatic juice and pancreatic tissue were examined for presence of LPL activity.

* Aided by NIAMD, NIH, USPHS Grants.

† Present address: Dept. of Medicine, Jewish General Hospital, Montreal, Canada.