Table I—Assay Results[a] on Ointments

Ointment	Assay Results, % I	II
Lot A	0.0	99.6
Lot B	99.4	99.8
Lot C	97.9	99.2
Average deviation (based on six readings)	±2.1	±2.8

[a] Average of three.

0.1 *N* NaOH solution and 60 ml of water. Heat almost to boiling and cool until the base congeals at the top of the aqueous layer. Then decant and transfer the aqueous layer to a 250-ml volumetric flask, wash the beaker with small portions of water, and transfer the rinsings to a volumetric flask to bring the mixture to volume. Filter if necessary and inject 10–20 μl.

For comparison purposes, inject an identical volume of the appropriate standard solution after the assay sample is eluted.

Calculations—Since preliminary investigations indicated that the peak area of each ingredient was directly related to the concentration (range of 4–8 μg for I and of 2–4 μg for II), the results on I and II were calculated by direct comparison of the peak areas:

$$\frac{\text{corrected } A_a}{A_s} \times 100 = \text{percent of label claim} \qquad \text{(Eq. 1)}$$

where corrected A_a = peak area of the assay × (1.667/weight of sample in grams), and A_s = peak area of the standard solution.

A typical liquid chromatogram is shown in Fig. 1, and the assay results are presented in Table I.

DISCUSSION

The results (Table I) indicate that I and II in combinations can be assayed directly by high-pressure liquid chromatography. The separation of I from II is excellent (Fig. 1). The method is accurate, rapid, and simple. The three ointment bases used did not interfere with the assay; other bases may interfere. Any other base should be checked for interference by using the assay procedure on the plain base. It is necessary to run both the assay and the standard with the same lot of the chromatographic solvent, since slight differences in pH may change the area of the peak.

REFERENCES

(1) V. D. Gupta, *J. Pharm. Sci.*, **61**, 1625(1972).

(2) V. D. Gupta and A. N. Deleon, *Indian J. Hosp. Pharm.*, **10**, 141(1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 28, 1975, from the *College of Pharmacy, University of Houston, Houston, TX 77004*

Accepted for publication February 13, 1976.

Effects of Aspirin and Acetaminophen on Fetal and Placental Growth in Rats

W. C. LUBAWY ˣ and R. J. BURRISS GARRETT

Abstract □ Aqueous suspensions of aspirin or acetaminophen (125 and 250 mg/kg/day) were administered orally to pregnant Sprague–Dawley rats on Days 8–19 of gestation. On gestation Day 20, each rat was sacrificed and the uterus was examined *in situ*. Each fetal–placental unit was resected and examined. Fetuses from rats given 125 or 250 mg/kg/day of aspirin were shorter and weighed less than those obtained from control rats. In animals receiving the higher dose of aspirin, the placentas were smaller and the number of fetal resorptions was increased. Acetaminophen (250 mg/kg/day) did not affect fetal length or weight or the incidence of resorptions. Acetaminophen interfered less with the normal growth of the rat fetus and placenta than did aspirin.

Keyphrases □ Aspirin—effects on fetal and placental growth, rats □ Acetaminophen—effects on fetal and placental growth, rats □ Fetal growth—effects of aspirin and acetaminophen, rats □ Placental growth—effects of aspirin and acetaminophen, rats

The antigonadal and antireproductive activities of the two most commonly used nonprescription analgesic drugs were reported previously. Chronic administration of acetaminophen to rats produced a decrease in testicular weight and impaired spermatogenesis (1). Also, chronic administration of acetaminophen significantly decreased fecundity in mice, but similar doses of aspirin were ineffective (2). Aspirin administration interfered with the rapid increase in ovarian size following hemicastration (3) and also caused embryotoxicity and teratogenecity in rats (4).

Many studies document the effects of salicylates on fetal growth and development (4–10); however, no studies are known that directly compare the effects of aspirin to those of acetaminophen. Since aspirin and acetaminophen may influence gonadal function, normal reproduction, and the growth of rapidly developing tissue, the effects of these widely used analgesics on fetal and placental growth were compared.

EXPERIMENTAL

Female Sprague–Dawley rats[1] were used. For gravid rats, the sperm-positive date indicated by the supplier was designated Day 0 of pregnancy. If allowed to progress to term, parturition occurred on Day 21. Animals were randomized, housed three or four to a wire mesh bottom cage, and fed rat chow[2] and water *ad libitum*.

From the 8th through the 19th day of gestation, groups of 21 sperm-positive rats were weighed and received aspirin[3] or acetaminophen[4] at 0, 125, or 250 mg/kg by gavage. Groups of nonmated female rats, comparable in age, were treated according to the same regimen. Each drug was suspended fresh daily in 0.5% methylcellulose with a homogenizer[5] at a concentration such that 1 ml was administered for every 200 g of body weight. Drugs were administered without anesthesia, since Kimmel *et al.* (5) observed abnormalities in offspring from rats anesthetized with ether.

[1] Holtzman Co., Madison, Wis.
[2] Purina.
[3] Merck and Co., Rahway, N.J.
[4] Provided by McNeil Laboratories, Fort Washington, Pa.
[5] Potter–Elvehjem.

Table I—Effect of Aspirin and Acetaminophen on Weight Gain in Pregnant and Nonpregnant Rats[a]

Body Weight	Vehicle (0.5% Methylcellulose)	Aspirin (125 mg/kg/day)	Aspirin (250 mg/kg/day)	Acetaminophen (125 mg/kg/day)	Acetaminophen (250 mg/kg/day)
Nonpregnant rats:					
Initial weight[b], g	214.8 ± 5.4	212.3 ± 2.7	220.7 ± 6.4	219.9 ± 2.1	219.3 ± 3.3
Weight[b] after 12 days, g	242.2 ± 3.5	241.0 ± 2.5	238.0 ± 4.5	243.1 ± 3.5	236.4 ± 2.9
n	10	7	9	7	8
Pregnant rats:					
Initial weight[b], g	240.6 ± 4.6	238.7 ± 2.6	239.9 ± 4.7	240.5 ± 4.7	237.5 ± 4.4
Weight[b] after 12 days, g	332.6 ± 8.4	336.3 ± 4.9	298.9 ± 9.6[c]	320.4 ± 7.6	347.3 ± 4.8
n	18	21	15	17	16

[a] Drugs were administered by gavage once daily for 12 days. [b] Mean ± *SE*. [c] Differs from control at $p < 0.05$.

On Day 20 of gestation, the sperm-positive animals were sacrificed with ether overdosage. The uterus was examined *in situ* for implantation sites, and fetal viability was determined. Each fetal–placental unit was resected. The chorioallantoic placenta was dissected free of the fetus, and both were blotted and weighed. The length of each fetus was measured from crown to rump.

Continuous variables from fetuses and placentas grouped according to treatment were analyzed using an analysis of variance or a *t* statistic. Discrete variables were subjected to the Fisher Exact Probability Test or the χ-square test according to the criteria outlined by Siegel (11).

RESULTS

Among the nonpregnant rats, no significant differences occurred in average body weights between the various treatment groups and comparable controls at the end of the 12-day treatment period (Table I). Although the actual amount of weight gained during the experimental period by the high dose (250 mg/kg/day) aspirin-treated nonpregnant rats was approximately 10 g less than the average weight gain of the control animals, this difference was not statistically significant. Pregnant rats given aspirin at 250 mg/kg/day gained less weight than those receiving the vehicle, and some of this difference was probably due to the effect of the aspirin on the fetuses and placentas.

Data presented in Table II indicate some effects of aspirin and acetaminophen on fetal and placental development. Four of 17 pregnant rats given 125 mg/kg/day of acetaminophen and 12 of 15 pregnant rats given 250 mg/kg/day of aspirin had at least one resorption site. This incidence was statistically different from that seen in rats given the vehicle only in the aspirin-pretreated group. In this group, 44% of the fetuses were resorbed and the weight and length of the remaining fetuses were reduced. Furthermore, placental growth was depressed in these animals. Acetaminophen at a comparable dose level (250 mg/kg/day) did not affect fetal length, fetal weight, or placental weight.

In rats receiving 125 mg/kg/day of aspirin, fetal growth was depressed but placental weight was not influenced. The incidence of resorptions was not increased over control values. In contrast, 125 mg/kg/day of acetaminophen produced more resorptions than occurred in the control group, 16 *versus* 0%, respectively, and also shorter fetuses. At this dose, acetaminophen did not affect either fetal or placental weight.

DISCUSSION

After 12 days of drug administration, no difference resulted in the average body weight of vehicle-treated and analgesic-treated nonpregnant animals. Thus, it seems unlikely that the small fetal and placental sizes in the aspirin-treated rats resulted from reduced food consumption by the dam. This interpretation is only speculative, however, since the compounds may affect the appetite of pregnant rats differently than that of nonpregnant rats. Fetal growth inhibition produced by aspirin could be a result of some of the many biochemical actions of the salicylates such as altered protein synthesis (12–14). Other actions of the salicylates such as uncoupling oxidative phosphorylation (15) and interfering with prostaglandin synthesis (16) may also be involved.

In contrast to aspirin, acetaminophen had little effect on fetal and placental growth. Shorter fetuses did occur in pregnant rats receiving the lower dose of acetaminophen than in those receiving the high dose. No rigorous conclusions about these results should be drawn since the data were obtained from a single experiment. However, lack of a classical dose–response relationship has been reported for other actions of acetaminophen. Dikstein *et al.* (17) reported that doses of 2–10 mg/kg/day of acetaminophen produced a greater increase in the width of the female rat tibial epiphysial cartilage than did doses of 20 mg/kg/day.

As with all animal experiments, these results cannot be extrapolated to other species. Mechanisms involved in fetal development and teratogenesis are so complicated that any conclusions concerning the relative safety of each analgesic during human pregnancy must be drawn with caution. Women taking large doses of salicylates during pregnancy may have a more complicated delivery (18), but even daily

Table II—Effect of Aspirin and Acetaminophen on Placental Weight and Fetal Resorption, Viability, Weight, and Length

Parameter	Vehicle (0.5% Methylcellulose)	Aspirin (125 mg/kg/day)	Aspirin (250 mg/kg/day)	Acetaminophen (125 mg/kg/day)	Acetaminophen (250 mg/kg/day)
Number of pregnant rats	18	21	15	17	16
Number of implant sites	140	211	145	149	155
Number of resorption sites	0	3 (1%)	64 (44%)[a]	24 (16%)[a]	0
Number of viable fetuses	140	208	79	125	155
Number of rats with at least one resorption	0	3 (14%)	12 (80%)	4 (24%)	0
Average fetal weight, g[b]	3.86 ± 0.03	3.54 ± 0.03[a]	2.55 ± 0.06[a]	3.82 ± 0.04	3.89 ± 0.04
Average fetal length, cm[b]	3.73 ± 0.02	3.63 ± 0.02[a]	3.21 ± 0.03[a]	3.65 ± 0.01[a]	3.77 ± 0.02
Average placental weight, g[b]	0.63 ± 0.01	0.61 ± 0.01	0.52 ± 0.01[a]	0.64 ± 0.01	0.64 ± 0.01

[a] Differs from control at $p < 0.05$. [b] Mean ± *SE*.

doses of 6.5 g taken during an entire pregnancy have resulted in the birth of a normal healthy neonate (19). However, in an animal such as the rat in which a wide variety of compounds can affect fetal growth, acetaminophen in the doses used in this study seems to produce less of an adverse effect than does aspirin.

REFERENCES

(1) E. M. Boyd, *J. Clin. Pharmacol.*, **10**, 222(1970).

(2) H. N. Wright, *Toxicol. Appl. Pharmacol.*, **11**, 280(1967).

(3) M. Goldman and C. A. Poppens, *ibid.*, **24**, 159(1973).

(4) J. D. McColl, M. Globus, and S. Robinson, *ibid.*, **7**, 409(1965).

(5) C. A. Kimmel, J. G. Wilson, and H. J. Schumacher, *Teratology*, **4**, 15(1971).

(6) K. S. Larsson and H. Bostrom, *Acta Paediatr. Scand.*, **54**, 43(1965).

(7) S. C. Smith and I. W. Monie, *Teratology*, **2**, 1(1969).

(8) K. T. Szabo, S. M. Free, H. A. Birkhead, Y. J. Kange, E. Alston, and M. Henry, *Toxicol. Appl. Pharmacol.*, **19**, 371(1971).

(9) J. Warkany and E. Takacs, *Am. J. Pathol.*, **35**, 315(1959).

(10) E. Takacs and J. Warkany, *Teratology*, **1**, 109(1968).

(11) S. Siegel, "Non Parametric Statistics," McGraw-Hill, New York, N.Y., 1956, p. 110.

(12) R. D. Pawkins, B. J. Gould, and M. J. H. Smith, *Biochem. J.*, **99**, 702(1966).

(13) K. Janakidevi and M. J. H. Smith, *J. Pharm. Pharmacol.*, **22**, 51(1970).

(14) *Ibid.*, **22**, 249(1970).

(15) J. T. Miyahara and R. Karler, *Biochem. J.*, **97**, 194(1965).

(16) J. R. Vane, *Nature*, **231**, 232(1971).

(17) S. Dikstein, M. Grotto, V. Zor, M. Tamari, and F. G. Sulman, *J. Endocrinol.*, **36**, 257(1966).

(18) R. Lewis and J. Shulman, *Lancet*, **2**, 1159(1973).

(19) L. Garrettson, J. A. Procknal, and G. Levy, *Clin. Pharmacol. Ther.*, **17**, 98(1975).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 8, 1974, from the *College of Pharmacy, University of Kentucky, Lexington, KY 40506*

Accepted for publication February 9, 1976.

Supported in part by University of Kentucky Research Committee Grant 201-12-83100-63039.

The authors acknowledge the assistance of Mr. Michael Chestnut, Mr. Douglas Droz, Ms. Kay Gaines, and Mr. John Triplett with the manipulative procedures.

[x] To whom inquiries should be directed.

Baeocystin in *Psilocybe semilanceata*

DAVID B. REPKE [*x] and DALE THOMAS LESLIE [‡]

Abstract □ Baeocystin and psilocybin were found in extracts of a variety of *Psilocybe semilanceata*. Psilocybin (but not baeocystin) was also detected in extracts of a related species, *Psilocybe pelliculosa*. Traces of psilocin were present in these two species. The structures of the isolated compounds were corroborated using mass spectrometry and UV spectroscopy.

Keyphrases □ Baeocystin—isolated from extracts of *Psilocybe semilanceata*, identified by mass spectrometry and UV spectroscopy □ Psilocybin—isolated from extracts of *Psilocybe semilanceata* and *P. pelliculosa*, identified by mass spectrometry and UV spectroscopy □ *Psilocybe semilanceata*—extracts of carpophores, baeocystin and psilocybin isolated, identified by mass spectrometry and UV spectroscopy

The genus *Psilocybe* contains many species with hallucinogenic properties (1). Their use for religious purposes in Mexico was first recorded 4 centuries ago (2). However, only in the last 35 years has serious study been devoted to this subject (3). In 1959, two active principles were isolated from several members of this genus (4): the 4-hydroxy-3-(2-dimethylaminoethyl)-indole (psilocin) and its corresponding phosphate ester (psilocybin). Since then psilocin and psilocybin have been detected in other members of *Psilocybe* and related genera (5).

Heim (6) suggested that the hallucinogenic mushroom described in a 16th century English report was *Psilocybe semilanceata* (Fr.) Kummer (Strophariaceae), considered the type species of the genus (7, 8). Investigators have verified the presence of psilocybin (but not psilocin) in several European collections of *P. semilanceata* (9–12).

The presence of the psilocybin analogs 4-phosphoryloxy-3-(2-methylaminoethyl)indole and its demethyl counterpart (baeocystin and norbaeocystin, respectively) have been reported only in *P. baeocystis* Singer and Smith (13). These compounds were not found in *P. attrobrunnea* (Lasch) Gillet, *P. pelliculosa* (Smith) Singer and Smith, *P. caerulipes* (Peck) Sacc., or *P. strictipes* Singer and Smith (14).

During investigations of the indole alkaloids of the genus *Psilocybe*, a compound with a mobility slightly slower than psilocybin was consistently detected on thin-layer chromatograms of extracts of a Pacific Northwest variety of *P. semilanceata*. This compound was observed in all collections of this species examined over several years.

EXPERIMENTAL[1]

Freeze-dried carpophores of *P. semilanceata*[2], 824 mg, were ground to a powder and extracted by shaking with methanol at room tem-

[1] TLC was carried out using 0.25- and 1.0-mm layers of silica gel GF on glass plates. The solvent system used was 1-propanol–5% ammonium hydroxide (5:2) (13). Mass spectra were obtained with an Atlas CH-4 spectrometer *via* direct inlet. The probe temperature was 300°, and the ion source potential was 70 ev. A Cary model 15 spectrophotometer was used to determine UV spectra.

[2] The collections are in the herbarium of the Escuela Nacional de Ciencias Biológicas, I. P. N., Mexico, D. F., Mexico, and some are also on deposit at the herbarium of the University of Michigan, Ann Arbor, Mich. Carpophores of *P. semilanceata* were collected in pastures at the following locations: Grays Harbor County, Wash., 1972–1975 [LESLIE 1351 (also at MICH), 1807 (also at MICH), 2426 (also at MICH), and 2647]; Jefferson County, Wash., 1973–1975 [LESLIE 1843 (also at MICH), 2409 (also at MICH), and 2664]; Randle, Wash., 1975 (LESLIE 2710); and Sixes, Ore., 1975 (LESLIE 2781). Collections of *P. pelliculosa* were found in forests on wood debris and sawdust in Grays Harbor County, Wash., 1973 (LESLIE 1822) and Maytown, Wash., 1975 (LESLIE 2758). The taxon, designated here as *P. semilanceata*, conforms to published descriptions of the European *P. semilanceata*, but some doubt remains concerning this specific placement which further study may clarify.