influences. IV. Notes on the physicochemical and immunological characteristics of an enterovirus that depresses the growth of mice. J. Exp. Med., *130:* 955 (1969).

21. Lee, C. J., and Dubos, R.: Lasting biological effects of early environmental influences. VII. Metabolism of adenosine 3',5'-monophosphate in mice exposed to early environmental stress. J. Exp. Med., *135:* 220 (1972).

22. Lee, C. J., and Dubos, R.: Lasting biological effects of early environmental influences. VIII. Effects of neonatal infection, perinatal malnutrition and crowding on catecholamine metabolism of brain. J. Exp. Med., *136:* 1031 (1972).

23. Lefkowitz, R. J., and Haber, E.: A fraction of the ventricular myocardium that has the specificity of the cardiac beta-adrenergic receptor. Proc. Nat. Acad. Sci. U.S.A., *68:* 1773 (1971).

24. Lefkowitz, R. J., Haber, E., and O'Hara, D.: Identification of the cardiac beta-adrenergic receptor protein: Solubilization and purification by affinity chromatography. Proc. Nat. Acad. Sci. U.S.A., *69:* 2828 (1972).

25. Lefkowitz, R. J., Sharp, G. W. G., and Haber, E.: Specific binding of β-adrenergic catecholamines to a subcellular fraction from cardiac muscle. J. Biol. Chem., *248:* 342 (1973).

26. Miledi, R., Molinoff, P., and Potter, L. T.: Isolation of the cholinergic receptor protein of *Torpedo* electric tissue. Nature, *449:* 554 (1971).

27. Molinoff, P. B., and Axelrod, J.: Biochemistry of catecholamine. Ann. Rev. Biochem., *40:* 465 (1971).

28. Moore, B. W.: A soluble protein characteristics of the nervous system. Biochem. Biophys. Res. Commun., *19:* 739 (1965).

29. Moore, B. W., and Perez, V. J.: In: F. C. Carlson: Physiological and Biochemical Aspects of Nervous Integrity, p. 343 (Prentice-Hall, Englewood Cliffs, N.J., 1968).

30. Moore, S., and Stein, W. H.: Chromatographic determination of amino acids by the use of automatic recording equipment. Methods Enzymol. *6:* 819 (1963).

31. Munro, H. N.: Metabolic regulation in relation to cell development. Fed. Proc., *29:* 1490 (1970).

32. Naeye, R. L., Blanc, W., and Paul, C.: Effect of maternal nutrition on the human fetus. Pediatrics, *52:* 494 (1973).

33. Schramm, M., Feinstein, H., Naim, E., Lang, M., and Lasser, M.: Epinephrine binding to the catecholamine receptor and activation of the adenylate cyclase in erythrocyte membranes. Proc. Nat. Acad. Sci. U.S.A. *69:* 523 (1972).

34. Spackman, D. H., Stein, W. H., and Moore, S.: Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem., *30:* 1190 (1958).

35. Tell, G. P. E., and Cuatrecasas, P.: β-Adrenergic receptors: Stereospecificity and lack of affinity for catechols. Biochem. Biophys. Res. Commun., *57:* 793 (1974).

36. Vincendon, G., Waksman, A., Uyemura, K., Tardy, J., and Gombos, G.: Ultracentrifugal behavior of beef brain S-100 protein fraction. Arch. Biochem. Biophys., *120:* 233 (1967).

37. Weber, K., and Osborn, M.: The reliability of molecular weight determination by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem., *244:* 4406 (1969).

38. Winick, M., and Rosso, P.: The effect of severe early malnutrition on cellular growth of human brain. Pediat. Res., *3:* 181 (1969).

39. Winick, M., Rosso, P., and Brasel, J. A.: Malnutrition and cellular growth in the brain: Existence of critical periods. In: Lipids, Malnutrition and the Developing Brain, p. 199 (Ciba Foundation Symposium, 1971).

40. Charles River Breeding Laboratories, Inc., Wilmington, Mass.
41. Dietrich & Gambrill, Inc., Frederick, Md.
42. Pharmacia Fine Chemicals, Inc., Piscataway, N. J.
43. Eastman Kodak Co., Rochester, N. Y.
44. Schwarz BioResearch, Inc., Orangeburg, N. Y.
45. Calbiochem, Los Angeles, Calif.
46. ICI America, Inc., Bridgeport, Conn.
47. Union Carbide Corp., Chicago, Ill.
48. Fisher Scientific Co., Fair Lawn, N. J.
49. Buchler Instrument, Inc., Fort Lee, N. J.
50. Sigma Chemical Co., St. Louis, Mo.
51. Worthington Biochemical Corp., Freehold, N. J.
52. Brinkmann Instruments, Westbury, N. Y.
53. Requests for reprints should be addressed to: C.-J. Lee, Sc.D., Bureau of Biologics, Bldg. 29, National Institutes of Health, Bethesda, Md. 20014 (USA).
54. Accepted for publication April 10, 1975.

Inhibition of RNA Synthesis by Acetyl Salicylate and Actinomycin D during Early Development in the Mouse

ANIL B. MUKHERJEE, MARIA CHAN, RICHARD WAITE, MARTIN I. METZGER, AND SUMNER J. YAFFEE[29]

Department of Pediatrics, School of Medicine, State University of New York at Buffalo, and Children's Hospital, Buffalo, New York, USA

Extract

Experiments were designed to ascertain and compare the effects of acetyl salicylate and actinomycin D on RNA synthesis in mouse oocytes *in vitro* and *in vivo*. After exposure to the drugs the effects on RNA synthesis were measured by incorporation of [³H]uridine and autoradiography. The results indicate that acetyl salicylate inhibits RNA synthesis in the treated oocytes as does actinomycin D. The only difference in the effects of these two drugs is that salicylate inhibits RNA synthesis to a much lesser degree than does actinomycin D. Effects from a short exposure to salicylate may be reversible; the same effects with actinomycin D cannot be reversed. *In utero* exposure of the female fetus may lead to partial or total sterility (depending on the dose and time of exposure) of that fetus and/or abnormal development of the progeny from those mice (F2).

These results suggest that RNA synthesis in early oogenesis is a vital part of later development of the oocytes in adult mouse ovary. Inhibition of RNA may be one of the causes of malformations and sterility.

Speculation

During early stages of development a species of RNA (known as masked messenger RNA) is synthesized by the oocyte nucleus and stored in the cytoplasm. After fertilization this RNA is utilized for the production of proteins necessary for the development of the embryo. If this RNA synthesis is inhibited by some agent (e.g., drugs) malformation or infertility could ensue in the female progeny.

Information on the molecular mechanisms by which some drugs cause malformation in the developing fetus is virtually nonexistent. There is increasing evidence, however, that regulation of early animal development may be dependent on sequential synthesis of different messenger RNA molecules (mRNA). These mRNA molecules are stored as stable cytoplasmic ribonucleoprotein particles, which subsequent to fertilization are utilized for the production of specific proteins for a limited time during development (7). This species of nucleic acid is known as masked messenger RNA. To date its presence has been documented in amphibian and sea urchin eggs (4). One possible mechanism leading to induction of malformation is by specific inhibition of such transcription and/or translation of this r asked messenger RNA during development. It is already known t at an inhibitor of RNA synthesis, such as actinomycin D, caus s developmental defects in amphibian (4) as well as in mammali. 1 eggs (2, 23). It would seem reasonable to believe that a block i RNA synthesis for a short period of time during development t ther directly or indirectly may be responsible for at least some of t e large number of anomalies induced by drugs in experimental an mals.

The present investigations were designed to ascertain and compare the effects of acetyl salicylate upon RNA synthesis in mouse oocytes with those of actinomycin D, which is a known inhibitor of DNA-dependent RNA synthesis. Additionally, we administered each of these drugs at a specific time during pregnancy to ascertain the immediate effects on the first generation (F1) and to determine long term effects on the second generation progeny produced by the mating of F1 females and normal untreated males.

MATERIALS AND METHODS

OOCYTE COLLECTION AND CULTURE

Follicular oocytes were obtained from the ovaries of 8-10-week-old randomly bred ICR/Ha strain mice. The ovaries were dissected out and placed on a depression slide containing a mixture of one-half normal saline and one-half Whitten and Bigger's oocyte culture media (22). They were then teased with a 20-gauge needle and fine forceps to release the oocytes into the media. Subsequent to this procedure, the oocytes with well-defined germinal vesicles were collected into a micropipette and put into a plastic Petri dish containing full strength media under oil. All the oocytes needed for one experiment were collected and put into culture as quickly as possible to avoid the difference in culture time. Twelve to 15 mice were killed each time to obtain approximately 600 oocytes. The cultures were than incubated at 37° in 5% CO_2 in air. Four types of experiments were performed.

In Vitro Treatment of Oocytes with Actinomycin D and Acetyl Salicylate. Bloom and Mukherjee (2) have shown that actinomycin D at a concentration of 1×10^{-1} $\mu g/ml$ or higher prevented maturation of oocytes. However, at a concentration of 1×10^{-2} and 1×10^{-3} $\mu g/ml$, oocytes reached metaphase II, indicating apparent maturity. We have used these three concentrations of actinomycin D (1×10^{-1}, 1×10^{-2}, and 1×10^{-3} $\mu g/ml$). After exposure to actinomycin D for 30 min, oocytes were transferred to media containing [³H]uridine (5 $\mu Ci/ml$ sp act; 1,900 mCi mM (26) and left in this media for 4 hr. Similarly, oocytes were treated in acetyl salicylate concentrations ranging from $1 \times 10^{-1} \mu g/ml$ to 10 $\mu g/ml$ for 30 min, followed by exposure to [³H]uridine (5 $\mu Ci/ml$) for 4 hr. The control cultures received equal volumes of the solvent, normal saline. The oocytes were then washed thoroughly in saline, fixed on the slide with 1:3 glacial acetic acid and absolute methanol, and air dried. All slides were extracted with trichloroacetic acid (5%) at 4° for 30 min to eliminate the unincorporated isotopic pool. After this, they were coated with Kodak NTB_2 nuclear track liquid emulsion and exposed in the dark at 4° for 3 weeks before developing. They were stained in buffered Giemsa. All slides were coded and scored blindly by one

person. Some slides were digested with RNAase and subsequent autoradiography revealed no radioactivity, indicating the incorporation of [³H]uridine solely in the RNA.

In Vivo Treatment of Oocytes with Actinomycin D and Acetyl Salicylate. For this purpose actinomycin D and acetyl salicylate were injected (1.5 ml/20 g body wt) intraperitoneally 1 hr before injection of [³H]uridine (50 $\mu Ci/20$ g body wt). The control group received the same volume of solvent plus [³H]uridine. For each treatment, six mice were used. The oocytes were isolated from the ovary and autoradiographs prepared as described above. They were coded and scored blindly by one individual for labeled and nonlabeled oocytes.

In Vitro Fertilization and Development of Treated Oocytes. From our previous experience in treatment of mouse oocytes with actinomycin D and acetyl salicylate and their subsequent development, and from the data presented by Bloom and Mukherjee (2), we arrived at a dosage of these drugs which consistently prevents RNA synthesis without apparent morphologic change in the maturation process of the oocyte. To find out whether or not these oocytes have full developmental potential, they were utilized for *in vitro* fertilization and subsequent development to the blastocyst stage. The concentration of the two drugs used in *in vitro* experiments were actinomycin D, 1×10^{-3} $\mu g/ml$, and acetyl salicylate, 10 $\mu g/ml$. The oocytes were exposed to these drugs for 1 hr and then transferred to fresh drug-free media and allowed to grow to maturity for 10-12 hr. They were then transferred to the fresh culture media with capacitated sperm suspension for *in vitro* fertilization as described by Mukherjee (12). The number of oocytes undergoing cleavage was recorded as evidence for fertilization.

Inhibition of RNA Synthesis in Fetal Ovary and Its Consequences. Since we obtained clear indication that acetyl salicylate does inhibit RNA synthesis in maturing oocytes, we wanted to see the consequences of this inhibition of RNA synthesis in the fetal ovary. In our previous studies we found that RNA synthesis in fetal ovaries takes place as early as 16-18 days of gestation with cytoplasmic storage in the oocyte (14). The control animals, which were at their 16th-18th day after the appearance of vaginal plug, received an intraperitoneal injection of 1.5 ml normal saline divided into three equal injections followed in 1 hr by 50 μCi of [³H]-uridine/mouse. The experimental group received a total of 1.5 ml 10 $\mu g/ml$ acetyl salicylate/mouse in three equal injections on the 16th day and again on the 17th day when peak RNA synthesis occurs. At this time 50 μCi/mouse of [³H]uridine was also administered. Similarly, 1.5 ml actinomycin D at a concentration of 1×10^{-2} $\mu g/ml$ was injected into each mouse in three equal doses before the injection of [³H]uridine as described for acetyl salicylate. When these progeny were born they were allowed to grow to maturity. At this time, the ovaries were taken out, oocytes were isolated, and autoradiographs were prepared. In another group of animals only the drugs were injected into the mother on the 16th-18th day of gestation but no isotope was administered. When these mothers delivered, the progeny were allowed to grow to maturity. At 6-8 weeks of age the female progeny were mated with males of proven fertility to see whether normal pregnancy resulted. These data were compared with that of the control progeny whose mothers received equal volumes of the drug solvents on the same days as the drug was introduced to the experimental group.

RESULTS

The normal process of maturation of oocytes *in vitro* has been published elsewhere (13). Approximately 9 of the oocytes reached second metaphase of meiosis at 16-17 hr after initiation of the cultures. In the labeling experiments with [³H]uridine it has been observed that appreciable isotope incorporation took place between 2 6 hr. Therefore, in all experiments a 4-hr pulse was used. incorporation of [³H]uridine was indeed into the RNA of the oocytes. This was confirmed because (1) the isotope was not
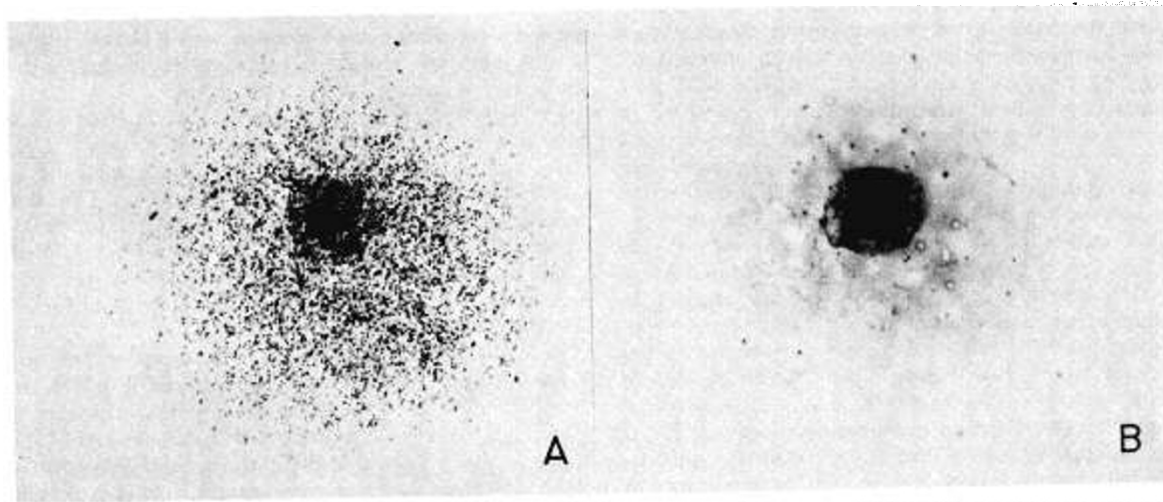
Fig. 1. RNA synthesis in oocytes maturing *in vitro*. *A:* [³H]uridine incorporation in a mouse oocyte. The oocytes were extracted with cold trichloroacetic acid (5%) before emulsifying for autoradiography. *B:* the same oocyte was digested with RNase and the emulsion stripped off and subsequently re-emulsified. When developed 20 days after exposure in the dark, no appreciable grains could be detected. This indicates that the [³H]uridine label was incorporated solely into the RNA.

Table 1. *In vitro effects of actinomycin D and acetyl salicylate on RNA synthesis in mouse oocytes*

Drug	Dose, μg/ml	No. oocytes treated	No. labeled	No. unlabeled	labeled, %
Actinomycin D	1×10^{-1}	276	4	272	1.4
	1×10^{-2}	258	17	241	6.6
	1×10^{-3}	250	28	222	11.2
Control	Culture media	280	235	45	83.9
Acetyl salicylate	10	269	6	263	2.2
	1×10^{-1}	280	21	259	7.5
	1×10^{-3}	256	44	212	17.1
Control	Culture media	260	229	31	88.0

Table 2. *In vivo treatment of oocytes with actinomycin D and acetyl salicylate*

Drug	Dose (1.5 ml), μg/ml	No. mice treated	No. oocytes studied	No. labeled oocytes/No. unlabeled	Labeled oocytes, %
Actinomycin D	1×10^{-1}	10	320	9/320	2.8
	1×10^{-2}	10	300	31/269	10.3
	1×10^{-4}	10	332	68/264	20.4
Control	N saline	10	315	300/15	90.5
Acetyl salicylate	10	10	330	16/314	4.8
	1×10^{-2}	10	325	36/289	11.0
	1×10^{-3}	10	336	79/257	23.5
Control	N saline	10	328	312/16	95.1

extractable with trichloroacetic acid and (2) treatment with RNAase removed all radioactivity from the oocyte (Fig. 1).

Table 1 compares the *in vitro* effects of actinomycin D and salicylate on RNA synthesis in the oocytes. After an hour of treatment with salicylate, oocytes were washed and put into fresh culture media. They resumed RNA synthesis, although to a lesser extent than normal. This treatment of actinomycin D-treated oocytes did not revive their RNA synthetic capability. However, both actinomycin D and acetyl salicylate inhibited RNA synthesis in the oocytes (Tables 1 and 2).

The most important biologic assay for the effects of inhibition of

RNA synthesis came from our observations on *in vitro* and *in vivo* fertilization. The results of *in vitro* fertilization using the treated oocytes and capacitated sperm are provided in Table 3. It is clear from these data that actinomycin D, at a concentration which allows apparent progression of the oocytes to the second metaphase, does affect the process of fertilization, as well as normal cleavage and development of the embryo. Also, acetyl salicylate, like actinomycin D, prevented normal fertilization and developmental processes in the mouse.

The RNA synthesis in the fetal ovary during 16th–18th days of gestation was inhibited when actinomycin D and/or acetyl salicy-

Table 3. *In vitro fertilization and development of in vitro treated oocytes*

Drug	Dose, μg/ml	No. oocytes treated	Oocytes matured		No. used for fertilization	Two-cell embryos		No. blastocysts
			No.	(%)		No.	(%)	
Actinomycin D	1×10^{-3}	916	380	(41.4)	320	68	(21.2)	0
Control		858	825	(96.1)	750	445	(59.3)	218
Acetyl salicylate	10	1,120	578	(51.6)	550	160	(29.0)	4
Control		1,018	998	(98.0)	964	494	(51.2)	300

Table 4. *Effects of actinomycin D and acetyl salicylate on RNA synthesis in fetal ovarian oocytes in mice*

Drug	Dose/mouse (20 g), μg/ml	No. pregnant mice injected	No. progeny born	No. ovarian oocytes labeled/ total no. oocytes studied	No. oocytes unlabeled	Oocytes labeled, %
Actinomycin D	1×10^{-2} (1.5 ml)	10	86 (50 ♀, 36 ♂)	36/500	464	7.2
Control (N saline)	(1.5 ml)	10	90 (48 ♀, 42 ♂)	476/520	44	91.7
Acetyl salicylate	10	10	92 (42 ♀, 50 ♂)	53/426	373	12.4
Control	(1.5 ml)	10	89 (40 ♀, 49 ♂)	410/448	38	91.5

Table 5. *Reproductive capacity of female progeny treated in utero and mated when mature*

Treatment	Group	No. *in utero-* treated mice mated (vaginal plug)	No. mice pregnant (18 days)	No. normal progeny	No. abnormal progeny
Actinomycin D[1]					
1×10^{-2} g/ml	1	20	6	12	14
1×10^{-2}	2	25	4	0	12
Total		45	10 (22%)	12	26
Salicylate[1]	1	25	10	14	16
10 μg/ml	2	25	11	17	19
Total		50	21 (42%)	31	35
Control, N saline	1	26	24	216	0
	2	25	23	183	1 (?)
Total		51	47 (92.1%)	399	1 (?)

[1] Dose is the same as that used in the *in vitro* experiments.

late had been administered to the dam (Table 4). Subsequently, when these progeny were tested for fertility, they were found to have lower capacity to reproduce than the controls. Although some morphologically normal progeny were born to the dams who received the drug, they were nonetheless definitely affected by the drugs as judged by their reproductive capability. It is quite evident from Table 5 that *in utero*-treated females had less ability to reproduce and gave birth to more abnormal progeny when compared with the controls. Various abnormalities in the first generation included extreme reduction in size of the fetuses in the treated group compared with nontreated controls (Figs. 2 and 3). However, a majority of the female progeny that received the drugs *in utero* had extreme reabsorption of the embryos (Fig. 4) or they were completely sterile (depending on the dose of the drug).

DISCUSSION

The pattern of RNA synthesis in oocytes in general has been studied previously by Oakberg (16) and by Baker (1). Bloom and Mukherjee (2) have demonstrated that treatment of the oocytes with actinomycin D prevents their maturation. However, the effects of inhibition of RNA synthesis on the developmental

potential of mouse oocytes, presented in this report, have not been shown previously. The demonstration in the present investigation that acetyl salicylate, like actinomycin D, inhibits RNA synthesis in oocytes during development is important from the standpoint of fetal malformations in other species, perhaps including man, because with a few exceptions every prenatally ingested medication crosses the placenta (11) and in the United States mothers take an average of 4–5 medications during pregnancy (15, 17).

The teratogenic effects of salicylate in the mouse (9, 19) and in the rabbit (5, 19) are well documented. However, a review of the literature on the effects of salicylate on early development in mammals shows that very few studies have been performed concerning the effects on the preimplantation stages of embryos (6). Young *et al.*, in 1972 (25), have shown toxic effects of sodium salicylate on preimplantation mouse embryos in a preliminary study. In Young's study only morphologic criteria of survival of embryos were studied. The present investigations point to one of the mechanisms by which malformations and/or lethal effects of some drugs may be exerted during early development, *e.g.*, by inhibition of RNA synthesis in the oocyte. Aspirin and other salicylates have been recognized as teratogens in rats since the first report by Warkany and Takaes (21). Recently, Janakidevi and

Fig. 2. Effect of salicylate (10 g/ml, 1.5 ml/24 hr) administered on the 16th and 17th day of gestation. Note the size of the fetuses on the 21st day of gestation when they were removed from the uterus. *Left:* control; *right:* treated.
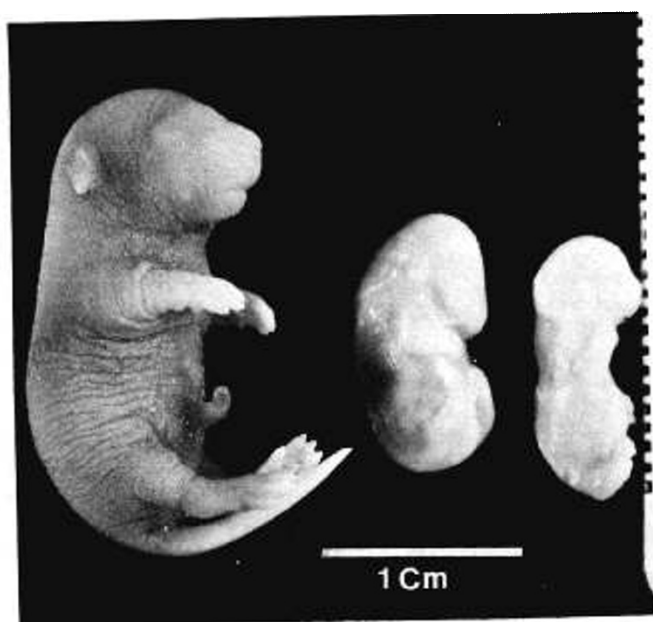

Fig. 3. Comparison of the progeny derived from mothers who received actinomycin D (1×10^{-3} μg/ml), acetyl salicylate (10 μg/ml), and control. *Left:* control; *middle:* salicylate; *right:* actinomycin D.

Smith (8) have elucidated the molecular nature of salicylate action on living cells by pointing out that this drug indeed inhibits the nucleic acid polymerases *in vitro*. Our observations on the basis of autoradiographic evidence support the finding of those authors and further suggest that RNA synthesis in the maturing oocytes is an essential and integral part of the process of development and differentiation. Additionally, these results point to the possibility
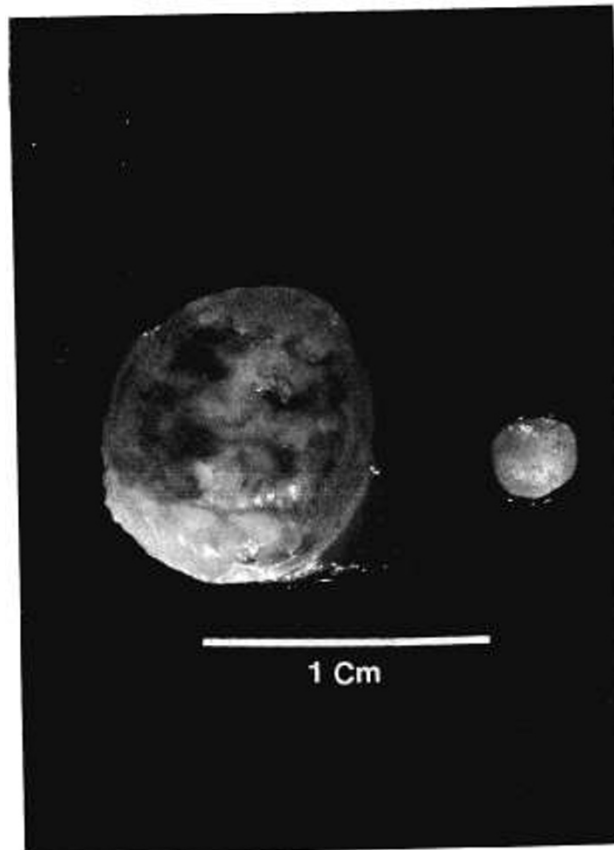

Fig. 4. A normal placenta compared to the reabsorbed fetus recovered from a mouse which was exposed to salicylate *in utero* during 15–18 days of gestation.

that the inhibition of nucleic acid synthesis may be one of the reasons for the teratogenic effect and the reduction of fertility in the female mouse when exposed to acetyl salicylate *in utero*.

The reduction in fertility or complete sterility (depending on the dose) because of salicylate treatment *in utero* of the female fetus may be taken as indirect evidence of the inhibition of masked messenger RNA synthesis in the developing oocytes. It has been shown by Rudkin and Griech (18) and Borum (3) that the oocytes contained in the adult mouse ovary persist from fetal life and that the last round of DNA synthesis takes place in these oocytes during the 12th–15th day of gestation. Our autoradiographic data obtained by injecting [³H]uridine into pregnant mice on the the 16th–18th day of gestation and recovering oocytes from adult progeny whose cytoplasm contained labeled RNA suggest that perhaps this is the time during which the fetal ovary synthesizes masked messenger RNA, which is then stored in oocyte cytoplasm for future use after fertilization. This view has been further supported by the fact that the developmental potential of the oocytes of the progeny that received salicylate *in utero* during the 16th–18th day of gestation is indeed reduced (Table 5). This effect of salicylate had been reported by Wright (24) who studied the toxicity of various analgesic and antipyretic drugs for five generations in the mouse.

In conclusion, the present investigations suggest a possible mechanism for the adverse effects of salicylate in concentrations which fall within the therapeutic range in man, on the normal maturation and development of mouse oocytes. Extrapolation of these findings to humans must be considered with caution, in view of the difference in the process of oocyte maturation in human as compared with the mouse. However, the results of this study may be used to illustrate that drugs may affect progeny (at least in the mouse) adversely in one of three ways: (*1*) by directly affecting the offspring in the early developmental processes such as organogenesis; (*2*) by antagonizing the RNA synthesis of their own oocytes during maturation in the ovary, and (*3*) by preventing RNA synthesis in the oocytes and/or normal maturation of the oocytes in their female offspring, thereby imposing sterility or the production of malformations in the subsequent generation.

SUMMARY

The effects of acetyl salicylate and actinomycin D on RNA synthesis in the mouse oocyte were compared by *in vitro* and *in vivo* experiments. After exposure to the drugs the effects on RNA synthesis were measured by incorporation of [³H]uridine and autoradiography. The results indicate that acetyl salicylate inhibits RNA synthesis in the treated oocytes in a manner similar to actinomycin D. The effects from a short exposure to salicylate may be reversible, whereas, the same effects with actinomycin D cannot be reversed. *In utero* exposure of the female fetus may lead to partial or total sterility (depending on the dose and time of exposure) of that fetus and/or abnormal development of subsequent progeny.

These results suggests that RNA synthesis in early oogenesis plays a vital role in the later development of the oocytes in adult mouse ovary. Inhibition of this early RNA synthesis may be one of the causes of subsequent malformations and sterility in future offspring.

REFERENCES AND NOTES

1. Baker, T. A., Beaumont, H. M., and Franch, L. I.: The uptake of tritiated uridine and phenylalanine by the ovaries of rats and monkeys. J. Cell. Sci., *4:* 655 (1962).
2. Bloom, A. M., and Mukherjee, B. B.: RNA synthesis in maturing mouse oocytes. Exp. Cell Res., *74:* 577 (1972).
3. Borum, K.: Oogenesis in the mouse: A study of the origin of the mature ova. Exp. Cell Res., *45:* 39 (1966).
4. Brachet, J., Denis, H., and de Vitry, F.: The effects of actinomycin D and puromycin on morphogenesis in amphibian eggs and *acetabularia mediterranea*. Dev. Biol., *9:* 398 (1964).
5. Early, P. A., and Hayden, J.: Effect of acetyl salicylic acid on fetal rabbits. Lancet, *i:* 763 (1964).
6. Eriksson, M.: Salicylate induced fetal damage late in pregnancy. Acta Pediat. Scand. Suppl., *211:* 5 (1971).
7. Gross, P. R.: RNA metabolism in embryonic development and differentiation. I. Fertilization and after. N. Engl. J. Med., *276:* 1239 (1967).
8. Janakidevi, K., and Smith, M. J. H.: Inhibition of nucleic acid polymerases by salicylate *in vitro*. J. Pharm. Pharmacol., *21:* 401 (1969).
9. Larsson, K. S., Bostrom, H., and Ericson, B.: Salicylate induced malformations in mouse embryos. Acta Pediat., *52:* 36 (1963).
10. McColl, J. D., Golbus, M., and Robinson, S.: Effect of some therapeutic agents on the rabbit fetus. Toxicol. Appl. Pharmacol., *10:* 244 (1967).
11. Moya, F., and Thorndike, V.: Passage of drugs across placenta. Amer. J. Obstet. Gynecol. *84:* 1778 (1962).
12. Mukherjee, A. B.: Normal progeny from *in vitro* fertilization of mouse oocytes matured in culture and spermatozoa capacitated *in vitro*. Nature *237:* 397 (1972).
13. Mukherjee, A. B.: *In vitro* embryogenesis: An experimental model for the understanding of reproductive physiology and development in mammals. Canad. J. Genet. Cytol., *16:* 11 (1974).
14. Mukherjee, A. B.: RNA synthesis in developing oocytes (unpublished manuscript).
15. Nora, J. J., Nora, A. H., Sommerville, R. J., Hill, R. M., and McNamara, D. G.: Maternal exposure to potential teratogens. J. Amer. Med., Ass., *202:* 1065 (1967).
16. Oakberg, E. F.: Relationship between stage of follicular development and RNA synthesis in the mouse oocytes. Mutat. Res., *6:* 155 (1968).
17. Peckham, C. H., and King, R. W.: A study of intercurrent conditions observed during pregnancy. Amer. J. Obstet. Gynecol. *87:* 609 (1963).
18. Rudkin, A. T., and Griech, H. A.: On the persistence of oocyte nuclei from fetus to maturity in the laboratory mouse. J. Cell Biol., *12:* 169 (1962).
19. Trasler, D. G.: Aspirin induced cleft lip and other malformations in mice. Lancet, *i:* 606 (1965).
20. Tyler, A.: Masked messenger RNA and cytoplasmic DNA in relation to protein synthesis and process of fertilization and determination in embryonic development. Dev. Biol. Suppl., *1:* 170 (1967).
21. Warkany, J., and Takaes, E.: Experimental production of cogenital malformations in rats by salicylate poisoning. Amer. J. Pathol., *35:* 315 (1959).
22. Whitten, W. K., and Biggers, J. D.: Complete development *in vitro* of the preimplantation stages of mouse in a simple chemically defined medium. J. Reprod. Fertil., *17:* 399 (1968).
23. Wilson, J. G.: Effects of acute and chronic treatment with actinomycin D on pregnancy and the fetus in the rat. Harper Hosp., Bull., *24:* 109 (1966).
24. Wright, H. N.: Chronic toxicity studies of analgesic and antipyretic drugs and congeners. Toxicol. Appl. Pharmacol., *11:* 280 (1967).
25. Young, L. M., Mukherjee, A. B., Cohen, M. M., and Yaffe, S. J.: Effects of sodium salicylate on preimplantation mouse embryos cultured *in vitro*. Recent Commun. Chem. Pathol. Pharm., *3:* 579 (1972).
26. Schwarz BioResearch, Orangeburg, N. Y.
27. We wish to thank Dr. Ronald Davidson for suggestions and critical review of the manuscript. We gratefully acknowledge the technical assistance of Ok Kim and the editorial assistance of M. Short.
28. This investigation was supported by grants from Department of Health, Education and Welfare Maternal and Child Health Service (Project 417), National Institutes of Health, (RR-05493, HD-05187, HD-06321, and HD-04287), and the Lalor Foundation.
29. Requests for reprints should be addressed to: S. J. Yaffe, M.D., Children's Hospital, Division of Pharmacology, 219 Bryant St., Buffalo, N. Y. 14222 (USA).
30. Accepted for publication April 10, 1975.