the mammary glands of the rats at sacrifice.

Summary. 1. The effect of TR, TEM and TEPA on rat fetus and litter *in utero* was studied with 2 doses of the 5 LD_{50} on 4th and 5th, or 7th and 8th, or 11th and 12th days of gestation. 2. TR had no effect on the rat litter and can be regarded as a control series to TEM. 3. TEM had its maximum effect at the time of implantation, but failed to destroy the entire litters. An increase in dosage from .3 mg/kg to .5 mg/kg given at the time of implantation led to 100% litter destruction. 4. Progesterone administration protected 40% of the fetuses against otherwise lethal doses of TEM. 5. Rats treated with TEM displayed uniformly smaller placentas than normal. 6. TEM in combination with AZS given on the 15th and 16th days of gestation led to much stunting of the fetuses, to 32% of fetal death, but in no instance to complete litter destruction. 7. Freshly prepared TEPA destroyed all litters when given on the 7th and 8th, or 11th and 12th days of gestation. 8. The malformations observed with TEM and TEPA consisted of cranial defects and abnormalities of facial structures. 9. TEM and TEPA in doses used affected adversely the bone marrow and lymphoid system of the mother animals.

1. Biesele, J. J., *et al., Nature,* 1950, v166, 1112.
2. Haskin, D., *Anat. Rec.,* 1948, v102, 493.
3. Karnofsky, D. A., *et al., Arch. Intern. Med.,* 1951, v87, 477.
4. Murphy, M. L., and Karnofsky, D. A., *Cancer,* 1956,
5. Philips, F. S., and Thiersch, J. B., *J. Pharm. and Exp. Therap.,* 1950, v100, 398.
6. Sykes, M. P., *et al., Cancer,* 1953, v6, 142.
7. Thiersch, J. B., *Ann. N. Y. Acad. Sci.,* 1954, v60, 220.

Received September 11, 1956. P.S.E.B.M., 1957, v94.

Effect of 2-6 Diaminopurine (2-6 DP): 6 Chlorpurine (ClP) and Thioguanine (ThG) on Rat Litter *in Utero.** (22850)

J. B. THIERSCH
Department of Pharmacology, University of Washington School of Medicine and Institute of Biological Research, Seattle.

2-6-DP, CIP and ThG were synthesized as members of a larger group of purine analogues(1,4). Their biological activity was extensively investigated in microorganisms (6), as well as in the embryo of *Rana pipiens* (2), adult mice, and rats(8) and experimental tumors(4). Following the study of 6 Mercaptopurine (6-MP) which showed that the dosage of 10 mg/kg and more would destroy most of the fetuses of the pregnant rat *in utero* at the time of implantation(9), the 3 compounds were investigated for their action on the rat litter.

Procedure. Long Evans strain of rats was used exclusively in the present experiments. All rats were 6 months old, had one previous satisfactory litter, and weighed 200 g or more. For details of reproductive performance of the strain of rats used, diet and technics employed, see previous publication(9). The onset of gestation was determined by finding of vaginal sperm. All animals were injected intraperitoneally strictly on a mg/kg basis. Experimental animals and controls were sacrificed on the 21st day of gestation, the day before littering, to avoid loss of fetuses or placentas. The internal organs of the mother animals were sectioned, the uteri removed *in toto,* and their contents noted. Placenta and fetuses were counted, measured and weighed, and many of the fetuses sectioned or cleared.

Toxicity data. Single and 5 LD_{50} for each compound is listed as given by Philips for the Wistar rat(8).

Rat toxicity in mg/kg:

	2-6-DP	ClP	ThG
Single LD_{50}	100	500	300
5 LD_{50}	50	100	10

* This investigation was supported in part by a research grant by the Population Council and PHS Grant.

TABLE I. Effect of 2,6-Diaminopurine on Rat Litter *In Utero*, 50 mg/kg IP.

No. of rats		Day of dose	Mother's wt gain, %	Total implants	Fate of litter: Live	Malf. or stunt	Dead	Res.	Mothers with all resorb. litters	Avg litter wt	Avg single fetus: Wt, g	Length, cm
21	a	4, 5	+13.9	167	42			125	12	21.8	4.7	4.6
	b			8.0	2.0			6.0				
	c				25.2			74.8	57.2			
23	a	7, 8	+25.1	167	143	17	3	21		29.8	4.8	3.9
	b			7.3	6.23	0.74	0.13	0.91	0			
	c				85.6	11.9	1.8	12.6				
20	a	7, 8 +	+32.3	165	148	5		17		39.5	5.3	4.1
	b	lactate		8.25	7.4	0.25		0.85	0			
	c				89.7	3.8		10.3				
26	a	11, 12	+26.3	220	208		2	10		40.0	5.0	3.8
	b			8.5	8.0		0.1	0.4	0			
	c				94.6		0.9	4.5				

a = total No. b = per mother rat. c = % of total implants. d = % of live.

Results. To avoid toxicity to the mother animals, the compounds were injected intraperitoneally in 2 doses of the 5 LD_{50}, only. Groups of pregnant rats were injected on the 4th and 5th, or the 7th and 8th, or the 11th and 12th days of gestation to determine the effects of the drugs before implantation, at the time of implantation, and at midterm. 2-6-DP was prepared with lactate to render the compound more water-soluble and easier to inject. 2-6-DP had a peak of action when given before implantation. 75% of all fetuses were resorbed, and 57% of all litters completely destroyed (Table I). On the 7th and 8th days of gestation, the drug failed to destroy a single litter, but induced malformation and stunting in 12%, and resorption of 12% of all fetuses (Table I, 2). In a second series, treated in the same time period, 2-6-DP was given without lactate. Now only 4% of the fetuses were found to be stunted, and only 10% resorbed. At midterm 2-6-DP had statistically no effect on the litter (Table I, 4). ClP when given before implantation induced 32% of complete litter destruction, with 70% resorption of all fetuses (Table II). Following implantation, it destroyed all the litters (Table II, 2). At midterm only 10% of the fetuses were resorbed, and not a single litter completely destroyed. However, 53% of the

TABLE II. Effect of 6-Chloropurine on Rat Litter *In Utero*, 100 mg/kg IP.

No. of rats		Day of dose	Mother's wt gain, %	Total implants	Fate of litter: Live	Malf. or stunt	Dead	Res.	Mothers with all resorb. litters	Avg litter wt	Avg single fetus: Wt, g	Length, cm
25	a	4, 5	+21.9	247	76	8		171	8	20.9	4.7	3.9
	b			9.9	3.0	0.32		6.9				
	c				30.4	10.5		69.6	32.0			
24	a	7, 8	+ 3.5	242				242	24			
	b			10.1				10.1				
	c							100.0	100.0			
25	a	11, 12	+23.3	203	174	93	7	22	0	23.9	3.4	3.4
	b			8.1	7.0	3.7	0.3	0.8				
	c				85.8	53.2	3.4	10.8	0			
9	a	7, 8 +	+ 1.3	94	0			94	9			
	b	proges-		8.5				8.5				
	c	terone						100	100			

a = total No. b = per mother rat. c = % of total implants. d = % of live.

TABLE III. Effect of Thioguanine on Rat Litter *In Utero*, 10 mg/kg IP.

No. of rats		Day of dose	Mother's wt gain, %	Total implants	Fate of litter: Live	Malf. or stunt	Dead	Res.	Mothers with all resorb. litters	Avg litter wt	Avg single fetus: Wt, g	Length, cm
20	a	4, 5	+18.8	142	31	6	4	107	6	13.7	4.2	5.3 live
	b			7.1	1.6	0.3	0.2	5.3		5.0	3.1	2.5 dead
	c				21.8	19.3	2.8	75.4	10.3			
24	a	7, 8	+ 5.2	202				202	24			
	b			8.4				8.4				
	c							100.0	100.0			
23	a	11, 12	+17.2	184	46	17	25	113	10	14.1	2.9	3.2 dead & live
	b			8.0	2.0	0.7	1.1	4.9				
	c				25.0	37.0	13.6	61.4	43.5			
6	a	7, 8 +	+ 5.6	50	9			41	5			
	b	proges-		8.3	1.5			6.8		47.0	4.2	5.2
	c	terone			18.0			82.0	83.5			

a = total No. b = per mother rat. c = % of total implants. d = % of live.

surviving fetuses were stunted or malformed (Table II, 3). Three doses of 10 mg progesterone given intramuscularly on the 7th, 8th and 9th days of gestation before the ClP administration (on the 7th and 8th days only) failed to protect the litters (Table II, 4). *ThG,* when given before implantation, destroyed 75% of all fetuses, but only 10% of all the litters. Stunting was seen in 19% of the survivors (Table III, 1). At the time of implantation, ThG destroyed all litters completely (Table III, 2). At midterm, 61% of all the fetuses were resorbed, and still 43% of all litters destroyed. Thirteen per cent of all the fetuses were found dead, and 37% of the survivors stunted or malformed, indicating the great toxicity of the drug to the fetuses (Table III, 3). Three doses of 10 mg progesterone given intramuscularly on the 7th and 8th and 9th days of gestation, before the doses of ThG on the 7th and 8th days, did not protect the fetuses (Table III, 4).

Action of the drugs. None of the drugs prevented decidua formation or implantation, as the histological studies of the uteri showed. The drugs had little effect on the placentas, as many surviving organs demonstrated. The maternal ovaries carried intact corpora lutea of pregnancy. The mammary glands of most of the animals treated at the time of the 7th implantation or midterm showed glandular proliferation and early lactation, as seen in pregnancy—*ruling out pituitary inhibition by the drugs.* From this, one *must* conclude that the drugs acted primarily on the fetuses. The maternal organs, especially bone marrow, intestinal tract and lymphoid system, were found to be *intact* at the time of sacrifice in the mother rats. If lesions had occurred with drug therapy, the animals had recovered at the time of sacrifice.

Placentas. In many instances the placentas survived the fetuses. A number of uteri containing only placentas of varying sizes, but no fetuses, were noted. Remarkable was the absence of surviving placentas in the groups treated on the 7th and 8th days of gestation with ClP and ThG. Giant placentas, twice normal size, connected with normal size fetuses, were noted 4 times in uteri where the remainder of the litter was destroyed. The percentage of surviving placentas without fetuses in each experimental group of rats is listed below.

% of surviving placentas without fetuses:

Drug	Days of drug administration: 4th & 5th	7th & 8th	11th & 12th
2-6-DP	12	35	25
6 ClP	60		5.5
ThG	13		72

The *malformations* noted with all 3 compounds consisted of general edema and anasarca; general stunting of the skeleton; cranial defects, with and without hydrocephalus; ventral hernia; situs inversus; incom-

plete development of fore and hind legs.

Weight changes of mother animals. The weight changes of the experimental animals corresponded to those found in control animals, and indicated that no severe intoxication of the experimental animals occurred.

Discussion. The 3 compounds listed in the present experiment differ in their action on the adult mouse and rat tissue. 2-6-DP affects bone marrow, intestinal epithelium and to some degree the lymphoid system. ClP acts on bone marrow and intestinal epithelium; while ThG affects in corresponding doses primarily the bone marrow only(8).

2-6-DP was introduced into experimental chemotherapy of the leukemias(3) and a preliminary trial with the 2 other compounds was carried out(7). For this reason, knowledge of their action on the fetus is important. The findings confirm the studies of Bieber and others on the embryo of *Rana pipiens*(2). By comparison to 6 Mercaptopurine, ClP and ThG match its effects in total litter destruction at the time of implantation; ThG exceeds the action of 6 Mercaptopurine on the rat litter, especially at midterm. All 3 compounds investigated here were more effective on the 4th and 5th days of gestation than 6 Mercaptopurine(9).

Summary. 1. 2-6-DP, ClP and ThG were given to groups of pregnant rats in 2 doses of 5 LD_{50}, not toxic to the mother animals. The compounds were given on the 4th and 5th, or on the 7th and 8th, or on the 11th and 12th days of gestation. 2. 2-6-DP showed a peak of action on the fetuses and litters when given before implantation, destroying 75% of all the fetuses, but only 57% of all the litters. 3. ClP showed a peak of action on the 7th and 8th days, destroying all fetuses and litters. Treatment on days 4 and 5 of gestation destroyed 69% of the fetuses, but only 32% of all litters. At midterm, no litter was completely destroyed, and only 10% of all the fetuses were resorbed. However 50% of all survivors were stunted and malformed. 4. ThG was the most toxic compound to the fetuses. At the time of implantation it destroyed all fetuses. On days 4 and 5 of gestation, it led to resorption of 75% of the fetuses, but only to 10% complete litter destruction. At midterm it still destroyed 61% of all fetuses, and 43% of all litters, while stunting 37% of all survivors. 5. The lethal action of ClP and ThG on the rat litter at implantation time could not be prevented by progesterone administration.

1. Bendich, A., Russell, P. J., Jr., and Fox, J. J., *J. Am. Chem. Soc.,* 1954, v76, 6073.

2. Bieber, S., Bieber, R., and Hitchings, G. H., *Ann. N. Y. Acad. Sci.,* 1954, v60, 207.

3. Burchenal, J. H., *et al., Cancer,* 1951, v4, 554.

4. Clark, D. A., *et al., Ann. N. Y. Acad. Sci.,* 1954, v60, 235.

5. Elion, G. B., Hitchings, G. H., and Vanderwerff, H., *J. Am. Chem. Soc.,* 1951, v73, 5235.

6. Sniger, S., and Hitchings, G. H., *Ann. N. Y. Acad. Sci.,* 1954, v60, 201.

7. Hall, B. E., *et al., Proc. Am. Assn. Cancer Research,* 1955, v2, 21.

8. Philips, *et al., Ann. N. Y. Acad. Sci.,* 1954, v60, 283.

9. Thiersch, J. B., *ibid.,* 1954, v60, 220.

Received September 11, 1956. P.S.E.B.M., 1957, v94.