

**Effect of 2,4,6, Triamino-"S"-Triazine (TR), 2,4,6 "Tris" (Ethyleneimino)-  
"S"-Triazine (TEM) and N, N', N"-Triethylenephosphoramidate (TEPA)  
on Rat Litter *in Utero*.\* (22849)**

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Previous experiments had shown that some antimetabolites introduced into chemotherapy of cancer have an adverse effect on fetal development when given to the mother rats in the first trimester of pregnancy (7). In the present experiments, TR, TEM and TEPA were given to pregnant rats in order to determine their action on the growing fetus and litter.

**Procedure.** The experimental procedures employed are mentioned in detail elsewhere (7). **Methods.** Groups of pregnant female rats were given the compounds on the 4th and 5th, or the 7th and 8th, or the 11th and 12th days of gestation, counting the morning of massive sperm findings in the vagina as day 0. The rats were kept in single cages, fed *ad lib.*, the "White Breeding" diet of Evans and Long, and were sacrificed under ether anesthetic on the 21st day of gestation, or the day before the expected littering. Only rats with known breeding performances were used. All animals had one previous litter, were approximately 6 months old, and weighed between 200 and 300 g at the time

of mating. Weight curves of the animals were kept. The internal organs were inspected, the sternum and spleen fixed and sectioned. The uteri were removed *in toto*, opened longitudinally, and the placentas and fetuses measured, weighed, recorded, x-rayed and then fixed in 10% formalin, stained and cleared. **Dosage.** According to F. S. Philips (5,6), the 5 LD<sub>50</sub> for the Wistar rats, for Triazine is approximately 70 mg/kg, for TEM is .3 mg/kg, and for TEPA 5 mg/kg. The 5 LD<sub>50</sub> was given twice on subsequent days. TR and TEM were injected intraperitoneally, TEPA intramuscularly.

**Results with TR.** TR administration had only a minor effect on the fetuses or litters of the experimental rats. The number of resorptions in the group treated on the 4th and 5th days of gestation exceeded those of the controls, but is not significant (Table I). The findings demonstrate that TR, itself forming the basic molecule of TEM, is harmless to the rat fetus.

**TEM.** The compound induced 78% fetal resorption but only 25% total litter destruction

TABLE I. Effect of Triazine on Rat Litter *In Utero*, 70 mg/kg Intraperitoneally.

| No. of rats | Day of dose | Mother's wt gain, % | Total implants | Fate of litter—<br>d |      |             |      | Mothers with all resorb. litters | Avg litter wt | Avg single fetus |            |
|-------------|-------------|---------------------|----------------|----------------------|------|-------------|------|----------------------------------|---------------|------------------|------------|
|             |             |                     |                | Live                 | Dead | Gross malf. | Res. |                                  |               | Wt, g            | Length, cm |
| 21          | a           | 4, 5                | +28.2          | 176                  | 160  | 1           | 13   |                                  |               |                  |            |
|             | b           |                     | 8.4            |                      | 7.6  | .57         | .62  | 0                                | 41.0          | 5.0              | 4.2        |
|             | c           |                     |                | 91                   |      |             | 7.4  |                                  |               |                  |            |
| 26          | a           | 7, 8                | +39.9          | 221                  | 213  | 2           | 6    |                                  |               |                  |            |
|             | b           |                     | 8.5            |                      | 8.2  | .07         | .23  | 0                                | 46.5          | 5.7              | 4.1        |
|             | c           |                     |                | 96.4                 | .9   |             | 2.7  |                                  |               |                  |            |
| 26          | a           | 11, 12              | +33.3          | 230                  | 224  | 6           | 6    |                                  |               |                  |            |
|             | b           |                     | 8.8            |                      | 8.6  |             | .23  | 0                                | 42.7          | 4.4              | 4.1        |
|             | c           |                     |                | 97.4                 |      |             | 2.6  |                                  |               |                  |            |

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

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TABLE II. Effect of TEM on Rat Litter *In Utero*.

| No. of rats | Day of dose | Dose, mg/kg       | Mother's wt gain, % | Total implants | Fate of litter |       |      |       |      | Mothers with all resorb. litters | Avg single fetus |            |
|-------------|-------------|-------------------|---------------------|----------------|----------------|-------|------|-------|------|----------------------------------|------------------|------------|
|             |             |                   |                     |                | Live           | Stunt | Dead | Malf. | Res. |                                  | Avg litter wt    | Length, cm |
| 24          | a           |                   |                     | 202            | 41             | 5     | 2    |       | 159  | 6                                |                  |            |
|             | b           | 4, 5              | +12.5               | 8.4            | 1.7            | .2    | .1   |       | 6.6  |                                  |                  |            |
|             | c           |                   |                     |                | 20.3           | 2.5   | 1.0  |       | 78.8 | 25                               | 11.9             | 4.4 3.6    |
| 26          | a           |                   |                     | 233            | 49             | 23    | 1    | 1     | 183  | 9                                |                  |            |
|             | b           | 7, 8              | +19.0               | 9.0            | 1.9            | .9    | .4   | .4    | 7.0  |                                  | 8.2              | 4.3 3.5    |
|             | c           |                   |                     |                | 21.0           | 47.0  | .4   | 2.0   | 78.6 | 34.6                             |                  |            |
| 26          | a           |                   |                     | 245            | 201            | 13    | 4    | 1     | 40   |                                  |                  |            |
|             | b           | 11, 12            | +30.4               | 9.4            | 7.7            | .5    | .2   | .4    | 1.5  | 0                                | 35.2             | 4.6 3.9    |
|             | c           |                   |                     |                | 82.5           | 6.5   | 1.6  | .5    | 16.3 |                                  |                  |            |
| 18          | a           |                   |                     | 153            |                |       |      |       | 153  | 18                               |                  |            |
|             | b           | 7, 8              | + 6.9               | 8.5            |                |       |      |       | 8.5  |                                  |                  |            |
|             | c           |                   |                     |                |                |       |      |       | 100  | 100                              |                  |            |
| 21          | a           |                   |                     | 163            | 111            | 66    | 29   | 19    | 23   |                                  |                  | 3.5 live   |
|             | b           | 15, 16            | +14.0               | 7.8            | 5.3            | 3.1   | 1.4  | .9    | 1.1  | 0                                | 22.0             | 3.3        |
|             | c           | .3 + AZS          |                     |                | 68.1           | 59.5  | 17.8 | 13.6  | 14.1 |                                  |                  | 2.9 dead   |
| 14          | a           |                   |                     | 123            | 52             | 9     | 1    |       | 71   | 5                                |                  |            |
|             | b           | 7, 8              | +18.3               | 8.8            | 3.7            | .64   |      |       | 5.0  |                                  | 23.5             | 4.1 3.6    |
|             | c           | .5 + progesterone |                     |                | 42.2           | 17.3  |      |       | 57.2 | 35.7                             |                  |            |

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

TABLE III. Effect of TEPA on the Rat Litter *In Utero*, 5 mg/kg Intramuscularly.

| No. of rats | Day of dose | Mother's wt gain, % | Total implants | Fate of litter |                   |       |       | Mothers with all resorb. litters | Litter wt, g | Avg single fetus |            |
|-------------|-------------|---------------------|----------------|----------------|-------------------|-------|-------|----------------------------------|--------------|------------------|------------|
|             |             |                     |                | Live           | Malform. or stunt | Dead  | Res.  |                                  |              | Wt, g            | Length, cm |
| 24          | a           | 4, 5                | +18.3          | 211            | 36                | 6     | 175   | 10                               | 13.0         | 5.0              | 4.1        |
|             | b           |                     |                | 8.8            | 1.5               | 0.24  | 7.3   |                                  |              |                  |            |
|             | c           |                     |                |                | 17.1              | 16.7  | 82.9  |                                  |              |                  |            |
| 19          | a           | 7, 8                | +15.9          | 146            | 30                | 7     | 116   | 10                               | 13.2         | 3.9              | 3.7        |
|             | b           |                     |                | 7.7            | 1.6               | 0.37  | 6.1   |                                  |              |                  |            |
|             | c           |                     |                |                | 20.5              | 23.3  | 79.5  |                                  |              |                  |            |
| 22          | a           | 11, 12              | +10.0          | 191            | 15                | 15    | 176.0 | 10                               | 4.5          | 3.1              | 3.3        |
|             | b           |                     |                | 8.7            | .7                | .7    | 8.0   |                                  |              |                  |            |
|             | c           |                     |                |                | 7.9               | 100.0 | 92.1  |                                  |              |                  |            |
| 23          | a           | 11, 12              | + 8.6          | 209            |                   |       | 209   | 23                               |              |                  |            |
|             | b           |                     |                | 9.1            |                   |       | 9.1   |                                  |              |                  |            |
|             | c           |                     |                |                |                   |       | 100.0 |                                  |              |                  |            |
| 19          | a           | 7, 8                | + 5.9          | 178            |                   |       | 178   | 19                               |              |                  |            |
|             | b           |                     |                | 9.4            |                   |       | 9.4   |                                  |              |                  |            |
|             | c           |                     |                |                |                   |       | 100.0 |                                  |              |                  |            |

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

before implantation. The maximum effect of two doses of .3 mg/kg on the litter was found at the time of implantation. 78% of all the fetuses were found dead or resorbed, but only 38.5% of all litters were destroyed (Table II, 2). An increase in dosage from 2 x .3 mg/kg to 2 x .5 mg/kg given at this sensitive period induced 100% litter destruction, while still permitting a weight gain of 6.9% of the mothers (Table II, 4), within the normal range of untreated control rats. At midterm 2 doses of .3 mg/kg failed to destroy a single litter and induced only 16.3% of fetal resorptions. Attempts to destroy the litter *in utero* on the 15th and 16th days of gestation with 2 x .3 mg/kg TEM and 2 x 10 mg/kg of Azaserine (AZS) failed entirely (Table II, 5). Not a single litter of 21 pregnant rats was destroyed, only 17.8% of all fetuses were found dead *in utero*, and only 14% of all embryos resorbed; however, nearly 60% of all living fetuses were found to be stunted or malformed. An attempt was made to protect the litter of rats treated on the 7th and 8th days of gestation with .5 mg/kg TEM, by giving 10 mg progesterone intramuscularly 1 hour before the TEM administration on the 7th and 8th days, as well as further 10 mg on the 9th day. Progesterone permitted 42% of the fetuses to survive and reduced the litter

effect to 35%. Stunting was not prevented (Table II, 6). *Stunting*. The smallest live fetus measured 2.6 cm in length, and was observed in an animal treated with .3 mg/kg on the 7th and 8th days of gestation. In the same group, 10 other fetuses measured only 3 cm in length. The group of rats treated with TEM and AZS on the 15th and 16th days of gestation showed 35 fetuses varying in length from 2.5 to 3 cm, the remainder of the stunted embryos varying from 3 to 3.4 cm in length. *Malformations*. The malformations were predominantly cranial defects, harelip, anencephaly, encephalocele, abnormalities of the facial structures, and general edema and anasarca of the body.

*TEPA*. The results are listed in Table III, and indicate that this compound was most active in litter destruction from the 7th and 8th to the 11th and 12th days of gestation. The first series of experiments (Table III, 1, 2, 3) was carried out with the compound suspended in sesame oil for 22 months. However, when the experiments were repeated with a fresh compound (Table III, 4, 5) 100% litter destruction was obtained. Animals treated on the 11th and 12th days had a tendency to bleed from the uterus. Several died spontaneously on the 20th day of gestation. The effect on the litter and fetuses be-

fore implantation exceeded that of TEM with 41% of total litter destruction and 83% of all fetuses resorbed. *Stunting.* Of 15 surviving fetuses of the group of rats treated on the 11th and 12th days of gestation, 6 measured only 3.3 cm and the remainder 3.4 cm. Four fetuses of the group treated at the time of implantation varied in size from 2.5 to 3 cm. Four others were less than 3.5 cm. In the group of rats treated on the 4th and 5th days of implantation, one measured only 2.3 cm and one other only 3.4 cm in length. *Malformations.* Two fetuses with malformations of the cranium, one anencephalus with ventral hernia, and one fetus with a facial defect, were observed.

*Mother animals.* The appetite of the mother rats was not impaired during or after drug administration. Weight loss or diarrhea did not occur. However, a number of rats were lost from uterine hemorrhages on the 20th day of gestation, especially in the group of rats treated with TEPA on the 11th and 12th days of gestation. *Microscopic Examination.* Bone marrow and spleens of rats treated with TR were found to be normal at sacrifice. The bone marrow and spleen of the rats treated with TEM were found to be normal with 2 exceptions showing a 30% depletion of the total marrow cellularity. The histological findings of bone marrow and spleens were in most cases those of an actively regenerating erythro- and myelopoiesis—the animals having completely recovered from the marrow injury. The groups of rats treated with TEPA still showed at sacrifice definite evidence of marrow depression, ranging from 15% to 35%, the normally active cellular marrow being replaced by hemorrhages and fat.

*Placentas.* The average discoid placenta of our control fetuses weighed .8 g and measured .9 x .4 cm in diameter. The placentas of surviving fetuses of rats treated with TEM were uniformly smaller than those of the controls, but the fetuses were of normal size and weight. The placentas of the group of rats listed in Groups 2 and 3 of Table II weighed only .4 g, and measured only .4 x .2 cm in diameter. Some of the placentas of

group 4 in Table II weighed only .6 g and measured .6 x .3 cm in diameter.

% of surviving placentas without fetuses:

| Drug      | Days of drug administration |           |             |             |
|-----------|-----------------------------|-----------|-------------|-------------|
|           | 4th & 5th                   | 7th & 8th | 11th & 12th | 15th & 16th |
| TR        | 100                         | 100       |             |             |
| TEM       | 14                          | 75        | 40          | 94          |
| TEPA      | 14                          | 5.1       | 28          | 94          |
| TEM & AZS |                             |           |             | 74          |

The generally decreased size of placentas in the TEM treated rats and the protective action of progesterone on the fetuses against the otherwise lethal doses of TEM suggest strongly a TEM effect on the placenta. Rats treated with TEPA had larger placentas, corresponding to those found in the controls. The percentage of placentas surviving the fetuses also differed from the TEM groups.

*Discussion.* Nitrogen mustard was previously shown to have a very deadly effect on the 10-day-old rat embryos(2,4). TEM and TEPA are compounds very similar in action to the nitrogen mustards(5). Their effect on the bone marrow, lymphoid system, intestinal and testicular epithelium has been described (5,6). The compounds were also shown to induce chromosomal abnormalities producing bridging and linkage of chromosomes in mitosis(1). TEM and TEPA are used in palliative chemotherapy and Hodgkin's disease and lymphoma in men(3,6). It was therefore of great interest to study the effect of TEM and of TEPA on the rat litter *in utero*. The results have shown that TEPA in 2 doses of the 5 LD<sub>50</sub> would destroy with greater certainty the entire litter of rats at the time of implantation, but also that by comparison to TEM, TEPA induces a more lasting bone marrow depletion and intoxication of the rat. This might be due to a slower rate of resorption from the site of injection. TEM appeared to inhibit the growth of the placenta to a minor degree. Its effect on the fetus was surprisingly counteracted by simultaneous doses of progesterone. The findings suggest that TEM acts therefore not only on the fetus but also on the placenta. The pituitaries of the present rats treated were not affected, judged on the secretory activity of

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<sub>50</sub> 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.

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### Effect of 2-6 Diaminopurine (2-6 DP): 6 Chlorpurine (CIP) and Thioguanine (ThG) on Rat Litter *in Utero*.<sup>\*</sup> (22850)

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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<sub>50</sub> for each compound is listed as given by Philips for the Wistar rat(8).

Rat toxicity in mg/kg:

|                         | 2-6-DP | CIP | ThG |
|-------------------------|--------|-----|-----|
| Single LD <sub>50</sub> | 100    | 500 | 300 |
| 5 LD <sub>50</sub>      | 50     | 100 | 10  |

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