scribed for this organ by Wessels(3) and Hamilton(5). The small rise of activity at stage 30 coincides with the brief mitotic pulse accompanying mesodermal condensations. The first large rise is simultaneous with feather germ outgrowth, reaching a maximum at stage 36. The dip at stage 37 occurs at the time of barb-ridge formation which may represent a rearrangement rather than a new growth. At stage 39, there is an increase in specific activity of polymerase occurring simultaneously with feather elongation. Elongation ceases with the onset of keratinization at stage 40, and polymerase activity drops abruptly at the same time.

The possibility that variation in DNase activity plays a role in the observed DNA polymerase assays was tested by the data given in Fig. 2. Neutral DNase activity increases approximately 2-fold by stage 36, and then drops again to original values by stage 40. A doubling of the DNase activity would not account for the 9-fold increase in polymerase activity found in the stage 36 extracts(10). Furthermore, the second rise of polymerase specific activity found at stage 39 is not related at all to neutral DNase activity.

Acid DNase activity mirrors the activity of neutral DNase until stage 36, after which acid DNase rises to a maximum by stage 40 while neutral DNase activity decreases uniformly.

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Mitigated Teratogenicity of Thio-TEPA in Goldthioglucose Obese Mice.* (31961)

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Recently much attention has been paid to the effect of maternal metabolic states upon the susceptibility of the offspring to a teratogen(1-6). Kalter(6) demonstrated that heavier mice are more resistant to the cleft-palate inducing properties of cortisone than lighter animals and related this finding to maternal fat metabolism. Nevertheless, obesity induced by goldthioglucose (GTG) in mice did not alter the frequency of fetal abnormalities(7). However, the susceptibility of the fetuses of GTG obese mice to a known teratogen, triethylene thiophosphoramide (thio-TEPA) has not been examined and these results are here presented.

Materials and methods. Colony bred ICR-JCL mice from Japan CLEA Co. (Tokyo) were used. They were fed OA-2 pellets made by Japan CLEA Co. and given fresh water *ad libitum*. Virgin females of 12 weeks of age were caged separately and after one week were randomly divided into 4 groups. Two groups were given GTG in a single

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TABLE I. Effect of Thio-TEPA Given to GTG Obese or Non-Obese Pregnant Mice.

Group	No. of litters	Fetuses: Total No. (litter size)	Number resorbed‡	Number macerated‡	Externally malformed %§	Body wt mean ± S.E. (g)	Sex ratio ♂/♀
1. GTG + thio-TEPA	22	292 (13.3)	18	13	11.8*	1.09*± .008	1.25
2. Thio-TEPA	10	136 (13.6)	13	2	26.4	1.15 ± .011	1.47
3. GTG ± saline	23	308 (13.4)	26	10	3.7	1.19†± .008	1.18
4. Saline	10	133 (13.3)	10	2	2.5	1.33 ± .010	1.47

* Significantly different ($P < 0.05$) as compared with thio-TEPA group.
† Significantly different ($P < 0.05$) as compared with saline group.
‡ Dead fetuses larger than the size of their placentas were classified as "macerated" and smaller dead or resorbed conceptuses were called "resorbed".
§ % of live fetuses.

intraperitoneal injection of 400 mg/kg at 13 weeks of age; GTG was dissolved in physiological saline at a concentration of 4%. Body weight of the treated females was measured 3 days after treatment. One week later, the GTG treated females were caged overnight (16 hours) with males. The day when the vaginal plug was found was designated as day 0 of gestation. Mating was also initiated at 14 weeks of age in 2 groups of untreated mice. Only those who copulated by 17 weeks of age were used. On day 10 of gestation, 3 mg/kg of thio-TEPA was administered to mice with and without prior GTG treatment. A single intraperitoneal injection of 0.03% solution in physiological saline of thio-TEPA was used. Controls were treated with physiological saline. The pregnant mice were weighed every day. Daily food and water intake was also measured.

All the animals were autopsied on day 17 of preganacy and the fetuses, live or dead (resorbed or macerated) were counted and removed. Live fetuses were weighed and examined externally. After gross inspection, two-thirds of the fetuses were fixed in Bouin's fluid to study internal visceral abnormalities. Free hand razor blade sections described by Wilson and Warkany(8) were made on the heads and defects in the palate, nasal cavities, eyes or brain were sought. Furthermore, the thoracic and abdominal regions were carefully dissected under the stereomicroscope. The remaining one-third of the fetuses were fixed in 95% alcohol, cleared and stained with alizarin red S(9). Examinations for skeletal deviations including grade of ossification were performed on these specimens.

Results. Body weights of GTG-treated mice was significantly increased within a week after injection, and the body weight of GTG-treated mice at the time of successful copulation exceeded that of the untreated mice at the corresponding week of age by 5.0 to 9.0 g. During the later half of pregnancy, however, body weight of the untreated animals increased more rapidly than that of the obese mice and at autopsy no significant difference in body weight was noted. Food intake of obese mice was more than that of the untreated mice in the early period of gestation, but the relation was reversed later in pregnancy. There was no difference in the daily water intake among the 4 groups excepting a transient reduction probably due to thio-TEPA injection.

No significant difference was observed in the average litter size, fetal mortality and sex ratio despite GTG and thio-TEPA treatment, though a relatively high incidence of macerated fetuses was found in the obese mice (Table I). Mean body weight of live fetuses in the two obese groups was less than the corresponding non-obese groups.

The teratogenicity of thio-TEPA was clearly exhibited in obese and non-obese mice. However, it was noted that the incidence of malformations was significantly reduced in the obese group as compared with that in the non-obese group. Obese mice exhibited a reduced incidence of oligodactyly, brachidactyly and polydactyly on the forelimb,

TABLE II. Type and Number of Externally Observed Malformations in Fetuses of Thio-TEPA-Treated GTG Obese or Non-Obese Mice.

	GTG + thio-TEPA	Thio-TEPA	GTG + saline	Saline
No. of live fetuses	261	121	272	121
No. of malformed fetuses	31	32	10	3
Open eyelid	1	0	0	0
Cleft palate	4	0	1	0
Oligodactyly† finger	8*	20	0	0
toe	0	0	0	0
Brachydactyly† finger	0*	4	0	0
toe	0	0	0	0
Syndactyly† finger	7*	17	0	0
toe	0*	7	0	0
Polydactyly† finger	0*	4	0	0
toe	11	2	0	0
Club foot	4	5	6	3
Umbilical hernia	0	0	1	0
Short tail	8	8	0	0
Waving tail	2*	9	2	0

* Significantly different ($P < 0.05$) as compared with thio-TEPA group.
† Number of limbs with that anomaly is presented.

TABLE III. Type and Number of Skeletal Deviations in Fetuses of GTG Obese or Non-Obese Mice Treated with Thio-TEPA.

	GTG + thio-TEPA	Thio-TEPA	GTG + saline	Saline
No. of fetuses examined	83	40	93	41
Non-fused supraoccipital	0	0	0	1
Split or bifurcated atlas or axis	0	3	0	0
Ossified caudal vertebrae	5.62*	6.45	6.13	6.80
(mean ± S.E.)	±.18	±.25	±.17	±.32
Cervical rib	6	1	7	8
14. rib	17	6	8	5
Sternebrae with delayed ossification	12	0	19	7
Hand with non-ossified midphalanges	40*	8	48†	11
Non-ossified calcaneus	78	37	84†	22
Foot with non-ossified midphalanges	74	34	85	33

* Significantly different ($P < 0.05$) as compared with thio-TEPA group.
† Significantly different ($P < 0.05$) as compared with saline group.

syndactyly on the hind limb and waving tail (Table II). On the other hand, a small number of cleft palates were found in the obese groups only.

Examination of the internal structure of the fetuses disclosed 2 cases of hydronephrosis in the obese thio-TEPA-treated group, and one case in the obese not thio-TEPA-treated group. Many fetuses had a left umbilical artery instead of the normal right one but the incidences of such cases could not be related to drug treatment.

No skeletal malformation was found, except these associated with external malformation such as cleft palate or polydactyly. Fetuses of the obese mice have a tendency toward retardation in ossification and there is neither potentiative interaction nor interference between the induced obesity and thio-TEPA with respect to that tendency (Table III).

Discussion. The present experiment revealed: 1. the GTG induced obese ICR-JCL mice with or without thio-TEPA treatment produce fetuses which are smaller and exhibit retardation in ossification as compared to the corresponding non-obese mothers; 2. the teratogenicity of thio-TEPA was mitigated in

the obese mice. The former findings may be attributed simply to the fact that both groups of obese mothers showed in spite of their greater body weight a tendency toward a decreased food intake in the later period of pregnancy as compared with the non-obese mothers.

It is presumed that teratogenicity of thio-TEPA(10,11) may be attributed to cytotoxic effects inducing an inhibition of nucleic acid metabolism, especially DNA synthesis in embryonic tissues. The reduction of teratogenicity of thio-TEPA in the obese mice is probably caused by interaction of those effects of the compound with certain altered metabolism in the obese mothers and the embryos. It is generally agreed that GTG obese animals are characterized by an increased lipid deposition associated with lesions in the hypothalamus as well as increase in non-lipid constituents of many organs (12). Also, it was reported that those animals show several physiological changes such as increased oxygen consumption, minor elevation of blood glucose, increased inactivation of insulin by the liver, altered function of the kidney and affected pituitary-ovarian axis (12,13). However, the complicated biochemical mechanisms operating in the obese pregnant animals consisting of two different biological systems, mothers and embryos, have not been elucidated.

Summary. Teratogenicity of 3 mg/kg of triethylene thiophosphoramide (thio-TEPA), given intraperitoneally on day 10 of gestation, was examined in obese and non-obese mice. Obesity was induced with goldthioglucose (GTP). No difference was observed in fetal mortality, but frequency of fetuses with malformations caused by thio-TEPA was reduced in the obese mothers. Live fetuses in the obese group showed slight growth retardation.

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Bile Acid Synthesis in Normal and Hypophysectomized Rats: A Rate Study Using Cholestyramine.* (31962)

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Bile acid and sterol metabolism in hypophysectomized rats differs from that in normals both qualitatively and quantitatively (1,2). Specifically, sterols are metabolized to bile acids in the liver at about one-third the rate found in normal rats; the bile acid spectrum differs; bile acids are eliminated *via* feces more slowly; and sterol synthesis and excretion are retarded. When hypophysectomized rats are fed commercial rat diets which contain little cholesterol, tissue cholesterol concentrations are maintained at about

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