Discussion. Types 12, 14 and 18 were rapidly inactivated at 56°C, complete inactivation occurring with types 12 and 18 after only 8 minutes. These findings agree closely with those of Ginsberg(1) for types 1 to 4. Types 12, 14 and 18 show no decrease in titer after at least 3 months at 4°C. No loss in infectivity was evident with types 12 and 18 after 30 freeze-thaw cycles or type 14 after 15 cycles. These data attest to the stability of types 12, 14 and 18 and agree essentially with data reported by other investigators for other adenovirus types.

The pH inactivation kinetics at 37° under the above experimental conditions elucidate an important property of the adenoviruses studied, *viz.*, a marked increase in stability on the acid side of neutrality. Ginsberg(1) reported decreased stability at alkaline values for type 3; however, it was not determined if optimal stability was attained at acid pH, in particular, pH 6.0 as we have observed with types 12, 14 and 18. Denny and Ginsberg(4) observed only a slight loss in titer with type 14 after 7 days at pH 7.5. The results reported here are in agreement, although a marked loss occurs in 7 days at pH 8.0.

As ordinarily prepared, adenovirus harvests from cell cultures have a pH of 7.0 to 8.0; and if care is not taken to prevent loss of CO_2 the pH may be even higher. The present results show that there may be rapid loss of infectivity at these high pH values, and that the pH must be adjusted to 6.0 for maximum stability.

These findings indicate a need for a systematic examination of the pH stability of animal viruses. Hamparian *et al* (6) have shown that animal viruses fall into two groups on the basis of their stability at pH 3.0. Extension of these studies may reveal many more detailed systematic differences between classes of viruses with respect to pH stability.

Summary. Adenovirus types 12, 14 and 18 are relatively quite stable when exposed to 4° and 37°C. At 56°C types 12 and 18 are completely inactivated and type 14 more than 99% inactivated after 8 minutes. No inactivation occurs with type 14 after 15 freeze-thaw cycles, and types 12 and 18 after 30 cycles. The 3 types studied show maximal stability at pH 6.0. Infectious virus is rapidly inactivated on the alkaline side of neutrality. Types 14 and 18 demonstrate greater stability than type 12 at all pH values studied.

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Teratogenesis: Effects of Substituted Purines and the Influence of 4-Hydroxypyrazolopyrimidine in the Rat.* (29345)

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Certain substituted purines are recognized as potent teratogenic agents in experimental animals. For example, 6-mercaptopurine (6-MP) given to pregnant rats on the 12th day of pregnancy (62 mg/kg) produces

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TABLE I. Effects of Various Purine Derivatives on Placental Weight.

Treatment	No. animals	No. placentas	Placental wt (g)	Malformations
Controls (no injection)	16	164	.58 ± .07*	
Controls (CMC)	22	194	.63 ± .12	—
Thioguanine (25 mg/kg)	3	26	.25 ± .11 p <.001	+
6-chlorpurine (60 mg/kg)	7	56	.51 ± .18	—
Adenine (60 mg/kg)	5	52	.55 ± .09	—
2,6-diaminopurine (60 mg/kg)	7	66	.56 ± .06	—
6-MP (60 mg/kg)	23	192	.31 ± .14 p <.001	+

All injections were made on 12th day in a carboxymethyl cellulose vehicle (CMC, 10 mg/ml). Controls were not injected or were given CMC alone.
* ± S.D.

stunting, shortened extremities, fused digits and rudimentary legs in the off-spring(1). Others also affect embryonic development, depending on the stage of gestation at time of administration of the drug(2).

In studying the mechanism of experimental teratogenesis, a decrease in weight of the chorioallantoic placenta was noted, in addition to the decreased fetal weight which accompanied the malformations. In view of this, a series of experiments was conducted on pregnant rats to assess the influence of various purine derivatives on placental weight. Also, 4-hydroxypyrazolo-(3,4-d)-pyrimidine (HPP), a xanthine oxidase inhibitor(3), was investigated for its ability to potentiate the teratogenic effects of 6-MP.

Materials and methods. Pregnant albino rats (Sprague-Dawley) were obtained from Holtzman Rat Co. The day that the rats were sperm positive was counted as gestation day zero. They were housed in air-conditioned quarters and fed Rockland rat chow and tap water *ad libitum*. All purine derivatives were purchased from Nutritional Biochemicals. HPP was obtained from Aldrich Chemical Co.

On gestation day 12, all animals were injected intraperitoneally with a 1.0 ml suspension of the compound under investigation. On the 20th day of gestation, the rats were sacrificed by cervical dislocation and the fetuses delivered by cesarean section. The placentas were separated from the fetuses, blotted with filter paper and weighed on a Mettler semi-micro balance. All fetuses were examined for gross anomalies, weighed, and stained for skeletal visualization(4).

Results. Those purine derivatives which caused malformations (6-MP and thioguanine) also caused decreases in placental weight (Table I). Adenine, 6-chlorpurine and 2,6-diaminopurine produced no malformations and had no effect on placenta weight, the latter two in contrast to previous reports (1).

At a dosage of 15 mg/kg, 6-MP was nonteratogenic (Fig. 1), although placental weights were lower (Table II). HPP at 30 mg/kg likewise produced no malformations, and no change in placental weight was noted. However, when the 2 drugs were combined at these dosages, malformations occurred which resembled those produced by the teratogenic dosage (60 mg/kg) of 6-MP alone (Fig. 1), and placental weights were significantly decreased (Table II). Furthermore, although HPP at 120 mg/kg caused neither malformations nor placental weight change, its combination with the teratogenic dosage of 6-MP caused fetal death in utero.

Discussion. The results reported herein indicate that the presence of fetal malformations is accompanied by a decrease in placental weight, at least when certain substituted purines are administered. In the absence of malformations, differences between weights of control and experimental placentas are negligible. The only exception is seen following administration of 6-MP at a non-

TABLE II. Effect of 6-MP and HPP on Placental Weight.

Treatment	No. animals	No. placentas	Placental wt (g)	Malformations
6-MP (15 mg/kg)	9	83	.44 ± .08* $p < .001$	—
HPP (30 mg/kg)	10	101	.60 ± .06	—
6-MP (15 mg/kg) HPP (30 mg/kg)	9	96	.35 ± .06 $p < .001$	+
HPP (120 mg/kg)	5	48	.63 ± .09	—
6-MP (60 mg/kg) HPP (120 mg/kg)	5	57	Resorbed	

All injections were made on 12th day in a carboxymethyl cellulose vehicle (CMC, 10 mg/ml). Controls were not injected or were given CMC alone.
* ± S.D.

teratogenic level; no malformations occur, but placentas weigh less, which suggests that placental weight may be more indicative of drug action than is the presence of malformations. In any event, it is conceivable that in addition to any direct effect on the fetus, teratogenic agents may exert an effect on the placenta. This possibility has previously been offered to explain the teratogenic effect of 5-hydroxytryptamine on fetal mice (5,6).

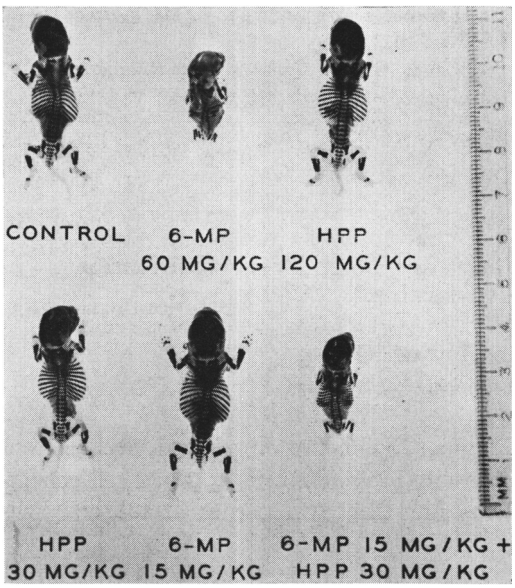

FIG. 1. Effects of 6-MP and HPP. In animals receiving 6-MP (60 mg/kg), there was generalized stunting of growth throughout fetal skeleton, with proportionally greater stunting of caudal end and gross shortening of tail and hind extremities. In addition, there was retardation or absence of digital and metacarpal or metatarsal ossification on fore and hind paws; absence of one of lower leg bones; marked branching and fusion of ribs; and fusion or absence of lateral processes of lumbar and sacral vertebrae with divergence and double vertebral centra. Animals receiving 6-MP (15 mg/kg) and HPP (30 mg/kg) showed identical malformations but to a somewhat lesser degree. No other dosage regimen produced malformations.

That placental size is directly related to functional capacity must not be assumed. The extent to which decreased placental weight is associated with abnormal physiologic processes remains to be determined. However, this investigation suggests the possibility that such an association does exist.

Of added interest are the findings when HPP is used in combination with 6-MP. The former compound is known to be an *in vivo* inhibitor of xanthine oxidase, the enzyme responsible for biotransformation of 6-MP to 6-thiouric acid, and has been shown to potentiate the inhibitory action of 6-MP against both cancer and the immune response in mice(3). The data reported above indicate that HPP has a similar ability to potentiate the teratogenic action of 6-MP. This finding draws an interesting parallel between the action of 6-MP against rapidly growing, relatively undifferentiated cells, out of control, as in malignancy, and under control, as in the developing fetus.

That anomalies produced following 6-MP administration are due to 6-MP rather than one of its intermediary metabolites(7) has not been proven, but this investigation indicates the excretory end product, 6-thiouric acid, is not the responsible agent. If it were, addition of HPP would prevent or lessen the anomalies obtained, since 6-thiouric acid would not be formed enzymatically from

6-MP. On the other hand, if 6-MP or one of its metabolites from pathways other than that involving xanthine oxidase were the teratogenic agent, addition of HPP would increase the severity of the anomalies or increase the effectiveness of 6-MP at a lower dosage. Both were the case. When HPP at 120 mg/kg was added to the teratogenic dosage of 6-MP, fetal death occurred shortly after administration, as demonstrated by small fetal size and extensive resorption. Fetal anomalies obtained with 6-MP (15 mg/kg) in combination with HPP (30 mg/kg) were similar to those obtained with 6-MP (60 mg/kg) alone. In the latter experiment placental weights were significantly lower than those obtained with 6-MP at 15 mg/kg alone ($p<0.05$), providing additional evidence of the relationship between placental growth inhibition and congenital malformations. Further studies are being carried on to ascertain the nature of this relationship.

Summary. In addition to producing anomalies in rats, 6-mercaptopurine and thioguanine cause a decrease in placental weight. Other purine derivatives which produce no anomalies have no effect on placental weight. These findings suggest that a relationship exists between malformations and placental growth inhibition. Administration of 4-hydroxypyrazolo-(3,4-d)-pyrimidine (a xanthine oxidase inhibitor) with non-teratogenic dosages of 6-mercaptopurine results in anomalies and decreased placental weight. Furthermore, combination of the former drug with teratogenic dosages of the latter causes fetal death.

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Serum Albumin Supplemented Medium for Long Term Cultivation of Mammalian Fibroblast Strains.* (29346)

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Though many improvements have been made in cell culture technique in the past years, it is still not possible serially to cultivate mammalian cell strains† unchanged for very long periods; either they eventually die out, or they change their properties and develop into established cell lines. In commonly employed media (such as Eagle's medium(1) or some modification thereof) supplemented with 10% serum, human fibroblasts die out after from 50-70 cell generations(2,3); Syrian hamster fibroblasts under the same conditions have a much shorter lifetime(4); mouse fibroblasts decline considerably in growth rate by 20-30 cell generations but then give rise to established lines (5,6).

One respect in which nearly all cell culture media differ from the fluid environment of cells in the animal is in their much lower protein concentration. Most culture media contain 3-12 mg protein per ml (5-20% serum) while plasma contains about 65 mg/ml and lymph or extracellular fluid about 30 mg/ml. Though certain cell lines have been evolved that are able to grow in serum-free medium(7,8), and cell strains may be propagated in protein-free medium to a limited extent, both grow much better in

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† Terminology of Hayflick aund Moorhead(2).