Sections in the plane *zz'* for the normal and abnormal eyes were comparable except for the smaller size of the abnormal optic cup. The total volume of the 2 lenses put together and the 2 optic cups put together was less than that of the single normal lens and single normal cup, respectively. However, the maximum circumference of the normal cup and that of the abnormally divided cup, as seen in serial cross sections, was comparable. A layer of dead cells lined the divided optic cup on its inner face while the cells on the outer side appeared healthy (Figure 2 C). Only dead and degenerating cells were seen in the wall of the abnormally divided optic cup in experiment C (Figure 2 D).

Discussion. Haemorrhage in the heart as observed in *Calotes* has been one of the major lesions produced in X-ray irradiation experiments with new-born pig[5]. Our observations further indicate that haemorrhage is perhaps the major but not the only factor leading to death, since we also observed dead embryos without heart haemorrhage. Death characterized by haemorrhage and general circulatory system breakdown has also been reported in chick embryos during the first day after irradiation[9].

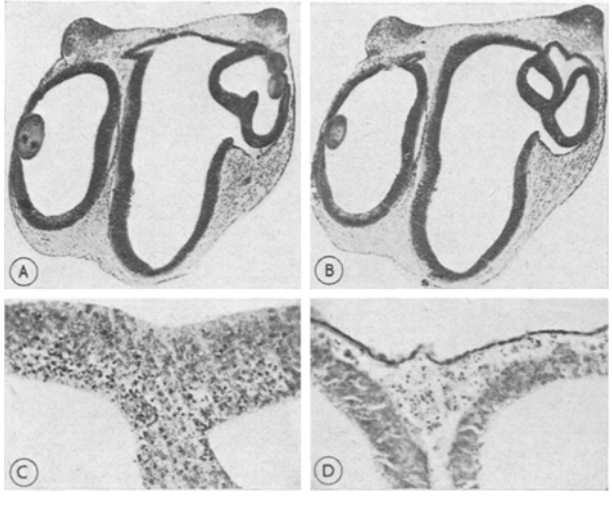

Fig. 2. Sections through experimental embryos showing abnormal optic cup and the lens. A) Partially divided optic cup and lens of the left side (experiment A, ×90). B) Complete division of the optic cup of the embryo in Figure A (×90). C) Magnified view of the mesial region of the divided optic cup of experimental embryo in Figure A showing the necrotic cells on the inner surface of the cup (×500). D) Magnified view of the mesial region of the divided optic cup of experimental embryo (experiment C) showing optic wall consisting of only necrotic cells (×500).

The unilateral nature of the defects is by now a widely reported observation[3,10–12]. PIEAU and VASSE[3] attribute unilaterality of defects in their experiments with *Lacerta viridis* to the position of the embryo in the egg. However, it appears that the position of the embryo in the egg may not have a direct bearing on the unilaterality of the defects: in both *Lacerta* and *Calotes* the embryos are recumbent on the left side but in the former the defects are more pronounced on the right side[3] whereas in the latter these are more so on the left side[11].

The manner of genesis of a 'double optic cup' and a 'double lens' remains uncertain but it seems probable that the alteration in the shape and division of the optic cup results from an inwardly directed collapse of the mesial wall of the cup; the retinal layer in the eye of the right side is also detached from the outer border at about the point of collapse on the left side. This event was followed by cell death beginning along the inner face of the optic cup as noticed in experiment A, and finally resulting in experiment C in the optic cup formed of only dead and degenerating cells. The absence of the eye defect in the experiment B may possibly be due to the embryos, at the time of exposure to γ-rays, being younger as compared with those in the experiments A and C.

Summary. The γ-ray irradiation causes mortality, retardation in development and growth rate, haemorrhage, axial defects and unilateral microphthalmia. Histological examination of microphthalmic embryos revealed a partial division of the left optic cup and a 'double lens'.

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[14] The authors wish to thank Professors L. MULHERKAR and H. J. ARNIKAR for the laboratory facilities and to Mr. E. D. ROBERTS for drawing the Figure 1.

Establishment of Normal Diploid and Malignant Heteroploid Cell Lines from Non-Treated and Benzo(a)pyrene Treated Hamster Embryo Cell Cultures

Various investigators have reported a limited life-span for normal cells and an indefinite growth period for transformed cells[1–6] in culture. Despite the wealth of information on the in vitro growth of hamster cells, stable diploid cell lines grown for extended periods have not been reported. During the present study, we have been able to establish two stable diploid cell lines from non-treated and a heteroploid malignant cell line from benzo(a)pyrene (a tobacco smoke component) treated hamster embryo cells.

Primary cultures were raised by growing cells from 12–14-days-old hamster embryos in Eagle's minimal essential medium supplemented with glucose, sodium bicarbonate, tryptose phosphate broth, non-essential amino acids, glutamine, Hepes and a 10% mixture of different sera[7]. Secondary mass cultures were prepared in plastic flasks, without feeder cells, and treated for 24 h with benzo(a)py-

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rene (0.1 μg/ml) dissolved in acetone and suspended in the culture medium. After this period, the supernatant was replaced by a fresh carcinogen-free medium. The control cells were fed with a medium containg 0.5% acetone and grown under similar conditions.

Control and treated cells were subcultured every 2–3 weeks. The normal or malignant state of the cells was systematically checked through s.c. injection into newborn to 1-week-old hamsters.

For chromosome analysis, exponentially growing cultures were treated for 4 h with colcemid (8 μg/10 ml medium). The medium was then discarded, the cultures washed, trypsinized (1 ml, 0.25% trypsin, 10 min), centrifuged (800–1000 rpm, 10 min) and supernatant aspirated. The cells were resuspended in phosphate buffered saline for 6–8 min; equal amount of fixative added to it and centrifuged (800 to 1000 rpm, 10 min); washed in fixative (1 part glacial acetic acid: 3 parts absolute ethanol); centrifuged and resuspended in fresh fixative for 10 min. The concentrated cell suspension was dropped on cold, wet slides and allowed to air dry for at least 48 h. The slides were stained with Giemsa solution

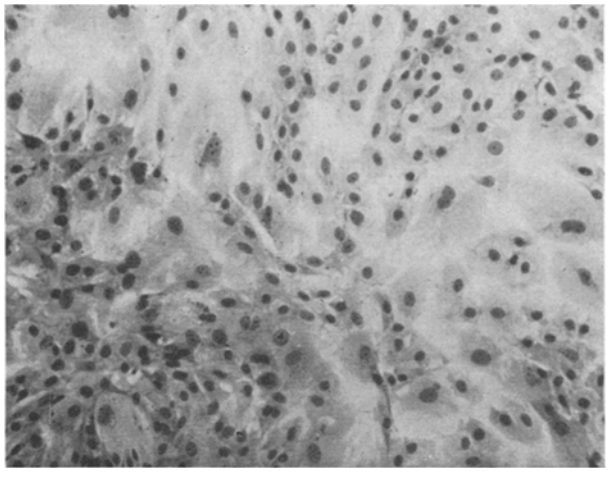

Fig. 1. T_1 control cell line showing large cells forming a monolayer (21st passage in vitro; 18 month in culture; May-Grunwald-Giemsa staining). ×100.

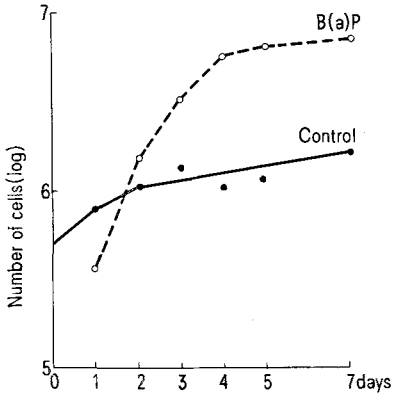

Fig. 3. In vitro growth curves of the T_1 control and of benzo(a)pyrene [B(a)P] transformed cells.

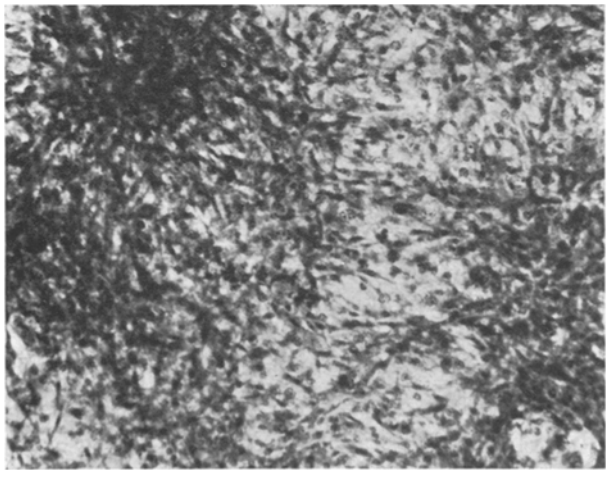

Fig. 2. Benzo(a)pyrene in vitro transformed malignant cell line showing criss-cross pattern of growth (22nd passage; 12 months in culture). ×100.

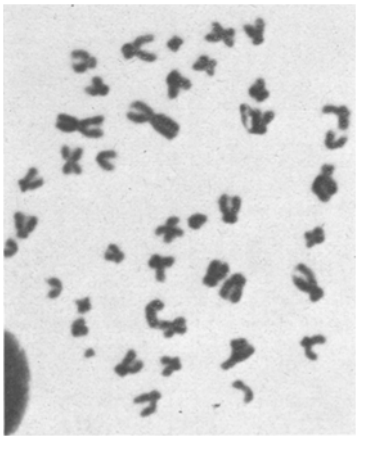

Fig. 4. Normal diploid cell from the 15th passage of T_2 control line (42 autosomes + 2 sex chromosomes). ×400.

Table I. Effects of grafting of control and benzo(a)pyrene [B(a)P] transformed cell lines into newborn hamsters

Cell line	Passage	Days in culture	No. of cells grafted/animal	Days of observation after grafting	No. of tumor bearing animals/No. of animals tested
T_1 (control)	25	560	2×10^6	150	0/6
T_2 (control)	15	180	2×10^6	150	0/10
B(a)P (transformed)	12	210	2×10^6	60	8/8

Table II. Chromosome analysis of control and chemically transformed hamster embryo cell lines

Cell line	Passage number	Age (months)	State	Total cells counted	No. of dividing cells	Mitotic index (4 h cumulative)	Diploid cells	Polyploid cells Tetraploid	Aneuploid
T_1 (control)	27	20	Normal	1016	70	0.069	65	1	4
T_2 (control)	15	6	Normal	1834	54	0.034	50	1	3
B(a)P (transformed)	25	14	Malignant	1122	92	0.082	16	12	64

diluted 1:50 with phosphate buffer at pH 6.8, washed with distilled water and air dried. Observations on the percentage of chromosomal abnormalities and the cumulative mitotic indices (4 h in colcemid) were recorded. Mitotic index is the ratio of the number of dividing cells per total cells counted.

We obtained 2 control cell lines, T_1 and T_2, which have been growing in vitro for 20 months and 27 passages and 6 months and 15 passages respectively. Both retained their normal slow, orderly growth and formed contact-inhibited monolayers (Figure 1). In contrast the cells originating from a culture treated with 0.1 µg/ml of benzo(a)pyrene (B(a)P) exhibited morphological changes 3 months after the treatment. However, these transformed cells could not induce tumors in hamsters before 7 months following the treatment. The transformed cells exhibited a criss-cross growth pattern resulting in several layers of heavily stainable fibroblastic cells (Figure 2). These cells grow 8 times faster than the control cell line T_1 (Figure 3).

From grafting experiments (Table I) it is evident that neither of the control lines induced tumors in hamsters, while the B(a)P transformed cells produced tumors in all hamsters receiving these cells.

The normal chromosome complement for Syrian hamster somatic cells consists of 2 sex chromosomes and 21 pairs of autosomes[6]. We have observed that in T_1 and T_2 cultures 93% of the cells had a normal diploid chromosome complement (Figure 4) and only 7% showed tetraploidy and hypertetraploidy (Table II). This was in distinct contrast to the situation in the transformed cell line where only 17% of the B(a)P transformed cells were diploid, and 83% with chromosomal anomalies which include hypo-diploid (Figure 5), hypotetraploid, tetraploid (Figure 6), hypo-octaploid (Figure 7), and octaploid cells.

In this paper we would like to emphasize the importance of control cultures in chemical transformation studies. Previous reports using hamster cells[5,6] have not included such controls. Other studies, using human cells[1-4] have expressed various degrees of doubt that such cells could be maintained in culture for prolonged periods without 'spontaneous' transformation. The experiments described here demonstrate the feasibility of maintaining non-transformed, diploid control cells. These cells have retained normal cell morphology, slow and orderly growth with contact inhibition and a normal diploid chromosome

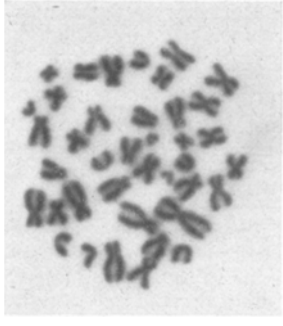

Fig. 5. Hypo-diploid cell from the 25th passage of B(a)P transformed line (39 autosomes + 1 sex chromosome). ×400.

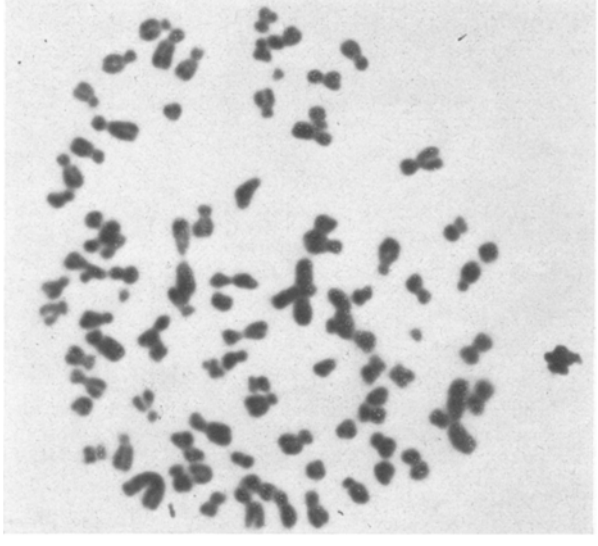

Fig. 6. Tetraploid cell from the same transformed line as in Figure 5 ×400.

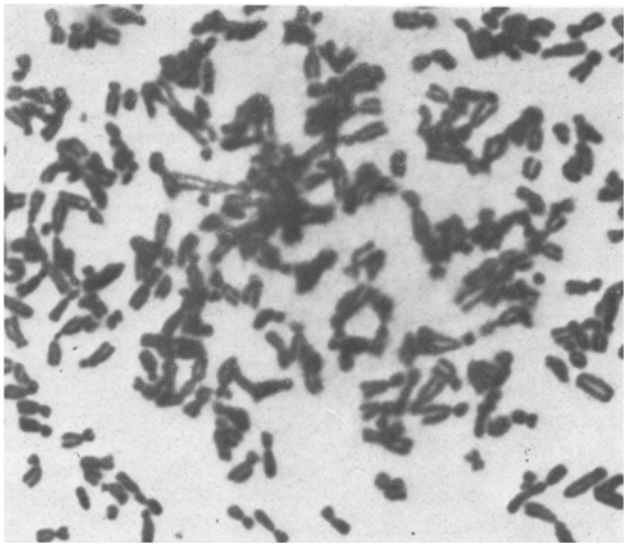

Fig. 7. Hypo-octaploid cell from the same transformed line as in Figure 5. ×400.

complement. Further, the cells consistantly fail to provoke tumors in appropriate hosts, and have been maintained for up to 20 months and 27 pasages in culture. These normal characteristics have been retained despite the fact that the only difference in treatment of these control cells from that of the transformed cells was the presence or absence of benzo(a)pyrene.

DIPAOLO et al.[8,9] performed chromosomal analyses of chemically transformed hamster and rat cultures and of tumors induced by these cells. They found that the cells were, generally, near-diploid. Analysis of our transformed cultures show chromosomal anomalies in 83% of the dividing cells, including 5 different types of aberrations. We are currently engaged in chromosomal analysis of other transformed hamster cell lines to determine if these aberrations are specific for B(a)P induced transformation or are common to other transformations in culture.

In the present work, the successful maintenance of normal characteristics in the control cell lines, grown for extended periods, may be due to the utilization of an improved Eagle's medium and extended time between passages. Both of these factors apparently facilitate adaptation of hamster embryo cells to culture conditions.

Summary. Two normal diploid control cell lines and a heteroploid malignant transformed cell line from B(a)P treated hamster embryo cell cultures were established. The 14-month-old B(a)P transformed cell line grew 8-times faster than the 20-month-old control cell line. The control cell line showed normal diploid chromosome complement in 93% cells and heteroploidy in 7% cells while B(a)P treated line showed 83% heteroploid cells and only 17% diploid cells. This is the first report on the establishment of diploid hamster cell cultures grown for extended period.

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Potency of Thyroid Hormone Analogues in Suppressing Prolactin-Mediated Mammary Growth in Thyroidectomized Rats

It has been recently demonstrated that following thyroidectomy the mammary epithelium of the rat undergoes extensive growth due to an increased sensitivity to endogenous prolactin[1]. Administration of replacement doses of thyroxine to such animals prevents this exaggerated mammary growth response. A similar prolactin-thyroxine antagonism may be implicated in the pathogenesis of human beast cancer[2,3]. BERN et al.[4,5] and MITTRA[1] have proposed that prolactin-thyroxine interaction at the level of the mammary epithelium may be analogous to that known to occur in amphibian tissues at metamorphosis. It was therefore of interest to investigate the effect on mammary epithelium of two thyroid hormone analogues, 3,5,3′-triiodothyropropionic acid (TRIPROP) and 3,5,3′-triiodothyroacetic acid (TRIAC), which have relatively weak calorigenic and goitre prevention activities in the rat[6], while their potency in inducing tadpole metamorphosis is comparable to[7], or even greater than[8], that of the natural thyroid hormones thyroxine (T_4) and triiodothyronine (T_3).

Material and methods. Virgin, female Sprague-Dawley rats weighing about 200 g were used for the experiment and were fed on a commercial diet. All rats were given oestradiol-17β (in 50% propylene glycol) 8 μg s.c. daily from Day 1 to Day 10 and killed at the termination of the experiment on Day 16. Rats in Group I (Figure 1) received no further treatment and were allowed tap water *ad libitum*. The remainder were surgically thyroidectomized on Day 1 and, to ensure complete endogenous thyroid hormone deficiency, were concurrently started on 3-amino-1,2,4-triazole (0.1% solution) in the drinking water until completion of the experiment. This anti-thyroid drug has been shown to have little effect on the peripheral deiodination of T_4[9]. The thyroidectomized animals were divided into 9 groups (Group II–X, Figure 1). Group II received no other treatment while the remainder received variously T_4, T_3, TRIPROP and TRIAC at dose levels of 2.0 and 0.2 μg/100 g body wt., i.p. daily from Day 1 to Day 15 (Figure 1). At completion of the experiment the left thoracic mammary glands were excised and processed for whole mount preparation. The degree of mammary gland development was rated on the standards shown in Figure 2 by an independent observer. The mammary scores of rats in the various groups were compared with those of Group II using a one-way analysis of variance of normal scores[10].

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