block of DNA synthesis, as well as a delayed cell death, is revealed by the progressive decrease of DNA content.

These effects of $K_2Cr_2O_7$ on DNA synthesis in BHK cultures are confirmed by the values for ^{3}H-dT incorporation into DNA only after 10^{-3} *M* and 10^{-6} *M* treatments, while the intermediate doses, which have been shown either to inhibit DNA synthesis (10^{-4} *M*) or to leave it unaffected (10^{-5} *M*), lead to a considerable increase of DNA specific activities (Figure 2B). The discrepancy between the two sets of data can be overcome by taking into account the fact that $K_2Cr_2O_7$ also interferes with ^{3}H-dT uptake into the intracellular pool, that is stimulating it when used at concentrations higher than 10^{-6} *M* (Figure 2A). DNA specific activity changes are therefore induced (Figure 2B) which do not directly depend on the actual DNA synthesis rates.

Since the intracellular pool becomes saturated with ^{3}H-dT in a much shorter time than our incubation time[16], the DNA specific activities have been normalized by dividing their original values by the corresponding ^{3}H-dT specific activities. Such normalized values (Figure 2C) therefore express the actual rates of ^{3}H-dT incorporation into DNA and represent the net levels of the DNA synthesis after $K_2Cr_2O_7$ treatment. By this procedure, it becomes evident that the DNA synthesis inhibition induced by protassium dichromate is almost complete and irreversible when cells are exposed to 10^{-3} *M* concentrations, while on the other hand it is more or less pronounced and reversible, according to the duration of treatment, when 10^{-4} *M* concentrations are used. This effect on DNA duplication can be attributed to the action of reduced trivalent chromium, as this represents the only chromium oxidation state present inside the cell even after treatment with hexavalent chromium compounds[13,17]. Moreover, DNA synthesis inhibition is the primary chromium effect on cell macromolecular syntheses, since higher $K_2Cr_2O_7$ doses or longer exposures are required to reduce RNA and protein syntheses to comparable levels[15].

The present results point out that potassium dichromate also independently affects ^{3}H-dT incorporation into the intracellular pool, increasing its uptake across the plasma membrane and thereby producing higher ^{3}H-dT specific activities. This effect is not observed after treatment with trivalent chromium compounds[17] and is most probably related to the oxidizing action of hexavalent chromium on the plasma membrane, which leads to coordination complexes directly involving cell ligands. As in the present experimental conditions potassium dichromate does not inhibit endogenous nucleotide synthesis, unlabelled thymidine concentration in the intracellular pool thus remaining unchanged[15], the observed increase in ^{3}H-dT specific activity seems to be due to an actual stimulation of the mechanisms responsible for nucleoside transport across the plasma membrane[18].

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Effect of photoperiod on early changes in the neonatal rat pineal gland

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Summary. It is concluded that photoperiod has little direct effect on the mitotic activity or morphological development of the pineal parenchymal cell of the rat in the early postnatal period.

If rats are blinded, development of gonads is impeded in intact animals but not in pinealectomized animals[2]. This indicates that pineal function is influenced by the presence or absence of light. At birth, the rat pineal is not complete in its development, since mitotic activity of parenchymal cells is still occurring[3], parenchymal cells have not reached their full size[4] and do not have their full complement of enzymes[5–7]. The following experiment was performed to determine whether or not conditions of lighting could influence some of the anatomical aspects of pineal gland maturation in the immediate postnatal period in the rat.

Materials and methods. Newborn albino rats and their mothers were exposed to one of 3 lighting conditions: normal (12 h of light; 12 h of dark); continuous light (24 h of light) or dark (1 h of light; 23 h of dark). All rooms were air conditioned (25–26 °C) and food and water were provided ad libitum. Tritiated thymidine was injected intraperitoneally at a dosage of 0.5 μC/g body weight on day 1, 7, 14 or 21. Rats were killed 6 h, 7 or 14 days after injection. At euthanasia the pineal gland was fixed in Bouin-Hollande fixative for 24 h and then processed for paraffin sectioning at 5 microns. The sections were dipped in Kodak NTB2 liquid emulsion for autoradiography, exposed for 6–8 weeks, developed and then stained in hematoxylin.

Over 6000 cells from sections through the middle of each pineal gland were studied to determine the percentage of parenchymal cells labeled with tritium and the percentage of parenchymal, neuroglial, endothelial and ependymal cells present. By counting the number of parenchymal cells in 10 ocular grid fields and using the percentage of parenchymal cells present, a rough estimate of the average parenchymal cell size was determined. Results were tested for significance using unpaired Student's *t*-test and correlation coefficients.

Results. Rat pups kept in the dark showed the best weight gain and by day 14 were significantly ($p < 0.01$) heavier than pups from the other groups. Pups kept in

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constant light were significantly ($p < 0.02$) lighter in body weight at day 7 than normal pups. No significant differences in body weight remained at day 21.

The percentage of parenchymal cells labeled with ^{3}H-thymidine 6 h after injection was 6.61% at day 1, 1.16% at day 7, 0.52% at day 14 and 0.10% at day 21 for normal animals. Data from the dark and light groups were not significantly different. Results from animals killed 6 to 14 days after injection substantiated the fact that mitotic activity continued, but decreased with age.

The percentage of parenchymal cells in the pineal gland decreased significantly ($p < 0.001$) from 98.44% at day 1 to 96.62% at day 21 for normal pups. There were no significant differences between groups. The percentage of neuroglial (normal, $p < 0.01$; dark, $p < 0.02$; light, $p < 0.05$) and endothelial (normal, $p < 0.01$; dark, $p < 0.001$; light, $p < 0.01$) cells increased significantly with time, but a significant increase in ependymal cells occurred only in the dark group ($p < 0.001$).

Parenchymal cell area increased in all groups, but some differences in timing occurred. For normal animals, a sudden and significant ($p < 0.01$) increase occurred between day 7 and 14. For rats in the dark group, significant increases in size occurred between day 1 and 7 ($p < 0.02$) and between day 14 and 21 ($p < 0.05$). For rats kept in constant light, no change in size of parenchymal cells occurred from day 1 to 7, but significant increase occurred between day 7 and 14 ($p < 0.02$) and day 14 and 21 ($p < 0.01$). On day 7 parenchymal cells of the dark group were significantly larger than those of the light group ($p < 0.01$) and at day 14 the parenchymal cells of the light group were significantly smaller than those of the normal group ($p < 0.05$).

Discussion. KERENYI and VON WESTARP[8] showed that constant darkness delayed the transformation of the pineal gland of rabbits, based on morphology. Our results using rats indicate that neither long or short photoperiods could significantly alter the postnatal mitotic activity of pineal parenchymal cells or the percentages of parenchymal, neuroglial, endothelial and ependymal cells from normal. The parenchymal cell area from rats in the dark group were increased at day 7 and for rats in the light group were decreased at day 14, but no significant differences were found on day 21. We conclude that length of photoperiod has little direct effect on the morphological maturation of the rat pineal gland in the early postnatal period, since the differences seen could be the result of alterations in the body weight gain.

However, the significant differences in the body weights of the pups in the different groups seems to indicate that photoperiod has a rapid and marked effect on lactation of the mother rats in the immediate postpartum period and that this may be a time when the pineal best shows its control of a hormonal pathway.

The decrease in weight gain of pups in the dark group between day 14 and 21 may indicate that the dramatic inhibition of growth hormone production and release by the pituitary gland in blinded rats shown by SORRENTINO, REITER and SCHALCH[9] for young rats may start as early as day 14.

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Immunological and radioimmunological studies of membrane antigen(s) from human breast carcinomas and non-tumoral breast tissues. I.

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Summary. The authors extracted and partially purified a pool of antigens from primary breast carcinomas. The antigens responded to anti-CEA antibody in a radioimmunoassay (R. I. A.) and were not detected in non-tumoral breast tissues used as controls. Antisera were obtained by immunizing rabbits.

Introduction. In over 1000 cases investigated[3,4] by our direct R.I.A. of plasma carcinoembryonic antigen (CEA)[4,5] in patients with malignant tumors differing in type from adenocarcinomas of the gastroenteric tract, only 18 out of 243 cases gave positive results. Of these, 10 were carcinomas of the breast with metastasis (M^+). No case of adenocarcinoma of the breast without metastasis (M_0) was positive. The antigen used was extracted from hepatic metastasis of adenocarcinomas of the colon[3,6].

The small percentage of M^+ breast carcinomas positive to our R.I.A. suggests that in this pathology the circulating antigens show little reaction with our antibody. Therefore, the result is positive only in the few cases in which the blood level of these antigens is high.

Although no case of M_0 breast carcinoma was positive to our R.I.A. for CEA, we postulate that primary tumors of this type might have antigenic determinants that could be recognized by our antibody, however low the affinity. It will thus be possible to use this anti-CEA antibody to monitor the extraction and initial purification of antigens that are tumor-associated with primary breast carcinoma.

Materials and methods. A pool of histologically different primary breast carcinomas obtained by biopsy was used (MK). No metastatic tissue was used, as opposed to other

1 The authors want to express their appreciation to Dr Sergio Orefice, Prof. Carlo Mor and Dr Luisa Amante for their collaboration.

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