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A fraction of labelled mitoses study on adrenal chromaffin tissue in the newborn mouse and the effect of hydrocortisone

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INTRODUCTION

It is now accepted that the influence of corticosteriods is important in the fate of neural crest derivatives, some of which form chromaffin cells, and in the modulation of the catecholamine synthetic pathway. Exogenous corticosteroids increase the size and delay the disappearance of extra-adrenal chromaffin tissue in the newborn rat (Lempinen, 1964), and Monkhouse & Coupland (1985) demonstrated the hyperplastic effect of hydrocortisone on extra-adrenal chromaffin cells of the mouse para-aortic body. The mechanism of this effect on extra-adrenal chromaffin tissue is not clear but recent work using the fraction of labelled mitoses technique (Monkhouse & Chell, 1987) has demonstrated that hydrocortisone renders unobtainable the constituent phase lengths of the cell cycle in the para-aortic body of the newborn mouse. Whether or not glucocorticoids have any effect on the cell cycle of developing adrenal medullary chromaffin cells is not known although there is evidence that mature adrenal chromaffin cells are insensitive to hydrocortisone (Kent, Monkhouse & Coupland, 1981). Newborn animals provide a convenient model during a period which is important in the destiny of neural crest derivatives and in the present work the fraction of labelled mitoses technique has been used in the hope of deriving basic data concerning the cell cycle of adrenal chromaffin cells of the newborn mouse and the effect on this of hydrocortisone.

MATERIALS AND METHODS

Pregnant CS1 mice (*Mus musculus*) were supplied by Nottingham University Medical School Animal House. They were bred from closed colonies and were kept under standard conditions of hygiene, temperature and lighting (12 hours light, 12 hours darkness), and were allowed free access to water and a normal diet (R and M breeding diet, Heygate & Sons Ltd, Northampton).

Hydrocortisone (total daily dosage 40 mg/Kg/day) was delivered orally in a medium of water and evaporated milk to gravid mice from the thirteenth day of pregnancy until term. It was prepared and administered as described by Monkhouse & Coupland (1985) except that it was given twice daily at roughly twelve hourly intervals (not thrice daily).

Newborn animals within the first 24 hours of postnatal life were injected intraperitoneally at 10 a.m. with 10 μCi (370 KBq) [6-^{3}H]thymidine, specific activity

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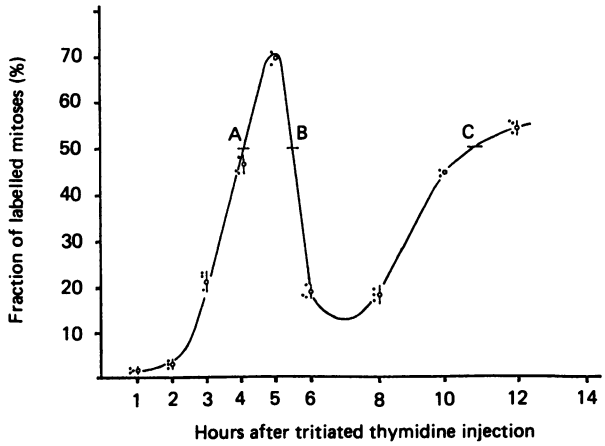

Fig. 1. Fraction of labelled mitoses curve for adrenal medullary chromaffin cells from untreated newborn mice. Solid circles show results from individual animals, open circles show the mean, and vertical bars represent standard error of mean ×2. Curve fitted visually.

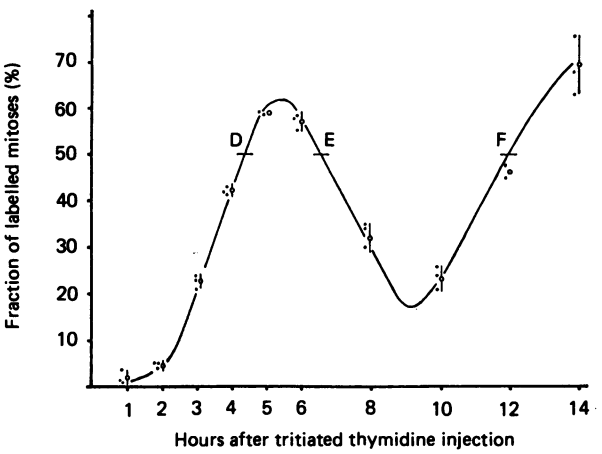

Fig. 2. Fraction of labelled mitoses curve for adrenal medullary chromaffin cells from hydrocortisone-treated newborn mice. Solid circles show results from individual animals, open circles show the mean, and vertical bars represent standard error of mean ×2. Curve fitted visually.

21 Ci/mmol (777 GBq/mmol; Amersham International plc). In both normal and hydrocortisone-treated groups, mice were killed at various time intervals up to 14 hours (hourly up to 6 hours, then two hourly) after injection of tritiated thymidine. Where possible, three animals were used at each time interval. Animals were killed by decapitation and were eviscerated. A block of tissue including the adrenal glands was fixed by immersion in 10% buffered formalin (pH 6·8) for 24 hours, washed in buffer, dehydrated in graded alcohols, cleared in xylene and embedded in paraffin wax. Each block was sectioned serially at 6 μm using a Cambridge rotary rocking microtome and sections were dried overnight onto gelatinised slides before being dewaxed in xylene and rehydrated.

Autoradiographs were prepared by dipping in Ilford K2 photographic emulsion at 43 °C under an Ilford safelight F904. The emulsion was allowed to gel on a cold plate and the slides were exposed in dry conditions at 4 °C in light-tight boxes for 5 days. Autoradiographs were developed for 4½ minutes in Kodak D19 at 16 °C, washed in distilled water and fixed for 3 minutes in 1:10 'Hypam':water. Sections were stained

in Mayer's haemalum for 4½ minutes, blued in tap water, dried in air overnight and mounted in Picolyte resin.

Specimens were examined using a × 100 (oil immersion) objective and the numbers of both labelled and unlabelled mitoses noted. Where possible, at least 100 mitoses were counted in each specimen using a randomised sampling system. In about one quarter of the specimens, however, 100 mitoses could not be found and in these cases all mitoses seen were counted. All stages of mitosis were counted and the fraction of labelled mitoses was expressed as a percentage of the total number, both labelled and unlabelled. For each time interval the mean and (where appropriate) standard error of the mean were calculated. The results are shown in Figures 1 and 2: the single lines were fitted visually to the plotted data.

RESULTS

Chromaffin tissue was presented in many specimens as separate collections scattered throughout the central region of the adrenal gland, rather than aggregated to form a discrete medulla. However, in the hydrocortisone-treated group, the proportion of distinct, well formed medullas was higher than in the control group. This effect of hydrocortisone was reported by Monkhouse & Coupland (1985). The data for untreated and hydrocortisone-treated newborn mice produced typical fraction of labelled mitoses curves. These curves are not trapezoidal but rounded due to damping as a result of the variation of the cell cycle times around a mean value. For comment on this, together with a discussion of other methodological considerations relevant to this work, see Monkhouse & Chell (1987).

Control group (Fig. 1)

The total cell cycle time, measured between equivalent points on the two peaks, is (taking points A and C on the ascending limbs) 7 hours. Mitoses are seen after 1 hour and therefore the duration of G_2 is 1 hour or less. The curve reaches a peak at 5 hours and the duration of mitosis may be measured between the time of first appearance of mitosis and the point at which the first peak is reached; this is 4 hours (this method is not employed by all workers: some simply assume the duration of mitosis to be 1 hour). The S phase is measured as the time interval between the 50% levels on the ascending and descending limbs of the first peak (points A and B) and is, in this case, 1½ hours. By subtraction, this leaves the duration of G_1 as ½ hour.

Hydrocortisone-treated group (Fig. 2)

Using the same methods, the total cell cycle time (between points D and F) is 7½ hours; the duration of G_2 is 1 hour or less; the duration of mitosis 4 hours, the S phase (between points D and E) 2 hours, and G_1 phase ½ hour. These results show that exogenous hydrocortisone administered to the gravid mouse during the last week of pregnancy has no effect on the cell cycle of adrenal medullary chromaffin cells.

DISCUSSION

Monkhouse & Coupland (1985) showed that the procedure used in this work to administer hydrocortisone is itself without effect on the size and the S-phase labelling indices of both intra- and extra-adrenal chromaffin tissue. It was therefore considered unnecessary in the present work to include a group of mice that had received the milk and water mixture only, without hydrocortisone.

The absence of any profound effect of hydrocortisone on intra-adrenal chromaffin cells if the newborn mouse is in contrast to its much greater effect on extra-adrenal chromaffin tissue (Monkhouse & Coupland, 1985; Monkhouse & Chell, 1987). It is possible that intra-adrenal chromaffin tissue is, and has been for some time, exposed to such high concentrations of endogenous adrenal steroids that the administration of exogenous steroids, along with the likely feedback effects resulting in reduced secretion of ACTH, may well have no overall effect on the proliferative activity of the medullary cells. Hydrocortisone may affect the cell cycle of intra-adrenal chromaffin tissue only at an earlier developmental stage rather than the immediate postnatal period and fraction of labelled mitoses experiments on the effect of hydrocortisone on late fetal adrenal chromaffin tissue of mice would thus yield interesting results.

These results mean that in the immediately postnatal stage, the cell cycle of mouse adrenal chromaffin, unlike that of extra-adrenal chromaffin tissue, is not susceptible to the influence of corticosteroids. Glucocorticoids have been shown to be essential for the maintenance of healthy adrenal chromaffin cells *in vitro* (Doupe, Landis & Patterson, 1985), but our results suggest that any functional switching mechanisms which corticosteroids may induce in cells of neural crest origin have been completed, as far as mouse adrenal chromaffin cells are concerned, before birth.

Previous work on mitotic activity in the adrenal glands of newborn animals is scarce and conflicting. Jackson (1919) and Mitchell (1948) reported that mitoses were absent in the rat adrenal medulla after 60 days and Coupland (1957) in the rabbit observed no mitotic activity in transplants of adrenal chromaffin tissue to the anterior chamber of the eye. Malvaldi, Mencacci & Viola-Magni (1968), using colchicine as a stathmokinetic agent, reported 12–16 mitoses in 60–80 days old rat adrenals and 6–7 mitoses in 360 days old rat adrenals.

As a supplement to the present work we performed a similar fraction of labelled mitoses experiment on intra-adrenal chromaffin tissue of newborn Wistar rats. This was unsuccessful: there were simply not enough mitoses, labelled or unlabelled, to allow meaningful results to be gathered. In many sections no mitoses were seen, and the greatest number of mitoses seen in one section was four. The adrenal medulla of the newborn rat is relatively more advanced than that of the newborn mouse with a well formed medulla being present in a higher proportion of specimens in the rat than the mouse. This is supported by unpublished work being undertaken by Tomlinson in this Department using high-resolution electron microscopic studies. The degree of maturity of the rat adrenal medulla advances rapidly during the first two days of postnatal life, and it may thus be more fruitful to perform fraction of labelled mitoses studies on prenatal rat adrenal glands rather than on newborn animals.

SUMMARY

Hydrocortisone was administered to gravid mice from the thirteenth day of pregnancy until term. *In vivo* fraction of labelled mitoses experiments on intra-adrenal chromaffin tissue of newborn mice were performed to assess the effect of hydrocortisone on the cell cycle in this tissue. In untreated mouse intra-adrenal chromaffin tissue the total cell cycle time was 7 hours, being made up as follows: S phase $1\frac{1}{2}$ hours, G_2 phase 1 hour, M phase 4 hours (by analysis of results, not by assumption) and G_1 phase $\frac{1}{2}$ hour (by subtraction). Hydrocortisone administration was without marked effect on these values. These results are discussed in the context of the influence of corticosteroids on the development biology of chromaffin tissue, both intra- and extra-adrenal.

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