Discussion. The gradual transition from macro- to microchromosomes, apparently typical in the Picidae, was also found in *D. major* and *D. medius.* It appears that the Piciformes have among the highest chromosome numbers for birds[2,4,6] and this is supported by our study.

The Z-chromosome is usually not the largest element in the chromosome sets of birds; however, exceptions have been found in the woodpeckers *D. major, D. medius* (this study), *Picoides mahrattensis* and *Dinopium benghalense*[2] as well as in some species of the Alaudidae (Passeriformes)[6].

Among the Piciformes, the Z-chromosome in *Megalaima haemacephala* (Capitonidae) is the 2nd largest and in *Picus viridis* (Picidae) only the 4th largest element[1,2].

In *M. haemacephala,* all larger chromosomes (1-7) are biarmed while in *D. benghalense,* all larger chromosomes (1-11) are telocentric. In the genus *Dendrocopos,* in which *P. mahrattensis* is included by various taxonomists (e.g. Voous[5]), *D. mahrattensis* and *D. medius* show 7 large biarmed chromosomes, but *D. major* only 1. *Picus viridis* has 4 biarmed chromosomes. Kaul and Ansari[2] have suggested that the telocentric chromosomes of *D. benghalense* have been produced by an extensive fission of biarmed chromosomes of the type of *D. mahrattensis.*

1 Hammar, B., Hereditas *65* (1970) 29.
2 Kaul, D., and Ansari, H.A., Genetica *48* (1978) 193.
3 Hoffmann, R., J. Orn. *113* (1972) 334.
4 Bloom, S.E., J. Hered. *60* (1969) 217.
5 Voous, K.H., Ibis *115* (1973) 612.
6 Bulatova, N.S., Acta scient. nat. Brno *15* (1981) 1.

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Effects of 5-bromodeoxyuridine on in vitro development of mouse embryos in the early somitic stage

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Summary. The inhibitory effect of 5-bromodeoxyuridine on the early somitic stages of mouse embryos was largely prevented in the presence of excess thymidine but only partially prevented by deoxycytidine.

The teratogenic action of 5-bromodeoxyuridine (BUdR) on the development of rodent embryos has mainly been investigated using pregnant animals[1,2]. However, rapid degradation of BUdR administered to them by the maternal liver[3] did not allow examination of the full potential of BUdR action. Recent progress in the whole-embryo culture of rodent embryos during organogenesis has enabled us to maintain their growth for a limited period of time almost as well as in vivo[4,5]. We report the inhibitory effects of BUdR on mouse embryos at the early somitic stage.

Materials and methods. Mouse embryos of the STD-ddy strain, at the late presomitic to early somitic stage, were extracted from the uterus on day 7 of gestation. The decidua and extraembryonic membranes were removed[4]. 3 embryos were placed in a flask containing 3 ml of medium and rotated at 30-40 rpm at 38 °C. At 0.5-3 h after the initiation of the culture (somitic stages 1-4), each flask was supplemented with 15 µl of nucleoside solution in a Hanks BBS. Untreated controls were supplied only with the same volume of Hanks BBS. After 20 h embryos were transferred into a flask containing 6 ml of fresh medium and cultured for another 24 h. On termination, the heartbeat, visceral yolk sac circulation, axial rotation, somite number and cranial neural tube closure were examined in all the embryos. Some of them were then cleaned of membranes and the protein content was measured by the Lowry method[6]. The gas phase was 5% O_2/5% CO_2/90% N_2 until the exchange of the medium, followed by 20% O_2/5% CO_2/75% N_2 until the termination of the culture. The medium consisted of 80% rat serum and 20% Earle supplemented with streptomycin sulfate (50 µg/ml) and glucose (final concentration 2.0 mg/ml). The osmolarity increase caused by addition of glucose was corrected by diluting the medium with distilled water. The rat serum was prepared by immediate centrifugation of the blood freshly drawn from a female STD-Wistar rat after an 18 h fast and heat-inactivated at 56 °C for 30 min[7]. The serum glucose content was measured by the glucose oxidase method[8].

Results and discussion. The inhibitory effect of BUdR on the mouse embryos of the early somitic stage was dose-

Effects of various concentrations of BUdR and inhibition of BUdR actions by thymidine(TdR) and deoxycytidine(CdR) in explanted mouse embryos of the early somitic stage

	Number of embryos explanted	Somites[a]	Protein[a] (µg)	Percent of embryos showing Heart-beat[b]	Yolk sac circulation[b]	Rotation completed	Open cranial neural tube
Untreated control	21	28.0±0.2	274.3±6.6	100	100	100	0
5 µg/ml BUdR	21	27.5±0.2	259.9±8.7	95.2	95.2	95.2	28.6
25 µg/ml BUdR	21	–[c]	186.4±8.9*	66.7	19.0	19.0	100
100 µg/ml BUdR	21	–[c]	86.7±3.0*	0	0	0	100
TdR control	15	27.7±0.2	262.1±6.0	100	95.2	95.2	0
TdR+BUdR	15	27.7±0.2	243.8±6.1**	100	100	100	6.7
CdR control	15	27.2±0.2	258.1±5.3	93.3	86.7	86.7	0
CdR+BUdR	15	26.9±0.1	203.8±7.6*	100	80.0	80.0	86.7

[a]Values represent mean±SE. [b]Including those with active heartbeat and yolk sac circulation. [c]Could not be counted because of poor definition. *Significant at $p<0.001$. **Significant at $0.05<p<0.02$.

dependent. At a concentration of 5 µg/ml, only 28.6% of the embryos showed an open cranial neural tube. At 25 µg/ml of BUdR, however, all the embryos examined had an open cranial neural tube with a significantly lower amount of protein as compared with the controls ($p < 0.001$, table). A concentration of 100 µg/ml of BUdR was highly toxic and none of the embryos had the visceral yolk sac circulation. At concentrations above 10^{-7}–10^{-6} M the analog has previously been reported to be lethal to preimplantation mouse embryos[9]. 10-day embryos generally survived free from effects for 24 h in the presence of 100–150 µg/ml of the analog[10]. Therefore, the mouse embryos of the early somitic stage seem to be more sensitive than the 10-day embryos.

The effects of 3 M excess thymidine and deoxycytidine on the inhibition of development by 25 µg/ml of BUdR were investigated. As shown in the table, thymidine almost completely reversed the morphological effects of the analog. A similar result has been reported on the inhibition of development of cleavage stages of mouse embryos[11]. In contrast to thymidine, deoxycytidine produced only a marginal effect in reversing the inhibition of the cranial neural tube closure. The result obtained by cotreatment with thymidine suggests that the cranial neural tube defects probably result from BUdR incorporation into DNA in place of thymidine. On the other hand, the effect of deoxycytidine can better be explained in terms of the nucleotide pool[12].

BUdR has been reported to inhibit differentiation in a number of different in vitro culture systems[13,14], whereas in vivo experiments have shown that the analog can cause many different types of abnormalities[1,2]. The mode of action of BUdR has not yet been satisfactorily explained. Nevertheless, the analog appears to be a valuable tool for investigating the mechanism of teratogenesis.

1 Ruffolo, P.R., and Ferm, V.H., Lab. Invest. *14* (1965) 1547.
2 Skalko, R.G., Packard, D.S., Schwendimann, R.N., and Raggio, J.F., Teratology *4* (1971) 87.
3 Kriss, J.P., and Révész, L., Cancer Res. *22* (1962) 254.
4 New, D.A.T., Coppola, P.T., and Cockroft, D.L., J. Embryol. exp. Morph. *36* (1976) 133.
5 Sadler, T.W., J. Embryol. exp. Morph. *49* (1979) 17.
6 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
7 Steele, C.E., and New, D.A.T., J. Embryol. exp. Morph. *31* (1974) 707.
8 Miwa, I., Okuda, J., Maeda, K., and Okuda, G., Clinica chim. Acta *37* (1972) 538.
9 Garner, W., J. Embryol. exp. Morph. *32* (1974) 849.
10 Agnish, N.D., and Kochhar, D.M., J. Embryol. exp. Morph. *36* (1976) 623.
11 Golbus, M.S., and Epstein, C.J., Differentiation *2* (1974) 143.
12 Goz, B., Pharmac. Rev. *29* (1977) 249.
13 Stockdale, F., Okazaki, K., Nameroff, M., and Holtzer, H., Science, N.Y. *146* (1964) 533.
14 Abbott, J., and Holtzer, H., Proc. natl Acad. Sci. USA *59* (1968) 1144.

0014-4754/83/020202-02$1.50 + 0.20/0

Peroxidase activity (PA) and progesterone receptors (PgR) in normal rat mammary glands

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Summary. PA and PgR appear to be reliable markers of estradiol action in normal female rat mammary glands, as seen by simultaneous expression of both proteins between the ages of 61 and 131 days. However, expression of PA at certain periods, when PgR was undetectable suggests, that the extent of availability of both estradiol and progesterone at target sites may act as controlling factors in synthesis of one protein over another.

As far back as 1955, Lucas et al.[3] observed that estradiol could stimulate peroxidase activity (PA) in the uteri of immature rats. However, it was only after 1972 that various groups of investigators[4-7] established that PA was a reliable indicator of estrogen stimulation of uterine growth, both by biochemical and histochemical methods. Further, this enzyme could only be induced by estradiol in target glands such as uterus, vagina and cervix of immature rats[5-7]. Our laboratory was the first to report that physiological doses of estradiol could induce PA in yet another target tissue, the mammary gland of rats, the highest inducibility of PA by estradiol being noted between 40 and 90 days of age[8].

Furthermore, as estradiol was the only hormone that could induce PA in a dose-dependent way in the mammary tissue, it appeared to be a reliable biochemical end-product of estrogenic action. Similar results have been recently reported in mammary glands of virgin mice[9].

Progesterone receptor (PgR) is another protein that is considered to be a marker of estradiol action in target tissues[10-12]. Very recently, Haslam and Shyamala[13] showed that in mammary glands of virgin mice, PgR is augmented by estradiol administration in a manner similar to that observed in the uterus. In this investigation we have simultaneously determined in the mammary glands of young, pubertal and adult intact female Holtzman rats PA and PgR formed as a result of endogenous estrogen stimulation, to see if there exists any correlation between these 2 parameters.

Materials and methods. Virgin female Holtzman rats aged 21–171 days with free access to food and water were used independently of their estrous status. For each value presented, mammary tissues from 3 to 6 rats were individually analyzed. The rats were sacrificed by an overdose of ether.

For the assessment of PA, the method of Lyttle and DeSombre[14], was essentially followed, except that crude enzyme extract (5000 g supernatant) of the mammary tissue was used. An enzyme unit was defined as the amount of enzyme required to produce an increase of 1 absorbance unit per min, and activity was expressed as units per g of protein.

The entire procedure for PgR assay was carried out at 0–4 °C. Freshly excised mammary tissue, or tissues stored in liquid nitrogen, were homogenized in 2 vol. of chilled 10 mM Tris-HCl buffer, pH 7.4 containing 1 mM Na_2EDTA and 2 mM mercaptoethanol and centrifuged for 1 h at 2 °C in a Beckman ultracentrifuge at 150,000 × g to obtain the clear cytosol. Concentrations of the specific ligand, R5020 (17,21-dimethyl-19nor-4,9 pregnadiene-3,20-dione [6,7^{3}H], 51.4 Ci/mmole) varying from 0.5 to 20 pmoles/ml, in triplicates, were added to glass tubes and to each tube,