lateral asymmetry in mouse embryo chromosomes. A possible explanation for our observations, which is consistent with current theories, is that lateral asymmetry may occur through the process of sister chromatid exchange which may involve unequal crossing over. Such an event would occur whenever an unequal number of base pairs are exchanged between sister chromatids during a crossing over event. Indeed, a theory proposed by Smith[10] suggests that the evolution of repeated DNA sequences could have developed by the mechanism of unequal crossing over while Bostock and Christie[8] have suggested that unequal sister chromatid exchange could also be implicated in the occurrence of asymmetric banding in mitotic chromosomes. The developmental significance of our observation remains to be determined.

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Inheritable precocious opening of the vagina in laboratory rats exposed to 300 R and 200 R of X-rays on day 6 of their intrauterine life

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Summary. A highly significant inheritable advancement in opening of the vagina in laboratory rats has been induced by the X-ray application to pregnant females of different filial generations originating from the X-irradiated F_0 embryos. The reaction to X-rays has so far been followed through 9 filial generations.

As far as we know there are no data to show how X-irradiation acts on the factors controlling the onset of puberty in filial generations of laboratory animals, as measured by opening of the vaginal orifice. Mandel and Griesewood[1] have claimed that the vagina of rat opened precociously after irradiation of the ovarian region, but Beaumont[2] has failed to confirm this finding. On the other hand, we know that advanced opening of the vagina can be effected by more than one experimental approach, but the phenomenon is not transmitted to filial generations[3-6].

Material and methods. The animals used in this experiment belonged to a strain of close-bred Wistar rats, in our possession since 1929. All matings were carried out between animals belonging to the same litter. The X-irradiation was performed on etherized animals with the use of a Siemens set under the following conditions: 200 kV, 14 mA, Cu 0.5 mm filter, 42 cm FSD, dose rate 100 R/min. The region of the uterus of normal pregnant females was exposed to 300 R of X-rays on day 6 of pregnancy. The young of these animals represented F_0 generation. All members of the F_2 generation and a number of F_5 offspring were also exposed to X-rays at day 6 of their intrauterine life, using this time dose of 200 R. The opening of the vaginal orifice was followed in all animals through 9 filial generations, starting from F_0. Only those

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Opening of the vagina in experimental rats through a number of filial generations

	F_0 E_1	F_1	F_2 E_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
	1*	5*	16*	17*	50*	91*	114*	109*	129*	60*
I		42.0 ± 1.3	35.5 ± 0.9	34.3 ± 1.1	34.0 ± 0.9	40.4 ± 0.6	34.7 ± 0.5	31.6 ± 0.4	31.4 ± 0.5	31.0 ± 0.6
II		38–46	30–40	28–41	22–53	27–59	24–52	23–46	22–48	21–39
III		0.00	18.75	41.18	40.00	6.59	36.84	63.30	59.69	60.00
						E_3				
						13*	19*	41*	37*	20*
					I	35.9 ± 1.4	31.5 ± 1.2	31.9 ± 0.7	28.9 ± 1.0	27.2 ± 1.1
					II	30–45	23–42	24–40	19–37	20–37
					III	38.46	57.89	63.41	72.97	80.00

F_{0-9}, Filial generations; ● E_1, E_2, E_3, exposure to X-rays. *No. of rats; I, vaginal opening – days (mean ± SE); II, range; III, percent of rats with advanced opening of the vagina.

animals in which the vagina opened on day 32, assumed to be extreme lower time limit characteristic of our strain, or before that day, were considered to show an advanced opening of the vagina.

Results. As can be seen from the figure, it is clear that advanced opening of the vagina, appearing after the exposure of F_2 embryos to X-rays, has proved to be (figure, E_2) and inheritable phenomenon which for 7 subsequent generations, so far studied, showed no signs of even gradual return to the state characteristic of normal animals. After the 3rd exposure (figure, E_3) the mean values for opening of the vagina shifted for a 2nd time in the same direction to a new position and the precocity reached what appeared to be its extreme time limit, i.e., day 19, recorded in 2 animals. At the same time, cases of extreme precocity of opening of the vagina (days 22, 21, 20, 19) increased in number. The mean age at vaginal opening of 540 normal rats, recorded in our stock over a period of the last 6 years, was found to be 41.5 ± 0.9 days, ranging from 33 to 66 days.

The b.wt of rats in which the vagina opened precociously, as would be expected, was below normal, but with aging it reached its normal value. It is worth noting that repeated exposure to X-rays on the whole in no way affected the reproductive capacity of animals with precocity or their litter mates (table).

Mean values for the first litter size of animals belonging to all filial generations

Vaginal opening	No. of rats	Litter size (mean ± SE)	Range
Advanced	114	7.0 ± 0.2	2–14
Littermates within the normal values	122	7.0 ± 0.2	2–13
Controls	620	7.0 ± 0.1	2–12

Purification and immobilization of human carbonic anhydrase B by using polyacrylamide gel

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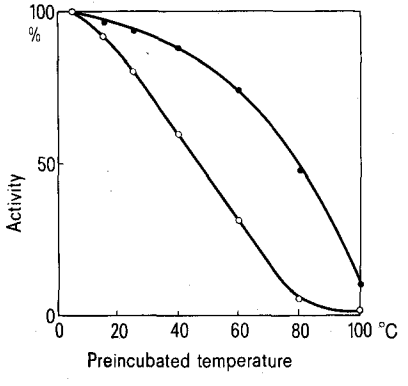
Summary. Human erythrocyte carbonic anhydrase B was purified and immobilized in polyacrylamide gel. As compared to the soluble enzyme, the immobilized enzyme was considerably more resistant to heat and sulphanilamide action.

Recently the study of the theoretical and practical aspects of matrix bound enzymes has gained a lot of interest[2–4]. A number of enzymes and isoenzymes have been purified by biospecific affinity chromatography by using different types of matrixes[3]. Because of the non-availability of preexisting functional groups, the use of polyacrylamide gel matrix has been limited[5]. Falkbring et al.[6] purified carbonic anhydrase B and C by using sulphanylamide-linked Sepharose as the specific matrix. It is known that the rate of the catalytic reaction of erythrocyte carbonic anhydrase is high after isolation from the cells. Chang first noted that, after encapsulation, the enzyme in nylon microcapsules the rate can be maintained as high as in the intact cells[7]. The present investigation deals with the affinity chromatographic purification and immobilization of carbonic anhydrase B by using polyacrylamide gel.



Effect of heat on soluble and immobilized carbonic anhydrase B activity. The soluble and immobilized enzymes were preincubated at different temperatures for 90 min prior to incubation at 25 °C for 10 min. Enzymatic activity was expressed as percentage of the respective controls (5 °C preincubation for 90 min). ○—○ soluble enzyme; ●—● immobilized enzyme.

Materials and methods. P-Nitrophenylacetate was prepared by acetylation with acetic anhydride in pyridine and recrystalized from dilute alcohol[8]. Polyacrylamide gel beads were prepared[9], pulverized and 100–200 mesh sieved materials were used as the matrix for affinity chromatography. The beads were activated to acyl azide derivative and coupled with sulphanilamide at 0–5 °C for 30 min. Partially purified enzyme was obtained by ethanol-chloroform extraction method[8] and dialyzed against 0.1 M Tris-sulphate buffer, pH 7.5, prior to couple with sulphanilamide-linked polyacrylamide gel. The coupling was conducted at 20 °C for 0.5 h and washed with the same buffer until no protein was detected in the washings. Carbonic anhydrase B was eluted with buffer containing 0.2 M KI and dialyzed against 0.05 M Tris-sulphate buffer, pH 7.5. In all these cases, soluble protein was estimated according to Lowry et al.[11]

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