which is a chlorine-deficient analogue of W-7 which only weakly interacts with calmodulin[21], did not inhibit the TPA-stimulated 2DG uptake at concentrations up to 100 μM. Prenylamine, a N-diphenylpropyl derivative of amphetamine, which was reported to antagonize calmodulin[22], also inhibited 2DG uptake stimulated by TPA. IC_{50} of prenylamine was about 25 μM (decrease in 2DG uptake: 10 ± 2.2 pmoles/mg protein/min; $n = 3$; $p < 0.05$). These inhibitory effects of calmodulin antagonists on TPA-stimulated 2DG uptake correlate well with the IC_{50}-values for inhibition of Ca^{2+}-dependent phosphodiesterase activity reported previously[18,21].

To analyze the effect of TPA and calmodulin antagonists on hexose transport, kinetic studies were carried out. Lineweaver-Burk plots of the initial rates of 2DG uptake in the presence or absence of TPA are shown in the figure, A. Chick embryo fibroblasts treated with TPA had significantly decreased K_m for 2DG transport compared to controls (1.43 ± 0.02 vs 2.77 ± 0.25 mM; $n = 4$; $p < 0.01$), but the V_{max}-values were not significantly different (22.7 ± 0.24 vs 23.5 ± 1.4 nmoles/mg protein/min). Thus, in chick embryo fibroblasts, TPA stimulates hexose transport by changing the affinity for the substrate, but not by affecting the number of hexose functional carriers. This is different from the properties in virus-transformed chick embryo fibroblasts in which V_{max} is increaed without change of K_m[23].

For analysis of the kinetics of the inhibitory action of calmodulin antagonists on TPA-stimulated 2DG uptake, W-7 was selected as a reliable antagonist because among calmodulin antagonists only W-7 was proved to penetrate through cell membrane and be distributed in cytoplasm[21]. The figure B shows Lineweaver-Burk plots of the initial rates of TPA-stimulated hexose transport in the presence or absence of W-7. In TPA-treated fibroblasts, W-7 significantly increased K_m compared to control (1.91 ± 0.13 vs 1.43 ± 0.02 mM; $n = 4$; $p < 0.01$) without affecting V_{max} (23.4 ± 1.0 vs 22.7 ± 0.24 nmoles/mg protein/min). In other words, a calmodulin antagonist W-7 reversed the decreased K_m induced by TPA. Therefore, W-7 may specifically affect the mechanism through which TPA induces the stimulation of hexose transport.

From the results obtained in this study, we suggest that the effect of TPA on hexose transport in chick embryo fibroblasts may be mediated by Ca^{2+}-calmodulin system.

1 Acknowledgment. This work was supported in part by grants-in-aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

2 Diamond, L., O'Brien, T.G., and Baird, W.M., Adv. Cancer Res. *32* (1980) 38.
3 Driedger, P.E., and Blumberg, P.M., Cancer Res. *37* (1977) 3257.
4 Lee, L.S., and Weinstein, I.B., J. Cell Physiol. *99* (1979) 451.
5 Nishino, H., Nishino, A., Fujita, K., Hasegawa, T., and Iwashima, A., Cell Struct. Funct. *5* (1980) 265.
6 Becker, J.H., and Cook, J.S., Cancer Res. *41* (1981) 4512.
7 Dicker, P., and Rozengurt, E., Biochem. biophys. Res. Commun. *100* (1981) 433.
8 Wenner, C.E., Hackney, J., Kimelberg, H.K., and Mayhew, E., Cancer Res. *34* (1974) 1731.
9 Fisher, P.B., and Weinstein, I.B., Carcinogenesis *2* (1981) 89.
10 Nishino, H., Fujita, K., Inoue, F., Hasegawa, T., and Iwashima, A., J. Kyoto Pref. Univ. Med. *90* (1981) 547.
11 Cheung, W.Y., Science *207* (1980) 19.
12 Murray, A.W., Jones, M.J., Guy, G.R., and Froscio, M., Biochem. int. *3* (1981) 1.
13 Froscio, M., Guy, G.R., and Murray, A.W., Biochem. biophys. Res. Commun. *98* (1981) 829.
14 Kwong, C.H., and Mueller, G.C., Cancer Res. *42* (1982) 2115.
15 Adams, R.L.P., in: Cell culture for biochemists, p.79. Ed. R.L.P. Adams. Elsevier/North-Holland Biomedical Press, Amsterdam 1980.
16 Markwell, M.A.K., Hass, S.M., Bieber, L.L., and Tolbert, N.E., Analyt. Biochem. *87* (1978) 206.
17 Wilkinson, G.N., Biochem. J. *80* (1961) 324.
18 Hidaka, H., and Tanaka, T., in: Calmodulin and intracellular Ca^{2+} receptors, p.19. Eds S. Kakiuchi, H. Hidaka and A.R. Means. Plenum Press, New York 1982.
19 Verma, A.K., and Boutwell, R.K., Biochem. biophys. Res. Commun. *101* (1981) 375.
20 Naito, E., Kodama, T., Takino, T., and Nishino, H., J. Kyoto Pref. Univ. Med. *92* (1983) 83.
21 Hidaka, H., Sasaki, Y., Tanaka, T., Endo, T., Ohno, S., Fujii, Y., and Nagata, T., Proc. natl Acad. Sci. USA *78* (1981) 4354.
22 Honda, F., Katsuki, S., and Sakai, N., Jap. J. Pharmac., suppl. *23* (1973) 27.
23 Inui, K., Tillotson, L.G., and Isselbacher, K.J., Biochim. biophys. Acta *598* (1980) 616.

0014-4754/83/091036-02$1.50 + 0.20/0

Effect of X-ray irradiation on developing eggs of the silkworm

S. Mathavan

School of Biological Sciences, Madurai Kamaraj University, Madurai-625 021 (India), March 3, 1982

Summary. Eggs of *B. mori* were exposed to X-ray irradiation at different times after laying, and the changes in fertilization, hatchability and mortality of the eggs were observed. Percentage of fertilization and hatchability increased with increasing age of the egg. Statistical analyses of the data show that the observed changes are age-dependent.

Insects, particularly silkworms, have been considered to be good tools for observation of genetic and nongenetic effects of ionizing radiations[1,2]. Several workers have exposed the eggs and larvae of the silkworm to varying doses of X-ray, γ-ray and UV-irradiations to obtain a dominant mutant as well as to demonstrate the radio-sensitivity of different stages of the silkworm[3-6]. Eggs of the silkworm *Bombyx mori* (Indian race) were exposed to different doses of X-ray irradiation for mutation studies and the present paper reports the observations made on the effect of X-ray irradiation on the eggs of the silkworm, at various stages of development.

Materials and methods. The silkworm chosen for the present study was an Indian race, Pure Mysore. Layings were prepared on egg cards from the freshly emerged healthy moths. Disease free layings of 1-, 3-, 6-, 9-, 12- and 24-h-old eggs were chosen for irradiation studies. The eggs laid by the moth during the first hour from the initiation of egg laying were described as 1-h-old, and the age of the eggs was determined similarly in other cases. In fact, 1- and 3-h-

Table 1. Effects of X-ray irradiation on fertilization, hatchability and mortality of the developing eggs of *B. mori*

X-ray dose (R)	Age of egg (h)	Eggs exposed (number)	Fertilized* (%)	Hatched** (%)	Killed** (%)
1000	1	2890	2	0	100
	3	2962	36	16	84
	6	3531	75	20	80
	9	3540	80	32	68
	12	3412	97	61	39
	24	3879	97	88	12
1500	1	1690	3	0	100
	3	3079	23	0	100
	6	3051	64	6	94
	9	3238	88	40	60
	12	3220	90	72	28
	24	4021	98	93	7
2000	1	2740	8	0	100
	3	3411	43	3	97
	6	2969	59	7	93
	9	3840	81	27	73
	12	3322	80	38	62
	24	3620	98	83	17
2500	1	2110	6	0	100
	3	3105	42	9	91
	6	3202	54	16	84
	9	3133	68	19	81
	12	3196	77	44	56
	24	3871	96	82	18
3000	1	2250	3	0	100
	3	3072	25	3	97
	6	3992	46	9	91
	9	3771	90	9	91
	12	3930	95	36	64
	24	4001	98	83	17

*Represented as percent of total eggs. ** Represented as percent of fertilized eggs.

Table 2. Analysis of variance for percentage of fertilization (A), hatchability (B) and mortality (C) of eggs of *B. mori*. The variance was calculated considering the age of the egg and dose of X-ray exposure

Source of variation	SS	df	MS	F
A Total	30397	29		
Between age of egg	28583	5	5716	69.7 p 0.0005*
Between X-ray dose	174	4	435	0.53 p 0.5**
Remainder	1640	20	82	
B Total	27727	29		
Between age of egg	25353	5	5070	65.94 p 0.0005*
Between X-ray dose	836	4	209	2.72 p 0.5**
Remainder	1538	20	76	
C Total	31333	29		
Between age of egg	26726	5	5345	14.03 p 0.0005*
Between X-ray dose	2368	4	592	1.5 p 0.5**
Remainder	7639	20	381	

*Highly significant. **Not significant.

old layings did not have the maximum number of eggs since the egg cards were removed before the moths completed egg laying. The eggs were exposed to the following doses of X-ray irradiations: 1000, 1500, 2000, 2500 and 3000 R. 50 layings of 1-h-old eggs were prepared and 10 layings were exposed to each dose of treatment. Equal numbers of layings were prepared with eggs of other age groups, and treated in a similar way. Immediately after irradiation the layings were incubated and development and hatching were observed. Data obtained were subjected to a test for the analysis of variance[7].

Results. Fertilized and unfertilized eggs were identified in each laying exposed to different doses of treatment, and the percentage of these eggs was calculated. Unfertilized eggs remained pale yellow in color throughout the incubation period, and the fertilized eggs attained the blue egg stage prior to hatching. Of the fertilized eggs, some did not hatch, and these were considered as 'eggs killed' due to irradiation and the percentage of mortality was calculated relating it to the fertilized eggs.

Table 1 presents data on the number of eggs (different ages) exposed to 1000, 1500, 2000, 2500 and 3000 R X-ray irradiation and the percentage of eggs fertilized, hatched and killed. Irrespective of the dose of X-rays, only about 4% of 1-h-old eggs were fertilized; while about 97% of the eggs were fertilized in 24-h-old eggs. Fertilization percentages of the other age groups lie between these 2 values. The trend obtained on the effect of X-ray irradiation on hatching is similar to the one obtained for fertilization. 1-h-old eggs failed to hatch after any of the X-ray treatments tested. Hatching percentage was very low in the early developmental stage and it increased with the increasing age of the egg. Maximum hatching of about 85% was observed in 24-h-old eggs exposed to different doses of X-ray treatment. It is clear from the table that mortality was almost 100% in the case of 1-h-old eggs and it decreased with increasing age of the egg. The minimum mortality of about 11% was observed in the 24-h-old eggs.

Further, to find out the most potent factor for the changes in the developing eggs of *B. mori*, data obtained on the effects of irradiation on the percentage of fertilization, hatchability and mortality of eggs of different ages were subjected to an analysis-of-variance test. The analysis revealed that the observed differences in the effects on the developing eggs are significantly dependant on the age of the egg ($p < 0.0005$; table 2).

Discussion. The degree of radio-sensitivity of insect eggs is dependant upon the stage and rate of developement[1]. The

high sensitivity of the eggs to X-rays has been correlated to the high mitotic activity of the developing eggs[8]. Freshly laid eggs of *B. mori* have only yolk, and the sperm received from the male moth through the micropyle. The egg completes the maturation division, the sperm and egg nuclei unite and the egg is fertilized. The whole process is accomplished in about 3.5–4.5 h after egg deposition[9]. After fertilization the cleavage nucleus undergoes active repeated division and within 24 h the germ band appears in the blastoderm and the embryo develops. It is therefore clear that the mitotic activity in the silkworm egg is at a higher rate during the early phase of development; the highest percentage of lethal effects (low fertilization, low hatchability and high mortality) of X-ray irradiation observed in the present study on 1- and 3-h-old eggs of *B. mori* can be easily correlated with this fact.

The low fertilization percentage and 100% mortality of the fertilized eggs observed in 1-h-old eggs are due to the interference of X-rays with the developing eggs of *B. mori*. Kobayashi[6] has shown that in *B. mori* the germ band formed at the irradiated region shows perforations and is irregular in shape. It is said that the process of fertilization must normally be completed within a period of 3.5–4.5 h after egg deposition. However, in the present study, a certain percentage of eggs is fertilized in 3-h-old eggs and a certain percentage of eggs is not fertilized even in 6–9-h-old eggs exposed to varying X-ray treatments. Astaurov[9] showed that the maturation division of some eggs of *B. mori* prior to fertilization may be accelerated or delayed. It is not known whether this explains the above observation. However, the lethal effects of X-rays decreased with increasing age of the egg. Working on the effects of X-ray irradiation on the developing eggs of *Manduca sexta*, Ely and Jungreis[4] demonstrated 100% mortality in 96-h-old eggs; they exposed the eggs to higher dose (40,000–50,000 R) treatment. The doses of X-ray irradiation used in the present study were not sufficient to inflict any major damage on 12–24-h-old eggs.

1 O'Brien, R.D., Radiation, radioactivity and insects. Academic Press, New York 1945.
2 Murakami, A., Int. J. Radiat. Biol. *10* (1971) 167.
3 Miki, M., and Murakami, A., J. seric. Sci., Tokyo *48* (1979) 59.
4 Ely, M.J., and Jungreis, A., J. Insect Physiol. *23* (1977) 95.
5 Kobayashi, Y., J. seric. Sci., Tokyo *49* (1980) 197.
6 Kobayashi, Y., J. seric. Sci., Tokyo *50* (1981) 51.
7 Zar, J.H., Biostatistical analysis. Prentice-Hall, Inc., 1974.
8 Packard, C., Radiology *25* (1935) 223.
9 Astaurov, B.C., Adv. Morphogen. *6* (1967) 199.

0014-4754/83/091037-03$1.50 + 0.20/0

Heterochromatin blocks in the karyotype of the pencil-tailed tree-mouse, *Chiropodomys gliroides* (Rodentia, Muridae)[1]

H.S. Yong

Department of Genetics and Cellular Biology, University of Malaya, Kuala Lumpur 22-11 (Malaysia), February 15, 1983

Summary. Metaphase chromosomes of *Chiropodomys gliroides* (2n = 42) were studied by G- and C-banding. One arm of both pairs of biarmed autosomes and the Y-chromosome is totally heterochromatic. Most of the other autosomes and the X-chromosome have large pericentromeric C-bands.

Additions of heterochromatin and pericentric inversions have been suggested to be the principal mechanisms involved in the evolution of the karyotypes of the New World rodent genera *Peromyscus, Onychomys* and *Baiomys*[2,3]. The phenomenon of heterochromatin addition has not been reported in the Old World murid rodent genera *Mus* and *Rattus*[4–6]. The present report concerns the presence of heterochromatic blocks in the karyotype of an Old World murid rodent, *Chiropodomys gliroides* (Blyth).

Chiropodomys Peters, 1868 is considered to be one of the most primitive murid genera, making the transition between the *Lenothrix* and the *Parapodemus* groups[7]. It is probably closely related to *Vernaya, Vandeleuria* and *Micromys*. The species *C. gliroides* is a relict form which occurs from north-eastern India, Burma and southern China, southwards to Sumatra, Java and Borneo. The mitotic and meiotic chromosomes of *C. gliroides* have been previously described[8]. That report, however, did not include G- and C-banding.

Recently a male and 2 female specimens of *C. gliroides* collected from Peninsular Malaysia were available for study. Bone-marrow preparations and G- and C-bandings were performed by conventional methods.

The karyotype of the present material is identical to that reported previously - 18 pairs acrocentric, 1 pair metacentric and 1 pair submetacentric autosomes (m and sm, respectively, in the figures), metacentric *X* and submetacentric *Y* sex chromosomes. Figures 1 and 2 illustrate the G- and C-banding patterns respectively.

In both the G- and C-banded metaphase plates, one arm of the metacentric and the short arm of the submetacentric autosomes are wholly heterochromatic. Likewise, the *Y*-chromosome is entirely heterochromatic. Most of the other autosomes and the *X*-chromosome have large pericentromeric C-bands. The *X*-chromosome also possesses a terminal C-band block on the arm with a pericentromeric C-band.

The occurrence of whole-arm heterochromatin blocks in both the biarmed autosomes, the presence of large pericentromeric heterochromatin in most autosomes and the completely heterochromatic *Y*-chromosome of *C. gliroides* are very similar to the situation reported for the closely-related genus *Micromys*[9]. This phenomenon of large heterochromatin blocks in the autosomes is, however, not found in the other closely-related genus *Vandeleuria*[10].

It is evident from comparison between the karyotypes of *C. gliroides, Vandeleuria oleracea* and *Micromys minutus* that different mechanisms were involved in the karyological evolution of these closely-related murid genera. In the case of *Chiropodomys* and *Micromys,* the addition of heterochromatin plays an important role, as is the case in some New World rodent genera[2,3].