substantial fraction of the total outward current between −20 mV and +100 mV which has been identified as a Ca-dependent K^+ current[21,22]. Substitution of Ba^{2+} for external Ca^{2+} did not restore the Ca-dependent component and further reduced the total outward current at all potential levels (closed circles, figure 2, B).

Our results demonstrate that internal Ba^{2+} blocks the Ca-dependent, as well as the voltage-dependent K^+ current. Out findings agree with recent results from squid axon[23,24] where internal perfusion of Ba^{2+} ions blocks the voltage-dependent K^+ current and with a variety of results from muscle fibres[13,14,18]. Our finding that internal Ba^{2+} blocks the Ca-dependent K^+ current is not consistent with previous reports from other molluscan neurons[3]. We have no explanation for this conflict in results except for the possibility that cells differ in their response to Ba^{2+} ions. The present results with the effects of external Ba^{2+}, as well as previous findings with other nerve cells[19,20,22,25] are, however, more consistent with our Ba^{2+} injection results than with the possibility that internal Ba^{2+} activates a Ca-dependent K^+ current. We have no information about the absolute internal Ba^{2+} concentration necessary to block K^+ channels. We find, however, that the Ba^{2+} block occurs at internal concentrations that do not alter substantially the Ca-dependent K^+ current reversal potential and, therefore, at concentrations that presumably do not alter the intracellular K-concentration. Unless internal Ba^{2+} exchanges for some other cation (e.g. Na^+ or Mg^{2+}) the concentration necessary to block K channels must be small. It has been suggested that Ba^{2+} ions can enter but not pass through the K^+ channel because of steric factors[13,14]. Our finding that *both* components of the K^+ current are blocked by internal Ba^{2+} suggests that the 2 components of the K^+ current may represent different aspects of the same K^+ channel.

1 R. Whittam, Nature *219*, 610 (1968).
2 V.L. Lew, J. Physiol. *206*, 35 (1970).
3 R.W. Meech, Symp. Soc. exp. Biol. *30*, 161 (1976).
4 E.F. Barrett and J.N. Barrett, J. Physiol. *255*, 737 (1976).
5 G. Isenberg, Nature *253*, 273 (1975).
6 R.W. Meech, Comp. Biochem. Physiol. *48A*, 397 (1974).
7 T.K.S. Jansen and T.G. Nicholls, J. Physiol. *229*, 635 (1973).
8 M.V. Thomas and A.L.F. Gorman, Science *196*, 531 (1977).
9 W.T. Clusin and M.V.L. Bennett, J. gen. Physiol. *69*, 145 (1977).
10 A.M. Brown and H.M. Brown, J. gen. Physiol. *62*, 239 (1973).
11 R.W. Meech, J. Physiol. *237*, 259 (1974).
12 R.W. Meech and R.C. Thomas, J. Physiol. *265*, 867 (1977).
13 R. Werman and J. Grundfest, J. gen. Physiol. *44*, 997 (1961).
14 N. Sperelakis and D. Lehmkuhl, J. gen. Physiol. *49*, 867 (1966).
15 W.T. Frazier, E.R. Kandel, I. Kupferman, R. Waziri and R.E. Coggeshall, J. Neurophysiol. *30*, 1288 (1967).
16 A.L.F. Gorman and M.V. Thomas, J. Physiol. *275*, 357 (1978).
17 D.L. Kunze, H.M. Walker and H.M. Brown, Fed. Proc. Fed. Am. Soc. exp. Biol. *30*, 255 (1971).
18 S. Hagiwara, Adv. Biophys. *4*, 71 (1973).
19 R. Eckert and H.D. Lux, J. Physiol. *254*, 129 (1976).
20 M. Gola and C. Ducreux, Experientia *33*, 328 (1976).
21 R.W. Meech and N.B. Standen, J. Physiol. *249*, 211 (1975).
22 C.B. Heyer and H.D. Lux, J. Physiol. *262*, 349 (1976).
23 S.R. Taylor and C.M. Armstrong, J. Biophys. *21*, 164a (1978).
24 D.C. Eaton and M.S. Brodwick, J. Biophys. *21*, 164a (1978).
25 J.S. Magura, J. Memb. Biol. *35*, 239 (1977).

Pigment aggregation by melatonin in the retinal pigment epithelium and choroid of guinea-pigs, *Cavia porcellus*

S.F. Pang and D.T. Yew[1]

Department of Physiology and Department of Anatomy, Faculty of Medicine, University of Hong Kong (Hong Kong), 13 June 1978

Summary. The treatment of pigmented guinea-pigs with melatonin aggregates pigmented cells in the retinal pigment epithelium and choroid of the eye. This suggests that melatonin may regulate eye pigmentation in vertebrates.

Melatonin (N-acetyl-5-methoxytryptamine), a derivative of the putative neurotransmitter, serotonin, was isolated and identified from the bovine pineal gland[2,3]. Subsequently, melatonin was found in the pineal gland of all the animals studied[4,5]. Pineal melatonin is secreted into the circulation with a high activity at night and a low or undetectable activity in the daytime[6-10], and is suggested to have an antigonadal function[11-13]. In addition to the well-established antigonadal function of melatonin, in the lower vertebrates such as the frog, melatonin is the best melanophore aggregating agent known[3]. However, melatonin appears to have little effect on mammalian pigmentation[14]. Recently, melatonin has been identified in the nervous tissue, retina, Harderian gland and intestine of chicks and rats[15-18]. The function of melatonin in the above tissues remains to be elucidated. In this report, the effect of melatonin on the retinal pigment epithelium of the guinea-pig was investigated, and melatonin was found to have a potent pigment aggregating action on the retinal pigment epithelium and choroid of the eye.

Materials and methods. Male and female pigmented (coloured) guinea-pigs *Cavia porcellus* (200–450 g), obtained from the animal house, Faculty of Medicine, University of Hong Kong, were used. All experiments were performed under 300–400 lux illumination and a temperature of 23–26 °C. The animals were anaesthetized with 60 mg/kg sodium pentobarbital (50 mg/ml, Sigma). The corneas of the 2 eyes were removed and the lens were taken out. A pair of blunt forceps was used to remove as much vitrous humor as possible. Operated animals were placed in a prone position. Melatonin solution (1 ng/ml, 10 ng/ml, 100 ng/ml and 1 µg/ml in 0.9% saline) was perfused into one of the operated eye cups while the other eye cup was perfused with carrier, both at a rate of 1.5 ml/min. There were at least one left eye cup and one right eye cup perfused by melatonin solution in each group. After 20 min of melatonin or carrier perfusion (20 min was used because it takes 20–90 min for the completion of physiological color changes in lower vertebrates), the eyes were removed and fixed in Bouin's solution. The fixed materials were embedded in paraffin, sectioned at 6 µm, stained with hematoxylin and eosin and observed under light microscope. The above experiment was repeated with N-acetylserotonin (1 µg/ml, Sigma) and 5-hydroxytryptamin (1 µg/ml, Sigma).

In other experiments, guinea-pigs were anaesthetized and 50 µl of melatonin solution (2 µg/ml, 20 µg/ml, 200 µg/ml and 2 mg/ml) were injected into the posterior chamber of

one eye while the other eye was injected with 50 µl of carrier. After 30 min, the eyes were removed and processed as noted. There were a minimum of one left eye and one right eye for each experimental or control group.

In the last groups of experiments, conscious guinea-pigs with intracarotid cannula implanted 4 h earlier were used. Melatonin solution (0.1 mg/ml) was perfused through the intracarotid cannula for 45 min at a rate of 1.25 ml per h. The animals were decapitated right after perfusion and the eyes were removed and processed as noted. Untreated animals were used as control.

Results. In all experiments, the eyes of carrier-treated or untreated animals displayed heavy pigmentation in pigmented layers and around blood vessels in choroidal regions. The melanin in pigmented epithelium dispersed out in pigmented cells (figure 1). The eyes of melatonin-treated guinea-pigs, on the other hand, had aggregated pigmented cells and the sections appeared lighter in contrast. In choroids, only limited areas around blood vessels were pigmented and the pigment granules in the chromatophores clumped together so that most of these cells displayed a much rounder appearance. In the pigmented epithelium, melanin aggregated in the apical regions of pigmented cells, thus unmasking the nuclei of the pigmented cells (figure 2). Results of the experiments are summarized in the table. The aggregation effect of melatonin on the eye pigmentation could be achieved by intraocular perfusion, intraocular injection or intracarotid perfusion. When intraocular perfusion was used, pigment aggregation could be observed at a concentration as low as 1 ng of melatonin/ml. However, intraocular perfusion of 5-hydroxytryptamine, the precursor of melatonin, has no effect at a concentration as high as 1 µg/ml and intraocular perfusion of 1 µg/ml of N-acetylserotonin, another melatonin precursor, resulted in pigment dispersion.

Fig. 1. Choroid and pigment epithelium of guinea-pig perfused with carrier for 20 min. Note slender chromatophores surrounding the choroidal vessels (arrows). Also note rather even distribution of pigmentation in the cells of the pigment epithelium (P). (6 µm section, stained with hematoxylin and eosin; × 100.)

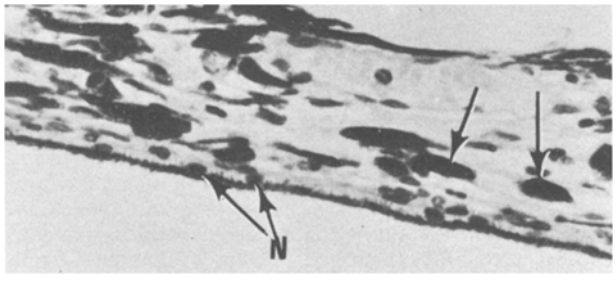

Fig. 2. Choroid and pigment epithelium of guinea-pig perfused with 1 ng/ml melatonin for 20 min. Note: 1. Few chromatophores surrounded the vessels. 2. The chromatophores were rounder in contour (arrows) and darker in contrast. The pigment granules in the pigment epithelium were located at the apical regions of the cells, thus unmasking their nuclei (N). (6 µm section, stained with hematoxylin and eosin: × 100.)

Discussion. Melatonin blanching ability on the pigmentation of adult frog or on tadpole melanophores has been studied extensively[15,19,20]. In concentrations as low as 10^{-10} to 10^{-11} g/ml, melatonin causes lightening in tadpole melanophores[8,21] and in concentration as low as 10^{-12} g/ml, melatonin prevents or reverses the darkening actions of MSH, ACTH and caffeine on the frog skin[22]. Although melatonin has an exceptional potency in melanophore pigment aggregation of many lower vertebrates, attempts using melatonin to modify human or mammalian pigmentation have met with little success[14,23,24]. In this experiment, it is found that intraocular perfusion of melatonin, at a concentration of 10^{-9} g/ml, aggregates pigments in the eye of guinea-pigs. The concentration of 10^{-9} g/ml is comparable to the concentration needed to aggregate dermal melanophores in frogs.

The presence of melatonin in rat and chicken retina has been established by bioassay[25] and radioimmunoassay[16]. Using an immunohistochemical technique, melatonin has been found in the outer nuclear layer of the rat retina[18]. The above findings, together with the demonstration of hydroxy-indole-O-methyl-transferase[14,26], and N-acetylserotonin[16] in the retina, suggest that melatonin may be synthesized de novo in the retina[16]. The facts that melatonin is present in the retina and that exogenous melatonin has a lightening effect on the eye pigmentation, as demonstrated in this study, suggest that endogenous melatonin in the retina and/or the circulating melatonin from the pineal may regulate eye pigmentation under physiological conditions. Recently a diurnal variation of eye pigmentation was observed in pigmented guinea-pigs. The pigments in the eye of guinea-pigs appear to aggregate in the dark period but disperse in the light period[27]. With the above findings, we would like to suggest that, in guinea-pigs and possibly in other animals, during the daytime, the pineal and the hyperpolarized photoreceptors secrete little or no melatonin, and the pigments in the eye disperse in the absence of melatonin and/or under the influence of MSH. The dispersed pigments may prevent or minimize the diffusion of light in the visual layer and/or the reflection of light from the choroid. In darkness, the depolarized photoreceptors secrete melatonin which acts locally on the pigment melatonin secreted by the pineal[6–8] may also be important in eye

Effect of melatonin, N-acetylserotonin and 5-hydroxytryptamine on guinea-pig eye pigmentation

Treatment		Results
A. Intraocular perfusion		
Melatonin	1 ng/ml (2)*	Pigment aggregation
	10 ng/ml (2)	Pigment aggregation
	100 ng/ml (2)	Pigment aggregation
	1 µg/ml (3)	Pigment aggregation
N-acetylserotonin	1 µg/ml (2)	Pigment dispersion
5-hydroxytryptamine	1 µg/ml (2)	No detectable change
B. Intraocular injection		
Melatonin	100 ng/eye (2)	Pigment aggregation
	1 µg/eye (2)	Pigment aggregation
	10 µg/eye (2)	Pigment aggregation
	100 µg/eye (2)	Pigment aggregation
C. Intracarotid perfusion		
Melatonin	0.1 mg/animal (3)	Pigment aggregation

* Number of animals studied in parentheses.

pigment aggregation. Aggregation of pigments in the pigment cells of the retina and the choroid would allow light to diffuse out and sensitize more photoreceptors, or permit light that passed the retina to be reflected back and sensitize the photoreceptors again.

1 We thank Mr M.K. Yip, Mr C.Y. Wat and Miss P. Yip for assistance and Prof. A. Hsieh and Prof. J. Widdicombe for reading the manuscript.

2 A.B. Lerner, J.D. Case, Y. Takahashi, T.H. Lee and W. Mori, J. Am. Chem. Soc. *80,* 2587 (1958).
3 A.B. Lerner, J.D. Case and R.V. Heinzelman, J. Am. Chem. Soc. *81,* 6084 (1959).
4 R.J. Wurtman, J. Axelrod and D.E. Kelly, The Pineal. Academic Press, New York 1968.
5 C.L. Ralph, Am. Zool. *16,* 35 (1976).
6 R.W. Pelham, C.L. Ralph and I.M. Campbell, Biochem. biophys. Res. Commun. *46,* 1236 (1972).
7 R.W. Pelham, Endocrinology *96,* 543 (1975).
8 S.F. Pang and C.L. Ralph, J. exp. Zool. *193,* 275 (1975).
9 J. Arendt, L. Pannier and P.C. Sizonenko, J. clin. Endocr. Metab. *40,* 347 (1975).
10 G.M. Vaughan, R.W. Pelham, S.F. Pang, L.L. Laughlin, K.M. Wilson, K.L. Sandock, M.K. Vaughan, S.H. Koslow and R.J. Reiter, J. clin. Endocr. Metab. *42,* 752 (1976).
11 F.W. Turek, C. Desjardins and M. Menaker, Science *190,* 280 (1975); F.W. Turek, C. Desjardins and M. Menaker, Proc. Soc. exp. Biol. Med. *151,* 502 (1976).
12 J.E. Martin and D.C. Klein, Science *191,* 301 (1976).
13 L.C. Ellis, Am. Zool. *16,* 67 (1976).
14 W.B. Quay, Pineal Chemistry. Thomas, Springfield 1974.
15 S.F. Pang, C.L. Ralph and D.P. Reilly, Gen. Comp. Endocr. *22,* 499 (1974).
16 S.F. Pang, G.M. Brown, L.J. Grota, J.W. Chambers and R.L. Rodman, Neuroendocrinology *23,* 1 (1977).
17 G.A. Bubenik, G.M. Brown, I. Uhlir and L.J. Grota, Brain Res. *81,* 233 (1974).
18 G.A. Bubenik, G.M. Brown and L.J. Grota, Brain Res. *118,* 417 (1976a); G.A. Bubenik, G.M. Brown and L.J. Grota, J. Histochem. Cytochem. *24,* 1173 (1976b); G.A. Bubenik, G.M. Brown and L.J. Grota, Experientia *32,* 579 (1976c).
19 A.B. Lerner and J.D. Case, J. invest. Derm. *32,* 211 (1959).
20 W.B. Quay and J.T. Bagnara, Archs Inst. Pharmacodyn. Ther. *150,* 137 (1964).
21 C.L. Ralph and H.J. Lynch, Gen. Comp. Endocr. *15,* 334 (1970).
22 R.M. Wright and A.B. Lerner, Endocrinology *66,* 599 (1960).
23 R.S. Snell, J. invest. Derm. *44,* 273 (1965).
24 J.S. McGuire and H. Moller, Nature *298,* 493 (1965).
25 D. Mull and C.L. Ralph, Am. Zool. *12,* 674 (1972).
26 W.B. Quay, Life Sci. *4,* 983 (1965).
27 S.F. Pang, D.T. Yew and H.W. Tsui, Neurosci. Lett., in press (1978).

Sensitivity of human lymphocytes to bleomycin increases with age

R.S. Seshadri, A.A. Morley, K.J. Trainor and J. Sorrell[1]

Department of Haematology, Flinders Medical Centre, Bedford Park (South Australia 5042), 21 August 1978

Summary. The sensitivity of human peripheral blood lymphocytes to bleomycin and mitomycin-C was assessed by measuring the inhibition of phytohemagglutinin stimulated proliferation. The sensitivity to bleomycin, and not to mitomycin-C, increased with the age.

Aplastic anaemia, a marrow disorder characterized by chronic marrow failure, shows some features which have led to the suggestion that it represents premature or induced ageing of haemopoiesis[2]. We recently observed[3] that lymphocytes from some patients with the disorder were abnormally sensitive to bleomycin and, since this agent acts by causing strand breaks in DNA[4-6], we suggested that abnormality of DNA structure or repair was involved in the disease. During the course of these studies we observed that lymphocytes from normal individuals showed an increasing sensitivity to bleomycin with age and in this paper we report these observations and discuss their possible significance for the aetiology of ageing.

Studies were performed in 89 individuals. Cord blood was obtained at the time of delivery from 16 full-term normal babies and venous blood was obtained from 32 males and 41 females between the ages of 2 and 86 years. All of the subjects studied were healthy and, except for some females on oral contraceptives, none were taking any medication. Lymphocytes were stimulated with phytohaemagglutinin (PHA) and the proliferative response was measured after 72 h by pulsing with ^{3}H-thymidine. Lymphocytes from all 89 subjects were exposed to bleomycin and lymphocytes from 63 subjects to mitomycin. Repeat studies with bleomycin were performed on a total of 44 occasions in 15 individuals.

The drug concentrations producing 50% inhibition of ^{3}H-thymidine uptake were logarithmically transformed and the relationship with age was analysed by linear regression analysis. There was a significant correlation between the sensitivity of lymphocytes to bleomycin and the age of the individual ($r = -0.54$; $p = < 0.0001$) (figure 1). The variance about the regression line was 0.33 whereas the variance of repeated estimations in 15 individuals was 0.18; these results suggest that, of the observed variation in bleomycin sensitivity unrelated to age, approximately 55% was due to within-individual experimental variation and 45% was due to true between-individual variation. There was no correlation between the sensitivity of lymphocytes to mitomycin-C and the age of the individual (figure 2) ($r = -0.17$; $p =$ not significant).

The observed increase in the sensitivity of lymphocytes to bleomycin with age could be due to a membrane phenomenon since an age-related increase in passive diffusion or active transport of the drug across the cell membrane would result in an age-related increase in sensitivity to it. Such an explanation would be of great interest for the understanding of ageing but it seems unlikely since there is little other evidence that a primary membrane alteration is involved in ageing (reviewed in Masoro[7]). Most hypotheses on the nature of ageing have stressed the importance of alteration of critical cellular macromolecules, and particularly of DNA. There is evidence to suggest that ageing is associated with an increase in DNA strand breaks[8-10] and a decrease in DNA replication[11-13]. Several observations can conceivably be interpreted as indicating a decrease in DNA-protein cross-linking with age[14-16], and Hart and Setlow reported that the extent of DNA repair in a variety of animal species was related to longevity of those species[18]. Since DNA is the primary target for bleomycin, the increased sensitivity to bleomycin observed in lymphocytes from aged individuals suggests an age-related alteration in DNA in these cells. This could be a structural alteration of DNA which by some mechanism results in production of a