Anaphylactic reactions in groups of 5 guinea-pigs immunized s.c. with 50 µg of $DNP_{48}BGG$ in FIA

No. of *C. pyrenoidosa* emulsified with the antigen in FIA	Anaphylactic reactions to the hapten on day		Anaphylactic reactions to the carrier on day		Foot pads inflammation on day	
	8	12	8	12	8	12
0	13.0±1.7	15.2±1.0	0	0	+	+
4×10^6	12.0±1.5	14.4±1.4	0	12.6±2.9	+	+
4×10^7	9.6±1.2 (1)	12.6±0.8 (3)	0	12.4±1.9	+++	++
4×10^8	11.0±1.4 (2)	11.8±1.7 (3)	0	0	++++	+++

The results are expressed as Evans blue extravasation diameters in mm (±SE) after i.d. injections of 10 µg of antigen. Degree of foot pads inflammation are quoted from + to ++++. (1) $p<0.05$. (2) $0.1>p>0.05$. (3) $p<0.02$.

C. pyrenoidosa, which have been reported to protect C_3H mice against sarcoma BP8, appeared, when injected in FIA, to modulate the antibody synthesis induced by immunization with a hapten-carrier complex. Their effects were transient, observed only at the beginning of the response and could not be directly correlated with the inflammation at the site of injection. On the other hand, the effects of *C. pyrenoidosa* depended on the dose injected. A heavy dose led to a depression of the antibody synthesis while a light dose led to a very small depression of reactions to the hapten but to the induction of antibody synthesis to the carrier. A medium dose of *C. pyrenoidosa* appeared to depress antibody synthesis to the hapten and to induce antibody synthesis to the carrier. The possible immune response to algal antigens could not account for these results. It could be assumed that *C. pyrenoidosa* initiate an antigenic competition between hapten and carrier determinants in antibody synthesis without concomitent variation of DH during the early response to a hapten-carrier complex. If this is true, as antigenic competition between hapten and carrier moieties of the antigen molecule has been previously shown to occur at the macrophage level[13], it could be speculated that *C. pyrenoidosa* modulates antibody synthesis through the macrophage.

1 Acknowledgments. This work was supported by the Secrétariat d'Etat aux Universités and by the DGRST, grant No. 77.71347.
2 Correspondence to Prof. C. Vermeil, Laboratoire de Parasitologie UER de Médecine, F-44035 Nantes Cédex, France.
3 L.J. Old, D.A. Clarke and B. Benacerraf, Nature *184*, 291 (1959).
4 B.N. Halpern, G. Biozzi, C. Stiffel and D. Mouton, Nature *212*, 853 (1966).
5 Z.T. Mankowski, I.C. Diller and M.E. Fisher, Cancer Res. *17*, 382 (1957).
6 P. Lemonde, R. Dubreuil, A. Guindon and G. Lussier, J. natl Cancer Inst. *47*, 1013 (1971).
7 C. Vermeil and O. Morin, C. r. Soc. Biol. *170*, 646 (1976).
8 B. Benacerraf and P.G.H. Gell, Immunology *2*, 53 (1959).
9 J.H.L. Playfair, Clin. exp. Immunol. *8*, 839 (1971).
10 B. Benacerraf and W.E. Paul, in: Cellular interactions in the immune response, p. 298. Ed. S. Cohen, G. Cudkowitz and R.T. McCluskey. Karger, Basel 1971.
11 H. Wigzell, Transplant. Rev. *5*, 76 (1970).
12 G.A. Voisin and F. Toullet, Annls Inst. Pasteur *111*, 376 (1976).
13 P.J. Neveu, Immunology *33*, 269 (1977).

Pineal N-acetyltransferase depression in rats exposed to heat

I. Nir and N. Hirschmann

Department of Pharmacology and Experimental Therapeutics, Hebrew University Schools of Medicine and Pharmacy, Jerusalem (Israel), 24 April 1978

Summary. Exposure of adult male rats to increased temperature of 33±1 °C for 3 and 10 days brought about decreases in pineal N-acetyltransferase activity. These and previous findings of pineal HIOMT inhibition under similar conditions support the postulation of a possible thermoregulatory role for the pineal gland.

It has been established that the mammalian pineal is a neuroendocrine gland transducing external environmental stimuli into certain hormonal and behavioural processes. Among the external stimuli influencing the metabolic and hormonal activity of the pineal gland, the effect of environmental lighting has been most thoroughly investigated. Exposure to continuous light, or to long daily photoperiods, was found to decrease the weight[1], protein and RNA content[2] of the rat pineal and to inhibit strongly the activity of N-acetyltransferase (NAT)[3], and of hydroxyindole-O-methyltransferase (HIOMT)[4] responsible for the biosynthesis of the pineal specific hormone melatonin. Little is known about the relationship of the pineal to environmental stimuli other than light, such as temperature, sound, etc. Brief and prolonged exposure of rats to low temperature produced pineal hypertrophy and increased metabolic activity[5,6], while high temperature decreased pineal contents of protein and RNA[7] as well as HIOMT activity[8]. Since NAT activity was found to be highly sensitive - much more so than HIOMT - to changes in environmental lighting, and as NAT is the primary enzyme in the synthesis of melatonin, we decided to investigate the effect of high environmental temperature on this pineal enzyme.

Materials and methods. Male rats weighing 160-180 g were divided into 3 groups of 6-8 animals each and housed 3 or 4 to a cage. 2 groups were exposed to a constant heat of 33±1 °C, one for 3 and the other for 10 days; the 3rd group, which served as control, was kept at a temperature of 24±1 °C. Light was switched on at 06.00 h and off at 18.00 h each day. After exposure to their respective thermoenvironments for 3 or 10 days, the rats were decapitated either between 22.30 h and 23.00 h or between 10.30 h and

11.00 h. The pineal glands were removed rapidly, rinsed in cold saline solution and frozen by liquid air.

N-acetyltransferase activity was assayed by a modified version of the method of Deguchi and Axelrod[9] using a 2 dimensional TLC systems for separating the radioactive compounds produced. 1 pineal gland was homogenized in 30 µl 0.1 M sodium phosphate buffer pH 6.5. Aliquots of 15 µl homogenate were placed in incubation tubes each containing 5 µl freshly prepared 0.1 µM serotonin creatinine sulfate in phosphate buffer pH 6.5 and 5 µl acetyl-1 ^{14}C coenzyme A (2 µmoles = 100 µCi) sealed with parafilm and incubated for 15 min at 37 °C. To stop the reaction, 25 µl ethanol containing N-acetyl-serotonin and melatonin at a concentration of 1 mM were added to the tubes. The radioactive N-acetylserotonin and melatonin formed were separated from the radioactive acetyl coenzyme A by TLC. A 40 µl aliquot of the reaction mixture was applied to the left corner of a precoated plastic 20×10 cm sheet (Polygram: Sil G/UV_{254}, Macheray Nagel & Co., Düren, Germany) under a continuous stream of nitrogen. Sheets were developed in darkness in 2 directions: 1stly, in a solvent system of ethylacetate and ethanol (85:15) at the long dimension; then, after the sheets had been rapidly dried, a strip containing the adherent spots of N-acetylserotonin and melatonin was cut out from the chromatograph and was run in a 2nd direction in a system composed of isopropanol methylacetate/ammonium hydroxide 25% (45:35:20). The latter step was necessary in order to free the product obtained from any adherent radioactive impurities. The adherent radioactive common spots of N-acetylserotonin and melatonin, which are located near the top of the strip, were marked under a UV-lamp, cut out from the sheet and each placed in a counting vial to which 10 ml scintillation fluid were added. The samples were then counted by standard scintillation techniques. Indoleamines and ^{14}C acetylcoenzyme A (sp. act. 54 mCi/mmole) were obtained from Sigma Chemical Co. and New England Nuclear respectively. The procedure used gave reproducible results linear with time of incubation and with quantity of pineal homogenate taken and with much lower background (less than 100 cpm) than any other method tried.

Results. From the table, it can be seen that after exposure to 33±1 °C heat for 3 days a significant (41%) decrease in pineal NAT take place in rats killed during the day. When heat exposure is prolonged to 10 days, the NAT is still significantly depressed when compared to that of animals kept in room temperature (24±1 °C), although to a lesser extent (32%). Rats exposed to heat for 3 or 10 days and killed at night (when pineal NAT activity is at its peak) also demonstrated decreased activity of the enzyme, which corresponds with the findings observed in animals killed during the day. The differences, however, never reached obvious significance due to the magnitude of the individual variations in the values in each group.

Effect of continuous heat (33±1 °C) on rat pineal N-acetyltransferase activity*

Time of sacrifice	Days of exposure 3	10	Control (23±1 °C)
10.30–11.00 h	9.9± 1.2** (24)	11.5± 1.2*** (26)	16.9± 1.2 (18)
22.30–23.00 h	517.3±82.5 (18)	605.8±102.7 (22)	835.6±161.4 (16)

*pmole melatonin/h/pineal± SEM; ** $p < 0.001$; *** $p < 0.005$ compared to control. Figures in parentheses = number of samples.

Discussion. We have previously shown that exposure of rats to heat for 1–3 days resulted in significant decrease in pineal HIOMT activity[8]. In the present work we found that the same treatment depresses pineal NAT activity to an even greater degree than it does that of HIOMT. Similar to the effect on HIOMT activity, extension of the heat exposure to 10 days did not increase the degree of depression of pineal NAT. Moreover, after 10 days' heat exposure a tendency towards a more moderate decrease in pineal NAT was observed, which could indicate that the heat-induced depression may further decline or even disappear with time, should the animal's exposure to heat be extended even further.

The effect of elevated ambient temperature on the pineal metabolism appears to occur in 2 or 3 phases. 1stly, a few h exposure to 33±1 °C strongly decreases the activity of NAT without affecting that of HIOMT or the serotonin content of the pineal gland, at least in suckling rats[10]. 2ndly, after 24 h exposure to 33±1 °C, a decrease in activity of the 2 melatonin-forming enzymes NAT and HIOMT occurs[8], but not always reaching significance. The decrease reaches its fullest extent on or just after the 3rd day of exposure, tending to diminish at 10 days' exposure. During these phases, no apparent changes could be recorded in pineal RNA, DNA or protein contents nor in the weight of the gland[7]. Later, with continuous exposure of the animal to heat, increasing depression of pineal protein and RNA contents becomes apparent, suggesting that the basic metabolism of the gland is affected too[7]. Similarly, exposure of maturing rats to 33±1 °C for 20 days strongly depressed body, liver, kidney and thyroid weights[11]. However, the much earlier response of pineal NAT (and HIOMT) than of RNA/protein metabolism to heat stimuli, may allow us to assume that the effect of environmental heat on the melatonin-forming enzyme is not a result of a general inhibition of the body and pineal metabolism. Analogous to our findings are those that exposure of rats to cold had a stimulatory effect on pineal activity[5,6], with strongly decreased gonadal weight and activity in male and female rodents, although pinealectomy did not affect the regressive changes in the gonads of animals exposed to cold[12,13].

The enzymic and general metabolic changes recorded in the pineals of rats exposed to altered environmental temperatures encouraged a postulation implicating the pineal gland in thermoregulatory functions of some mammals and birds.

1 V. Fiske, G.K. Bryant and J. Putnam, Endocrinology *66*, 489 (1960).
2 I. Nir, N. Hirschmann, J. Mishkinsky and F.G. Sulman, Life Sci. *8*, 279 (1969).
3 H. Illnerova, Neuroendocrinology *16*, 202 (1974).
4 J. Axelrod, R.J. Wurtman and S.H. Snyder, J. biol. Chem. *240*, 949 (1965).
5 R. Miline, V. Deverčerski, N. Šijački and R. Krstić, Hormones *1*, 321 (1970).
6 C.D. Bucana, M.J. Nadakavukaren and J.L. Frehn, J. Neurocyt. *2*, 237 (1973).
7 I. Nir, N. Hirschmann and F.G. Sulman, Experientia *28*, 701 (1972).
8 I. Nir, N. Hirschmann and F.G. Sulman, Experientia *31*, 867 (1975).
9 T. Deguchi and J. Axelrod, Analyt. Biochem. *50*, 174 (1972).
10 R. Ulrich, A. Yuwiler, L. Wetterberg and D. Klein, Neuroendocrinology *13*, 255 (1973/74)
11 S. Dikstein, Y. Kaplanski, Y. Koch and F.G. Sulman, Life Sci. *9*, 1191 (1970).
12 R.J. Reiter, J. Reprod. Fert. *16*, 217 (1968).
13 Ch.-Ch. Lin and J.L. Frehn, Experientia *29*, 1507 (1973).