TABLE 2. NEONATAL AFFINITY (pD_2) AND RELATIVE HYPOSENSITIVITY TOWARDS FURTRETHONIUM AT VARIOUS AGE DAYS

Age	pD_2* ± S.E.	Est/org.†	Significance‡	Relative hyposensitivity§
Adult	6·49 (±0·06)	62/26	—	1·0
1 day	5·72 (±0·20)	32/14	$P < 0·01$	−5·8
6 days	5·79 (±0·20)	12/5	$P < 0·01$	−5·0
9 days	6·28 (±0·22)	21/9	$P < 0·05$	−1·6
12 days	6·28 (±0·18)	21/9	$P < 0·05$	−1·6
18 days	6·43 (±0·21)	8/4	n.s.	1·0

* pD_2 (affinity): negative logarithm of the concentration of agonist required to produce 50 per cent of the maximal response obtained in the system.

† Est/org.: number of cumulative dose-response curves/number of organs used.

‡ Significance levels of the neonatal affinity in comparison to that of the adult.

§ Relative hyposensitivity expressed as factor of the affinity in the adult animal (= 1·0; inverse log. scale).

This is shown by the experiments done with HFur, drug acting on receptor systems other than that of serotonin and exhibiting a certain degree of minor affinity in the newborn rat. But the more important reason of this hyposensitivity must lie in other causes, located probably at level of the receptor system for the 5-HT (sialic acids?) and of the receptor-5HT interaction, as indicated by preliminary data.

Acknowledgement—We are indebted to Dr. H. Green (S.K. & F., Philadelphia, U.S.A.), for the generous supply of furtrethonium (Furmethide).

Institute of Pharmacology, University of Trieste, Trieste, Italy

ANDREA VACCARI
FRANCO CUGURRA

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Biochemical Pharmacology, Vol. 17, pp. 828–830. Pergamon Press. 1968. Printed in Great Britain

Hydroxyindole-*O*-methyltransferase in several avian species

(*Received* 9 *October* 1967; *accepted* 16 *November* 1967)

THE MAMMALIAN pineal gland has the capacity to *O*-methylate *N*-acetylserotonin to form the gonad-inhibiting compound, melatonin.[1] The formation of melatonin is catalyzed by an enzyme, hydroxyindole-*O*-methyltransferase (HIOMT), which is uniquely localized in the mammalian pineal gland.[2] The enzyme was found to be present in the chicken[3] and in other animal species.[4] This report describes some properties of HIOMT in the Japanese quail (*Coturnix coturnix japonica*) and in other avian species that are different from the mammalian enzyme.

Previous work has shown that in mammals, *N*-acetylserotonin was by far the best methyl acceptor for pineal HIOMT.[1] A comparison of the ability to *O*-methylate a variety of hydroxyindoles indicated that the hydroxyindole-*O*-methyltransferase from adult quail (male and female) pineal glands lacked the specificity of that from the cow pineal gland (Table 1). Serotonin was as good a substrate in the

TABLE 1. SUBSTRATE SPECIFICITY OF HYDROXYINDOLE-*O*-METHYLTRANSFERASE IN QUAIL AND COW PINEAL GLANDS*

Substrate	Quail	Cow
N-acetylserotonin	1·63	1·42
Serotonin	1·75	0·07
Bufotenine	0·51	0·05
5-Hydroxytryptophol	2·38	0·25
6-Hydroxy-N-acetyltryptamine	0·24	0·007
4-Hydroxy-N-acetyltryptamine	0·30	0·007

* Frozen pineal glands were homogenized with an all-glass homogenizer in 5 ml of ice-cold 0·05 M (pH 7·9) phosphate buffer and a 0·2-ml aliquot was incubated with 1 mμmole S-(^{14}C)-adenosylmethionine (60,000 cpm) and 0·1 μmole substrate in a final volume of 0·3 ml. After incubation at 37° for 30 min, 0·5 ml of 0·5 M (pH 10) borate buffer was added and the ^{14}C-O-methylated products were extracted into organic solvents as previously described.[1] Results are expressed as mμmoles of product formed per mg pineal gland.

quail as *N*-acetylserotonin, whereas in the cow *N*-acetylserotonin was 20 times more effective as a methyl acceptor. *O*-methylated products of serotonin and *N*-acetylserotonin formed enzymatically had the same R_f value as 5-methoxytryptamine and melatonin, respectively, in several solvent systems. Other 5-hydroxyindoles such as bufotenine (*N*,*N*-dimethyl-5-hydroxytryptamine) and 5-hydroxytryptophol also served as effective methyl acceptors in the quail pineal gland. Indoles with hydroxy groups on the 4- or 6-position were poor substrates for both the quail and cow pineal enzymes.

When individual pineal glands were examined for ability to *O*-methylate serotonin and *N*-acetylserotonin, the activity of serotonin was found to range from one-half the effectiveness of *N*-acetylserotonin to twice the activity. These observations suggested that HIOMT might be more than a single enzyme. To examine this possibility, the capacity of the quail pineal extract to *O*-methylate serotonin and *N*-acetylserotonin after various times of heating was investigated. Before heating, serotonin was a more effective methyl acceptor, but after heating the pineal extract for 4 min at 48°

TABLE 2. *O*- AND *N*-METAYLTRANSFERASE IN AVIAN PINEAL GLANDS

Species	No.	HIOMT (N-acetyl-serotonin)	HIOMT (Serotonin)	HNMT (Histamine)	PNMT (Normetane-phrine)	COMT (Noradrena-line)
Quail (male and female)	5	1·53	1·42	1·04	1·01	0·12
Turkey (male)	2	2·30	0·46	0·18	0·06	0·03
Kahki-Campbell duck (female)	3	4·21	0·33	0·29	0·06	0·25
Leghorn chicken (female)	2	5·11	1·20	0·68	0·12	0·17
Pigeon (male)	2	0·73	0·02	0·22	0·05	0·04

Pineal glands (prepared as described in Table 1) were incubated with 1 mμmole S-(^{14}C)-adenosylmethionine and 0·1 μmole substrate in a final volume of 0·3 ml. After 30 min, of incubation, the *N*- or *O*-methylated product was extracted into organic solvents as previously described for histamine-*N*-methyltransferase (HNMT)[2], catechol-*O*-methyltransferase (COMT)[6], and phenylethanolamine-*N*-methyltransferase (PNMT)[7]. Results are expressed as mμmoles of ^{14}C-methylated product formed per mg pineal gland. Snbstrates were shown in parentheses.

in 0·1 M (pH 7·9) phosphate buffer, *N*-acetylserotonin was almost twice as active as serotonin. These observations suggested the presence of a second, more heat-labile, HIOMT-like enzyme.

HIOMT activity was also examined in the pineal glands of a number of adult avian species kept in diurnal lighting (Table 2). With the exception of the pigeon, all the birds studied had as much enzyme activity as mammals or more. In addition, bird pineal extracts *O*-methylated serotonin with varying degrees of effectiveness. HIOMT was also assayed in various brain areas and tissues (eyes, liver, lung, heart and gut) of quail and found to be undetectable. The high activity of HIOMT in bird pineal glands indicates that this species has a considerable capacity to synthesize melatonin. This indole has been found to inhibit gonadal activity in mammals and to lighten the skin of amphibians.[8] Recently it has been reported that small doses of melatonin also inhibit gonad growth in the Japanese quail[9] under certain lighting conditions.

Other *O*- and *N*-methyltransferase enzymes such as histamine-*N*-methyltransferase, catechol-*O*-methyltransferase, and phenyl-ethanolamine-*N*-methyltransferase (the enzyme that *N*-methylates noradrenaline to adrenaline) were also measured in bird pineal glands (Table 2). The pineal glands of quails and leghorn chickens had relatively high histamine-*N*-methyltransferase activity. The quail pineal glands had large amounts of the adrenaline-forming enzyme, even more than the adrenal gland where this enzyme is highly localized.

Laboratory of Clinical Science,
National Institute of Mental Health,
Bethesda, Md., U.S.A., and
Department of Zoology,
University of Alberta,
Edmonton, Canada

JULIUS AXELROD
JEAN K. LAUBER

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Biochemical Pharmacology, Vol. 17, pp. 830–833. Pergamon Press. 1968. Printed in Great Britain

Interaction with heparin of deoxyribonucleoprotein complex damaged *in vivo* by nitrogen mustards

(*Received* 3 *October* 1967; *accepted* 23 *October* 1967)

THE TREATMENT with biological alkylating agents simulates in many regards the effect of irradiation.[1–3] In experiment *in vitro* they damage DNA and other important biological molecules by alkylating and cross-linking them in various groups.[4, 5] The primary site of reaction[5] responsible for the effect observed *in vivo* remains, however, in spite of intensive study, still obscure.[6]

The treatment of animals with alkylating agents, similarly as irradiation, leads to profound changes in the lymphatic tissues, causing intermitotic death of lymphocytes, followed by rapid cellular depletion of the tissues.[1, 7] Among the first biochemical changes in lymphatic tissues after both treatments is damage to the nucleoprotein complex of cell nuclei which is seen as an increase in the amount of