proportion of infectious particles (*5*), but only about 1 in 6 to 8 of these particles are infectious. These data suggest that only particles containing the largest amount of DNA are infectious, but there has been no good explanation for the fact that 83 to 88 percent of even the densest particles are noninfectious. Incorporation of two short DNA strands (both presumably incompletely coded and therefore noninfectious genomes) into a single particle might account for a particle's being dense without being infectious. This might explain the earlier observations (*5*). A second explanation for variation in strand lengths from particles of uniform density would be a lack of uniformity in the density of individual strands.

Green and Pina, in studying the chemical composition of adenovirus type 2 DNA, obtained sedimentation data which are compatible with a cyclic structure (*7*). The data presented here strengthens this interpretation. If a value of 2.5 μ for the average length of DNA from the densest particles (1.357 to 1.358) is applied and if the mass per unit length is the same as in strands of the sodium salt of DNA (*8*), this length corresponds to a molecular weight of about 5×10^6.

The possibility of disrupting viruses gently and measuring the lengths of their DNA strands now permits quantitative studies of the relation between DNA strand lengths within virus particles and the infectivity of the particles. In the case of tumorigenic adenoviruses, lengths of DNA strands within particles might also be correlated with subsequent production of malignant transformations of cells either in vivo or in vitro. This physical approach might also be usefully applied to the study of a recently recognized phenomenon: integration of two virus types within the protein coat of a single particle (*9*).

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Indole Compounds: Isolation from Pineal Tissue

Abstract. *Five indole compounds have been isolated from bovine pineal tissue and characterized as 5-methoxytryptophol, N-acetyl-5-methoxytryptamine (melatonin), 5-hydroxytryptophol, 5-methoxyindole-3-acetic acid, and 5-hydroxyindole-3-acetic acid. Pineal hydroxyindole-O-methyltransferase, with S-adenosylmethionine, converts 5-hydroxytryptophol to 5-methoxytryptophol.*

The presence of 5-hydroxyindole-3-acetic acid, 5-methoxyindole-3-acetic acid, and *N*-acetyl-5-methoxytryptamine (melatonin) in pineal tissue has been reported (*1*). Another compound, or compounds, which gave an atypical green color on paper when sprayed with Ehrlich's reagent and which antagonized the myotropic action of serotonin has also been noted (*2*). Because some β-carbolines have these properties (*3*) and can be derived from corresponding tryptamines (*4*), it was tentatively suggested that a tetrahydro-β-carboline might be present in pineal tissue (*2*). Bioassay by antagonism to serotonin indicated that pineal tissue was a particularly rich source of the unknown material.

Pineal glands removed from cattle immediately after slaughter were quick-frozen in an airtight container surrounded by dry ice. Tissue was always shipped and stored under the same conditions, namely at −10°C, with light and air excluded. Extracts were prepared and chromatographed in an atmosphere of nitrogen; the extracts were stored in the dark at −10°C. Approximately 50 kg of pineal tissue was extracted in batches of 1 kg.

Tissue (1 kg) was homogenized in 2 liters of redistilled ethyl acetate and filtered. Ethyl acetate in the filtrate was removed at reduced pressure and at 40°C with a rotary evaporator; the residue was partitioned between hexane and water to remove cholesterol. The aqueous phase was extracted twice with equal volumes of ethyl acetate; the extracts were combined, dried, and freed of solvent. The residue was dissolved in a minimum amount of ethanol for chromatographic separation. In silica-gel thin-layer chromatography (chloroform:methanol, 9:1; solvent A), five xanthydrol-positive compounds consistently appeared. These had R_F values of 0.70, 0.60, 0.35, 0.10, and 0.05 (Table 1). Two other xanthydrol-positive compounds were often seen: unknown No. 1 with R_F 0.95, and unknown No. 3A with R_F of 0.58.

Fifty derivatives of serotonin were used as reference compounds for the preliminary comparative identification of the indoles in pineal extracts, and it was necessary to synthesize some new compounds (*5*). Chromatography, electrophoresis, and ultraviolet spectra were used to characterize the unknowns.

Fractions were eluted from chromatoplates, and ultraviolet spectra were obtained. Evaporation of the eluates to dryness yielded gums. Several milligrams of unknowns, Nos. 5 and 6, were obtained, and recrystallization from toluene yielded 0.5 mg of each in crystalline form sufficiently pure for melting-point determination. Though each compound had a range of 10°C, the melting point of 5-methoxyindole-3-acetic acid when mixed with No. 5 was not depressed, nor did mixture with No. 6 depress the melting point of 5-hydroxyindole-3-acetic acid (Table 1).

The gum containing unknown No. 3 was dissolved in 0.2 ml of chloroform, and 0.5 mg of picric acid in chloroform was added. Standing for 24 hours at 4°C produced 0.5 mg of crystalline material which was removed by centrifugation; the material had the same melting point as *N*-acetyl-5-methoxytryptamine picrate and gave matching infrared spectra.

A neutral picrate of unknown No. 4 was obtained in the same manner; although it amounted to only 0.2 mg, its low solubility in chloroform permitted its isolation. It was identical with synthetic 5-hydroxytryptophol picrate.

We failed to identify unknown No. 3A, although some similarities were

Table 1. Characteristics of indoles from pineal tissue.

	Color reaction*		R_F in solvent†					Electrophoretic mobility‡	UV max. (mμ)	Melting point (°C)		Antagonism to serotonin
	Xanthydrol	Ehrlich	A	B	C	D	E			Picrate	Compound	
Unknown No. 1	Bl-Gr	Pale Bl	0.95						262			
Unknown No. 2	Bl	Gr-Bl	.70	0.42	0.60	0.83	0.82	?				+
5-Methoxytryptophol	Bl	Gr-Bl	.70	.40	.60	.85	.84			113		+
Unknown No. 3	Bl	Bl	.60	.15	.50	.85	.82	−10	278	130–5§		
N-Acetyl-5-methoxytryptamine	Bl	Bl	.60	.15	.50	.85	.84	−10	278	137		
Unknown No. 3A	Mauve		.58	.02	.25	.80	.86	?	?	?	?	?
1-Benzyl-1,2,3,4-tetrahydro-β-carboline	Mauve	None	.62	.02	.26	.82	.90	−38	272			+
Unknown No. 4	Bl	Gr-Bl¶	.35	.10	.30	.75	.76	−17	278	150–2§		+
5-Hydroxytryptophol	Bl	Gr-Bl¶	.35	.10	.30	.77	.76	−17	278	151		+
Unknown No. 5	Bl	Bl	.10	.45	.10	.85	.30	+106	278		140–8§	
5-Methoxyindole acetic acid	Bl	Bl	.10	.45	.10	.85	.30	+106	278		150	
Unknown No. 6	Bl	Bl¶	.05	.10	.05	.75	.20	+60	278		150–60§	
5-Hydroxyindole acetic acid	Bl	Bl¶	.05	.10	.05	.75	.20	+60	278		165	

* Sprays used for detecting compounds were: Xanthydrol, 0.2 percent in ethanol plus concentrated HCl; Ehrlich, *p*-dimethylaminobenzaldehyde, 0.5 percent solution in acetone with concentrated HCl; Ninhydrin, 0.2 percent in acetone with a few drops of pyridine; Gibb's reagent, 2 percent ethanolic solution of 2,6-dichloroquinonechloroimide, followed by saturated aqueous $NaHCO_3$. Gr, green; Bl, blue. † Solvent systems used were: A, chloroform:methanol (9:1); B, chloroform:acetic acid (95:5); C, chloroform:methanol (93:7) on silica-gel thin-layer chromatography; D, butanol:acetic acid:water (4:1:5); and E, propanol:ammonia (8:2) for paper chromatography. ‡ Electrophoretic mobility expressed as millimeter migration from the origin toward cathode (−) or anode (+) after 16 hours at 100 volts, 5 ma (*p*H 8.6). § Mixed melting point, no depression. ¶ Also gave positive color reactions for phenolic group. UV max., ultraviolet maximum.

noted between its chromatographic behavior and that of a β-carboline.

Attempts to isolate unknown No. 2 in crystalline form were unsuccessful, although in all other respects it appeared to be the same as 5-methoxytryptophol.

The presence of hydroxyindole-*O*-methyltransferase in pineal tissue has been described, and its substrate specificity has been explored (*6*), the optimum substrate being *N*-acetyl serotonin (*6*), a metabolite of serotonin (*7*). Other hydroxyindoles including serotonin were only one-tenth as reactive as *N*-acetyl serotonin. 5-Hydroxytryptophol has been reported as a metabolite of serotonin, in liver tissue, by Kveder *et al.* (*8*).

Hydroxyindole-*O*-methyltransferase was prepared from beef pineal tissue and purified (*6*). 5-Hydroxytryptophol and 5-methoxytryptophol were synthesized (*9*). Tritiated *S*-adenosylmethionine (specific activity, 40 mc/mmole, Tracerlab) was diluted with cold compound and used as methyl donor.

N-Acetyl serotonin and 5-hydroxytryptophol were compared as substrates under identical conditions. The substrate (5 μmole), *S*-adenosylmethionine-H^3 (5 μmole), and purified hydroxyindole-*O*-methyltransferase were incubated (0.2*M* phosphate buffer, *p*H 8) for 2 hours at 37°C; the mixture was then extracted with ethyl acetate. The ethyl acetate extract was dried under reduced pressure, and the residue was dissolved in 0.2 ml ethanol. Known amounts of this solution were used for chromatography, and the radioactive chromatograms were scanned to determine the amount of product. This method was more accurate than measuring directly the total activity in the extract, since an unidentified degradation product of *S*-adenosylmethionine-H^3 was also extractable in the solvent. Unreacted *S*-adenosylmethionine did not interfere with the analysis; it was not soluble in ethyl acetate and remained at the origin in solvent A. The degradation products had an R_F of 0.5 in solvent A, and did not interfere with estimation of melatonin (R_F 0.80) and 5-methoxytryptophol (R_F 0.85).

The product of hydroxyindole-*O*-methyltransferase acting on the substrate 5-hydroxytryptophol was identified as 5-methoxytryptophol by its color reaction (blue-green Ehrlich), negative reaction with phenolic reagents, its R_F values in four solvent systems (Table 1), and its radioactivity. 5-Hydroxytryptophol appeared to be a good substrate, though not as good as *N*-acetyl serotonin.

These data confirm the presence of 5-hydroxyindole-3-acetic acid, 5-methoxyindole-3-acetic acid, and *N*-acetyl-5-methoxytryptamine in pineal tissue as originally described by Lerner *et al.* (*1*). 5-Hydroxytryptophol and 5-methoxytryptophol are both present in pineal tissue, 5-hydroxytryptophol being a substrate for hydroxyindole-*O*-methyltransferase. The presence of these tryptophols in pineal extracts explains the atypical green Ehrlich color reactions and the activity antagonistic to serotonin (*2*).

The physiological importance of the methoxyindoles is indicated by reports that (i) *N*-acetyl-5-methoxytryptamine affects thyroid function (*10*), estrus, and ovarian weight (*11*); and (ii) 5-methoxytryptophol inhibits incidence of estrus and retards ovarian growth (*12*).

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