BIOSYNTHESIS OF PTERIDINES IN D. MELANOGASTER

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Evidence is accumulating to support the concept that a guanosine-5'-phosphate might be a direct precursor of the folic acid group and biopterin-like compounds. (2-Amino-4-hydroxy-6-pteridinyl) glycerol phosphate was isolated from E. coli (Goto and Forrest, 1961), and this compound was believed to be closely related to the first pteridine intermediate. However, the evidence to demonstrate this concept is still meager. This paper reports experiments using D. melanogaster which show that 2-amino-4-hydroxy-6-(D-erythro-1', 2', 3'-trihydroxypropyl) pteridine (neopterin) is a precursor of biopterin. Reduced 2-amino-4-hydroxypteridine is also incorporated into biopterin. A possible metabolic pathway of pteridines is discussed in the light of these results.

EXPERIMENTAL

Materials. 2, 4, 5-Triamino-6-hydroxypyrimidine-sulfate monohydrate-4-^{14}C was prepared from potassium cyanide (1.5 mc/mM) by the method of Korte and Barkemeyer, (1957). Guanosine-^{14}C (U)-5'-monophosphate (293 mc/mM) was purchased from Dai'ichi Chemical Co. Ltd. 2-Amino-4-hydroxy-6-(D-erythro-1', 2', 3'-trihydroxypropyl) pteridine-8a-^{14}C (Weygand et al., 1964), 2-amino-4-hydroxy-6-hydroxymethylpteridine-8a-^{14}C (Forrest and Walker, 1949), 2-amino-4-hydroxypteridine-8a-^{14}C (Mowat

et al., 1948) were prepared from 2, 4, 5-triamino-6-hydroxypyrimidine-sulfate monohydrate-4-^{14}C by known methods. The materials were purified by chromatography on cellulose columns and a Dowex 1X8 ($HCOO^-$) column. 2-Amino-4-hydroxypteridine-8a-^{14}C was hydrogenated before use over Adams' catalyst in 0.1 N sodium hydroxide for 3 hours; the hydrogenation solution was neutralized with acetic acid, and lyophylized; a slight amount of 7, 8-dihydroxanthopterin, which could be neglected, was produced. Radioactivity was measured with a gas flow counter and concentrations of compounds were determined from UV-absorption measurements.

Feeding, and Isolation of the Radioactive Compounds from D. melanogaster. The radioactive compound was suspended in a little water, and 4 ml. of the medium (yeast 15%, agar 1%, water 76%, sugar 8% and a little propionic acid; the whole heated for 20 minutes at 120° C) was added carefully. D. melanogaster larvae were allowed to develop on this food; the pteridines were extracted from the adult flies as follows: the flies were powdered with a mixture of 50% ethyl alcohol and 1% ammonia (1:1) in a mortar. The suspension was centrifuged and the precipitate was extracted 3 more times with the same solvent. The combined extracts were evaporated to a small bulk in vacuo. Compounds were purified by paper chromatography in four solvent systems; isopropyl alcohol, 1% ammonia (2:1), isopropyl alcohol, 2% ammonium acetate (1:1), n-butyl alcohol, acetic acid, water (4:1:1), and 3% ammonium chloride. Final chromatograms were cut into sections of 1 cm along vertical lines and the material from each fraction was eluted with water. Each eluate was evaporated to dryness below 30° C in vacuo, and the residue dissolved in a little ammonia water (1%). After measurement of the UV-spectra, the solutions were evaporated to dryness in vacuo, and the residue was counted; the positions of radioactive compounds were determined graphically both from UV-absorption and radioactivity. The purification procedures described above were repeated until a constant specific radioactivity was obtained. The experimental results are summarized in Table 1.

Radioisotopes fed to flies			Pteridines isolated from flies			
				Radiochemical data		
Compounds	Specific radioactivity and amount	Species; No. of larval	Compounds	c.p.m.	Amount x 10^{-8} Mole.	Specific radioactivity x10^6c.p.m./mM
G.M.P.	293.mc/mM 0.068mg	(ry^2) 1016	Biopterin	495.6 ± 5.2*	12.9	3.84
			AHP	437.4 ± 3.3	9.0	4.86
Neopterin	1.5 1.98	(+) 400	Neopterin	2006.3 ± 15.8	4.9	40.8
			Biopterin	5.21 ± 0.14	2.7	0.19
			AHP	3.85 ± 0.21	2.1	0.18
			Ixp	5.37 ± 0.19	3.7	0.15
Neopterin	1.5 1.85	(ry^2) 604	Neopterin	4008. ± 21.8	3.5	114.
			Biopterin	10.38 ± 0.32	6.3	0.16
			AHP	25.14 ± 0.39	14.8	0.17
P-6-CH_2 OH	1.5 3.7	(ry^2) 383	P-6-CH_2 OH	7587. ± 43.	23.6	32.1
			Biopterin	1.49 ± 0.19	2.5	0.058
			AHP	310.4 ± 2.64	11.3	2.75
			Lumazine	9.24 ± 0.12	--	--
AHP (reduced)	1.5 6.0	(ry^2) 1062	Biopterin	10.86 ± 0.23	9.5	0.11
			AHP	13424. ± 57.	16.9	79.4
			Lumazine	603.5 ± 6.1	4.9	12.3
AHP (reduced)	1.5 2.4	(+) 643	Biopterin	0.02 ± 0.10	2.5	(0.0008)
			AHP	3.35 ± 0.15	3.0	0.11
			Ixp	983.1 ± 7.5	19.8	49.7

* Statistical standard deviation

DISCUSSION

As shown in Table 1 GMP is an effective precursor of biopterin and 2-amino-4-hydroxypteridine (AHP); the specific radioactivities of the products are approximately the same. Neopterin is also converted into biopterin and 2-amino-4-hydroxypteridine, and again the specific radioactivities of the products are essentially identical. This evidence supports the postulation that neopterin, or its phosphate, or a reduced form of either, might be the focal compound for the synthesis of both biopterin and 2-amino-4-hydroxypteridine.

2-Amino-4-hydroxy-6-hydroxymethylpteridine (P-6-CH_2OH) was effectively converted into 2-amino-4-hydroxypteridine, and to a lesser extent into biopterin (the specific radioactivity of biopterin is 2.1% of that of 2-amino-4-hydroxypteridine). Reduced 2-amino-4-hydroxypteridine was effectively converted into isoxanthopterin (Ixp) (wild-type flies) and lumazine (ry^2 flies), but to a lesser extent into biopterin. If 2-amino-4-hydroxy-6-hydroxymethylpteridine could function as a common precursor of 2-amino-4-hydroxypteridine and biopterin, the specific radioactivities of both 2-amino-4-hydroxypteridine and biopterin must be the same, as is the case with GMP and neopterin. This possibility was, however, ruled out, since the results show that 2-amino-4-hydroxy-6-hydroxymethylpteridine is first converted into 2-amino-4-hydroxypteridine, which is then incorporated into biopterin.

The evidence presented here suggests that there exists a <u>main</u> catabolic pathway for neopterin, i.e. Neopterin $\longrightarrow$ P-6-CH_2OH $\longrightarrow$ AHP $\longrightarrow$ Isoxanthopterin (+) or Lumazine (ry^2).

A possible biosynthetic mechanism for biopterin, which is consistent with these results, is as follows: the glycerol side-chain of neopterin is entirely replaced with unknown C_3-fragment, as suggested by Forrest and Nawa (1964). Reduced 2-amino-4-hydroxypteridine itself is, however, probably not a free intermediate in this enzymatic process, but it can act as a substrate in the biopterin synthesizing enzyme system. A biological analogy for this

mechanism is the biosynthesis of tryptophan from indolglycerol phosphate (Yanofsky, 1960).

A plausible mechanism for the biosynthesis of biopterin, 2-amino-4-hydroxy-6-hydroxymethylpteridine, 2-amino-4-hydroxypteridine, isoxanthopterin, and lumazine in D. melanogaster is outlined in Fig. 1.

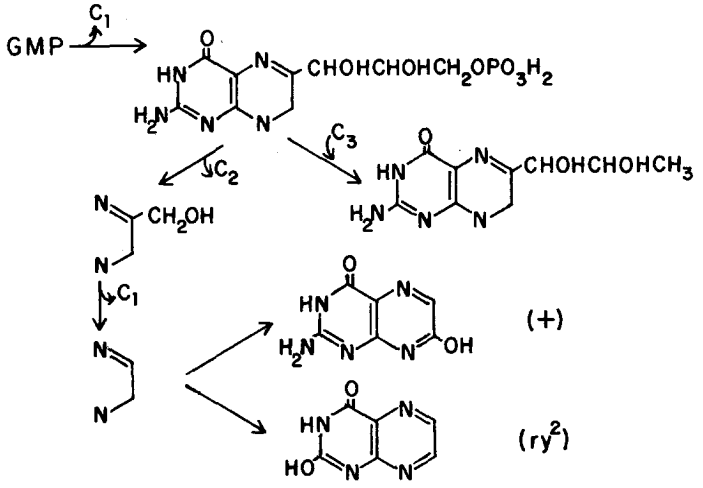

Fig. 1.

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