Influence of Aminopterin Administration to Dams upon Single Carbon Transfer into Purines of Fetal Brains*

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The ^{14}C incorporation into purine bases of fetal brains was investigated when DL-serine-3- ^{14}C , L-histidine-2- ^{14}C , or formate- ^{14}C was injected intraperitoneally to dams with or without pre-treatment with aminopterin. The radioactivity of ^{14}C in fetal brain tissues was estimated on the 18th day of gestation. On the 11th day of gestation, DL-serine-3- ^{14}C , L-histidine-2- ^{14}C , or formate- ^{14}C was injected to dams intraperitoneally. In the case of aminopterin treatment, aminopterin, 0.076 mg/kg, was injected intraperitoneally to dams on the 10th day of gestation.

The results in these experimental conditions showed that the ^{14}C incorporation from formate- ^{14}C into adenine of fetal brains and that from DL-serine-3- ^{14}C into guanine of fetal brains were impaired significantly by aminopterin injection to dams prior to injections of the labeled materials.

Mental retardation, brain atrophy and dilatation of cerebral ventricles were found commonly in both the formiminotransferase deficiency and N^5 methyltetrahydrofolate transferase deficiency syndromes.^{1,2} We are of the opinion that the brain atrophy and ventricular dilatation observed in these syndromes may result from an impaired purine biosynthesis of the brain in the fetal life due to a functional deficiency of folate.^{1,2}

Our conception mentioned above seemed to be supported by an experimental production³ of brain atrophy associated with ventricular dilatation without any other gross malformations in rat offsprings when dams were given 6-mercaptopurine, 20 mg/kg, intraperitoneally on the 11th day of gestation.

In the present study the ^{14}C incorporation into fetal brain purines is examined when dams are given on the 11th day of gestation an intraperitoneal dose of DL-serine-3- ^{14}C , L-histidine-2- ^{14}C , or formate- ^{14}C , and it is estimated comparatively when dams are injected with aminopterin on the 10th day of gestation, just prior to the day when the labeled materials are given.

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METHODS AND MATERIALS

Female rats of the Wistar strain were used and maintained on a standard diet purchased from the Oriental Co. Tokyo, Japan.

The onset of pregnancy was determined by examining the vaginal content of sperm, and the morning of massive sperm findings in the vagina was regarded as day zero of gestation.

Fetalized animals were divided into two groups (Groups A and B): Group A consisted of 19 rats and served as the control group; Group B consisted of 9 rats and all the rats of this group were given an intraperitoneal dose of aminopterin, 0.076 mg/kg, on the 10th day of gestation.

On the 11th day of gestation, rats of Group A were divided into three subgroups (Subgroups A-a, A-b and A-c); and those of Group B were also divided into three subgroups (Subgroups B-a, B-b and B-c), and labeled materials were injected in the following way: To each of rats of Subgroups A-a and B-a, 25 μ c of DL-serine-3- 14 C (specific activity of 38.9 mc/mM) were injected intraperitoneally. To each of rats of Subgroups A-b and B-b, 25 μ c of L-histidine-2- 14 C (specific activity of 41.5 mc/mM) were given intraperitoneally. And to each of rats of Subgroups A-c and B-c, 25 μ c of sodium formate- 14 C (specific activity of 4.01 mc/mM) were injected intraperitoneally.

On the eighteenth day of pregnancy all the dams were decapitated and fetuses were removed from the placenta. The brains from fetuses of one and the same dam were collected and weighed, then subjected to the extraction procedure of nucleic acids from brain tissues, followed by an estimation of the radioactivity of 14 C incorporated into fetal brain purine bases.

Extraction of acid soluble fractions and nucleic acids from brain tissues was carried out according to Schneider *et al.*'s method.^{4,5} Brain tissues, 0.3–1.5 g, were homogenized by a Potter's blender with approximately 8 ml of 0.5 N perchloric acid, and the final volume of the homogenates was made up to 10.0 ml with an addition of 0.5 N perchloric acid. The homogenates were kept at 95°C for 15 minutes,⁶ then centrifuged. The deposits were again subjected to the extraction procedure in the same way as described above. The supernatants were combined together and added with 1.0 ml of 1 N silver nitrate solution. The mixture was left to stand overnight, and then centrifuged. To the precipitate 20 ml of 1 N hydrochloric acid were added and the mixture was kept in boiling water bath for 60 minutes. After completion of hydrolysis, the hydrolysate was placed on a Dowex 50 $\times$ 8 (H⁺) column⁷ (1 $\times$ 10 cm). After washing the column with 60 ml of deionized water, purine bases were washed out from the column with 60 ml of 0.5 N ammonium hydroxide. All the eluate was dried up on a steam bath, and the residue was dissolved in a small amount of 0.5 N ammonium hydroxide.

The resulting ammonium hydroxide solution was subjected to one dimensional paper chromatography of purine bases using the Toyo-filter paper No. 50 and a solvent⁸ consisting of n-butanol: acetic acid: water (2:1:1, v/v).

The radioactivity of 14 C in spots corresponding to those of adenine and guanine was measured by an automatic radioisotope scanner.

RESULTS AND DISCUSSION

The averages of the radioactivity of 14 C incorporated into fetal brain purine bases from DL-serine-3- 14 C, which had been given intraperitoneally to dams on the 11th day of pregnancy, were shown in Fig. 1, indicating that they were lower in Group B (fetuses from dams with aminopterin injection) than in Group A (fetuses from dams without aminopterin injection). On a statistical examination a significant difference was found only in the radioactivity of 14 C incorporated into guanine fraction between Groups A and B (cf. Tables 1 and 2).

Fig. 1. The averages of the ^{14}C -incorporation into adenine and guanine of fetal brains from DL-serine-3- ^{14}C injected intraperitoneally to dams on the 11th day of gestation. Black columns were values obtained from fetuses of dams to which aminopterin injection was done on the 10th day of gestation (Group B), and white columns from dams without aminopterin injection (Group A).

TABLE 1. Incorporation of ^{14}C into purine bases of fetal brains from serine-3- ^{14}C , histidine-2- ^{14}C , and formate- ^{14}C injected intraperitoneally into dams on the 11th day of pregnancy

Group A	No. of dams	Fetuses in number	Total wet weight, g, of fetal brains gathered from the same dam	^{14}C of purine bases of fetal brains (cpm/g)		
				Adenine	Guanine	Total
Subgroup A-a (serine-3- ^{14}C)	1	10	0.9650	77	58	133
	2	8	0.7330	48	48	96
	3	9	0.8385	54	33	87
	4	6	0.4680	47	60	107
	5	11	1.1055	87	63	150
	6	10	0.9400	33	47	80
	7	9	0.7085	112	137	249
	8	9	0.7745	90	117	207
Average				69	70	138
Subgroup A-b (histidine-2- ^{14}C)	9	7	0.4765	151	104	254
	10	10	0.9120	200	44	244
	11	5	0.5490	204	77	281
	12	8	1.0705	288	90	378
	13	10	0.8830	220	85	305
	14	7	0.3880	172	27	199
	15	3		152	111	263
Average				198	76	274
Subgroup A-c (formate- ^{14}C)	16	11	1.1200	154	57	211
	17	11	0.9620	716	53	769
	18	11	1.0960	202	39	241
	19	8	0.8650	217	21	238
Average				322	43	368

TABLE 2. Incorporation of ^{14}C into purine bases of fetal brains from serine-3- ^{14}C , histidine-2- ^{14}C , and formate- ^{14}C injected intraperitoneally to dams on the 11th day of pregnancy. Aminopterin, 0.076 mg per kg of body weight, had been injected intraperitoneally to dams on the 10th day of pregnancy

Group B	No. of dams	Fetuses in number	Total wet weight, g, of fetal brains gathered from the same dam	^{14}C of purine bases of fetal brains (cpm/g)		
				Adenine	Guanine	Total
Subgroup B-a (serine-3- ^{14}C)	20	9	0.7730	53	25	78
	21	10	0.9450	60	18	78
	22	7	0.6200	60	26	86
	Average			58	23	80
Subgroup B-b (histidine-2- ^{14}C)	23	9	0.8000	329	46	375
	24	11	1.1875	193	31	224
	25	13	1.2265	100	42	142
	Average			207	40	247
Subgroup B-c (formate- ^{14}C)	26	9	1.0100	48	32	80
	27	8	0.8510	27	13	40
	28	13	1.3510	40	27	67
	Average			38	24	62

Fig. 2. The averages of the ^{14}C incorporation into adenine and guanine of fetal brains from L-histidine-2- ^{14}C injected intraperitoneally to dams on the 11th day (cf. notes of Fig. 1).

Fig. 3. The averages of the ^{14}C -incorporation into adenine and guanine of fetal brains from ^{14}C -formate injected to dams on the 11th day of gestation (cf. notes of Fig. 1).

As for the radioactivity of ^{14}C incorporated into purine bases of fetal brains when dams were injected with L-histidine-2- ^{14}C on the 11th day of pregnancy, there was no significant differences between Groups A and B (cf. Fig. 2, and Tables 1 and 2).

In the case of formate- ^{14}C injection to dams on the 11th day of pregnancy, there were statistically significant differences between Groups A and B in the radioactivity of ^{14}C incorporated into adenine and adenine plus guanine fractions of fetal brains (cf. Fig. 3, Tables 1 and 2).

From the result of the present study, it is likely that the incorporation of single carbons into purine bases of fetal brains was more severely affected when either formate- ^{14}C or DL-serine-3- ^{14}C was used as the single carbon precursor than when L-histidine-2- ^{14}C was used, in a condition where the labeled materials were injected on the 11th day of pregnancy to dams to which aminopterin injection was done on the 10th day of pregnancy.

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