6-Azauridine in Brain Tissue During Chick Embryonal Development

Attention has been paid in recent years not only to the cytostatic but also to the neurotoxic effects of 6-azauracil (6-AzU), 6-azauracilriboside (6-azauridine, 6-AzUR) and some other 6-azapyrimidines. The basic compound, 6-AzU, was already withdrawn from clinical use because of its strong neurotoxicity. Also 6-AzUR showed neurotoxic effects when administered orally, since it was degraded in the intestines to the neurotoxic 6-AzU[1,2]. It is now established that 6-AzUR exerts practically no toxicity when administered to adult humans i.v. However, some undesirable side effects of 6-AzUR were observed in animals; e.g. neurotoxic activity, or induction of malformations and/or deaths in new-born mice and in chick embryos[3–8]. Measurements of brain 6-AzUR concentration could not be performed in our previous work concerned with the effects of 6-AzUR on chick embryonal development, because other use of the brains was made[8]. Such measurements were realized later in similar experiments and the results are reported here.

Fertile Leghorn eggs were kept at 37.5 °C and constant air humidity (maintained by spontaneous evaporation of water in the incubator). The eggs were treated by 6-AzUR either on the fourth or the eighth day of incubation. Each of them was injected (in a sterile way and under UV-light) 10 mg of 6-AzUR contained in 0.2 ml of its sterile solution in 0.1 *M* phosphate buffer. The pH of the solution was controlled to be 7.2 in every run. Embryos were killed on the fourteenth, seventeenth and twentieth day of incubation. Brain surfaces were carefully but quickly dried by touching them gently with an edge of filter paper and the brains were weighed. In a part of brains dry weights were estimated.

Each brain destined for the drug determination was homogenized in 5 ml of ethylalcohol and the homogenate was filtered through a small filter paper. The tube and the filter were twice washed with 2 ml of ethylalcohol. The combined extracts were evaporated on a water bath, the residue taken into a small volume of water and quantitatively transferred on Whatman No. 3 paper. Chromatographic separation and determination of 6-AzUR was described in detail previously[9].

Brain dry weights and brain 6-AzUR concentrations

Day of 6-AzUR injection	Day of embryo killing	Brain dry weight (% from wet weight)	6-AzUR in the brain (μg/1 g of wet weight)	6-AzUR in the brain (μg/1 g of dry weight)
4	17	11.82 ± 0.55 (20)	93.53 ± 39.00 (20)	815.11 ± 339.64 (21)
8	14	10.80 ± 0.39 (7)	101.96 ± 20.35 (13)	944.95 ± 188.63[a] (13)
8	17	12.22 ± 1.42 (7)	89.09 ± 25.34 (20)	729.05 ± 207.36[a] (20)
8	20	14.31 ± 0.63 (9)	101.22 ± 30.66 (9)	707.37 ± 214.26[b] (9)

Results as mean values ± standard deviation. No. of animals is given in brackets. a: $P < 0.005$; b: $P < 0.025$.

The dry weight of brains (see Table) increased with the time of egg incubation, as would be expected. On a wet weight basis, 6-AzUR content of brains did not differ from 14–20 days of incubation. However, when calculated on the dry weight basis, it decreased with increasing age of embryos. There was no difference in the 6-AzUR content of brains when eggs were injected either on the fourth or on the eighth day, and the embryos killed on the seventeenth day of incubation. Neurotoxic 6-Azauracil was not detected in the brains. If present at all, its quantities must have been lower than those detectable by the chromatographic procedure used.

As far as the effects of 6-AzUR in central nervous system are concerned, we wish to refer to the report of JANKU et al.[6] who observed deaths in mice and motor incoordination in mice and cats due to i.p. and/or intraventricular application of the drug. Various regions of the cat brain contained amounts of 6-AzUR practically equal to those described here. KOENIG et al.[3] and WELLS et al.[4] also described neurological disturbances in cats which were given 6-AzUR intraventricularly. The authors found that the drug interferred with the formation of uridine nucleotides in the brain. Hence, the same biochemical mechanism seems to be responsible for the carcinostatic and central activities of 6-AzUR. However, the mechanism of the teratogenic effect of 6-AzUR is unclear and deserves further studies.

Résumé. 10 mg d'azauridine-6 ont été injectés dans les œufs au 4è et au 8è jour d'incubation. Dans nos précédentes expériences, cette dose a provoqué des malformations dans les embryons du poulet. La concentration de l'azauridine-6 a été déterminée dans le cerveau des embryons et on a trouve qu'elle varie de 0,48–1,16 mg pour 1 g du poids sec du cerveau, du 14è au 20è jour d'incubation.

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