O\textsubscript{6}-Alkylguanine-DNA Alkyltransferase in the Chick Embryo during Development

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In the whole embryo, O\textsubscript{6}-alkylguanine-DNA alkyltransferase (AT) activity increased until day 9 of development and declined sharply after day 13. AT activity of the liver was greatest between day 12 and day 20 and decreased fast after hatching. In the brain, AT activities reached a maximum at day 17 and 18 and declined sharply after hatching. At two developmental stages with different AT activities (day 10 and day 17) DNA alkylation in the brains was estimated 6, 12, 24 and 48 h after administration of N-methyl-N-nitrosourea in ovo by viscometric measurement of DNA fragmentation. The high AT activities of the chicken embryo brain at the 17th day of development correlated with minor DNA fragmentation following a repair period of 12-24 h. It is suggested that the high basal level of AT in the chick embryo might have a protective function against the persistence of the genotoxic lesion O\textsubscript{6}-methylguanine during development.

Introduction
Alkylating substances form an important class of DNA damaging agents that includes a number of environmentally important N-nitroso-compounds. O\textsubscript{6}-alkylguanine is considered as the major genotoxic lesion produced by these agents, which may give rise to base mispairing during DNA replication or to miscoding during transcription [1,2]. The lesion can be repaired by the action of a protein termed O\textsubscript{6}-alkylguanine-DNA alkyltransferase (AT), which catalyses the transfer of the alkyl group to a cysteine residue in its own amino acid sequence [3]. Many studies in animals have correlated the level and persistence of O\textsubscript{6}-alkylguanine with target organ transformation (see, e.g., [4,5]). Therefore, the constitutive or inducible repair activity of AT may be a critical factor — among others — in determining the vulnerability of organs, species and, with particular interest, of the embryo to alkylating agents.

The chick embryo offers a rapid, sensitive and inexpensive model [6], which allows investigations independent of maternal influences, and does not conflict with animal protection laws. The present study demonstrates — to our knowledge for the first time — presence and changes of AT activities of the whole embryo and of liver and brain during development. Furthermore, it is suggested that AT activities in the brains of 17-days-old embryos correlated with greater DNA repair following treatment with N-methyl-N-nitrosourea (MNU). Therefore, the chick embryo could be a suitable vivo system to investigate the importance of AT during development in greater detail.

Materials and Methods
AT was measured in the whole embryo as well as in liver and brain at different stages of development (day 1 = first day of incubation at 37.8°C and 60% humidity). We used fertile eggs obtained from white leghorn chickens. Tissue extraction and direct AT assay were performed as described by Stammberger et al. [5]. The method based on the transfer of [\textsuperscript{3}H]-labelled methyl groups from the O\textsubscript{6}-position of guanine in the substrate DNA to the acceptor protein. The amount of tritium was determined in the acid insoluble protein fraction by liquid scintillation counting. AT activity was expressed as fmol O\textsubscript{6}-methylguanine (O\textsubscript{6}-meG) per 1 mg protein, which was measured by the Bradford method (Bio Rad kit, Munich, F.R.G.). The specific activity for O\textsubscript{6}-meG was considered to be the same as for the methylation of the substrate-DNA with [\textsuperscript{3}H]methylthioate (94.7 × 10\textsuperscript{8} Bq/mmole).

N-methyl-N-nitrosourea (MNU) (Sigma Chemie, F.R.G.) was administered to 10- and 17-days-old embryos in 0.1 ml 0.1 m acetic acid via the air cell of the eggs onto the inner shell membrane (1 mg per egg). The controls received only acetic acid. The embryos were sacrificed at various times (6, 12, 24, 48 h) after treatment. As measurement of DNA fragmentation is useful to estimate, though in non-specific manner, the level of DNA-alkyl adducts [7], alkaline lysates from the brains were measured by low-shearing glass capillary viscometry as previously described [8]. The signifi-
cance of the difference between AT activities (%) of 10- and 17-days-old embryos was determined using the non-parametric Mann-Whitney test.

**Results and Discussion**

As presented in Fig. 1, AT activity in the whole embryo increased until day 8 and declined after day 13 of development. AT activity of the liver was greatest between day 12 and day 20 (200.7 ± 15.5 fmol/mg protein at day 20) and decreased fast after hatching to 52.6 ± 0.8 fmol/mg protein (3-day-old chickens) (Fig. 2). In the brain, AT activities increased reaching a maximum at day 17 and 18 (151.3 ± 6.9 and 151.9 ± 11.0 fmol/mg protein, resp.) and declined sharply to 50.4 ± 3.6 fmol/mg protein in 3-days-old chickens (Fig. 2).

Measurement of DNA fragmentation is a useful alternative to the investigation of the DNA binding level of alkylating substances [7]. Therefore, MNU-induced strand breaks were determined by viscometric measurement [8] of alkaline lysates of the brains to estimate – in a preliminary manner – DNA alkylation at developmental stages with different AT activities (10- and 17-days-old embryos, AT activities 71.4 ± 10.6 and 151.3 ± 6.9 fmol/mg protein, resp.). As presented by Fig. 3, DNA frag-

![Fig. 1. O\(^\text{6}\)-alkylguanine-DNA alkyltransferase activity expressed as transfer of fmol O\(^\text{6}\)[\(^3\)H]meG per 1 mg extract protein of chick embryos at different developmental stages. Each data point represents the mean of at least five individuals (± standard deviation).](image1)

![Fig. 2. O\(^\text{6}\)-alkylguanine-DNA alkyltransferase activity expressed as the transfer of O\(^\text{6}\)[\(^3\)H]meG per 1 mg extract protein in liver (– • –) and brain (— O–) of chick embryos at different developmental stages. Each data point represents the mean of at least five experiments (± standard deviation).](image2)

![Fig. 3. Viscosity of alkaline cell lysates of the brains of 10 days (– ○–) and 17 days (– ●–) old embryos at different times after N-methyl-N-nitrosourea (MNU) treatment *in ovo* (1 mg/egg). Values in per cent as related to untreated controls. Each data point represents the means of at least 8 experiments (± standard deviation). Using the Mann-Whitney test, the values 6 and 12 h following MNU administration were not significantly different, whereas the values at 24 h differ with p < 0.001.](image3)
mentation showed no significant difference between 10- and 17-days-old embryos 6 h after treatment. Therefore, one may conclude that the initial degree of DNA alkylation was the same at both developmental stages as previously shown for rat embryos [9]. After 12 h about 60% of the viscosity of the untreated controls were reached. In 10-days-old embryos, however, no further DNA repair occurred between 12 and 24 h, whereas repair seemed to be complete in 17-days-old embryos 24 h following MNU-administration. After 48 h, there were no significant differences between 10- and 17-days-old embryos (not shown in Figure).

Alkyl adducts of the DNA are removed by a number of repair proteins the most important being AT. Repair of O<sup>6</sup>-alkylguanine via AT occurs stoichiometrically without regeneration of the alkyl acceptor site. Therefore, after an initially rapid rate of repair, the AT reaction becomes saturated until new enzyme protein is synthesized [3]. With respect to the present results, it is highly probable, therefore, that the differential persistence of DNA fragmentation in the brains of chick embryos at different stages of development reflects, at least partly, the persistence of DNA alkyl adducts, i.e., the enzymatic DNA repair throughout embryo- and fetogenesis.

In adult hens AT activities are in the same range as in young chickens [10]. Previous investigations in rats [5] also demonstrated higher AT activities in fetal liver (1.4-fold) and fetal brain (2.3-fold) than in the corresponding adult organs. There is some evidence that more rapidly proliferating systems – like the embryo or the fetus – have increased capacity for self-correction [11]. This might be due to a greater accessibility ("openness") of active chromatin for DNA repair proteins [12] and/or an increase of DNA repair enzymes. The level of AT activity also appears to be dependent on the proliferative state of the cell being high in the late G<sub>1</sub> and early S phase [13–15]. Rabes <i>et al.</i> [15] concluded that this "might result in partial protection of DNA synthesizing cells from base-mispairing".

Therefore and from our results, it can be suggested that the relatively high constitutive AT activities in the chick embryo might have a protective function against the persistence of the genotoxic lesion O<sup>6</sup>-meG caused by alkylating agents occurring in the environment and/or produced by endogenous [16] reactions. The chick embryo may be an interesting model to investigate the importance of AT during development in greater detail.

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