Influence of 6-Azauridine on the Calcium Content of Plasma and Bones in New-Born Chicks

6-Azauridine (6-AzUR) is an antimetabolite of pyrimidine bases of nucleic acids. It is used not only in the treatment of malignant tumours[1] but also in the treatment of some types of psoriasis[2] and rheumatic arthropathies[3]. Its use as an abortifacient was also considered[4-6], however, malformations were observed caused by 6-AzUR in the developing chick embryo[7] and in rats[8]. In our investigations of the teratogenic effects of 6-AzUR on the chick embryo X-ray examinations of hatched chickens were also made. We could observe differences in the density of roentgenograms of the long bones in chicks influenced by 6-AzUR, as compared with controls. Therefore we dealt in detail with the quantitative determination of calcium and phosphorus in the bones and plasma of such chicks as described below.

Material and methods. In the experiments fertile eggs of Leghorn strain were used and divided into 3 groups: Group I: the eggs were incubated without any intervention. Group II: each egg was injected on the eighth day of incubation 0.2 ml of phosphate buffer (pH 7.2) with sterile technique. Group III: the eggs were injected on the eighth day of incubation 10 mg of 6-AzUR dissolved in 0.2 ml phosphate buffer (pH adjusted to 7.2).

The eggs were incubated for 21 days at 37.5 °C at a humidity of 75%. The blood of new-born chicks was obtained under ether anaesthesia by intracardiac puncture and the plasma calcium content determined complexometrically[9]. The bones (femur and tibia) of both extremities were carefully cleaned from soft tissue, their diaphyses dried and the dry substance was ashed.

The weighed ashes were first dissolved in a small amount of 1 *N* HCl and this solution was made up to 10 ml so as to be 0.6 *N* in final acidity.

Since it is known that the presence of substantial amounts of phosphates interferes with the estimation of calcium[10-12], we determined calcium and phosphorus in the solutions only after separation of the ions on a column of the ion-exchange resin Amberlite IR 120[13,14]. First we eluted phosphorus from the ion exchange resin[13] which was then estimated spectrophotometrically according to GOLDENBERG[15]. Subsequently calcium was eluted[13] and estimated complexometrically[9]. The content of both ions was expressed in % from dry weight or ash. All results were evaluated statistically using the *t*-test.

Results and discussion. The analysis of bones revealed (Table) that there was no difference between the 2 control groups (I and II) as regards the investigated parameters, i.e. the dry weight, ash and the calcium and phosphorus content. In chicks of group III (after administration of 6-AzUR) a statistically significant increase of bone dry weight and ash contents as well as of the calcium contents (Table) was found in comparison with both control groups. The phosphorus content of bones was not altered by the administration of 6-AzUR.

Our experiments also showed (Figure) that 10 mg of 6-AzUR injected into fertile eggs on the eighth day of

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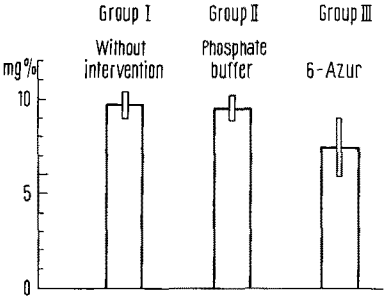

Plasma calcium levels.

The content of calcium and phosphorus in the bones of new-born chicks

Group	Dry weight % from wet weight	Ash % from wet weight	P % from dry weight	% from ash	Ca % from dry weight	% from ash	Ratio Ca:P
I not injected	41.98 ± 4.12 (13)	17.22 ± 2.06 (13)	8.15 ± 0.93 (13)	19.18 ± 1.36 (13)	14.03 ± 0.83 (13)	33.40 ± 1.48 (13)	1.73 ± 0.20 (13)
II phosphate buffer	39.28 ± 4.59 (10)	16.55 ± 2.13 (10)	8.60 ± 1.06 (10)	20.34 ± 0.86 (10)	14.17 ± 1.08 (10)	33.69 ± 1.97 (10)	1.65 ± 0.19 (10)
III 6-AzUR	44.39 ± 4.11 (13) $P < 0.005$	19.57 ± 1.89 (13) $P < 0.005$	8.58 ± 0.85 (13)	19.32 ± 1.03 (13)	16.53 ± 1.48 (13) $P < 0.001$	37.46 ± 2.64 (13) $P < 0.005$	1.93 ± 0.16 (13) $P < 0.005$

All values are means ± S.D. Statistical evaluation calculated against group II. No. of animals in brackets.

incubation reduced the plasma calcium levels in new-born chicks (mean 7.3 mg%) as compared to controls (mean 9.7 mg%).

The results of our experiments indicate that 6-AzUR affects the metabolism of calcium during embryonal development of the chick. Exact interpretation of the mechanism by which 6-AzUR exerts this action is not possible, so far. However, an interference of 6-AzUR with the function of endocrine organs could be considered. It is known[16–18] that parathormone regulates exchange of the calcium ion between blood and bones and maintains in this way a constant level of calcium in plasma (Ca 10 mg%). After removal of the parathyroid glands a rapid drop of the plasma calcium level occurs (to Ca 7 mg%). According to one theory[16] parathormone controls in the bones the concentration of citric acid, which forms a soluble complex with calcium and enables the transfer of calcium ions from bone to blood. Parathormone also stimulates the formation of osteoblasts which participate in the resorption of calcium from the bones. According to another theory[16] parathormone influences the degree of polymerization of mucopolysaccharides in the ground substance of bones and thus influences the release and uptake of mineral salts. Our finding of the drop of plasma calcium level and of the rise of bone calcium content would be in agreement, therefore, with the hypothesis that 6-AzUR might reduce resorption of bone calcium by interference with the action of parathormone.

Another possible interpretation of the described changes could be a direct effect of 6-AzUR on the bones during chick embryonal development by a stimulation of the formation of osteoblasts which could lead to enhanced utilization of calcium from the shell. It is known[19] that the egg (without the shell) contains 250 mg of P_2O_5 and 38.6 mg of CaO, the ratio of Ca:P being 0.2:1.0. However, hatched chicks contain on the average 255 mg of P_2O_5 and 209 mg of CaO, the ratio of Ca:P being 1.34:1.0. It is apparent from these data that chick embryos cover 82% of their calcium requirement from the shell. Although the shell contains also a small amount of phosphorus (14 mg/egg), the embryos utilize only the phosphorus contained inside the egg. 6-AzUR thus could influence the activity of osteoblasts and promote deposition of calcium in bones which would result in an increased ash content and Ca:P ratio, with unaltered content of phosphorus, as observed in our chicks.

So far we do not have definite evidence for any of the possible interpretations. An exact explanation of the interference of 6-AzUR with the metabolism of calcium will be possible, therefore, only after collection of further data.

Zusammenfassung. Eine Dosis von 10 mg 6-AzUR, die am 8. Tage der Inkubation in befruchtete Eier von Leghornhennen injiziert wurde, verursachte bei neugeborenen Hühnern die folgenden Änderungen: (a) eine Erniedrigung des Plasma-Kalzium-Spiegels, (b) eine Erhöhung des Trockengewichtes und des Aschegehaltes der Diaphysen der langen Beine, (c) eine Erhöhung des Kalziumgehaltes der Knochen. In Übereinstimmung mit diesen Tatsachen fand man auch eine Erhöhung des Ca:P-Quotienten in den Knochen nach Verabreichung von 6-AzUR.

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Zur Wirkung von Strahlenschutzstoffen an Thymuszellen

In früheren Untersuchungen konnte mehrfach gezeigt werden, dass im Thymus die Lymphozyten der Rinde strahlenempfindlicher sind als die entsprechenden Zellen des Markanteils[1]. Von Trowell[2] wird angenommen, dass die Lymphozyten des Marks durch die verhältnismässig grosse Anzahl der sie umgebenden Retikulumzellen geschützt werden. Es wird unter anderem ein ATP-Transfer von diesen Zellen zu den Lymphozyten vermutet. Andere Autoren[3] nehmen an, dass 2 unterschiedlich strahlenempfindliche Lymphozytenpopulationen vorliegen.

Untersuchungen zur Wirkung von Strahlenschutzstoffen am Thymus im Hinblick auf Veränderungen des Organgewichtes während der ersten 24 h nach Bestrahlung ergaben keine Unterschiede gegenüber unbehandelten, bestrahlten Kontrollen[4–7]. Es wurde eine schnellere Erholung der Organe beobachtet. Hingegen hat eine Reihe von Experimenten gezeigt, dass Thymuslymphozyten bei Bestrahlung in vitro geschützt werden können[8–11].

In den hier dargestellten Versuchen wurde die Wirksamkeit von 2 strahlenschützenden und einer nicht schützenden SH-Substanz an den Lymphozyten sowie den Retikulumzellen des Thymus untersucht. Rinden- und Markanteile wurden getrennt von jeweils 30 Tieren ausgewertet. Versuchsbedingungen: 5 Wochen alte männliche Mäuse erhielten Ganzkörperbestrahlungen von 200, 600 oder 800 R (150 kV, 20 mA 148 R/min). 5 min vor Bestrahlung wurden entweder 4 mg Cystein, 3 mg Cysteamin oder 6 mg Isocystein, bei den Kontrollen physiologische NaCl-Lösung i.p. injiziert.

Wie aus Tabelle I zu ersehen ist, kann nach einer Ganzkörperbestrahlung von 200 R weder in der Rinde

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