The differences are statistically significant (analysis of variance: $p < 0.01$ day 4 and $p < 0.05$ day 6 and 8). It should be noted that the cytology of neutrophils scored from blood of $PbAc_2$-treated and control mice was indistinguishable.

Results obtained from the labeling studies are shown in figure 2. On day 4 the percentage for $PbAc_2$-injected mice is about 28 while the control percentage value is approximately 12. The differential of 16% would represent the control-corrected stimulatory effect of $PbAc_2$ on neutrophil production. Similarly, the differences between experimental and controls are 10 and 7% on days 6 and 8, respectively. Experimental data for days 4 and 6 are significantly greater than control percentages (analysis of variance: $p < 0.01$ and $p < 0.05$, respectively). Only those cells in the process of DNA synthesis would incorporate ^{3}HTdR. Thus, the increased labeling percentage observed for the $PbAc_2$-injected mice is due to a greater number of cells in the DNA-synthesizing phase of their production cycle. The decline in the time course for neutrophil labeling for experimentals and controls parallel one another. This would be expected, since ^{3}HTdR is only available for incorporation shortly after its injection.

The day for the maximum neutrophilic granulocytosis and that for the appearance of ^{3}HTdR-labeled neutrophils appears to be the same, i.e. 4 days after $PbAc_2$. The time course for the appearance of the maximum ^{3}HTdR-labeling of that of others[4,5,7]. Lead has a stimulatory effect on neutrophil production, but it is not a pronounced one. Viewing experimental and control labeling percentages, it is apparent that only the magnitude of production is affected by lead and not the time course of production. Experimental and control neutrophil labeling percentage values show the same trend for the appearance of the label in peripheral blood. This indicates that lead has little, or no effect, on the time for progression and subsequent ejection of neutrophils following their proliferative phase in bone marrow.

There are at least two possibilities to account for lead's stimulatory effect, namely 1) lead may directly influence marrow progenitor cells, increasing the number of such cells entering the neutrophil line of production; and 2) the stimulatory effect may be secondary to the action of leukocytosis-inducing factor (LIF). This substance has been reported to increase in serum levels following tissue trauma, providing injury is sufficiently great, and to stimulate the production and release of granulocytes[8]. The second postulate does not seem as attractive as the former because of the observed time course. Peak labeling in peripheral blood was observed four days following $PbAc_2$. Tritiated thymidine was injected only 6 h after lead and its incorporation into DNA-synthesizing neutrophils would have occurred immediately after injection. Thus, it does not seem plausible that significant tissue trauma would have occurred between the injections of lead and ^{3}HTdR to induce the formation of LIF with the subsequent LIF-induced stimulation of neutrophil production. The direct stimulatory action of $PbAc_2$ has been proposed before[2].

It has been published that the time course for changes in the number of circulating neutrophils is highly variable depending upon the extent of the insult and the mechanism of action of the agent causing the insult[3]. Mobilization of neutrophils from reserve sites to a site(s) of injury via the blood may be quite rapid, i.e. within hours[9] or considerably slower[3]. A proposal is made here that there is an accumulation of the $PbAc_2$-induced injury occurring over several days, and once the level of injury reaches a critical point, neutrophil-attracting substances are released in sufficient quantity to mobilize neutrophils from reserve deposits.

From these data, it is concluded that the marked neutrophilic granulocytosis detected on day 4 post-$PbAc_2$ is due primarily to mobilization of neutrophils from storage sites. The smaller component of the increase in the number of neutrophils (only about 16%) is due to a stimulatory effect of $PbAc_2$ on neutrophil-producing tissue.

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Biosynthesis of ascorbic acid in chick embryos

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Summary. Biosynthesis of ascorbic acid was found in the kidneys (mesonephros and metanephros) of the chick embryo as well as in the yolk sac membrane. The activity of L-gulonolactone oxidase in the yolk sac membrane suggested that it was the major source of ascorbic acid in the chick embryo.
Key words. Ascorbic acid; L-gulonolactone oxidase; biosynthesis; chick embryo.

The fertile eggs of chicken *(Gallus gallus)* contain no detectable ascorbic acid (AA). Upon incubation, however, AA content in the egg rapidly increases and high concentrations of AA are found in various embryonic tissues[2]. It has been established that in chickens, the location of AA synthesis is in the kidneys and not in any other tissues[3,4]. But in the early stages of embryonic development, the kidneys (mesonephros and metanephros) seemed too small and primitive to be the only tissues capable of supplying the AA needed by the rapidly growing embryo. Although there has been speculation that different embryonic tissues might be capable of synthesizing AA[5] so far, no information is available on AA synthesis in chick embryos. This report summarizes a study of possible sources of AA in chicken embryos during different stages of development.

The activity of L-gulonolactone oxidase was used as an indicator of capacity of AA synthesis[6]. Different embryonic and extra-embryonic tissues at various stages of development were dissected, weighed, and immediately homogenized in phos-

Ascorbic acid synthesis in chick embryos

Age	Mesonephros	Metanephros	Livers	Yolk sac membrane
6-day	–	–	–	0.78 (2,194.0)
11-day	1.46 (85.5)	0.05 (3.0)	0.36 (114.1)	1.33 (11,180.0)
14-day	6.14 (518.7)	2.04 (353.3)	0.35 (309.0)	1.03 (15,489.0)
18-day	1.32 (57.4)	5.79 (2,788.1)	0.06 (141.2)	1.87 (25,197.0)

Ascorbic acid synthesis is measured in μmoles/g wet wt/h, and μg/day/embryo (in parenthesis). Values represent averages of four determinations.

phate-buffered solution containing sodium deoxycholate and incubated with/without the substrate, L-gulonolactone according to the method of Ayaz, Jenness and Birney[7]. AA concentration was determined by the method of Roe and Kuether[8]. L-Gulonolactone oxidase activity was measured by the net increase of AA in the tissue homogenates.

Tissues studied for activity of AA synthesis included the egg white, yolk, allantoic sac membrane, yolk sac membrane, brain, liver, mesonephros, and metanephros. The liver, mesonephros and metanephros were too small and fragile for the analyses until the embryo was 11 days old. It was necessary to combine tissues from several embryos to obtain sufficient samples for the study.

Our results are summarized in the table. In spite of the high concentrations of AA found in the embryonic brains, no AA synthesis was indicated in the brain tissues. No AA synthesis was detected in yolk, white or the allantoic sac membrane.

L-Gulonolactone oxidase activity was found in mesonephros of 11-, 14-, and 18-day chick embryos. But in the 18-day chick embryos, the enzyme activity of mesonephros had decreased, coinciding with its degeneration. While very low enzyme activity was found in the metanephros of 11-day chick embryos, it increased as the embryo matured.

Interestingly, L-gulonolactone oxidase activity was consistently found in the yolk sac membrane throughout the development of the chick embryos. The capacity of total daily AA synthesis in yolk sac membrane was much higher than those found in the mesonephros or metanephros of the chick embryo. The yolk sac has been regarded as the reservoir of nutrients. Our finding suggested that yolk sac membrane also actively synthesizes the AA required by the rapidly growing chick embryo.

Although no AA synthesis was reported in livers of chicken, low levels of L-gulonolactone oxidase activity was detected in the livers of the chick embryos during various stages of development. This result seemed to support Rinaldini's speculation[5] that some embryonic tissues might be capable of AA synthesis but loss the capacity during the course of differentiation. Different species of birds have been shown to synthesize AA in kidneys or in livers according to their phylogenetic trend[9]. It seemed probable that AA synthesis in chick during embryogenesis parallels the phylogenetic patterns.

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C-band variability in some Lacertidae (Sauria, Reptilia)

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Summary. The chromosome C-banding pattern has been studied in four lacertid species possessing the same karyotype. The results obtained show a remarkable interspecific variability both in the amount and distribution of C-banded heterochromatin. This leads us to the speculation that alleged conservativeness in their karyology is probably due to inadequate resolution by the conventional cytological techniques. Moreover, it has been hypothesized that these variations of the C-bands play an important role in the evolution of this saurian family.

Key words. C-band; heterochromatin; lacertid lizards; chromosomes.

In the investigations carried out with standard cytological techniques, the lizards from the family *Lacertidae* are commonly considered to be conservative from a karyological viewpoint[2-5]. However, it has been observed that in several animal groups, karyotypes which are apparently the same from the point of view of conventional morphology, have proved to be extremely different when examined by banding techniques[6-8].

In this regard we have studied the C-banding pattern in four lacertid species possessing similar karyotypes, with 36 uni-armed macrochromosomes and 2 microchromosomes. The aim of this research was to test whether the apparent homogeneity in karyology by conventional techniques corresponds to a homogeneity in the distribution of heterochromatin.

Material and methods. The C-banding pattern was investigated in five female specimens of *Lacerta dugesii* from the island of Madeira; two male specimens of *Lacerta trilineata* from the Balkans; one female and two male specimens of *Podarcis sicula sicula* from the surroudings of Naples and three female and two male specimens of *Takydromus sexlineatus* from Thailand.

The animals were stimulated with two doses of phytohemagglutinin (0.02 ml/g b.wt) then they were treated with colchicine (0.01 ml/g b.wt) and sacrificed after 6–19 h under anesthesia