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Neutrophilic granulocytosis following lead acetate in female mice

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Summary. Lead induces an abrupt neutrophilic granulocytosis with the peak response detected 4 days after treatment. Using the incorporation of tritiated thymidine as an index of neutrophil production, autoradiographic analysis revealed that only about 16% of the total neutrophil increase is associated with a stimulatory effect on production. The remainder of the increase appears to be linked to the panhistotoxic action of lead, promoting migration of neutrophils from reserve sites in the wake of tissue damage.
Key words. Lead toxicity; neutrophils; mice.

It has been demonstrated that the various salts of lead exert a wide range of biological effects[1]. Among these was the induction of a transient, yet striking, leukocytosis. The major component of the increase was the neutrophilic granulocyte, i.e. neutrophil, where approximately 65% of the total white blood cell increase was due to a neutrophilic granulocytosis[2]. The primary objective of the research presented here was to explore further the neutrophilic granulocytosis in regard to whether the marked increase in the number of circulating neutrophils was due to 1) a stimulatory effect on neutrophil production in bone marrow, and/or 2) a mobilization of neutrophils from reserve sites. In relation to the latter possibility, neutrophils are known to be very chemotaxic and capable of migration in the wake of tissue damage inflicted by a variety of factors, including exogenous substances such as lead[3]. Tritiated thymidine was used to test for the possible stimulatory effect. The incorporation of this radioactive DNA precursor is known to be an accurate indicator of neutrophil production[4,5]. Only those cells that are in the process of DNA synthesis at the time of tritiated thymidine administration, would incorporate the labeled precursor. Subsequently these cells would appear in peripheral blood as 'labeled'.

Young adult female mice of the ICR strain were used throughout these studies. 10 mice with an average weight of 28 g comprised each subgroup within the experimental and control groups; animal weights ranged between 21 and 37 g. Preparation of and injection procedures for lead acetate ($PbAc_2$) have been reported by others[6]. Control mice received 0.3 ml isotonic saline; this volume represented the average injection volume for the $PbAc_2$-treated mice. 6 h after $PbAc_2$ (day 0), mice received 200 µCi/kg of tritiated thymidine (^{3}HTdR, methyl-^{3}H-thymidine, aqueous, New England Nuclear, Boston, MA). 1, 2, 4, 6, and 8 days following lead, animals were sacrificed by cervical dislocation, and cardiac blood was removed using sodium heparin as the anticoagulant. From the sample, the total white blood cell count (wbc/mm^3), differential leukocyte count, and the number of neutrophils/mm^3 were determined for all experimental and control samples. In addition, the percentage of ^{3}HTdR-labeled neutrophils was found for each sample using an autoradiographic technique that was slightly modified from procedures published by others[7].

The temporal relationship between administration of $PbAc_2$ and neutrophilic granulocytosis is shown in figure 1. An abrupt increase in the number of circulating neutrophils is seen by day 4 and continues through day 8. Although not completely shown, the number of neutrophils after day 4 continues to decline progressively to control values by day 12. On days 4, 6, and 8 the number of circulating neutrophils for the experimental group are approximately 215, 170, and 100% greater than controls.

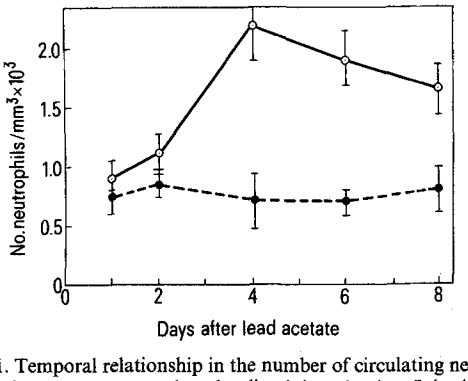

Figure 1. Temporal relationship in the number of circulating neutrophils between lead acetate-treated and saline-injected mice. Injections were conducted on day 0 using a total of 50 animals for experimental and control groups. 10 animals were sacrificed for each group on each day indicated.

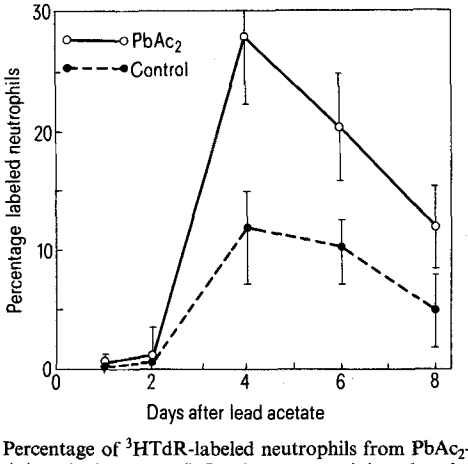

Figure 2. Percentage of ^{3}HTdR-labeled neutrophils from $PbAc_2$-treated and saline-injected mice (control). Lead acetate was injected on day 0 with ^{3}HTdR administered 6 h later. 50 animals were treated initially for each group, with 10 animals sacrificed on the designated days/group.

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-