

Mitochondrial Lesions in Reversible Erythropoietic Depression Due to Chloramphenicol

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ABSTRACT The mechanism underlying the reversible depression of erythropoiesis by chloramphenicol has been investigated in rabbits in which hemolytic anemia had been induced by phenylhydrazine so that the compensatory erythroid hyperplasia would provide a situation where abnormalities in the bone marrow cells reflected predominantly those of erythroid precursors.

Maintenance of chloramphenicol in the serum of these animals at concentrations in the order of 15 µg/ml resulted in erythropoietic depression after several days. The onset of this depression corresponded to the development of a cellular respiratory defect in the erythroid precursors which was associated with an abnormality in the composition of the mitochondrial respiratory pathway. The abnormality took the form of a selective depletion of cytochromes $a + a_s$ and b which can be explained by an inhibitory effect of the antibiotic on their formation by the mitochondrial protein-synthesizing system. The relationship between the mitochondrial lesion and the depression of proliferative activity was further indicated by the correlation between the restoration of the cytochrome deficit and the recovery of erythropoiesis after chloramphenicol administration was ceased.

The features of the reversible depression of erythropoiesis corresponded closely to those in man, so that a specific action of chloramphenicol on mitochondrial formation provides a reasonable explanation for this important manifestation of chloramphenicol toxicity.

INTRODUCTION

Chloramphenicol therapy has been frequently connected with toxicity in hemopoietic tissue, and the most commonly encountered form of toxicity in animals and man

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is a reversible depression of erythropoiesis (1-3). This depression of erythropoiesis develops regularly in man when the concentration of free chloramphenicol in the serum is maintained at or above 15-20 µg/ml for several days (2). As recovery of erythropoiesis takes place rapidly after the suspension of chloramphenicol administration, the antibiotic has been considered to exert a reversible and concentration-dependent toxic effect in erythroid precursors (2, 3). Several biochemical processes in bone marrow cells are known to be inhibited by chloramphenicol in vitro (4-8), but the process whose inhibition by chloramphenicol is responsible for the reversible depression of erythropoiesis in vivo has not yet been identified.

The purpose of the present study has been to determine whether a specific effect of chloramphenicol on the formation of mitochondria is related to the depression of erythropoiesis. Such a mechanism was considered because chloramphenicol has been shown to selectively inhibit the synthesis of the mitochondrial cytochromes $a + a_s$ and b in HeLa cells at concentrations similar to those producing erythropoietic depression in man (20 µg/ml). The physiological significance of this action of the drug was that it eventually led to impaired function of the mitochondrial respiratory pathway which in turn resulted in depression of proliferative activity after the cells had completed two divisions in the presence of chloramphenicol (9).

This study has been performed in the rabbit with the object of determining the relationship between chloramphenicol-induced changes in the mitochondrial respiratory pathway of erythroid precursors and the state of erythropoietic activity. It was considered that the task of examining mitochondria from erythroid cells would be greatly simplified by inducing erythroid hyperplasia in the experimental animals, as mitochondria isolated from bone marrow tissue under these conditions would essentially represent those of erythroid precursors. Erythroid hyperplasia was induced as a compensatory

response to hemolytic anemia produced by the regular administration of phenylhydrazine. It was also decided to maintain the concentration of free chloramphenicol in the serum of the experimental animals at approximately 15 $\mu\text{g/ml}$ in order to provide a comparable situation with that in man where sustained concentrations of this order produce erythropoietic depression (3). Due consideration was given to the relatively greater capacity of the rabbit to eliminate chloramphenicol, which necessitated the administration of larger doses of the drug on a body weight basis to produce similar serum chloramphenicol concentrations to those in man (10, 11).

METHODS

Drug administration. The study was performed in young adult New Zealand white rabbits. Hemolytic anemia was induced by single daily subcutaneous injections of phenylhydrazine HCl in a dose of 4 mg/kg body weight (12). Chloramphenicol was administered in a depot form by single daily subcutaneous injections of microcrystalline chloramphenicol suspended in 0.5% carboxymethylcellulose, at a dose of 600 mg/kg body weight.

Measurement of hematological indices. The packed erythrocyte volume (PCV)¹ and reticulocyte count were estimated in venous blood using conventional techniques and the films of bone marrow particles were stained with Leishman's stain.

Preparative procedures. Animals were sacrificed by exsanguination via cardiac puncture and the blood collected using sodium citrate at a final concentration of 0.38% as the anticoagulant. Erythrocytes were sedimented by centrifugation at 200 g for 10 min. The supernate and buffy coat were carefully discarded to reduce leukocyte contamination. The erythrocyte pellet was washed twice in isotonic saline and the supernate and buffy coat discarded as before. Fewer than 200 leukocytes/mm³ were present in the final erythrocyte pellet after completion of the washing steps. Red bone marrow was obtained from the femur, humerus, and upper third of the tibia. Bone marrow was suspended in a medium consisting of 0.25 M sucrose, 0.1 mM EDTA, and 10 mM tris buffer, pH 7.4, and gently homogenized in a Potter-Elvehjem homogenizer to disperse the cells. The cell suspension was then centrifuged at 600 g for 10 min and the floating layer of fatty tissue discarded. Sedimented bone marrow cells were resuspended in the same medium for cell fractionation, or in 137 mM NaCl, 3 mM KCl, and 10 mM Na phosphate, pH 7.4 for estimation of respiratory activity.

Mitochondrial fractions were isolated from peripheral blood erythrocytes which comprised 30–35% reticulocytes, and from bone marrow cells. The cells were disrupted by high speed homogenization which was continued until examination of the homogenate by phase contrast microscopy indicated that greater than 95% of the cells had been ruptured. Mitochondrial fractions were prepared by sedimentation at 8000 g for 15 min from the supernate remaining after low speed centrifugation of the homogenate had removed intact cells, nuclei, and large membrane fragments (13). The mitochondrial fractions were washed twice under the same conditions for biochemical studies,

and three times before the determination of amino acid incorporation into protein.

Assay procedures. Respiratory activity of bone marrow cells was determined polarographically with an oxygen electrode (Rank Brothers, Bottisham, U. K.). Respiration was supported by succinate at a concentration of 5 mM. Amino acid incorporation into protein by isolated mitochondria was assayed at 30°C during a 30 min period by previously described methods (14) using uniformly labeled L-leucine-¹⁴C (151 $\mu\text{Ci}/\mu\text{mole}$) obtained from C.E.A., Gif-sur-Yvette, France. Aseptic techniques, autoclaved equipment, and millipore-filtered solutions were used throughout this part of the study to minimize contamination by bacteria. Total bacterial counts were determined routinely in the final incubation mixtures. Activities of the following enzymes were determined at 30°C: malate dehydrogenase (15), fumarase (16), succinate dehydrogenase (17) and cytochrome oxidase (18). Mitochondrial cytochromes were estimated by difference spectroscopy using the extinction coefficients of Estabrook and Holowinsky (19). Protein was measured by the method of Lowry, Rosebrough, Farr, and Randall (20). The concentration of chloramphenicol in the serum was determined as the free form by the method of Levin and Fishbach (21).

RESULTS

Effect of drug administration on hematological status

Stimulation of erythropoiesis by phenylhydrazine administration. Daily phenylhydrazine administration to control animals produced hemolytic anemia with a progressive fall in the PCV until compensation of the hemolytic state developed on the 5th treatment day and subsequently maintained the PCV at approximately 24% (Fig. 1). The reticulocyte counts rose to between 30 and 40% on the 4th treatment day and remained at this level while phenylhydrazine was being administered. Section of the long bones on the 5th and 7th days demonstrated that red marrow had extended below the normal lower limit in the upper half of the femur and humerus to the midshafts of the distal long bones. Smears of red marrow from various sites in the long bones at these times revealed hypercellular particles and erythroid hyperplasia in which erythroid precursors comprised approximately 80% of the nucleated cells (Table I), an increase from the value of approximately 50% in normal animals.

Serum chloramphenicol concentrations during chloramphenicol administration. The serum chloramphenicol concentration was found to be $17 \pm 4 \mu\text{g/ml}$ (mean \pm SE) 6 hr after the daily injections of 600 mg chloramphenicol/kg body weight in rabbits receiving phenylhydrazine. 24 hr after the injections, the value was $12 \pm 2 \mu\text{g/ml}$, and when chloramphenicol administration was ceased, the concentration fell to $9 \pm 4 \mu\text{g/ml}$ after 48 hr and 0 $\mu\text{g/ml}$ after 72 hr, indicating a relatively slow rate of elimination under these circumstances.

Effect of chloramphenicol on erythropoiesis in phenyl-

¹ Abbreviation used in this paper: PCV, packed erythrocyte cell volume.

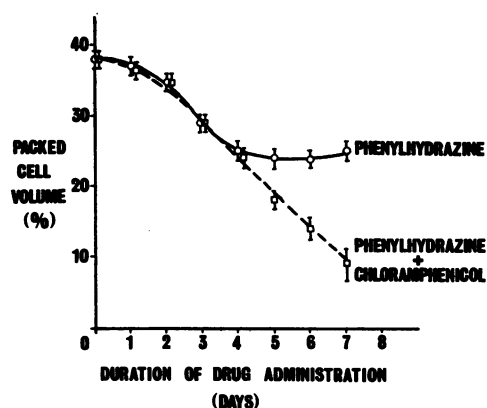


FIGURE 1 Effect of chloramphenicol administration on the erythrocyte packed cell volume in the phenylhydrazine-treated rabbit. Values are means \pm SE from six experimental animals in each category.

hydrazine-treated rabbits. The PCV fell to a mean of 23% and the reticulocyte counts rose to 30–40% during the first 4 days of chloramphenicol administration to phenylhydrazine-treated rabbits. This was identical with the sequence of events in the control animals (Fig. 1), and indicated that chloramphenicol did not prevent the initial reticulocyte response to phenylhydrazine-induced hemolytic anemia.

However, it is evident that continued administration of chloramphenicol prevented the development of a compensated hemolytic state after this period. The PCV fell steadily after the 4th day in the chloramphenicol-treated animals and they became progressively weaker and died when the PCV had fallen to values in the order of 8%. During this phase the reticulocyte count remained between 30 and 40%, so that with the fall in the PCV there was a proportional decline in the number of reticulocytes in the circulation.

Section of the long bones on the 5th and 7th days demonstrated the same degree of extension of the red marrow as in the control animals. Cellularity of aspirated particles from the red marrow was also similar to that in control bone marrow, and erythroid precursors comprised approximately 80% of the nucleated cell population. In contrast to the distribution of erythroid precursors in control bone marrow, there was a decrease in the proportion of orthochromatic and polychromatic normoblasts and an increase in the proportion of less mature erythroid precursors (Table I). This was similar to the picture of arrested maturation in the erythroid series of patients immediately after the onset of erythropoietic depression due to chloramphenicol (2). Cytoplasmic vacuolation was present in some basophil normoblasts, pronormoblasts, and myeloid precursors as reported in chloramphenicol-affected human bone marrow (2).

TABLE I
Effect of Chloramphenicol on the Bone Marrow Cell Population in Phenylhydrazine-Treated Rabbits

| | Proportion of erythroid and myeloid precursors* | | | |
|--------------------------------------|---|----------------------|----------------|-------------------------------------|
| | Ortho-chromatic and polychromatic normoblasts | Basophil normoblasts | Pronormoblasts | Myeloid series and other cell lines |
| Control bone marrow | 44 \pm 4 | 28 \pm 3 | 7 \pm 2 | 21 \pm 2 |
| Chloramphenicol-affected bone marrow | 28 \pm 5 | 40 \pm 4 | 10 \pm 3 | 22 \pm 2 |

Values are means \pm SE from four experimental animals in each category. Differences between the control and chloramphenicol-affected bone marrow were statistically significant in the proportions of orthochromatic and polychromatic normoblasts ($0.02 < P < 0.05$) and basophil normoblasts ($0.02 < P < 0.05$), but were not statistically significant in the case of pronormoblasts and other cell series ($P > 0.05$).

* Per cent of total cell population.

Recovery of erythropoiesis after chloramphenicol withdrawal. When chloramphenicol and phenylhydrazine administration was suspended, the PCV continued to fall for several days at a similar rate to that in animals whose treatment had been continued. The PCV eventually began to rise but the delay before this occurred was considerably greater than after the suspension of phenylhydrazine administration to the control animals (Fig. 2). During the fall in the PCV after a 5 day course of chloramphenicol and phenylhydrazine as illustrated in Fig. 2, the reticulocyte count remained between 30 and 40% for 3 days until the nadir was reached and persisted at this level for 4–5 days during the subsequent rise in the PCV. There was thus a progressive fall in the number of reticulocytes in the circulation before

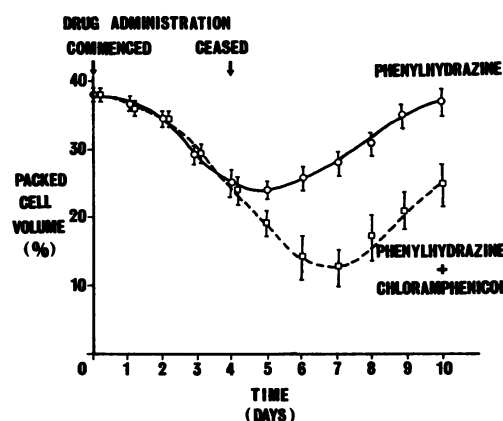


FIGURE 2 Response of the erythrocyte packed cell volume to a limited course of drug administration in the rabbit. Values are means \pm SE from four experimental animals.

the number began to increase 4 days after the suspension of chloramphenicol administration.

On the 1st day that the PCV and number of circulating reticulocytes began to rise, bone marrow aspiration revealed that the pattern of maturation in erythroid precursors had been restored to that in the corresponding controls. The degree of erythroid hyperplasia and the proportions of the various erythroid precursors were identical with those in control animals on the 5th day of phenylhydrazine treatment as illustrated in Table I. Cytoplasmic vacuolation was no longer present.

Suspension of treatment after a longer period of chloramphenicol and phenylhydrazine administration resulted in the PCV falling to levels which were incompatible with survival. Hence studies on the recovery of erythropoiesis were performed on animals whose treatment was limited to a 5 day course.

Mitochondrial and respiratory abnormalities in chloramphenicol-affected erythroid tissue

Two stages in the response of erythropoiesis to chloramphenicol were selected for evaluating changes in mitochondrial composition and respiratory activity in erythroid tissue. These were when erythropoietic depression had become established, and when recovery of erythropoiesis had clearly developed after the suspension of chloramphenicol administration. The hematological picture on the 5th treatment day was considered representative of the former situation as compensation of anemia could not be sustained, the circulating number of reticulocytes was declining in spite of worsening anemia, and a picture of arrested erythroid maturation was present in the bone marrow. The hematological picture on the 4th day after the suspension of a 5 day course of chloramphenicol administration was considered consistent with resumption of active erythropoiesis as the PCV and numbers of circulating reticulocytes had started to rise and the pattern of erythroid maturation in the bone marrow had reverted to that in control animals.

Respiratory activity of chloramphenicol-affected erythroid tissue. The rate of oxygen consumption in chloramphenicol-affected bone marrow cells was $0.88 \pm 0.07 \mu\text{l O}_2/10^6$ nucleated cells/hr (mean \pm SE, four experiments), and represented a statistically significant depression ($P < 0.01$) of 27% in the value of $1.21 \pm 0.05 \mu\text{l O}_2/10^6$ nucleated cells/hr for bone marrow from control animals on the 5th treatment day. 4 days after the suspension of treatment the values were $1.28 \pm 0.05 \mu\text{l O}_2/10^6$ nucleated cells/hr in bone marrow from the animals which had received chloramphenicol, and $1.27 \pm 0.06 \mu\text{l O}_2/10^6$ nucleated cells/hr in the controls.

Mitochondrial composition in chloramphenicol-affected erythroid tissue. The level of a number of respiratory chain cytochromes was significantly reduced in mito-

chondria from the bone marrow of chloramphenicol-treated rabbits on the 5th treatment day as illustrated in Table II. This change involved cytochromes $a + a_3$ and b , and the decrease in the content of cytochromes $a + a_3$ demonstrated by difference spectroscopy was corroborated by a similar decrease in the specific activity of cytochrome oxidase in keeping with its role as the enzymatic function of cytochromes $a + a_3$.

On the other hand, there was no significant change in the content of the c group cytochromes or in the specific activities of other representative mitochondrial enzymes assayed, such as fumarase and malate and succinate dehydrogenases. There was also no detectable decrease in the total protein content of the mitochondrial fraction from chloramphenicol-affected bone marrow cells ($8\text{--}10 \text{ mg}/10^6$ nucleated cells), so that the decreased content of cytochromes $a + a_3$ and b represents an alteration in mitochondrial composition rather than an over-all decrease in the mitochondrial content of chloramphenicol-affected bone marrow cells. It is of interest that the cytochrome deficit appeared to be specific for cytochromes $a + a_3$ and b , as the c group cytochromes were present in normal amounts.

4 days after suspension of drug administration, the cytochrome levels and enzyme-specific activities in mitochondria isolated from the bone marrow of animals which had received chloramphenicol were identical with those from control animals. There was no statistically significant difference between these values and the values in the mitochondria of control animals on the 5th treatment day as illustrated in Table II.

Mitochondrial composition in peripheral blood reticulocytes of chloramphenicol-treated animals. Cytochrome oxidase activity was significantly decreased in mitochondria isolated from peripheral blood erythrocytes of chloramphenicol-treated animals on the 5th treatment day (Table II). The mitochondria originated from the reticulocytes which comprised from 30 to 40% of the erythrocytes in the cells which had been subjected to fractionation after leukocytes had been extensively removed by repeated washing. This decrease in cytochrome oxidase activity was similar to that in the bone marrow mitochondria, and the selective nature of the defect was again illustrated by the lack of change in the specific activity of succinate dehydrogenase. As in the bone marrow mitochondria, the specific activity of cytochrome oxidase in reticulocyte mitochondria had reverted to control values on the 4th day after the suspension of chloramphenicol administration.

Action of chloramphenicol on amino acid incorporation into protein by isolated bone marrow mitochondria. Chloramphenicol inhibited leucine- ^{14}C incorporation into protein by mitochondria isolated from control bone marrow on the 5th treatment day. This effect was evi-

TABLE II
Mitochondrial Composition in Chloramphenicol-Affected Erythroid Tissue

| Source of mitochondria | Nature of mitochondrial component | Cytochrome levels and enzyme-specific activities in mitochondria | | |
|-------------------------------|--|--|---------|------------------------|
| | | Chloramphenicol affected | Control | <i>P</i> value |
| Bone marrow cells | Cytochromes <i>a</i> + <i>a</i> ₃ | 0.023 | 0.074 | <0.01 |
| | Cytochrome <i>b</i> | 0.117 | 0.146 | 0.01 < <i>P</i> < 0.02 |
| | Cytochromes <i>c</i> + <i>c</i> ₁ | 0.149 | 0.166 | >0.05 |
| | Cytochrome oxidase | 0.32 | 1.15 | <0.01 |
| | Succinate dehydrogenase | 0.29 | 0.30 | >0.05 |
| | Malate dehydrogenase | 0.81 | 0.71 | >0.05 |
| | Fumarase | 0.41 | 0.40 | >0.05 |
| Peripheral blood Erythrocytes | Cytochrome oxidase | 0.28 | 1.21 | <0.01 |
| | Succinate dehydrogenase | 0.35 | 0.36 | >0.05 |

These values are the means from four experimental animals in each category. The levels of the cytochromes in the mitochondria are expressed as micromoles per milligram protein in the mitochondrial fraction. Enzyme activity is expressed as micromoles substrate utilized/minute for malate dehydrogenase and fumarase, as the Δ OD at 600 m μ /min for the decolorization of phenazine methosulphate (0.17 mg/ml) for succinate dehydrogenase, and as the first order rate constant/minute for cytochrome oxidase. Specific activities are per milligram protein in the mitochondrial fraction.

dent at a concentration of 20 μ g/ml and the degree of inhibition increased with the concentration of the antibiotic (Table III). Cycloheximide, an inhibitor of amino acid incorporation into protein by mammalian cytoplasmic ribosomes (22), did not affect the process which indicated that contaminating cytoplasmic ribosomes did not contribute to the amino acid incorporation. The very low contamination by bacteria in the final incubation systems (routinely less than 40 organisms/ml) indicated that amino acid incorporation was also not due to contaminating bacteria, as such small numbers of organisms do not significantly contribute to amino acid in-

corporation into protein under the experimental conditions employed (23). The lack of effect of lincomycin on amino acid incorporation indicates that the sensitivity to chloramphenicol does not represent sensitivity to antibiotics in general, in keeping with the limited antibiotic sensitivity of mitochondrial protein-synthesizing systems in the tissues of other mammalian species (14).

Action of chloramphenicol on bone marrow cell respiration. The capacity of chloramphenicol to directly affect cellular respiration was also examined using bone

TABLE III
Antibiotic Sensitivity of Amino Acid Incorporation into Protein by Isolated Bone Marrow Mitochondria

| Antibiotic | Concentration | Specific activity of mitochondrial protein | Inhibition of amino acid incorporation |
|-----------------|---------------|--|--|
| | μ g/ml | cpm/mg | % |
| Nil | — | 198 \pm 10 | — |
| Chloramphenicol | 20 | 140 \pm 5 | 27 |
| Chloramphenicol | 50 | 121 \pm 7 | 38 |
| Chloramphenicol | 100 | 71 \pm 12 | 64 |
| Lincomycin | 100 | 197 \pm 8 | 0 |
| Cycloheximide | 100 | 200 \pm 11 | 0 |

Values are means \pm SE from three experiments.

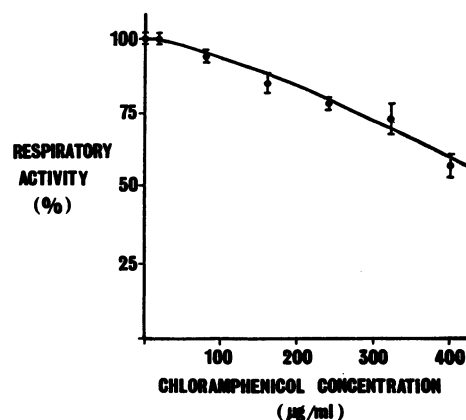


FIGURE 3 Action of chloramphenicol on the respiratory activity of bone marrow cells isolated from the phenylhydrazine-treated rabbit. Values are means \pm SE from four experimental animals.

marrow cells from control animals on the 5th treatment day. Chloramphenicol did inhibit respiratory activity, but this effect was manifested only at concentrations greater than those attained in the serum of the experimental animals (Fig. 3). The degree of inhibition was only 5% at a concentration of 80 μ g/ml but increased progressively to 30% at a concentration of 320 μ g/ml.

DISCUSSION

Chloramphenicol administration has been shown to produce a selective depression in the content of certain respiratory enzymes in the mitochondria of erythroid precursors in the rabbit. The significance of this mitochondrial lesion is indicated by its close relationship to the reversible depression of erythropoiesis by chloramphenicol.

The experimental approach involved the treatment of the rabbits with phenylhydrazine in order to produce hemolytic anemia and compensatory erythroid hyperplasia in the bone marrow. Under these conditions the proportion of erythroid precursors increased to approximately 80%, so that the mitochondrial and respiratory characteristics of the bone marrow tissue predominantly reflected those of erythroid precursors. Administration of chloramphenicol was commenced with the phenylhydrazine so that erythroid precursors would be exposed to chloramphenicol from the start of their active proliferative response to the hemolytic anemia. It was reasoned that development of erythropoietic depression under these conditions would be manifested by abrupt changes in the peripheral blood picture which would clearly indicate a suitable time to evaluate underlying changes in the erythroid precursors.

The number of circulating reticulocytes relative to that in the control animals did in fact begin to decline abruptly on the 5th day after the institution of chloramphenicol treatment, and the underlying arrest of maturation of erythroid precursors indicated that this response was due to depression of reticulocyte production. Studies on the bone marrow at this stage demonstrated that cellular respiration was depressed, and it was shown that this did not simply represent a direct effect of free chloramphenicol on the cells. A specific cause for depressed respiratory activity was present in the form of an abnormality in the mitochondrial respiratory chain, as there was a considerable decrease in the mitochondrial content of cytochromes $a + a_s$ and b , and in the specific activity of cytochrome oxidase. Cytochrome oxidase activity in the reticulocyte mitochondria was also considerably reduced, and as the reduction was equivalent to that in bone marrow mitochondria it was evident that mitochondrial composition had been affected throughout the erythroid series in the chloramphenicol-treated animals.

The mechanism of oxidative energy production by mitochondria is involved by such a lesion in the respiratory pathway, which indicates how the abnormality can interfere with proliferation of erythroid precursors. As a similar degree of depletion of these cytochromes from the mitochondria of HeLa cells is responsible for the depression of proliferative activity of these cells by chloramphenicol (9), it has to be considered that erythropoietic depression was also produced by this means. When turnover rates of erythroid precursors are taken into account, it would be expected by analogy with the response of HeLa cells that proliferation of pronormoblasts and basophil normoblasts would become depressed after two divisions, which would occur after approximately 2 days (24). However, the maturation of polychromatic and orthochromatic normoblasts which occurs without further cell proliferation would still continue to yield reticulocytes for a further 2-3 days before reticulocyte production became depressed. Such a course of events is consistent with that observed in the experimental animals. After the suspension of chloramphenicol administration, the period before the number of circulating reticulocytes began to rise is also consistent with that required for maturation of cells derived from the resumption of basophil normoblast proliferation after chloramphenicol had been cleared from the serum. On the grounds that the mitochondrial lesion was causally related to erythropoietic depression, its recovery throughout the erythroid series should have occurred at the stage when the cohort of newly formed reticulocytes had reached the circulation, and this is in agreement with the experimental findings.

The means by which chloramphenicol selectively interferes with the synthesis of cytochromes $a + a_s$ and b in erythroid precursors is suggested by findings in studies with other mammalian tissues. It had been shown in these studies that the drug inhibited the synthesis of a small proportion of mitochondrial proteins in the absence of an inhibitory effect on the synthesis of the majority of mitochondrial and other cellular proteins (9, 13, 22). Cytochromes $a + a_s$ and b were among the mitochondrial proteins whose synthesis was affected by chloramphenicol. The selective nature of the inhibitory effect was attributed to the capacity of chloramphenicol to inhibit protein synthesis by mitochondria but not by cytoplasmic ribosomes. Such a mechanism is also applicable to the erythroid precursors as protein synthetic activity in the isolated mitochondria was inhibited by chloramphenicol *in vitro*, and isolated cytoplasmic ribosomes from this tissue have been shown elsewhere to be insensitive to the drug (25). The ability of chloramphenicol to affect protein-synthetic activity in isolated mitochondria has been known for some time, and provided the basis for the speculation that such an effect was re-

sponsible for toxicity (7, 13, 23, 26, 27). The importance of the present findings is in providing evidence that an effect of chloramphenicol on this system is operative in erythroid precursors in vivo.

Development of such highly specific changes in the mitochondria is not consistent with the consequences of certain alternative mechanisms proposed to account for chloramphenicol toxicity, because the suggested mechanisms invoked an inhibition of major cellular synthetic pathways which would have led to a generalized rather than specific inhibition of the synthesis of mitochondrial components. These include the inhibition of protein synthesis by cytoplasmic ribosomes proposed by Weisberger and Wolfe (4), and the inhibition of ribonucleic acid synthesis proposed by Ward (5). Depression of ferrochelatase activity (8) also cannot account for the changes produced in the mitochondria, as this would have interfered with heme synthesis and thereby generally inhibited the formation of cytochromes. Such a situation was shown not to be the case as the *c* group cytochromes were formed in normal amounts as in other studies on the action of chloramphenicol in mammalian tissues (9, 13).

Erythropoietic depression due to chloramphenicol in this experimental situation closely resembles that in man under clinical conditions in the pattern of development and recovery, as well as in the morphological changes in erythroid precursors (2, 3). The effective chloramphenicol concentration in the rabbit is also comparable with the level of 15–20 $\mu\text{g}/\text{ml}$ at or above which depression regularly develops in man (3). The serum concentration rather than the administered dose of chloramphenicol is believed to be the determining factor in the development of erythropoietic depression (3, 11), and it was only to compensate for the greater capacity of the rabbit to eliminate chloramphenicol that a relatively greater dosage on a body weight basis was administered to them (10). Thus, in all essential features, the response in the rabbit resembles that in man, so that a similar mechanism can be proposed to account for the reversible erythropoietic depression by chloramphenicol in man.

Chloramphenicol also produced a direct effect on bone marrow cell respiration at concentrations which were considerably greater than those attained in the experimental animals, and this second type of effect may be related to a different form of human toxicity manifested only at very high serum concentrations of the drug. Inhibition of cell respiration has also been observed at high concentrations in other mammalian tissues (14, 22, 28), and was shown to be due to inhibition of NADH dehydrogenase which in turn led to depression of energy-dependent synthetic processes (28). This mechanism provides an explanation for the potentially lethal generalized

toxicity known as the Grey syndrome, which develops when chloramphenicol accumulates to very high concentrations (75–180 $\mu\text{g}/\text{ml}$) in the serum of neonates as a consequence of their impaired capacity to detoxify the drug (29).

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