Chloramphenicol toxicity in infants

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CHLORAMPHENICOL TOXICITY IN INFANTS

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Submitted in Partial Fulfillment for the Degree of Doctor of Medicine

College of Medicine, University of Nebraska

February 1, 1964

Omaha, Nebraska
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INTRODUCTION

Prior to 1959 chloramphenicol had been considered relatively free from side effects other than those of bone marrow depression (1) and bacterial or fungal superinfection. (2) The concern regarding the increasing incidence of nursery infections had resulted in widespread use of antibiotics for prophylaxis as well as therapy.(3,4) Chloramphenicol gained popularity in this field because of its greater effectiveness against those bacterial agents which more commonly cause trouble in the premature nursery, i.e. staphylococci and gram negative rods as well as streptococci and pneumococci. However, prior to the Parke, Davis and Company announcement of neonatal chloramphenicol intoxication in 1959,(5) the recommended dose was 100 to 150 mg per kilogram of body weight per day for children of 15 kilograms or less.(6-9)

It is the purpose of this paper to present not only the findings of the various investigators who made the initial observations, but also the work of those who presented a physiologic explanation of the drug's toxicity when given to newborn and premature infants in excessive doses.
STATEMENT OF THE PROBLEM

In 1959 workers in a number of newborn nurseries observed an increase in toxic reactions and death rates, especially among premature infants, following the administration of this antibiotic.(10-14) These infants died in acute circulatory collapse.

In some instances sepsis was blamed for these deaths; however, no specific evidence of infection was found. Van Gelder (15) questioned sepsis as the cause of death because of the frequency of myocardial failure in these infants. Previous reports had not listed myocardial disease as a common feature of neonatal sepsis. Antibiotic dosages were increased and additional deaths resulted until the common etiology was recognized. Thereafter dosages were reduced and no more unexplained deaths occurred. Post mortem findings attributable to the use of chloramphenicol were absent in all organ systems including the hematopoietic system.

Symptoms first appeared two to five days after continued therapy with chloramphenicol doses of 100 mg per kilogram per day or more. In many cases the symptoms appeared in the order of abdominal distention, with or without emesis, progressive pallid cyanosis, vasomotor collapse, frequently accompanied by irregular respiration, and sometimes death within a few
hours after the onset of these symptoms. This has been referred to in some institutions as the "gray syndrome". The progression of symptoms from onset to death is accelerated with higher dose schedules.

The delay in discovering the toxic manifestations of chloramphenicol appears to be due to the fact that the drug was not used extensively in newborn infants prior to 1957 and the earlier reports involved short-term therapy without toxicity (16,17). Also, St. Geme pointed out that the symptoms of chloramphenicol toxicity are difficult to distinguish from those of overwhelming neonatal bacterial and viral infections (18).

This clinical picture, i.e. acute circulatory collapse, had been previously noted by European observers (19-21) in adults and children following three to five days of high doses of chloramphenicol, but with few exceptions (9) this has been associated with therapy of salmonella infections. Viti in 1956 reported vasomotor collapse during the administration of chloramphenicol in the absence of bacterial infection. He suggested that chloramphenicol had an adverse effect on carbohydrate metabolism, having found fasting hypoglycemia in normal adults after five to ten days of oral therapy and a return to normal ten days after the drug was discon-
continued. He was unable to determine the exact cause of this hypoglycemia but believed it involved a toxic action in the liver. The acute cardiovascular collapse was believed to be directly related to this hypoglycemic action. Groh (22) found delayed glucose breakdown in infants during chloramphenicol therapy. This delay was reversed when B vitamins were administered during treatment. He ascribed this delay to a decrease in B vitamin coenzymes in the G. I. tract. Patterson (23) observed anaphylactoid shock and vasomotor collapse during the administration of chloramphenicol. The patient developed pruritis thirty minutes following ingestion of the first dose and in a few minutes developed angioneurotic edema with laryngeal involvement. Blood pressure was unobtainable but treatment with epinephrine, theophylline, and diphenhydramine resulted in recovery in a few days. This reaction was also reported by Chatterjee (20) and Stephens (24) in 1950 and by Welch (25) in 1957 who observed typhoid fever deaths after initial improvement with chloramphenicol. This was considered to be a Jarisch-Herxheimer reaction from endotoxins released from destroyed virulent bacilli which overwhelmed the already weakened patient. Deaths in children under the age of three resulting from bacterial superinfection following chloramphenicol therapy have been mentioned (2).
These followed vomiting and diarrhea as a result of the superinfection by resistant staphylococci.

Substitution of the acid in the ester could produce an increased toxicity if the absorption rate was increased, but this alone has had no qualitative effect on toxicity since similar deaths have been reported with the palmitic acid and sodium succinate esters and the microcrystalline suspension.

Studies were performed to determine if there exists any difference in the metabolic products of chloramphenicol in the serum and urine of newborn infants from those in the adult. Dill (27) found the nitro compounds to be identical to those in the adult. He also discovered a new metabolite of chloramphenicol, a glycolic acid analogue, which possesses a low order of toxicity and is unlikely to have produced the side reactions seen in infants.

Sutherland (10) considered methemoglobinemia because of the gray pallor. Although no determinations for methemoglobin were done this possibility was discounted because of the absence of the characteristic slate cyanosis and chocolate colored blood and tissue during life or at necropsy. Mongelli and Carrozzini (28) suggested that chloramphenicol has a primary toxic effect on the autonomic nervous system.

The mechanism of action is unknown but there is con-
siderable evidence in the literature which suggests that the toxic manifestations of chloramphenicol in infants are related to poor liver and kidney function. This reduced detoxication and excretion leading to retention of the free drug results in toxic levels of 100 micrograms per milliliter after three days of continued therapy with high doses.

REPORT OF CASES

Two abstracted cases will be presented to illustrate both the typical onset of symptoms and the insidious way in which this toxic effect manifests itself.

CASE 1. The patient was a 3,320 gm infant born of a Negro, married, Group A, Rh-positive, primipara with negative serologic test for syphilis at 44 weeks' gestation. The mother had had no prenatal care and was admitted in labor with albuminuria and a systolic blood pressure over 200 mm. Hg.

After a five-and-one-half hour labor with no medication the infant was delivered by vertex presentation under nitrous-oxide and oxygen anesthesia with a mediolateral episiotomy. At birth the infant had an Apgar score of 6 and was noted to be meconium stained.

A foul odor was encountered on physical examination. The time of rupture of the maternal membranes was unknown, and 50,000 units of procaine penicillin and 250 mg of chloramphenicol were started intramuscularly every eight hours. Menadione sodium bisulfite 5 mg was given. At 8 hours the infant nursed at the breast. At 2 days rectal temperature was 99 F. The infant did not seem ill and he was circumcised.

At 4 days of age the infant developed a gray color and a cold moist skin in spite of a temperature of 99 F. The infant died at 106 hours of age, 8 hours after the onset of vascu-
lar collapse. He received 250 mg of chloramphenicol 9 times in 72 hours, or 230 mg per kilogram per 24 hours.

At postmortem examination the lungs weighed 40 gm, and there was lobular consolidation. The heart weighed 21 gm and was moderately dilated. On microscopic examination there was patchy atelectasis and emphysema without an apparent causative factor. There was a trivial pneumonitis. Quite extensive focal myocardial degeneration with pyknosis was seen. There was some vacuolization of proximal renal tubules. No bone-marrow changes were noted.

CASE II. (13) This 1,490 gram, Negro, female infant was born following a pregnancy which was complicated by vaginal bleeding during the third trimester. This was the fifth pregnancy for this 25-year-old mother with type "O", Rh-positive blood, who had been in good health. A normal spontaneous delivery produced a small weak infant with poor respirations and a weak Moro response. There was no period of apnea.

The infant was placed in an incubator in an atmosphere of high humidity and 35% oxygen. The infant's vigor and strength improved and the oxygen was discontinued at the end of 24 hours. Color, cry and respirations were labelled "good" by the attending nurses on repeated occasions, and there was no record of difficulty during the second day of life.

At 24 hours of age, 5% glucose in water was given orally by means of a bottle and nipple. This was repeated at 3-hour intervals on four occasions. A formula of milk was given at 36 hours of age and repeated every 3 hours.

The first dose of chloramphenicol (93 mg) was given at 48 hours of age. At 62 hours of age, the infant's color was recorded as being "dusky" and oxygen was administered for 5 minutes.

This type of episode occurred at 14 and 16 hours, respectively, after the first and second doses of chloramphenicol. There followed a 12-hour period without difficulty, during which time the third and fourth doses of chlor-
amphenicol were given. At 74 hours of age, the infant became cyanotic and the house physician was called. Oxygen was prescribed, with proper restrictions, and the chloramphenicol was continued. The infant ate well at the next feeding, but she became "very cyanotic" and her respirations were very irregular and shallow. The milk formula was replaced with glucose in water which she took without difficulty for two feedings.

At 84 hours of age (36 hours after the first dose of medication), she had a momentary period of apnea. However, she took the next four routine feedings without trouble, and her color and respirations were good; there were no reports of difficulty during this period. At 100 hours of age (52 hours after the first dose of chloramphenicol), respirations suddenly ceased and she could not be revived. She had received 560 mg Chloromycetin Palmitate divided in six doses during a period of 52 hours.

The post-mortem examination showed immature pulmonary structures. There were scattered areas of focal atelectasis. There was no lesion demonstrable which seemed to be of sufficient magnitude to be the cause of death.

CLINICAL STUDY

As a result of the increased mortality rates in 1958 and 1959, following both prophylactic administration and treatment with chloramphenicol, several series of these unexplained deaths were collected. Burns et al. (7) studied four groups of premature infants treated after premature rupture of membranes.

Group I received no antibiotic, group II received chloramphenicol alone 100-165 mg per kilogram per day; group III received penicillin 150,000-600,000u per day and streptomycin
50 mg per kilogram per day. Group IV received all antibiotics.

The mortality rates of the groups given chloramphenicol were markedly elevated from the other two groups. Interestingly, the mortality of the non-treated group was identical to the penicillin-streptomycin group. The infants treated with chloramphenicol followed a typical clinical course similar to that presented earlier.

A retrospective study by Lischner (22) et al. in March and April of 1958 included a search for the common etiology in these deaths. There were common factors in nine of the ten infants who died. Chloramphenicol in doses of over 113 mg per kilogram was given early for prevention or treatment of infection. After two to five days of treatment initial improvement of symptoms was followed by vascular collapse and sudden death. Extensive microbiological studies had been carried out by both bacteriologic cultures and virus isolation studies on HeLa cells, monkey kidney and live animals. One of their 42 infants in this series had a single colony of Staphylococcus aureus and all the viral studies were negative. Post mortem examination failed to reveal a cause of death.

Van Gelder (15) et al. reported on two outbreaks of neonatal deaths but the association with chloramphenicol did not become evident until the toxicity was announced by Parke,
Davis & Company. One of these deaths was in an infant 25 days of age. The maximum danger period for chloramphenicol toxicity has not been established, but Lischner indicates that infants three weeks and older can tolerate 200 mg per kilogram per day provided they are not severely ill or dehydrated and further weakened by chloramphenicol therapy. The current recommended dosage by Parke, Davis, & Co. for premature and newborn infants is 25 mg per kilogram per day. Full term infants may receive 25-50 mg per kilogram per day.(29)

Only one case of the "gray syndrome" has been reported in older infants. Morton (30) describes abdominal distention, progressive cyanosis, vasomotor collapse with irregular respiration and finally apnea in a six week old infant following a dosage of 750 mg intramuscularly in the first twelve hours, or 166 mg per kilogram. She ascribes the reason for toxicity at this age to anuria.

Chloramphenicol toxicity has been extensively studied in animals. The report of Kent (31) et al. illustrates the desirability of obtaining toxicity data on newborns as well as in the adult by their study on 394 newborn mice and two groups of adult mice. Their test animals received 230-2400 mg per kilogram of chloramphenicol for five successive days and the control animals received 0.1 molar sodium succinate in 5% glucose.
The LD$_{50}$ for newborn mice in this experiment was $315 \pm 22.6$ mg per kilogram per 24 hours and the LD$_{50}$ for adult mice was $1,675 \pm 35.6$ mg per kilogram per 24 hours. This is 5.3 times the LD$_{50}$ of newborns.

Similar studies by Smith et al. showed the LD$_{50}$ for adult mice to be 245 mg per kilogram of body weight. They also were able to produce a drop in blood pressure following intravenous doses of chloramphenicol. With high doses, following the fall in blood pressure, respiratory failure and death resulted.(32)

**BLOOD LEVEL STUDIES**

Following publication of numerous reports in 1959, the Research Division of Parke, Davis and Company published a summary of the blood levels of different forms of chloramphenicol in infants.(33)

A colorimetric procedure was used to determine the total nitro compounds and aryl amines in the samples. This procedure was described by Glazko (34) in 1949 and is not specific for active chloramphenicol since the inactive degradation products are included in the determination. The method devised is based on reduction of the aromatic nitro group to a primary amine with titanous chloride or metallic zinc, followed by diazotization and coupling of the resulting amines. All results are expressed in chloramphenicol equivalents.
Determinations of the free and unconjugated portion of chloramphenicol provide data on the effective levels of chloramphenicol and are performed by means of a turbidimetric procedure against Shigella sonnei. This method is, however, less valuable than the colorimetric procedure for absorption and excretion studies.

Chloramphenicol palmitate is a water insoluble ester which is absorbed slowly and poorly following hydrolysis in the intestine. An initial dose of 100 mg per kilogram and 25 mg per kilogram per 6 hours is necessary to maintain a minimal therapeutic blood level of 10 micrograms per milliliter. Following the control of particle size, satisfactory blood levels could be maintained with the recommended doses.

The series included infants under forty-eight hours of age and children ranging from one to twelve years of age. A single dose of 50 mg per kilogram was administered. The older age group obtained maximum blood levels in two hours and by four hours half of the maximum level remained. In the newborn the maximum was reached in twelve hours indicating reduced hydrolysis and absorption. The half-life was prolonged to twenty-eight hours.

The microcrystalline form of chloramphenicol was designed for intramuscular use. The slow rate of absorption
and rapid excretion produces low but prolonged blood levels. A single dose of 50 mg per kilogram was administered to 9 infants under forty-eight hours of age. The maximum blood level with this form was reached in twenty-three hours. This was followed by a slow rate of fall and later by an acceleration of the rate of removal which was believed due to either less absorption in the later periods or interval maturation of the infants hepatic and renal systems.

This series showed that in most cases the infants one to four days of age had significantly higher blood levels than the infants that were fourteen to twenty-three days of age.

Chloramphenicol sodium succinate is the preferred and only suitable parenteral form for intramuscular, intravenous, or subcutaneous use. Hydrolysis occurs in the tissues with liberation of free chloramphenicol. The study included children four to five years of age, infants ten to sixteen days of age and newborn infants one to two days of age. The dosage was 50 mg per kilogram except for the ten to sixteen day age group which received 25 mg per kilogram.

High levels were obtained within a few hours in all groups indicating rapid absorption; however the half-life was twenty-six hours in the newborn group compared to ten hours in the second group and four hours for the older children.
The prolonged half-life in the newborn group indicates that elimination of the nitro compounds is slow.

Chloramphenicol blood levels in newborns were studied by Wiltse (35) at the University of Nebraska Hospital. A total of 32 infants was incorporated into this study and divided into groups according to weight. Infants weighing 2500 grams or less received 25 mg per kilogram per 24 hours and those weighing over 2500 grams received 50 mg per kilogram per 24 hours. All infants received an initial loading dose of 50 mg per kilogram. Chloramphenicol succinate was given intramuscularly in divided doses every 12 hours.

Chemical assay of heparinized plasma samples was done by the colorimetric procedure of Glazko (34) et al. The resulting blood levels were expressed in micrograms per milliliter of chloramphenicol equivalents which included total nitro compounds.

One group included 19 infants and was divided into 4 subgroups according to weights of the infants. All these infants received only a single dose of 50 mg per kilogram per milliliter. Table I compares the half-lives of four weight ranges, i.e. 1000-2500 grams, 2500-3000 grams, 3000-3500 grams, and weights over 3500 grams. The half-lives were calculated from the 6-18 hour "K" value, on the assumption that first-order kinetics apply. A graph of the mean values of all
patients in table I is shown in figure 1. There appears to be a significant increase in the half-life with increasing weight of the infant as shown in figure 1. This may be due to increased amounts of adipose tissue in larger babies. Glazko (36) found that chloramphenicol was widely distributed in all body tissues but did not include adipose tissue in his analysis.

Table II represents another group of newborns divided into 2 subgroups according to weight. Plasma levels were obtained on samples drawn 6 hours after the first dose on days 1, 3, and 5, i.e. at 6, 54, and 102 hours.

The mean values for the larger infant group are approximately twice those of the smaller group indicating that full term babies probably do not eliminate the antibiotic any more satisfactorily than the prematures. This suggests that increasing the dose in infants weighing 2500 grams or more is not justified and can even be dangerous. Sutherland (37) recently recommended doses of 25 mg per kilogram per 24 hours for premature and full term infants under one week of age and 50 mg per kilogram per 24 hours for all infants over one week of age. Day 3 is the most significant with two blood levels within the toxic range. Both of these infants were large babies well over 2500 grams. Lowering the dose schedules below 25 mg per kilogram per 24 hours would result in inef-
fective blood levels of 10 micrograms per milliliter or less in some cases. In this series one sample was below 10 micrograms at the 25 mg per kilogram per 24 hours dosage.

The wide standard deviation indicates that toxic levels can be reached with these doses in some cases, thus use of this antibiotic in newborn infants requires closely monitored blood levels to assure safe administration.
<table>
<thead>
<tr>
<th>Patient number</th>
<th>Birth Weight (kg)</th>
<th>6-18 hr. &quot;k&quot;</th>
<th>Half-life (hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.984</td>
<td>15.4</td>
<td>10.7</td>
</tr>
<tr>
<td>2</td>
<td>1.210</td>
<td>21.1</td>
<td>14.6</td>
</tr>
<tr>
<td>3</td>
<td>1.990</td>
<td>21.9</td>
<td>15.2</td>
</tr>
<tr>
<td>4</td>
<td>2.168</td>
<td>22.9</td>
<td>15.9</td>
</tr>
<tr>
<td></td>
<td><strong>1.838</strong></td>
<td></td>
<td><strong>Mean 14.1</strong></td>
</tr>
<tr>
<td></td>
<td><strong>SD±2.6</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.533</td>
<td>27.6</td>
<td>19.1</td>
</tr>
<tr>
<td>6</td>
<td>2.573</td>
<td>21.4</td>
<td>14.8</td>
</tr>
<tr>
<td>7</td>
<td>2.520</td>
<td>21.8</td>
<td>15.1</td>
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<tr>
<td></td>
<td><strong>2.542</strong></td>
<td></td>
<td><strong>Mean 16.3</strong></td>
</tr>
<tr>
<td></td>
<td><strong>SD±2.4</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.020</td>
<td>33.7</td>
<td>23.4</td>
</tr>
<tr>
<td>9</td>
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<td>16.3</td>
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<td>10</td>
<td>3.225</td>
<td>24.8</td>
<td>17.2</td>
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<td>11</td>
<td>3.422</td>
<td>26.1</td>
<td>18.1</td>
</tr>
<tr>
<td>12</td>
<td>3.290</td>
<td>41.9</td>
<td>29.0</td>
</tr>
<tr>
<td>13</td>
<td>3.232</td>
<td>26.2</td>
<td>18.2</td>
</tr>
<tr>
<td>14</td>
<td><strong>3.223</strong></td>
<td>16.1</td>
<td><strong>Mean 19.1</strong></td>
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<tr>
<td></td>
<td><strong>SD±5.7</strong></td>
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<tr>
<td>15</td>
<td>3.500</td>
<td>25.5</td>
<td>17.7</td>
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<tr>
<td>16</td>
<td>3.512</td>
<td>22.4</td>
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<td>17</td>
<td>3.561</td>
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<td>3.885</td>
<td>37.0</td>
<td>25.6</td>
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<td>19</td>
<td><strong>4.104</strong></td>
<td>26.0</td>
<td><strong>Mean 21.4</strong></td>
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<td></td>
<td><strong>3.712</strong></td>
<td></td>
<td><strong>SD±7.2</strong></td>
</tr>
</tbody>
</table>

**TABLE I. Chloramphenicol Half-lives in Newborn Infants**
FIGURE 1.

A = Mean half-lives of total nitro compounds for infants 6-18 hours after intramuscular administration of chloramphenicol succinate in a single dose of 50 mg per kilogram.

B = Variations to 1 standard deviation.
TABLE II. Chloramphenicol Plasma Levels (micrograms per milliliter) in Newborn Infants following Doses of 25mg/kg/24hrs. in Group I, and 50mg/kg/24hrs. in Group II.

<table>
<thead>
<tr>
<th>Weight (kg)</th>
<th>Dosage mg/kg/24hrs.</th>
<th>Day 1 (6 hrs.)</th>
<th>Day 3 (54 hrs.)</th>
<th>Day 5 (102 hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.707</td>
<td>23.4</td>
<td>29.5</td>
<td>37.7</td>
<td>16.7</td>
</tr>
<tr>
<td>2.205</td>
<td>22.7</td>
<td>29.0</td>
<td>24.5</td>
<td>4.7</td>
</tr>
<tr>
<td>2.290</td>
<td>21.8</td>
<td>17.1</td>
<td>34.2</td>
<td>51.3</td>
</tr>
<tr>
<td>2.245</td>
<td>26.7</td>
<td>19.0</td>
<td>32.7</td>
<td>33.1</td>
</tr>
<tr>
<td>Mean</td>
<td>23.8</td>
<td>Mean 32.3</td>
<td>Mean 26.4</td>
<td></td>
</tr>
<tr>
<td>SD±6.3</td>
<td></td>
<td>SD±5.8</td>
<td>SD±20.2</td>
<td></td>
</tr>
<tr>
<td><strong>Group II</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2.760</td>
<td>50.7</td>
<td>----</td>
<td>57.8</td>
<td>59.0</td>
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<td>2.915</td>
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<td>28.1</td>
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<td>3.130</td>
<td>47.9</td>
<td>76.8</td>
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<td>43.1</td>
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<td>72.5</td>
<td>128.9</td>
<td>64.6</td>
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<td>3.225</td>
<td>49.6</td>
<td>50.7</td>
<td>58.1</td>
<td>36.5</td>
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<td>3.339</td>
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<td>57.1</td>
<td>99.3</td>
<td>46.6</td>
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<td>4.907</td>
<td>48.9</td>
<td>58.1</td>
<td>51.1</td>
<td>54.0</td>
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<tr>
<td>Mean</td>
<td>51.9</td>
<td>Mean 62.2</td>
<td>Mean 43.4</td>
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</tr>
<tr>
<td>SD±19.6</td>
<td></td>
<td>SD±33.3</td>
<td>SD±14.7</td>
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</tr>
</tbody>
</table>
FIGURE 2a. Chloramphenicol plasma levels for infants weighing less than 2500 grams following repeated injections of chloramphenicol succinate in doses of 25 mg per kilogram per 24 hours. Dotted lines represent variations to 1 standard deviation.

FIGURE 2b. Chloramphenicol plasma levels for infants weighing more than 2500 grams following repeated injections of chloramphenicol succinate in doses of 50 mg per kilogram per 24 hours. Dotted lines represent variations to 1 standard deviation.
METABOLIC DISPOSITION OF CHLORAMPHENICOL

It is apparent that several functions are quantitatively less well developed in the infant as compared to the adult. These include reduced glomerular filtration and tubular secretion as well as a number of enzymatic reactions. Glazko et al. (38) reported that chloramphenicol was inactivated through conjugation with glucuronic acid. Brown and Zuelzer (39,40) later showed that the fetal liver at mid-gestation possesses no glucuronide conjugating activity. The newborn liver has about one-half the activity of the adult, but by 15-20 days of life the enzyme systems are comparable to the adult. They speculated that this inability to form glucuronide may have resulted in the formation of an alternate and more toxic metabolic product.

The deficiency in the newborn liver involved two enzymatic steps of the glucuronide conjugation pathway. (41) Uridine diphosphate glucuronic dehydrogenase and glucuronyl transferase were deficient resulting in absence of the uridine diphosphoglucuronic acid (UDPGA). The UDPGA, combining with the agent to be detoxified (bilirubin in these experiments), results in the bilirubin glucuronide, or the direct reacting bilirubin.

Billing (42) demonstrated that the capacity to conjugate bilirubin with glucuronic acid is not entirely confined to the
liver and suggested that the formation of the monoglucuronide may be effected in the kidney. In vitro studies showed that the kidney has as high as one-fourth the conjugating activity of the liver.

The amount of a dose of chloramphenicol recovered in twenty-four-hour urine approaches 90% in the adult human. Only 5-15% of this is microbiologically active or free chloramphenicol and about 90% is excreted as inactive nitro compounds, mainly the glucuronide. The remainder of the dose excreted includes a small amount of products of hydrolysis, less than 3% excreted as aryl amines into the bile, and less than 1% excreted into the feces as aryl nitro and amine derivatives. These mechanisms of degradation of the chloramphenicol molecule occur at the sites illustrated:

To determine the potential toxicity of chloramphenicol and its metabolic products, the glucuronide was given in large amounts to rats without side effects. Indirect studies on other metabolites also failed to produce toxicity.
indicating that the toxicity of chloramphenicol in newborns was probably due to the free antibiotic.

In infants the amount of free chloramphenicol excreted in the urine is similarly 5-10% but the excretion of the glucuronide is considerably less than normal (50% of the dose). There is also a higher proportion of the glucuronide in the blood of newborns in addition to high, prolonged levels of free chloramphenicol. This appears to be the result of both poor renal function and a deficient glucuronide conjugating system.

Analogous studies to those in the newborn were performed on adult patients with hepatic cirrhosis which revealed that the half-life for active chloramphenicol was markedly prolonged. However, the defect was in the rate of conversion to the glucuronide not the total amount converted. In patients with acute renal failure the half-life for active chloramphenicol was not prolonged but high levels of the chloramphenicol glucuronide were found in the blood. No toxicity occurred. This prolonged serum half-life and accumulation of metabolic products in these anuric patients indicates that removal other than by renal mechanisms is slow (less than 1% per hour).(43) The glucuronide conjugate is excreted by renal tubular secretion while the free drug is excreted by glomerular filtration.(36)
Experimental work with creatinine excretion suggests that the tubules of newborn infants have not yet developed the ability to excrete this substance. (45) Driscoll reported infant glomerular filtration rates for inulin, mannitol and creatinine to be 30 to 50 per cent of adult levels. The clearances of para-aminohippurate and iodopyrce were 20 to 40 per cent those in the adult. (46)

DISCUSSION

Following the numerous reports of the "gray syndrome" in 1951 to 1959, the physiologic explanation of chloramphenicol toxicity was based on the excessive doses used between 1951 and 1959 together with reduced glucuronide conjugation and impaired renal excretion. This resulted in toxic blood levels above 100 micrograms per milliliter. This potential toxicity was an important consideration until the infant became approximately three weeks of age. The conclusions of these authors were that the free drug was the toxic component, and although the mechanism of action was unknown, the toxic reactions were related to the impaired glucuronide conjugation and impaired renal excretion.

These conclusions do not satisfy the question why the excessive concentration of the chloramphenicol molecule results in the clinical picture of cardiovascular collapse. Further research in the literature revealed that Japanese
writers in 1952 studied the effects of various antibiotics on blood pressure and respiration in the rabbit. Chloramphenicol was the most potent drug. Large doses produced slight to moderate decreases in blood pressure, bradycardia, and decreased amplitude of respiration which did not parallel the dosages used. The blood pressure drop was believed to be peripheral in origin and chiefly responsible for the cardiac action. They also noted that chloramphenicol in concentration of 1:5000 to 1:4000 stimulated a segment of frog intestine but in concentrations of 1:10,000 produced inhibition only.

Swain also found chloramphenicol the most potent cardiac depressant as compared with other antibiotics. Using a dog heart-lung preparation he induced heart failure with a 100 to 200 mg dose of chloramphenicol. The failure was slowly reversed by ouabain and promptly reversed by 100 mg of calcium chloride. Successive episodes of heart failure required smaller and smaller doses of chloramphenicol and progressively larger doses of calcium chloride to reverse it. The drug's cardiac depressant actions were found to be indistinguishable from pentobarbital, and on a molar basis chloramphenicol was more potent.

A technique for titrating calcium ions was developed to determine if the agents bound the ion. By this method Swain concluded that chloramphenicol acted by removing ionized cal-
cium from the environment of the heart rather than binding
the calcium ion, because calcium chloride would not completely
reverse several consecutive episodes of heart failure. Al-
though the experiment is significant, his conclusions do not
consider that there may be other mechanisms for the cardiac
failure.

Kretchmer speculated that the toxicity in infants could
be explained by the action of chloramphenicol on protein syn-
thesis. Chloramphenicol is a selective inhibitor of pro-
ten synthesis. The RNA formed in the presence of chloram-
phenicol is abnormal being unstable following the drugs re-
moval. The hypothesis is that chloramphenicol blocks the
conversion of the unstable RNA polymer to a stable ribonucleo-
protein.

Roblin has shown that the antibacterial action of
small concentrations of chloramphenicol toward Escherichia
coli is reversed by phenylalanine which has a similar struc-
ture. This appears to be a non-competitive inhibition between
the chloramphenicol and phenylalanine molecules.
Although antibiotics are not generally believed to function as metabolic antagonists, it could be speculated that the chloramphenicol molecule might have an inhibitory effect on the synthesis of catecholamines since tyrosine, the initial step in the pathway to epinephrine, is formed from phenylalanine. The structures of chloramphenicol and epinephrine are also similar.

This possibility seems more likely as a cause for cardiovascular collapse when it is recalled that the opposite effects of catecholamines were obtained with the heart-lung preparation, frog intestine and rabbit experiments. Disturbed carbohydrate metabolism with fasting hypoglycemia was noted as well as the clinical course of the infants affected.

SUMMARY

Chloramphenicol toxicity in the newborn, i.e. the "gray syndrome" is described and the findings of the various workers are presented. Two case reports describe the typical onset of symptoms and the insidious way in which this toxicity manifests itself.
As a result of the increased mortality rates in newborn nurseries several series of these deaths were collected and animal studies were obtained to compare the tolerance of newborn animals to adult animals.

Blood level studies by Parke, Davis and Company are presented and the different forms of the antibiotic are considered. Additional unpublished data on chloramphenicol succinate is also presented.

Emphasis is placed on the metabolic disposition of chloramphenicol because of the development of hepatic and renal function in the infant.

Because the data obtained did not adequately explain why cardiovascular collapse occurred in these infants, a discussion of the effects of chloramphenicol on the cardiovascular system and speculation of the possible mechanisms involved are presented.

CONCLUSIONS

Chloramphenicol is toxic to premature infants and newborns up to three weeks of age when blood levels exceed 100 micrograms per milliliter. This toxicity is manifested by circulatory collapse and termed the "gray syndrome". Effective blood levels of 10 micrograms per milliliter are usually obtained with doses of 25 mg per kilogram per day for premature and newborn infants. Full term infants may receive from 25 to
50 mg per kilogram per day, but the data suggests that full term infants probably do not handle the drug any more effectively than the prematures. Most of the deaths occurred following doses of 100 to 150 mg per kilogram per day, although the experiments show that toxic levels may be reached with 50 mg per kilogram.

The mechanism of this toxicity is unknown but the evidence in the literature indicates that it is related to reduced detoxication by the glucuronide conjugation pathway and poor renal excretion resulting in abnormally high blood levels of the free antibiotic.

An attempt to explain why this toxicity is manifested by cardiovascular collapse resulted in speculation by several authors. Chloramphenicol produced a decrease in blood pressure and respiration in rabbits which was thought to be peripheral in origin. It produced cardiac failure in a dog heart-lung preparation which was thought to occur as a result of a decrease in calcium ion available to the myocardium.

There is some evidence that chloramphenicol and phenylalanine are antagonistic in biological systems. Speculation along these lines includes the possibility that chloramphenicol may exert some antagonism to the synthesis of catecholamines in the pathway from phenylalanine to epinephrine. This hypothesis could perhaps be supported with animal experiments.
which include the determination of urinary catecholamines
during chloramphenicol administration. There is no indication
in the literature that this has been done. Further work
utilizing data from Swain's experiments may aid in identifying
the cause of the "gray syndrome".

ACKNOWLEDGEMENT

I am indebted to Dr. H. E. Wiltse for permitting me to
use his experimental data and also for his suggestions and
kind assistance in the preparation of this paper.
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