However, 2-aminoethanethiosulfuric acid has good protective ability (2), whereas the cyanomethanethiosulfuric acid has none (19). It may be more significant that the thiosulfates can stabilize Cu(I) ion by complex formation; it has been proposed that one mechanism of radiation protection involves stabilization of the valence state of copper in copper-containing enzymes (20) or other macromolecules (21).

REFERENCES

(1) B. Holmberg and B. Sorbo, *Nature*, **183,** 832(1959).

(2) D. L. Klayman, M. M. Grenan, and D. P. Jacobus, *J. Med. Chem.*, **13,** 251(1970).

(3) W. O. Foye and J. Mickles, in "Progress in Biochemical Pharmacology," vol. 1, Butterworths, Washington, D. C., 1965, p. 152.

(4) W. O. Foye, *J. Pharm. Sci.*, **58,** 283(1969).

(5) L. G. Sillen and A. E. Martell, "Stability Constants of Metal-Ion Complexes," The Chemical Society, London, England, 1964, p. 224.

(6) W. O. Foye and M. C. M. Solis, *J. Pharm. Sci.*, **58,** 352(1969).

(7) W. O. Foye and J. M. Kauffman, *ibid.*, **57,** 1614(1968).

(8) A. Kaluszyner, P. Czerniak, and E. D. Bergmann, *Radiat. Res.*, **14,** 23(1961).

(9) D. M. G. Armstrong, *Chem. Ind.*, **1955,** 1405.

(10) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Wiley, New York, N. Y., 1962.

(11) H. Flood and V. Loras, *Tidsskr. Kjemi Bergv. Met.*, **5,** 83 (1945).

(12) A. Albert, *Biochem. J.*, **47,** 531(1950).

(13) *Ibid.*, **54,** 646(1953).

(14) B. C. Haldar and S. Banerjee, *Proc. Nat. Inst. Sci. India*, **14,** 1(1948).

(15) S. E. Livingston, *Quart. Rev.*, **19,** 386(1965).

(16) D. I. Ryabchikov, *Omagiu Raluca Ripan*, **1966,** 485; through *Chem. Abstr.*, **67,** 39696(1967).

(17) M. J. Janssen, *Rec. Trav. Chim.*, **75,** 1411(1956).

(18) E. Gonick, Ph.D. thesis, Pennsylvania State University, University Park, Pa., 1951.

(19) D. P. Jacobus and T. R. Sweeney, private communication.

(20) J. Schubert, *Radiat. Res.*, **14,** 499(1961).

(21) M. Anbar, *Nature*, **200,** 376(1963).

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Serum Levels of Chloramphenicol in Children, Rhesus Monkeys, and Cats after Administration of Chloramphenicol Palmitate Suspension

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Abstract □ The absorption of amorphous and polymorph A forms of chloramphenicol palmitate suspensions made with polysorbate 80 was investigated in children, rhesus monkeys, and cats. In children, both forms were absorbed. While polymorph A was absorbed at a slower rate, maintaining the blood level of chloramphenicol for 6 hr., the amorphous form was absorbed maximally at 2 hr. followed by a profound fall in the blood level. Although the absorption of the amorphous form was definitely superior, the polymorph A form in suspension with a surface-active agent was also absorbed to a considerable extent in children, maintaining blood levels of chloramphenicol for a prolonged period. In experiments with cats, polysorbate 80 increased the absorption of both forms of chloramphenicol palmitate, with the amorphous variety having superior absorption. In rhesus monkeys, there was little absorption of the polymorph A form of chloramphenicol palmitate in suspension, possibly indicating the absorption as species specific.

Keyphrases □ Chloramphenicol serum levels—following chloramphenicol palmitate suspension administration, in children, rhesus monkeys, cats □ Polysorbate 80—effects on absorption of chloramphenicol palmitate (amorphous and polymorph A forms), in cats □ Serum levels, chloramphenicol, amorphous *versus* polymorph A forms—comparison in children, rhesus monkeys, cats

Three polymorphic forms of chloramphenicol palmitate have been described; two are crystalline, termed α and β, and one is amorphous. In the solid state, transition occurs from α to β and is irreversible (1). These crystalline forms differ in their physicochemical properties (2). The IR spectrum of the amorphous form is identical to that of the α-type of crystals; but that of the β-type crystals, also termed polymorph A, is different. By using a relative absorbancy ratio of 862–864 $cm.^{-1}$, characteristic bands for α- and β-type crystals and the relative amounts of both types in a mixture can be determined (3).

Contradictory reports appear about the absorption of the different forms of chloramphenicol palmitate or stearate. Altmann *et al.* (4) determined blood levels of chloramphenicol in human adults after feeding chloramphenicol and chloramphenicol palmitate, as large or small crystals, and chloramphenicol palmitate incorporated with surface-active agents. They observed that both the administration of chloramphenicol and chloramphenicol palmitate with surface-active agents yielded the highest blood levels of chloramphenicol, whereas the blood levels following chloramphenicol palmitate as large crystals were definitely lower for the first 4 hr. There was no appreciable difference in the blood levels of chloramphenicol at 6- and 8-hr. intervals following administration of chloramphenicol and the various preparations of chloramphenicol palmitate.

Table I—Serum chloramphenicol (mcg./100 ml.) of Children and Rhesus Monkeys Administered Suspensions of Chloramphenicol Palmitate Equivalent to 250 mg. Chloramphenicol

Chloramphenicol Palmitate Suspension Used[a]	Hours after Feeding Chloramphenicol Palmitate Suspension			
	2	4	6	8
Children				
Amorphous, 100%	1018 ± 127	604 ± 39	417 ± 43	263 ± 16
Range of values	793–1607	429–776	258–518	240–296
	(7)	(7)	(7)	(4)
Polymorph A, 100%	344 ± 22	347 ± 56	567 ± 66	234 ± 24
Range of values	263–481	207–484	422–762	180–337
	(10)	(4)	(7)	(7)
Rhesus Monkeys				
Amorphous, 100%	675 ± 120	389 ± 83	183 ± 12	
Range of values	454–1243	245–806	132–227	
	(7)	(7)	(7)	
Polymorph A, 100%	223 ± 38	173 ± 21	170 ± 44	
Range of values	108–394	108–276	108–282	
	(7)	(7)	(7)	

[a] Values = mean ± standard error. Figures in parenthesis indicate the number of subjects or animals.

Menachenoff (5) observed that crystals of chloramphenicol palmitate that could be hydrolyzed within 45 min. were absorbed to give blood levels comparable with chloramphenicol *per se* at equivalent dosage. Dony and de Roeck (6) reported that certain suspensions where the palmitate appeared under the microscope as fine needles or submicro crystals were hydrolyzed completely by pancreatin in 4 hr. and were well absorbed by the rabbit. In experiments with rats, Almirante *et al.* (7) observed that only the amorphous form of chloramphenicol stearate was hydrolyzed in the organism, liberating the antibiotic, whereas the crystalline form was therapeutically inert. They also reported that blood levels obtained in man after administration of a syrup containing chloramphenicol stearate with polysorbate 80 as emulsifier were equal to those obtained with a tablet of chloramphenicol. Aguiar *et al.* (8) reported that suspensions containing only α or amorphous chloramphenicol palmitate gave higher blood levels in adult male human volunteers following a single oral dose than did suspensions containing only β crystals of chloramphenicol palmitate (termed polymorph A). They did not find any influence of surface-active agents on the absorption of chloramphenicol palmitate. They observed that increasing the particle size of chloramphenicol palmitate to approximately 25 μ had little effect on the blood levels. According to the U. S. Food and Drug Administration Regulations (9), the limit of polymorph A (β-crystals) in chloramphenicol palmitate suspension should not exceed 10%.

In view of the conflicting reports about the absorption of chloramphenicol palmitate and in the absence of any report on such studies in children where palmitate suspensions are ordinarily used due to the absence of the bitter taste of chloramphenicol, this study was conducted on the absorption of amorphous and polymorph A forms of chloramphenicol palmitate in children. Comparative studies were also undertaken in rhesus monkeys and cats.

EXPERIMENTAL

Materials—Amorphous chloramphenicol palmitate was prepared from commercial chloramphenicol palmitate (10). Polymorph A chloramphenicol palmitate was prepared by dissolving commercial chloramphenicol palmitate in benzene and recrystallizing in the cold condition (11). The purity of the substance was determined by IR spectrophotometry. Suspensions of both 100% amorphous and 100% polymorph A forms of chloramphenicol palmitate were prepared using polysorbate 80 at the 2% level as the dispersing agent. The particle-size ranges of the various drug lots were 5–10 μ.

Children—Suspensions of chloramphenicol palmitate, equivalent to 250 mg. chloramphenicol, were administered orally to children aged 5–7 years in the basal condition. Blood samples were collected at 2-hr. intervals for 8 hr. after the administration of the suspension.

Rhesus Monkeys—Rhesus monkeys of 5 kg. average weight were fed suspensions of chloramphenicol palmitate equivalent to 250 mg. chloramphenicol in the early hours of the morning after a fast for 16 hr. Blood samples were withdrawn from the femoral vein at intervals of 2 hr. for 6 hr. after the administration of chloramphenicol.

Cats—Six cats, weighing 2.5–3 kg., were anesthetized by intramuscular injection of sodium phenobarbital, 150 mg./kg., to facilitate the feeding of the drug and the collection of blood samples. Through a stomach tube they were fed chloramphenicol, chloramphenicol palmitate amorphous or polymorph A, and suspensions of amorphous or polymorph A forms of chloramphenicol palmitate prepared with polysorbate 80 at the 2% level. The dose of chloramphenicol or chloramphenicol palmitate was equivalent to 42 mg. chloramphenicol/kg. body weight. Blood samples were withdrawn from the femoral vein at intervals of 2 hr. for 6 hr. after drug administration.

Serum of the blood samples was used for the estimation of chloramphenicol (12).

RESULTS

Children—The rise in the blood levels of chloramphenicol after administration of suspensions containing the polymorph A form of chloramphenicol palmitate was much less at 2 and 4 hr. as compared to blood levels with amorphous palmitate suspension. At 6 hr. the blood level of chloramphenicol was higher with the polymorph A suspension. At 8 hr. there was no difference between the blood levels with either form of chloramphenicol palmitate. The results are shown in Table I.

Monkeys—There was little rise in the blood levels of chloramphenicol when suspensions of polymorph A chloramphenicol palmitate were fed to monkeys (Table I).

Cats—The rise in the blood levels of chloramphenicol was less marked and of comparable degree after the feeding of raw amorphous or polymorph A forms of chloramphenicol palmitate. Suspensions of these preparations with polysorbate 80 produced higher blood levels of chloramphenicol. Suspensions of amorphous chloramphenicol palmitate produced blood levels of chloramphenicol much higher than those produced by either suspension of polymorph A chloramphenicol palmitate or chloramphenicol itself. The results are given in Fig. 1.

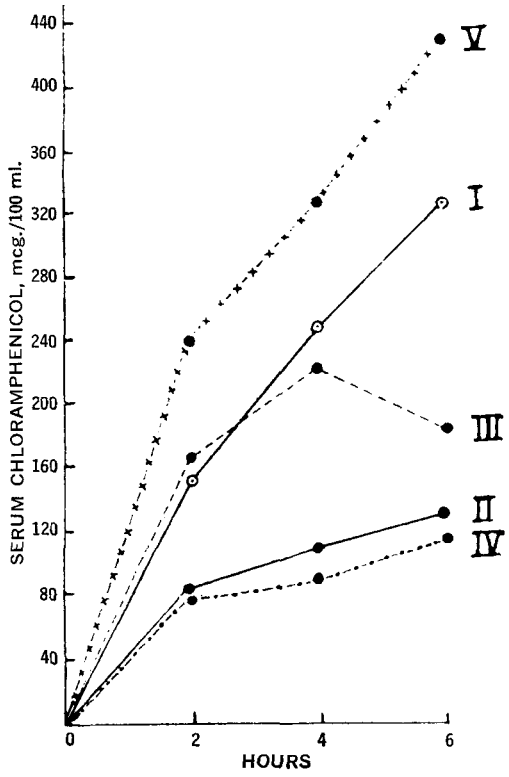

Figure 1—*Serum chloramphenicol after administration of chloramphenicol or its equivalent of chloramphenicol palmitate in cats. Key: I, animals fed chloramphenicol; II, animals fed chloramphenicol palmitate, polymorph A crystals; III, animals fed chloramphenicol palmitate, polymorph A suspension with polysorbate 80; IV, animals fed chloramphenicol palmitate, amorphous; and V, animals fed chloramphenicol palmitate, amorphous suspension with polysorbate 80.*

DISCUSSION

There were biological variations in the individual blood levels of chloramphenicol in both children and animals studied. But these variations were not inordinately high or low, as indicated by the standard error of the mean values and the range of values given in Table I.

The presence of chloramphenicol in blood of children after administration of suspensions of either amorphous or polymorph A varieties of chloramphenicol palmitate indicated that both these forms were absorbed. With polymorph A the rise in the blood chloramphenicol was gradual, and the maximum was reached 6 hr. after administration. With the amorphous suspension the rise was maximum at 2 hr. followed by a gradual decline. Due to practical difficulties in the collection of urine from children, urinary excretion of chloramphenicol could not be studied. This would have furnished further information about the absorbability of different forms of chloramphenicol.

Experiments with cats indicated that surface-active agents had a profound influence on the absorption of different forms of chloramphenicol palmitate. While the amorphous form was superior to crystalline polymorph A in raising the blood level of chloramphenicol, the latter was absorbed considerably when administered with a surface-active agent such as polysorbate 80.

Observations with children and cats were contrary to those of Aguiar *et al.* (8) in adult human males. Possibly the mechanism of absorption of chloramphenicol palmitate was different for adult males (8) and for the adult rhesus monkeys in this study where chloramphenicol palmitate polymorph A crystal administration with surface-active agents resulted in little absorption.

REFERENCES

(1) C. Tamura and H. Kuwano, *Yakugaku Zasshi*, **81,** 755 (1961); through *Chem. Abstr.*, **55,** 25417(1961).

(2) H. Negoro, S. Ueda, T. Suyama, and T. Hoshi, *Takamine Kenkyusho Nempo*, **12,** 141(1960); through *Chem. Abstr.*, **55,** 8765 (1961).

(3) M. Maruyama, N. Hayashi, and M. Kishi, *ibid.*, **13,** 176 (1961); through *Chem. Abstr.*, **56,** 11708(1962).

(4) S. Altmann, B. Bogokovsky, H. Boichis, and B. Cohen, *Harokeach Haivri*, **9,** 745(1963); through *Chem. Abstr.*, **61,** 1127 (1964).

(5) E. Menachenoff, *ibid.*, **10,** 300(1964); through *Chem. Abstr.*, **63,** 6180(1965).

(6) J. Dony and A. de Roeck, *J. Pharm. Belg.*, **20,** 475(1965); through *Chem. Abstr.*, **65,** 1230(1966).

(7) L. Almirante, I. DeCarneri, and G. Coppi, *Farmaco (Pavia) Ed. Pract.*, **15,** 471(1960); through *Chem. Abstr.*, **55,** 905(1961).

(8) A. J. Aguiar, J. Krc, Jr., A. W. Kinkel, and J. C. Samyn, *J. Pharm. Sci.*, **56,** 847(1967).

(9) "Regulations for Tests and Method of Assay of Antibiotic Drugs," U. S. Food and Drug Administration, 1965, part 141 d, p. 306.

(10) T. Kimura and S. Hashimoto, Japanese pat. 5798(1960); through *Chem. Abstr.*, **55,** 5878(1961).

(11) C. M. Anderson, *Australas. J. Pharm.*, **45,** 544(1966).

(12) D. C. Grove and W. A. Randall, "Assay Methods of Antibiotics, a Laboratory Manual," Medical Encyclopedia, New York, N. Y., 1955, p. 74.

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