18. Miller, S. E., Textbook of Clinical Pathology, Williams & Wilkins Co., Baltimore, 1955.

19. Rowley, D. A., J. Exp. Path., 1963, v44, 284.

20. Shih, H. E., Jung, T. S., Proc. Soc. Exp. Biol. and Med., 1931, v29, 243.

21. Levin, J., Beck, E., to be published.

22. Fine, J., Rutenberg, S., Schweinburg, F. B., J. Exp. Med., 1959, v110, 547.

23. McCluskey, R. T., Zweifach, B. W., Antopol, W., Benacerraf, B., Nazler, A. L., Am. J. Path., 1960, v37, 245.

24. Rich, A. R., Bull Johns Hopkins Hosp., 1944, v74, 1.

25. Smith, R. T., Thomas, L., Proc. Soc. Exp. Biol. and Med., 1954, v86, 806.

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## Cobalt Salts in Acute Cyanide Poisoning.* (30652)

CHARLES L. ROSE, ROBERT M. WORTH, KENZO KIKUCHI AND K. K. CHEN
*Department of Pharmacology, Indiana University School of Medicine, Indianapolis*

Antal, in 1894(2), advocated the use of cobalt nitrate for the treatment of cyanide poisoning after his favorable experience in rabbits and dogs. Confirmatory results were published by Meurice(3) on pigeons and rabbits. Based on the same concept of precipitation, Martin and O'Brien(4) employed cobalt chloride to convert potassium cyanide into insoluble cobalt cyanide in the stomach of rabbits. Rozhkov *et al*(5) demonstrated the prophylactic and therapeutic effect of cobalt nitrate, chloride, and sulfate in mice poisoned with sodium cyanide. The mortality dropped from 83-85% to 7-11%. After Mushett *et al*(6) showed that hydroxo-cobalamin alleviated the toxic signs of mice due to KCN injection by conversion of the hydroxo group to a cyano group bound to the cobalt atom, a renewal of interest in studying the antidotal action of various cobalt salts against cyanide poisoning occurred. Paulet (7,8) showed that cobalt gluconate or glutamate could protect the dog anesthetized with chloralose from 5 lethal doses of NaCN. Mercker and Bastian(9) claimed that cobalt histidine and dicobalt ethylenediamine-tetraacetic acid ($Co_2EDTA$) offered advantages in treatment of experimental cyanide poisoning. Recent work from the same laboratory by Grützmacher and Friedberg(10) appeared to indicate that cobalt desferrioxamine was even more suitable for the detoxification of NaCN in guinea pigs. $Co_2EDTA$ was also investigated by Terzic and Milosevic(11) and Evans(12). Another compound assessed in mice was sodium cobaltinitrite by Goldenberg and Mann(13).

Since 1933 we have been interested in cyanide antidotes(14). Our suggestion of nitrite-thiosulfate has resulted in saving of human lives(15). Any new proposal such as cobalt therapy prompted us to examine its merit, because cyanide poisoning is so rapidly fatal that an improvement in the treatment will benefit humanity.

*Methods.* Twenty-seven mongrel dogs were procured from the same source. Their average weight was 11 kg. Each animal was starved for 16 hours before the day of experimentation. Freshly prepared solutions of NaCN, 1-10%, were injected subcutaneously, immediately followed by the i.v. injections of cobalt salts or the chelate, $Co_2EDTA$. Cobalt chloride, $CoCl_2$, was used in 5% solution and sodium cobaltinitrite, $Na_3Co(NO_2)_6$, 10%. Our $Co_2EDTA$ was prepared, with the assistance of Aldrich Chemical Co., Milwaukee, by titration of $Na_2CoEDTA$ with a stoichiometric amount of cobalt chloride in an aqueous solution using eriochrome indicator for the end point. A 5.9% solution was employed throughout. Two dogs, non-anesthetized, were given i.v. injection of $Co_2EDTA$ without NaCN to test for any untoward effects. Four additional dogs were anesthetized with secobarbital sodium, 25 mg/kg i.v., and their respiratory movements, carotid

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TABLE I. Action of Cobalt Compounds in Non-anesthetized Dogs Poisoned with NaCN.

| Dog No. | Sex | Wt (kg) | NaCN (mg/kg) | Cobalt compound (mg/kg) | Results |
|---|---|---|---|---|---|
| | | | | $Na_3Co(NO_2)_6$ | |
| 1 | ♂ | 8.8 | 6 | 30 | recovery in 23 hr |
| 2 | ♂ | 7.3 | 12 | 66 | " " 22 " 45 min. |
| 3 | ♂ | 8.9 | 18 | 60 | " " 22 " 24 " |
| 4 | ♂ | 7.4 | 24 | 84 | death in 6 hr 30 min. |
| 5 | ♂ | 8.1 | 24 | 84 | " " 6 " 26 " |
| 6 | ♀ | 7.6 | 24 | 42 | " " 1 " 24 " |
| 7 | ♂ | 8.9 | 30 | 45 | " " 2 " 10 " |
| | | | | $CoCl_2$ | |
| 8 | ♀ | 10.7 | 12 | 54 | death in 2 hr 55 min. |
| 9 | ♀ | 5.8 | 12 | 15 | " " 40 min. |
| 10 | ♂ | 13.8 | 12 | 51 | " " 17 hr 55 min. |
| 11 | ♂ | 13.5 | 18 | 50 | " " 3 " 59 " |
| | | | | $Co_2EDTA$ | |
| 12 | ♂ | 13.6 | 7 | 40 | recovery in 7 hr 20 min. |
| 13 | ♂ | 10.9 | 7 | 40 | " " 6 " 57 " |
| 14 | ♂ | 10.0 | 7 | 20 | death in 1 hr 41 min. |
| 15 | ♂ | 16.8 | 9 | 40 | recovery in 6 hr 45 min. |
| 16 | ♂ | 13.6 | 12 | 40 | " " 6 " 32 " |
| 17 | ♀ | 12.4 | 12 | 80 | death in 7 hr 17 min. |
| 18 | ♀ | 12.6 | 18 | 40 | " " 2 " 55 " |
| 19 | ♀ | 12.3 | 18 | 40 | " " 2 " 5 " |
| 20 | ♀ | 12.0 | 24 | 80 | " " 4 " 15 " |
| 21 | ♀ | 13.4 | 24 | 80 | " " 2 " 8 " |

blood pressure and electrocardiograms were recorded with a Physiograph.

*Results.* The data on the non-anesthetized dogs poisoned with NaCN and treated with cobalt compounds are listed in Table I. Sodium cobaltinitrite saved the lives of 3 dogs receiving 6-18 mg/kg of NaCN; it took almost 23 hours each. Three dogs on 24 mg/kg of NaCN died. Similarly the dog on the largest dose, 30 mg/kg, did not survive the poison. The dose of sodium cobaltinitrite was given either singly in 30 mg/kg or divided into fractions. In our previous paper we found(16) that a dose of 6 mg/kg of NaCN was lethal to all 5 dogs. It thus appears that sodium cobaltinitrite is capable of detoxifying 3 LD's but not 4 or 5 LD's.

Cobalt chloride in a single dose of 15 mg/kg or in divided doses was unable to antidote 2-3 LD's of NaCN. All 4 dogs died. It is not certain that this ionizable cobalt salt can detoxify one LD of NaCN.

$Co_2EDTA$ has a demonstrable value of antagonizing the toxic effects of NaCN. The initial i.v. dose was either 20 or 40 mg/kg and the subsequent doses were multiples of 20 mg/kg. Two out of 3 dogs receiving 7 mg/kg of NaCN recovered. One out of 2 animals on 2 LD's survived. A single dog on 1.5 LD also lived by this treatment. All 4 dogs, 2 on 3, and 2 on 4 LD's of NaCN, died in spite of an increase of the total dose of $Co_2EDTA$ for dogs numbered 20 and 21.

Of the 4 anesthetized dogs only 2 showed comparable results to those published by Paulet(8) and Mercker and Bastian(9). NaCN was infused at the rate of 0.1 mg/kg/min. As soon as the arterial pressure started to fall and the respiration almost stopped, cyanide infusion was discontinued and $Co_2$-EDTA, 8 mg/kg, was injected i.v. There was a slow rise of blood pressure and a resumption of respiration. The electrocardiogram did not show special changes except that the decreased rate returned to the initial level after $Co_2EDTA$. The remaining 2 anesthetized dogs did not respond favorably and died. The 2 non-anesthetized dogs, one receiving 20 mg/kg $Co_2EDTA$, and the other 40 mg/kg, vomited, defacated and became lethargic, a condition which lasted more than an hour, and made the animals obviously uncomfortable.

*Discussion.* Our criteria of observations

follow the pattern of Barcroft's work(17) and simulate clinical conditions. To assess the optimal effect of an antidote it is injected i.v. immediately after subcutaneous injection of NaCN. When a dog recovers, it stands up, walks, accepts food, and answers calls—normal in every respect. If the toxic signs persist, the antidote is repeated. By such a standard procedure, sodium cobaltinitrite is unequivocally effective in detoxifying cyanide ions. Dogs survive 1-3 LD's but not 4-5 LD's (Table I). In contrast with this salt, cobalt chloride is incapable of antagonizing 2-3 LD's. It is fully possible that the $NO_2^-$ portion of the molecule is responsible for detoxification of NaCN. There is evidence that $Co_2$EDTA may save dogs from 1-2 LD's of NaCN. The mechanism of cobalt action has been speculated upon by Paulet(8) and Bartelheimer *et al*(18). The toxicity of cobalt chelates has been discussed by Bartelheimer (19) and Tauberger and Klimmer(20). Some synergistic action of $Co_2$EDTA and sodium thiosulfate is demonstrated by Terzic and Milosevic(11) in rats, but the protection is limited to 5 LD's of KCN.

According to our experience, the combination of sodium nitrite and sodium thiosulfate is more effective than cobalt compounds in detoxifying NaCN in dogs(15,21). This animal is the most sensitive of the quadrupeds and more susceptible to CN ions than man, as proved by Barcroft(17). The nitrite-thiosulfate therapy is capable of detoxifying 20 LD's, namely, 20 by 6 mg/kg.

Any proposal for the treatment of acute cyanide poisoning from animal experimentation must be validated by clinical confirmation. One of us (K.K.C.) reviewed the first 50 human cases treated with the nitrite-thiosulfate therapy at a meeting in Japan(22). This has been followed by favorable reports of Done(23), Hirsch(24), Sayre and Kaymakçalan(25), and Dall and Hannah(26), totalling 73 cases with 72 recoveries. Evans (12) mentioned a human case of cyanide poisoning treated with $Co_2$EDTA, but stated, "the interpretation was not clear-cut, as other remedies were used as well." It appears that nitrite-thiosulfate is still the best antidote for the acute cyanide poisoning.

*Summary.* Three cobalt compounds have been tested in non-anesthetized dogs poisoned with subcutaneous injection of NaCN. Sodium cobaltinitrite i.v. detoxifies 3 LD's. Cobalt chloride does not seem to be effective. $Co_2$EDTA at its best antidotes 2 LD's.

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1. Rose, C. L., Chen, K. K., Tox. appl. Pharmacol., 1965, v7, 496.
2. Antal, J., Ungar. Arch. Med., 1894, v3, 111.
3. Meurice, J., Arch int. pharmacodyn., 1900, v7, 11.
4. Martin, C. J., O'Brien, R. A., Intercolon. Med. J. Austral., 1901, v6, 245.
5. Rozhkov, V. M., Stepanenko, N. S., Usova, K. M., J. Physiol. (USSR), 1935, v19, 582; Chem. Abst., 1936, v30, 11195.
6. Mushett, C. W., Kelley, K. L., Boxer, G. E., Richards, J. C., Proc. Soc. Exp. Biol. and Med., 1952, v81, 234.
7. Paulet, G., Compt. rend. soc. biol., 1957, v151, 1932.
8. Paulet, G., L'intoxication cyanhydrique, Masson & Cie, 1960, p62.
9. Mercker, H., Bastian, G., Arch. exp. Path. Pharmak., 1959, v236, 449.
10. Grützmacher, J., Friedberg, K. D., ibid., 1965, v250, 252.
11. Terzic, M., Milosevic, M., Therapie, 1963, v18, 55.
12. Evans, C. L., Brit. J. Pharmacol., 1964, v23, 455.
13. Goldenberg, M. M., Mann, D. E., Jr., J. Am. Pharmaceut. Sci., 1960, v49, 210.
14. Chen, K. K., Rose, C. L., Clowes, G. H. A., Proc. Soc. Exp. Biol. and Med., 1933, v31, 250.
15. Chen, K. K., Rose, C. L., J.A.M.A., 1952, v149, 113.
16. Rose, C. L., Worth, R. M., Chen, K. K., Life Sciences, 1965, v4, 1785.
17. Barcroft, J., J. Hyg., 1931, v31, 1.
18. Bartelheimer, E. W., Friedberg, K. D., Lendle, L., Arch. int. pharmacodyn., 1962, v139, 99.
19. Bartelheimer, E. W., Arch. exp. Path. Pharmak., 1962, v243, 254.
20. Tauberger, G., Klimmer, O. R., Arch. int. pharmacodyn., 1963, v143, 219.
21. Chen, K. K., Rose, C. L., Clowes, G. H. A., Am. J. Med. Sci., 1934, v188, 767.
22. Chen, K. K., Proc. 15th Jap. Med. Congress, 1959, v1, 363.

23. Done, A. K., Clin. Pharmacol. Therap., 1961, v2, 750.
24. Hirsch, F. G., Arch. Environ. Health, 1964, v8, 622.
25. Sayre, J. W., Kaymakçalan, S., N. Engl. J. Med., 1964, v270, 1113.
26. Dall, J. L. C., Hannah, W. M., Brit. Med. J., 1964, v2, 33.

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## An Indirect Fluorescent Antibody Technique Using Soluble Antigens for Serodiagnosis of *Trypanosoma cruzi* Infection. (30653)

ANDRE J. TOUSSAINT, CARL J. TARRANT AND ROBERT I. ANDERSON
(Introduced by Earl H. Fife, Jr.)
*Department of Serology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.*

Recently described(1) methodology for an indirect fluorescent antibody technic employing a soluble somatic protein schistosome antigen and an artificial matrix has certain advantages over the normally employed whole organism tests. The advantages include: 1) objective selection of the antigen; 2) precise fluorometric reading of test results; 3) elimination of fade-out of the reaction during reading of tests; and 4) correction of nonspecific fluorescence contributed by substances in the patient's serum (*e.g.*, therapeutic drugs) and/or by free fluorescein in the conjugated antiserum.

The present study was conducted to determine whether the soluble antigen fluorescent antibody (SAFA) technic could be employed for the serorecognition of infections with *Trypanosoma cruzi* and to ascertain if antigens other than somatic schistosome protein could be used with the artificial matrix.

*Materials and methods. Serum specimens.* Sera from 82 individuals who had clinical, and/or serological evidence of Chagas' disease were used to evaluate the sensitivity of the SAFA technic. The specificity of the procedure was determined with sera from 54 healthy persons and 10 patients with each of the following infectious diseases—syphilis, schistosomiasis, amoebiasis, malaria, trichinosis, and filariasis. In addition, 9 sera from patients with leishmaniasis were tested. The sera had been collected and preserved under various conditions: some were preserved with either merthiolate or sodium azide whereas others were collected and shipped under aseptic conditions without a preservative. Upon receipt in the laboratory, all sera were stored at —20°C. When required for tests the sera were thawed at room temperature, and tested without heating.

*Antigens.* Three antigens of *T. cruzi* (exo, somatic protein and somatic carbohydrate) were employed in initial studies. The exoantigen was prepared by published methods (2) and was a pH 4.6 soluble fraction of the dialyzed, organism-free supernates from cultures of *T. cruzi* grown inside cellulose sacs. The somatic protein and somatic carbohydrate antigens were separated by chloroform-gel fractionation(3) of the trypanosomes recovered from the same cultures used to produce the exoantigen. All 3 antigens had been previously studied(2,3) in a complement fixation test.

*SAFA technic.* Tests were performed as previously described(1). Briefly, the method consisted of incubating each serum (0.2 ml of a 1:2 dilution in Tris buffered saline) with a cellulose acetate filter paper disc (¼″ dia., 0.45 μ porosity) impregnated with test antigen optimally diluted in 1% bovine serum albumin (BSA). A control for each serum was tested concurrently with a 1% BSA impregnated disc. Following the incubation period (18 hours at 3°-6°C) the discs were washed in saline and incubated for 30 minutes at room temperature in an optimal dilution of fluorescein tagged antihuman globulin. The discs were again washed and