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# EXPERIMENTS WITH THE TRYPANOCIDAL COMPOUND "528" IN WEST AFRICA

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Studies have been made on the use of the chloride salt of "528" against cattle trypanosomiasis in Nigeria. Toxic effects, terminating in death, were produced in cattle receiving the drug at 5 mg./kg. and above. The maximum permissible dose for field use in Nigeria was found to be 2 mg./kg. The drug had an appreciable curative action against a syringe-transmitted strain of *T. congolense*, but had no curative effect against two strains of *T. vivax*. It is concluded that "528" would be of very limited value in the treatment of cattle in West Africa, where *T. vivax* is the more important cause of cattle trypanosomiasis.

The existence of activity against *Trypanosoma congolense* in cinnoline derivatives was first described by Keneford, Lourie, Morley, Simpson, Williamson, and Wright (1948). The synthesis of one of these compounds ("528"; $N^1:N^3$-bis(4′-aminocinnolyl-6′)-guanidine dimethiodide) was subsequently described by Lourie, Morley, Simpson, and Walker (1951). This substance had high activity against *T. congolense* infections in mice; it was approximately one half as toxic and one half as active as antrycide methylsulphate. It appeared from these preliminary observations that "528" was worthy of trial against cattle trypanosomiasis in Africa. The commonest species of trypanosome encountered in West African cattle is *T. vivax* (Unsworth, 1953), and it frequently causes death of the infected animals. For any drug to be considered of use for bovine trypanosomiasis in West Africa, it must possess high activity against both *T. vivax* and *T. congolense*.

## MATERIALS AND METHODS

*"528."*—The drug used was a sample of the chloride kindly supplied for trial by Dr. F. Hawking. The substance was dissolved in water at 80° C.; the orange-coloured solution was allowed to cool to room temperature and water added to adjust the concentration to 2%. When solutions of the drug were exposed to light, the colour darkened somewhat after two weeks, but no changes in toxicity or trypanocidal activity were detected in mice after one month. It was therefore concluded that solutions of the drug were reasonably stable.

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*Toxicity Test in Infected Cattle.*—Seventeen Zebu cattle infected a few days previously with *T. congolense* (Nigerian strain A or B) were injected subcutaneously with doses of "528" ranging from 36 mg./kg. to 1 mg./kg. The dose was given on one side of the neck, or, if the volume was large, on both sides of the neck or into the brisket. The animals were observed continuously for 2–3 hr., and then on regular occasions until nightfall. Survivors were retained for 2–3 months and grazed out of doors during the day in order to observe any delayed toxic effects of the kind observed with phenanthridine derivatives (Goodwin and Chandler, 1951). The rectal temperature was taken before and at intervals after giving the drug. This test also served to give an indication of the curative activity of the drug.

*Effect against* T. congolense.—Fourteen Zebu cattle were inoculated subcutaneously with mouse blood containing *T. congolense* (Nigerian strain B). This organism had been maintained for one year in laboratory mice. Trypanosomes were detected in the blood of the cattle 12–15 days after inoculation. On the 18th day of the infection, doses of drugs were given subcutaneously. One group of animals was given antrycide methylsulphate as a standard, and a further group was kept untreated as controls. Fresh blood films were examined daily, and the site of inoculation was observed for evidence of local reaction.

*Effect against* T. vivax.—Twelve Zebu cattle were inoculated subcutaneously with the "Toro" strain of *T. vivax*. This strain was originally isolated from a horse and had been maintained by serial passage in sheep. Just before the experiment began the strain was transmitted from a sheep through *Glossina palpalis* to a cow. Blood from this cow was used to inoculate the experimental cattle.

Trypanosomes appeared in the blood of all the animals 7 days after inoculation, and treatment was given on the 8th day. Injections of drugs were given subcutaneously to groups of three cattle. The cattle were kept in screened houses throughout the experiment, and blood films were examined daily for 6 weeks or longer. The site of injection of the drug was observed for local reaction.

A further experiment was made with a more recently isolated strain of *T. vivax*. This strain ("Bokkos") was passaged from the original bovine host by syringe into a sheep. Blood from the sheep was transferred to a cow, which was used as the donor of infected blood for the main experiment. (The donor was eventually treated with antrycide methylsulphate, and included in the results of the experiment.)

Eleven cattle were inoculated, and trypanosomes were detected in their blood 5 days later. Doses of drugs were given subcutaneously on the 9th day after infection. Six animals were given "528"; three, including the donor, were given antrycide methylsulphate; three were kept untreated as controls.

*Development of Drug Resistance.*—An attempt was made to produce a strain of *T. congolense* resistant to "528," using splenectomized mice. At each passage the infection was treated with a dose of drug sufficient to cause temporary disappearance of parasites from the blood. When the infection relapsed trypanosomes were transferred to clean, splenectomized mice. A total of 18 passages were made in this way, and the sensitivity of the trypanosome to the drug re-determined.

## RESULTS

### *Toxicity*

The local reaction at the site of injection of "528" was always comparatively mild and was similar to that caused by antrycide methylsulphate. A swelling, which was not much larger than the volume of drug given, remained for 3 or 4 days and then resolved slowly. The chief toxic effects observed immediately after injection were profuse salivation, muscular tremors, and incoordination. Animals often lay down and were unable to rise unassisted for 10 min. or more.

All animals given 8 mg./kg. or more died, the survival time varying from 12 hr. to 9 weeks. Of the five cattle which received 5 mg./kg. of "528," two died in 4–8 weeks. Doses of 3, 2, and 1 mg./kg. did not cause death; but the immediate toxic effects of 3 mg./kg. were serious enough to make the use of such doses undesirable in the field.

Post-mortem examination of the cattle which died showed the main lesion to be an acute nephritis similar to that produced by toxic doses of antrycide methylsulphate. Red streaks, due to masses of erythrocytes in the tubules, were visible macroscopically in both cortex and medulla. The glomeruli were packed with erythrocytes; the cells of the convoluted tubules showed karyolysis and many had ruptured into the lumen; a hyaline substance was present in many tubules. The collecting tubules of the medulla showed similar but less marked changes.

### *Curative Effect against* T. congolense

During the toxicity trials in infected cattle, it was observed that *T. congolense* disappeared from the blood of cattle injected with 1 mg./kg. of "528." The doses used in the subsequent experiment gave the following results:

1 mg./kg. or 0.5 mg./kg. of "528": Apparently cured the three cattle treated at each dose level.

0.25 mg./kg.: One animal was apparently cured; one showed a scanty infection (on one day only) four weeks after treatment; the third relapsed 14 days after treatment and died of trypanosomiasis seven weeks later.

0.5 mg./kg. of antrycide methylsulphate cured one of the two animals treated; the other relapsed four weeks after treatment. Untreated control animals showed regular but not heavy infections; one died after 8 weeks, one after 5 months, and the third was killed to provide histopathological specimens. It is clear that "528" is effective against this strain of syringe-transmitted *T. congolense* and has a wide margin of safety.

### *Effect against* T. vivax

With the "Toro" strain, two of three cattle relapsed, 10 and 16 days respectively, after a dose of 2 mg./kg. of "528." The third animal was apparently cured.

After a dose of 1 mg./kg. all three cattle relapsed within nine days of treatment, and all showed large numbers of parasites in the blood.

After treatment with "528," trypanosomes remained in the blood on the day following the dose, and then disappeared until relapse occurred.

Three cattle treated with 5 mg./kg. of antrycide methylsulphate were apparently cured.

Three controls showed heavy blood infections during the first four weeks, after which the disease became chronic in type.

With the "Bokkus" strain, all of six cattle injected with 2.5 mg./kg. of "528" relapsed within 11 days of treatment, and one died. Three animals which were given 5 mg./kg. of antrycide methylsulphate were apparently cured and no trypanosomes were observed again in the peripheral blood; one of these cattle died 18 days after drug treatment, and showed lesions of helminthiasis and unresolved trypanosomiasis.

Two of three control animals died of the acute trypanosome infection; the third was killed during the acute infection to obtain specimens. The "Bokkus" strain of *T. vivax* was virulent, and gave very heavy blood infections.

These experiments show clearly that "528" has no appreciable curative effect against *T. vivax*, even when used in doses approaching the toxic level.

*Drug Resistance*

Doses up to 1 mg./kg. of "528" given to normal mice infected with *T. congolense* were insufficient to prevent relapse. After 18 treated passages in splenectomized mice, relapses occurred after a dose of 4 mg./kg. However, after one passage in normal mice, resistance was hardly detectable. No information is available upon the readiness with which the drug produces resistance in infected cattle.

## Discussion

The results of this investigation show clearly that, although "528" had an appreciable action against a syringe-transmitted strain of *T. congolense*, it had no curative effect against two strains of *T. vivax*. "528" would therefore be of very limited value in the treatment of cattle trypanosomiasis in West Africa, where *T. vivax* is the more important cause of cattle disease.

The drug is of interest because, like the phenanthridinium derivatives and antrycide, it is a quaternary heterocyclic compound, and it has toxic effects which are similar to those of antrycide.

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## References

Goodwin, L. G., and Chandler, R. L. (1952). *Brit. J. Pharmacol.*, **7**, 591.

Keneford, J. R., Lourie, E. M., Morley, J. S., Simpson, J. C. E., Williamson, J., and Wright, P. H. (1948). *Nature, Lond.*, **161**, 603.

Lourie, E. M., Morley, J. S., Simpson, J. C. E., and Walker, J. M. (1951). *Brit. J. Pharmacol.*, **6**, 643.

Unsworth, K. (1953). *Ann. trop. Med. Parasit.*, **47**, 361.