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THE TRYPANOCIDAL ACTION OF HOMIDIUM, QUINAPYRAMINE AND SURAMIN

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Homidium, quinapyramine, and suramin (Group II compounds) produce their trypanocidal effect *in vivo* only after a latent period of 24 hr. or more, during which time the trypanosomes may continue to multiply; this is in contrast to trivalent arsenical and diamidine compounds (Group I compounds), which begin to act immediately. Group II compounds also differ from Group I compounds in that (*a*) they have only a slight tendency to combine with trypanosomes, (*b*) they have little trypanocidal action *in vitro*, but (*c*) they make trypanosomes non-infective to fresh subinoculated mice. To explain these features it is postulated that homidium, quinapyramine, and suramin first combine in small amounts with some receptor on the trypanosome and then block some biochemical system which produces a hypothetical substance X which is needed for cell division of the trypanosome; the trypanosome is supposed to contain a preformed store of this substance X sufficient for several divisions to take place; and it is only when this store is exhausted that cell division is prevented and the trypanosome eventually dies.

The purpose of this paper is to describe certain characteristics of the trypanocidal action of homidium (Ethidium ; B.Vet.C.Supp. 1959, p. 27), quinapyramine (Antrycide ; B.Vet.C., 1955, p. 553), and suramin, and to offer a hypothesis to explain them. These compounds differ from other trypanocidal compounds such as trivalent arsenicals and diamidines in that their trypanocidal action *in vivo* is manifested only after a long latent period. Experiments were undertaken to study quantitatively the behaviour of the trypanosomes during this latent period.

METHODS

Rats or mice were infected with trypanosomes by intraperitoneal inoculation When the blood contained scanty trypanosomes, blood from the tail was diluted in a W.B.C. or R.B.C. pipette with a fluid which lysed the erythrocytes and stained the trypanosomes ; the trypanosomes were then counted in a haemocytometer slide under the microscope. A suitable fluid for this purpose was: 1% methylene blue, 2 ml. ; glacial acetic acid, 0.25 ml. ; water, 50 ml. In the case of *Trypanosoma evansi*, the number of trypanosomes in a drop of blood from the tail was probably the same as that in the main volume of circulating blood. *T. congolense*, on the other hand, tends to accumulate in the capillaries of the tail, and the count recorded may often have been much greater than that in the circulating blood ; presumably, however, the two were proportional, and this discrepancy would not affect the general argument. The animal was then treated intraperitoneally with a dose of the drug approximately double the dose which was sufficient to remove all visible trypanosomes from the blood within four days. Counts of the trypanosomes were made at suitable intervals (usually morning and evening) until no further trypanosomes were found.

The trypanosomes used were *T. evansi*, Mathura strain, described in a previous paper (Sen, Sharma, and Hawking, 1960) which was studied in rats ; and *T. congolense* (N.I.M.R. strain) which had been maintained in mice for some years and which was very sensitive to quinapyramine and homidium.

RESULTS

The results of typical experiments are shown in Fig. 1 in which the time is plotted horizontally and the logarithm of the number of trypanosomes in the blood is plotted vertically. In the case of *T. evansi*, the trypanosomes of the untreated control rat increase about 25 times (average) every 24 hr., that is, 4.7 divisions per 24 hr., or 1 division approximately every 5 hr. In the case of *T. congolense*, the trypanosomes of untreated control mice increased approximately 8 times every 24 hr, that is, approximately 1 division

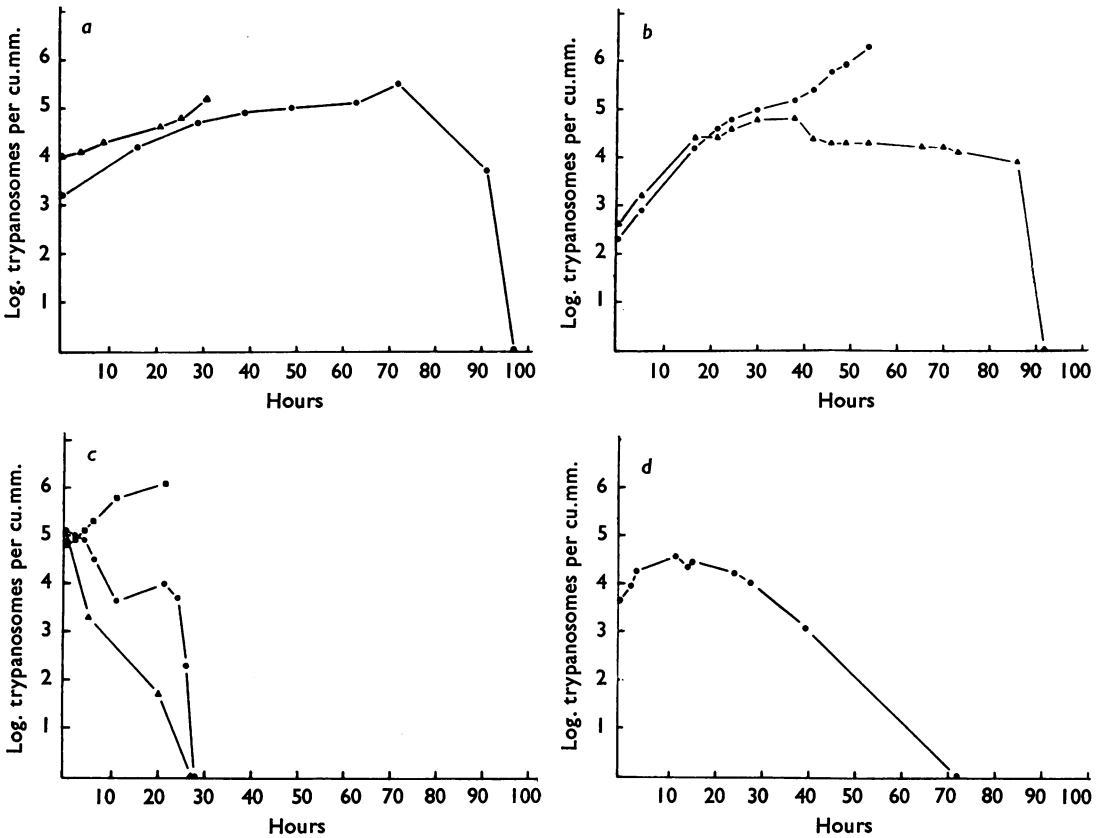

FIG. 1.—The vertical scales show the log. of the number of trypanosomes per cu.mm. tail blood; failure to see trypanosomes is arbitrarily indicated as log. 0. The horizontal scales show the time in hours after treatment. Drugs were given by intraperitoneal injection. Suramin 0.03 mg./100 g. ●—● (control ▲—▲) in (*a*). Quinapyramine 0.01 mg./100 g. ▲—▲ (control ●—●) in (*b*); melarsoprol 0.01 mg./100 g. ▲—▲, stilbamidine 0.2 mg./100 g. ●—● (control ■—■) in (*c*); were tested on *T. evansi* in rats. Homidium 0.004 mg./20 g., ●—●, in (*d*), was tested on *T. congolense* in mice.

every 8 hr. After treatment with two compounds (melarsoprol and stilbamidine), which are believed to exert a direct lethal action on the trypanosomes, the number of trypanosomes begins to fall immediately after the treatment, and reaches zero in about 27 hr. After treatment with homidium, quinapyramine, and suramin the trypanosomes continue to multiply for 24 to 72 hr.; then there is a period during which the trypanosomes remain approximately constant in number; finally they diminish rapidly and disappear. Depending on the dose, the drug, and the sensitivity of the trypanosome, these three phases vary in duration, but they can still be seen even when the dose is raised considerably. The essential point is that the trypanosomes continue to multiply for a considerable period after they have come into contact with the drug. In the case of suramin and quinapyramine the number may increase by 128 times, that is, 7 divisions; in the case of homidium it increased 8 times, that is, 3 divisions.

DISCUSSION

Two types of trypanocidal compounds can be recognized, according to their biological action as summarized in Table I.

It is considered that the first group—trivalent arsenicals (for example, trivalent tryparsamide, melarsoprol), acriflavine, diamidines (for example, stilbamidine, berenil)—are specifically absorbed by the trypanosomes, so that a high concentration is reached inside the cells, and that they then cause the death of the trypanosomes by direct action on some vital mechanism (Hawking,

TABLE I

TYPES OF TRYPANOCIDAL COMPOUNDS

	Group I Trivalent Arsenical Compounds	Group II Homidium and Other Phenanthridines, Quinapyramine, Suramin
Absorption by trypanosomes	Marked and immediate	Inconspicuous
Trypanocidal action *in vitro*	Marked	Slight or absent
Effect on infectivity ..	Slight	Marked
Trypanocidal action *in vivo*	Immediate	Manifest only after a period of multiplication

1938). The other compounds (homidium, quinapyramine, and suramin) are less simple in their mode of action.

Little absorption of the compound by the trypanosome, either *in vitro* or *in vivo*, can be shown by direct chemical methods, although a slight absorption probably does occur. Suramin could not be shown to be absorbed by trypanosomes, when a somewhat insensitive method was used (Hawking, 1939). Prothidium (a phenanthridine compound with pyrimidine side chain) is absorbed to a slight extent according to Taylor (1960). By contrast trivalent arsenicals and acriflavine are absorbed by trypanosomes in large amounts (Hawking, 1938) and the same is true of stilbamidine (Hawking, 1944; Fulton and Grant, 1955); this absorption can easily be demonstrated *in vitro* and *in vivo* and can be studied quantitatively.

The trypanocidal action *in vitro* of homidium and quinapyramine is moderate and that of suramin is slight. Suramin kills *T. rhodesiense* (old laboratory strain) (37°, 24 hr. exposure) only at 1/3,000 (Hawking, 1939). Under the same conditions quinapyramine kills *T. equiperdum* at 1 in 1 to 4 million (Hawking and Thurston, 1955) and dimidium kills *T. rhodesiense* (old laboratory strain) 1 in 1 million (Lock, 1950). These concentrations are all greater than are reached *in vivo* after therapeutic doses. By contrast the trivalent arsenicals and similar compounds under the same conditions kill trypanosomes at low concentrations like 1 in 50 million or 1 in 200 million.

When trypanosomes are exposed *in vivo* or *in vitro* for several hours to homidium, quinapyramine or suramin in suitable concentrations the trypanosomes appear unharmed and may continue to wriggle actively for 24 hr.; but if they are inoculated into fresh animals, no infection develops, showing that some profound change has been produced in them (Hawking, 1938; Lock, 1950; Ormerod, 1951; Hawking and Thurston, 1955). By contrast, when trypanosomes are exposed to arsenicals and similar compounds, their infectivity is not usually diminished, unless all the trypanosomes have been obviously killed or damaged before they are inoculated.

As demonstrated above, the *in vivo* trypanocidal action of homidium, quinapyramine, and suramin is exerted only after a latent period during which 3 to 7 cell divisions may occur, while the *in vivo* trypanocidal action of trivalent arsenicals and diamidine is manifested almost immediately.

It is suggested that these special features of the action of Group II compounds might be explained by a hypothesis on the following lines.

The drug first combines in small amounts with some receptor on the trypanosome. It then blocks some biochemical system which produces a hypothetical substance X which is needed for the cell division (multiplication) of the trypanosome. It is postulated that the trypanosome contains a preformed store of this substance X sufficient for several divisions to take place; when this store is exhausted, no further divisions can occur (perhaps the cell cannot even continue to function), and after a further period all the trypanosomes die.

The chief evidence for combination between group II drugs and trypanosomes (fixation) is that if trypanosomes are exposed to these drugs *in vitro* or *in vivo* they become non-infective to fresh animals, although they continue to wriggle for long periods. In its early stages part of this combination is probably reversible, but, judging by the period of exposure needed to make most of the trypanosomes non-infective, an irreversible combination seems to occur with dimidium in 0.5 hr. (Lock, 1950), with quinapyramine in 2.5 hr. (Ormerod, 1951), and with suramin in 1 to 2 hr. (Hawking, 1939). Newton (1957) found that *Strigomonas oncopelti* exposed to homidium (10 μg. per ml.) absorbed 3 μg. per 10^8 organisms in the first 3 hr., and that this combination was irreversible.

The phenomena of drug resistance show that homidium, quinapyramine, and suramin do not all act on the same site in the trypanosome (although there may be some overlapping between homidium and quinapyramine). Accordingly it may be supposed that either the compounds are

first fixed on different receptors (drug resistance depending on a diminished avidity of such receptors) after which all three act on the same biochemical system; or homidium blocks the production of a substance X, quinapyramine blocks the production of a similar substance Y, and suramin the production of a substance Z.

Substance X (or Y or Z) might be imagined to be a compound concerned with the synthesis of ribonucleic acid. In this connexion it may be noted that homidium rapidly inhibits the synthesis of deoxyribonucleic acid in *Strigomonas oncopelti* while the synthesis of ribonucleic acid and protein continues for several hours after addition of the drug (Newton, 1957). McIlwain (1946) has pointed out that in bacteria the molecules of many important substances, such as vitamins and co-enzymes, are limited in number per cell, for example, a cell may contain only 200 to 1,200 molecules of folic acid, and possibly only a few molecules of certain fundamental enzymes. Something the same may be true of trypanosomes, and substance X may be one of the molecules which are present only in limited numbers.

According to fluorescent appearances quinapyramine (Ormerod, 1951) and prothidium (Taylor, 1960) usually collect in the kinetoplast and in granules, in the cytoplasm of trypanosomes, and not in the nucleus to any large extent. (A similar distribution is shown by stilbamidine and acriflavine, which kill trypanosomes in a more direct manner). Either the hypothetical biochemical processes blocked by quinapyramine and prothidium take place mainly in the kinetoplast rather than in the nucleus, or only a small proportion of the drug absorbed by the trypanosome is responsible for the death of the organisms, the greater proportion being diverted into inactive combinations.

The morphological effects produced on trypanosomes by the administration of homidium, quinapyramine, or suramin to infected rats consist of: (i) Diminution in the percentage of dividing forms. (ii) Sometimes the occurrence of giant multinuclear trypanosomes in which the nucleus has divided once, or twice, but the cytoplasm has not; this is evidence of disordered cell division, but the disorder is greater in the cytoplasm than in the nucleus: this occurs with *T. rhodesiense* but it is rare with *T. equiperdum* or *T. congolense*. (iii) The appearance in the trypanosome cytoplasm of basophilic inclusion bodies which are best developed after 24 hr. Apparently these contain ribonucleic acid protein and drug (Ormerod, 1951). (iv) Diminished mobility in the trypanosomes, which can be seen under the microscope to wriggle less actively than usual (Mr. P. Walker, unpublished observation).

In discussing the mode of action of homidium upon *S. oncopelti* with particular reference to the metabolism of ribonucleic acid and deoxyribonucleic acid, Newton (1957) postulates that there is first a rapid combination of the drug with "primary binding sites" which does not interfere with growth and cell division, and then a slower combination with "secondary binding sites" which become available to the drug during growth; it is this secondary uptake which progressively inhibits growth. The conception of a rapid combination with primary binding sites (fixation), followed by a slower combination with secondary binding sites, is supported by many phenomena in trypanocidal action, and it is generally accepted. The suggestion that these secondary binding sites for homidium, etc., only become available during growth is an alternative hypothesis to the one submitted above in the attempt to explain the biochemical mechanism by which a drug can produce its inhibitory effect only after a latent period which may be 24 hr. or more. Whatever hypothesis is eventually proved to be correct, it is to be expected that it should apply to quinapyramine and suramin as well as to the phenanthridine compounds.

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