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A STUDY OF GRANULES AND OTHER CHANGES IN PHASE-CONTRAST APPEARANCE PRODUCED BY CHEMOTHERAPEUTIC AGENTS IN TRYPANOSOMES

BY

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The morphological changes produced by four series of organic trypanocidal drugs have been studied by quantitative and by qualitative methods using phase-contrast and fluorescence microscopy. Basic drugs were absorbed rapidly into the region of the kinetoplast; acidic drugs did not affect this region. Faint granules, which were present in some trypanosomes before the administration of drugs, absorbed the drugs and increased in contrast relative to the cytoplasm. Hydroxystilbamidine, quinapyramine, related compounds, and possibly also suramin produced additional granules which did not contain drug. These additional granules are similar to the granules (volutin granules) which occur in trypanosome infections (not treated with drugs) when trypanosomes are about to be cleared from the blood. Homidium did not produce additional granules.

In a series of papers (Ormerod, 1951a, 1951b, 1952) one of us described basophilic inclusions produced in the cytoplasm of trypanosomes by trypanocidal drugs. Later (Ormerod, 1958) it was pointed out that these granules were closely related to the volutin granules which appear in the cytoplasm of some strains of trypanosomes without their having received previous drug treatment; both are identical in appearance and both contain protein and ribonucleic acid. The only observed difference between the two sorts of granules was that drug was present in one but not in the other. It was suggested (Ormerod, 1961) that they might have the same function and be connected, as the volutin granules appeared to be connected, with the removal of trypanosomes from the blood.

Since the demonstration of granules produced by drugs in trypanosomes in 1951, no general description of the phenomenon has been published as had been done for the volutin granules of trypanosomes (Ormerod, 1958). The present paper describes the granules produced by drugs and their mode of formation, together with other changes which occur in the trypanosome cell under the action of drugs.

As with volutin granules the granules which are produced by drugs stain irregularly with eosin-methylene blue compound (Romanowsky) stains; because of this, the description of the changes which occur will be given in terms of the appearance of the trypanosomes under phase-contrast microscopy and by fluorescence microscopy which demonstrates the actual distribution of the drug in the trypanosome.

METHODS

A strain of *Trypanosoma rhodesiense* isolated many years ago and passaged in mice by injection at the Liverpool School of Tropical Medicine was used in this work. This strain showed no tendency to " polymorphism " ; it produced acute infections which killed rats and mice within 5 days. Volutin granules did not appear until the 3rd day of infection when granules appeared faintly (see below, for example Fig. 2a), but they only became conspicuous enough to be identified without doubt when the maximal stage of infection had been reached.

Drugs were dissolved in distilled water, and given at 70 hr after inoculation and by intraperitoneal injection, except where otherwise stated. Examination of living, motile trypanosomes was made on agar films using the technique of Ormerod (1958) mainly with a Cooke, Troughton & Sims phase-contrast microscope (series M 20,000) but the observations were checked and the photographs taken with a Zeiss phase-contrast microscope with greater resolving power.

Quantitative studies. These were carried out using rats which were infected at the same time, in each experimental group, with the same number of trypanosomes. The trypanosomes, which had been stored at −79° C by the method of Polge & Soltys (1957), were thawed rapidly and counted living and motile in a haemocytometer ; the necessary dilutions were made with distilled water containing 0.9% (w/v) of sodium chloride and 10% (v/v) of horse serum. The course of the infection, before and after treatment, was followed by counting and examining both the trypanosomes and the granules that they contained by the method of Ormerod, Healey & Armitage (1963). Each experiment was performed several times using eight to twenty-nine rats for each drug ; illustrative examples of these experiments are given in the text and in the figures.

Qualitative studies. These were carried out in mice. It was found necessary to use heavy infections since it was difficult to examine enough affected trypanosomes in a light infection to draw adequate conclusions about their appearance. Consequently large (sometimes toxic) doses of drugs had to be given near the peak of the infection.

Fluorescence microscopy was performed using an assembly similar to that described by Taylor (1960), but the microscope was the same as that used for the phase-contrast study with the phase-contrast condenser removed and replaced by an Abbé-type quartz condenser. This arrangement enabled individual living trypanosomes to be observed first by phase-contrast and then, after changing the condenser, by fluorescence microscopy. In later experiments a Reichert " Zetopan " was used, combined with fluorescence and reversed phase-contrast equipment. Quartz slides were used for fluorescence microscopy both as a mount for agar preparations for the study of living motile trypanosomes and for the air-dried blood films which were examined unfixed and unstained. Four types of compound were studied ; the suramin, the diamidine, the quinapyramine and the phenanthridinium groups (Table 1).

RESULTS

Comparison of controls with drug-treated animals

In two control rats (Figs. 1a and 5a) no granules were observed but in one control rat (Fig. 2a) a small number of trypanosomes contained granules. In this rat the mean number of granules per trypanosome was 1 to 1.5 when the calculation was based only on those trypanosomes which contained granules (this is referred to as the " G-mean "), but if the calculation was based on the *total* number of trypanosomes (referred to as " T-mean ") a much smaller mean—approximately 0.1 granules per trypanosome—was obtained. One of the characteristics of an untreated infection with this very rapidly growing strain is the scarcity of trypanosomes which contain granules ; in some instances, for example Figs. 1a and 5a, no trypanosomes containing granules were seen, but in the result shown in Fig. 2a where a few trypanosomes with granules were present, a wide divergence between the G-mean

TABLE 1

NAMES AND FORMULAE OF DRUGS USED, WITH DOSES OF DRUGS USED IN THE QUALITATIVE EXPERIMENTS

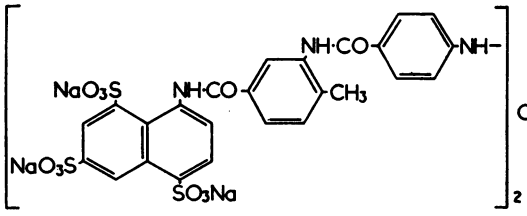
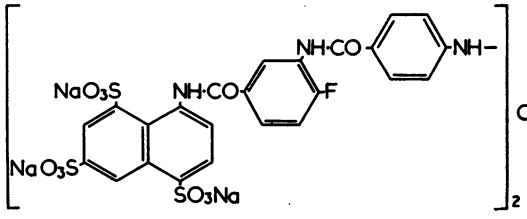
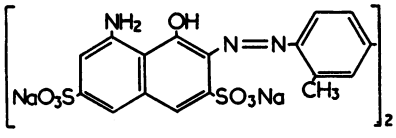
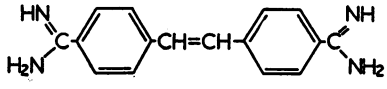
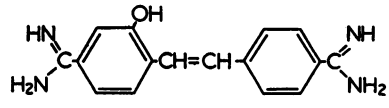
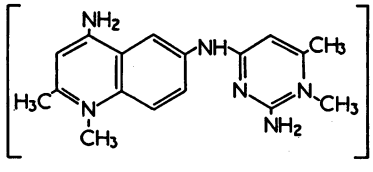
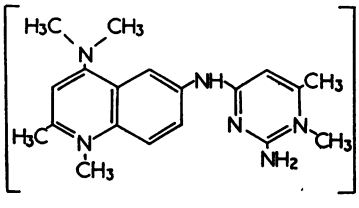
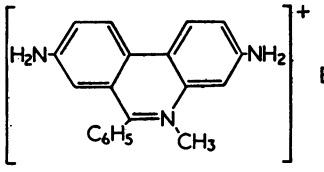
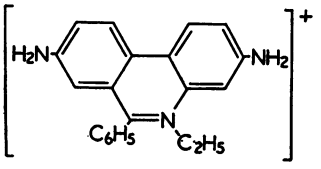
Drug	Formula	Dose (mg/kg of mouse)
Suramin series		
Suramin (Antrypol)		1·25 0·60
8154		12·5 6·0
Trypan blue		50·0
Diamidine series		
Stilbamidine		50·0
Hydroxy-stilbamidine		50·0 25·0
Quinapyramine series		
Quinapyramine (Antrycide)		10·0 2·5 1·25
7419		10·0 5·0

TABLE 1—*continued*

Drug	Formula	Dose (mg/kg of mouse)
Phenanthridinium series		
Dimidium		15·0 10·0
Homidium (Ethidium)		15·0 10·0

and the T-mean indicated that the few trypanosomes which did contain granules contained them in considerable numbers (comparable to those in the drug-treated infections). In the result shown in Fig. 7a, where the rat survived 140 hr after inoculation with trypanosomes, a larger proportion of trypanosomes contained granules, indicated in Fig. 7a by the higher T-mean at 138 hr.

In the drug-treated infections a variable degree of convergence of the G-mean and T-mean was shown ; this was often complete, as in Fig 1c, indicating that all trypanosomes present contained granules. The different degrees of divergence of the G-mean and T-mean in drug-treated infections is of particular interest and will be discussed in more detail later in this paper.

Suramin series

The quantitative results of an experimental infection treated with suramin are shown in Fig. 1. The control rat (Fig. 1a) died at about 100 hr ; the last count which was made at 93 hr showed no granules under phase-contrast. Counts from the experimental rats dosed with suramin showed a proportion of granular trypanosomes which increased with the dose: 18 hr after a dose of 1.97 mg/kg (Fig. 1b), 72% of trypanosomes contained granules. Eighteen hours after a dose of 6.05 mg/kg (Fig. 1c), 100% of trypanosomes contained granules.

The first qualitative change occurred about 6 hr after a dose of suramin when a few dense granules appeared, probably as a result of an increase in contrast of faint volutin granules already present ; these granules increased in size and contrast as did the number of granules during the next 12 hr. At the same time there was a steady decrease in the numbers of trypanosomes in the blood. As the granules increased in size and contrast they remained spherical and clear cut in outline ; there was no evidence of change in the appearance of the surrounding cytoplasm, nor of the nuclear region. There was no change in the region of the kinetoplast, which remained the same throughout, in all observations of trypanosomes treated with suramin.

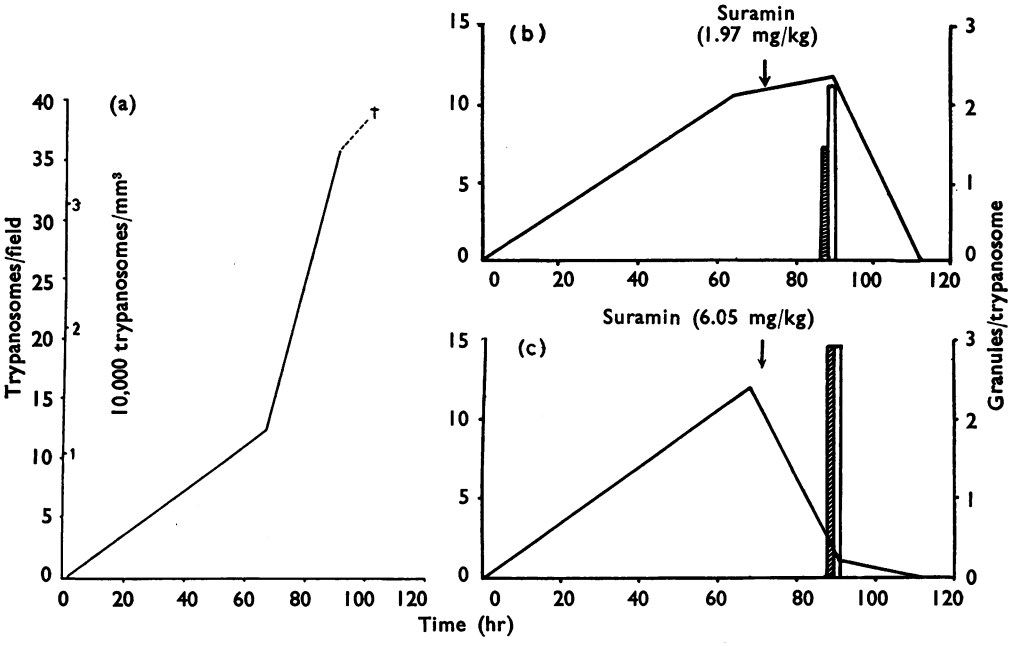


Fig. 1. Action of suramin. Each graph represents the infection in a single rat, which received 6×10^5 trypanosomes. Ordinates on left of graphs: number of trypanosomes/field converted in (a) into 10,000's/mm³ by the factor for this strain given by Ormerod, Healey & Armitage (1963). Ordinates on right of graphs: number of granules/trypanosome. Hatched columns= mean number of granules/trypanosome (based on total number of trypanosomes), the T-mean. Open columns=mean number of granules/trypanosome (excluding trypanosomes without granules), the G-mean. †=rat died. Abscissae: time (in hr) from the initial infective dose of trypanosomes. (a): control rat (121 g). No granules seen. (b): rat (127 g) which received 1.97 mg/kg of suramin. 18 hr after the dose the T-mean was less than the G-mean. This indicates that a proportion of circulating trypanosomes did not contain granules. (c): rat (124 g) which received 6.05 mg/kg of suramin. 18 hr after the dose the G-mean and the T-mean were the same, that is, all the trypanosomes contained granules.

The same morphological changes were observed with other acidic trypanocidal drugs (for formulae see Table 1). Compound 8154 (the fluorine derivative of suramin) produced the same appearances but at a higher dose level. Trypan blue was not curative but reduced the number of trypanosomes in the blood; it also produced granules which were similar under phase-contrast to those produced by suramin. By microscopic observation under direct illumination the granules could be seen to be stained a faint blue by the dye. No changes could be seen in the region of the kinetoplast or in the nucleus.

Diamidine series

A quantitative study using hydroxystilbamidine is shown in Fig. 2. In the control rat (Fig. 2a) a small proportion of trypanosomes contained granules, 3.5% at 74 hr and 1.1% at 80 hr from the initial infection of the rat. Fig. 2b shows the result of an infection treated ineffectively with hydroxystilbamidine at a dose of 0.25 mg/kg.

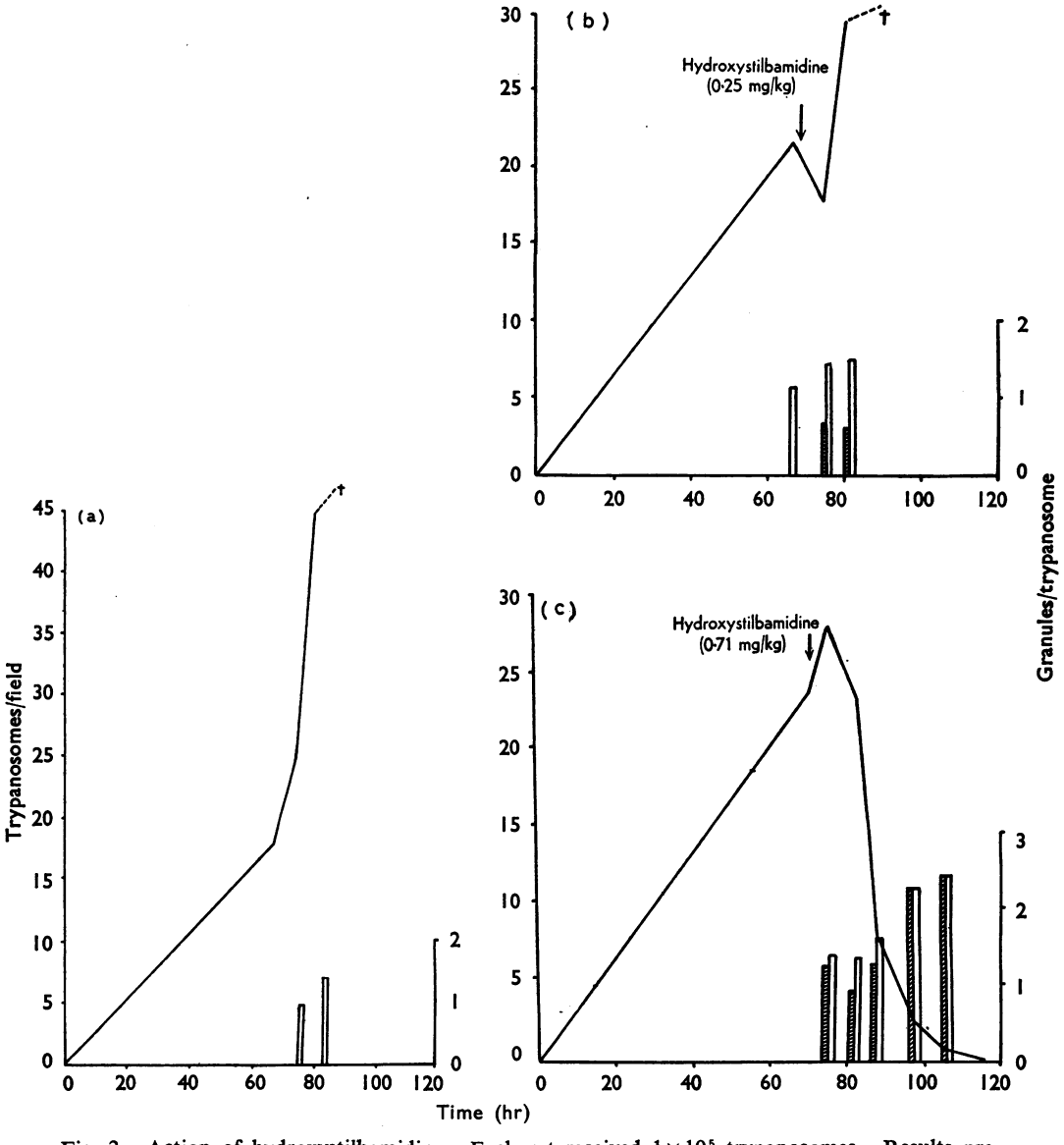


Fig. 2. Action of hydroxystilbamidine. Each rat received 1×10^5 trypanosomes. Results presented as in Fig. 1. (a): control rat (121 g). A small number of trypanosomes (1 to 4%) contained on average 1 to 2 granules. (b): rat (134 g) which received 0.25 mg/kg of hydroxystilbamidine, an ineffective dose. Considerable divergence between the G-mean and the T-mean is shown. (c): rat (140 g) which received 0.71 mg/kg of hydroxystilbamidine, an effective dose. The divergence between the G-mean and the T-mean indicates that from the time of the dose until approximately 15 hr after it trypanosomes with granules were being replaced in the circulation by trypanosomes without granules. However, at 25 hr from the dose all trypanosomes contained granules.

This dose produced a transient fall in the numbers of circulating trypanosomes, but the infection soon began to increase and the rat died. The result of effective treatment is shown in Fig. 2c ; 4 hr after 0.71 mg/kg, 83% of trypanosomes contained granules and this high proportion was continued (69% at 12 hr, 77% at 17 hr, 100% at 27 hr, and 100% at 33 hr from the dose). The transient fall in the proportion of trypanosomes which contained granules is shown in Fig 2c as a divergence of the G-mean and T-mean ; this phenomenon occurred also in other experiments with hydroxystilbamidine. This divergence records the observation that for a time after dosage there was a tendency for the trypanosomes which contained granules to disappear from the circulation and to be replaced by trypanosomes without granules ; this tendency was, however, later reversed so that eventually all circulating trypanosomes contained granules. Quantitative experiments with stilbamidine did not differ in any significant way from those with hydroxystilbamidine.

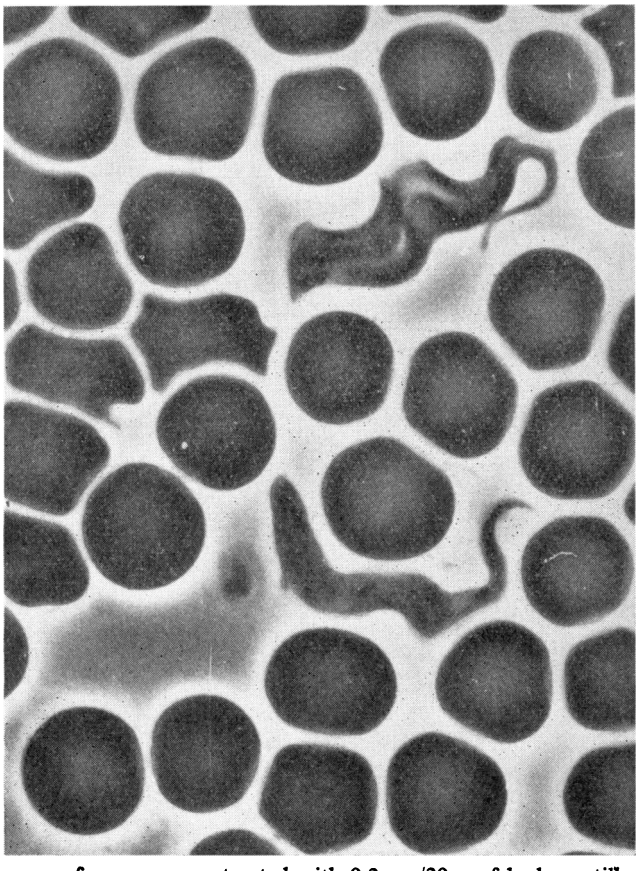


Fig. 3. Trypanosomes from a mouse treated with 0.2 mg/20 g of hydroxystilbamidine 2 hr previously. Irregular granules are shown in the region of the kinetoplast. Photomicrographs taken with a Zeiss phase-contrast and electronic flash equipment at 30 watt/sec intensity. A X-100 fluorite objective was used without filters and at maximum aperture. The film was Adox KB17. The trypanosomes, which were fully motile, were flattened on agar as described in the text.

Qualitative experiments with stilbamidine and hydroxystilbamidine also gave similar results. The first change occurred within 1 min of intraperitoneal injection of the drug, and consisted of the appearance of a slightly darker area in the region of the kinetoplast. Within 5 min a marked darkening of this region had occurred; this did not appear to be confined to the kinetoplast itself, but was diffuse and appeared to involve the whole of the posterior end of the trypanosome. It did not, however, involve the basal vacuole which, although it became distended in many trypanosomes, retained its negative contrast with the cytoplasm. Within 15 min an increase in contrast of such granules as were already present had occurred, and a dark spot had appeared at the centre of the nucleus which appeared to be the nucleolus; at the same time a granule began to form in the region of the kinetoplast and during the following hour the granule extended to form an irregular scar-like mark (Fig. 3). The other dense granules which had formed in the cytoplasm also formed scar-like marks. Under the Zeiss microscope these scar-like marks appeared to be formed of a close aggregation of small granules. Not all granules seen at this time were irregular in outline, and the number of trypanosomes so affected appeared to decrease as the number of granules per trypanosome increased, so that from approximately 14 hr onwards granules with irregular outline were not observed (Fig. 4). From 18 hr onwards, especially at lower dose levels, the multinuclear forms described by Ormerod (1951a) were a constant feature.

Observations with the fluorescence microscope were made using hydroxystilbamidine rather than stilbamidine because of the more intense fluorescence that trypanosomes which had absorbed the drug exhibit when irradiated at 3,650 Å. All the phase-contrast changes observed in the first 2 to 3 hr after a dosage of hydroxystilbamidine were accompanied by the characteristic orange fluorescence associated with the combination of the drug with acidic groups in the structure of the organism (Ormerod, 1952). Initially the fluorescence was diffuse except in the region of the

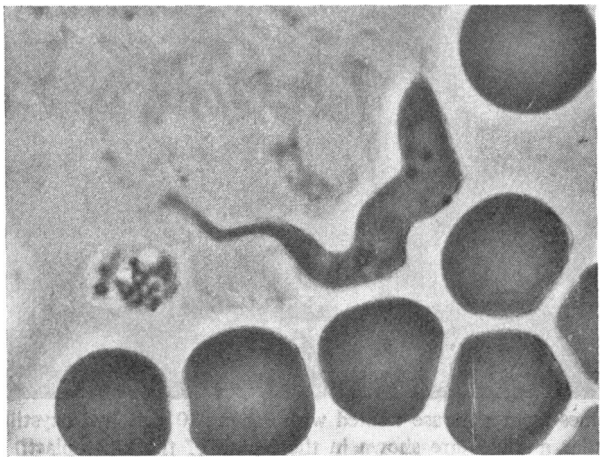


Fig. 4. Trypanosome from a mouse treated with 0.1 mg/20 g of hydroxystilbamidine 20 hr previously. Spherical granules of high contrast are shown. Photomicrograph prepared as for Fig. 3.

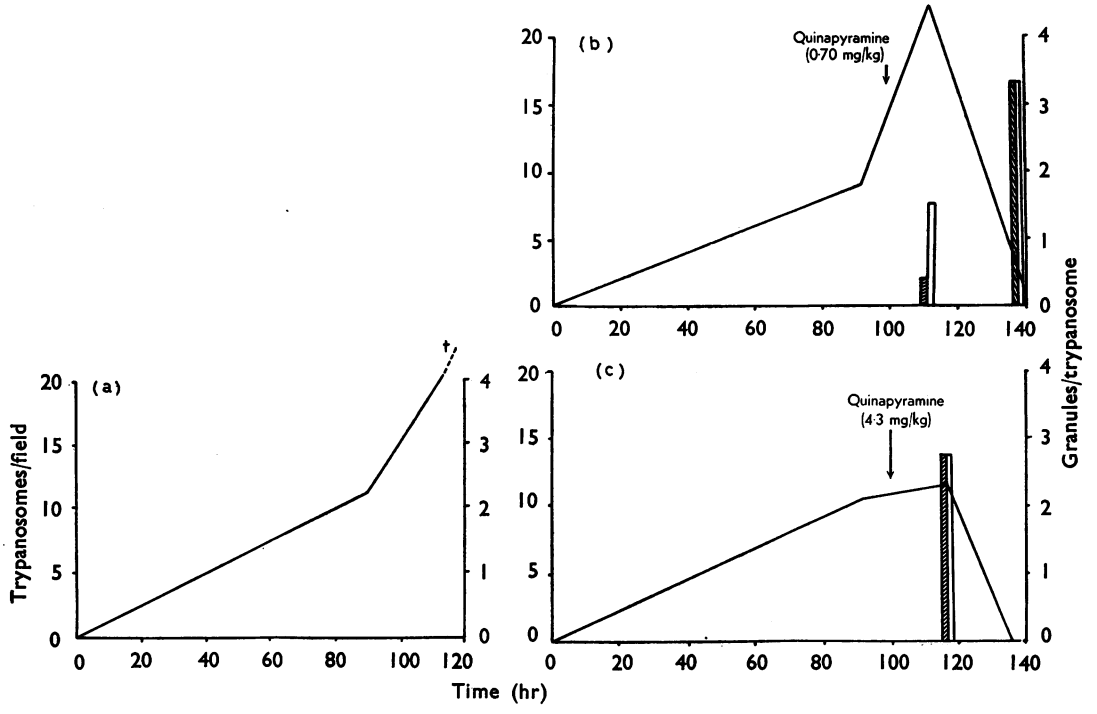


Fig. 5. Action of quinapyramine. Each rat received 2×10^4 trypanosomes. Results presented as in Fig. 1. (a): control rat (124 g). No granules seen. (b): rat (144 g) which received 0.70 mg/kg of quinapyramine. 40 hr after the dose all the trypanosomes contained granules. (c): rat (115 g) which received 4.3 mg/kg of quinapyramine. 14 hr after the dose all the trypanosomes contained granules.

kinetoplast where it was intense in all observations, but after 15 min it became concentrated also in granules in the cytoplasm and in the nucleolus.

Since granules continued to be observed under phase-contrast and to increase in number as long as trypanosomes were observed in the blood, it was expected that fluorescence would also continue, but this did not occur. An examination of individual living and motile trypanosomes was made both by phase-contrast and by fluorescence microscopy 12 hr after an effective dose of hydroxystilbamidine, a time when all, or nearly all, circulating trypanosomes contained granules. By this means it could be seen that only a small proportion of trypanosomes fluoresced, but their fluorescence was as intense as in other observations at 2 to 3 hr from the dosing; the great majority of trypanosomes showed no sign of fluorescence.

Quinapyramine series

A quantitative study using quinapyramine is illustrated in Fig. 5. In the control rat (Fig. 5a) no granules were observed throughout the infection. Fourteen hours after 0.70 mg/kg (Fig. 3b) 18% of trypanosomes carried granules, and the proportion rose to 100% at 40 hr after the dose. At a higher dose level, 4.3 mg/kg (Fig. 5c), 14 hr after the dose all circulating trypanosomes contained granules. As Ormerod

(1951a) and Hawking & Sen (1960) have already shown, the infection continues to rise for some time after an effective dose of quinapyramine ; this is also demonstrated in Fig. 5.

The first qualitative change to occur, as with the diamidine drugs, was a darkening in the region of the kinetoplast. This change began to occur 10 to 15 min after intraperitoneal injection of the drug and it was followed in 20 to 30 min by an increase in contrast of existing volutin granules and of the nucleolus. From this time, there was a gradual increase in the number, size and contrast of the granules, which were larger and more numerous than with any other drug used in this study. As with suramin they retained their even shape and contrast and did not form scar-like marks like those produced by hydroxystilbamidine (Fig. 6). From

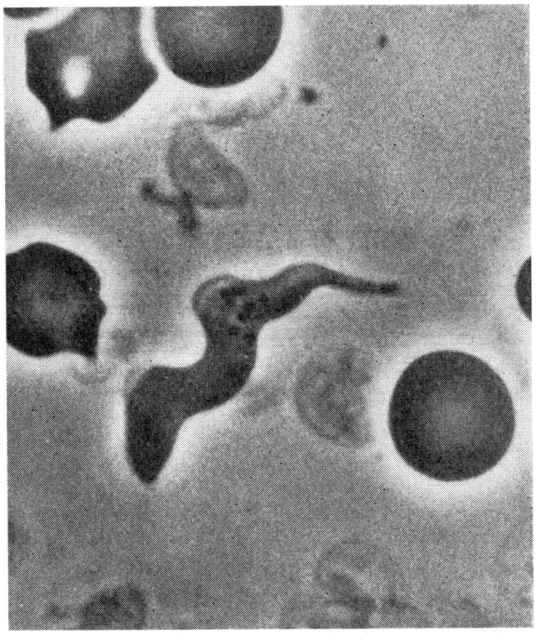


Fig. 6. Trypanosome from a mouse treated with 0.1 mg/20 g of quinapyramine 27 hr previously. The spherical granules in the anterior of the trypanosome show the typical appearance of a trypanosome treated with quinapyramine; the arrangement in rows is typical of volutin granules in *T. rhodesiense*. The large granule at the posterior end is not typical but can often be seen in *T. rhodesiense* treated with quinapyramine and in strains which develop volutin granules in the course of normal development. Photomicrograph prepared as for Fig. 3.

approximately 18 to 30 hr, multinuclear forms were frequently seen and at the lower dose levels as many as 5% of circulating forms were of this type.

Compound 7419, a derivative of quinapyramine (for formula see Table 1), produced similar changes in trypanosomes although at a higher dose level.

Quinapyramine is a fluorescent substance and, although the fluorescence of trypanosomes treated with this drug is less intense than that of trypanosomes treated with hydroxystilbamidine, the distribution was found to be similar: the region of

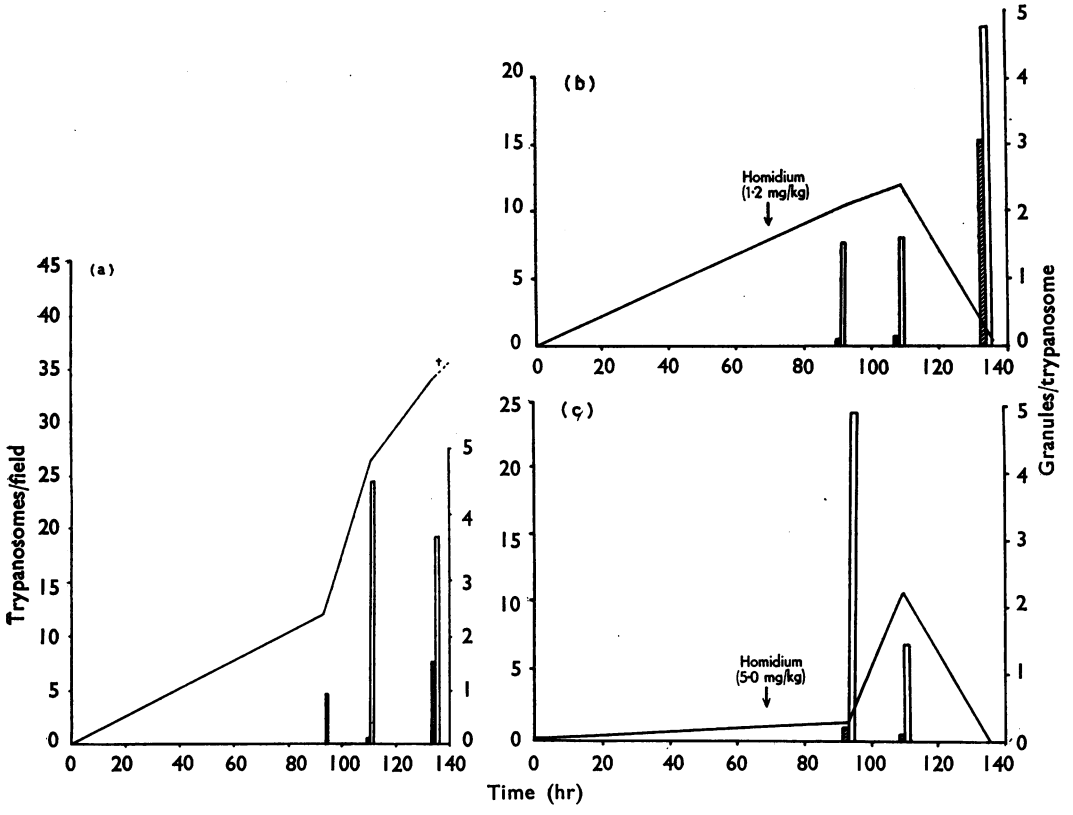


Fig. 7. Action of homidium. Each rat received 2×10^5 trypanosomes. Results presented as in Fig. 1. (a): control rat (157 g). At 110 hr a small proportion of trypanosomes (7% of 510 trypanosomes counted) carried a large number of granules, hence the wide divergence between the T-mean (4.7) and the G-mean (0.2). At 138 hr (an unusually long survival for an untreated infection) 45% of trypanosomes carried granules. (b): rat (221 g) which received 1.2 mg/kg of homidium. Only at 138 hr (68 hr from the dose) did the proportion of trypanosomes with granules rise to 67%. (c): rat (170 g) which received 5.0 mg/kg of homidium. A low blood infection was obtained. After dosage only a small proportion of trypanosomes contained granules.

the kinetoplast, the granules and the nucleolus all showed fluorescence 0.5 to 1 hr after injection. As with hydroxystilbamidine, the fluorescence disappeared while trypanosomes which contained granules were still circulating ; but as the fluorescence of trypanosomes treated with quinapyramine was less intense than those treated with hydroxystilbamidine, it is possible that it was the fluorescence that had faded rather than the fluorescent trypanosomes that had disappeared.

Phenanthridinium series

The quantitative study (Fig. 7) shows the action of two effective doses of homidium given at 90 hr from the time of inoculation. In Fig. 7c the level of infection was lower than in the control (Fig. 7a) but the level of infection shown in Fig. 7b was similar. As the control rat survived longer than is usual for this strain, the last

count made at 138 hr showed a larger proportion (45%) of trypanosomes containing granules than the other control rats. In the two experimental animals the infection continued to rise for at least 20 hr after the dosing and trypanosomes were eliminated from the blood 70 hr after the dosing. In Fig. 7b, at 138 hr (68 hr after the dose) 67% of circulating trypanosomes contained granules, but at the higher dose level the proportion of trypanosomes that contained granules remained low throughout the experiment. There is no evidence from this experiment that the pattern of granule formation is changed by the action of homidium.

On qualitative examination trypanosomes reacted in the same way both to homidium and to dimidium. At 15 min after the dose the region of the kinetoplast had become darker but no dense granule formed subsequently in this region as with the other basic trypanocidal substances examined. Existing volutin granules tended to increase in contrast but not in a striking manner (for example, the experiment with the control rat illustrated in Fig. 7a, where granules were seen at 95 hr, about the time when the rats were dosed); from 1 to 6 hr after the dose the trypanosomes became paler and more mottled in appearance and tended to become vacuolated on standing; many were misshapen, with shortened or kinked flagellae, but showed no specific changes such as had been observed after treatment with the other substances. After 10 hr there was a general increase in the number of granules present, but there always remained a proportion of trypanosomes which did not contain granules.

DISCUSSION

These experiments show that the basic trypanocidal compounds, hydroxystilbamidine, quinapyramine, homidium and substances related to them, first affect the posterior end of the trypanosome in the region of the kinetoplast; this action seems to be a characteristic of basic trypanocidal substances which has often been demonstrated previously (for example, Fischl & Singer, 1934). Hydroxystilbamidine is absorbed rapidly, and changes can be seen less than 1 min after intraperitoneal injection. Quinapyramine and homidium are absorbed less rapidly, but they penetrate the cell more rapidly than had previously been supposed (Ormerod, 1951b). No information is available on the rate of penetration of the trypanosome by suramin and other related acidic compounds; since they do not fluoresce and do not affect the posterior end of the trypanosome, no changes are observed until granules begin to appear about 6 hr after the dose. It is not known how the absorption of drugs occurs, but recently Baker, Bird, Healey & Ormerod (1961) have demonstrated by electron microscopy the existence of a structure resembling a tube that appears to connect the kinetoplast with the exterior at the posterior end of the organism; if this structure does in fact represent a connection between the kinetoplast and the exterior it might enable drugs to penetrate rapidly into this organelle and allow basic drugs to come into contact with the band of deoxyribonucleic acid which lies therein (Baker, 1961). Basic drugs might be expected to combine with the phosphate groups of the deoxyribonucleic acid whereas acidic drugs would not be likely to do so.

The next stage in the distribution of drugs is their diffusion in the cytoplasm and their concentration in a granule, in or near the kinetoplast, in the nucleolus and in any granules which are already present in the cytoplasm. With hydroxystilbamidine

trypanocidal action appears to begin as soon as absorption has taken place, but with quinapyramine, suramin and homidium the parasites continue to multiply albeit at decreased rate for some hours after the dose. The close aggregation of small granules which have absorbed hydroxystilbamidine is a most characteristic appearance of trypanosomes treated with diamidines but not with other groups of drugs, and it is probable that this is in some way related to the direct trypanocidal effect which is more marked with the diamidines than with the other groups of drugs.

Suramin, hydroxystilbamidine and quinapyramine all appear to promote the appearance of new granules in the trypanosome as distinct from their producing an increase in the size and contrast of existing granules; homidium differs in promoting no further production of granules beyond those that would have been produced by the trypanosomes had no drug been administered. The fact that granules are produced when an infection with an otherwise acute and granule-free strain of trypanosomes is prolonged beyond the normal period of survival of the host animal led to the previous erroneous conclusion (Ormerod, 1951b) that dimidium produced granules in trypanosome infections in the same way as quinapyramine. It is now clear that homidium and dimidium do not produce new granules but exert the part of their trypanocidal activity which occurs after the initial absorption in some other way.

The new granules which are produced by stilbamidine, hydroxystilbamidine and quinapyramine appear several hours after the absorption of the drug. Hawking (1944) and Spinks (1950) have shown respectively that stilbamidine and quinapyramine are removed rapidly from the blood so that the drug available in the circulation is at this time at a low concentration which is unlikely to be effective against the relatively large numbers of trypanosomes that may still remain in the circulation. Besides this, there is additional evidence. The drug itself, as demonstrated by fluorescence, is no longer in the trypanosomes when the new granules have formed. In experiments with hydroxystilbamidine, after the first increase in granules, a divergence occurred between the G-mean and the T-mean followed later by convergence; this appears to indicate that between the absorption of drug and appearance of new granules, other trypanosomes have been produced in the circulation without absorbing the drug.

There are therefore two possible causes for the formation of new granules after diamidines and quinapyramine:

1. The low concentration of drug may have produced them. We think that this is unlikely because the granules do not fluoresce although the possibility remains of there being a non-fluorescent trypanocidal metabolite.

2. That a process occurs analogous to the formation of similar granules which appear in certain strains of trypanosomes without their being treated with drugs. The granules in these strains accumulate in large numbers before the removal of trypanosomes from the blood (Ormerod, 1961). When trypanosomes have been treated with quinapyramine and diamidine they first develop characteristic granules which contain the drug and, with the diamidines, have a distinctive scar-like appearance; these characteristic granules then disappear and are replaced by others which do not contain the drug and are identical morphologically with the granules

(previously called volutin granules) which appear in untreated infections. Suramin produces granules which are morphologically similar and it is possible that the chemotherapeutic process is also similar, but the absence of fluorescence and retention of high blood levels of this drug make the formation of granules by direct action of this drug more likely. The granules which form in trypanosomes which have not been treated with drugs appear to be associated with the immune reaction of the host (Ormerod, 1961). There is now much evidence that a substantial part of the trypanocidal action of stilbamidine (Fulton & Grant, 1956), quinapyramine and suramin (Soltys, 1958) is due to an immune response of the host to trypanosomes which have been affected (perhaps killed) by the initial activity of the drug, and we believe that the new granules which appear after administration of these drugs are related to the immune response rather than to the direct action of the drugs themselves. Homidium, on the other hand, has a different mode of action which does not stimulate the production of volutin granules; the granules accumulate in the same way as they would have done in an untreated infection.

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REFERENCES

BAKER, J. R. (1961). The distribution of nucleic acids in *Trypanosoma evansi*. *Trans. roy. Soc. trop. Med. Hyg.*, **55**, 518–523.

BAKER, J. R., BIRD, R. G., HEALEY, P. & ORMEROD, W. E. (1961). Electron micrographs of the kinetoplastic region in *Trypanosoma* spp. *Trans. roy. Soc. trop. Med. Hyg.*, **55**, 304.

FISCHL, V. & SINGER, E. (1934). Die Wirkungsweise chemotherapeutisch verwendeten Farbstoffe. *Z. Hyg. Infekt.-Kr.*, **116**, 348–355.

FULTON, J. D. & GRANT, P. T. (1956). Experiments on the mode of action of stilbamidine. *Ann. trop. Med. Parasit.*, **50**, 381–384.

HAWKING, F. (1944). Absorption of 4:4′-diamidinostilbene (stilbamidine) by trypanosomes and its concentration in the blood of animals. *J. Pharmacol. exp. Ther.*, **82**, 31–41.

HAWKING, F. & SEN, A. B. (1960). The trypanocidal action of homidium, quinapyramine and suramin. *Brit. J. Pharmacol.*, **15**, 567–570.

ORMEROD, W. E. (1951a). The mode of action of Antrycide. *Brit. J. Pharmacol.*, **6**, 325–333.

ORMEROD, W. E. (1951b). A study of basophilic inclusion bodies produced by chemotherapeutic agents in trypanosomes. *Brit. J. Pharmacol.*, **6**, 334–341.

ORMEROD, W. E. (1952). A study of resistance to Antrycide in a strain of *Trypanosoma equiperdum*. *Brit. J. Pharmacol.*, **7**, 674–684.

ORMEROD, W. E. (1958). A comparative study of cytoplasmic inclusions (volutin granules) in different species of trypanosomes. *J. gen. Microbiol.*, **19**, 271–288.

ORMEROD, W. E. (1961). The study of volutin granules in trypanosomes. *Trans. roy. Soc. trop. Med. Hyg.*, **55**, 313–332.

ORMEROD, W. E., HEALEY, P. & ARMITAGE, P. (1963). A method of counting trypanosomes allowing simultaneous study of their morphology. *Exp. Parasit.*, **13**, 386–394.

POLGE, C. & SOLTYS, M. A. (1957). Preservation of trypanosomes in the frozen state. *Trans. roy. Soc. trop. Med. Hyg.*, **51**, 519–526.

SOLTYS, M. A. (1958). Immunity in trypanosomiasis and its effect on chemotherapy. *Vet. Rec.*, **70**, 657–660.

SPINKS, A. (1950). Absorption and persistence of Antrycide. *Brit. J. Pharmacol.*, **5**, 445–454.

TAYLOR, A. E. R. (1960). The absorption of prothidium by *Trypanosoma rhodesiense*. *Brit. J. Pharmacol.*, **15**, 230–234.