# THE MODE OF ACTION OF BAYER '205' ON TRYPANOSOMES

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Since its introduction the drug Bayer '205' has excited the interest of various workers engaged in the study of trypanosomes or of general chemotherapeutic problems. This interest is due partly to its active trypanocidal properties and partly to the peculiarity of its behaviour *in vivo* and *in vitro*. *In vivo* it excites a profound reaction, modifying the coagulability of the blood (Steppuhn, Zeiss u. Brychonenko, 1923) injuring the red blood cells (Sei, 1923), stimulating a lymphocytic response (Kligler & Weitzman, 1924), and, in larger doses, producing marked toxic effect on the kidneys (Duncan and Manson-Bahr, 1924). Unlike most other drugs it is retained in the body in active form for weeks after the injection (Mayer u. Zeiss, 1920, and Ruppert, 1923). Another striking peculiarity is the apparent difference in its trypanocidal power *in vivo* and *in vitro*; *in vivo* it is active in small doses, while *in vitro* it is apparently ineffective.

All those who have experimented with the drug agree as to its profound effect on the parasites in animals; but there is a considerable amount of controversy as to the mode of action on the trypanosomes. Morphological studies by Steffan (1922) and by Hesselbach (1922) indicated a direct effect on the cell protoplasm, and observations by Mayer and Zeiss (1920) and Shintake (1923) suggested an influence on the process of division. The work by Haendel and Yotten (1920) showed that there is a direct combination between the trypanosomes and the drug and that the latter cannot be released by washing. Ruppert (1923), on the other hand, concluded from his experiments that Bayer in its active form is not fixed *in vitro* although it does exert some effect, and that the action of the drug is indirect.

The nature of the action of the drug is of more than theoretical importance. If its effect is really an indirect one, it follows that the usual *in vitro* estimation of the parasiticidal property of a drug is of little value as an indication of the behaviour of the drug in the animal body. It seemed of interest, therefore, to investigate further the effect of the drug on the trypanosomes *in vitro* and to ascertain whether there is any relation between its effect *in vitro* and *in vivo*.

As our experiments were drawing to a close Nauck (1925) published a paper on the same subject. This article does not, therefore, present any new information, but our experiments serve to supplement as well as confirm Nauck's findings.

Nauck worked with a strain of nagana trypanosome and used mice as his culture medium; infecting rabbits, treating them with Bayer, and then, at varying intervals after treatment, infecting mice with the blood of the treated rabbit. Nauck used large doses of Bayer and carried on most of his experiments *in vivo*.

We used a strain of *Tr. evansi* and our procedure differed from Nauck's in that the exposure of the trypanosomes to the drug were made *in vitro* and only the effect on the organisms tested by inoculation into animals to determine loss of virulence. Our experiments were also designed to obtain an approximate quantitive comparison of the trypanocidal power of the drug *in vitro* and *in vivo*.

The following is a brief presentation of the principal experiments bearing on this question.

The object of the first series of experiments was to ascertain whether exposure to the drug in any way affected the virulence of the trypanosomes. After exposure of the organisms for varying lengths of times in varying dilutions of the drug, the trypanosomes were sedimented and injected into guinea-pigs or rabbits. Adequate controls were always made.

EXPERIMENT 1.—The first experiment consisted in exposing suspensions of trypanosomes in serum to which varying dilutions of Bayer were added. The suspension was kept three hours at 25° C. centrifugalized, the supernatant solution containing the drug was decanted, and the sediment inoculated into rabbits and guinea-pigs. The details of this experiment are given in the protocols.

*Protocol a*, Experiment 1. Date 23.9.24.

4 c.c. blood from a guinea-pig containing 4 trypanosomes per microscopic field, defibrinated; 1 c.c. saline added; centrifuged at 700 revolutions for 5 minutes; opalescent fluid withdrawn; divided into 3 parts; to 1 part added Bayer in concentration 1/200; to 1 part 1/400; 1 part-control; kept 3 hours at 25° C. (incubator); centrifuged; fluid decanted, sediment shaken in 1 c.c. saline, injected half into a rabbit (R.) and half into a guinea-pig (G.p.).

R. 12. Injected tryps. in Bayer 1/200; after 5 days positive.

R. 13. Tryps. in Bayer 1/400; after 9 days positive.

R. Control. After 9 days positive.

G.p. 38. Tryps. in Bayer 1/200, after 5 days positive.

G.p. 39. Tryps. in Bayer 1/400, after 10 days positive.

G.p. Control. After 9 days positive.

*Protocol b, Experiment* 1. Date, 2.10.24.

Guinea-pig punctured; numerous parasites; 3½ c.c. blood taken; defibrinated by means of beads; 1 c.c. saline added, centrifuged. To the plasma added Bayer to concent. 1/100, 1/200, 1/400; ½ c.c. quantum taken, kept 3 hours, centrifuged, serum decanted; to sediment added ½ c.c. saline and 0·2 c.c. injected into each animal.

R. 15. Bayer 1/100; negative, observed 37 days; 10.11.24 injected 10 c.c. oil; observed 18 days; negative; superinfected; positive after 7 days.

R. 16. 1/200; died after 5 days; intercurrent infection.

R. 16a. 1/400; died after 5 days; intercurrent infection.

R. 17. Control. Died after 5 days; intercurrent infection.

G.p. 41. Bayer 1/100; observed 37 days; results negative. 10.1.24 injected 4 c.c. oil, observed 18 days; negative. 18.11.24 superinfected; positive after 4 days.

G.p. 42. Bayer 1/200; negative; history same as g.p. 41.

G.p. 43. Bayer 1/400; positive after 14 days.

G.p. Control. Positive after 10 days.

5.10.24 preparation of material the same as that of 2.10.24 and injected again into

R. 18. 1/200.

R. 19. 1/400. To replace R. 16 and R. 16a.

R. 18. 1/200, negative; observed 35 days. 10.1.25, 10 c.c. oil; observed 18 days; negative; superinfected; positive after 5 days.

R. 19. 1/400 positive after 14 days.

It appears that contact of trypanosomes for three hours with a 1 : 100 dilution of the drug is sufficient to destroy their virulence; a 1 : 200 dilution gave variable results; in one experiment the organisms were still infective, in the other not; a three-hour exposure to 1 : 400 dilution did not completely destroy the virulence, but the incubation period was prolonged, indicating a certain degree of injury.

EXPERIMENT 2.—This experiment was similar to No. 1, except that the exposure was for twenty-four hours. The results as shown in the protocol were negative, even in a dilution of 1 : 400.

*Protocol a, Experiment* 2. Date, 13.10.24.

Two guinea-pigs punctured; 3½ c.c. and 2½ c.c. blood taken; positive 7 per field; defibrinated; 2 c.c. saline added; centrifuged; supernatant fluid containing tryps. withdrawn; Bayer added to dilution 1/200, 1/400 in quant. of ½ c.c.; fluid left for control. After 24 *hours* suspensions examined; tryps. alive; sluggish motion; tubes centrifuged; clear fluid decanted saline added to sediment and injected with glass capillaries intraperitoneally.

R. 25. 1/200; negative; observed 30 days. 15.11.24 injected 10 c.c. oil; negative. 28.11.24 superinfected; positive; 11 days incubation.

R. 24. 1/400; negative; observed 30 days; 15.11.24 injected 10 c.c. oil; negative. 28.11.24 superinfected; positive; incubation 9 days.

R. 25. Control; positive after 6 days.

*Protocol b, Experiment* 2. 12.1.24.

Two guinea-pigs bled; 3½ c.c. blood; defibrinated; centrifuged slow speed until fluid opalescent.

| 0.25 c.c. susp. of tryps.<br>0.25 c.c. 1/100 Bayer | 0.25 c.c. susp. of tryps.<br>0.25 c.c. 1/200 Bayer | 0.25 c.c. susp. of tryps.<br>0.25 c.c. saline |
|---|---|---|
| 0.50 c.c. 1/200<br>After 24 hours at 25° C.—1/200; Alive; persistaltic movements of the undulating membrane. Motion sluggish. | 0.50 c.c. 1/400<br>Control for motility; 1/400; Active movement. | Control<br>Control; Active movement. |

Tubes centrifuged 10 minutes at highest speed; clear serum decanted; added ½ c.c. saline to each tube; injected 0·2 c.c. into each animal.

R. 27. 1/200 negative; observed 44 days; superinfected; positive; 5 days incubation.

R. 28. 1/400 negative; after 44 days superinfected; positive after 5 days.

R. 26. Control; positive after 5 days.

EXPERIMENT 3.—This was a repetition of Experiment 1, namely, a three-hour exposure, but a smaller number of trypanosomes was injected; the results showed that even an exposure of three hours to 1 : 400 dilution of the drug renders the organisms non-infective.

G.p. 34. 3 c.c. (tryps. 1 per field) taken; defibrinated; added 1 c.c. saline; centrifuged; dilutions to 1/200, 1/400 Bayer made as in experiment date 12.1.25; kept 3·15 hours in incubator at 25° C.; centrifuged, clear fluid decanted; to sediment added ½ c.c. saline; shaken; 0·2 c.c. injected into each animal.

R. 34. 1/200 negative; observed 19 days; superinfected 1.3.25; positive after 7 days.

R. 35. 1/400; negative; observed 19 days; 1.3.25 superinfected; positive; incubation 5 days.

R. Control. Positive; incubation 5 days.

EXPERIMENT 4.—This experiment was a repetition of Experiment 2 (twenty-four hours exposure), except that higher dilutions of the drug were used (800 and 1,600). The results indicated that even as low a concentration of the drug as 1 : 1600 is sufficient to destroy the virulence of the organisms. The control trypanosomes in each case were put through the same manipulations as the drug-exposed organisms, so that the possibility of loss of virulence through mechanical injury was eliminated.

*Protocol Experiment* 4. 7.2.25.

Two guinea-pigs bled 2½ c.c. ; tryps. 3 per field ; defibrinated ; added 1 c.c. saline ; centrifuged at 750 revolutions ; opalescent fluid still containing few r.b.c used. Dilutions with Bayer made ; opalescent fluid taken ; 0·9 c.c., ½ c.c., ½, etc., to the first tube added ; 0·1 c.c. 10% Bayer ; dilution obtained 1/100 ; ½ c.c. transferred to second tube ; etc. Final dilutions 1/100, 1/200, 1/400, 1/800, 1/1600 ; tubes left for 24 hours in the incubator at 25° C. After 24 hours examined ; 1/100—slight undulant movement ; 1/200 sluggish movement
1/400 active movement
1/800 active movement
1/1600 active movement
Control active movement.

Tubes 1/800, 1/1600 and control ; centrifuged ; clear fluid decanted ; sediment diluted in ½ c.c. saline ; tryps. still active ; injected 1/800 into rabbit 48 ; 1/1600 into rabbit 49 ; control into rabbit 50.

R. 48. Observed 30 days ; negative.
R. 49. Observed 30 days ; negative.
R. Control. Positive after 6 days ; heavy infection.

This series of experiments showed that Bayer ' 205 ' has a marked effect on trypanosomes *in vitro*. Ordinarily this effect is overlooked because the result is judged by the motility of organisms. The motility is not, however, an index of protoplasmic injury and the principal effect of the drug lies in a lowering or destruction of virulence due presumably to cell injury.

On the basis of these experiments made *in vitro*, it appears that the action of the drug *in vivo* is also direct and that the therapeutic as well as prophylactic action of the drug depends on the concentration of the drug in the body and the rate of elimination by a given host.

Previous therapeutic experiments showed clearly that the drug is active in certain proportional doses, at least in so far as rabbits and guinea-pigs are concerned. A dose of 0·1 gm. per kilo cured all animals ; 0·05 gms. per kilo gave about 80 per cent. cures, while 0·005 gms. per kilo was not effective.

This relation of dose to effect is further illustrated by the following experiment. The purpose of this experiment was to see whether an infection can be aborted by a dose of Bayer smaller than the therapeutic dose. As is seen from the protocol below, the abortive dose is the same as the therapeutic dose ; 0·05 gm. per kilo aborted the infection, while 0·005 gm. did not.

*Protocol Experiment* 5. 13.12.24.

Two rabbits of same weight infected 13.12.24 ; on 16.12.24 rabbit 015 given 0·005 gm. Bayer per kilo and rabbit 016 was given 0·05 gm. per kilo. R. 015, 28.12.24, blood positive. R. 016, blood negative ; observed 32 days and continued negative.

The next series of experiments dealt with the prophylactic property of the drug. The object was to ascertain whether there was any relation between dose and the duration of protection. In other words, we tried to determine the relation between concentration of the drug and prevention of infection.

*Experiment* 6. Three rabbits injected with different doses of Bayer and at varying intervals ; after treatment the animals were infected.

R. 017. 0·05 gm. Bayer injected 16.12.24 ; infected 35 days later ; negative.

R. 018. 0·05 gm. Bayer injected 16.12.24 ; infected 35 days later ; negative. Infected again after three months ; positive, after incubation of 15 days.

R. 019. Injected 0·1 gm. Bayer per kilo ; one month later infected ; negative ; reinfected after another month ; trypanosomes appeared in the circulation after a delay of three weeks.

This experiment indicates that Bayer apparently confers protection only so long as the drug remains in the body in a concentration sufficient to affect the parasites. This relation between concentration of drug and protection is further emphasised by the subsequent experiment.

Experiment 7.—The object of this experiment was to determine whether the minimal protective dose corresponds to the minimal therapeutic dose. In this experiment the infection was given within a week or two after the injection of the drug. It is evident from the results that a dose of ·005 gm. per kilo failed to give any protection just as this dose is devoid of any therapeutic effect.

R. 022. Given ·005 gms. Bayer ; two weeks later infected ; positive after ten days.

R. 023. Given 0·01 gm. Bayer per kilo and infection followed 8 days later ; results negative ; animal observed two months.

R. 024. Given ·005 gms. Bayer per kilo ; infected 10 days later ; positive after 7 days.

Analysis of Experiments.—The various experiments described above bring out two facts. First that contrary to our previous belief that Bayer exerts little trypanocidal action *in vitro*, it appears that the drug has a marked effect on the cell so that a dilution of 1 : 1600 is sufficient to destroy the virulence of the organisms. The other fact is that, in rabbits at least, the therapeutic, abortive and prophylactic doses are similar.

It is difficult to make comparisons between *in vitro* and *in vivo* effect, because it is not possible to determine the amount of drug which remains in circulation. The work of Mayer and Zeiss indicates that the drug is bound in the blood stream, by the serum, and is thus retained for many weeks. If the weight of the blood is accepted as approximately 1/15 the total body weight, it is possible to make a rough estimate of the effective dilution of the drug in the circulation. Our experiments show that doses of 0·005 gm. per kilo, or a dilution of 1 : 3000, fails either to protect or cure an animal while 0·01 gms. per kilo, or a dilution of 1 : 1500, is effective in a proportion of cases. Even if we assume that only 50 per cent. of the drug is bound in the serum, the effective doses *in vivo* correspond fairly well with those *in vitro*. A still further correspondence is the fact that when small doses of the drug are given the trypanosomes disappear from the circulation only sixteen to eighteen hours after treatment.

The rational conclusion then is that the therapeutic property of Bayer ' 205 ' is due to a direct injury to the trypanosomes which renders them avirulent for the host and thus readily destroyed and eliminated. The difference observed in different hosts are probably due to the rate of elimination of the drug, or in other words, to the residual concentration of the drug in the circulation.

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