ACTION OF THE COLOURS OF BENZIDINE ON MICE INFECTED WITH TRYPANOSOMA DIMORPHON.

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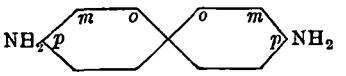
The investigations which form the subject of this paper were undertaken at the Pasteur Institute, on the suggestion of M. Mesnil. It is a pleasure to acknowledge my great indebtedness to M. Mesnil for the help and advice he so willingly rendered me whilst I worked in his laboratory. M. Nicolle and he very kindly placed at my disposal their unique collection of colours, which had been supplied them by various firms (notably the Farbenfabriken of Elberfeld). By their suggestions, without which this work would not have been accomplished, they saved me much time and labour. MM. Nicolle and Mesnil had been studying the influence of a large number of colours on various trypanosomes, chiefly *Trypanosoma Brucei* and to a less extent *T. Evansi*, *T. equinum*, and *T. Gambiense*. Their work was undertaken after the publication of Ehrlich and Shiga's paper on the action of Trypanroth, one of the benzidine colours, on *Trypanosoma equinum*. M. Mesnil suggested that I should investigate the action of these colours on *T. dimorphon* which differs in many ways from the above trypanosomes.

Chemistry of the benzidine colours.

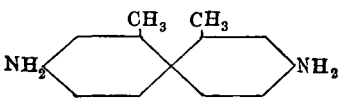
Before proceeding with the question of their action, it will be first necessary to explain briefly the somewhat complicated constitution of these colouring matters.

As regards its chemical constitution, each colour may be considered as consisting of two parts, a base and a side-chain. The base, which is

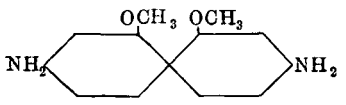
benzidine, or one of its homologues or derivatives, contains two amido (NH_2) groups which occupy the positions indicated in the figure. Benzidine is composed of two benzene rings, united apex to apex, in which two atoms of hydrogen have been replaced by two amido groups. With reference to the point of union of the two benzene rings, substituting groups may enter the nucleus at the ortho, meta, or para (*o*, *m*, *p*) positions. In benzidine the two amido groups have taken up the para position. In other bases, all of which have amido groups in the para position and may be referred to benzidine as their type, other substituting groups are found in the ortho and meta positions.



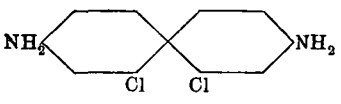
Benzidine.



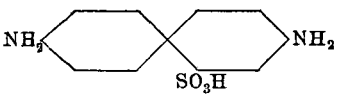
Tolidine.



Dianisidine.

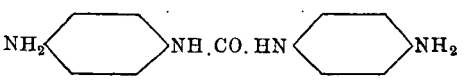


O. Dichlorbenzidine.



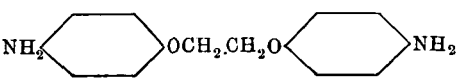
Benzidine orthomonosulphonic acid.

In some cases the two benzene rings are not directly united to one another, but are joined by an intermediate group. This is the case with the base para diamidodiphenylurea where the group urea



p. diamidodiphenylurea.

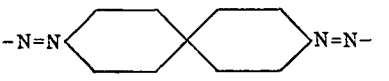
($NH_2.CO.NH_2$) is the connecting link. In another case, the group glycol ($OH.CH_2.CH_2.OH$) forms the intermediate link, giving rise to the compound para diamidophenylglycolether.



p. diamidophenylglycolether.

Whatever their constitution, all these bases agree in having the two amido groups in the para positions. These amido (NH_2) groups

may be converted into diazo (N = N –) groups. With benzidine this would give diazotised benzidine, a compound which only theoretically exists, and which unites directly it is formed with some other group. By diazotising benzidine in the presence of one of the side-chains presently to be described, the benzidine may be caused to unite to this side-chain, and thus to give rise to one of the benzidine colours in which there is a benzidine nucleus united to a side-chain in each of its para positions.

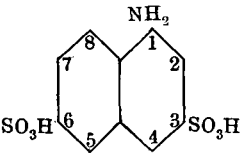


Diazotised benzidine.

The following is a list of the bases which have been employed in these experiments. After each one is placed the abbreviation which is used in the complete list of colours given below.

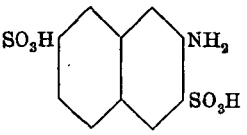
1. Benzidine (B).
2. Dianisidine (D).
3. Tolidine (T).
4. Dichlorbenzidine (Di.Cl.B).
5. Dichlordianisidine (Di.Cl.D).
6. Benzidineorthomonosulphonic acid (B.o.m.s).
7. Benzidinemetamonosulphonic acid (B.m.m.s).
8. Benzidineorthodisulphonic acid (B.o.di.s).
9. Benzidinemetadisulphonic acid (B.m.di.s).
10. Paradiamidodiphenylurea (P.d.d.u).
11. Diamidophenylglycolether (D.p.g.e).

The side-chains are sulphonic acid derivatives of naphthylamine. It was shown by Nicolle and Mesnil that unless the side-chain contained at least one amido (NH_2) group, and at least two (SO_3H) groups it would be inactive against trypanosomes. Accordingly, the simplest active side-chain would be one of the type α naphthylamine disulphonic acid 136. By varying the position of the two (SO_3H) groups other α naphthylamine disulphonic acid compounds may be obtained as the types 148, 147, 146, 138, 137, etc. If the amido (NH_2) group be in the 2 position the β naphthylamine disulphonic acids are obtained as the types 237, 236, 257, etc. Similarly occur compounds with two amido groups, the naphthalenediamine disulphonic acids, as the form 1836, which may be considered to be derived from the α naphthylamine

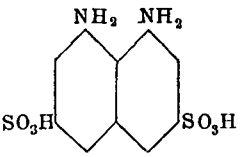


α naphthylamine disulphonic acid 136.

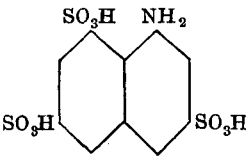
disulphonic acid 136 by the introduction of a second amido group in position 8. There are also α and β naphthylamine trisulphonic acids as



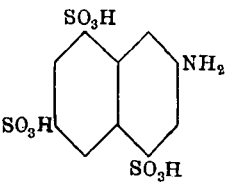
β naphthylamine disulphonic acid 237.



Naphthalenediamine disulphonic acid 1836.

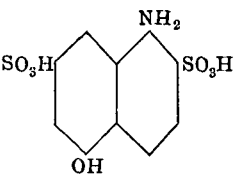


α naphthylamine trisulphonic acid 1368



β naphthylamine trisulphonic acid 2468.

Amidonaphthol disulphonic acids also occur in which a hydroxyl group (OH) has entered the nucleus. Of such a type is the compound amidonaphthol disulphonic acid 1527. Those side-chains which contain other groups besides the necessary one (NH_2) and two (SO_3H) groups, may be considered as being derived from either the α or β naphthylamine disulphonic acids by the introduction of these other substituting groups. The realisation of this fact is important, for if the action of a compound of the type α naphthylamine disulphonic acid 136 is known, and also that of a compound of the type α naphthylamine trisulphonic acid 1368, any difference in the actions of the two compounds must be due to the fact that in the first, there is wanting the third (SO_3H) group, which is present in position 8 in the second. As stated above, each colour is composed of a base united to two side-chains. Any one of the above-mentioned bases may be united to any one of the side-chains. In naming these colours the side-chain is named first, and then the base to which it is combined. Thus the colour amidonaphthol disulphonic acid 1836 + dichlorbenzidine is the base dichlorbenzidine united to two molecules of the side-chain amidonaphthol disulphonic acid 1836.



Amidonapthol disulphonic acid 1527.

In the case of the amidonaphthol disulphonic acids it happens that the union between base and side-chain may be effected in an acid

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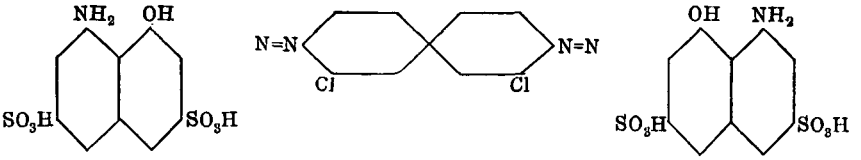
as well as in an alkaline medium. In other cases the union is only brought about in alkaline medium. When the union can take place in both media, the compound resulting from the union in an acid medium differs from that obtained from a union in an alkaline medium. This difference is due to the fact that in one medium the side-chain unites to the base by one position (position 2 for instance) while in the medium of opposite reaction the side-chain is, as it were, turned completely round, and unites to the base by the corresponding position on the opposite side. For position 2 in one medium it would be position 7 in the other medium. The resulting compounds, though differing so slightly in their chemical constitution, show marked differences in their therapeutic actions.



Amidonaphthol disulphonic acid 1836 + dichlorbenzidine.

In the list of colours given below, where the union between base and side-chains has been in acid medium (ac.) is inserted, and where one side-chain has been united in acid and the other in alkaline medium (ac. alk.) is inserted. In all other cases the union of both side-chains has been in alkaline medium.

These sulphonic acids are not employed as free acids, but chiefly as salts in union with sodium.

For further particulars as to the chemistry of these complex colours readers are referred to the papers by Nicolle and Mesnil (1906) and to treatises on industrial chemistry.

Irregularity of the infection produced by Trypanosoma dimorphon.

In the treatment of mice infected with *T. dimorphon*, one encounters a difficulty which is not present in the treatment of the infection produced by *T. Brucei*. The strain of *T. Brucei* used by Nicolle and Mesnil gave rise to an infection of such regularity that mice died in exactly three days after trypanosomes appeared in the peripheral blood, so that it was possible to appreciate a survival under treatment of less than 24 hours. *T. dimorphon*, on the contrary, produces in mice an irregular infection which brings about the death of the animal in

any time between two weeks and three months, or even more. Rarely do the trypanosomes disappear from the peripheral blood when once they have been found. When this does occur, usually the trypanosomes are absent only for a few days, while very exceptionally there may be a complete and permanent disappearance of the parasites. These irregularities in the infection compared with that produced by *T. Brucei*, make it much more difficult to judge of the action of any drug, and also, in this instance, render the criterion used by Nicolle and Mesnil impracticable. It is impossible to appreciate any increase in the length of life of the animals, since death takes place at such varying intervals after infection. For this reason I noted the number of days during which trypanosomes remained absent from the peripheral blood after treatment with any colour. This was taken as an index of the activity of the drug in question. The mice were treated on the second day of the infection. The incubation period is very irregular, the average for 121 mice being ten days. In some cases the mice were naturally immune and resisted several inoculations. The duration of the infection is, generally speaking, in proportion to the number of trypanosomes present in the blood. Where multiplication is rapid, and the blood becomes quickly crowded with large numbers of trypanosomes, death will take place more rapidly than in those cases where multiplication is slow. Death appears to be due more to the mass of trypanosomes present than to any toxic influence they may have. With enormous numbers of trypanosomes present in the blood, mice may appear practically normal. They will then usually die quite suddenly after convulsions, which suggest some sudden stoppage to the cerebral circulation. In sections of the brain of these animals the cerebral vessels are seen to be crowded with trypanosomes to such an extent as would quite explain the convulsions and death, apart from any toxic influence. That there is some toxic influence is well shown by the enormous hypertrophy of the spleen that takes place in the more chronic cases. In these the spleen forms a large tumour in the abdomen, and may increase to such a size as to be one-eighth of the weight of the whole body.

Method of conducting the experiments.

The manner in which the experiments were carried out was as follows:—Mice were inoculated subcutaneously with blood from an infected animal. The mice were examined from day to day and, on

TABLE I.

Name of colour		No. of mice	*T. dimorphon*	*T. Brucei*
α naphthylamine disulphonic acid	148 + B.	2	6	0
,, ,,	148 + T.	1	0	0
,, ,,	147 + B.	2	27	1
,, ,,	147 + D.	1	0	0
,, ,,	147 + T.	1	0	0
,, ,,	146 + B.	1	0	0
,, ,,	138 + B.	2	5	1
,, ,,	137 + B.	1	0	0½
,, ,,	136 + B.	1	0	0
,, ,,	168 + B.	2	5	0
,, ,,	168 + D.	1	0	0
,, ,,	157 + B.	6	14	14½
,, ,,	157 + D.	1	0	0
β naphthylamine disulphonic acid	237 + B.	2	4	1½
,, ,,	237 + T.	1	2	0
,, ,,	236 + T.	1	12	8
,, ,,	236 + Di.Cl.B.	1	0	4
,, ,,	236 + B.o.m.S. (Trypanroth)	5	14	8
,, ,,	236 + B.m.m.S. ...	1	6	0
,, ,,	236 + B.o.di.S. ...	1	2	1
,, ,,	236 + B.m.di.S. ...	1	0	0
,, ,,	257 + B.	3	6	5
,, ,,	257 + T.	1	2	0
α naphthylamine trisulphonic acid	1468 + B.	2	2	0
,, ,,	1368 + Di.Cl.B.	2	0	1
β naphthylamine trisulphonic acid	2367 + B.	1	0	2
,, ,,	2367 + T.	1	0	0
,, ,,	2468 + T.	1	0	0½
Amidonaphthol disulphonic acid	1527 + B.	2	0	1
,, ,,	1527 + D.	1	0	0
,, ,,	1836 + B.	1	0	7½
,, ,,	1836 + D.	2	0	14
,, ,,	1836 + T.	2	5	8
,, ,,	1836 + T. (ac. alk.) ...	4	0	8
,, ,,	1836 + Di.Cl.B. (alk. alk.) ...	4	4	8
,, ,,	1836 + Di.Cl.B. (ac. ac.) ...	1	0	5
,, ,,	1836 + Di.Cl.D.	2	5	10
,, ,,	1836 + D.p.g.e.	2	3	11
,, ,,	1836 + P.d.d.u.	4	4	18
,, ,,	2517 + B.	1	0	5
,, ,,	2517 + T.	1	4	1
,, ,,	1846 + B.	1	3	5½
,, ,,	1846 + T.	2	5	18
,, ,,	1824 + D.	1	4	3
,, ,,	2368 + B.	1	6	1
,, ,,	2836 + B.	2	1	0
Glycine of amidonaphthol disulphonic acid	1836 + B.	2	8	8
,, ,,	1836 + D.	1	0	2½
,, ,,	1836 + T.	2	6	3
Acetyl of amidonaphthol disulphonic acid	1836 + D.	1	0	0
Naphthalenediamine disulphonic acid	2736 + B. (Alpha) ...	4	8	8
,, ,,	1836 + B.	1	0	0
,, ,,	1836 + D.	1	0	0½
,, ,,	1836 + T.	2	8	1

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the second day of the infection, each mouse received under the skin of the back about 1 c.cm. of a 1% solution of the colour in distilled water. Physiological saline solution was not used, as the salt is liable to cause precipitation of the colour. The dose of colour for a mouse of 15 to 20 grammes weight was usually one centigramme in 1% solution. In the case of Trypanroth the dose was half this amount. With larger mice the dose was increased according to their size. To obtain the best results a careful selection of mice must be made. They should if possible be quite 20 grammes in weight and in healthy condition. Small mice are much more susceptible to the toxic influences of the colours than the larger ones. Where a colour is having a favourable action the mice will often increase in weight, while the reverse is the case with the inactive colours. It may be possible to predict a coming relapse by the continued loss of weight of the animals.

Treatment of mice by a single dose of colour.

In Table I will be found a complete list of the colours employed in these experiments. In the first column is given the number of mice used for each colour tested. In the second column is recorded the maximum result obtained in any case, being the number of days

TABLE II.

Name of colour		*T. dimorphon*	*T. Brucei*	*T. equinum*	*T. Evansi*	*T. gambiense*
α naphthylamine disulphonic acid	157+B.	14	14½	15	—	5
β naphthylamine disulphonic acid	236+B.o.m.S. ...	14	∞	∞	∞	10 (Monkeys) 20 (Rats)
,, ,, ,,	236+Di.Cl.B. ...	0	4	2	—	—
Amidonaphthol disulphonic acid	1836+B.	0	7½	3	—	—
,, ,, ,,	1836+D.	0	14	13	—	—
,, ,, ,,	1836+T.	5	∞	∞	∞?	—
,, ,, ,,	1836+T. (ac. alk.) ...	0	∞	∞	∞?	10
,, ,, ,,	1836+Di.Cl.B. ...	4	∞	∞	∞	22
,, ,, ,,	1836+Di.Cl.B. (ac. ac.)	0	5	5½	—	—
,, ,, ,,	1836+P.d.d.u. ...	4	18	14	20	30
,, ,, ,,	1836+D.p.g.e. ...	3	11	—	—	26
,, ,, ,,	1846+T.	5	18	7	—	—
Naphthalenediamine disulphonic acid ...	2736+B.	∞	∞	∞	28	8

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during which trypanosomes remained absent from the blood after treatment. In the third column are given the results obtained by Nicolle and Mesnil with *T. Brucei*. It will be seen that with only one colour (naphthalenediamine disulphonic acid 2736 and benzidine) was a permanent cure obtained, and this in only one mouse out of four. The great majority of these colours have little or no action, while only five gave results over ten days.

In Table II the colours found by Nicolle and Mesnil to be best for the trypanosomes of Nagana, Surra, Mal de Caderas, and Sleeping Sickness are compared with the results obtained for *T. dimorphon*. It must be remembered that except for *T. dimorphon* the results represent the number of days of survival of the animals, and are not therefore strictly comparable with the results for *T. dimorphon*.

Action of side-chains and bases and comparison with results obtained for T. Brucei by Nicolle and Mesnil.

Having employed a very large number of colours in their treatment of Nagana, Nicolle and Mesnil were able, by a method of comparison, to arrive at some of the points of chemical constitution which regulate the action of the colours on trypanosomes. One of these laws has already been mentioned. I refer to the necessary presence of at least one (NH_2) group and two (SO_3H) groups in the side-chain. Another is the method of union of the two benzene rings in the base. Any other method of union of the two rings than by their apices produces an inactive base.

1. *Side-chains.* The side-chains may be divided into families according to the position of the (SO_3H) groups. Each family contains as its type a side-chain which has one (NH_2) group and two (SO_3H) groups. All other members of this family have two (SO_3H) groups in the same position as in the type and the (NH_2) group in the α or β position; a third (SO_3H) group, a second (NH_2) group, or an (OH) group may be present also. α naphthylamine disulphonic acid 157 is the type of one family, while in this same family are also β naphthylamine disulphonic acid 257, and all other side-chains which have the (SO_3H) groups in the positions 57. The action of α naphthylamine disulphonic acid 157 being known, any difference in the activity of the β compound must be due to the fact that the (NH_2) group has changed position from α to β. In this manner all the colours in one family can be compared, and the influence of the sub-

stituting groups arrived at, as any variation in their activity from that of the type must be due to the presence of the other groups. To do this completely a much larger number of colours would have to be tried than are here recorded, but there are sufficient to illustrate some of the points.

Sulphonic acid groups 5, 7. The side-chains which have their (SO_3H) groups in this position give better results for *T. dimorphon* than for *T. Brucei*. The α naphthylamine disulphonic acid 157 + B. is more active than the β compound, where the amido group has changed from the 1 to the 2 position. This is true as well for *T. dimorphon* as *T. Brucei*. It is not, however, a general rule that the 1 position is better than the 2 position for the amido group. With other arrangements of the (SO_3H) groups the reverse is the case.

Sulphonic acid groups 3, 6. These are generally less active for *T. dimorphon* than for *T. Brucei*. However, the best side-chain for *T. dimorphon*, as also for *T. Brucei*, is in this family. The best side-chain for *T. dimorphon* must be naphthalenediamine disulphonic acid 2736 (see Table I).

The union of the base Di.Cl.B with amidonaphthol disulphonic acid 1836 in acid medium gives a colour which is quite inactive against *T. dimorphon*, though with *T. Brucei* it prolonged life for five days. This same side-chain united with tolidine, first in acid, and then in alkaline medium, is also quite inactive against *T. dimorphon*. Thus the effect of uniting a base to a side-chain in acid, instead of alkaline medium, is to produce a greater diminution of curative power with *T. dimorphon* than with *T. Brucei*.

With β naphthylamine disulphonic acid 236 + T the results for *T. dimorphon* and *T. Brucei* are 12 and 8. If a third (SO_3H) group is introduced in position 7, as in β naphthylamine trisulphonic acid 2367 + T, the activity is reduced to *nil* for both trypanosomes. Here then the third (SO_3H) group is a disadvantage, and this is in agreement with Nicolle and Mesnil, who say that the introduction of a third (SO_3H) group is more an inconvenience than advantage.

The compound β naphthylamine trisulphonic acid 2367 + B gives 0 for *T. dimorphon* and 2 for *T. Brucei*. In the compound naphthalenediamine disulphonic acid 2736 + B one of the (SO_3H) groups of the trisulphonic acid has been replaced by (NH_2). This change results in a great increase in activity, the colour then giving ∞ for both trypanosomes. This example illustrates very well how a slight change in chemical constitution may be followed by a marked change in

therapeutic action. On the contrary, the introduction of an (OH) group into the nucleus of α naphthylamine disulphonic acid 136 + B producing amidonaphthol disulphonic acid 1836 + B is no improvement for *T. dimorphon*, but is for *T. Brucei*, while further, the introduction of a second amido group into the same compound, giving naphthalene-diamine disulphonic acid 1836 + B is no improvement, giving 0 for both trypanosomes.

The acetyl derivative of the side-chain amidonaphthol disulphonic acid 1836 is inactive for *T. dimorphon* as it was for *T. Brucei* while the glycine derivative has the same action on both trypanosomes.

Sulphonic acid groups 6, 8. These give better results than with *T. Brucei.*

The introduction of a third (SO_3H) group into the compound α naphthylamine disulphonic acid 168 + B giving α napthylamine trisulphonic acid 1468 + B causes here again a reduction in the activity.

Sulphonic acid groups 3, 7. With this arrangement of the (SO_3H) groups the β position of the amido group is better than the α position, the contrary of what is found with the 5, 7 positions of the (SO_3H) groups. This is true for both trypanosomes.

Sulphonic acid groups 4, 6. The compound α naphthylamine disulphonic acid 146 + B gives 0 for both trypanosomes. With the addition of a third (SO_3H) group in position 8, there is a slightly better action on *T. dimorphon*, this being an exception to the usual rule. The introduction of an (OH) group into the same compound, giving amidonaphthol disulphonic acid 1846 + B, markedly increases the action on both trypanosomes.

Sulphonic acid groups 4, 8. The compounds α naphthylamine disulphonic acid 148 + B and the α naphthylamine trisulphonic acid 1468 + B again illustrate the unfavourable action of the third (SO_3H) group.

The other arrangements of the (SO_3H) groups were not tried in a sufficient number of colours to enable any comparisons to be made.

The best arrangement for the (SO_3H) groups for *T. dimorphon* in order of merit are 3, 6, 4, 7 (bad for *T. Brucei*), 5, 7 and 4, 8 (bad for *T. Brucei*).

2 *bases.* A complete list of the bases used has been given in the chemical part of this paper.

A comparison between the activities of the different bases may be obtained by comparing their activities when united to the same side-chain. As pointed out by Nicolle and Mesnil, it does not follow that a good base united to a good side-chain will give rise to a good

colour. There exists some relation between the base and the side-chain with which it is combined, that regulates the action of the colour produced. One of the laws which is true for the influence of the base on the side-chain was discovered by Nicolle and Mesnil for *T. Brucei.* It is also true for *T. dimorphon.* If a side-chain with a (SO_3H) group in position 6 is tried in combination with benzidine, dianisidine, and tolidine, it will be found that the action on the trypanosomes is greatest in the combination with tolidine, and that it decreases towards benzidine, dianisidine occupying an intermediate position. If, however, the side-chain has a (SO_3H) group in position 7 the order of the activities is reversed so that the combination with benzidine has the greatest power, and with tolidine the least. With *T. dimorphon* there is one exception to this law where the result obtained with amidonaphthol disulphonic acid 2517 + B = 0 and amidonaphthol disulphonic acid + T = 4.

Benzidine orthomonosulphonic acid, and benzidine orthodisulphonic acid are better than the corresponding meta compounds. (This is in combination with β naphthylamine disulphonic acid 236.) Dichlorbenzidine is better than benzidine (in combination with amidonaphthol disulphonic acid 1836). These results are in agreement with those obtained for *T. Brucei.* On the contrary, however, dichlordianisidine is better than dichlorbenzidine for *T. dimorphon.*

Comparison with other trypanosomes.

It was found by Nicolle and Mesnil that the best side-chain for the four trypanosomes *T. Brucei, T. equinum, T. Evansi,* and *T. Gambiense* is amidonaphthol disulphonic acid 1836, which gave an ∞ result (except with *T. Gambiense*). The next is β naphthylamine disulphonic acid, except for *T. Gambiense* and *T. Brucei,* where naphthalenediamine disulphonic acid 2736 is second. This last side-chain is the third in value for *T. equinum* and *T. Evansi.*

In their reaction to the side-chains just mentioned these four trypanosomes show a close agreement. *T. dimorphon* varies considerably in its reaction to these side-chains. For this trypanosome the best side-chain is naphthalenediamine disulphonic acid 2736, and is the only one which gave an ∞ result. Then come the side-chains α naphthylamine disulphonic acid 147 and 157, the glycine of amidonaphthol disulphonic acid 1836, and finally amidonaphthol disulphonic

acid 1836 itself, which we have seen was the best for the other trypanosomes.

For the four trypanosomes mentioned above the best base is dichlorbenzidine, except that for *T. Gambiense* para diamidodiphenylurea is superior. Tolidine is as good, or nearly so, for *T. Brucei*, but is inferior for the other three.

For *T. dimorphon* dichlorbenzidine is generally a very bad base. In combination with β naphthylamine disulphonic acid 236 benzidine-orthomonosulphonic acid is superior to the other benzidine sulphonic acids as is true for *T. Brucei*.

As regards their reaction to the colours the four trypanosomes, which have just been considered, show marked differences from *T. dimorphon*. The colours which were found to be the most active for these four trypanosomes were blue, while the red colours were inferior to these, though still active. With *T. dimorphon* it was found that the blue colours had usually no action whatever in causing the trypanosomes to disappear, and it often happened that the animals treated died more quickly than if they had been left alone. In one or two cases the trypanosomes disappeared for a day or two after the treatment with a blue colour, but the best colour for the three trypanosomes *T. Brucei*, *T. equinum*, and *T. Evansi*, namely, amidonaphthol disulphonic acid 1836 + Di.Cl.B only gave a result of four days with *T. dimorphon*. The same result was obtained for *T. dimorphon* with the colour, also blue, which was found to be most active against *T. Gambiense* (see Tables I and II). With *T. dimorphon* it was the red colours which were most active, though only one of these gave an ∞ result. This colour, naphthalenediamine disulphonic acid 2736 + B, also gave the same result in the case of *T. Brucei* and *T. equinum*, and was active to a less extent with the other two trypanosomes. It thus appears that *T. dimorphon* as regards its reaction to the red colours is allied to these trypanosomes, but as regards its reaction to the blue colours, is widely separated from them. No reason can be offered as to the cause of this difference between the red and blue colours. As a rule the blue colours are found amongst the amidonaphthol compounds, while the red ones occur amongst the others.

Treatment of relapses.

This was not in any way satisfactory. The further treatment of a mouse after having relapsed, though it might cause a second disappearance of the trypanosomes, was no more successful in bringing about a cure than the first treatment. The time during which the trypanosomes remained absent from the blood after the second treatment was always shorter than after the first treatment. Usually a third treatment at the second relapse would kill the mice, but in the few cases that the mice survived a third treatment, a third disappearance of the trypanosomes would take place, and this for a shorter time than either after the first or second treatments. In one mouse treated with Trypanroth a third relapse took place, and a fourth treatment, followed by a fourth disappearance of the trypanosomes, was made. This mouse, however, died, evidently from the toxic effect of the drug, two days later. This diminution in the length of time during which trypanosomes remained absent from the blood as the relapses increased in number, is in agreement with the observations of Nicolle and Mesnil on the trypanosomes with which they worked. They, however, found that in certain cases after a number of relapses and treatments, the colour became quite inactive against the trypanosomes.

In the treatment of the relapses of *T. dimorphon* the colour employed was always the same as had been given at the first treatment. It might be possible to obtain better results by using for the second treatment a different colour. No attempt was made to treat the relapses unless five days had elapsed since the first treatment. If trypanosomes were found to have reappeared in the blood on the sixth day, half the initial dose of colour was at once administered. This dose was increased with the interval of time from the first treatment.

Treatment by repeated doses of colour.

With single doses of colour, we have seen that only with the colour naphthalenediamine disulphonic acid 2736 + B did a permanent cure result. With four other colours results over ten days were obtained (see Table I). With these five colours attempts were made to treat the mice by giving repeated doses of colour so as to prevent, if possible, the relapses. For each colour three mice were used. In one series treatment was made on the 2nd, 5th, 8th, and 11th days of the infection, in another series on the 2nd, 7th, and 12th days,

TABLE III.

Name of colour	No. of experiment	Days of treatment	Doses on corresponding days (centigrams)	Results
α naphthylamine disulphonic acid 147 + benzidine	1	2, 5, 8, 11	1·2, ·3, ·3, ·3	Relapse 21 days after 1st treatment.
	2	2, 7, 12	1·3, ·5, ·5	,, 19 ,, ,, ,,
	3	2, 10	1·2, ·8	,, 5 ,, ,, ,,
α naphthylamine disulphonic acid 157 + benzidine	1	2, 5, 8	1·2, ·3, ·3	Died without relapse 7 days after 1st treatment.
	2	2, 7, 12	1·2, ·5, ·5	,, ,, ,, 21 ,, ,, ,,
	3	2, 10	1·2, ·8	,, ,, ,, 14 ,, ,, ,,
β naphthylamine disulphonic acid 236 + tolidine	1	2, 5, 8, 11	1·2, ·3, ·3, ·3	Died without relapse 12 days after 1st treatment.
	2	2, 7, 12	1·0, ·5, ·5	,, ,, ,, 14 ,, ,, ,,
	3	2, 10	1·2, ·8	,, ,, ,, 13 ,, ,, ,,
β naphthylamine disulphonic acid 236 + benzidine orthomonosulphonic acid (Trypanroth)	1	2, 5, 8, 11	·5, 1·5, 1·5, 1·5	Relapse 14 days after 1st treatment.
	2	2, 7, 12, 17	·6, ·25, ·25, ·25	,, 24 ,, ,, ,,
	3	2, 10, 18	·6, ·4, ·2	No relapse.
naphthalenediamine disulphonic acid 2736 + benzidine	1	2, 5, 8, 11	1·2, ·3, ·3, ·3	Died without relapse 14 days after 1st treatment.
	2	2, 5, 8, 11	1·2, ·3, ·3, ·3	No relapse.
	3	2, 7, 12	1·2, ·5, ·5	,,
	4	2, 10, 18	1·2, ·8, ·4	,,

and in the last series on the 2nd and 10th days. The details of the treatment are given in Table III. The second and third colours in this table caused such extensive necroses of the skin that all the mice died from this cause without having relapsed. The fourth colour (Trypanroth) was able to bring about a permanent cure in the third mouse. With the last colour all the mice were cured with the exception of the first, which died from the toxic effects of the colour. This last colour, which was the one which gave an ∞ result with the single dose, is evidently superior to the other colours as far as its action on *T. dimorphon* is concerned.

Other strains of T. dimorphon.

To further test the results obtained with the colour naphthalene-diamine disulphonic acid 2736 + B, two other strains of *T. dimorphon* were used. These were very kindly given me by Dr Gustave Martin, who had brought them from French Guinea. One strain was from a pig and the other from a dog. In each case the colour brought about a permanent disappearance of the parasites.

Harmful influence of the colours on mice.

In treating mice with these colours two difficulties have to be encountered, their toxicity and their property of causing local necrosis. The latter can, to some extent, be avoided by careful inoculations, but in spite of all precautions, some of the colours will cause sloughing of large areas of skin. It is probable that this is more readily produced in infected than in uninfected mice and also, what at first sight is not so apparent, that where the drug has a curative action, it is not so likely to occur.

The toxicity of the colour causes the death of the animals with or without any local necrosis. If a large dose is given the animals may die in a few hours. With smaller doses death does not take place for two or three days, and it may be after the complete disappearance of the trypanosomes. As shown by Dr Bouffard (1906), the kidney is one of the three places in the body where the colour is deposited in the form of granules. In sections of the kidney of some of my mice which had died from the toxic effects of the drugs, the cells of the convoluted tubules were packed with granules of the colour, and many of them were in a condition of necrosis. It is probable that in these cases the mice had died from the nephritis. Fortunately, as regards

both these dangers, the colour found to be most active against *T. dimorphon* is very well tolerated by the mice.

Concluding remarks.

As regards the changes produced in the trypanosomes themselves by treating mice with these colours, there is nothing more to add to the description given by Nicolle and Mesnil. Soon after the injection of the colour, distorted forms of trypanosomes appear in the blood. The proportion of these forms increases, and finally the trypanosomes disappear completely from the blood if the colour is sufficiently active. What becomes of the trypanosomes between the time of their disappearance and the relapse is not known. It is a question allied in many ways to the relapses in some diseases, for instance in relapsing fever, where the disappearance of the parasites from the blood is probably due to some substance present in the blood, which is active against these parasites. As regards the benzidine colours, a glance at Tables I and II is sufficient to show that *T. dimorphon* is more resistant than the other trypanosomes. This is in conformity with the observations of other workers. Laveran, working with human serum, found *T. dimorphon* more resistant than the trypanosomes of Nagana, Surra, and Mal de Caderas, though with trypanosomes fairly numerous in the blood, a sufficient dose of human serum would cause their temporary disappearance. Thomas and Breinl (1905) found *T. dimorphon* "harder to combat with atoxyl, or any other form of arsenic," than the other trypanosomes mentioned in this paper. These authors found that "animals infected with *T. dimorphon* do not react well to arsenical treatment by itself; Trypanroth medication only causes the parasites to temporarily disappear; the combination of arsenic and Trypanroth causes the parasites to be absent for a longer period." In testing the action of atoxyl and arsenic I obtained for the former a result of only two days, and for the latter, in the form of sodium arsenite, a result of six days[1].

T. dimorphon has thus proved more resistant to all forms of medication that have hitherto been tried, than other trypanosomes. The only drug which promises success being the naphthalenediamine disulphonic acid 2736 + benzidine (named alpha). So far this colour has only been tried in mice and here only gives successful results when given in repeated

[1] See p. 280, *et seq.* This refers to the number of days during which trypanosomes remained absent from the blood after treatment.

doses. It still awaits trial in larger animals, where it is hoped that still more successful results may be obtained.

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