THE DEGENERATIVE APPEARANCES OBSERVED IN *PIROPLASMA CANIS* AND IN *TRYPANOSOMA BRUCEI* FOLLOWING UPON DRUG TREATMENT.

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(2 Diagrams.)

In a lecture delivered recently in Cambridge, I had occasion to demonstrate the degenerative changes undergone by *P. canis* and *Tr. brucei* in the blood of animals which had been subjected to curative treatment with drugs. These changes have not as yet been depicted. They are, in my opinion, of more than passing interest, since they may help us to distinguish normal from abnormal parasites in untreated animals. Without doubt there occurs a certain death-rate amongst blood parasites under natural conditions; what this death-rate is we do not know, but appearances which cannot be regarded otherwise than as degenerative are not infrequently encountered. On the other hand, there is always a danger that false interpretations may be placed upon abnormal or degenerative forms, and of this I fear there is ample evidence in current literature. In publishing this note I merely desire to draw attention to what may prove to be a useful method of differentiating some of the normal from the abnormal appearances presented by Haematozoa.

I. *The degeneration observed in Piroplasma consequent upon Trypanblue treatment.*

In our papers on the successful drug treatment of canine and bovine piroplasmosis (*Parasitology*, vol. II. 1909, Nuttall and Hadwen, pp. 163, 190, 249, 265; Nuttall, pp. 418, 432) we described how *P. canis* and *P. bovis* degenerated under the influence of trypanblue, the appearances observed in both species of parasites being similar. To repeat,

the essential changes observed consist (*a*) in the disappearance of the typical intracorpuscular pyriforms, whilst (*b*) the surviving parasites appear rounded or irregular prior to their disappearance from the peripheral circulation. In stained blood films (Giemsa) the degenerating parasites often show a pale, ragged or irregular appearance, and masses of chromatin are frequently extruded. Viewed in fresh films, the parasites usually appear rounded. The changes undergone by *P. canis*, and this holds equally for *P. bovis*, consequent upon the treatment of an animal with trypanblue, are depicted in the accompanying diagram.

Diagram 1, Figs. 1—4, represent normal and common types of parasites occurring in the blood of a dog *before treatment*: a rounded form (O), a pair of pyriforms (PP), two pairs of pyriforms (PPPP) and a dividing form (D). Frequent reference has been made to these types

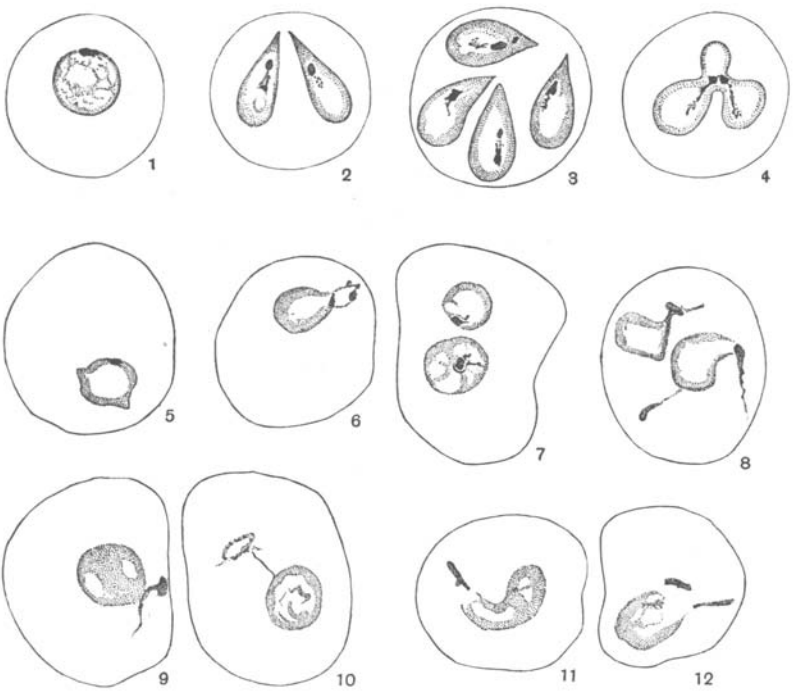

Diagram 1.

Showing the degeneration of *Piroplasma canis* consequent upon the treatment of a dog with trypanblue. Figs. 1—4, normal types of parasites (see text). Figs. 5—12, parasites from the same dog, 6 hours after the injection of trypanblue; parasites degenerating. After coloured figures, from Giemsa-stained blood films, drawn with a Winkel camera lucida and Zeiss 2 mm. objective with eyepiece 18 (× 3000).

of *Piroplasma* in the papers referred to above. The remaining figures (Figs. 5—12) show the appearances of the parasites in the same animal six hours after the injection of a curative dose of trypanblue. In Fig. 5 we have a common type, the parasites being irregular or rounded in form and shrunken. Pairs of pyriforms are no longer encountered or they are very scarce. Where two parasites occur in a corpuscle they appear rounded or irregular (Figs. 7, 8). In Fig. 8 the chromatin is drawn out like a flagellum to one side, reminding one of certain parasites which various authors have wrongly regarded as flagellate forms of *Piroplasma*. In most parasites the protoplasm stains faintly blue, and frequently, as is shown in the figures, the chromatin assumes fantastic shapes and is extruded. Figs. 6 and 7, 9 and 10, 11 and 12 represent pairs of corpuscles containing degenerating parasites and lying side by side in the blood-film from which the drawing was made.

In untreated dogs, it is rare to encounter parasites extruding their chromatin. We have noted this elsewhere (Nuttall and Graham-Smith, 1906, *Journ. of Hygiene*, VI. p. 597, Diagram 7; p. 598, Diagram 9, Figs. 21, 23).

II. *The degeneration observed in Trypanosoma brucei consequent upon Arsenophenylglycin treatment.*

The effect of arsenophenylglycin upon *Tr. brucei* in treated mice is very marked, and is recorded in the following tables. The dose of the drug administered subcutaneously was 1 c.c. of a 1:70 aqueous solution per 20 gramme mouse.

Mouse I.

Time	No. of tryps. per 1000 r.b.c.	% of tryps. showing: No granules	% of tryps. showing: Granules	% of tryps. containing: 1-2 granules	% of tryps. containing: 3-5 granules	% of tryps. containing: Many granules	% pale	% rounded or breaking up	% pointed	% blunt
Before treatment:										
1 hr.	12	95	5	4	0	1	0	0	92	8
After treatment:										
1 hr.	10	5	95	19	44	31	1	0	0	100
2 hrs.	8	0	100	4	22	69	1	4	0	96
3 ,,	0·6	0	100	2	16	62	11	9	0	91
5 ,,	0·2	—	—	—	—	—	—	—	—	—

The number of trypanosomes counted for every 1000 red blood corpuscles in a stained film of peripheral blood gives an adequate idea of the degree of infection both before and at various times after treatment.

Thus in Mouse I. there were 12 trypanosomes per 1000 r. b. c. present in the blood before treatment. Two hours after treatment the proportion was 8 : 1000, and after 5 hours only 0·2 : 1000. No parasites were subsequently discovered.

Whereas before treatment 95 % of the trypanosomes contained no dark red or purplish spherical granules in their blue-staining protoplasm, and 92 % showed blue-staining protoplasm protruding beyond the blepharoplast in the characteristic beak-like manner (Diagram 2, Fig. 1), already one hour after treatment only 5 % of the parasites were free from granules and none showed the beak-like process posteriorly. After two hours all of the trypanosomes showed granules (Diagram 2, Figs. 2, 3). As will be seen from the table, the proportion of trypanosomes containing 1—2 or 3—5 granules was very high one hour after treatment, but their number became less subsequently. On the other hand, the proportion of trypanosomes containing many granules increased after the second hour and decreased slightly after the third hour. After three hours no less than 12 % of the trypanosomes stained faintly (Fig. 4), some taking on a violet tint, whilst 9 % had become rounded or were breaking up. After five hours (Figs. 5—7) only a few pale-staining parasites, fragments of parasites, or loose flagella could be found. During the first three hours after the administration of the drug 21 to 29 % of the trypanosomes were found in various stages of longitudinal division as evidenced by the existence of two blepharoplasts and partial separation into two of the flagellar filament. These dividing forms, however, showed the same appearances of degeneration as those described above for the ordinary single parasites.

A second experiment was now carried out as follows. Two mice, each weighing about twenty grammes were inoculated at 10 a.m. on 15. II. 1910 with a small amount of blood obtained from the tail of a mouse infected with *Tr. brucei*. A very few trypanosomes were detected in the blood of both mice on 17. II. Counts of the number of trypanosomes in the blood of these mice were made commencing on 18. II., blood-films being prepared at intervals as stated in the protocols. One mouse (II.) was treated with arsenophenylglycin while the other (III.) was left untreated. There were 10 trypanosomes per 1000 r.b.c. present in the blood of the mouse (II.) immediately before treatment; blood-films

were prepared from this mouse every half-hour and up to 7 hours after the injection of the drug. Mouse III. served as a control; blood-films were prepared at longer intervals in this case. All of the blood-films were stained intensely by Giemsa, *i.e.* for 2 hours at 25° C., and only the central part of the films was examined for the purpose of making the enumerations.

After 5½—6 hours only a few parasites could be detected; they were granular. After 6½ hours two parasites were detected, the one

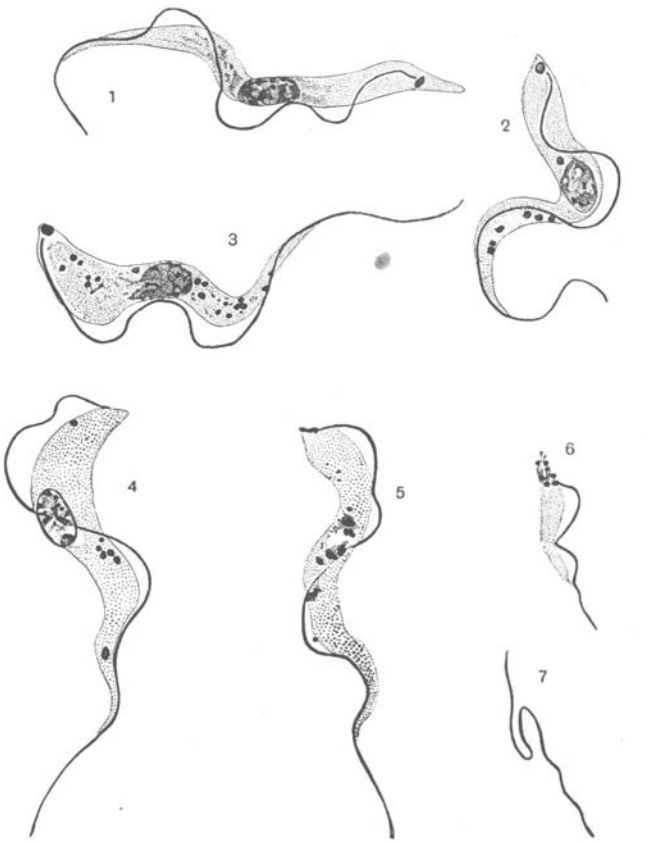

Diagram 2.

Showing the degeneration of *Trypanosoma brucei* consequent upon treatment of a mouse with arsenophenylglycin. Fig. 1, normal trypanosome. Figs. 2—3, parasites 2 hours after treatment. Fig. 4, a parasite 3 hours after treatment. Figs. 5—7, parasites and detritus 5 hours after treatment. After coloured figures from Giemsa-stained blood-films, drawn in the same manner as the figures in Diagram 1.

granular, the other rounded and breaking up. After 7 hours only one parasite was found per 30,000 r.b.c. enumerated; this parasite was pale, rounded and breaking up.

Treated Mouse II.

Time	No. of tryps. per 1000 r.b.c.	% of tryps. showing: No granules	% of tryps. showing: Granules	% of tryps. containing: 1-2 granules	% of tryps. containing: 3-5 granules	% of tryps. containing: Many granules	% pale	% rounded or breaking up	% pointed behind	% rounded behind	Parallel observations on the living parasites
Before treatment, 18.II.'10:											
10.40 a.m.	10	86	14 f *	8	4	2	0	0	100	0	—
After treatment:											
½ hr.	10	28	72 ffc	10	30	32	2	0	18	82	Tryps. more active than normal.
1 ,,	7	2	98 fc	4	40	52	0	2	16	82	Still more active.
1½ hrs.	6·5	0	100 fcc	0	10	90	14	6	2	92	Less active, appear granular.
2 ,,	5	6	94 ffc	8	26	60	48	6	0	94	Ditto.
2½ ,,	4	2	98 fffc	2	18	78	94	18	0	82	Ditto, but more granular.
3 ,,	4	42	58 fffc	28	16	14	100	6	0	94	Ditto.
3½ ,,	2·5	44	56 fffc	22	24	10	100	14	0	86	Ditto.
4 ,,	1·0	0	100	0	0	100	100	12	0	88	Still less active, granular.
4½ ,,	0·16	0	100	0	4	96	100	.	0	.	Ditto.
5 ,,	0·14	0	100	0	16	84	100	16	0	.	Much degenerated, very granular, flagellum moving slowly.
5½ ,,	0·13	0	100	.	.	.	.	.	.	.	Ditto (only 2 tryps. seen).
6 ,,	0·06	.	.	.	.	.	.	.	.	.	Ditto (ditto).
6½ ,,	0·06	.	.	.	.	.	.	.	.	.	No tryps. found.
7 ,,	0·03	.	.	.	.	.	.	.	.	.	Ditto.

* f = granules all fine, fc = granules growing coarser, ffc = granules mostly fine, fcc = granules mostly coarse, fffc = granules mostly very fine.

Owing possibly to the intense staining process to which the blood-films were exposed a somewhat lower percentage (86 %) of the trypanosomes was found to be free from granules in Mouse II. before treatment was commenced. Following upon treatment the percentage of granular parasites rose rapidly, but after 3—3½ hours many pale trypanosomes were noted which contained no granules. After 1½ hours no trypanosomes with pointed posterior extremities were enumerated. All of the parasites appeared pale after 3 hours, and after 2½ hours a considerable

number appeared rounded or breaking up. As in the previous experiment the number of trypanosomes had begun to decrease already 1 hour after the drug was injected. It will be noted that the granules grew coarser up to 1½ hours after treatment and subsequently appeared to grow finer. In a number of cases the coarser granules took on almost a blackish tint and were arranged in rows in the degenerating trypanosomes.

The trypanosomes, viewed under the microscope in fresh blood, showed increasingly active movement up to 1 hour after the drug was injected, but after 1½ hours they appeared granular and their movements grew slower. After 5 hours only the flagellum was seen to move slowly. Whereas there were 10 trypanosomes present per 1000 r.b.c. before treatment, there was but 1 trypanosome per 30,000 r.b.c. present 7 hours after treatment.

In the case of the untreated mouse it is interesting to note that the percentage of granular typanosomes increased rapidly on the day on which the animal died. The proportion of trypanosomes containing many granules also increased as the time of death approached. Four hours before the mouse died 12 % of the trypanosomes no longer showed pointed extremities. It appears therefore as if similar degenerative appearances occur in trypanosomes both in treated and untreated animals, in the latter case only on the approach of the host's death.

Untreated Mouse (Control to Mouse II.).

Time	No. of tryps. per 1000 r.b.c.	% of tryps. showing		% of tryps. containing			% pale	% rounded or breaking up	% pointed behind	% rounded behind
18. II. '10		No granules	Granules	1-2 granules	3-5 granules	Many granules				
10.40 a.m.	2	74	26	10	6	10	0	0	100	0
2.30 p.m.	11	90	10	2	4	4	0	0	94	6
4.30 p.m.	10	90	10	10	0	0	0	0	98	2
6 p.m.	10	98	2	2	0	0	0	0	98	2
19. II. '10										
9.45 a.m.	48	22	78	24	28	26	0	0	100	0
1 p.m.	24	2	98	0	28	70	0	0	88	12
5 p.m.	Mouse died.									

The observations above recorded indicate that the following changes take place in the trypanosomes consequent upon the drug treatment:— They retract their "beaks," and deep-staining reddish or purplish granules

appear in the protoplasm especially toward the flagellar end. The number and size of these granules increase rapidly, after which they disappear with the progressive dissolution of the parasite. After a time only nuclear detritus, portions of the ectoplasm and the flagellar filaments remain, and finally all traces of the parasites disappear. The red- or purple-staining rounded granules doubtless owe their origin to the breaking up of the nuclear chromatin, but the large number of granules observed in some cases suggests that they may also have some other origin. Similar changes occur in trypanosomes in dying animals which have been left untreated. Future investigations will no doubt determine more regarding the origin and nature of these granules which react to chromatin stains[1].

In conclusion, I desire especially to thank Geheimrath Ehrlich for placing a supply of arsenophenylglycin[2] at my disposal.

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[1] With regard to these granules we may note the following: Woodcock (1906, p. 228, Fig. 21) gives a brief description of the involution forms in which chromatolysis vacuolisation and change of form occur. In chromatolysis, according to Woodcock, the chromatic constituents of the nucleus pass out into the protoplasm or else direct fragmentation of the nucleus occurs.

Swellengrebel (1909, p. 87 *et seq.*) evidently refers to similar extranuclear granules occurring in *T. gambiense* and which he observed in the later stages of infection. He does not regard them as consisting of chromatin but of a substance allied to volutin. They stain red by Giemsa and are scarce in the early period of infection. Some of his figures much resemble what I have seen.

[2] See in this connection Roehl, W. (11. III. 1909). Heilversuche mit Arsenophenylglycin bei Trypanosomiasis, *Zeitschr. f. Immunitätsforsch. u. exper. Therapie*, I. pp. 633—649.