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The Toxicity of Trypan Blue.

By DOUW G. STEYN, B.Sc., Dr.Med.Vet., D.V.Sc., Veterinary Research Officer, Onderstepoort.

THIS investigation was prompted by the fact that information was sought in regard to whether, or not, solutions of Trypan Blue became toxic when left standing for a few days before injection.

The following experiments conducted by du Toit (1928) are of interest as far as the toxicity of freshly prepared solutions of Trypan Blue, injected intravenously, are concerned:—

(*a*) An eighteen-month old calf received 39 gm. of Trypan Blue (in 1 per cent. solution) in the course of twenty-nine days. The initial dose was 1·5 gm. and the last dose on the twenty-ninth day 4 gm. Trypan Blue. On the fiftieth day after the first injection of Trypan Blue the animal died in a state of advanced cachexia and weakness. It should be mentioned that the animal was suffering from anaplasmosis and that the time of death anaplasmata were still present in the heart blood.

(*b*) Calf 316 ($2\frac{1}{2}$ years old) received 32·5 gm. (in 1 per cent. solution) Trypan Blue in ten injections in the course of thirty-two days. The initial dose was 1·5 gm. and the last dose 4 gm. The blood of the animal remained normal throughout the experiment. On the 9th, 14th and 20th day of the experiment the animal showed a temperature ranging from 105 tot 105·8° F.

(*c*) Calf 426 ($5\frac{1}{2}$ months old) received 8·5 gm. Trypan Blue in the course of twenty-seven days. The injections were commenced with 0·5 gm. (in 1 per cent. solution) and ended with 0·75 gm. No symptoms of poisoning were noticed, the animal however developed anaplasmosis and gonderiosis. Du Toit considers that the injection of Trypan Blue brought about a relapse of these two diseases, from which the animal had suffered some time prior to the experiment with Trypan Blue.

(*d*) Each of two calves 709 and 607 ($2\frac{1}{2}$ and $4\frac{1}{2}$ months old respectively) received twenty-six injections of Trypan Blue in a 1 per cent. solution in the course of fifty days. The initial injection was 0·5 gm.; the dose was increased to 0·75 gm., then 1·0 gm., then 1·5 gm., 2·5 gm., 3·0 gm., 4·0 gm., and finally 5·0 gm. Each calf received 36·5 gm. Trypan Blue altogether. No symptoms of poisoning were discernible with the exception of a high temperature (above 106° F.) which occurred on the 9th day after the first injection.

(*e*) Calf 424 (2 years old) a carrier of anaplasmosis received fourteen injections of Trypan Blue in a 1 per cent. solution in the course of sixty-seven days, commencing with a dose of 1·0 gm. and ending with 2·5 gm. No symptoms of poisoning except a slight rise in temperature, were discernible.

(*f*) Calf 755 (1½ years old), a carrier of anaplasmosis, received fifteen injections of Trypan Blue in a 1 per cent. solution in the course of sixty-seven days, commencing with a dose of 1·0 gm. and ending with one of 3 gm. No symptoms of poisoning were discernible.

Ryrie (1933) who investigated the curative effect of coal-tar dyes in cases of leprosy, states that different samples of the same dye exhibit marked differences in their immediate effect on the patient. He tested the following dyes:—Chrysoidine, Bismark brown, Trypan Blue, brilliant green, Malachite green, Crystal violet, Methyl violet, Auramine, Eosin, Fluorescin, Rhodamine, Methylene blue, Toluidine blue and Indigo Carmine. He describes the toxic effects of these dyes in human beings as follows:—cardiac pain, epigastric palpitation, gastric and rectal irritation, feeble pulse, and signs of shock, and, in severe cases coma and even temporary failure of respiration and loss of radial pulse. He states that solutions tend to become more toxic if left for a few days before injection. He found that a number of dyes, including Trypan Blue, if injected in sufficient quantity show almost at once a selective affinity for the endothelial tissues of the lesions. In most cases patients tolerated 25 cc. of a 4 per cent. solution, whilst some develop symptoms of poisoning. Some patients even tolerated 75 cc. of a 4 per cent. solution. Ryrie states that it is important to filter the solution before injection.

Anderson, Emerson and Fisher (1934) determined the toxic effects of Trypan Blue, Crystal violet and Brilliant green on mice, rats, rabbits and guinea-pigs. Guinea-pigs were found to be more susceptible than mice, rats and rabbits. Three out of five rabbits injected intravenously with 0·15 gm. Trypan Blue per kg. bodyweight in a 5 per cent. aequeous solution succumbed. When administered per os. Trypan Blue is absorbed very slowly and is almost non-toxic. The authors were unable to elucidate the mechanism of the toxic action of the dyes. They suspect Trypan Blue of causing depression of the central nervous system. In all fatal cases there were marked congestion of the lungs. Trypan Blue appeared to cause less damage to the liver than brilliant green and gentian violet. Repeated injections of Trypan Blue into leprous rats are better tolerated than those of gentian violet and brilliant green.

In another article Anderson, Fisher and Emerson (1934) refer to the toxicity of Trypan Blue and state that " mice survive 300 mgm. per kilogram intraperitoneally, while 400 mgm. per kilogram of a 1 per cent. solution kills all animals. Intravenously the dye is about twice as toxic, being lethal for 3 of 5 mice at 200 mgm. per kilogram. Rats tolerate oral amounts to 1·0 gram per kilogram and do not become blue, indicating no absorption by this route. A subcutaneous dose of 400 mgm. per kilogram kills 4 of 5 animals.

Intraperitoneally all rats die when given 350 mgm. per kilogram of a 2 per cent. solution of Trypan Blue. Three hundred mgm. per kilogram is lethal for rats on intravenous administration of a 2 per cent. solution. The dye is slightly more toxic for guinea-pigs killing 3 of 5 animals on subcutaneous injection of 300 mgm. per kilogram, while intraperitoneally half of six guinea-pigs die with 250 mgm. per kilogram of a 2 per cent. solution. Rabbits tolerate larger amounts intraperitoneally surviving 300 mgm. per kilogram, but die when given 400 mgm. per kilogram. Intravenous doses of 100 mgm. per kilogram kill 1 of 5 animals, while 150 mgm. per kilogram of a 5 per cent. solution is lethal for 3 of 5 rabbits."

Gousseff and Sudzilowsky (1934) ascertained the effect of medicinal doses of Trypan Blue manufactured by the Bayer-Meister-Lucius factory) on the horse. They recommend that horses injected intravenously with Trypan Blue should not be worked for a day after the injection. In the case of subcutaneous and intramuscular injections the animals should not be worked for four to five days after the injection.

They state that Trypan Blue had no effect on the bilirubin content and alkali reserve of the blood.

ONDERSTEPOORT EXPERIMENTS.

Rabbit No.	Weight in Kg.	Manufacturer of Trypan Blue.	Amount of Trypan Blue injected intravenously.	Result.
A	2·05	I "Casella" Trademark; manufactured by Leopold Casella & Co., Frankfurt a. M.; Germany	5·0 cc. of a freshly prepared 2 per cent. aequeous solution	No symptoms developed.
B	1·75		10·0 cc. of a freshly prepared 2 per cent aequeous solution	Slight transient accelerated respiration.
C	2·15		15·0 cc. of a freshly prepared 2 per cent aequeous solution	Died 36 hours after injection.
D	2·3		5·0 cc. of a 10-day old 2 per cent. aequeous solution	Laboured respiration, which lasted about four hours.
E	2·4		10·0 cc. of a 10-day old 2 per cent. aequeous solution	Died ¾ hour after injection.
F	1·6		10·0 cc. of a 10-day old 2 per cent. aequeous solution	Died 1½ hours after injection.
G	2·14	II "Ciba" Trademark; Gesellschaft für Chemische Industrie, Basel, Schweiz. (Per Carl Bittmann, 31 Petersgraben, Basel.)	5·0 cc. of a freshly prepared 2 per cent. aequeous solution	No symptoms developed.
H	2·0		10·0 cc. of a freshly prepared 2 per cent. aequeous solution	Died 3½ days after injection.
I	2·14		It was intended to inject 15·0 cc. of a freshly prepared 2 per cent. aequeous solution, but animal collapsed and died after 10 cc. had been injected	Died.
J	1·6		5·0 cc. of a 10-day old 2 per cent. aequeous solution	Slight transient, laboured respiration.
K	1·6		10·0 cc. of a 10-day old 2 per cent. aequeous solution	Pronounced transient, laboured respiration.
L	1·9		15·0 cc. of a 10-day old 2 per cent. aequeous solution	Pronounced transient, laboured respiration.

ONDERSTEPOORT EXPERIMENTS (*cont.*).

Rabbit No.	Weight in Kg.	Manufacturer of Trypan Blue.	Amount of Trypan Blue injected intravenously.	Result.
M	1·6	III Dr. G. Grübler & Co., Leipzig, Germany. (Batch 10,33.)	5·0 cc. of a freshly prepared 2 per cent aeqeous solution	No symptoms developed.
N	2·1		10·0 cc. of a freshly prepared 2 per cent. acqeous solution	Transient laboured respiration.
O	2·0		15·0 cc. of a freshly prepared 2 per cent. aeqeous solution	Laboured respiration set in immediately after injection. Animal died about 12 hours after injection.
P	1·75		5·0 cc. of a 10-day old 2 per cent. aeqeous solution	Died 3 hours after injection.
Q	1·9		10·0 cc. of a 10-day old 2 per cent. aeqeous solution	Died 2 hours after injection.
R	1·6		It was intended to inject 15·0 cc. of a 10-day old 2 per cent. aeqeous solution. When 12 cc. were injected, severe convulsions set in and at 14 cc. the animal collapsed and died.	

Solutions of the above brands of Trypan Blue were prepared and injected immediately. The remaining quantities of the solution were injected after having been allowed to stand for ten days in flasks fitted with dry plugs of cotton wool. Trypan Blue I solution was violet and not blue in colour. The specimens of Trypan Blue used in the author's experiments were taken from the original containers issued by the respective manufacturers. The solutions were injected at the same rate into all the animals, namely, 5 c.c. per minute.

From the above table it is evident that the solutions of Trypan Blue I and III markedly increased in toxicity after having been left standing for ten days, whilst this was not the case with the solution of Trypan Blue II.

SYMPTOMS OF POISONING.

After intravenous injection of toxic and lethal amounts of Trypan Blue the rabbits developed the following symptoms: Laboured respiration, accelerated and weak heart-action, and convulsions. Those animals which survived for a while were in a paralytic state after the attack of convulsions had passed off. In peracute cases of poisoning methaemoglobinaemia was present and was detectable both macroscopically and spectroscopically.

The autopsy revealed no lesions of a characteristic type.

SUMMARY.

It has been definitely proved that two out of three brands of Trypan Blue tested markedly increased in toxicity after the solutions had been left standing for ten days before injection. It is therefore obvious that only freshly prepared solutions of Trypan Blue should be used.

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