

THE ERDMANN NEW CULTURE MEDIUM FOR PROTOZOA.

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It is a well-known fact that the first culture *in vitro* of a pathogenic trypanosome (*Trypanosoma Brucei*) was obtained by Novy and MacNeal¹ in 1903. The medium used was a meat extract agar plus two parts of defibrinated rabbit's blood. Of fifty animals tested only 4, or 8% positive cultures resulted. In 1905 Smedley², using a similar medium, found that three out of ten attempts, or 30%, were successful.

Because of the inconsistent results we deemed it advisable to attempt an improvement of the medium. The first attempts along these lines were in 1909³. The media with their per cent. of positive growths are as follows:

1	Novy MacNeal blood agar.....	25%
1A	Novy MacNeal blood.....	0%
2	Bean and pea extract blood agar.....	53%
2A	Bean and pea extract blood.....	0%
3	Nicolle blood agar.....	48%
3A	Nicolle blood	0%
4	Dialyzed meat extract blood agar.....	80%
4A	Dialyzed meat extract blood.....	0%
5	Dialyzed meat extract dilute serum agar.....	100%
5A	Dialyzed meat extract dilute serum.....	0%
6	Dialyzed meat extract inactivated serum agar.....	100%
6A	Dialyzed meat extract inactivated serum.....	0%
7	Dialyzed meat extract dilute red blood cells agar.....	38%
7A	Dialyzed meat extract dilute red blood cells.....	0%
8	Dialyzed meat extract Ascitic fluid agar.....	0%
8A	Dialyzed meat extract Ascitic fluid.....	0%
9	Veal extract blood minus white blood cells agar.....	100%
9A	Veal extract blood minus white blood cells.....	0%

¹ Jour. Amer. Med. Assn., 1903, 41, p. 1266; Jour. Infect. Dis., 1904, 1, p. 1.

² Jour. Hyg., 1905, 5, p. 38.

³ Jour. Infec. Dis., 1914, 15, 1, p. 4.

The above table indicates that successful cultures ranging from 25 to 100 per cent. are obtained when the solid type of medium is employed and that in every case where the liquid medium is used negative results occurred. In the successful cultures growth always resulted in the water of condensation after a period of incubation from one to four weeks at a temperature ranging from 25° to 28° C.

We therefore naturally were very much interested when in 1914 Rh. Erdmann¹ announced a new liquid culture medium for *Trypanosoma Brucei*. Erdmann states that by using the plasma of the host as the medium she grew *Trypanosoma Brucei* in hanging-drop cultures and kept them in normal condition for an indefinite period. The technique employed in brief was as follows: The plasma was obtained by the method of Harrison², Burrows³, and Walton⁴. "The blood from the infected rat was taken and put into a small drop of plasma on a cover-glass and then this was further diluted with plasma in order to reduce the number of blood corpuscles in the hanging-drop which was taken from this." The cover glass with hanging-drop was either placed on a depression or regular slide and sealed. Precautions to secure aseptic conditions were taken.

We attempted to follow the technique thus outlined as nearly as possible. These cultures showed no signs of bacterial contamination at the end of forty-five days. In only a few instances were actively motile survivals in evidence for more than five days when kept at 10°C. In preparations incubated at 20°C, or above no survivals were observed after forty-eight hours.

In the course of an extensive series of attempts using heterologous and homologous sera under various conditions we found it impossible at any time to obtain a second generation by the Erdmann method. The homologous sera used were rat and guinea pig. The heterologous sera were human, horse, beef, sheep, pig, rabbit and chicken. These sera were used in a dilute one to one, inactivated, and normal form and the preparations were incubated at temperatures of 10, 15, 20, 25, 28, 30, 35, 37½, and 40°C. Ascitic fluid was also used without success.

It is true that trypanosomes will multiply and remain actively

¹ Soc. Exp. Biol. and Med., 1914, XII, p. 57.

² Proc. Soc. Exp. Biol. and Med., 1907, IV, p. 40; Jour. Exp. Zool., 1910, IX, p. 787.

³ Jour. Amer. Med. Assn., 1910, LV; Jour. Exp. Zool., 1911, X, p. 63.

⁴ Proc. R. S. L., Ser. B., 87, p. 452.

motile when first placed in a medium such as described by Erdmann. We have especially noticed this in connection with our work with solid media. Good survival forms of other pathogenic trypanosomes as those causing human sleeping sickness, dourine, and mal de caderas were observed as late as the twenty-eighth day, but in no case did these forms result in positive growth or second generation when transplanted to similar medium under similar conditions.

In summing up our work we can positively say that at no time, under no conditions were we able to obtain a positive culture using the Erdmann cultural medium. As a matter of fact the easily cultivated trypanosome of Lewis would not develop successfully on this medium.

