The Cultivation of Trypanosomes in Vivo.

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Practically all of the common laboratory animals are susceptible to trypanosomal infection. For the following reasons white rats and guinea pigs are the ones most frequently used; (a) they require but little space, (b) they are easily kept, and (c) in them the infection runs an acute or sub-chronic course. It is essential to know at all times the status of the infection and the condition of the animal, and there are certain routine procedures which are followed in making the observations on the culture. These routine procedures as carried out in the laboratories at Purdue University will be described more or less in detail.

The infection is transferred from animal to animal by the hypodermatic injection of infected blood. The injections may be made subcutaneously or intraperitoneally. The former method is usually used when it is desired to have the course of the infection proceed slowly; and only small quantities of blood are injected for that reason. The intraperitoneal method is used when it is desired that the climax of the infection come quickly. In this case larger quantities of the virulent blood are injected.

To transfer the infected blood a small hypodermatic needle is used, which has been boiled for ten minutes in a saturated solution of borax, and then rinsed thoroly with a sterile physiological salt solution. About 0.5 cc. of the sterile salt solution is then drawn up into the syringe. A drop of the virulent blood is then drawn up and mixed with the salt solution in the barrel of the syringe, and all or any fraction of it is injected into the animal, as it has been shown that a single trypanosome when injected will cause a typical infection.

Methods of Obtaining the Virulent Blood.

Rats are bled from the tip of the tail and Guinea pigs from the ear. The rats are kept in large battery jars which have weighted wire covers. The cover is held slightly to one side and the rat's tail is drawn thru the opening thus made until the rump is snugly against the edge of the jar and the rim of the cover. The tail is taken in the left hand, the left forearm holding the cover in place, and with a sharp pair of sterile scissors a bit is cut cleanly from the end of the tail. It is important that this cut be made cleanly, for if the tail is lacerated or if any shreds of tissue remain, the blood will run back among the stubby hairs which cover the tail and will not form a drop on the end of it. This drop which collects is drawn into the syringe, mixed with the salt solution and injected. In the case of the guinea pig the ear is held between the thumb and forefinger of the left hand. A clean cut is made in the edge of the ear and the drop of blood which forms is taken into the syringe in the same manner as the drop from the rat's tail.

When a rat is to be injected intraperitoneally, the skin on the back of the neck or shoulders is seized with a pair of self-locking rat forceps. The tail and hind legs are pulled well down and held in the left hand, along with the forceps. The needle, held in the right hand, is inserted thru the skin and muscular wall in the median portion of the abdomen and the desired amount of the contents of the syringe injected. In making a subcutaneous injection the rat is held in the same manner, the needle being inserted just thru the skin in the thoracic region. Guinea pigs do not offer the vicious resistance to this treatment which is characteristic of rats, and consequently it is not necessary to handle them with forceps. When making a subcutaneous injection it is advisable to lift the point of the needle slightly after it has been inserted, in order to determine definitely that it has not entered the muscular tissue.

Beginning with the next day or the second day after an animal is injected, daily or bi-daily examinations of its blood are made. A drop of blood is obtained in the manner described above, but instead of being taken into a syringe, is touched to a clean glass slide and immediately covered with a clean coverglass. The cover-glass is pressed down until a layer of blood of about the thickness of one red blood corpuscle remains under it. Care is taken not to push the coverglass sidewise as this causes rapid plasmolysis of the red cells. This "fresh preparation" of the blood is examined under the 4mm. objective with a No. 10 ocular and 160 mm. tube length, and the number of trypanosomes per field is counted and recorded.

The following method of keeping the records of the animals and cultures has proven most satisfactory. The inoculated animals are kept in battery jars or small wire cages which are marked with gummed labels bearing the following information: the name of the organism, the number of the animal, the page in the note-book on which its record can be found, the date of inoculation and any mark that may be necessary to properly identify the animal in question. The latter item is only used when two or more animals are kept in the same jar or cage. For example:

Typanosoma Brucei.
Sp2 L
2-1-17

This label shows that this animal was inoculated with Trypanosoma Brucei on February 1, and that it is number eight on page two of the note-book. The 'L' indicates a notch cut in the left ear to distinguish it from another animal in the same cage. On page two, the following record of this animal is to be found:

6. Blk. and Wht. G. pig. ¼ ear drop from 5p2. Sub cut. inj.

1-2-17

1-5-17—0.5 per drop.
1-8-17—1-100 pf.
1-13-17—2 pf.
1-15-17—5 pf ¼ ear drop to 7p2.
1-17-17—10 pf.
1-20-17—0 pf.

		1-24-17—5 pf.
		1-27-17—100 pf.
		1-31-17—200 pf.
		2-1-17—Dead.*
7.	Brindle G. pig. ¼ ear drop from 6p2. Sub cut. inj.	1-17-17—0 pf.
		1-20-17—0 pf.
		1-22-17—1 per drop.
		1-24-17—5 pf.
		1-27-17—5 pf.
	1-15-17	1-29-17—10 pf.
		1-31-17—1 pf.
		2-1-17—2 pf ½ ear drop to 8p2.
		2-3-17—15 pf.
		2-6-17—65 pf.
		2-7-17—Dead.*
8.	Yellow G. pig. ½ ear drop from 7p2. Sub cut. inj.	2-3-17—0 pf.
		2-6-17—1 per drop.
		2-7-17—1 pf.
		2-10-17—5 pf.
		2-14-17—1-50 pf ½ eardrop to 9p2.
	2-1-17	2-16-17—3 pf.
		2-18-17—8 pf.
		2-19-17—75 pf 2 drops to 1p4.
		2-20-17—Dead.*

Note:

Blk.—Black. Sub cut. inj.—Subcutaneous injection.
Wht.—White.
pf—per field.

When the animal dies its label is crossed off and its record is marked with a large asterisk in the lower right hand corner. If any other disposition is made of the animal it is so noted in its record.

A careful observation of this record will show how the pedigree of a culture may be traced, and how it is possible to know at all times the condition of the infected animal.

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