

to cold at 4° C., in order to occlude the skin capillaries and drive the trypanosomes into the general circulation. Oocysts have also been studied in skin smears on supravital slides (neutral red) kept moist by addition of a drop of normal bovine serum. The oocyst appears to form sporoblasts, probably eight, which divide into innumerable sporozoites. The sporozoites break away from the oocyst body and swim actively away by a kind of rolling movement.

The fate of the sporozoite is uncertain. It is initially about 1–2 μ in diameter and its morphology has not been fully determined. It is probably leishmannioid, since larger forms have been seen of this type. It does not aggregate or develop in the tissues, though skin smears reveal developmental forms—leptomonad, crithidial and immature trypanosome. It is suspected that the macrophages are parasitized, since in skin, lymphatic gland and kidney sections, these cells have been found containing bodies, the larger of which are leishmannioid in appearance.

Trypanosomes treated with hypotonic saline in mouse blood react in three different ways and are plainly of three different forms as follows:

(1) Immature sexual or asexual. These become swollen by the saline, but show no other changes, though some may conjugate after forty-five minutes. (2) Mature sexual forms, which conjugate as described. (3) Infective (?) forms, which become changed into a variety of developmental types, usually associated with life in the tsetse fly, such as round forms, stumpy forms, elongated forms and so on.

These matters will shortly be described at greater length in a preliminary communication, and a full report published when all details have been studied.

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Multiplication *in vitro* of Koch Bodies of *Theileria annulata*

THE following simple method modified from Jacoby (1944) was found suitable for the growth *in vitro* of *Theileria annulata*. A small fragment of infected calf's spleen or lymphatic gland is placed on a sterile glass coverslip and a drop each of calf plasma and chickens' embryonic extract are added. After coagulation the coverslip is sealed to the bottom of a large Carrel flask by a drop of plasma and embryonic extract; 3–5 c.c. of a mixture of 30–40 per cent calf serum in Tyrode is then added and the flask closed and incubated. On this medium Koch bodies survive for at least twelve days, but do not multiply. If fragments of normal spleen are placed in juxtaposition to the infected fragment at intervals of 3–5 days, Koch bodies survive for 15–18 days, but there is no obvious multiplication. The addition of glutamine (3 γ per c.c.), pyridoxin (0.6 γ per c.c.), inositol (4 γ per c.c.) and riboflavine (0.04 γ per c.c.) to the mixture of serum and Tyrode induces multiplication of Koch bodies, which was observed in ten successive fragments of normal calf spleen during a period of two months. The addition of these factors to the transplant, in which Koch bodies have survived without multiplication for eighteen days, immediately induced multiplication of the surviving parasites.

It is interesting to note that, *in vitro*, good growths are obtained in spleen fragments of recovered animals

and overlying fluid containing 40 per cent of serum of the same animals. *In vivo* massive inoculation of infected blood or macerated infected lymph glands do not produce an attack of theileriosis in recovered calves, which always harbour a residual infection.

I have to thank Prof. Adler for his advice and Dr. Grossowitz for his kind help.

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July 3.

Histological Fixation of Locomotory Patterns

NORMAL histological methods usually fail to yield a satisfactory picture of the shape and form of a muscle at any given phase of its activity. For such purposes the following technique has been found useful in the case of small terrestrial or aquatic animals. Absolute alcohol is cooled to about -117° C. by submersion in liquid air. Small terrestrial animals moving over fine wire gauze or thin aluminium foil are quickly dipped into the alcohol, and aquatic animals moving in a small quantity of water contained in a spoon of aluminium foil are treated likewise.

Fixation for histological investigation can be achieved in a number of different ways, according to the material used; for example, dehydration in a frozen condition, gradual thawing in alcohol plus formic acid, etc.

Animals investigated so far include Planarians, Nemertines, Annelids, Gastropods, Myriapods, and small vertebrates. Fixation appears to be instantaneous; the tentacles of snails and slugs remain protracted, and the locomotory waves are clearly recognizable. The setae protrude in the longitudinally contracted segments of an earthworm, and are retracted in the elongated segments.

The details of the method will be discussed elsewhere. It is hoped that it may help to correlate, more satisfactorily than has been possible hitherto, structure and function within the animal body.

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A Modification of Slide-Culture Technique

A VERY simple and effective method of making slide-cultures for the study of moulds is described below. It has many advantages over the methods described by Henrici¹ (p. 30), Lewis and Hopper² (p. 221), Langeron³ (p. 957), and Smith⁴ (p. 176). The procedure, a slight modification of existing methods of slide-culture, was worked out by M. C. White and me jointly, while attached to the laboratories of the South African Institute for Medical Research, Johannesburg, on military duties.

Sterile nutrient agar is melted in a culture tube, in a water bath, and allowed to cool down to between 40°–45° C. This is inoculated fairly heavily with spores of the mould to be studied taken from a pure culture. A very small drop of the inoculated agar is transferred to a flamed slide by means of a platinum loop and is immediately covered over with a flamed coverslip. It should be noted that a hollow-ground