Phase numbers in the course of a blockage in the transition from prophase to metaphase. Using various inhibitors of nucleic acid synthesis in meristems, it was found that ethidium bromide at 10 µg/ml[6] increased the prophase index during the treatment, while the mitotic indices remained notably constant.

Analysis of the phase numbers in the course of continuous treatment with this drug shows an increase in the prophase number compared with other phase numbers, which all tend to be reduced to 0 (Figure 2). The fact that the mitotic index remains normal during the first 6 h of the treatment seems to indicate that all the cells are blocked at the transition from prophase to metaphase, i.e. that they accumulate in the prophase. This seems to be proved clearly by the observed kinetics of the prophase number.

Phase numbers in the course of a metaphase blockage. It has been known since 1934[7] that colchicine acts upon dividing cells by accumulating them in the metaphase, and the increase in the number of cells in metaphase per unit of time has been used by several authors as a way of measuring the speed of the cell division cycle. Analysis of the mitotic indices under 0.05% colchicine treatment[8] shows them to be normal during the first 4 h of treatment (Figure 3), while they increase subsequently through the accumulation of dividing cells. If we analyse the phase numbers under this treatment, we find that the prophase number remains constant, and that the increased mitotic index is due to the larger number of metaphases. The number of cells initiating mitosis is practically constant throughout the treatment, but 4 h after it has begun there are practically none issuing from it. The dynamics of the metaphase number clearly show the accumulating effect of colchicine from the first hour of treatment.

In short, the 3 effects studied – blockage in the interphase, in the prophase and in the metaphase – are easily detectable by the use of phase numbers and their analysis in sequence. The information thus obtained may be of use when a new drug is to be tested[9,10].

Resumen. Se determinan los números de fase en poblaciones meristemáticas en equilibrio dinámico, donde permanecen constantes a 1 o largo del tiempo. Se estudia su evolución en presencia de tres tratamientos que producen bloqueo en tiempos diferentes del ciclo de división celular – interfase, profase y metafase.

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In vitro Culture of *Toxoplasma* Infected Cat Intestine

Recent investigations by HUTCHISON et al.[1], FRENKEL et al.[2] and SHEFFIELD and MELTON[3] have shown that *Toxoplasma gondii* undergo schizogony and gametogony in the cat ileum giving rise to oocysts in the faeces of the infected animal. The oocysts are highly resistant to chemical treatment and can, therefore, be obtained in bacteriologically sterile condition by washing the faeces with various chemicals. The other intestinal forms of the parasite are, however, not resistant to chemicals and as such cannot be obtained in sterile condition from the lumen of the bowel like the oocysts. To overcome this problem we have used this method of organ culture involving the *Toxoplasma* infected cat ileum and find that the method is particularly suitable for obtaining bacteria-free merozoites in large numbers.

The strain of *Toxoplasma* used in this work was first isolated from a Malayan tree shrew (ZAMAN and GOH[4]). The cats used varied in age from 5–8 weeks and were reared in the laboratory, under pathogen free conditions. The infective material consisted of pooled mice brain containing a large number of cysts. The brain tissue was first chopped into small bits with scissors and fed to each cat by intragastric inoculation using a polythene tube. The faeces from each cat were collected daily and examined microscopically after zinc sulphate flotation. 6 cats passing a heavy concentration of oocysts, 7 days after the date of infection, were used for the study.

Laporotomy was performed on these animals and 4–5 inches of lower ileum was removed under sterile condi-

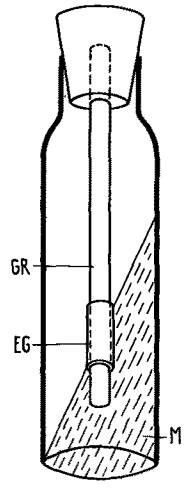

Fig. 1. GR, glass rod; EG, everted gut; M, medium. The container is kept at an angle in the roller drum to allow the everted gut to come in contact alternately with the medium and air in the tube.

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tions. This part of the intestine was then flushed with sterile 500 ml of Earle's balanced solution (BSS) by inserting a sterile pasteur pipette in one end of the lumen and allowing the solution to flow out from the other end. This procedure cleansed the intestine of all food contents and mucus. The segment was then dropped into a flask containing 200 ml of BSS + 500 units Bacitricin/ml + 50,000 µg Neomycin/ml. It was left in the antibiotic solution for 5–10 min and then transferred to a sterile petridish, where it was cut into 5 segments each about an inch long. These segments were then everted i.e. the mucous membrane was turned inside out. Various technique for eversion were tried. The most simple one was to use a thin 'alligator' forceps by which the cut ends of the segment were held from one side and then the forceps was gently pushed into the lumen till it emerged from the other side, thereby everting the segment. As long as the segments of the intestine were not too long eversion could be accomplished with no difficulty and with very little visible trauma. The everted intestine was again transferred to another flask containing 200 ml of BSS + 500 units Bacitricin/ml + 50,000 µg Neomycin/ml. It was left in the flask for 5–10 min, followed by 3 transfers of about 5–10 min each into 200 ml BSS + 100,000 units Penicillin/ml + 100,000 µg Streptomycin/ml. Finally, the sections were passed twice into BSS alone without antibiotics. At this stage the segments were ready for in vitro culture.

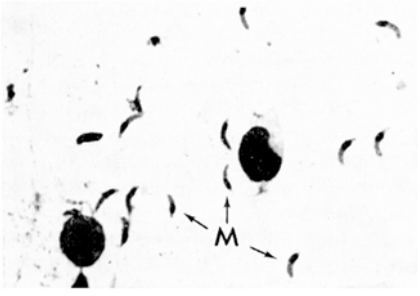

Fig. 2. M, merozoites. Alcohol fixed, Giemsa stained smears. × 1000.

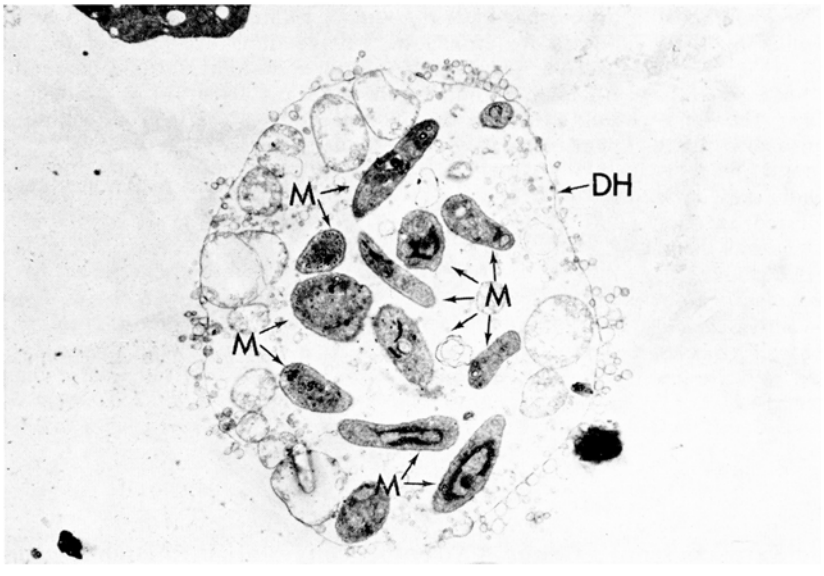

Fig. 3. M, merozoites; DH, degenerating host cell. Electronmicrograph. × 4400.

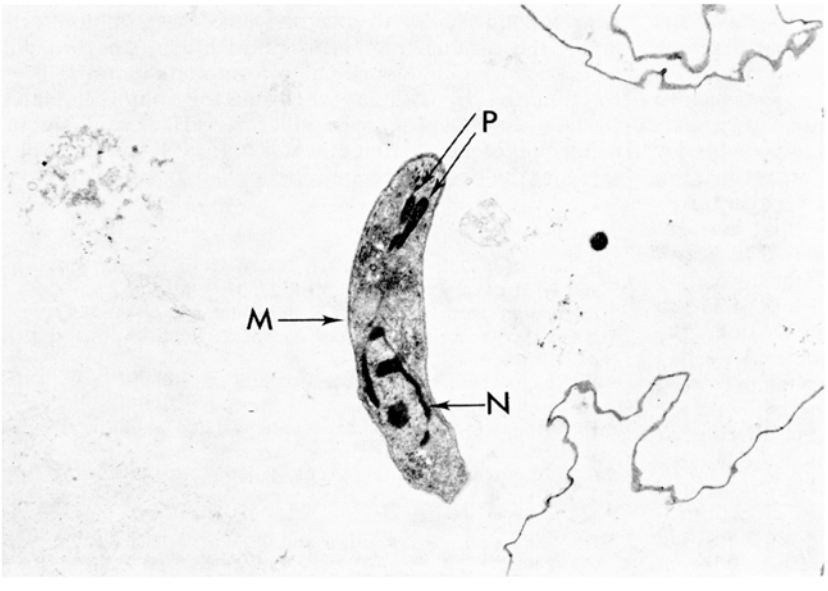

Fig. 4. M, merozoite; N, nucleus; P, paired organelle. Electronmicrograph. × 13,200.

The bottles used for in vitro culture were universal containers ('McCartney' bottles). The secrew cap of these bottles was replaced by rubber bung to which were attached glass rods. The glass rods extended to the lower one-third of the bottle. The everted intestine was then slipped on the glass rods. As long as the glass rods were of the right diameter the intestinal segment did not slip off and remained attached to the glass rod. Tissue culture medium which was then added to each bottle consisted of 5–6 ml of medium 199 in BSS (Difco) + 25% fetal calf serum + 25,000 U Penicillin/ml + 25,000 µg Streptomycin/ml. The bottles were placed in a roller drum and allowed to rotate slowly at a speed of about 1 revolution per 2 min. The angle in the tube was such that the fluid covered only one side of the intestine, at any given time, as shown in Figure 1. This allowed alternate exposure of the mucous membrane to the medium and the air in the tube. The medium was changed every 12 h and at the same time examined for the presence of bacterial growth. Only occasionally were the tubes found turbid due to bacterial or fungal growth, which were then discarded. The medium from the clean tubes was centrifuged and the sediment examined for the presence of parasites.

The examination consisted of observations in the light microscope under phase contrast and alcohol fixed Giemsa stained smears. The pooled sediments were also processed for electron microscopy.

The light microscopy showed the presence of merozoites in large numbers up to 3 days of the culture. The merozoites were motile and could be distinguished without any difficulty. Giemsa stained smears showed the morphology of the merozoites clearly. They were crescentic bodies 7–10 µm by 2.5–3.5 µm with a pointed anterior end and a rounded posterior end. The nucleus was ovoid and generally located towards the posterior end (Figure 2). In addition to the merozoites oocysts and macrogametocytes were also observed. All these stages were identical in appearance to the forms described previously (ZAMAN and COLLEY[5]). The number of merozoites fell sharply after 3 days but a few parasites continued to appear up to 7 days.

Sediments made from 12-h-old cultures and examined by electron microscope showed individual host cells containing different stages of parasites. Some of the parasites were degenerating but many were in excellent stage of preservation. As in the light microscopic observation they were predominantly merozoites. Apparently the host cells containing the mature schizonts break off from the mucous membrane and liberate the merozoites in the medium. Figure 3 shows a degenerating host cell containing merozoites. Figure 4 shows a merozoite lying outside the host cell and showing a nucleus and the paired organelles.

The technique described is a simple method for obtaining merozoites of *Toxoplasma gondii* in bacteria free conditions. The bulk of the parasites were obtained during the first 3 days, indicating that the development of the parasite did not continue for a longer time in the medium used. It is, however, possible that further modifications of the medium would enable the parasites to continue their life cycle for a longer period of time.

Résumé. Une technique, par laquelle l'intestin du chat infecté par le *Texoplasma* est débarassé des bactéries par l'antibiote et ensuite cultivé dans des éprouvettes est decrivée. Dans ces conditions, on peut obtenir des mérozoïtes en grande quantité durant 3 jours. Les mérozoïtes sont exempts de bactéries et viables. Cette technique peut être appliquée à d'autres espèces de coccidies.

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In vitro Anti-Tumor Activity of Lipid Extracts from a Group A *Streptococcus* against Ehrlich Ascites Carcinoma in Mice

In 1955, KOSHIMURA et al.[1] reported that washed cells of a group A *Streptococcus*, when premixed with tumor cells before inoculation, completely suppressed the development of ascitic tumors in mice. Their observations have been confirmed and extended by many researchers[2–5]. However, the nature of the factor responsible for the in vitro anti-tumor activity of the organisms has as yet been largely obscure. The present paper gives data on the in vitro anti-tumor effect of lipid extracts derived from a group A *Streptococcus hemolyticus*, strain Su, against Ehrlich ascites carcinoma cells in mice.

Streptococci were grown at 37 °C in 10 l lots of WOOD and GUNSALUS[6] medium. After 12 h incubation, the organisms were harvested by centrifugation and washed thoroughly in saline. The packed cells were then extracted 3 times (twice for 2 h and finally overnight) with 20 volumes of chloroform-methanol (2:1) by stirring at room temperature. The combined extracts were evaporated at 40 °C under a stream of nitrogen. The crude lipid extracts thus obtained were redissolved in chloroform-methanol and washed with saline to remove non-lipid materials as described by FOLCH et al.[7]. Upon concentration followed by drying in vacuo of the chloroform layer, the residual oily mass (total lipids) was brought to a known volume with chloroform-methanol and then fractionated by thin-layer chromatography on plates (20 × 20 cm) covered with silica gel H (1 mm thick), using chloroform-methanol-water (65:25:4)[8] as ascending solvent; the lipid extracts were applied as a narrow band

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