The RNA of Toxoplasma gondii[1] (34531)

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Evidence indicates that the protozoan *Toxoplasma gondii* replicates exclusively within cells of higher vertebrates (1). Little is known, however, of the metabolic capacity of free toxoplasma or of the role of the host cell at a biochemical level during toxoplasma replication.

With this in mind we have begun to study the RNA of toxoplasma and here report its isolation and initial characterization. Sedimentation of toxoplasma RNA in sucrose-density gradients demonstrated three distinct RNA species, and toxoplasma RNA could be distinguished from mouse macrophage RNA by sedimentation differences. It was also shown that ^{3}H-uridine is incorporated into the RNA of toxoplasma in the absence of host cells indicating that free toxoplasma are capable of synthesizing RNA.

Materials and Methods. The proliferative form of toxoplasma was obtained from peritoneal cavities of Swiss-Webster mice infected 3 days earlier with an intraperitoneal inoculation of the RH strain. The peritoneal fluid was collected and mixed with Hanks' balanced salt solution containing 100 units/ml of heparin. Toxoplasma were first released from peritoneal cells by forcing the fluid through a 27-gauge needle with a 10-ml syringe and then separated from unbroken peritoneal cells and large cell debris by filtering through a special sintered glass filter (Corning, medium-coarse porosity 15–25 μ) as described by Neimark and Blaker (2). In earlier experiments we observed that large numbers of toxoplasma free of all other detectable cells except for small numbers of red blood cells can be prepared by this method. After filtration the toxoplasmas were collected for further use by centrifugation in siliconized tubes at 2000 rpm for 10 min at 4°.

Peritoneal macrophages were obtained from normal mice, after sacrifice by cervical fracture, by washing the peritoneal cavities with 2 ml of heparinized (5 units/ml) Hanks' solution. The cells were then collected by centrifugation of the suspensions for 5 min at 800 rpm at 4°. Cells were washed three times in Hanks' solution and cultured in petri dishes in minimal essential medium (MEM) with 10% fetal calf serum. ^{3}H-uridine (specific activity 20 Ci/mmole) was added to the culture medium in a concentration of 5 μCi/ml and the cultures incubated for 24 hr at 37° in an atmosphere of 5% CO_2.

Macrophages were recovered from culture dishes by removing the culture medium and adding a solution containing 0.01 *M* Tris·HCl, pH 7.5, 0.10 *M* NaCl, 0.001 *M* disodium ethylenediaminetetraacetate (EDTA), and 1% sodium dodecyl sulfate (SDS). Preparations of toxoplasma were disrupted with the same buffer containing SDS. RNA was then prepared by phenol extraction and precipitation with alcohol as previously described (3).

Results. The RNA from toxoplasma was first characterized by rate zonal sedimentation in sucrose-density gradients. RNA from approximately 10^8 toxoplasma was mixed with a small amount of tritium-labeled RNA uninfected peritoneal cells prepared as described above, and the mixture was fractionated by sedimentation in a sucrose gradient. The results are shown in Fig. 1A where the direction of sedimentation was from right to left. The toxoplasma RNA is represented by the absorbency at 260 mμ and the mouse

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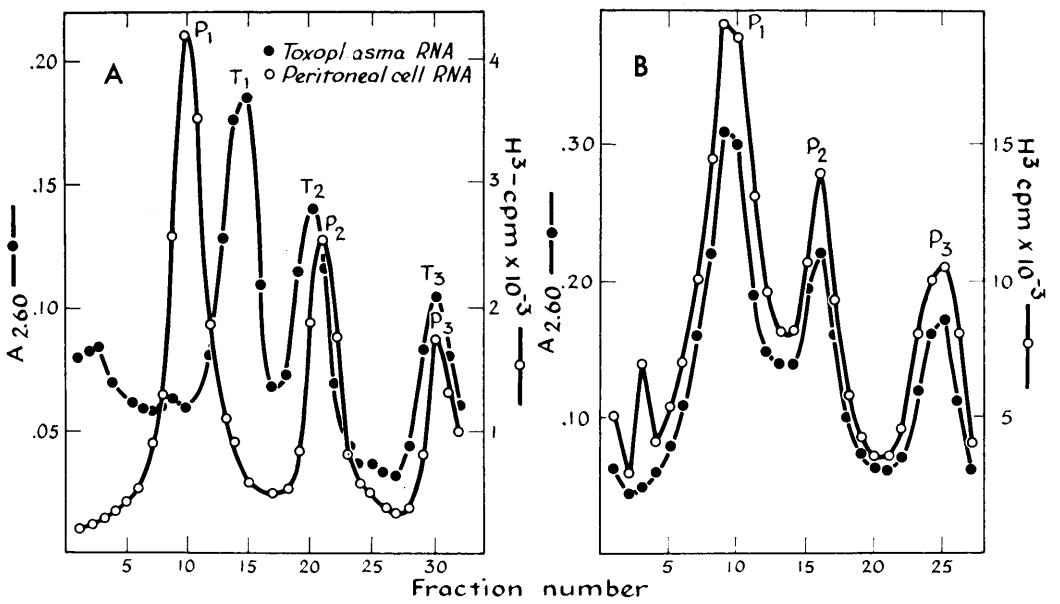

FIG. 1. Sedimentation of ^{3}H-RNA from mouse macrophages with unlabeled RNA from toxoplasma (A) and sedimentation of a larger amount of ^{3}H-RNA from mouse macrophages alone (B) in sucrose-density gradients. Mouse macrophage RNA labeled with tritium and unlabeled toxoplasma RNA were prepared as described in the text. A. The RNA from approximately 10^8 toxoplasma and a small amount of RNA from macrophages were mixed in 0.2 ml RNA buffer (0.01 *M*·Tris·HCl, pH 7.5, 0.10 *M*·NaCl, and 0.001 *M* EDTA), layered over a 13.5-ml 5–20% sucrose gradient containing the same buffer, and centrifuged at 40,000 rpm at 4° for 6 hr in the Spinco SW 40 T_1 rotor. Fractions were collected from the bottom of the tube and assayed for absorbency at 260 mμ (-●-) and TCA-precipitable tritium (-○-) as previously described (3). B. A larger amount of ^{3}H-RNA from mouse macrophages was sedimented alone in a sucrose-density gradient as described above.

macrophage RNA by the acid-precipitable tritium. The amount of macrophage RNA was insufficient to be detected by absorbency. The 28S, 18S, and 4S ^{3}H-RNA components from the mouse cells are designated P_1, P_2, and P_3, respectively. The RNA from toxoplasma has also been separated by sedimentation into three major components designated T_1, T_2, and T_3 in Fig. 1A. Component T_1 clearly differs in sedimentation rate from the mouse cell RNA components and can be estimated to have a sedimentation coefficient of 24S. This difference was observed in several separate experiments. RNA component T_2 from toxoplasma was found to sediment slightly faster than the 18S component from macrophages (P_2) and is designated 19S. The difference between the components T_2 and P_2, although small, was repeatedly observed. Component T_3 sediments in the 4–5S region of the gradient and is more heterogeneous than the other two components.

When the tritium-labeled peritoneal macrophage RNA used in the experiment in Fig. 1A was sedimented alone in a sucrose gradient in an amount that permitted detection of RNA by ultraviolet absorbency as well as by radioactivity, it was shown that the radioactivity corresponded to the major RNA components detected by absorbency (Fig. 1B). Thus, the differences between mouse macrophage RNA and toxoplasma RNA in Fig. 1A represent differences in the major RNA components of the two species.

To determine the survival time of toxoplasma in the absence of mouse cells, the organisms from peritoneal fluid were purified as described above and resuspended at 6×10^6 toxoplasma per milliliter in MEM with 10% fetal calf serum. The toxoplasma suspension

was incubated at 37° and at 1, 2, 4, and 6 hr aliquots were removed, and 0.2 ml of dilutions from 10^{-1} through 10^{-6} was inoculated into each of three mice. All mice died of toxoplasma infection within 10 days after inoculation of all dilutions of toxoplasma incubated for 4 hr or less. All mice survived after inoculation of all dilutions of toxoplasma incubated for 6 hr except for one animal which received the 10^{-1} dilution and one with the 10^{-3} dilution. Thus, it appears that under the conditions of these experiments almost all toxoplasma organisms incubated in tissue culture medium at 37° in the absence of vertebrate cells for their replication remain viable for at least 4 hr and lose viability before 6 hr.

In order to determine whether toxoplasma in the absence of mouse cells synthesize RNA, ^{3}H-uridine was added to the medium of cultured, cell free toxoplasma, and tritium incorporation into RNA was measured. Toxoplasma were purified from the formed elements of peritoneal fluid as described above and 10^8 toxoplasma were incubated in 5 ml of MEM with 10% dialized fetal calf serum, 5% dialized chick embryo extract, and 25 μCi ^{3}H-uridine (specific activity 20 Ci/mmole) at 37° for 4 hr, with continuous mixing with a magnetic stirring bar. Controls to exclude the possibility of small numbers of mouse macrophages in the toxoplasma culture accounting for any ^{3}H-uridine incorporation into RNA consisted of incubating 10^3 and 10^4 peritoneal macrophages under identical conditions with ^{3}H-uridine. This number of mouse macrophages was chosen because 10^4 macrophages is the smallest number which could be easily detected in the Nebaur-Levy hemacytometer. No mouse cells other than very small numbers of red blood cells were noted in the hemacytometer or on Giemsa-stained slides of purified toxoplasma preparations. The results in Table I show that almost 40 times as much ^{3}H-uridine was incorporated into RNA of the toxoplasma culture as into cells of the culture containing 10^4 macrophages, strongly suggesting that the ^{3}H-uridine was incorporated into RNA of the toxoplasma organisms rather than into small numbers of macrophages contaminating the toxoplasma preparation.

TABLE I. Incorporation of ^{3}H-Uridine into RNA of Toxoplasma or Peritoneal Cells.[a]

Culture[b]	Counts per minute
Toxoplasma (1.5×10^7)	19,130
Macrophages (1×10^3)	242
Macrophages (1×10^4)	531

[a] Cultures of toxoplasma and of mouse macrophages were prepared and incubated for 4 hr with ^{3}H-uridine as described in the text. At the end of the incubation period the cells were washed three times with buffer, and the cell RNA was recovered by the addition of SDS as described in the text. Aliquots of each RNA sample were taken for TCA precipitation and counting as previously described (3).

[b] Figures in parentheses represent the number of cells in culture.

Two experiments were done to confirm that ^{3}H-uridine was incorporated into the RNA of cell-free toxoplasma. First, the RNA was extracted from cells of the toxoplasma culture and the mouse macrophage culture and sedimented separately in sucrose-density gradients. The results of both sucrose gradients are plotted together in Fig. 2 and show that the tritiated RNA isolated from the cells of the toxoplasma culture sediments like toxoplasma RNA in that the fastest sedimenting component (T_1) sediments more slowly than the fastest component from the macrophage culture (P_1).

The second experiment was radioautography of preparation of the toxoplasma incubated with ^{3}H-uridine, and results in Fig. 2 show grains associated with toxoplasma.

Discussion. Fractionation of RNA extracted from *Toxoplasma gondii* by sedimentation revealed the presence of three major RNA components sedimenting at 24S, 19S, and 4S to 5S. The toxoplasma RNA components are analogous in sedimentation characteristics to the ribosomal and transfer RNA species known to be the major RNA components in all species from plants and bacteria to higher vertebrates (4). The differences in sedimentation rates of the mouse and toxoplasma RNA are no greater than the differences observed in ribosomal RNA of many different

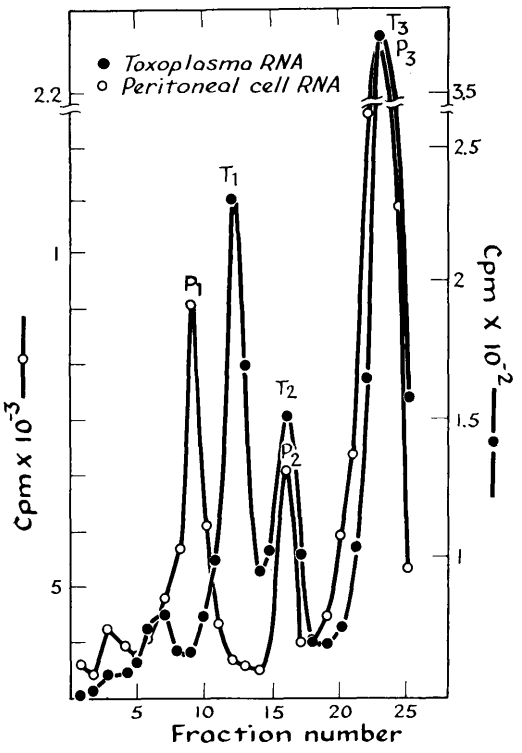

FIG. 2. Sedimentation of tritium-labeled RNA from cultured mouse macrophages (—○—) and from a preparation of toxoplasma free of mouse cells (—●—) in sucrose-density gradients. The tritium-labeled RNA from each culture was prepared as described in the text, and aliquots of each were sedimented in separate sucrose-density gradients as described in Fig. 1. The distribution of tritium in each gradient was plotted. The photographs are of autoradiographs of toxoplasma from the same experiment.

organisms in the phylogenetic scale (4). Other protozoans which replicate intracellularly, such as malaria, are known to contain ribosomes and transfer RNA which function in protein synthesis (5). Protein synthesis and protein synthetic machinery have not been studied in toxoplasma.

Our experiments also show that, although extracellular toxoplasma lose viability in tissue culture medium, they are capable of incorporating ^{3}H-uridine for a short time into the three major components of toxoplasma RNA. Thus, these organisms have the enzymatic capacity to synthesize RNA precursors and polymerize them into RNA.

The ability to isolate toxoplasma RNA and to differentiate it from host cell RNA should allow for studies designed to determine the reason for the apparent inability of toxoplasma to replicate in the absence of vertebrate cells and to learn the mechanism of the inhibition of toxoplasma replication by interferon which has been recently described (6).

Summary. Isolation and characterization of toxoplasma RNA revealed a ribosomal species which could be easily differentiated from mammalian host cell RNA. A species of transfer RNA also appeared to differ from that of the host cell. Extracellular toxoplasma were capable of incorporating ^{3}H-uridine into the major components of their RNA.

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