It is noted that while the M-P virus was infective in all mouse strains tested (Swiss and inbred strains) the HA for SRBC is stimulated only in $C_{57}Bl/6$ mice. A parallel was noted in that vaccinia virus grows in some hosts unaccompanied by a hemagglutinin (7).

Summary. The M-P virus (MPV) infection is accompanied by a sheep red blood cell hemagglutinin (HA) in $C_{57}Bl/6$ mice. The hemagglutinin titer is increased beyond the natural HA achieving a peak titer between the seventh and tenth day. The hemagglutinin, separable from the virus, occurred as an *in vivo* reaction of $C_{57}Bl/6$ mice against the infective virus and had the characteristics of an antibody different and separate from the antiviral antibody. The hemagglutinin was not produced in *in vitro* culture.

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The Entrance of Exogenous RNA into the Mouse Ascites Cell* (33063)

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During the past few years, a number of papers have appeared on the biological functions of exogenous RNA (1,2,5–7,11–16, 18–20,22, and others). It is of prime importance to make sure that the input RNA actually enters the recipient cell as an intact macromolecule, i.e., without apparent degradation. The aim of this paper is to provide experimental evidence for the entrance of RNA into the ascites cells as undegraded molecules.

Materials and Methods. Rats weighing about 200 gm were starved 15–24 hours. Each of 10–12 rats in a set of experiments received an intraperitoneal injection of 1 ml of saline containing 25 μC of adenine-^{14}C plus 100 μC of uridine-^{3}H, and immediately were allowed to feed. They were killed the next morning by a blow on the head. The liver was immediately removed and washed with ice-cold saline. The RNA was isolated from the pooled livers following the modified cold phenol procedure as reported by Hillman and Niu (7). Some of its properties are shown in Table I.

TABLE I. Characterization of the Double Labeled Rat Liver RNA.

1.	OD (mμ) 260/280 = 0.497
2.	OD (mμ) 260/230 = 2.43
3.	E(P) value (8) = 8000
4.	Protein contamination (10) = 1–2%
5.	DNA contamination (3) = nil
6.	Specific activity of orotic-5-^{3}H acid = 85,879 cpm /mg of RNA
7.	Specific activity of adenine-8-^{14}C = 14,688 cpm/ mg of RNA
8.	Proportion of ^{3}H and ^{14}C = 5.85

The Nelson mouse ascites tumor cells used in the present study were maintained in

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Swiss-Webster mice by weekly transfers. The cells were pooled from several mice after 5–7 days of transfer and washed 3–5 times with cold saline (0.15 *M* NaCl). Washed ascites cells were suspended in 20 vol of cold, sterile saline containing 50 OD/ml of labeled rat liver RNA. To assure maximal entrance of RNA and minimal ribonuclease activity, the suspension was slowly shaken overnight in a cold room (2–4°C). The cells were centrifuged in the cold room and resuspended in 20 vol of the cold incubation mixture (14) containing 50 OD/ml of the same labeled RNA. In order to determine the rate of RNA uptake, cell suspension was transferred to metabolic shaker at 37°C, and allowed 5 min for temperature equilibration. Aliquots were then taken at 0,25,30,40, and 55 min. Each sample was washed 4 times with 40 col of buffered saline (pH 7). The supernatant of the last wash did not show any appreciable radioactivity and absorbancy at 260 mμ. The residual labeled RNA left in the medium after overnight contact with the ascites cells at 2°C was also checked for degradation. It was found that during the overnight period, only about 1% of the exogenous RNA was detectable in the acid soluble fraction.

The samples were divided into two fractions. One fraction was fixed in 2% OsO_4 solution in Palade veronal buffer and processed according to the routine procedure for electron microscopy. Embedding was made in 4:1 butyl and methyl methacrylate mixture. Alcoholic solution of phosphotungstic acid 1% was used for staining in the preembedding state. Silver gray sections were chosen and mounted on electron microscopic grids. The procedure of Caro and Van Tubergen (4) for preparing the high resolution autoradiograms was followed by using L_4 liquid emulsion (Ilford), diluted to 1:7 (w/v) with distilled water. The grids, covered with emulsion, were kept dark and dry in a cold room for 21 days. They were developed with diluted Dektol (1:2 v/v) for 2 minutes. After fixation they were kept in NaOH (0.05 *N*) for 20 min, and washed thoroughly with H_2O. Each grid was stained with a saturated solution of uranyl acetate and alkaline lead citrate. Background grains were rarely found in any of the control girds, that is, sections of the ascites cells without radioactive material. For treatment with ribonuclease, the method of Leduc and Bernhard (9) was followed. The fixed cells were washed with cold 5% H_2O_2 for 20 min and then washed thoroughly with a buffered saline (pH 7). This treatment did not alter the electron microscopic picture with respect to the distribution of silver grains. Pancreatic ribonuclease was added to each of the samples at a concentration of 200 μg/ml in normal saline and incubated at 37°C for 1 hour. Following incubation, the cells were washed with ice-cold buffered saline. The rest of the procedure is the same as described above. It may be added here that identical preparations were also made for cytochemical observations of the disappearance of pyronin-positive basophilia from the cells after ribonuclease digestion.

The other fraction of the washed cells was used for the isolation of RNA. The RNA was isolated according to the technique of Ogur and Rosen (17). The double radioactivity (^{14}C and ^{3}H) of the RNA from various samples were recorded simultaneously by the Packard scintillation counter.

Results. During the incubation of the ascites cells at 37°C, an increase in the uptake was noted up to 40 min, and no further increase was observed (Table II). The specific activity of the RNA isolated from the ascites cells when compared with that of the exogenous RNA, shows that only 1.9% of the input RNA has entered into the ascites cells. The above experiment was reproducible when the exogenous RNA used was labeled with 1:3 proportions of adenine-^{14}C and uridine-^{3}H. When the ribonuclease digested RNA (85% of the RNA became acid soluble by the enzyme) was used, the maximum uptake of RNA, at 25 min of incubation, was reduced to about 0.4% of the hydrolyzed exogenous RNA.

Uptake of input RNA may occur in the form of intact RNA macromolecules (15, 21, 23) or degraded molecules. In the case of the former, the RNA isolated from the treated ascites cells should have a similar ratio of

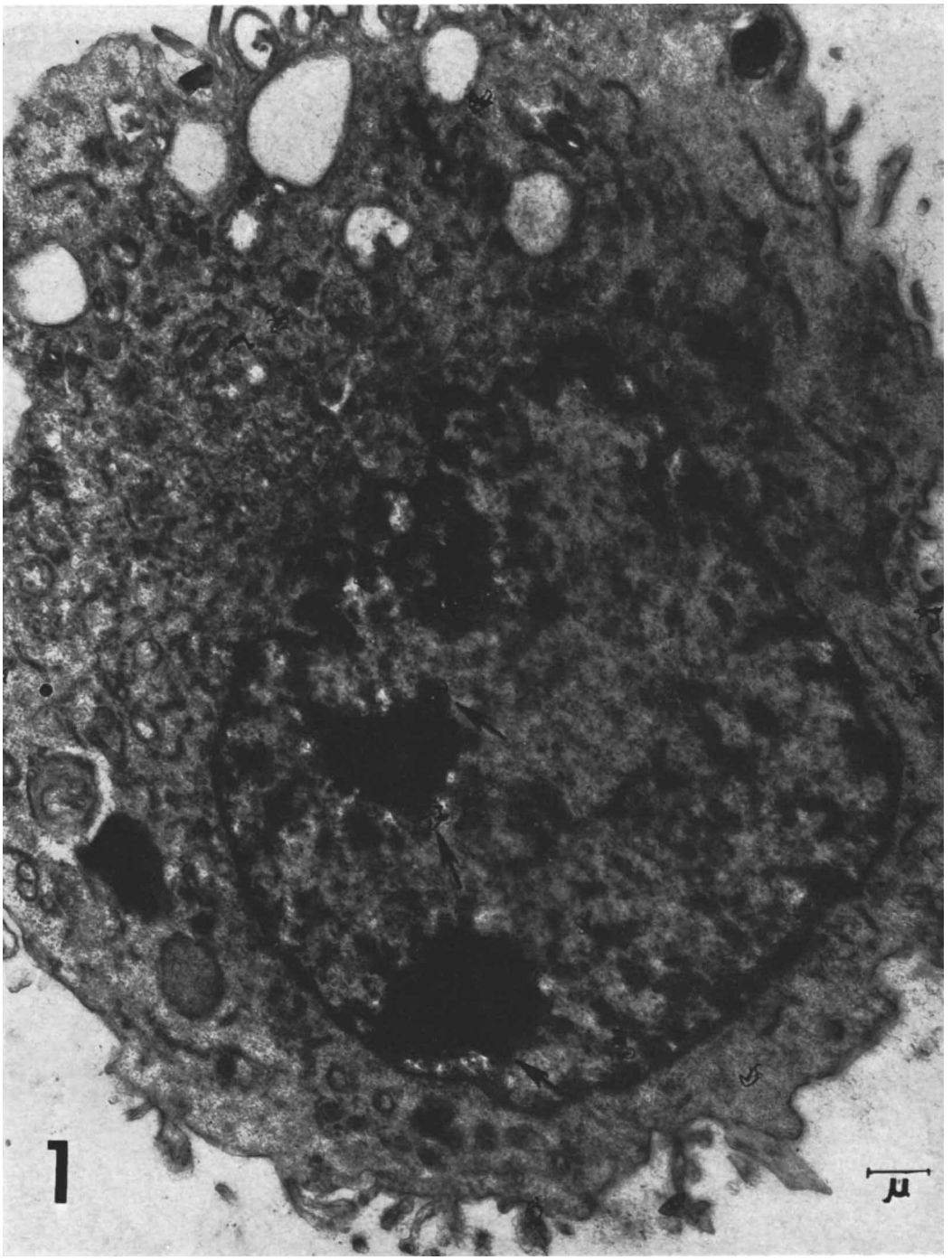

FIG. 1. High resolution autoradiogram of a thin section of the ascites cells after 20 min of incubation. The silver grains are due to the labeled exogenous RNA molecules after their entrance into the ascites cells. Arrows point to the grains over nucleolar material. 12,000 ×.

TABLE II. Summary of the Uptake of Exogenous Rat Liver ^{14}C and ^{3}H-RNA by Mouse Ascites Cells.

Samples	Incubation time at 37°C (min)	Specific activities (cpm/mg of RNA)		Ratio of $^{3}H/^{14}C$
		Uridine-^{3}H	Adenine-^{14}C	
Rat liver ^{3}H and ^{14}C-RNA[a]		85,879	14,688	5.85
Cells treated with double labeled rat liver RNA	0	793	128	6.17
	25	1367	207	6.60
	30	1480	220	6.73
	40	1600	225	7.11
	55	1618	225	7.14
Cells treated with ribonuclease digested, labeled RNA	0	127	39	3.26
	25	392	133	2.94

[a] Under similar conditions, double labeled RNA was isolated from ascites cells. In contrast to the rat liver RNA, its $^{3}H/^{14}C$ counting ratio was about 1.1.

radioactivity counts ($^{3}H/^{14}C$) as that of the rat liver RNA. If, on the other hand, the exogenous RNA is somehow degraded during the process of treatment in the cold (2–4°C) or at the time of incubation at 37°C, its hydrolytic products would contribute to the precursor pool and thus are used for resynthesis of RNA by the ascites cells. As a result, the ratio of radioactivity between ^{3}H and ^{14}C components of the RNA isolated from the ascites cells should differ from that of the ^{3}H and ^{14}C-liver RNA. In our present experiment about 5% of the exogenous RNA was degraded during the incubation period up to 25 min at 37°C and only 1% when incubated at 2°C overnight. Therefore, contamination due to such a small amount of degradation products of RNA can be neglected. It can be seen from Table II that at the 0 time of incubation which is, in fact, the end of RNA treatment at 2–4°C, the ratio given by exogenous RNA (5.85) is, within experimental error, the same as the RNA isolated from the treated cells (6.17) but differs remarkably from that of cells treated with the ribonuclease digested labeled liver RNA, whose ratio is 3.26. These data have led to the belief that macromolecular RNA enters or is adsorbed onto the surface without molecular degradation.

Direct proof that exogenous RNA actually enters the ascites cells came from the study of high resolution autoradiograms. Figures 1 and 2 show the silver grains inside the ascites cells after 20- and 25-min contact with the labeled RNA, respectively. The development of these grains is due to the presence of the labeled exogenous RNA, because they disappear after treatment with ribonuclease. The spatial distribution of the exogenous RNA in relation to the cytoplasmic and nuclear structure of the ascites cells cannot be specified at the present time. However, from the counts of silver grains in 50 electron micrographs, the distribution of grains in the cytoplasm was about three times more than that in the nucleus.

The presence of the radioactive RNA in the treated cells raises a question concerning the mechanism by which RNA macromolecules enter into the cells. Since pinocytosis is commonly believed to be responsible for the transport of macromolecules, special attention has been paid to locate pinocytosis vesicles. In our experience, however, labeling has been observed at cell surface but not over pinocytosis vesicles. The entrance of labeled RNA into the nucleus, presumably through nuclear pores, is indicated by the silver grains overlying the nuclear envelope (arrow, Fig. 2).

Summary. The entrance of RNA into ascites cells has been studied using double labeled rat liver RNA and high resolution electron microscope autoradiography. The evi-

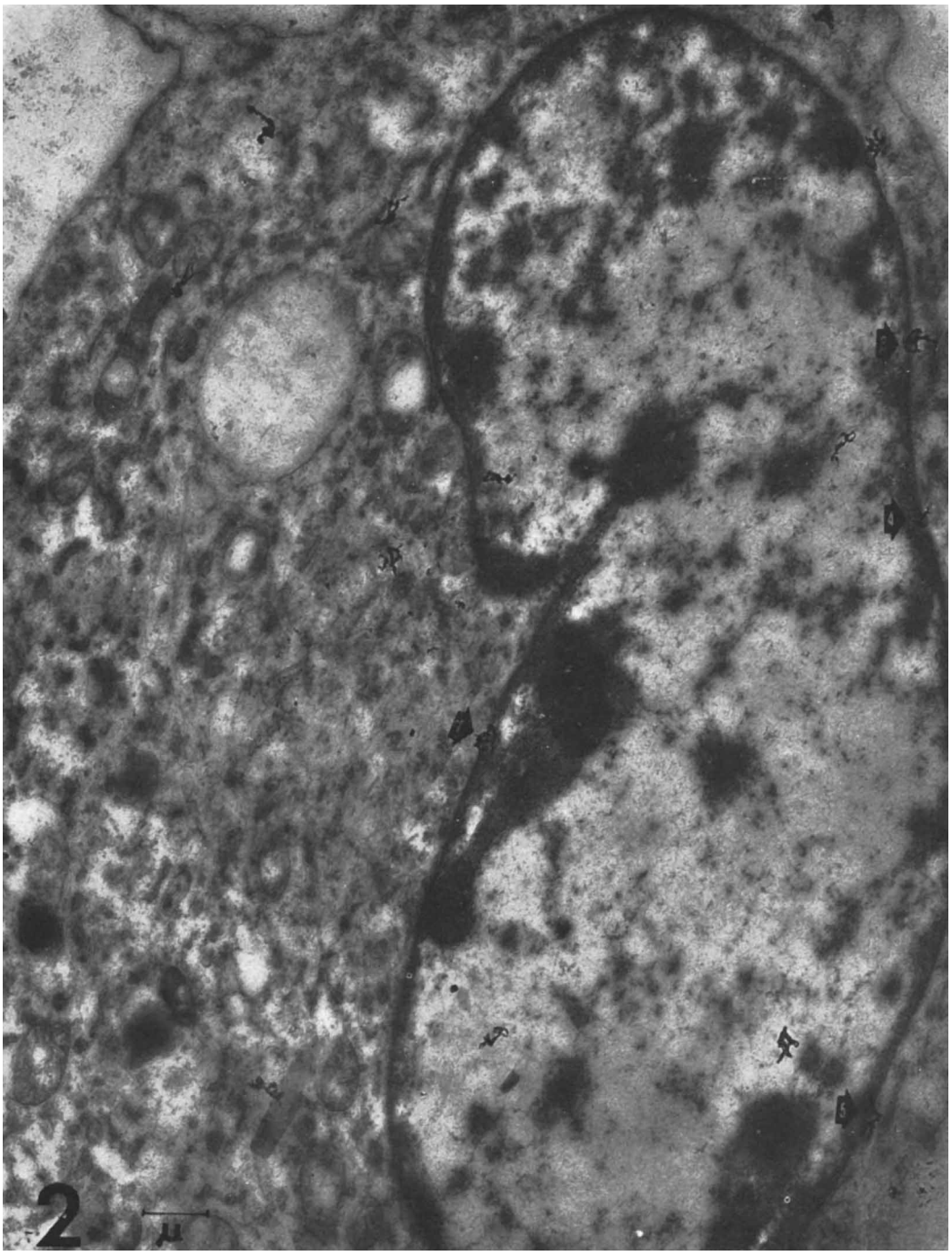

FIG. 2. High resolution autoradiogram of a very thin section of the ascites cells after 25 min of incubation. Note the silver grains overlying the nuclear membrane. 16,000 ×.

dence reported here indicates that RNA enters into the cells as an undegraded molecule.

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Magnesium and Calcium in the Cerebrospinal Fluid of the Rat (33064)

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Atomic absorption spectrophotometry is a specific, sensitive, accurate method for measuring numerous cations. This technique was used to obtain previously unavailable data on the content of magnesium and calcium in the cisternal cerebrospinal fluid (CSF) of the rat. The results are given in the present report.

Methods. Three groups of male albino rats (Sprague-Dawley strain) fed a stock laboratory diet and tap water *ad libitum* were studied. The concentrations of Mg and Ca were measured in plasma water (P), ultrafiltrate of plasma (UF), and CSF from animals weighing 120–130 gm and 320–380 gm. The P-Mg and CSF-Mg were also determined in a third group of animals weighing 150–220 gm.

Collections of samples. All glassware was cleaned with a 1:2 dilution of concentrated HNO_3 and rinsed thoroughly with distilled–deionized water prior to use.

CSF. Short-beveled no. 25 hypodermic needles (Becton, Dickinson and Co.) were removed from their hubs and soldered into the empty hubs from no. 22 needles so that the blunt end of the needle projected posteriorly approximately 0.5 cm into the hub. A removable 6-cm length of polyethylene tubing (B-D PX022) was inserted over the blunt end of the needle. Under ether anesthesia, hair was clipped from the posterior cervical and occipital areas of the rat, and the animal was pinned to a stand designed to

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