production of interferon-like activity in cell cultures infected with a rickettsia but did not describe the inhibition of rickettsial multiplication by this substance. In the accompanying paper(12) the characteristics of interferon induced by a TRIC agent are detailed.

Mordhorst's(2) failure to demonstrate susceptibility of TRIC agent to interferon can be attributed in part to the lack of an adequate quantitative method and in part to the use of a low potency interferon. In our studies the activity of mouse interferon was 10-fold lower against TRIC agent than against VSV titrated in the same cell strain. The reason for this lower susceptibility needs to be further explored.

TRIC agents produce protracted infections in the human eye. It cannot be stated what role, if any, locally produced interferon might have on the replication and persistence of the infectious agent. The present documentation of TRIC agent inhibition by interferon may play no practical role in disease but has important implications regarding the broad nature of interferon activity.

We wish to acknowledge the technical assistance of Miss H. Keshishyan.

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Received January 31, 1966. P.S.E.B.M., 1966, v122.

Characteristics of Interferon Induced *in vitro* and *in vivo* by a TRIC Agent.* (31151)

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Interferon has been induced by virtually all types of animal viruses in a number of mammalian cell species, both *in vivo* and in tissue culture(1). More recently, a number of nonviral agents have induced interferon production, including *Rickettsia tsutsugamushi*(2), brucella(3), pleuro-pneumonia-like organisms (Stinebring *et al*, personal communication), endotoxin(4), phytohemagglutinin(5), and statalon(6).

One attempt to demonstrate chick interferon production with trachoma-inclusion conjunctivitis (TRIC) agents *in ovo* and *in vivo* resulted in failure(7). These agents differ from true viruses in many important aspects of their structure and life cycle and, like the other members of the psittacosis-LGV-trachoma group, resemble rickettsiae and bacteria(8). Although nonviral agents induce interferon in serum which apparently has somewhat different physical characteristics compared to virus-induced interferon(9, 10), we thought it surprising if the TRIC agents lacked an interferon-inducing capacity. Therefore, appropriate experiments were performed in which interferon induction was successfully demonstrated.

Materials and methods. Interferon assay. A highly interferon-sensitive line of L cells obtained from Dr. J. Youngner was employed in these studies. These L cells are approximately 10 times more sensitive than mouse

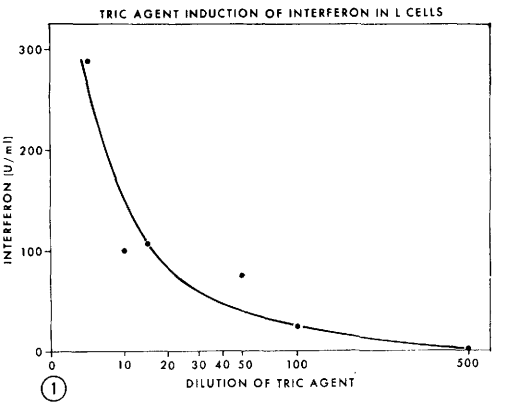
* Supported by grants from NIH (AI 05629, NB 00604) and from Burroughs Wellcome Fund.

embryo fibroblasts with a given dose of interferon acting on bovine vesicular stomatitis virus (VSV) (Youngner *et al*, personal communication). For measurement of interferon activity, a plaque inhibition method was employed using confluent monolayers in plastic dishes (60 × 15 mm) and 50 plaque forming units of VSV. Samples to be assayed were serially diluted in 4-ml aliquots and incubated for 16 hours over monolayers before virus challenge. The number of units of interferon in the given sample was defined as the reciprocal of the dilution that produced 50% reduction in the number of plaques. When sera were assayed, a 4-ml medium wash step was introduced before the virus challenge.

In vitro *interferon production.* The methods of growing L cells and infecting with the TRIC agent LB-1 are discussed in the accompanying paper(11). Monolayers were overlaid with infected yolk sac suspensions in concentrations ranging from 0.2% to 50%. The majority of experiments were performed with a 20% suspension of yolk sac and a 20-hour incubation period of infected monolayers at 35°C, followed by 4 hours at 38°C.

In vivo *interferon production.* After preliminary experiments, 0.2 ml of a 12% yolk sac suspension containing the TRIC agent LB-1 ($10^{7.8}$ ELD50/g of yolk sac) was inoculated intravenously into HA/ICR Swiss mice. The animals were bled at 6 hours, a time when interferon is detectable with most interferon inducers(10). None of the mice so challenged died before the end of the 6-hour period.

Results. Production of interferon. Both *in vivo* and *in vitro,* the amount of antiviral activity produced was dependent on the amount of agent employed for induction. Fig. 1 shows this concentration dependence in the production of interferon in the L cells *in vitro*. *In vivo* at 6 hours, enough residual TRIC agent was present in the mouse sera to interfere with plaque formation by the challenge VSV in our cell culture assay system unless the TRIC agent was removed by ultracentrifugation (100,000 *g* for one hour) before the antiviral activity was characterized. However, after this centrifugation, 100-



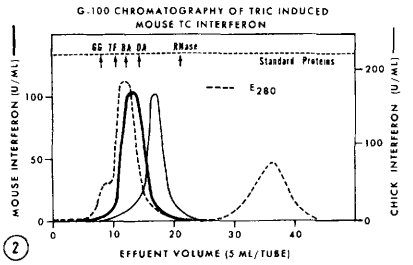


FIG. 1. *Induction of interferon in L cells by the TRIC agent.* The dilution of the TRIC agent employed is an expression of the dilution of the initial yolk sac suspension by tissue culture medium.

FIG. 2. *Molecular weight determination of TRIC-induced mouse interferon on G-100 Sephadex.* Emergence positions of the peak concentration of various standard proteins chromatographed on the same column are indicated at top of Figure (GG = bovine gamma globulin; TF = human transferrin; BA = bovine serum albumin; OA = ovalbumin; RNase = bovine pancreatic ribonuclease). The chick and mouse interferons were co-chromatographed in this experiment and concentrations of both assayed in each fraction.

500 units of interferon per 4-ml sample of serum from mice injected with LB-1 could be demonstrated. Control uninfected yolk sac suspensions or heat-inactivated LB-1 (56°C, one hour) at similar concentrations failed to induce antiviral activity in either *in vivo* or in cell culture. *In vitro* a TRIC agent (BOUR) which does not replicate significantly in cell culture also failed to induce antiviral activity when applied to monolayers of L cells at similar concentrations, even when the infective inoculum was driven into cells by means of centrifugal force by a modification (Hanna, unpublished data) of the methods of Gordon *et al*(12) and Padgett and Walker (13).

Characteristics of the TRIC-induced interferon. The antiviral activity induced by the TRIC agent was characterized by measuring its antiviral action on chick and human cells to assess species-specificity, employing a plaque inhibition assay(14). It was inactive against VSV in chick embryo fibroblasts and human neonate fibroblasts at 30-fold excess in the case of the L cell culture-induced material, and at 10-fold excess in the case of the *in vivo*-induced material. Both TRIC-induced activities were totally destroyed by trypsin (0.25 mg/ml, 38°C, one hour), but stable to pH 2 treatment and not sedimented by 100,000 $\times$ *g* for 2 hours. Unless exposure of cells was continued for the usual time necessary for appearance of interferon (6-24 hours), the antiviral effect could not be demonstrated. When the cell culture-induced material exhibiting antiviral activity was incubated directly with 10^5 VSV particles for one hour at 37°C, there was no decrease in plaque-forming units compared to controls. The cell species-specific nature of this inhibitor distinguishes it from the protein antiviral product of penicillium (cyclopin)(15), and its trypsin sensitivity distinguishes it from the several antiviral agents produced by yeasts or bacteria (statalon, helenin, etc.)(6, 16).

As has been pointed out(14,17), such methods of characterization are limited because they indicate only that the antiviral material is a protein acting on the virus through the cell. As the antiviral activity of cell culture preparations was high, gel filtration was employed to determine its molecular weight. The method of co-chromatography with chick interferon as an internal marker has been described(14,18). To avoid possible protein interaction artifacts, the chromatography was also carried out with phenol red as the only internal standard. Fig. 2 presents the results of chromatography of the *in vitro*-induced antiviral material and several individual standard proteins on the same column. We found TRIC-induced L cell culture interferon to have a molecular weight of approximately 50,000, both on a single co-chromatography with chick interferon and on 2 separate occasions when chromatographed alone on a standard column.

Discussion. The 50,000 molecular weight (mw) of the TRIC interferon appears to differ from that of 26,000 previously reported (9,18) for mouse interferon induced *in vivo* by Newcastle disease virus (NDV). Others (Lampson *et al,* personal communication) recently reported 2 species of mouse serum interferon induced by NDV (23,000 mw and 36,000 mw). Interferon induced by Chikungunya virus in mouse cell culture appeared to be 26,000 mw, and interferon induced in mouse cell culture by NDV appeared to be 31,000 mw(9). The differences among all these results may be within the error of the method. They differ significantly from the *in vivo*-induced heavy forms of interferon induced by endotoxin (90,000 mw)(9), statalon (85,000 mw)(10), or brucella (53,000 and 77,000 mw)(9). The interferon induced by TRIC agent in cell culture appears to be similar to the lighter of the 2 brucella interferons. A comparison of our results with those of Hallum, Youngner and Stinebring(9) indicates that a single cell line, the L cell, appears to be capable of responding with 2 different molecular species of interferon depending on the inducing agent: namely, a virus (NDV) or a TRIC agent (LB-1).

The failure of others(7) to demonstrate production of chick interferon either *in ovo* or *in vivo* by TRIC agents may have been partly due to inadequate dosage of the inducing agent. In contrast to our experiments which employed $10^{7.4}$ ELD50 per monolayer containing about 10^6 cells, and $10^{6.2}$ ELD50 per mouse, Mordhorst and Reinecke employed only 10^4 ELD50 per egg and 10^7 ELD50 per chicken(7). They also subjected their *in ovo* preparation to 57°C for one-half hour in an attempt to destroy the inducing agent. That temperature is known to decrease the biologic activity of both crude and purified chick interferon(1,18).

Others have observed a direct relationship between the interferon-inducing capacity of a virus and its sensitivity to interferon action (19). Hence, it is not surprising that this obligate intracellular parasite, the TRIC agent LB-1, was sensitive to interferon action

when high titer interferon and highly sensitive mouse cells were employed(11).

Summary. A TRIC agent (LB-1), a member of the psittacosis-LGV-trachoma group, can induce the production *in vivo* and *in vitro* of a material with antiviral activity similar to virus-induced interferon. The interferon induced in cell culture by LB-1 had a molecular weight of about 50,000 and hence appeared to differ significantly from virus-induced interferon prepared in cell culture or *in vivo*.

ADDENDUM: The peak of TRIC-induced interferon production in serum of mice is late (6-13 hours), similar to that of virus(3), brucella(3), or statalon(10), but distinct from the early (2 hour) peak appearing after endotoxin(4).

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Received January 31, 1966. P.S.E.B.M., 1966, v122.

Blood Preservation Solutions Containing Adenine, Phosphate, and Guanosine.* (31152)

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Although ACD (acidified citrate dextrose) solution has been in routine use for blood storage since World War II, there have been suggestions that this medium could be improved by addition of purine nucleosides such as adenosine or inosine (see review in ref. 1, also 2,3). Addition of adenine, with or without inosine, was also observed to have a beneficial effect(4,5,6). Other suggested alterations in the formulation include addition of phosphate(7,8) or control of pH(9,10). Added guanosine might be beneficial as an energetic purine nucleoside(11) or might have some additional role perhaps as a structural component(12). The present study embodies many suggestions for the improvement of ACD and emerges with what appears on the basis of *in vitro* testing to be an improved blood banking solution.

Methods and materials. The criteria for improved red cell storage media were prolonged maintenance of ATP(13,14) and nucleotide adenine (AMP + ADP + ATP). The latter measures the adenine pool available for ATP formation(15). Because of donor variability in ATP levels, the general plan of each experiment was to collect 100 ml of blood successively from the same donor into 5 vacuum bottles. These bottles† contained the various formulations to be tested

* Supported in part by USPHS AM-06367 and AM-05581 from Nat. Inst. of Arthritis & Metab. Dis.

† Baxter H-18 Plasma-Vac, 200 ml capacity, courtesy of Dr. L. D. Bechtol, Baxter Laboratories, Inc., Morton Grove, Ill.