TABLE IV. Comparison of Paper and Thin-Layer Chromatographic Methods Showing Distribution of 131-I in Rat Thyroid Homogenates.

	No. of rats	Origin	T_4	T_3	I	DIT	MIT	Alpha
TLC								
Isotopic equilibrium	17	3.9 ± 1.5	14 ± 3.3	2.7 ± 1.2	3.9 ± 1.6	51.8 ± 4.2	20.4 ±30	3.3 ± 1.6
4 hr after 10 μC 131-I	6	3.5 ± 0.4	7.9 ± 1.5	1.6 ± 0.2	5.3 ± 0.2	51.9 ± 2.2	27.2 ± 1.5	2.5 ± 1.9
Paper								
Isotopic equilibrium	15	5.3 ± 2.4	11.1 ± 3.9	1.6 ± 1.4	4.9 ± 1.3	50.7 ± 3.7	26.5 ± 4.4	—

hydrolysates using TLC. Although the separation achieved was excellent, iodide was not recovered quantitatively due to losses from the acidic formic acid : water system. Recovery of compounds averaged 76.0 ± 3.6% of the iodine containing compounds applied to the plates. West *et al*(4,10) measured serum T_4 levels after separation by TLC. The method used did not separate T_3 from T_4 and the published low Rf values seem to indicate that separation and quantitation of all moieties is technically difficult. The method of Shapiro and Gordon(6) gives recoveries similar to ours.

The modification of the CAMB reagent using acetone instead of distilled water prevented distortion of the spots on the chromatoplate. This stain was capable of detecting .001 μg of iodide; however, its use is associated with variable iodine loss, hence is not applicable to quantitative studies.

The advantages of this method are distinct separation of each spot with good resolution and excellent recovery and reproduceability. There was no loss of iodide or alteration of the compounds during chromatography.

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Interferon Production in Hamsters Experimentally Infected with Rabies Virus.* (31568)

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The virus inhibitor interferon has been shown to be produced by cells, both *in vivo* and *in vitro,* in response to several viruses and other agents(1). However, no work has

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been published showing that interferon is produced by cells infected with rabies virus. Kaplan *et al*(2) showed that medium from rabies virus infected chick embryo monolayers was capable of interfering with an heterologous virus, but they did not characterize the inhibitor, although they refer to the inhibition, in a later publication(3), as having been due to interferon. Later, Wiktor *et al*(4) were unable to isolate an interferon-like inhibitor from human diploid WI-38 strain cells infected with rabies virus, and Fernandes *et al* (5) were also unsuccessful in attempts to demonstrate interferon from rabbit endothelial cells infected with this virus. These observations have led Depoux(6) and others to question whether rabies virus is indeed capable of inducing interferon production in the cells it infects. Since studies on the pathogenesis of rabies are underway in this laboratory, it seemed desirable to include experiments which might clarify the status of rabies virus in relation to its interferon inducing capabilities.

Rabies virus is thought to migrate from the site of inoculation *via* nerves to the central nervous system and from there by a similar pathway to the various organs(7). Since inoculation of animals is followed by a rise in virus titer in the brain, which is then followed by appearance of the virus in various organs, it seemed likely that appearance of interferon in rabies infected animals would follow this pattern.

All the previous attempts to demonstrate interferon induction by rabies virus have involved *in vitro* systems(2,4,5), but since Finter(8) has shown that some viruses that give low interferon titers *in vitro* will give higher interferon titers *in vivo*, the latter system was employed in these studies.

Materials and methods. Animals. Weanling white Swiss mice were obtained from the Euers Farm, Austin, Texas. Weanling male golden hamsters weighing 25-30 g were obtained from our departmental hamster colony.

Viruses. Vaccinia virus was obtained from the American Type Culture Collection (ATCC) and was inoculated into chick embryos to prepare a stock virus suspension for use as challenge agent in hamster kidney (HK) cells. West Nile virus was obtained from ATCC and was inoculated into weanling mouse brains to prepare a stock suspension for use in subsequent experiments. Challenge Virus Standard (CVS) strain of rabies virus was obtained from ATCC and a stock suspension for use in hamster inoculations was prepared from infected mouse brains. This is the same strain of rabies virus that was used in the previously cited studies(2,4,5), and it is pathogenic for hamsters by either intramuscular or intracerebral routes of inoculation. The LD_{50} titer of this stock virus for weanling mice inoculated intracerebrally was $10^{-7.0}$. The Bell strain of rabies virus (59VI3B), isolated from naturally infected little brown bats was obtained from Dr. J. F. Bell in its third mouse brain passage and its titer for 3-week-old mice inoculated intracerebrally was $10^{-5.3}$. This strain of rabies virus is pathogenic for hamsters in intracerebral inoculation.

Tissue cultures. Primary cultures of chick embryo fibroblasts were prepared from 11-day-old embryos. Cells were grown in 60 mm plastic petri plates (Falcon) in Medium 199 with 5% calf serum and antibiotics. Plates inoculated with 1.0×10^7 cells in 5 ml medium were incubated at 37C in 5% CO_2 for 24-48 hours. Primary HK monolayers were prepared by inoculating 60 mm plastic plates with approximately 3.0×10^7 cells in 3 ml Medium 199 with 5% calf serum and antibiotics and incubating at 37C in 5% CO_2 for 4 days.

Interferon production and assay. To test for interferon (IF) production, hamsters were inoculated intramuscularly in the left thigh with approximately 60,000 weanling mouse intracerebral LD_{50} of the CVS strain of rabies virus. On each day post-inoculation, groups of 10 hamsters were sacrificed and the selected tissues were removed and immediately pooled and frozen. This process was repeated daily until death of the remaining animals. All tissues were stored at —70C until processed for IF. This was accomplished by grinding the frozen tissues with mortar and pestle and making 10% suspensions in Hanks' balanced salt solution. These suspensions were centrifuged at $1,000 \times g$ and the supernatant

fluids were dialyzed in the cold for 24 hours against pH 2.0 HCl-saline and then against pH 7.4 saline to neutrality. Precipitated materials were removed by centrifugation, and the supernatant fluids were then ultracentrifuged at 100,000 $\times$ *g* for one hour and the top three-fourths of the resulting supernatant fluids were again ultracentrifuged for an hour, and the top three-fourths of these supernatant fluids were stored at 4C until assayed for IF. Tissues from uninfected hamsters were processed in an identical manner, and the fluids so obtained were used as controls with each tissue IF assay.

Several hamsters were inoculated intracerebrally with the Bell strain of rabies virus to make preliminary determinations on the ability of this virus to induce IF. The brains of these animals were harvested near death and processed for IF as above.

An IF standard was prepared by inoculating a group of hamsters intracerebrally with West Nile virus and collecting their brains when they showed evidence of central nervous system disease. These brains were processed in the same manner as other tissues and this standard IF was used on each occasion of IF assay as a guide to the reproducibility of the assay system.

All preparations were assayed by the 50% plaque-reduction endpoint method(9). Washed primary HK monolayer cultures were incubated for 18 hours with 2 ml of appropriate dilutions of the preparations in culture medium. The cultures were then washed 3 times with phosphate buffered saline containing 0.1% bovine serum albumin (PBSA) (10), and approximately 100 PFU Vaccinia virus in 0.5 ml PBSA were added. After adsorption at 37C in 5% CO_2 for 90 minutes, overlay medium containing Medium 199, 2% calf serum, antibiotics, and 1% purified agar (11) was added, and plates were incubated at 37C in 5% CO_2 for 72 hours. The overlays were then removed and the cell sheets were stained with 0.2% crystal violet, washed in water, and the plaques counted. Five cultures were used for each dilution of each preparation. One unit of IF is defined as the amount in 2 ml of the dilution which reduces the plaque count to 50% of the control count. Using monolayers of the same age, which had been inoculated with the same number of cells, this assay system was found to be reproducible, giving the same IF titers on each occasion of assay.

Characterization of virus inhibitor as IF. All preparations assayed for interfering activity were first treated at pH 2.0 for 24 hours, ultracentrifuged at 100,000 $\times$ *g* for 2 hours, and exposed to an 8-watt G. E. germicidal lamp at 15 cm for 5 minutes(12). Samples were assayed for activity after treating with 0.1% trypsin for 1 hour at 37C; assayed for activity against both RNA and DNA viruses; assayed before and after storage at 4C for over 200 days; treated at 56C and 80C for one hour; assayed in heterologous cells; and tested for intracellular and extracellular activity by determining effect on virus adsorption to cells and direct inactivation of virus.

Results. Characteristics of the inhibitor. That the inhibitor involved in these studies is IF is shown by: 1) its failure to inactivate virus directly, as determined by mixing with virus inoculum prior to adsorption of virus to chick cells; 2) its failure to block adsorption of virus to treated cells; 3) its loss of activity after treatment with trypsin; 4) its activity against both Vaccinia and West Nile viruses in hamster cells (virus nonspecific), and 5) its lack of activity against either of these viruses in chick cells (species specificity); 6) its retention within dialysis tubing and failure to sediment at 100,000 $\times$ *g* for 2 hours; 7) its stability at 4C; 8) its insensitivity to ultraviolet irradiation; 9) its relative resistance to 56C but inactivation by 80C; 10) its resistance to pH 2; and 11) the inability to remove its activity from cells by repeated washings(1).

Results of tissue assays for IF. On the first day post-inoculation, none of the tissues showed any detectable IF. This is representative of a concentration of less than 10 units of IF per gram wet tissue (Table I). By the second day, there was a detectable amount of IF in the brain, but all of the other tissues were negative. This observation agrees well with the proposed route of rabies virus from bite to brain *via* nerve pathways. By the

TABLE I. Interferon Concentrations in Tissues of Hamsters Infected with Rabies Virus.*

Days post-inoc.	Brain	Blood	Spleen	Lung	Kidney	Brown fat	Liver
0†	—‡	—	—	—	—	—	—
1	—	—	—	—	—	—	—
2	10	—	—	—	—	—	—
3	20	10	—	—	—	—	—
4	80	20	10	10	10	10	—
5	3500	40	40	20	20	20	—
6	1500	20	20	20	20	20	—

* Animals received 60,000 weanling mouse LD_{50} of CVS strain intramuscularly.
† Normal uninfected animals.
‡ Interferon concentrations expressed in units/g wet tissue; — = IF concentration of less than 10 units/g wet tissue.

third day, the brain IF level had risen, and there was also detectable IF in the blood. The IF in the blood could not be explained as due to viremia, but may be due to a spill-over from the brain where IF was being produced. By the fourth day, a still greater increase was found in the brain and blood, and at this time detectable amounts of IF were present in the spleen, lung, kidney, and brown fat. Since IF was not found in the liver, it seems unlikely that its demonstration in the other tissues was due to their blood content. These findings can be interpreted as further evidence of the centrifugal spread of rabies virus from the brain to the organs, since virus also was detectable in brown fat, kidney, and lung, but not in liver. The amount of virus in these tissues, determined by intracerebral inoculation into weanling mice was considerably less than the virus level in the brain which had an LD_{50} titer of 10^{-7} for weanling mice inoculated intracerebrally. The virus levels in the various tissues assayed corresponded to their IF concentrations. Viral antigens were demonstrated in impression smears of representative samples of the various tissues by means of the fluorescent antibody technique using a fluorescein-conjugated rabies hyperimmune burro serum kindly provided by Dr. Keith Sikes, Communicable Disease Center, Atlanta, Ga. By the fifth day, the brain content of IF had risen to a very high level. The blood and spleen contents are equal at this time, and this is of interest as further evidence for the production of IF by the spleen itself, since Subrahmanyan and Mims(13) have shown that intravenously injected IF can be recovered from kidney, lung, and liver, but not from spleen. And again to rule out blood content as contributing significantly to the IF levels in the organs, the liver is still negative. By the sixth day, the brain IF level had fallen, as had the blood and spleen levels, with no change in the other tissues. Liver was still negative. All the remaining hamsters were dead before the seventh day.

The hamsters that had been inoculated intracerebrally with the Bell rabies strain were also found to contain IF in their brains. However, intramuscular inoculation of hamsters with this strain did not induce detectable IF in any of the tissues assayed, due to the failure of this virus to multiply following this route of inoculation.

Discussion. The results of these studies show that an interferon is produced by cells infected with rabies virus. In this system IF production was taken as an index of virus multiplication in the tissues and illustrates the pathway of rabies virus infection in the animal, spreading from the site of inoculation to the central nervous system and from there out along the nerves to various organs. The appearance of IF at the sites of virus multiplication indicates that rabies virus is capable of inducing IF production in several tissues.

The possible role of IF in the pathogenesis of rabies is indicated by the observations with the Bell rabies virus strain which is pathogenic for hamsters only if inoculated intracerebrally, or if given intramuscularly concurrent with a dose of cortisone(17). Interferon was demonstrated in the brains of hamsters inoculated intracerebrally with this strain, but if given intramuscularly virus does not reach the brain unless the animals receive cortisone; since

cortisone is known to depress IF production (14,15), IF may be the factor limiting this infection and preventing spread of virus to the central nervous system, thus determining the outcome of the infection. Also, the observations of Larke(16) on dose responses to viruses, together with these findings of rabies induced IF, suggest that IF production may be involved in the classical post-exposure antirabies prophylaxis (Pasteur treatment).

Summary. The induction of interferon by rabies virus was studied in order to establish the status of this virus as an inducer of interferon in the cells it infects. Previous investigators, using *in vitro* systems, have been unable to demonstrate interferon production from cells infected with rabies virus, and this has caused some confusion among researchers working on rabies. However, using an *in vivo* system, we were able to isolate and characterize rabies induced interferon from several tissues. The brain, in which the virus reached its highest titer, also contained the largest amount of interferon, with the other levels of interferon corresponding to the centrifugal spread of rabies virus from the brain to the organs.

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Effect of Adenosine Derivatives and Antihistaminics on Platelet Aggregation. (31569)

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It is well documented that ADP is a powerful platelet aggregator, both *in vivo* and *in vitro,* and that some adenosine analogues inhibit the ADP effect(1-6). Several adenosine derivates, prepared in our laboratories by Dr. Gerzon, were tested for inhibitory activity using rabbit platelet rich plasma.

O'Brien(7) reported that the antihistaminic, dephenylhydramine hydrochloride, had an inhibitory effect on platelet aggregation. Several antihistaminics were tested for their inhibitory activity on ADP induced aggregation to ascertain whether this was correlated to antihistaminic activity.

Materials and methods. Rabbits were used exclusively for this study and, unless otherwise stated, no anticoagulant was employed at any time. All surfaces coming in contact