

either immediately after washing away the first medium, or after incubation for 2 or 4 h in the absence of RNA. All the cultures were challenged by Sindbis virus 18 h after termination of the first, interference-inducing incubation. As Table 2 shows, the antagonistic effect of intact chick embryo RNA was also clearly demonstrable in this experimental design, indicating that direct interaction between effective and ineffective RNAs is not required.

Table 2. DIMINUTION OF RNA-INDUCED RESISTANCE TO VIRUS BY DELAYED ADDITION OF "INEFFECTIVE" RNA AFTER REMOVAL OF "EFFECTIVE" RNA

First incubation (7 h) with	Second incubation (18 h) with	Relative plaque No.
No RNA	No RNA	100
T1 RNase digest of CE RNA* (50 µg/ml.)	CE RNA (20 µg/ml.)	7
" " " " "	No RNA 2 h, then CE RNA	56
" " " " "	No RNA 4 h, then CE RNA	51
" " " " "	No RNA 4 h, then CE RNA	55

\* CE RNA = chick embryo ribosomal RNA.

Thus it is strongly suggested that the RNAs which are apparently ineffective in inducing viral interference exert on the cells an influence the nature of which is so far obscure. These experiments and others to be reported later indicate that the various RNAs and oligonucleotides tested act on chick embryo cells, either inducing resistance to virus (and physiological damage) or abolishing it. What structural features of the RNAs determine the direction of their action remains an interesting question for the future.

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## Incorporation of Tritium of <sup>3</sup>H-Arginine into DNA as the Explanation of "Late Synthesis of Protein" on the Human X Chromosome

CHERNICK'S<sup>1</sup> demonstration of apparent late synthesis of protein on the X chromosome of female lymphocytes labelled with <sup>3</sup>H-arginine<sup>1</sup> interested me—for several years ago I carried out a similar experiment. The studies were done on an individual with an iso-X chromosome so that there was no question of the identification of the late replicating X chromosome. Lymphocytes were grown in culture with phytohaemagglutinin and, after 72 h, 25 µCi/ml. of <sup>3</sup>H-arginine (Schwarz BioResearch, Inc.) and 0.1 µg/ml. of colchicine were added to the culture. Three h later the cultures were collected, treated with hypotonic, fixed in acetic acid-methanol and flame-dried. They were then stained with aceto-orcein, covered with Kodak AR-10 stripping film, and autoradiographed for 48 days. The iso-X chromosome was late labelled (Fig. 1) in a pattern identical to that seen with <sup>3</sup>H-thymidine. Similar studies with normal female lymphocytes also

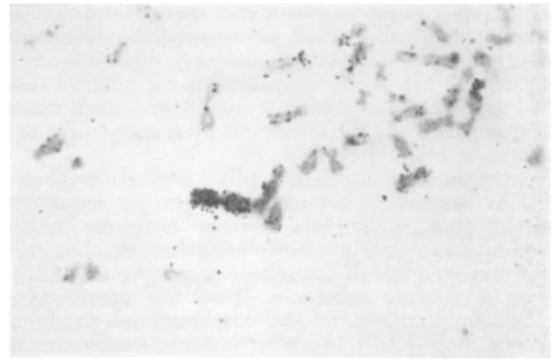


Fig. 1. Late labelling pattern of an iso-X chromosome labelled with <sup>3</sup>H-arginine. This typical DNA replication pattern disappeared after treatment with DNase. ( $\times 2,000$ )

showed a labelling pattern that mimicked that of <sup>3</sup>H-thymidine.

This result was initially exciting but seemed too good to be true. There was some non-specific label on the other chromosomes, typical of that seen with <sup>3</sup>H-lysine or <sup>3</sup>H-tryptophan labelling (my unpublished work). I also noted two populations of interphase cells, one very heavily labelled and one lightly labelled. The percentage of heavily labelled cells was similar to that in a culture labelled with <sup>3</sup>H-thymidine. The tritium of <sup>3</sup>H-arginine can readily gain access to the precursors of *de novo* DNA synthesis by the pathway arginine→ornithine→glutamic acid→the carbon pool of the citric acid cycle, and so what I observed was actually a roundabout method of labelling DNA. This was further indicated by the fact that treatment of these slides with DNase (0.5 mg/ml. in 2.0 µM MgCl<sub>2</sub>, 0.1 M phosphate buffer, pH 6.8, at 37° C for 1 h) completely removed the late labelling pattern and left only a non-specific pattern typical of chromosomal labelling by other tritiated amino-acids.

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## Luteinizing Hormone Releasing Activity of Crude Ovine Hypothalamic Extract in Man

A HYPOTHALAMIC luteinizing hormone releasing factor, which is active in rats and rabbits, has been purified and separated from other hypothalamic factors which control the function of the adenohypophysis<sup>1</sup>. In order to determine whether hypothalamic extracts of animal origin were capable of stimulating the release of pituitary hormones in man, a crude, thioglycollate-treated extract of ovine stalk-median eminence was administered to three children. This material elicited an increase in the plasma concentration of immunoassayable luteinizing hormone in each subject. The investigation was initiated to study the effect of such extracts on plasma growth hormone concentrations because of the potential use of these materials in patients with hypopituitarism secondary to hypothalamic dysfunction. A preliminary report of this aspect of the study has been presented<sup>2</sup>.

Two human infants with lethal chromosomal anomalies