In my study, PP-rib-P synthetase activity was measured by determining $^{14}CO_2$ release from [^{14}C-carboxyl]-orotidylic acid. Orotidylate and uridylate synthesis were catalyzed by the addition of a partially purified extract of yeast orotate phosphoribosyl transferase (E.C. 2.4.2.10; orotidine-5′-phosphate: pyrophosphate phosphoribosyltransferase) and orotidine-5′-phosphate decarboxylase (E.C. 4.1.1.23; orotidine-5′-phosphate carboxy-lyase). This method is suitable for measuring PP-rib-P synthetase activity in a one-step assay as well as for the two-step determination of PP-rib-P accumulation. Assays are linear with protein concentration and with time. Accurate results can be obtained with incubation periods ranging from 5 to 30 minutes. Longer incubation periods were not tested. A similar assay procedure was used by others for the determination of PP-rib-P synthetase activity in hepatoma cells and in fibroblasts (*13, 14*). PP-rib-P content, PP-rib-P synthetase activity, and PP-rib-P accumulation were determined on enzyme extracts prepared from lymphocytes in the logarithmic phase of growth, and studies were carried out on freshly harvested cells (Table 1).

PP-rib-P synthetase activity was strikingly increased in all HGPRT deficient cells (Table 1). Enzyme activity in mutant cell lines exceeded that of the control cells three to tenfold.

PP-rib-P accumulation in HGPRT deficient cells was also significantly greater than in control cells, but only a small percentage (3 to 10 percent) of the PP-rib-P produced, as measured by PP-rib-P synthetase activity, accumulated in the cells. PP-rib-P accumulation is dependent not only on synthesis, but also on utilization and catabolism. A small but constant amount of PP-rib-P accumulation could be sufficient to explain the strikingly high cellular concentration of this substrate in HGPRT deficient cells.

Findings are in agreement with those reported by Martin *et al.* on increased PP-rib-P synthetase in clones of mutagenized HGPRT deficient rat hepatoma cells and in cultured fibroblasts of patients with HGPRT deficiency (*14*). Martin *et al.* determined PP-rib-P synthetase activity by a one-step assay, similar to the one used in this study, based on the formation and decarboxylation of orotidylate in the presence of excess orotidylate phosphoribosyltransferase and decarboxylase.

The relation between HGPRT deficiency and PP-rib-P overproduction could reflect a compensatory mechanism for the production of inosinic acid, since an increase in available PP-rib-P could increase the catalytic efficiency of the abnormal HGPRT enzyme. The mechanism of this interaction between the function of the salvage pathway and PP-rib-P synthesis is unknown. This interaction could be genetic, with the HGPRT gene having a regulatory function on PP-rib-P synthetase activity. At present, it is not known whether PP-rib-P overproduction is an obligatory consequence of HGPRT deficiency. In lymphoblasts selected for thioguanine resistance, HGPRT deficiency was linked to elevated cellular PP-rib-P content and purine overproduction (*7*). The study of natural mutations appearing in man will reveal whether increase in PP-rib-P production and in purine biosynthesis is obligatory for the survival of HGPRT deficient cells. The increase in PP-rib-P synthetase activity observed in Lesch Nyhan cells could result in the abnormally high cellular PP-rib-P content of these cells and represents an additional metabolic and genetic abnormality in this syndrome.

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5-Iododeoxyuridine Potentiation of the Replication In Vitro of Several Unrelated RNA and DNA Viruses

Abstract. *Enhancement of the replication of unrelated viruses (three RNA viruses and one DNA virus), representative of four major virus groups, occurs in human, rodent, or avian cells treated in vitro with 5-iododeoxyuridine (IdU). The results suggest that the potentiation of viral replication by IdU is a widespread phenomenon.*

The normally inefficient replication of cytomegalovirus (CMV) is potentiated in cells treated with 5′-iododeoxyuridine (IdU) prior to the addition of virus (*1*). Adenovirus replication also is enhanced in semipermissive cells treated with IdU before the addition of virus (*2*). We now report the unexpected finding that IdU potentiates the synthesis of a number of unrelated RNA and DNA viruses [vesicular stomatitis, sindbis, mouse encephalitis (GD-7), and vaccinia]. These viruses replicate in apparent independence of nuclear control (*3*). Thus, IdU incorporation into host cell DNA may induce an alteration (or alterations) of general significance to viral replication.

In our experiments cells were grown in the presence of IdU for 48 hours, medium containing IdU was removed, and virus was added. Suspensions of 4×10^4 to 5×10^4 cells [mouse L, BHK-21, primary chicken embryo or human foreskin fibroblasts (HR203) (*4*)] in 0.1 ml of Eagle's medium supplemented with 10 percent fetal bovine serum (FBS) and antibiotics (*5*) were added to microtiter culture plates (plastic) (*6*). The IdU (*7*), in a final concentration of 10 μg/ml, was added to portions of the cell suspensions prior to their addition to culture plates. After 40 to 48 hours, culture supernatants were decanted. Virus dilutions were added to quadruplicate cultures of IdU-treated and untreated (control) cells and allowed to adsorb for 1 hour; the dilutions and medium were decanted, the cells were rinsed three times, and 0.2 ml of medium was added. Cultures were observed for the development of a viral cytopathic effect (CPE), or were frozen (−70°C) and thawed for titration of viral hemagglutinin (HA) (*8, 9*) or infectious virus. Titration of cultures for virus yields was performed in duplicate. Tissue culture infective doses, 50 percent effective ($TCID_{50}$), were determined by accepted techniques.

The effect of IdU treatment of cells on HA yield was determined for GD-7 virus in mouse L cells, and for Sindbis virus in HR203 fibroblasts (Fig. 1). The cells were infected at varying multiplicities of infection (MOI), and HA was determined 48 hours later. Consistently greater quantities of GD-7 virus HA were produced at all MOI's in IdU-treated cultures (Fig. 1A) as

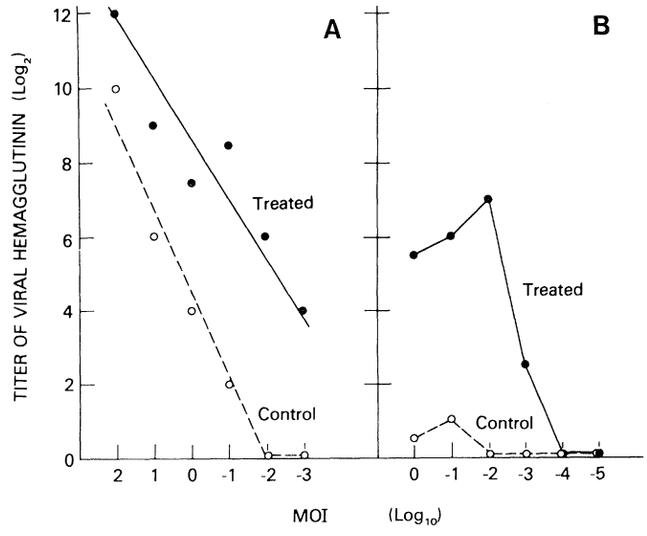

Fig. 1. Virus hemagglutinin synthesis in cultures with and without prior treatment with IdU: (A) L cells infected with GD-7 virus; (B) HR 203 cells infected with Sindbis virus. The medium in HR 203 cultures was replaced with reinforced modified Eagle's medium (*8*) without serum 1 hour after the addition of the viral dilutions. IdU (10 μg/ml) was present for 48 hours prior to the addition of virus. Hemagglutinin determinations were performed 48 hours after the addition of virus. ●——●, IdU-treated cells; ○----○, control cells.

compared with HA production in untreated cultures. The magnitude of this difference was least at the higher MOI and was greatest at the lower MOI (ranging from a minimum fourfold to a maximum 64-fold difference in HA yield between IdU-treated and control cultures). When examined microscopically, cultures infected at an MOI of 10 or greater had complete CPE at 16 hours (a single replication cycle), while cultures infected at an MOI of 1 or less underwent more than one cycle of replication during the 48-hour incubation period. Under the conditions of single-cycle replication, HA in IdU-treated cultures exceeded controls by fourfold or more. This finding confirms the increased yield of virus even when measured at the completion of viral replication.

Even greater differences in HA yield occurred in HR 203 cells infected with Sindbis virus (Fig. 1B), with the exception of MOI of -3 $\log_{10}$. In this experiment Sindbis virus replication occurred in the presence of serum-free medium required for HA determination (*8*). Replication of Sindbis virus, as judged by rate of development of CPE, is comparable in cultures containing medium with and without serum.

The IdU-treated and untreated cell cultures were examined for infectious virus at various intervals after challenge with either Sindbis virus, vesicular stomatitis virus, GD-7 virus, or vaccinia virus (Table 1). Prior treatment with IdU potentiated infectious Sindbis virus production in HR 203 and 204 cells, chicken embryo cells, and mouse L cells; GD-7 virus in mouse L cells and BHK-21 cells; vesicular stomatitis virus in BHK-21 cells; and vaccinia virus in mouse L cells and chicken embryo cells. Increases in virus yield ranged from 1 $\log_{10}$ for Sindbis in HR 203, and GD-7 virus in mouse L cells, to 2.5 $\log_{10}$ for vaccinia virus replicating in mouse L cells. This enhancement occurred for a range of MOI's from 0.001 to 10.

An accelerated rate of development of CPE occurred in human foreskin fibroblasts treated for 48 hours with IdU (10 μg/ml) prior to infection with Sindbis. End-titer readings ($TCID_{50}$), although initially higher for IdU-treated cultures, became equal by day 4 or 5 of the titration. These findings indicate that the rate of development of virus is more rapid in IdU-treated cells, but that these cells are not more susceptible to infection by Sindbis virus. Preliminary studies demonstrate the potentiation of several additional viruses, including mengo virus in L cells, influenza in monkey kidney cells, and dengue in HR 203 cells.

Our findings demonstrate potentiation by IdU of the replication in vitro of a DNA virus (vaccinia) and several unrelated RNA viruses in rodent, human, and avian cells. Enhancement was manifested as increased yields of viral hemagglutinin or of infectious virus, as well as more rapid development of CPE. In the case of Sindbis virus, the data suggest that enhancement is the result of increased or more rapid virus yield by IdU-treated cells, rather than increased susceptibility to infection in IdU-treated cells. The increased yield of Sindbis per infected cell differs from adenovirus which infects an increased number of semipermissive WI38 cells (*2*). In the case of the other viruses used in these studies the question of enhanced replication compared to increased efficiency of infection cannot be resolved by the available data. The enhancement of vaccinia replication in IdU-treated cells in which free IdU has been depleted by washing contrasts with the inhibition of DNA viruses when unincorporated IdU is present during viral replication.

Table 1. Production of infectious virus in IdU-treated cell cultures. Cells were treated with IdU (10 μg/ml) for 48 hours prior to the addition of virus. Cultures were examined microscopically at 12-hour intervals and frozen at −70°C pending assay for infectious virus. Cultures infected at low MOI's were frozen when there were demonstrable differences in CPE between IdU-treated cells and controls. Cultures infected at MOI's greater than 1 were frozen at fixed time intervals when the CPE was 100 percent in both IdU-treated cells and controls. The length of the incubation time allowed one or more cycles of viral replication.

Cell type	Challenge virus		Day of harvest	Virus yield [$TCID_{50}$ ($\log_{10}$)]		Increase virus yield ($\log_{10}$)
	Type	MOI		IdU	Control	
Human fibroblasts						
HR 203	Sindbis	1.0	1	9.0	7.0	2.0
		0.1	1	8.0	7.0	1.0
		0.001	1	5.0	4.0	1.0
HR 204	Sindbis	0.001	1	8.0	7.0	1.0
Hamster BHK-21	VSV	0.1	1	7.0	5.5	1.5
	GD-7	0.001	2	6.0	4.5	1.5
Mouse L929	GD-7	10.0	1	5.5	4.5	1.0
	Vaccinia	0.01	1	5.0	2.5	2.5
	Sindbis	10.0	0.5	6.5	5.0	1.5
Chick embryo fibroblasts	Sindbis	0.1	1	8.5	7.5	1.0
	Vaccinia	0.1	1	5.0	3.5	1.5

Previous investigators have reported the potentiating effect of IdU and its analog 5-bromodeoxyuridine (BrdU) on viruses with distinctive modes of replication such as the induction of type C viruses (*10*), λ prophage (*11*), SV40 (*12*), and Epstein-Barr virus (*13*); potentiation of adenovirus replication in normally restrictive cell types (*2*); and the enhancement of the normally inefficient multiplication of CMV in human fibroblasts (*1*). The previously studied viruses have been DNA viruses or viruses with a DNA dependent mode of replication. Some of the viruses used in our study contain RNA, and all four viruses apparently replicate independently of DNA synthesis and nuclear control (*3*), and are considered to multiply in an unrestricted fashion.

Currently the mechanism (or mechanisms) of IdU enhancement of the replication of RNA and DNA viruses with a cytoplasmic mode of synthesis is speculative. The diverse phenotypic changes produced by IdU, and BrdU (*14*), favors the existence of multiple mechanisms of viral enhancement. The demonstration of virus susceptibility to IdU potentiation in nonrestrictive cell types also should facilitate subsequent investigation of the general phenomenon of enhancement of viral replication by this agent, and suggests that the prior treatment of cells with the halogenated pyrimidines also may be applied to isolation, identification, and propagation of viruses.

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Thymus: Central Role in the Immune System of the Frog

Abstract. *In the leopard frog (*Rana pipiens*), the thymus profoundly influences immunologic development by providing virtually all the progenitor lymphocytic cells for the bone marrow and other peripheral lymphoid organs. The thymus houses a self-perpetuating population of lymphopoietic cells that originally self-differentiated in that gland.*

A widely held view is that the developing sites of lymphopoiesis in amniote vertebrates depend on colonization by circulating stem cells that originate in the yolk-sac blood islands (*1*). Lymphocytes of the thymus are thought to be derived from blood-borne mesenchymal precursor cells that enter the avian (or mammalian) thymic rudiment at an early stage of development. Likewise, lymphopoietic cells in the bone marrow apparently trace their origin to invading lymphoid stem cells derived from the yolk sac. It is difficult to believe that there can be a fundamental difference between the origin of lymphocytes in the amphibian and the chick (or mouse), but our experiments show persuasively that the lymphoid cells of both the thymus and bone marrow in the anuran frog (*Rana pipiens*) are *not* ontogenically derived from mesenchymal cells of the ventral blood islands (the counterpart of yolk-sac blood islands in amniotes). We established earlier (*2*) that thymic lymphocytes of the frog arise by direct transformation of the epithelial cells in the thymic rudiment itself. We now reinforce our previous finding with a new experimental approach and also show that lymphopoietic cells of the bone marrow, spleen, and kidney are specifically the progeny of immigrants from the thymus gland.

Our evidence is based on the interplay of the thymus and peripheral lymphoid organs in experimentally produced chimeric frogs. One of the boldest microsurgical procedures, dating back to the beginnings of experimental embryology (*3*), consists of cutting two embryos in half transversely and then interchanging the front halves. Anterior and posterior halves of different embryos can be united without flaw, and such a chimeric embryo can develop into a frog of normal form. Moreover, an embryo of triploid constitution ($3n = 39$), produced by pressure shock (*4*), can be combined with a diploid embryo ($2n = 26$). The use of chromosomally marked embryonic halves permits an analysis of the extent to which the histogenesis of organs in each half is influenced by cells migrating from one half to the other.

The operations were performed under sterile conditions on 3-mm embryos in the tail-bud stage of development (*5*). At this very early developmental period, 72 hours after fertilization, the embryo is completely avascular, and the thymus gland is wholly undifferentiated, existing only as an endodermal rudiment in the internal gill arch region. The operative mortality was very high, as might be expected from so delicate a surgical manipulation. When, however, anterior and posterior embryonic halves were perfectly aligned (Fig. 1A), only a seamlike line along the united margins was subsequently in evidence (Fig. 1B). In later development, frogs of chimeric origin were indistinguishable from normal (unoperated) frogs in general body form (Fig. 1C). There were no immunologic incompatibilities between tissues (*6*).

We were successful in rearing five chimeric larvae through the metamorphic climax when endochondral ossification occurs and bone marrow differentiates. Each of the five chimeric frogs was diploid anteriorly and triploid posteriorly. In postmetamorphic (terrestrial) life, the ploidy of the cells of the thymus, spleen, kidney, and bone marrow (of the humerus and femur) was determined in each chimeric frog. This was accomplished by making cell suspensions from these organs, staining them for DNA with the Feulgen reaction, and then determining the relative absorption of individually stained nuclei with a scanning integrating microdensitometer (type GN-2, Barr and Stroud) (*7*). Since the amount of stain absorbed by any given nucleus is proportional to its DNA content, measurements of the light absorbed by the stain reflected the DNA content (that is, ploidy) of that nucleus.

Bar diagrams (Fig. 2) show the percentages of diploid and triploid cells in the anteriorly located thymus gland and bone marrow of the humerus, and the posteriorly located spleen, kidney, and bone marrow of the femur. Both thymus glands were almost exclusively diploid; that is, at least 95 percent of the cells of each thymus developed in accord with the constitutional disposition of the diploid anterior half of the embryo. Evidently, thymic lymphocytes are derived from indigenous cells of the embryonic thymus primordium of the gill arch region. Any contribution of mesenchymal cells, or hemocytoblasts, from the ventral blood islands to the developing thymus was apparently minimal.

The ventral blood islands of the embryo are patches of splanchnic mesoderm that envelop the yolk mass. These vascular patches occupy the area immediately caudal to the primitive heart and extend posteriorly as far as the cloacal region. Ac-