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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References and Notes

1. S. St. Jeor and F. Rapp, *Science* **181**, 1060 (1973); *J. Virol.* **11**, 986 (1973).
2. S. P. Staal and W. P. Rowe, *Virology* **64**, 513 (1975).
3. E. Follett, *et al.*, *J. Virol.* **13**, 394 (1974); D. M. Prescott, J. Kates, J. Kirkpatrick, *J. Mol. Biol.* **59**, 505 (1971); F. Fenner, B. McAuslan, C. Mims, J. Sambrook, D. White, Eds., *The Biology of Animal Viruses* (Academic Press, New York, 1974), pp. 227, 236–239, and 258–262.
4. HEM Research.
5. Streptomycin, 100 μg/ml; penicillin, 100 unit/ml; achromycin, 100 μg/ml; and mycostatin, 5 μg/ml.
6. Falcon Plastics (catalog No. 3040).
7. Sigma Chemical Company.
8. D. H. Clarke and J. Casals, *Am. J. Trop. Med. Hyg.* **7**, 561 (1958).
9. L. S. Sturman and I. Tamm, *J. Immunol.* **97**, 885 (1966).
10. D. R. Lowy, W. P. Rowe, N. Teich, J. W. Hartley, *Science* **174**, 155 (1971); V. Klement, M. O. Nicolson, R. J. Huebner, *Nat. New Biol.* **234**, 12 (1971).
11. K. E. Price, R. E. Buck, J. Lein, *Antimicrob. Agents Chemother.* **4**, 505 (1964).
12. H. Rothschild and P. H. Black, *Virology* **42**, 251 (1970); D. R. Dubbs, S. Kit, R. A. deTorres, M. Anken, *J. Virol.* **1**, 968 (1967); J. F. Watkins, *J. Cell. Sci.* **6**, 721 (1970).
13. P. Gerber, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 83 (1972); B. Hampar, J. G. Derge, L. M. Martos, J. W. Walker, *ibid.* p. 78; R. Glaser and F. Rapp, *J. Virol.* **10**, 288 (1972).
14. W. J. Rutter, R. L. Pictet, P. W. Morris, *Annu. Rev. Biochem.* **42**, 601 (1973).

14 May 1975; revised 17 July 1975

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.

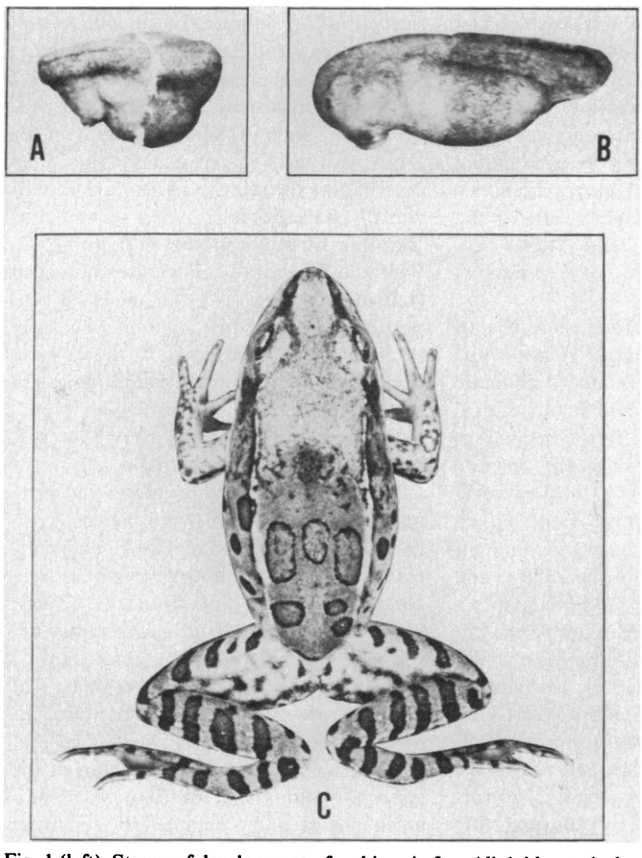
The ventral blood islands of the embryo are patches of splanchnic mesoderm that envelop the yolk mass. These vascular patches occupy the area immediately caudal to the primitive heart and extend posteriorly as far as the cloacal region. Ac-

cordingly, the ventral blood islands of a chimeric embryo would be constituted of both diploid and triploid hemocytoblasts. If stem cells from the blood islands were to invade the developing thymus, we should expect to find a fairly representative population of triploid cells in the thymus gland. This expectation was not realized. To sustain the notion that the ventral blood islands do make a cellular contribution to the thymus, we would have to postulate a selective migration of blood-borne mesenchymal elements strongly in favor of diploid cells.

The possibility of selective migration of blood-island cells is discounted by the chromosomal makeup of the cells of the bone marrow and spleen (Fig. 2). Although the posteriorly located femur and spleen developed in a constitutional milieu that was triploid (including the ventral blood islands), the cells of the femoral bone marrow and spleen were predominantly diploid. Specifically, 80 percent or more of the cells in the spleen and bone marrow of the femur were diploid in constitution. There is little doubt that the spleen and bone marrow of the femur are colonized extensively by diploid cells from the anteriorly located thymus (*8*). The thymus apparently is also the source of the large diploid component in the bone marrow of the humerus.

The kidney is an important site of erythropoiesis in anurans, and erythroid elements in this organ outnumber lymphoid elements by a ratio of at least three to one (*9*). It is of particular interest, then, that the incidence of diploid cells (of thymus origin) in the kidneys of chimeric frogs ranged from 16 to 25 percent (Fig. 2). It is likely that the primary contribution of the ventral blood islands was to the erythroid population of the kidney (and other peripheral tissues). Indeed, one of the first attempts at an experimental analysis of the blood islands in amphibians was made by Federici (*10*), who removed the primitive blood islands from embryos of *Rana fusca* before the circulation of the blood was established. The larvae which developed after this operation were found to be devoid of erythrocytes. The results were so striking that Goss (*11*) repeated the experiment on embryos of the axolotl *Ambystoma punctatum*. After the operation, the red blood cells of the axolotl were always reduced in number and sometimes were entirely absent. These results indicate that the ventral blood islands of amphibians contain the specific primordia of red blood cells. Our study points to the same conclusion (*12*).

In the early life of mammals, the thymus is a prominent source of circulating small lymphocytes. In later development, however, the bone marrow has been implicated as the principal contributor of lymphocytes or their precursors. One current view (*13*) holds that there is an inadequate resident population of progenitor lymphoid cells in the mammalian thymus in postnatal life, and that the postnatal thymus is continuously replenished by immigrant stem cells derived from the bone marrow. Under this view, lymphoid stem cells in the thymus are ultimately of bone-marrow origin. However, clear evidence for the ex-



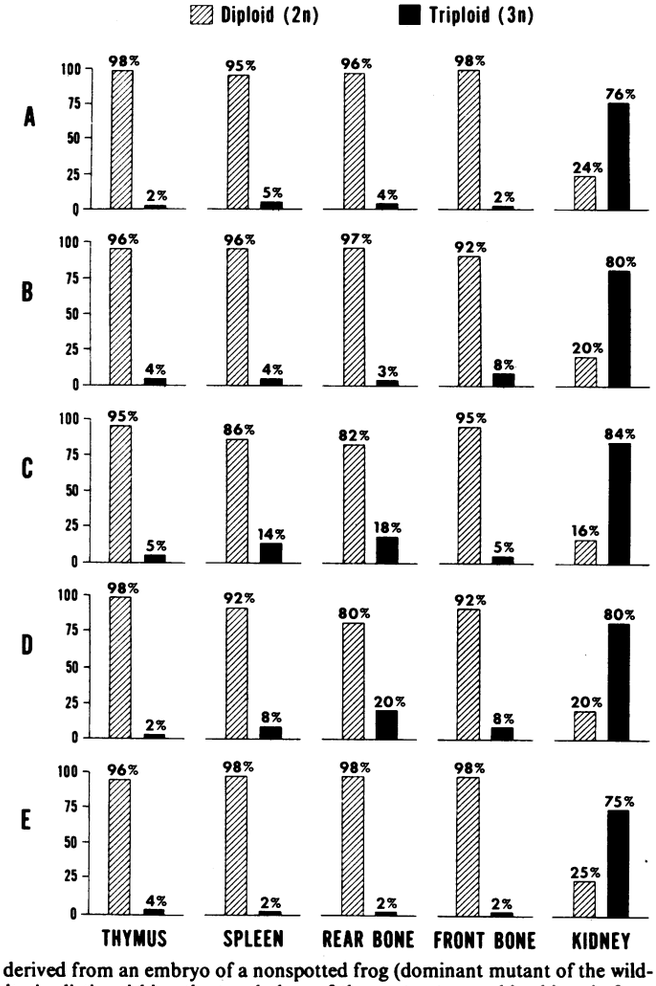


Fig. 1 (left). Stages of development of a chimeric frog (diploid anteriorly and triploid posteriorly). (A) One-half hour after operation. Some yolk cells are unavoidably extruded, but healing is faultless in favorable cases. (B) Embryonic period. The seamlike line along the united margins is barely perceptible. (C) Postmetamorphosis. The anterior half was typically derived from an embryo of a nonspotted frog (dominant mutant of the wild-type leopard-spotted frog) so that an observer would experience no difficulty in distinguishing the two halves of the postmetamorphic chimeric frog. Fig. 2 (right). Percentages of diploid and triploid cells as determined cytophotometrically in several different tissues of five experimentally produced chimeric frogs (A through E). In each chimeric animal, the anteriorly located diploid thymus is the source of the large diploid component of the rear (thigh) bone and spleen, both of which are located in the posterior triploid half of the chimera.

haustion and extensive replacement of thymus stem cells in mammals is wanting (*14*). Our position is that the thymus, at least in the frog, is an organ in which lymphopoiesis is genuinely self-sustaining. The thymus is the stem cell source of the diverse populations of peripheral antigen-reactive lymphocytes (T and B cells). Given the initially large indigenous population of stem cells in the developing thymus, there is no compelling reason to suppose that the functional thymus loses its reserve of undifferentiated, or immature, lymphoid cells. We suggest, therefore, that there is a self-perpetuating population of lymphoid stem cells in the intact thymus, and that such a resident population is neither replenished nor replaced by immigrant stem cells from the bone marrow. The important implication is that normal thymic involution in aging frogs is associated with a progressive impairment of the capacity to mount immune responses.

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References and Notes

1. D. Metcalf and M. A. S. Moore, *Haemopoietic Cells* (North-Holland, Amsterdam, 1971).
2. J. B. Turpen, E. P. Volpe, N. Cohen, *Science* **182** 931 (1973); *Am. Zool.* **15**, 51 (1975).
3. G. Born, *Arch. Entwicklungsmech. Org. (Wilhelm Roux)* **4**, 349 (1897); R. G. Harrison, *Arch. Mikroskop. Anat. Entwicklungsmech.* **63**, 35 (1904).
4. The formation of the second polar body is suppressed when the egg, 5 minutes after insemination, is subjected to hydrostatic pressure of 5000 lb/in² (350 kg/cm²) for 6 minutes. The resulting embryo is triploid. One set of chromosomes is paternal (the contribution of the sperm nucleus) and two sets are maternal (the normal maternal haploid contribution and the second polar body) [S. Dasgupta, *J. Exp. Zool.* **151**, 105 (1962)].
5. Stages of embryonic development of the frog are described → W. Shumway, *Anat. Rec.* **78**, 139 (1940). At the tail-bud stage (stage 17), there is no differentiation of blood cells.
6. The anterior and posterior halves are immunologically unresponsive to each other inasmuch as each half is essentially a massive, tolerance-inducing graft.
7. K. Bachman and R. R. Cowden, *Chromosoma* **17**, 22 (19(→ *Trans. Am. Microsc. Soc.* **86**, 454 (1967).
8. In the strictest interpretation of the experimental results, the only unconditional conclusion is that the spleen and bone marrow are colonized by lymphoid cells from the anterior portion of the chimeric embryo, not necessarily the thymus gland. It is reasonable, however, to implicate the thymus gland as the specific source of the colonizing lymphoid cells [see also (*2*)].
9. H. E. Jordan and C. C. Speidel, *Am. J. Anat.* **32**, 155 (1923).
10. H. Federici, *Arch. Biol.* **T36**, 466 (1926).
11. C. M. Goss, *J. Exp. Zool.* **52**, 45 (1929).
12. The conventional monophyletic concept is that the precursors of lymphoid, myeloid, and erythroid cell lines are derived from a single population of pluripotential stem cells. In anurans, however, it appears that lymphocytes and erythrocytes have different origins, the former arising in situ from elements in the thymic rudiment itself and the latter originating from blood-borne elements in the ventral blood islands. Our data thus favor the polyphyletic theory that recognizes two or more separate types of blood stem cells [see also J. G. Hollyfield, *Dev. Biol.* **14**, 461 (1966)].
13. J. E. Harris, C. E. Ford, D. W. H. Barnes, E. P. Evans, *Nature (Lond.)* **201**, 886 (1964).
14. G. Sainte-Marie, in *Contemporary Topics in Immunology*, ... J. S. Davies and R. L. Carter, Eds. (Plenum Press, New York, 1973), vol. 2, pp. 111–117.

28 July 1975; revised 8 September 1975

Neisseria gonorrhoeae and *Neisseria meningitidis*: Extracellular Enzyme Cleaves Human Immunoglobulin A

Abstract. *The gonococcus and meningococcus, which infect human mucosal surfaces, elaborate a highly specific proteolytic enzyme which cleaves the immunoglobulin A1 subclass of the principal mucosal antibody, immunoglobulin A (IgA). The susceptible Pro-Thr bond lies in a unique region of the IgA heavy chain; the IgA2 subclass, lacking this peptide bond, is enzyme resistant.*

Neisseria gonorrhoeae and *Neisseria meningitidis* are gram-negative diplococci that are strictly pathogenic for human beings in whom they cause gonorrhea and meningitis, respectively. In the initial stages of infection these microorganisms colonize mucosal surfaces and penetrate the epithelial membrane. In human beings the mucosal surfaces lining the respiratory, alimentary, and urogenital tracts have an elaborate immune system characterized by the secretion of antibody of the immunoglobulin A (IgA) class (*1*). Secretory IgA is synthesized by immunocompetent cells lying in the lamina propria of the mucosa beneath the epithelium, and these cells respond with antibody production to many microbial and macromolecular antigens present at the mucosal surface (*1*). The situation following gonorrheal infection is somewhat paradoxical, however, for although antigonococcal antibody of the secretory IgA type appears in the urethral secretions of patients recovering from infection, reinfection with the gonococcus may occur despite this seemingly adequate immunologic response (*2*). We report here that clinical isolates of *N. gonorrhoeae* and four groups of *N. meningitidis* grown in in vitro culture elaborate extracellular, highly specific, proteolytic enzymes that cleave human serum and secretory IgA to yield Fcα and Fabα fragments. This appears to be the first identification of extracellular enzymes from the pathogenic *Neisseria*, and their specificity for human IgA suggests that they may play a role in neisserial infections.

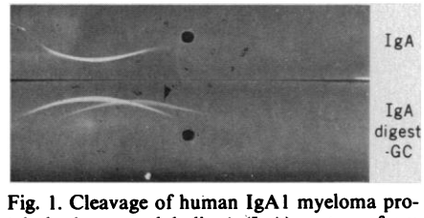

Fig. 1. Cleavage of human IgA1 myeloma protein by immunoglobulin A (IgA) protease from *Neisseria gonorrhoeae* (immunoelectrophoresis). The top well contains the purified, intact IgA; the bottom well shows the same protein following exposure to gonococcal enzyme (*GC*) for 6 hours at 37°C. The antiserum in the trough is unabsorbed goat antiserum to human IgA. The arrow indicates the Fcα fragment, which shows a precipitin reaction of immunologic nonidentity with the other fragment, Fabα. The anode is to the right.

Clinical isolates of *N. gonorrhoeae* (Kellogg colony types 1, 2, 3, and 4) and *N. meningitidis* (groups A, B, C, and Y) were examined for enzyme production. The organisms were inoculated into Difco GC base supplemented with Isovitalex (BBL, Cockeysville, Md.) and incubated, with gentle swirling, for 24 hours at 37°C under an atmosphere of expired air introduced by blowing through a sterile pipette plugged with cotton. The culture was centrifuged, the bacterial mass discarded, and proteins precipitated from the clarified supernatant by the addition of solid ammonium sulfate to a final concentration of 60 percent. The precipitate from 100 ml of supernatant was resuspended in 4 or 5 ml of phosphate buffer (*p*H 7.5, 0.05*M*), dialyzed against the same buffer, and examined for enzyme activity by incubation at 37°C with human IgA myeloma proteins at a concentration of 10 mg/ml in phosphate buffer. Serum IgA protein substrates of both subclasses, IgA1 and IgA2, were isolated from the plasma of patients with multiple myeloma, and colostral secretory IgA was isolated from human milk obtained 1 month postpartum (*3*). Proteolytic cleavage of IgA was detected by cellulose acetate electrophoresis, immunoelectrophoresis in which rabbit antiserums of known specificity were used, and by disc gel electrophoresis in 5 percent polyacrylamide gels, *p*H 9.5; these methods have been described previously in detail (*4*). Bacterial culture medium that had been incubated without the addition of bacterial inoculum was similarly treated to serve as a control.

Culture filtrates of the four colony types of *N. gonorrhoeae* and all four groups of *N. meningitidis* contained enzyme that rapidly cleaved all six human myeloma IgA proteins of the IgA1 subclass to yield two large fragments; a typical digest examined by electrophoresis is shown in Fig. 1. The IgA fragments were identified as Fabα and Fcα by criteria previously outlined (*4*, *5*), including electrophoretic mobility, reactivity with antiserums specific for light or heavy chains, and approximate molecular weight as determined by molecular sieve chromatography. Neither the gonococcal nor meningococcal enzymes were able to cleave any of a group of four human myeloma proteins of the IgA2 subclass. In addition, the enzymes could not