ity has been detected in the parent or mutant strains.

The HG-PRT locus is ideal for two-way mutation studies with human cells. Selection for reversions of mutants toward wild type can be effected in a medium where growth depends on the ability to utilize hypoxanthine, while selection for a variety of changes from normal to reduced amounts of HG-PRT can be achieved with AG. The latter selection provides a reasonable, genetically tidy model system for determining mutation rates in human cells and utilizes readily available, normal male fibroblasts in standard culture mediums. HG-PRT deficiency is not, by itself, deleterious to cultured cells. However, enzyme deficiency resulting from a deletion or inactivation of a sizable portion of the X chromosome containing the HG-PRT locus is likely to be associated with lethality in cells having one X. Therefore, viable cells deficient in HG-PRT selected for with AG will almost always result from small mutational changes in euploid chromosome complements. Our results so far indicate an incidence of mutant cells that is about 10^{-6}, which may be an underestimate. By using artificial mixtures of mutant and normal cells, we found that the recovery of mutants (strain 252) as clones of viable cells after selection with $2 \times 10^{-5} M$ AG is related to the number of normal cells (strain 52) that is present. The number of mutant clones that form is not reduced by 10^4 normal cells per 60-mm dish, but 10^5 normal cells reduces the number to about 10 percent of maximum and the recovery of mutant clones is almost negligible in the presence of 10^6 normal cells per dish. This interaction between cells is not yet understood.

If the suggested incidence (10^{-6}) of AG-resistant mutants represents their spontaneous incidence in vivo, one may expect that resistant mutants already exist in the cell population whenever 6-mercaptopurine is used to treat leukemia. This is one reason why the leukemias regularly become permanently resistant to the purine analog (*19*), but other bases for resistance apparently exist (*19, 20*). However, spontaneous mutant cells are not frequent enough to interfere with a new method of diagnosing heterozygotes for the Lesch-Nyhan mutation, which is based on the ability of cells deficient in HG-PRT in a heterozygous fibroblast population to proliferate in the presence of AG (*21*). HG-PRT variation may play a role in other medical contexts, such as immunosuppression, where purine analog substrates of the enzyme are used.

RICHARD J. ALBERTINI
ROBERT DEMARS
Department of Medical Genetics, University of Wisconsin, Madison 53706

References and Notes

1. J. W. Littlefield, *Proc. Nat. Acad. Sci. U.S.* **50**, 568 (1963).
2. W. E. Szybalski, E. H. Szybalska, G. Ragni, *Nat. Cancer Inst. Monogr.* **7**, 75 (1962).
3. E. H. Y. Chu, P. Brimer, K. B. Jacobson, E. V. Merriam, *Genetics* **62**, 359 (1969).
4. *Enzyme Nomenclature: Recommendations of 1964* (Elsevier, Amsterdam, 1965).
5. J. F. Henderson, W. N. Kelley, F. M. Rosenbloom, J. E. Seegmiller, *Amer. J. Hum. Genet.* **21**, 61 (1961); W. L. Nyhan, J. Pesek, L. Sweetman, D. G. Carpenter, C. H. Carter, *Pediat. Res.* **1**, 5 (1967).
6. W. L. Nyhan, *Fed. Proc.* **27**, 1027 (1968). This issue contains a useful collection of seminars on several aspects of the Lesch-Nyhan syndrome.
7. J. E. Seegmiller, F. M. Rosenbloom, W. M. Kelley, *Science* **155**, 1682 (1967).
8. R. DeMars and W. E. Nance, *Wistar Inst. Monogr.* **1**, 35 (1964).
9. Medium F10, as used in all of our experiments, differed from the original formulation (*10*) by lacking hypoxanthine, unless it was added as an experimental supplement. FCS-F10 is fetal bovine serum (15 volumes) freshly mixed with 85 volumes of F10 before each use. CS-F10 is calf serum (15 volumes) plus 85 volumes of F10. Calf serum is used in these experiments because some batches of fetal bovine serum contain substances that antagonize the inhibitory effect of AG and that allow Lesch-Nyhan fibroblasts to grow in the absence of added adenine or folic acid (*11*). These substances increase the minimum inhibitory concentration of AG in FCS-F10 about fivefold to tenfold compared to that in CS-F10. CS-F4 is calf serum (15 volumes) plus 85 volumes of F4 (*12*), which is used because of its low content of folic acid in comparison to medium F10.
10. R. C. Ham, *Exp. Cell Res.* **29**, 515 (1968).
11. J. S. Felix and R. DeMars, *Proc. Nat. Acad. Sci. U.S.* **62**, 536 (1969).
12. R. Ham and T. T. Puck, *Methods Enzymol.* **5**, 90 (1961).
13. R. J. Albertini and R. DeMars, unpublished results.
14. A. Wajntal and R. DeMars, *Biochem. Genetics* **1**, 61 (1967).
15. F. M. Rosenbloom, W. N. Kelley, J. F. Henderson, J. E. Seegmiller, *Lancet* **1967-II**, 305 (1967); B. R. Migeon, J. M. Der Qaloustian, W. L. Nyhan, W. J. Young, B. Childs, *Science* **160**, 425 (1968).
16. J. Salzmann, R. DeMars, P. Benke, *Proc. Nat. Acad. Sci. U.S.* **60**, 545 (1968).
17. J. F. Henderson, L. W. Brox, W. M. Kelley, F. M. Rosenbloom, J. E. Seegmiller, *J. Biol. Chem.* **243**, 251 (1968).
18. R. DeMars, unpublished results.
19. J. D. Davidson and T. S. Winter, *Cancer Res.* **24**, 261 (1964).
20. R. W. Brockman, *ibid.* **25**, 1596 (1965).
21. J. S. Felix and R. DeMars, in preparation.
22. Paper No. 1367 from the Genetics Laboratory, University of Wisconsin. Supported by PHS grants GM 06983 and GM 08217 and NIH postdoctoral fellowship AM 05313.

8 June 1970

Fetal Kidney in Organ Culture: Abnormalities of Development Induced by Decreased Amounts of Potassium

Abstract. *Kidneys from fetal mice (10 to 18 days' gestation) were grown in organ culture. Concentrations of potassium less than 9 milliequivalents per liter (10- to 14-day kidneys), and less than 6 milliequivalents per liter (14- to 18-day kidneys), produced abnormal branching, failure of nephron induction, and occasional cystic dilatations of the ureteral bud. These studies emphasize the importance of an environment with a high concentration of potassium for development of the fetal kidney.*

Perey *et al.* (*1*) showed that newborn rabbits and rats injected with adrenocortical steroids on the day of birth developed cystic abnormalities of the kidney. Their data suggested that hypopotassemia, induced by the mineralocorticoids, was responsible for the morphologic changes observed. Microdissection of the kidneys from cystic animals (*2*) showed that the ureteral buds in the nephrogenic zone of the cortex failed to branch normally, did not regularly induce nephron formation, and terminated as blind-ending cystic structures. Although hypopotassemia was a likely cause of the renal defect observed, other possibilities such as a direct effect of the steroid upon the developing kidney were not excluded.

We have studied the effects of mediums with low and normal amounts of potassium on 1000 fetal kidneys grown in organ culture. Fetal mouse kidneys were chosen because their small size and ease of handling minimized the problems of central necrosis, apparently due to hypoxia, which occur during prolonged organ culture. Timed pregnancies of the mice were interrupted at 10 or 11 to 18 days of gestation; the fetuses were removed quickly and placed in Hanks balanced salt solution (*3*). The kidneys were removed with cataract knives under a dissecting microscope and placed on nucleopore membrane filters (*4*), supported by a stainless steel wire mesh in plastic organ-culture dishes (*5*). The cultures were incubated in a water-jacketed incubator at 37°C in 95 percent O_2 and 5 percent CO_2. Some of the cultures

were oscillated on a rocker (*6*) to improve the circulation of the medium (two oscillations per minute). The medium consisted of 85 percent potassium-free (0.01 meq/liter) Medium 199 with Hanks base made on special order (*3*), 9-day chick-embryo extract (*7*) (4 percent), horse serum (10 percent), penicillin (200 unit/ml), streptomycin (200 mg/ml) (*3*), mycostatin (100 unit/ml) (*3*), and variable concentrations of potassium added as potassium chloride. Final concentrations of potassium in the medium were measured by flame photometry (the concentrations of sodium, chloride, and magnesium were also monitored in selected mediums). The culture medium was changed every 48 hours. Cultures were grown for 2 to 6 days in medium with potassium concentrations between 0.2 and 14 meq/liter. Tissue for serial standard histologic sections were fixed in Zenker's solution and embedded in paraffin. Thin (0.5 μm) serial sections were also prepared from tissue fixed in glutaraldehyde (1 to 2 percent) and embedded in Vestopal; they were stained by the method of Ghidon *et al.* (*8*). Similar histologic studies were made of normal kidneys removed from fetuses taken from the uterus at various gestational ages; these sections were compared with the organ cultures.

On the basis of growth characteristics in various concentrations of potassium and of age in vivo, cultures could be roughly divided into fetuses at 10 or 11 to 14 days of gestation and fetuses at more than 14 days' gestation.

Fetal kidneys (10 or 11 to 14 days) grew normally in a medium with a high concentration of potassium (9 to 12 meq/liter, 150 organs) (Fig. 1A). When the potassium concentration of the medium was reduced to 6 to 8 meq/liter (400 organs), the following defects were regularly noted: (i) a reduced number of branches of the ureteral buds; (ii) a failure of nephron induction at the site of branching; (iii) abnormal straight ureteral buds which grew through the metanephric tissue to the primative capsule and then curved toward the medulla; and (iv) occasional dilated ureteral buds (Fig. 1B). The fetal renal pelvis and calyces were normal. Cultures were not viable in mediums containing potassium at concentrations below 4.5 meq/liter.

Fetal kidneys obtained at 14 to 18 days grew normally in a potassium concentration of 6 to 12 meq/liter (150 organs). When the potassium concentration was reduced below 6 meq/liter (275 organs), defects similar to those described for the first group were noted. Growth did not occur when the medium contained less than 1.5 meq of potassium per liter.

Fetal kidneys grown at the higher concentrations of potassium showed branching of the ureteral bud and nephron formation at each dichotomous branching and were comparable to the normal control kidneys.

The potassium concentration is higher in the serum of most mammalian fetuses during mid-gestation than it is in maternal serum at the same time (*9*). Our data show that the potassium concentration in the serum of the mouse fetus at 16 days is also elevated to 9 to 10 meq/liter, or about twice the maternal amount. It thus seems likely that deviation from the normally high concentration of potassium in the serum of the fetus might have significant effects upon the development of fetal organs, including kidney.

Our study demonstrates that major developmental defects of the mouse kidney occur when the kidney is grown in vitro at potassium concentrations considered to be within normal or high concentrations for maternal serum. Amounts which induce abnormalities are about 4 to 6 meq/liter lower than the amounts present in fetal serum at comparable gestational ages. In the mouse embryo at 9 to 10 days (gestation period, 21 days), the ureteral bud has just encountered the metanephric cells along the posterolateral abdominal wall. The ureteral bud normally grows into the metanephric cell mass and, upon contact, branches repeatedly to form the pelvis, the calyceal system, and the embryonic collecting system. Further growth of the multiple extensions of the ureteral buds (now primitive collecting ducts) through the nephrogenic blastema results in the induction of nephrons (*10*).

Low concentrations of potassium in the medium of organ cultures appear to influence all phases of development of the ureteral bud beyond the initial branching and formation of the pelvis and calyces. The mechanism (or mech-

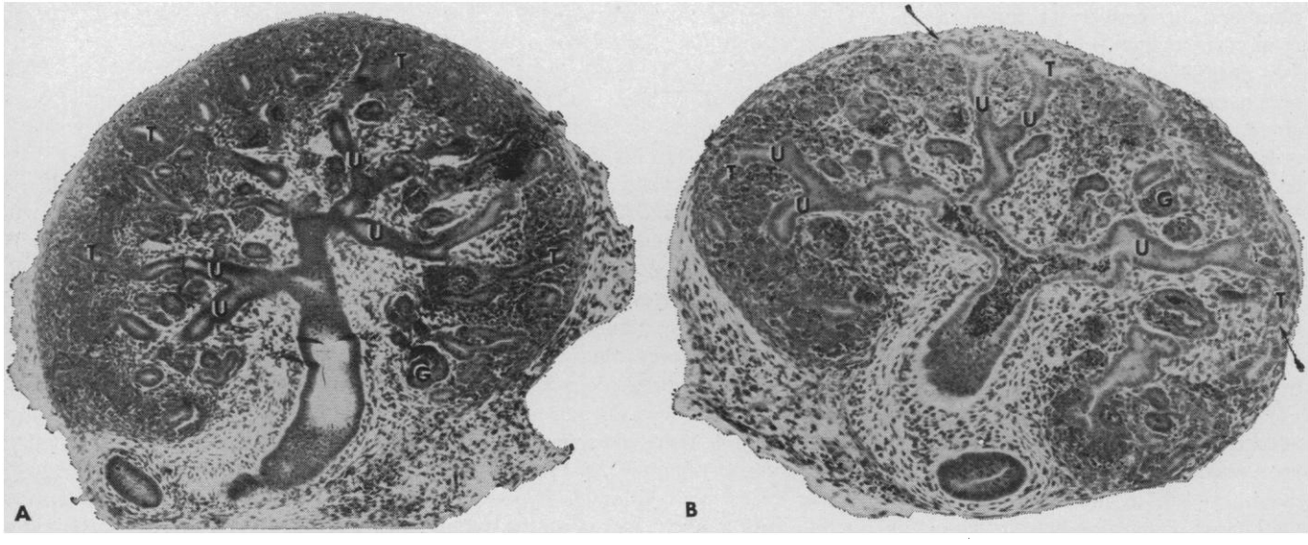

Fig. 1. Kidneys from 12-day-old mouse fetuses grown in culture for 48 hours. (A) Concentration of potassium in medium, 9.1 meq/liter; of sodium, 140 meq/liter. The ureteral buds (*U*) branch repeatedly; terminal branches (*T*) do not touch the capsule of the kidney, and there are many nephrons of glomeruli (*G*) in different stages of development. (B) Concentration of potassium in medium, 5.9 meq/liter; of sodium, 141 meq/liter. Ureteral buds branch less frequently, and there are fewer terminal branches (*T*) which often grow to the capsule and bend (arrows) without induction of glomeruli. The number of developing glomeruli (nephrons) is decreased compared with A (× 80).

anisms) which maintains a high amount of potassium in the fetus, the role of this ion in fetal development, and the possible general applicability of the concept of hypopotassemia as a cause of fetal kidney maldevelopment demand further study.

JOHN F. S. CROCKER
R. L. VERNIER

Department of Pediatrics,
University of Minnesota Hospitals,
Minneapolis 55455

References and Notes

1. D. Y. E. Perey, R. C. Herdman, R. A. Good, *Science* **158**, 494 (1967).
2. R. L. Vernier and F. G. Smith, in *Biology of Gestation*, N. S. Assali, Ed. (Academic Press, New York, 1968), vol. 2, pp. 225–260.
3. Grand Island Biologicals, Grand Island, N.Y.
4. Arthur H. Thomas Co., Philadelphia, Penn.
5. Falcon Plastics, Div. of B.D. Inc., Los Angeles.
6. Bellco Glass Inc., Vineland, N.J.
7. D. J. Merchant and R. H. Kahn, *Handbook of Cell and Organ Culture* (Burgess, Minneapolis, 1964), pp. 205–206.
8. J. J. Ghidon, M. M. Campbell, J. G. Adams, H. Thomas, E. E. Romas, *Proceedings of the Twenty-Sixth Annual Meeting of the Electron Microscopy Society of America*, C. J. Arceneaux, Ed. (Claitor's, Baton Rouge, La., 1968), p. 240.
9. J. Maniey, *Ann. Endocrinol (Paris)* **28**, suppl., 1 (1967); E. I. McDougall, *Biochem. J.* **45**, 397 (1949); H. Bakwin and H. Rivkin, *Amer. J. Obstet. Gynecol.* **13**, 68 (1927); E. F. Adolph and P. A. Hoy, *Amer. J. Physiol.* **204**, 3, 392 (1963); M. Christianson and I. C. Jones, *J. Endocrinol.* **15**, 17 (1957).
10. V. Osathanondh and E. L. Potter, *Arch. Pathol.* **76**, 271 (1963).
11. We thank Dr. R. Auerbach, University of Wisconsin, for assistance with the methods of organ culture. J.F.S.C. is a fellow of the Canadian Arthritis and Rheumatism Society and a postdoctoral fellow of the National Kidney Foundation. Supported by grants from the American Heart Association and PHS (AM 13254 and HE 06314).

11 June 1970

Antigen Receptor Molecules: Inhibition by Antiserum against Kappa Light Chains

Abstract. *Rabbit antiserum against mouse kappa chains, the predominant class of light chains found in mouse immunoglobulins, inhibits the immune response in vitro of mouse spleen cells to erythrocyte antigens. The inhibition becomes irreversible if the mouse spleen cells are treated briefly with both antiserum to kappa chains and complement.*

It is generally believed that certain lymphoid cells in immune responses carry antibody-like molecules on their surfaces and are responsible for specific antigen recognition. Sell and Gell, as reviewed by Gell (*1*), have presented ample evidence for the presence of immunoglobulin molcules on lymphoid cell surfaces; they used rabbit antiserum to rabbit immunoglobulin allotypes or sheep antiserum to rabbit light or heavy chains to stimulate blast transformation and DNA synthesis in rabbit lymphocytes. But their experiments do not establish any function of the immunoglobulin-bearing cells with respect to antigen recognition. Greaves, Torrigiani, and Roitt (*2*) have suggested a role for immunoglobulin-carrying lymphocytes in cell-mediated immune responses in vitro. The delayed hypersensitivity and homograft responses of human lymphocytes in vitro to tuberculin antigen (purified protein derivative) and histocompatability antigens, respectively, could be inhibited by the Fab (antigen binding) fragment of antibody against the light chain component of human immunoglobulin. They proposed that antibody to light chain binds to sites on the lymphocyte surface and blocks combination with antigen either by steric hindrance or by causing a configurational change in the receptor site. It is not known whether the immunoglobulins involved in antigen recognition contain both heavy and light chains or, if they contain heavy chains, whether they are of a known heavy chain class. We present evidence that mouse immunoglobulin molecules that contain light chains play a part in the generation of plaque-forming cells in response to heterologous eythrocytes in mouse spleen cell suspension cultures.

Suspensions of normal mouse spleen cells from BDF_1 (C57B1/6 × DBA/2) mice were cultured in a medium containing 5 percent fetal bovine serum (*3*). Thirty microliters of a 1 percent suspension of the erythrocyte antigen (about 3×10^6 cells) was added to each culture dish containing 1 ml of cell suspension at the density indicated. The number of spleen cells forming 19*S* antibody against sheep (S-RBC) or horse (H-RBC) erythrocytes was assayed after 4 days of culture by a modification (*3*) of the Jerne *et al.* plaque technique (*4*). Rabbit serum (5 percent) was added to cultures at time zero. This was either normal rabbit serum (NRS) from unimmunized rabbits, or rabbit antiserum to a mouse kappa chain obtained from mouse myeloma protein (*5*). The rabbit serums were extensively absorbed with sheep erythrocytes and filtered through a washed 0.22-μm Millipore filter. Normal mouse serum (NMS) was prepared from pools of blood collected from about 20 male and female BDF_1 mice; it was also absorbed with sheep erythrocytes and passed through a Millipore filter.

Table 1. Inhibition of in vitro response by antiserums to mouse kappa chains (anti-κ). Serums were added to cultures at time zero. Sheep erythrocytes were added at 1 hour. Results are given as the number of plaque-forming cells (PFC) per 10^6 cells recovered at day 4.

Additions	PFC at cell densities (cell/ml):		
	12×10^6	8×10^6	4×10^6
None (control)	897	754	554
5% anti-κ	32	5	0
5% anti-κ + 3% NMS	1446	532	426
5% NRS	1070	651	413
5% NRS + 3% NMS	1008	455	67
5% anti-κ at end	1103	733	654

Rabbit antiserum to mouse kappa chains, present throughout the culture period at a 1 : 20 dilution, inhibited the plaque-forming cell response to S-RBC almost completely. Normal rabbit serum in the same concentration stimulated cultures, so that the response was greater than that seen with 5 percent fetal bovine serum alone (Table 1). At the highest cell density in the cultures (1.2×10^7 cell/ml) inhibition was sometimes incomplete with 5 percent antiserum. Since higher serum concentrations were toxic to cultures, complete inhibition was obtained with spleen cell cultures of lower densities.

The addition of 3 percent normal mouse serum to the cultures was sufficient to reverse completely the inhibition by the antiserum to kappa chains, presumably by the binding of antibody against kappa chains to immunoglobulins in the mouse serum. The higher serum concentration (3 percent NMS plus 5 percent rabbit serum) was somewhat toxic, especially in cultures at low cell densities, giving low total cell yields and low plaque-forming cell yields as compared to yields from cultures with less serum added. In concentrations high enough to neutralize the antiserum to kappa chains, NMS is able to restore activity to inhibited cultures, the subsequent response being equal to that in NRS plus NMS control cultures.

Fuji and Jerne (*6*) and our colleagues, Hartmann *et al.* (*7*), have obtained very similar results with other