creasing antibody concentration up to 40 percent inhibition with 0.5 μl of γ-globulin preparation; complete inhibition was produced with 5 μl. Preimmune γ-globulin did not inhibit enzymatic activity. This experiment gives a quantitative confirmation of the specific cross-reactivity of the cystathionase protein in B_6-responsive lymphoid cell lines with the antibody developed to the normal liver enzyme. Taken together with the immunodiffusion results, which directly show cystathionase activity staining of the abnormal enzyme when PALP is added (Fig. 2C), this experiment demonstrates that the effect of PALP is on the abnormal protein and not on a possible alternative pathway of cysteine formation. In addition, chemical catalysis of the cleavage of cystathionine is ruled out because product formation is proportional to residual enzyme protein and not to the concentration of cofactor, which was the same in all assay tubes.

The presence of cross-reacting, inactive apoenzyme in the cells from the B_6-responsive patient and its absence from the cells from the B_6-unresponsive patient were further confirmed in the following manner (Table 1). Crude extracts of the two cystathionase-deficient cell lines, containing 0.5 to 0.6 mg of protein, were preincubated separately at 37°C for 40 minutes with the amount of the γ-globulin fraction of antiserum previously shown to produce approximately 50 percent inhibition of the cystathionase activity of an extract of normal cells. The normal cell extract (0.43 mg of protein per milliliter) was then added, and the mixture was incubated again for 40 minutes. After the second incubation, substrate was added and cystathionase was assayed without addition of PALP in order to measure only the normal enzyme. It was found that the extract from the B_6-responsive cells had partially neutralized the inhibitory action of the antibody on the normal enzyme, resulting in a decrease in inhibition from 54.2 to 39.8 percent. The extract from the B_6-unresponsive cells, on the other hand, did not alter the degree of inhibition of the normal enzyme by the antibody. When the experiment was repeated with twice the concentration of extract from the B_6-responsive cells, inhibition of the normal cell extract decreased further to 29.5 percent; however, doubling the concentration of the extract from the B_6-unresponsive cells had no effect. These experiments demonstrate that the protein from the B_6-responsive cells is capable of blocking the antibody-combining sites for the normal enzyme; protein from the B_6-unresponsive cells cannot do so.

Identification of a catalytically inactive, cross-reacting cystathionase protein in lymphoid cell lines from a patient with B_6-responsive cystathioninuria and the lack of cross-reacting material in the cells from a B_6-unresponsive patient indicate that the two forms of primary cystathioninuria arise from different mutations, which cause different molecular defects. The B_6-responsive form results from the synthesis of an aberrant enzyme protein exhibiting altered interaction with the coenzyme, thereby resulting in an inherited increase in the requirement for vitamin B_6. The B_6-unresponsive form of cystathioninuria results from the complete absence of cystathionase or its reduction to a level undetectable by the enzymatic or the immunological methods employed. It is also possible that there may be present an enzyme protein so greatly altered that it has lost both cystathionase activity (even in the presence of excess cofactor) and the determinants necessary for recognition by specific antibody.

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References and Notes

1. H. Harris, L. S. Penrose, D. H. H. Thomas, *Ann. Hum. Genet.* **23**, 442 (1959).
2. G. W. Frimpter, *Science* **149**, 1095 (1965).
3. K. Tada, T. Yoshida, Y. Yokoyama, T. Sato, H. Nakagawa, T. Arakawa, *Tohoku J. Exp. Med.* **95**, 235 (1968).
4. H. L. Levy, S. H. Mudd, P. M. Madigan, *Pediatr. Res.* 7, 390 (1973).
5. H. Eagle, C. Washington, S. M. Friedman, *Proc. Natl. Acad. Sci. U.S.A.* **56**, 156 (1966); A. H. Bittles and N. A. J. Carson, *J. Med. Genet.* **11**, 121 (1974).
6. K. N. F. Shaw, E. Lieberman, R. Koch, G. N. Donnell, *Am. J. Dis. Child.* **113**, 119 (1967).
7. N. G. Beratis and K. Hirschhorn, *Mamm. Chromosomes Newsl.* **14**, 114 (1973).
8. G. E. Gaull, D. K. Rassin, J. A. Sturman, *Neuropaediatrie* **1**, 199 (1969).
9. D. K. Rassin and G. E. Gaull, *J. Neurochem.* **24**, 969 (1975).
10. J. A. Schneider, K. H. Bradley, J. E. Seegmiller, *Pediatr. Res.* **2**, 441 (1968).
11. J. A. Sturman and L. T. Kremzner, *Biochim. Biophys. Acta* **372**, 162 (1974).
12. J. D. Finkelstein, W. E. Kyle, B. J. Harris, *Arch. Biochem. Biophys.* **146**, 84 (1971).
13. J. A. Sturman, G. E. Gaull, N. C. R. Räihä, *Science* **169**, 74 (1970); G. E. Gaull, J. A. Sturman, N. C. R. Räihä, *Pediatr. Res.* **6**, 358 (1972).
14. T. A. Pascal, B. M. Gillam, G. E. Gaull, *Pediatr. Res.* **6**, 773 (1972).
15. T. A. Pascal, H. H. Tallan, B. M. Gillam, *Biochim. Biophys. Acta* **285**, 48 (1972).
16. R. Arnon and E. Shapira, *Biochemistry* **6**, 3942 (1967).
17. J. Uriel, *Ann. N.Y. Acad. Sci.* **103**, 956 (1963).
18. J. D. Finkelstein *et al., Proc. Natl. Acad. Sci. U.S.A.* **55**, 865 (1966).
19. Supported by the New York State Department of Mental Hygiene, the Lalor Foundation, National Institutes of Health Genetics Center grant GM-19443, and Clinical Research Center Grant RR-00071. K.H. is a Career Scientist of the Health Research Council of the City of New York (I-513). We thank C. Parker and Y. Wada for providing blood for culture of lymphoid cell lines from patients with cystathioninuria and M. Kehoe and D. Pascal for helpful discussion and advice.

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Differentiation of T Cells in Nude Mice

Abstract. *Cells bearing the T-cell differentiation alloantigens TL and Thy-1 were enumerated in preparations of spleen and lymph node cells of* nu/nu *mice. Healthy* nu/nu *mice had few or no demonstrable* TL^+ *or* $Thy\text{-}1^+$ *cells whereas mice with viral hepatitis, including some born of* nu/nu × nu/nu *matings, had many. Healthy* nu/nu *mice were treated daily with the thymic hormone thymopoietin or with ubiquitin, polypeptides that induce the differentiation of* $TL^+Thy\text{-}1^+$ *cells from* $TL^-Thy\text{-}1^-$ *precursors in vitro. After 14 days, 20 to 40 percent of their spleen cells were of the* $TL^+Thy\text{-}1^+$ *phenotype typical of thymocytes and 10 to 25 percent of their lymph node cells were* $TL^-Thy\text{-}1^+$*, typical of later T-cell differentiation. Similar frequencies of such cells were found in untreated* nu/nu *mice suffering from severe viral hepatitis. These data conform to the current view that prothymocytes are preprogrammed cells whose maturation to thymocytes, normally induced in the thymus by thymopoietin, can be triggered by other agents under abnormal circumstances. Tests of T-cell function were not included in this study.*

The pleiotropic recessive mutant *nu* in mice (*1*) causes deficient growth of hair and failure to develop a normal thymus (*2, 3*). While there are reports of a thymic anlage in *nu/nu* embryos (*3*), this remains to be substantiated, and in postnatal life the thymus of *nu/nu* mice is absent or represented by a small epithelial rudiment lacking lymphocytes (*4*). The deficiency of T-cell function in *nu/nu* mice is attested by their inability to reject foreign skin grafts (*5*), with even avian skin being accepted for long periods (*6*).

The impaired development of T cells in *nu/nu* mice can be ascribed to lack of the maturation stimulus or stimuli normally provided by the epithelial thymus, because *nu/nu* mice have normal numbers of precursor cells capable of being induced to express the several cytotypic markers that distinguish members of the T-cell lineage. This is shown most incisively in the Komuro-Boyse (KB) assay in which cells from the spleen or bone marrow of *nu/nu* mice are induced in vitro to express these cell surface components, which include TL,

a marker for early T cells (thymocytes), and Thy-1, a marker present on all mouse T cells (*7*). Furthermore, functional T cells of host origin develop in *nu/nu* mice given thymic grafts (*4, 8*).

Cells of Thy-1$^+$ type are known to occur in *nu/nu* mice (*9*), their numbers ranging from 2 to 20 percent in spleen according to the criterion applied [allogeneic or xenogeneic T-cell antiserums: direct or indirect immunofluorescence (*9*); cytotoxicity test (*10*)]. These Thy-1$^+$ cells in athymic mice have been variously ascribed to thymic rudiments functioning during development or to the passage of T cells or thymic hormone from mother to fetus [reviewed in (*11*)]. But an alternative explanation is indicated by the data which we report here.

The T-cell differentiation step represented by conversion of the phenotype from TL$^-$Thy-1$^-$ to TL$^+$Thy-1$^+$ is known from the KB assay to be inducible in vitro by adenosine 3′,5′-monophosphate (cyclic AMP) and other agents, such as endotoxin or the polypeptide ubiquitin, which have no special relation to the thymus (*12–14*). This signifies that the induction of early T-cell differentiation—which we define here as the acquisition of cytotypic T-cell surface markers—involves the triggering of a "preprogrammed" or "determined" precursor, the "prothymocyte" (*12*). Thus *nu/nu* mice might be expected to harbor variable numbers of Thy-1$^+$ cells which have been triggered to differentiate from Thy-1$^-$ precursors by products of bacterial infection or tissue breakdown, an obvious explanation of the Thy-1$^+$ cells reported in the lymphoid tissues of *nu/nu* mice. Our observations, reported below, showed that whereas healthy *nu/nu* mice of our colony have few or no Thy-1$^+$ and TL$^+$ cells, substantial numbers of such cells were found in untreated *nu/nu* mice that were suffering from a severe disease similar to that described in the original *nu/nu* stock (*1*) and which we shall call hepatitis. These mice exhibited dehydration followed by wasting, hunched posture, and sometimes paralysis of their hind limbs. Their livers, spleens, and lymph nodes were enlarged at first but later shrunken. The most prominent late histological feature was massive hepatic necrosis. Mouse hepatitis virus (MHV) has been recovered from *nu/nu* mice (*15*) and has been isolated from sick *nu/nu* mice of our colony. Viral hepatitis would account for the liver pathology described in *nu/nu* segregants of the original stock (*1*), and MHV is probably endemic in mice derived from this stock. We and others (*1*) have not seen the overt hepatitis syndrome in +/*nu* segregants; the *nu/nu* immune-deficient segregants are doubtless more susceptible, as are neonatally thymectomized mice (*16*).

Table 1. Proportions of Thy-1$^+$, TL$^+$, and CR$^+$ cells in *nu/nu* mice treated with thymopoietin or ubiquitin, or suffering from hepatitis, as compared with healthy *nu/nu* mice. The livers of all mice were examined histologically. Mice scored as healthy had no gross or microscopic evidence of liver disease; mice scored as having hepatitis showed microscopic liver lesions, with or without gross macroscopic changes. Pooled axillary, inguinal, and mesenteric nodes of individual mice were examined. The percentage of the total cell population was calculated (i) for Thy-1$^+$ and TL$^+$ cells from the complement-dependent cytotoxicity assay according to the formula $100(a-b)/a$, where a is the percentage of viable cells after incubation with complement but no antiserum and b is the percentage of viable cells after incubation with antiserum (optimal concentration) and complement; and (ii) for CR$^+$ cells, from the number of rosettes with erythrocyte-antibody-complement as percentage of total cell count. Group 1 *nu/nu* mice were born of +/*nu*♀ × *nu/nu*♂ matings and were 6 to 9 weeks old; group 2 *nu/nu* mice were born of *nu/nu* ♀ × *nu/nu* matings and were 4 to 6 weeks old. All data are values for individual mice; NT, not tested.

Mice tested		Treatment	Spleen			Lymph nodes		
Genotype	Healthy or with hepatitis		Thy-1$^+$ (% of total)	TL$^+$ (% of total)	CR$^+$ (% of total)	Thy-1$^+$ (% of total)	TL$^+$ (% of total)	CR$^+$ (% of total)
+/*nu*	Healthy	None	25, 27, 29, 32, 35	<5, <5, <5, <5, <5	28, 29, 30, 32, 34	46, 48, 54, 56, 58	<5, <5, <5, <5, <5	15, 15, 17, 20, 22
nu/nu, group 1	Healthy	Medium 199	<5, <5, 7, 8	<5, <5, 6, 6	14, 14, 19, 21	<5, <5, <5	<5, <5, <5, <5	7, 10, 11, 13
	Healthy	Thymopoietin	33, 36, 37, 38	23, 24, 26, 27	49, 54, 56, 40	25, 28, 30, 30	5, 6, 6, 9	21, 30, 22
	Healthy	Ubiquitin	26, 27, 29	20, 21, 22	50, 63, 48	22, 28, 20	7, 11, 5	25, 29, 21
	Hepatitis	None	30, 31, 31, 36	19, 20, 28, 31	30, 30, 32, 38, 40	25, 26, 31, 38, 40	<5, <5, <5, <5, 7	29, 30, 31, 32, 33
nu/nu, group 2	Hepatitis	None	<5, 11, 17, 15	NT	12, 16, 28, 22	NT	NT	NT

Carefully selected healthy-appearing 4-week-old *nu/nu* mice were treated for 10 to 14 days with thymopoietin or ubiquitin to ascertain the capacity of these agents to induce TL$^+$ or Thy-1$^+$ cells in vivo. Our *nu/nu* mice are bred on a BALB/c background (thymocytes TL.2$^+$) from matings between *nu/nu* males and +/*nu* females. Thymopoietin (*17*) is a polypeptide hormone (molecular weight, 5562) of the thymus whose specificity of action is shown in the KB assay by its capacity to induce the differentiation of TL$^+$Thy-1$^+$ T cells but not CR$^+$ (positive for complement receptor) B cells. Ubiquitin (originally called ubiquitous immunopoietic polypeptide or UBIP) is a polypeptide (molecular weight, 8451) found not only in thymus but also in most or all living cells (*12*). In the KB assay, ubiquitin triggers the maturation not only of T cells but also of CR$^+$ B cells from CR$^-$ precursors; it evidently does so by reacting with β-adrenergic receptors (*12*), hence its nonselective differentiating capacity for preprogrammed B and T precursor cells.

Healthy-looking *nu/nu* mice were injected intraperitoneally each day with thymopoietin or ubiquitin (0.1 or 0.2 μg) in medium 199, controls receiving medium 199 alone. After 10 or 14 days of treatment, cell suspensions were made separately from spleen and from pooled lymph nodes, washed in medium 199, and tested for expression of TL and Thy-1 antigens by the cytotoxicity assay by using Thy-1.2 and TL.1,2,3 antiserums raised in congenic mouse strains [see table 3 of (*18*)]; CR$^+$ B cells were enumerated by rosetting with sheep erythrocytes coated with 19*S* immunoglobulin and complement in the presence of ethylenediaminetetraacetic acid (*19*). Untreated *nu/nu* mice with the hepatitis described above, in early stages of a chronic form of the disease (*20*), were tested similarly.

Our data are summarized in Table 1. The results in healthy *nu/nu* mice treated with either thymopoietin or ubiquitin resembled those in the untreated *nu/nu* mice with hepatitis in the following respects. In contrast to healthy untreated *nu/nu* mice, these three groups all showed (i) many TL$^+$Thy-1$^+$ cells in spleen; (ii) TL$^-$Thy-1$^+$ cells in lymph nodes; (iii) higher proportions of CR$^+$ cells in spleen and lymph nodes; and (iv) in histological examination, increased cellularity of lymphoid tissues, notably in the T-cell–dependent areas around the splenic central arteries (*21*) and around the postcapillary venules in lymph nodes (*16*).

Several points deserve mention.

1) Cells of the T-cell lineage, identified by TL and Thy-1 antigens, can be found in similar numbers in healthy *nu/nu* mice

treated with thymopoietin or ubiquitin and in untreated *nu/nu* mice suffering from hepatitis. This confirms previous indications that the presence of cells bearing TL and Thy-1 is not in itself evidence of thymic epithelial function in *nu/nu* mice, because these mice have normal numbers of prothymocytes which can be induced to differentiate by nonthymic agents such as ubiquitin not only in vitro but also in vivo. In view of the diversity of agents capable of inducing T cells in the KB assay (*13, 14*), one can only speculate as to the agent or agents responsible for inducing TL^+ and Thy-1^+ cells in the *nu/nu* mice with hepatitis. Ubiquitin must normally be inaccessible to T precursor cells because, although it is present in *nu/nu* tissues (*12*), healthy *nu/nu* mice lack demonstrable numbers of TL^+ or Thy-1^+ cells. But hepatic necrosis might lead to its release in quantities sufficient to induce many T-cell precursors. In the case of bacterial infection, endotoxin (another agent that is active in the KB assay) (*14*) could be responsible.

2) The rise in CR^+ cell numbers in mice treated with thymopoietin requires consideration because thymopoietin, unlike ubiquitin, does not induce CR^+ B cells in the KB assay. However, since the number of CR^+ cells in healthy *nu/nu* mice is reduced, the effect of thymopoietin in raising their numbers in vivo may be secondary to its action in inducing newly differentiated T cells that in turn can interact with the B-cell population (*22*).

3) TL^+ cells are usually restricted to the thymus, although for reasons given above it seems probable that TL^+ cells can in some circumstances be induced at other sites. Equal numbers of TL^+ and Thy-1^+ cells were found in the spleens of healthy treated and sick untreated *nu/nu* mice. Because the phenotype $TL^+Thy\text{-}1^-$ is unknown, we assume these all to be TL^+ Thy-1^+ cells such as are normally found in the thymus, which is in keeping with results in the KB assay in vitro. The finding that lymph node cells of the same mice included many Thy-1^+ cells and few TL^+ cells implies that cells of TL^-Thy-1^+ phenotype were being generated. This is the phenotype of the medullary thymocyte and peripheral T cell; thus, in vivo induction of prothymocyte differentiation in the three groups of *nu/nu* mice gave rise to cells not only of the thymic cortical type (TL^+Thy-1^+) generated in the KB assay in vitro but also of a later TL^-Thy-1^+ maturation stage. However, this gives no indication as to the function of the induced T cells, for the TL^-Thy-1^+ phenotype is common to all functional subclasses of T cells and may characterize intermediary differentiative stages between thymocytes and functional T cells. Prothymocytes are probably scarce or absent in lymph nodes, although this needs further study; it seems likely, therefore, that the TL^-Thy-1^+ lymph node cells were maturing migrants from the spleen rather than T cells induced in situ.

4) Among the *nu/nu* mice with hepatitis were some born of *nu/nu* × *nu/nu* matings, and 2 to 17 percent of spleen cells in these progeny were Thy-1^+; the induction of these cells cannot be ascribed to passage of thymic factors from the mother.

Our studies show that healthy *nu/nu* mice of our colony had few or no cells bearing T-cell markers and that T cells could be induced in vivo in these healthy mice by thymopoietin or ubiquitin injections. Our results indicating that *nu/nu* mice suffering from overt hepatitis had many cells bearing T-cell markers emphasize that the first recognizable step in T-cell differentiation (acquisition of surface markers) in *nu/nu* mice can be related to disease rather than to thymic mechanisms. There is no need to invoke the concept of a thymus briefly functioning during embryogenesis, or maternal transfer of thymic factors. In current studies, designed in accordance with the results reported here, we do not find a perceptible background of Thy-1^+ T cells in *nu/nu* mice reared under germfree conditions, although Thy-1^+ cells can be induced in these mice by thymopoietin and ubiquitin.

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References and Notes

1. S. P. Flanagan, *Genet. Res.* **8**, 295 (1966).
2. E. M. Pantelouris, *Nature (Lond.)* **217**, 370 (1968).
3. _____ and J. Hair, *J. Embryol. Exp. Morphol.* **24**, 615 (1970).
4. H. H. Wortis, S. Nehlsen, J. J. Owen, *J. Exp. Med.* **134**, 681 (1970).
5. J. Rygaard, *Acta Pathol. Microbiol. Scand.* **77**, 761 (1969); _____ and C. O. Povlsen, *ibid.*, p. 758; B. Kindred, *Eur. J. Immunol.* **7**, 384 (1972); E. M. Pantelouris, *Immunology* **20**, 247 (1971).
6. N. D. Reed and D. D. Manning, *Proc. Soc. Exp. Biol. Med.* **134**, 350 (1973).
7. K. Komuro and E. A. Boyse, *J. Exp. Med.* **138**, 479 (1973).
8. B. Kindred and F. Loor, *ibid.* **139**, 1215 (1974).
9. M. C. Raff, *Nature (Lond.)* **246**, 350 (1973); F. Loor and B. Kindred, *J. Exp. Med.* **138**, 1044 (1973); F. Loor and G. E. Roelants, *Nature (Lond.)* **251**, 229 (1974); G. E. Roelants *et al.*, *Eur. J. Immunol.* **5**, 127 (1975).
10. S. M. Kirov, *Eur. J. Immunol.* **4**, 139 (1975).
11. H. Pritchard and H. S. Micklem, in *Proceedings of the First International Workshop on Nude Mice* (Fisher, Stuttgart, 1974), p. 127–139.
12. G. Goldstein, M. P. Scheid, U. Hammerling, E. A. Boyse, D. H. Schlesinger, H. D. Niall, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 11 (1975).
13. M. P. Scheid, M. K. Hoffmann, K. Komuro, U. Hammerling, J. Abbott, E. A. Boyse, G. H. Cohen, J. A. Hooper, R. S. Schulof, A. L. Goldstein, *J. Exp. Med.* **138**, 1027 (1973).
14. M. P. Scheid, G. Goldstein, U. Hammerling, E. A. Boyse, *Ann. N.Y. Acad. Sci.* **249**, 531 (1975).
15. C. K. Hsu and E. C. Melby, paper presented at 25th annual session of American Association of Laboratory Animal Science, 1974.
16. J. East, D. M. V. Parrott, F. C. Chesterman, A. Pomerance, *J. Exp. Med.* **118**, 1069 (1963).
17. G. Goldstein, *Nature (Lond.)* **247**, 11 (1974).
18. H. Shiku, P. Kisielow, M. A. Bean, T. Takahashi, E. A. Boyse, H. F. Oettgen, L. J. Old, *J. Exp. Med.* **141**, 227 (1975).
19. C. Bianco, R. Patrick, V. Nussenzweig, *ibid.* **132**, 702 (1970).
20. T. Hirano and B. H. Ruebner, *Lab. Invest.* **14**, 488 (1965).
21. D. M. V. Parrott and M. A. B. De Sousa, *Clin. Exp. Immunol.* **8**, 663 (1971).
22. E. B. Jacobson, L. H. Caporale, G. J. Thorbecke, *Cell. Immunol.* **13**, 416 (1974); K. Okumura, N. Shimohara, M. Kern, *J. Immunol.* **113**, 2021 (1974).
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Cyclic Guanosine Monophosphate and Cellular Growth

Abstract. *The addition of serum to nongrowing cells decreases cyclic guanosine monophosphate and cyclic adenosine monophosphate levels and promotes cell growth.*

Cultured fibroblastic cells contain two cyclic nucleotides—cyclic adenosine monophosphate (AMP) and cyclic guanosine monophosphate (GMP). Cyclic AMP has the capacity to inhibit the growth of fibroblastic cells (*1*). It also regulates cell shape, motility, adhesiveness to substratum, and agglutinability by plant lectins (*1*). The role of cyclic GMP is unclear. One proposal that has received considerable attention is that cyclic GMP antagonizes the actions of cyclic AMP and promotes cellular growth (*2, 3*).

To gain more information about the role of cyclic GMP in cultured cells, we have measured concentrations of cyclic GMP in growing and nongrowing BALB 3T3 cells and in cells that have been stimulated to grow by the addition of serum. We find that cyclic GMP rises as cell growth slows and that the addition of serum to resting cells causes a prompt fall in both cyclic GMP and cyclic AMP. These data fail to support the proposal that cyclic GMP acts to promote cellular growth and raises the possibility that both cyclic AMP and cyclic GMP act as growth inhibitors.

BALB 3T3 cells clone B were grown in Dulbecco's modification of Eagle's medium containing either 10 percent calf serum or 5 percent calf plasma (Colorado Serum Company). The cells were propagated as described (*4*), except that the 37°C incubator was kept inside a 37°C warm room so that cells could be removed at frequent intervals without lowering their temper-