for 60 days secreted more insulin and glucagon when theophylline (10 m*M*) was present in the medium (Fig. 3). Likewise, attached islets with monolayer formation subjected to theophylline (10 m*M*) after 10 days in culture secreted more insulin in each of two 1-hour periods than those in control media.

It is customary to supplement nutrient medium used for the culture of living cells with serum that contains macromolecular components. These components, studied primarily in fibroblast cultures, have been shown to affect attachment of cells, cell stretching, DNA synthesis, mitosis, and cell multiplication, RNA synthesis, protein synthesis, and survival of cells (*6*). Fractionation of fetal calf serum has revealed the presence of fetuin and other α-globulins that are important for attachment and growth-promoting activity, as well as smaller molecular weight components, such as nonsuppressible insulin-like activity, multiplication activity, epidermal growth factor, and the somatomedins (*1*, *7*). Fetal calf serum, chick embryo extract, and rooster serum have been used as sources of these components in pancreatic endocrine cell culture systems (*8*).

The major advantage conferred by the use of homologous serum is either its ability to maintain the integrity of the islet capsule or to inhibit islet attachment. The result is a preparation suitable both for the relatively short-term maintenance of islets prior to their transplantation, and for the long-term maintenance of islets for use in extended physiologic studies.

When newly isolated islets were exposed to a medium that contained heterologous serum (FCS), the majority of the islets anchored rapidly, capsular integrity was quickly interrupted, and the production of endocrine cell monolayers followed. Here again those monolayer replicates maintained with HCS secreted significantly more insulin, but not glucagon, during the first 2 weeks in culture as compared to the replicates maintained with FCS, although this advantage disappeared during the third week of culture. This suggests that a further favorable factor is present in homologous serum. During the fourth week in culture, the monolayers of both HCS and FCS replicates disintegrate almost simultaneously. Nevertheless, the monolayer remains a useful preparation for morphologic, histochemical, and immunofluorescent studies.

H. GOLDMAN, E. COLLE
McGill University–Montreal Children's Hospital Research Institute, Montreal H3H 1P3, Quebec, Canada

References and Notes

1. E. B. Marliss, C. B. Wollheim, B. Blonder, L. Orci, A. E. Lambert, W. Stauffacher, A. A. Like, A. E. Renold, *Eur. J. Clin. Invest.* **3**, 16 (1973).
2. E. M. Leitner, *In Vitro* **9**, 421 (1974); A. Anderson, C. Hellerstrom, B. Peterson, *Z. Zellforsch. Mikrosk. Anat.* **82**, 110 (1967); I. Hilwig, S. Schuster, W. Heptner, E. Wasielewski, *ibid.* **90**, 333 (1968); I. A. Macchi and E. H. Blaustein, *Endocrinology* **84**, 208 (1969).
3. D. A. Hillman and E. Colle, *Am. J. Dis. Child.* **117**, 636 (1969).
4. Antiserum provided by Dr. Roger Unger; 30K is a specific pancreatic glucagon antiserum fraction derived from rabbit antiserum to beef-pork glucagon.
5. The antiserum to insulin was used at a final dilution of 1 : 600,000. Porcine insulin was used as a standard, and bound and free insulin were separated by charcoal. Identity of reaction between human and porcine insulin with this antiserum has been demonstrated. Dilution studies utilizing the media showed identity of displacement curves with the standard. Fractionation of the media on Sephadex G-100 indicated that more than 95 percent of the immunoreactive insulin was found in the same fractions as the ^{125}I-labeled porcine insulin. To assess the synthesis of insulin, 200 μc of [4,5-^{3}H]leucine (specific activity of 30 to 50 c/mmole; New England Nuclear) were added to the medium of islets maintained in culture as long as 60 days. After 24 hours under the usual conditions, the medium was removed, dialyzed against 0.05*M* phosphate buffer, *p*H 7, for 24 hours at 4°C, and then fractionated on columns of Sephadex G-100 (24 by 3 cm) at room temperature. Fractions corresponding to the second peak were combined and reacted with antiserum to insulin for 24 hours. (The amount of antiserum added is sufficient to bind 80 percent of ^{125}I-labeled insulin in the presence of 5 milliunits of insulin.) The reaction mixture was refractionated on Sephadex G-100, and 30 to 50 percent of the radioactivity of the second peak appeared in the bound form. This suggests synthesis of hormone rather than release of preformed insulin due to islet destruction.
6. H. M. Temin, R. W. Pierson, Jr., N. C. Dulak, in *Growth, Nutrition and Metabolism of Cells in Culture*, G. H. Rothblat and J. Cristofalo, Eds. (Academic Press, New York, 1972).
7. A. Lazarow, L. J. Wells, A. M. Carpenter, O. D. Hegre, R. J. Leonard, R. C. McEvoy, *Diabetes* **22**, 877 (1973).
8. E. R. Froesch, *Recent Prog. Horm. Res.* **23**, 565 (1967); R. W. Pierson, Jr., and H. M. Temin, *J. Cell. Physiol.* **79**, 319 (1972); S. Cohen and J. M. Taylor, *Recent Prog. Horm. Res.* **30**, 551 (1974); J. J. Van Wyk, L. [illegible] Underwood, R. L. Hintz, D. R. Clemmons, [illegible] J. Voina, R. B. Weaver, *ibid.*, p. 259.
9. Supported by grants from the Banting Research Foundation; by the McGill University–Montreal Children's Hospital Research Institute; and by the Juvenile Diabetes Foundation. We thank Ines Wong and Linda Waal for technical assistance, and the Blood Bank at the St. Mary's Hospital, Montreal, for providing us with a steady supply of umbilical cord serum.

3 November 1975; revised 26 January 1976

Direct Evidence for a Bone Marrow Origin of the Alveolar Macrophage in Man

Abstract. *Alveolar macrophages were obtained from 23 patients who had received marrow transplants for hematologic disorders. The presence of a Y body in macrophages of male origin was demonstrated by fluorescence microscopy. In those patients with a marrow donor of opposite sex the alveolar macrophages were shown to be of donor origin. The disappearance with time of host macrophages indicates a life-span, under the conditions, of approximately 81 days.*

Several studies of marrow transplantation in rodents (*1*) and one in the dog (*2*) have provided evidence that the lung macrophages derive from a bone marrow precursor. Other investigators, however, indicate that mouse alveolar macrophages may proliferate actively in vitro (*3*) and organ culture studies suggest that alveolar macrophages can be produced in vitro from a cell in the interstitium of the lung (*4*). In man, pulmonary macrophages exhibit a low but definite level of scheduled DNA synthetic activity in vitro, suggesting that they also

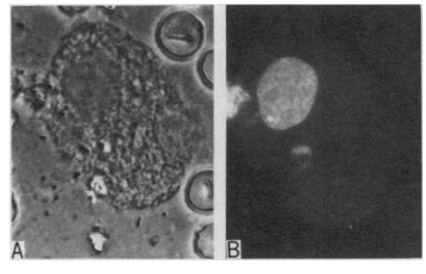

Fig. 1 (above). (A) Alveolar macrophage from a female patient given male bone marrow observed under phase contrast microscopy (×540). (B) The same macrophage observed under fluorescence microscopy. The Y body is the bright round spot at 7 o'clock in the cell nucleus (×540).

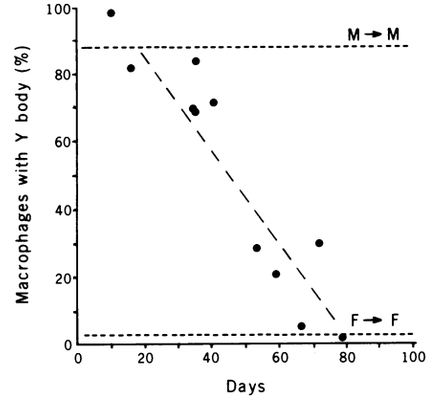

Fig. 2 (right). The dashed line $M \rightarrow M$ represents the average percentage of macrophages with a Y body in male to male transplants, and the dashed line $F \rightarrow F$, the average in female to female transplants. The closed circles represent the percentage of macrophages with a Y body in female to male transplants observed at various times after transplantation. The sloping dashed line was calculated by the method of least squares.

have proliferative capacity (5), but there is no information on the cellular origin of the human alveolar macrophage.

We studied a series of patients who had received bone marrow grafts from HLA matched sibling donors of opposite sex. Lung macrophages were examined at intervals after transplantation when the patients underwent lung biopsy or fiberoptic bronchoscopy during diagnostic evaluation of interstitial pneumonia, a common complication of allogeneic bone marrow transplantation in man (6). In 21 cases we obtained touch preparations of the biopsy material, and in two cases cells were retrieved for study by bronchopulmonary lavage performed through the fiberoptic bronchoscope. The cells were placed on slides, fixed in methanol, and stained for 15 minutes in a 0.5 percent solution of acranil dissolved in buffer, rinsed four times in buffer, and then mounted in buffer and sealed (7). They were then viewed with a Zeiss Photoscope II, equipped with an epi-illumination head, HBO 200/4 burner, FITC filter, and a 500 reflector; the barrier filter was set at 50. When possible, 100 morphologically typical macrophages were viewed for the presence or absence of a Y body. The pulmonary macrophages were identified in the fluorescence preparations and confirmed by switching to phase contrast (Fig. 1). The Y body was readily visualized in male macrophages. Cytogenetic studies were performed on donors and recipients prior to transplantation to exclude chromosomal anomalies.

Table 1 shows the results of the study. In four cases the patient and the donor were both female. The percentage of macrophages with an apparent Y body ranged from 1 to 4. In four cases, donor and recipient were both male, and the percentage of macrophages with Y bodies ranged from 83 to 92. Seven observations were made in the five cases where the donor was male and the recipient female. In four of these cases there were many macrophages with a Y body, and in the two observed at different time intervals after marrow grafting there was an increase in the percentage of macrophages with a Y body at the second examination. One patient (case 514) who did not show a significant increase in percentage of macrophages with a Y body had rejected her marrow graft approximately 40 days after transplantation. Thirteen observations were made in ten patients of male sex whose donor was female. The results are depicted graphically in Fig. 2. The disappearance of macrophages with a Y body is roughly linear and indicates a life-span, under these conditions, of approximately 81 days.

Table 1. Percentage of alveolar macrophages with Y bodies in 22 cases of human marrow graft recipients. Patients with leukemia were prepared for engraftment by the administration of cyclophosophamide, 60 mg/kg, on each of 2 days, followed 3 days later by 1000 rads midpoint tissue dose of total body irradiation from opposing ^{60}Co sources. Patients with aplastic anemia were prepared for engraftment by the administration of cyclophosphamide, 50 mg/kg, each of 4 days. Day 0 is the day of marrow transplantation. Abbreviations: AML, acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; AA, aplastic anemia; L, lymphoma; CY, cyclophosphamide; TBI, total body irradiation.

Patient number	Patient sex	Donor sex	Disease	Preparation for grafting	Day of study post grafting	Percentage of macrophages with Y body
516	F	F	AML	CY-TBI	44	4
504	F	F	AML	CY-TBI	46	2
506	F	F	ALL	CY-TBI	57	1
518	F	F	AA	CY × 4	109	4
532	M	M	AML	CY-TBI	35	91
505	M	M	AML	CY-TBI	53	92
513	M	M	ALL	CY-TBI	71	86
392	M	M	AML	CY-TBI	77	83
383	M	F	AML	CY-TBI	10	99
					35	70
530	M	F	ALL	CY-TBI	16	82
523	M	F	L	CY-TBI	36	69
535	M	F	AML	CY-TBI	36	84
528	M	F	ALL	CY-TBI	41	72
					72	30
396	M	F	AML	CY-TBI	54	29
393	M	F	AML	CY-TBI	59	21
					66	5
UCLA-1*	M	F	AA	CY × 4	231	0
500	M	F	ALL	CY-TBI	79	1
377	M	F	ALL	CY-TBI	186	5
519	F	M	ALL	CY-TBI	50	34
					66	48
384	F	M	AML	CY-TBI	51	45
					174	84
529	F	M	AA	CY × 4	58	28
UCLA-2*	F	M	AA	CY × 4	60	50
514	F	M	AA	CY × 4	70	5

*Sample obtained by bronchoscope; all other samples obtained by biopsy.

These studies indicate that lung macrophages are repopulated with donor-derived cells after allogeneic marrow transplantation in man. Repopulation occurred whether the patients were conditioned with cyclophosphamide or total body irradiation. Since chemotherapy should not be lethal to the lung macrophages (8), repopulation probably does not depend on the elimination of host cells. One thousand rads total body irradiation does not kill the lung macrophages but probably prevents their replication. The time curves of repopulation suggest that under the circumstances associated with transplantation the host macrophages are largely replaced in less than 100 days. This may relate in part to inflammatory processes in the lungs of some of the patients and may not reflect the life-span of the alveolar macrophages in the normal situation. Similarly, the kinetics of repopulation after transplantation probably cannot be related to the kinetics of lung macrophage replacement in normal man.

These observations indicate that the human alveolar macrophage population may be derived from marrow precursors. However, since the human alveolar macrophage has proliferative capacity, this population probably can survive for long periods if not indefinitely without depending on an influx of cells from the bone marrow. The present data provide direct evidence for a bone marrow derivation of the human alveolar macrophage and suggest that allogeneic transplantation in man may result in repopulation of large segments of the mononuclear phagocyte system.

E. DONNALL THOMAS
ROBERT E. RAMBERG
GEORGE E. SALE
Departments of Medicine and Pathology, University of Washington School of Medicine, Fred Hutchinson Cancer Research Center, Seattle 98104
ROBERT S. SPARKES
DAVID W. GOLDE
Departments of Hematology and Oncology, School of Medicine, University of California, Los Angeles 90024

References and Notes

1. J. J. Godleski and J. D. Brain, *J. Exp. Med.* **136**, 630 (1972); F. L. Shand and E. B. Bell, *Immunology* **22**, 549 (1972); R. van Furth, *Semin. Hematol.* **7**, 125 (1970); M. Virolainen, *J. Exp. Med.* **127**, 943 (1968).
2. P. L. Weiden, R. Storb, M. S. Tsoi, *RES J. Reticuloendothel. Soc.* **17**, 342 (1975).
3. D. H. Bowden and I. Y. Adamson, *Am. J. Pathol.* **68**, 521 (1972); ———, W. G. Grantham, J. P. Wyatt, *Arch. Pathol.* **88**, 540 (1969); D. H. Bowden, E. Davies, J. P. Wyatt, *ibid.* **86**, 667 (1968); S. C. Soderland and Y. Naum, *Nature (London)* **245**, 150 (1973).
4. M. A. Brunstetter, J. A. Hardie, R. Schiff, J. Lewis, C. Cross, *Arch. Intern. Med.* **127**, 1064 (1971); M. O. Pinkett, C. R. Cowdrey, P. C. Nowell, *Am. J. Pathol.* **48**, 859 (1966).
5. D. W. Golde, L. A. Byers, T. N. Finley, *Nature (London)* **247**, 373 (1974).
6. E. D. Thomas, R. Storb, R. A. Clift, A. Fefer, F. L. Johnson, P. E. Neiman, K. G. Lerner, H. Glucksberg, C. D. Buckner, *N. Engl. J. Med.* **292**, 832, 895 (1975).
7. P. L. Pearson, M. Bobrow, C. G. Vosa, *Nature (London)* **226**, 78 (1970); G. Moscetti, M. Petriaggi, C. G. Barbarossa, S. Tiberti, *Humangenetik* **12**, 56 (1971).
8. D. W. Golde, T. N. Finley, M. J. Cline, *N. Engl. J. Med.* **290**, 875 (1974).

9. This investigation was supported by grants CA 18029 and 15688 from the National Cancer Institute. E.D.T. is a recipient of research career award AI 02425 from the National Institute of Allergy and Infectious Diseases.

22 January 1976

Monoamine Oxidase Activity Decreased in Cells Lacking Hypoxanthine Phosphoribosyltransferase Activity

Abstract. *The Lesch-Nyhan syndrome in humans is characterized by lack of hypoxanthine phosphoribosyltransferase activity and neurologic abnormalities that suggest changes in catecholamine metabolism. Monoamine oxidase, which degrades biogenic amines, has decreased activity in noradrenergic murine neuroblastoma cell lines lacking hypoxanthine phosphoribosyltransferase activity and in skin fibroblasts from patients with the Lesch-Nyhan syndrome.*

The primary biochemical lesion in the Lesch-Nyhan syndrome is lack of hypoxanthine phosphoribosyltransferase activity (HPRT; E.C. 2.4.2.8), an enzyme involved in purine salvage (*1*). It is not clear why this lesion produces neurologic dysfunction, including mental retardation, spasticity, choreoathetosis, and compulsive self-mutilation (*2*). Other findings suggest that catecholamine metabolism may be altered in this disease: potentiation of catecholamine pathways in the brains of experimental animals leads to chorea, compulsive gnawing, and self-mutilation (*3*); and Lesch-Nyhan patients show peripheral changes in noradrenergic functioning (*4*). Our finding that monoamine oxidase (MAO; E.C. 1.4.3.4) activity is reduced in cells lacking HPRT activity could account for altered catecholamine metabolism in this disease. Monoamine oxidase is one of the major enzymes involved in degradation of biogenic amines throughout the body, and its decreased activity results in increased tissue levels of these neurotransmitters (*5*).

Murine neuroblastoma cells in culture have many properties of normal neurons (*6*) and provide a model system to explore the effects of HPRT deficiency on neuronal metabolism. We have used clonal lines of neuroblastoma that differ in their abilities to synthesize various neurotransmitters (*7*). Line N1E-115 is classified as noradrenergic in that it has high activities of two enzymes needed in norepinephrine synthesis, tyrosine 3-hydroxylase (E.C. 1.14.16.2) (*8*) and dopamine β-hydroxylase (E.C. 1.14.17.1) (*9*), as well as the ability to store catecholamines (*10*). This line also shows high MAO activity of the A type, which is the predominant form in sympathetic neurons (*11*). Cholinergic line NS-20 has high levels of choline acetyltransferase (E.C. 2.3.1.6) activity and synthesizes acetylcholine (*8*). Lines N-4 and N-18 are "inactive" with respect to synthesis of these transmitters as they have low levels of both tyrosine 3-hydroxylase and choline acetyltransferase (*8*).

6-Thioguanine (TG) resistant lines derived from these neuroblastoma clones (*7*) were found to have less than 1 percent of the HPRT activity (*12*) of the pa-

Table 1. Catecholamine metabolism in neuroblastoma lines with and without HPRT activity. The HPRT activities were measured in two different cultures of each line; 100 percent corresponds to 32 to 161 pmole/min per milligram of protein (*12*). Tyrosine hydroxylase (TH) and MAO activities (*13*) are expressed as the means (picomoles per minute per milligram of protein) ± standard mean error (S.E.): The numbers in parentheses refer to the number of separate homogenates tested; each homogenate was assayed in duplicate; only duplicates varying by < 20 percent were averaged and used here. Chromatographic identification of the metabolic products of [^{3}H]dopamine (*15*) are expressed as the percentage of radioactivity recovered from strips which comigrated with authentic standards; values are expressed as the means ± S.E., averaged from two separate experiments. Abbreviations: DA, dopamine; NE, norepinephrine; and MT, 3-methoxytyramine.

Cell line	HPRT (%)	MAO	TH	[^{3}H]Dopamine metabolism, radioactivity migrating as:			
				DOPAC (%)	NE (%)	MT (%)	DA (%)
			Noradrenergic				
N1E-115	100	103 ± 9 (5)	59 ± 7 (6)	9 ± 2	24 ± 1	4 ± 1	56 ± 1
N1E-115TG1	< 1	3.3 ± 2.8 (3)	64 ± 37 (4)	0	12 ± 2	9 ± 1	71 ± 4
N1E-115TG2	< 1	4.9 ± 3.3 (3)	48 ± 10 (3)	0	22 ± 9	7 ± 1	56 ± 8
N1E-115TG3	< 1	0.8 ± 0.5 (3)	21 ± 2 (4)	0	19 ± 6	7 ± 2	67 ± 8
N1E-115TG4	< 1	1.3 ± 0.6 (3)	50 ± 7 (2)	0	22 ± 3	7 ± 3	61 ± 2
N1E-115TG5	< 1	1.1 ± 0.5 (3)	24 ± 2 (3)	0	20 ± 4	6 ± 1	67 ± 3
N1E-115TG6	< 1	1.0 ± 0.5 (3)	29 ± 12 (5)	0	30 ± 8	7 ± 1	58 ± 9
N1E-115TG7	< 1	0.02 ± 0.01 (2)	40 ± 10 (4)	0	21 ± 5	6 ± 1	66 ± 8
N1E-115TG8	1	1.9 ± 1.1 (3)	17 ± 5 (4)	0	26 ± 3	3 ± 1	63 ± 4
			Cholinergic				
NS-20	100	132 ± 16 (4)	8 ± 3 (2)	8 ± 1	18 ± 1	4 ± 1	67 ± 2
NS-20TG7B	< 1	133 ± 18 (4)	15 ± 8 (2)	6 ± 2	10 ± 1	1 ± 1	78 ± 1
NS-20TG11E	< 1	85 ± 15 (4)	5 ± 3 (2)	10 ± 1	5 ± 1	1 ± 1	81 ± 2
			"Inactive"				
N-18	100	138 ± 20 (4)	9 ± 2 (3)	47 ± 6	4 ± 1	5 ± 2	35 ± 3
N-18TG2	< 1	75 ± 6 (5)	9 ± 4 (4)	42 ± 9	2 ± 1	9 ± 1	41 ± 6
N-4	100	112 ± 5 (2)	10 ± 8 (2)	31 ± 1	0	8 ± 1	50 ± 2
N-4TG1	< 1	136 ± 27 (4)	6 ± 3 (3)	32 ± 5	0	10 ± 3	45 ± 6