geneic mouse recipients of lymphoid cells from old donors, whereas grafting of cells from young donors had no effect (*6*).

This work demonstrated that also development of carcinoma as a result of DMBA treatment occurs with a higher incidence on skin from old as compared to young donors. In that three applications were made only 14 days apart, an influence of hair-cycle (*7*) on tumor development appears unlikely. In the above experiments, age-dependent alterations in general immune reactivity and hormone secretion can also be disregarded as contributing factors because the recipient mice of the various test groups were of the same age. An age-dependent alteration of graft permeability to cellular and hormonal host factors that could influence skin carcinogenesis is conceivable. It seems more likely, however, that skin cells themselves become more susceptible to carcinogen with increasing age. Tumors other than skin tumors were most numerous in mice bearing skin grafts from old donors. This can hardly be a reflection of a different concentration of DMBA in the bodies of the mice, since old skin is believed to retain the same or more DMBA than young skin (*8*).

P. EBBESEN

Institute of Medical Microbiology,
22 Juliane Maries Vej,
DK-2100 Copenhagen, Denmark

References and Notes

1. P. Ebbesen, *Nature (Lond.)* **241**, 240 (1973).
2. M. G. Hanna, *J. Natl. Cancer Inst.* **46**, 809 (1971); M. N. Teller and E. Marion, *ibid.* **39**, 231 (1967).
3. D. Metcalf, *Nature (Lond.)* **208**, 87 (1965); P. Ebbesen and M. J. Doenhoff, *Proc. Soc. Exp. Biol. Med.* **138**, 850 (1971).
4. V. M. Dilman, *Lancet* **1971-I**, 1211 (1971).
5. J. F. Danielli, in *Experimentelle Altersforschung*, F. Verzar, Ed. [*Experientia* (Suppl. 4) (1956)].
6. P. Ebbesen, *J. Natl. Cancer Inst.* **47**, 1241 (1971).
7. E. Andreasen and J. Engelbreth-Holm, *Acta Pathol. Microbiol. Scand.* **32**, 165 (1953).
8. I. Berenblum, N. Haran-Ghera, N. Trainin, *Br. J. Cancer* **12**, 23 (1958).
9. Aided in part by grants from the Danish Medical Research Council, the Danish Fund for the Advancement of Medical Science, the Danish Cancer Society, the P. Carl Petersens Fond, and Anders Hasselbalchs Fond til Leukaemiens Bekaempelse.

2 May 1973; revised 10 September 1973

Thymus Adenylate Cyclase Activity during Murine Leukemogenesis

Abstract. *Activity of thymus adenylate cyclase was more than three times higher in leukemic AKR mice than in nonleukemic AKR mice and CBA mice. Preleukemic AKR mice that had no evidence of leukemia but were expected to soon develop the disease exhibited similarly elevated activities of thymus adenylate cyclase.*

The postulate that adenosine 3,'5'-monophosphate (cyclic AMP) plays a role in the initiation of events leading to proliferation of lymphoid cells has been supported by observations that cyclic AMP increases (i) thymidine uptake by the nucleic acid fractions of spleen cells and thymocytes; (ii) uridine incorporation into RNA of peripheral lymphocytes; (iii) cell division of thymocytes in calcium deficient medium; and (iv) the number of antibody-forming cells after immunization (*1*). Smith *et al.* (*2*) reported that phytohemagglutinin, which stimulates the proliferation of lymphocytes, elevates intracellular concentrations of cyclic AMP and increases the activity of lymphocyte adenylate cyclase. Agents that have adjuvant effects on antibody formation also stimulate adenylate cyclase activity of spleen cells (*3*). Immunodeficient pituitary dwarf mice, with decreased thymic lymphopoiesis and inefficient splenic proliferative response to antigen, exhibit a defective lymphocyte adenylate cyclase that does not respond to epinephrine-induced stimulation (*4*).

In view of the role of cyclic AMP in lymphoid cell proliferation, the question was raised whether changes in cyclic AMP metabolism of thymic lymphocytes occurred during the development of leukemia in AKR mice. AKR mice have a high incidence of lymphoid leukemia that primarily affects the thymus and frequently involves other lymphoid tissues (*5*). The causative agent is a vertically transmitted oncogenic virus (Gross virus) (*6*) that apparently finds a suitable microenvironment in the AKR thymus for the induction of neoplastic transformation of lymphoid cells (*7*). This report describes our studies on adenylate cyclase of thymus cells during the development of leukemia in AKR mice.

Determination of adenylate cyclase activity in the 10,000*g* sediment fraction of thymus cell sonicates was performed with a modification (*8*) of the method of White and Zenser (*9*) and was based on the conversion of adenosine [α-^{32}P]triphosphate ([α-^{32}P]ATP) into cyclic [^{32}P]AMP. The 0.1-ml assay mixture contained 1 m*M* [α-^{32}P]ATP (2 to 3 $\times$ 10^6 count/min), 5 m*M* $MgCl_2$, 50 m*M* tris(hydroxymethyl)aminomethane (tris) HCl (*p*H 7.6), 0.1 m*M* cyclic [^{3}H]AMP (2 $\times$ 10^4 count/min), 10 m*M* theophylline, an ATP generating system consisting of 30 m*M* creatine phosphate and 30 μg of creatine kinase, and 25 μl of the 10,000*g* sediment of thymus tissue sonicate. Assays were performed at 30°C for 1 to 2 hours, and radioactive cyclic AMP was recovered through chromatography on alumina columns as described (*8*). One

Table 1. Adenylate cyclase activity of thymus cells from AKR mice during stages of leukemia development and from normal CBA mice. Values are geometric means ± standard error.

Group	Experiments (No.)	Age (days)		Cells per thymus (10^8 cells)	Protein in 10,000*g* sediment of sonicate of 10^{11} cells (g)	Enzyme per gram of protein in 10,000*g* sediment (units)	Enzyme per 10^{11} cells (units)	Increase of activity with 10^{-5}*M* epinephrine (%)
		Mean	Range					
Nonleukemic AKR	10	66	21–162	2.3 ± 0.3	0.58 ± 0.04	26 ± 4	15 ± 2	275 ± 27
Preleukemic AKR	10	231	200–268	1.1 ± 0.1	0.62 ± 0.08	80 ± 22	55 ± 18	190 ± 20
Leukemic AKR	5	224	200–252	6.1 ± 1.4	1.13 ± 0.17	88 ± 16	99 ± 23	100 ± 10
Young CBA	5	114	75–150	1.3 ± 0.1	0.49 ± 0.03	11 ± 2	5 ± 1	270 ± 33
Old CBA	3	241	228–253	0.6 ± 0.1	0.45 ± 0.05	11 ± 1	5 ± 1	110 ± 20

unit of adenylate cyclase is defined as the amount of enzyme that produced 1 nmole of cyclic AMP in 10 minutes under the above conditions.

The AKR mice were divided into three groups: a nonleukemic group of mice less than 5 months old with no evidence of leukemia; a preleukemic group of mice approximately 7 months old that had no leukemia but could be expected to soon develop the disease; and a leukemic group of AKR mice with thymic lymphomas and, frequently, enlarged spleens and lymph nodes. Groups of young (less than 5 months old) and old (more than 7 months old) CBA mice, which are not leukemia-prone, were also studied.

Thymus adenylate cyclase activities were markedly elevated in both preleukemic and leukemic AKR mice as compared to nonleukemic mice (Table 1). In view of the possible role of cyclic AMP in lymphoid cell proliferation (*1–4*), the high cyclase activity of leukemic cells may be related to the high rate of proliferation of these neoplastic cells. Elevated adenylate cyclase activity does not necessarily lead to higher concentrations of cyclic AMP, because the concentration of the nucleotide is also affected by cyclic nucleotide phosphodiesterase. Although it would be tempting to suggest that a mechanism involving elevation of adenylate cyclase may be generally operative in neoplastic cells, the role of cyclic AMP in the growth of both normal and neoplastic cells remains obscure. Furthermore, previous reports have not shown a consistent variation in the activities of adenylate cyclase following neoplastic transformation. A dimethylaminobiphenyl-induced mammary carcinoma in rats, certain rapidly growing rat hepatomas, and some in vitro virus-transformed cells contain elevated adenylate cyclase activity (*10*), but unchanged (*11*) and decreased (*12, 13*) cyclase activities in neoplastic cells have also been reported. However, these studies were done either by in vitro transformation of cells in culture or with transplantable tumors that might have undergone certain functional and biochemical changes during serial passages. As far as we are aware, the work on the dimethylaminobiphenyl-induced mammary carcinoma and our studies on AKR lymphoid leukemia are the only reported de novo neoplasms for which adenylate cyclase activity has been determined, and in both cases the activity of this enzyme was markedly elevated. However, other virally or chemically induced neoplasms should be studied before it can be established that adenylate cyclase activity is generally higher in de novo neoplasms.

The responsiveness of adenylate cyclase to epinephrine stimulation diminished progressively from the nonleukemic to the preleukemic and leukemic groups of AKR mice (Table 1). This decrease may be due to age-related involution of the thymus and is not necessarily related to the neoplastic change of thymus cells, because thymus adenylate cyclase stimulation was also less in old than in young CBA mice. No clear pattern of differences in the epinephrine-induced activation of adenylate cyclase of normal and neoplastic cells has emerged. The epinephrine responsiveness of adenylate cyclase has been reported to be increased (*11, 14*), unaltered (*10, 11*), and diminished (*13*) in neoplastic cells as compared to normal cells.

We found that the adenylate cyclase activity of thymus cells from preleukemic AKR mice was also markedly elevated as compared to that from normal thymocytes (Table 1). These preleukemic mice exhibited no leukemia, as indicated by the absence of enlargement of thymus or other lymphoid tissue. Furthermore, the number of cells per preleukemic thymus was in the normal range if the age-related involution of the thymus is considered. Preleukemic thymus cells appeared similar in size to nonleukemic thymus cells, as judged from microscopic examination and from the amount of particulate protein (10,000*g* sediment) recovered from each cell preparation (0.62 and 0.58 g per 10^{11} cells). In contrast, leukemic thymus cells were considerably larger than preleukemic and nonleukemic thymus cells. The increased adenylate cyclase activity of preleukemic thymus was not due to age-related involuntary changes of the thymus, inasmuch as old CBA thymus exhibited the same cyclase activity as young CBA thymus. The increased cyclase activity of preleukemic thymus cells may reflect an event during neoplastic transformation of thymus cells induced by Gross virus. With reference to the clonal nature of AKR lymphoid leukemia (*7*), it is possible that a small clone of leukemic cells with extremely high adenylate cyclase activity was responsible for the increased levels of adenylate cyclase in preleukemic thymus. The elevated adenylate cyclase activity of preleukemic thymus cells is perhaps more likely to be related to certain morphologic changes in the preleukemic thymus just before appearance of leukemic cells. These changes involve a loss of cortical thymocytes and extensive medullary hyperplasia, with the appearance of lymphoid follicles and even germinal centers that contain aggregates of large pyroninophilic cells exhibiting mitotic activity (*5, 15*). Although our studies indicated alteration in cyclic AMP metabolism, the exact nature of the cellular changes in thymus lymphoid cells during these preleukemic changes remains uncertain.

ROBERT G. KEMP
Department of Biochemistry, Medical College of Wisconsin, Milwaukee 53233

RENÉ J. DUQUESNOY
Department of Microbiology, Medical College of Wisconsin

References and Notes

1. M. Burger and A. Knyszynski, *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 1019 (1971); J. P. MacManus and J. F. Whitfield, *Exp. Cell Res.* **58**, 188 (1969); M. J. Averner, M. L. Brock, J.-P. Jost, *J. Biol. Chem.* **247**, 413 1972); M. Ishizuka, M. Gafni, W. Braun, *Proc. Soc. Exp. Biol. Med.* **134**, 963 (1970).
2. J. W. Smith, A. L. Steiner, W. M. Newberry, Jr., W. W. Parker, *J. Clin. Invest.* **50**, 442 (1971).
3. R. Winchurch, M. Ishizuka, D. Webb, W. Braun, *J. Immunol.* **106**, 1399 (1971); M. Ishizuka, W. Braun, T. Matsumoto, *ibid.* **107**, 1027 (1971); R. Winchurch and P. Actor, *ibid.* **108**, 1305 (1972).
4. R. G. Kemp, Y.-C. Huang, R. J. Duquesnoy, *ibid.*, in press.
5. D. P. McEndy, M. C. Boon, J. Furth, *Cancer Res.* **4**, 377 (1944); D. Metcalf, *Recent Results Cancer Res.* **5**, 100 (1966).
6. L. Gross, *Proc. Soc. Exp. Biol. Med.* **76**, 27 (1951).
7. D. Metcalf, *Adv. Cancer Res.* **15**, 181 (1972).
8. Y.-C. Huang, R. J. Duquesnoy, R. G. Kemp, *Int. J. Biochem.* **4**, 79 (1973).
9. A. A. White and T. V. Zenser, *Anal. Biochem.* **41**, 372 (1971).
10. H. D. Brown, S. K. Chattopadhyay, H. J. Spjut, J. S. Spratt, Jr., S. N. Pennington, *Biochim. Biophys. Acta* **192**, 372 (1969); H. D. Brown, S. K. Chattopadhyay, H. P. Morris, S. N. Pennington, *Cancer Res.* **30**, 123 (1970); C. V. Peery, G. S. Johnson, I. Pastan, *J. Biol. Chem.* **246**, 5785 (1970).
11. I. Schorr and R. L. Ney, *J. Clin. Invest.* **50**, 1295 (1971); D. O. Allen, J. Munshower, H. P. Morris, G. Weber, *Cancer Res.* **31**, 557 (1971); V. Macchia, M. F. Meldolesi, M. Chiariello, *Endocrinology* **90**, 1483 (1972).
12. D. Granner, L. R. Chase, G. D. Aurbach, G. M. Tomkins, *Science* **162**, 1018 (1968); R. R. Burk, *Nature* **219**, 1272 (1968); B. Weiss, H. M. Shein, R. Snyder, *Life Sci.* **10**, 1253 (1971); W. B. Anderson, G. Johnson, I. Pastan, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1055 (1973); V. Tomasi, A. Rethy, A. Trevisani, *Life Sci.* **12**, 145 (1973).
13. P. Emmelot and C. J. Bos, *Biochim. Biophys. Acta* **249**, 285 (1971).
14. S. N. Pennington, H. D. Brown, S. Chattopadhyay, C. Conaway, H. P. Morris, *Experientia* **26**, 139 (1970).
15. K. Arnesen, *Acta Pathol. Microbiol. Scand.* **43**, 350 (1958); R. Siegler and M. A. Rich, *Cancer Res.* **23**, 1669 (1963).
16. We thank A. Hsu and G. Pedersen for excellent help. The study was supported by American Cancer Society grant BC-114, Damon Runyon–Walter Winchell Cancer Fund grant DRG-1224, and PHS grant AM11410. R.G.K. is an established investigator of the American Heart Association.

30 July 1973; revised 24 September 1973