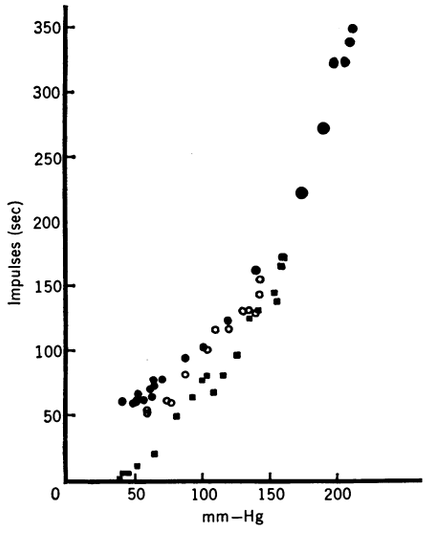

Fig. 3. Relation between afferent discharge rate and mean blood pressure. Values taken during short periods of constant pressure. Filled circles and squares, different trials on the same unit. Open circles, trials on a different unit.

periods of aortic clamping in our experiments.

Figure 3 shows the relationship between mean systemic blood pressure and firing rate from isolated afferent nerve fibers. The filled circles represent the response to intravenous injection of 100 μg of epinephrine; the filled squares represent the response to abdominal aorta clamping above the level of the adrenal artery, both recorded from the same afferent fiber. The open circles represent the response to intravenous injection of 30 μg of epinephrine in another afferent unit in a different animal. The high mean arterial blood pressure followed the injection of large amounts of epinephrine used to test the limits of the response. The relations between pressure and firing rate in these experiments are quite similar to those found for the carotid sinus (*3*).

We suggest that in the adrenal gland there are baroreceptors which send to the central nervous system information related to blood pressure within the gland. These receptors and those (*4*) in the rabbit kidney may represent the afferent limb of a set of reflex systems involved in the control of regional blood flow.

A. NIIJIMA*
D. L. WINTER
Department of Neurophysiology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C. 20012

26 JANUARY 1968

References and Notes

1. D. W. Bronk and G. Stella, *J. Cell. Comp. Physiol.* **1**, 113 (1932); S. Landgren, *Acta Physiol. Scand.* **26**, 1 and 35 (1951); C. Heymans and E. Neil, *Reflexogenic Areas of the Cardiovascular System* (Churchill, London, 1958).
2. J. C. G. Coleridge, A. Hemingway, R. L. Holmes, R. J. Linden, *J. Physiol. London* **136**, 174 (1957); J. C. G. Coleridge and C. Kidd, *ibid.* **150**, 319 (1960); A. S. Paintal, *Ergeb. Physiol.* **52**, 74 (1963); S. Homma and S. Suzuki, *Jap. J. Physiol.* **16**, 31 (1966).
3. C. M. Bloor, *J. Physiol. London* **174**, 163 (1964); J. W. Spickler and P. Kezdi, *Amer. J. Physiol.* **212**, 471 (1967).
4. A. Niijima, in *Proc. Int. Congr. Physiol. Sci. 23rd* **1965**, 381 (1965).
5. A.N. was supported by a China Medical Board fellowship.
* Present address: Department of Physiology, Niigata University School of Medicine, Niigata City, Japan.

26 September 1967

Parathyroid Hormone Production in vitro by Human Parathyroid Cells Transformed by Simian Virus 40

Abstract. *Cells from a human parathyroid adenoma were infected with simian virus 40 and maintained through 13 subcultures in monolayer tissue culture. For more than 9 months, these "transformed" cells continued to produce parathyroid hormone which was identified by radioimmunoassay and density-gradient ultracentrifugation.*

The production of parathyroid hormone (PTH) by parathyroid glands maintained as organ cultures has been described (*1*), but production of hormone by parathyroid cells grown as monolayer tissue cultures has not been reported. Certain oncogenic viruses, such as simian virus 40 (SV40), can alter the growth characteristics of certain mammalian cells in vitro, and these "transformed" cells retain some of the biochemical functions of the original cells (*2*). Production of a melatonin-forming enzyme by pineal tissue infected with SV40 (*2*) and synthesis of pituitary hormones in monolayer culture (*3*) have been reported. Our report describes the transformation (by simian virus 40) of tissue from a human parathyroid adenoma into a monolayer cell culture that produced parathyroid hormone; this hormone was identified by radioimmunoassay and density-gradient ultracentrifugation.

Tissue cultures of a human parathyroid adenoma were begun in prescription bottles (60-ml) with surgical specimens (10 mg, wet weight) of the tumor. The methods used have been previously described (*2, 4*). After 2 days, one of the cultures was infected with 2×10^7 $TCID_{50}$ (tissue culture infective dose, 50 percent effective) of SV40. By the 8th day, the infected cells showed the morphological characteristics of human cells transformed by SV40 (*5*). Uninfected cells grew poorly. Cells were separated from the surface of the bottle with trypsin and subcultured when the monolayers became confluent. The transformed cultures (Fig. 1) continued to grow, but the uninfected cells did not survive the first subculture. The SV40 "T" antigen was detected in more than 90 percent of the infected cells by immunofluorescence (*6*). The virus itself could be isolated from the transformed cells by growing them in contact with green-monkey kidney cells (*7*).

Tissue culture medium was assayed directly for PTH. Tissue culture cells were separated from the medium by centrifugation and assayed after extraction with urea. Parathyroid hormone was measured by radioimmunoassay (*8, 9*); charcoal (*10*) was used to separate the free and antibody-bound fractions

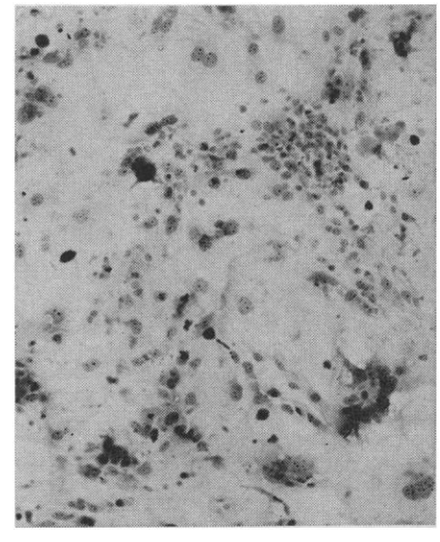

Fig. 1. Cell culture derived from human parathyroid adenoma and transformed in vitro by SV40 (tenth subculture, 8 months after infection). The cells are cuboidal and polygonal and vary considerably in size (fixed in formol-sublimate; stained with hematoxylin and eosin; magnification $\times$ 135).

Table 1. Production of parathyroid hormone by tissue culture cells.

Age (mo)	Hormone concentration in culture medium (pg/ml)	Cumulative production (pg)
	Subculture 4	
4	2400	
6	3000	
	Subculture 8	
7	350	4.2×10^7
	Subculture 12	
9	177	3.8×10^8
	Subculture 13	
9½	182	5.5×10^8

of parathyroid hormone labeled with ^{131}I (^{131}I-PTH). The hormone was readily detected in both the culture medium and cell extracts of each of the subcultures assayed. Control culture medium contained no detectable hormone. The radioimmunoassay is based on the competitive inhibition by unlabeled PTH of the binding of ^{131}I-PTH to specific antibody (*8*). Replicate determinations were made with multiple dilutions of each sample. Increasing amounts of culture medium in incubation mixtures containing labeled hormone and antibody caused a progressive decrease in the ratio of antibody-bound to free

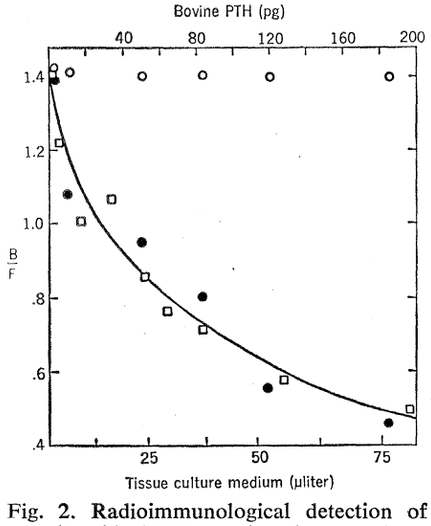

Fig. 2. Radioimmunological detection of parathyroid hormone in tissue culture medium of the fourth subculture of parathyroid cells. Increasing fractions of the medium (closed circles) and increasing amounts of bovine PTH (open squares) caused a similar decrease in the ratio of antibody-bound (B) to free (F) ^{131}I-PTH. The estimated concentration of PTH in the medium was 3000 pg/ml. Line represents standard curve; open circles represent control medium.

^{131}I-PTH, similar to the effect produced by the bovine PTH standard (*9*) (Fig. 2). The concentration of hormone in each sample could then be calculated by comparison with the standard curve derived from the displacement of antibody-bound ^{131}I-PTH by bovine PTH (Fig. 2). Density-gradient ultracentrifugation (*11*) provided additional evidence that the immunologically reactive material in the culture medium represented PTH; the immunological activity sedimented as a discrete substance with a sedimentation constant identical to that of pure bovine PTH.

Assays were begun with the fourth subculture (Table 1) when the tissue culture was well established. The cells of this subculture contained 12 to 13.6 ng/g (dry weight) or a total of 3000 pg of PTH; at least 1.8×10^5 times this amount of hormone was synthesized by the tissue culture thereafter (*12*). A minimum estimate of the cumulative production of PTH was made from the assumption that the media from subcultures 5 to 7 and 9 to 11 contained the same concentration of hormone as those from subcultures 8 and 12, respectively. The total production of PTH by each subculture was calculated by multiplying the concentration of hormone in the culture medium by the total volume of the medium for that generation. To eliminate any contribution from preformed hormone, estimates of cumulative production of PTH were begun with the hormone content of media from subculture 5 after subtracting the total content of hormone in the cells of subculture 4.

A more accurate calculation of the total parathyroid hormone production by the tissue culture should account for the rate of destruction of PTH once released into the medium. To estimate this, we added an excess of bovine PTH to the culture medium of the 13th subculture and measured its rate of disappearance by serial immunoassay; the half-life was 6 hours. This rapid turnover would greatly increase the estimate of total PTH produced by the tissue culture. However, the data in Table 1 do not include any correction for these findings, because the half-life was determined for the 13th subculture only and because it is not known whether the disappearance rate was influenced by binding of the added hormone by tissue culture cells.

Although the cells produced parathyroid hormone for 9½ months, the concentration of hormone decreased in successive subcultures. After 10 months, the cells began to grow poorly and showed degenerative changes similar to those observed in human embryo cells transformed by SV40 and cultured for prolonged periods of time (*13*). Hence, evaluation of such factors as the influence of low calcium media on hormone production were postponed. After 12 months, it was apparent that the culture would not survive, and hence it was terminated. In other studies with transformed human cells (*13*), permanent cell lines free of infectious virus but containing the SV40 "T" antigen were eventually established. A second culture line of parathyroid cells has been established; such a culture line should make possible detailed studies of the factors that influence the synthesis and secretion of parathyroid hormone in normal and neoplastic tissue.

L. J. Deftos, A. S. Rabson
R. M. Buckle*, G. D. Aurbach
J. T. Potts, Jr.
National Institutes of Health, Bethesda, Maryland 20014

References and Notes

1. L. G. Raisz, *Nature* **197**, 115 (1963); L. M. Sherwood, I. Herrman, G. M. Agosto, C. A. Basset, *J. Clin. Invest.* **6**, 1117 (1967).
2. S. A. Wells, Jr., R. J. Wurtman, A. S. Rabson, *Science* **154**, 278 (1966).
3. A. H. Tashjian, Jr., Y. Yasumura, L. Levine, G. Sato, in *Program of the 48th Meeting of the Endocrine Society* (Lippincott, Philadelphia, 1966), p. 27.
4. A. S. Rabson and R. L. Kirschstein, *Proc. Soc. Exp. Biol. Med.* **111**, 323 (1962).
5. M. M. Shein and J. F. Enders, *Proc. Nat. Acad. Sci. U.S.* **48**, 1164 (1962); H. Koprowski, J. Pontén, F. Jensen, R. G. Ravdin, P. Moorehead, E. Saksela, *J. Cell. Comp. Physiol.* **59**, 281 (1962); A. S. Rabson, R. A. Malmgren, G. T. O'Connor, R. L. Kirschstein, *J. Nat. Cancer Inst.* **29**, 1123 (1962).
6. R. A. Malmgren, A. S. Rabson, P. G. Carney, F. J. Paul, *J. Bacteriol.* **91**, 262 (1966).
7. P. Gerber and R. L. Kirschstein, *Virology* **18**, 582 (1962).
8. S. A. Berson, R. S. Yalow, G. D. Aurbach, J. T. Potts, Jr., *Proc. Nat. Acad. Sci. U.S.* **49**, 613 (1963).
9. J. T. Potts, Jr., G. D. Aurbach, L. M. Sherwood, *Recent Progr. Hormone Res.* **22**, 101 (1966).
10. V. Herbert, K. S. Law, C. W. Gottlieb, S. J. Bleicher, *J. Clin. Endocrinol. Metab.* **25**, 1375 (1965).
11. L. M. Sherwood, J. L. H. O'Riordan, G. D. Aurbach, J. T. Potts, Jr., *ibid.* **27**, 140 (1967); J. L. H. O'Riordan, P. G. Condliffe, J. T. Potts, Jr., G. D. Aurbach, *Endocrinology* **3**, 585 (1967).
12. Parathyroid adenomas assayed by radioimmunoassay typically contain 250 to 300 μg/g dry weight (*11*); the initial concentration of hormone in this adenoma was not measured.
13. A. J. Giardi, F. C. Jensen, H. Koprowski, *J. Cell. Comp. Physiol.* **65**, 69 (1965).
14. We thank Dr. R. A. Malmgren and Miss Frances Y. Legallais for assistance. R.M.B. supported by grant-in-aid from Armour Pharmaceutical Company.
* Present address: Southampton General Hospital, Southampton, England.

14 November 1967