does not cause a comparable differentiation of B cells. Purified bovine thymopoietin, which is active in diverse mammalian species, failed to induce differentiation over a concentration range from 0.001 to 5 μg/ml in this avian system. We conclude that we are observing the inductive effects of chicken thymopoietin in the extracts of chicken thymus and that, because of evolutionary divergence between the thymopoietin of birds and mammals, bovine thymopoietin is no longer effective in inducing T cell differentiation in birds.

Bursal extracts were also active in inducing differentiation in vitro (Table 2). This inductive activity was also demonstrable in extracts of bursa from newly hatched birds; these extracts were free of microorganisms and the inductive activity found could not be ascribed to contamination with bacterial endotoxin (*10*). Bursal extracts induced both Bu-1$^+$ and Th-1$^+$ cells but at lower concentrations induction of Bu-1$^+$ cells was always greater than that of Th-1$^+$ cells (Table 2). We suggest the name bursopoietin for the bursal substance inducing B cell differentiation. There are two possible explanations for the finding that bursal extracts can also induce Th-1$^+$ cells: (i) a single bursa-specific substance exists which is selective for B cell differentiation at lower (physiological) concentrations but at higher concentrations cross reacts with receptors on prothymocytes to induce T cell differentiation, or (ii) bursal extracts contain a substance that is selective for B cell induction plus an additional nonspecific inducing agent that is only detected at higher concentrations. These possibilities must now be resolved by isolation of bursopoietin and determination of its specificity in the dual induction assay.

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15. Cyclic AMP itself was used as the inducing agent because in the mouse it is as effective as dibutyryl cyclic AMP in inducing lymphocyte differentiation in vitro (M. P. Scheid, G. Goldstein, E. A. Boyse, unpublished results).
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Clonal Origin of Inherited Medullary Thyroid Carcinoma and Pheochromocytoma

Abstract. *A black female with inherited medullary thyroid carcinoma and pheochromocytoma was a mosaic for glucose-6-phosphate dehydrogenase types A and B in normal tissues (blood, thyroid, and adrenal gland); both the medullary carcinoma and pheochromocytoma tissue showed a B pattern only. This finding suggests a single clone origin for each of the tumors. Other inherited tumors similarly studied in man have appeared to be multiclonal in origin.*

One important approach to studying the pathogenesis of a given tumor is to establish whether it has a single or multiclonal cell origin. In general, it is postulated that tumors of monoclonal origin arise as a consequence of rare somatic mutations in a single or very small number of cells in the tissue of origin (*1, 2*). These mutations might arise as the result of spontaneous changes, viral transformations, or the effects of a carcinogen. Tumors with multiclonal origin may arise through processes that affect multiple cells in the target tissue; these might include the effects of certain carcinogens, a generalized susceptibility of a tissue to malignant change, or an abnormal response to hormonal stimulation or excessive hormonal stimulation of the target tissue cells (*1, 2*).

In general, most spontaneously arising tumors have been found to have a "clonal" or single cell origin. Examples include chronic myelocytic leukemia (*3*), leiomyomas (*4*), and lymphomas (*5*). Other tumors however, like carcinoma of the colon, appear to have a multiclonal origin (*5*). Inherited or genetically transmitted tumors are especially important as the focus for study of tumor pathogenesis; genetic tumors studied in man appear to be multiclonal in origin, possibly reflecting the inherited susceptibility of the target tissue cells to neoplastic transformation (*2, 6*). This evidence, however, is based on studies of only two inherited neoplasms, trichoepitheliomas (*7*) and inherited neurofibromas (*1*). With respect to these findings, Knudson has proposed, from retrospective statistical analysis, that inherited retinoblastomas (*8*) and inherited neuroblastomas and pheochromocytomas (*9*) arise from two mutational events. The first is an inherited mutation rendering the target cells susceptible to tumor formation. The second is a mutational event superimposed on the first and results in tumor formation. By these criteria, the final mutational event, if superimposed on a large population of susceptible cells, could result in inherited neoplasms of multiclonal origin such as found for trichoepitheliomas (*7*) and neurofibromas (*1*). In contrast, if the population of genetically susceptible cells arose from a single mutated cell (first mutational event) or if the second mutational event occurred only in a single susceptible cell, the resulting genetic neoplasm would be of monoclonal origin; biochemical data for a monoclonal hereditary tumor in man have not yet been reported.

We report on our study of the cell origin of medullary thyroid carcinoma and pheochromocytoma, two important tumors that can be inherited simultaneously in the same individual. This complex of inherited tumors, known as Sipple's syndrome (*10*), was diagnosed in a black family, and one female member underwent removal of both lesions. The fact that this patient was a mosaic for the two forms of the X-linked enzyme glucose-6-phosphate dehydrogenase (G6PD) allowed us to trace the clonal ori-

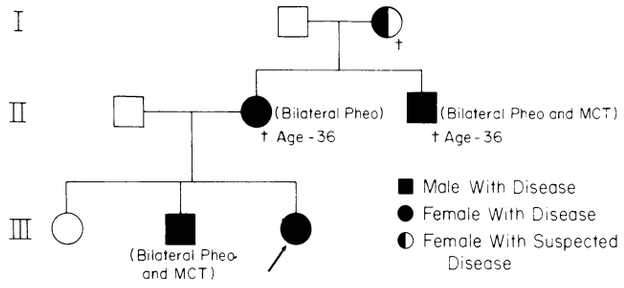

Fig. 1. Abbreviated pedigree of the subject (arrow) of this report, showing the family history of Sipple's syndrome; *Pheo*, pheochromocytoma; *MCT*, medullary carcinoma of the thyroid; †, deceased.

gin of her tumors. The findings suggest that multiclonal cell origin may not be the case for all inherited neoplasms in man.

Electrophoretic studies of the A and B forms of G6PD offer, at present, the only method for tracing the clonal origin of tumors (*1–7*). Such studies are limited to black individuals since polymorphism for G6PD types occurs almost exclusively in the black population, except for its occurrences in the Mediterranean area. The demonstration of tumor cell origin depends on the Lyon hypothesis (*11*). Inactivation of one X chromosome randomly occurs early in the embryonic development of all females and the inactivation is irreversible (*12*); thus, female individuals who are heterozygotes for an X-linked trait, such as the isoenzyme forms A and B of G6PD, will have approximately an equal number of cells containing one or the other isoenzyme in every normal tissue. Tumors that arise from a single clone of cells should contain only one enzyme form while those of multiclonal cell origin should resemble normal tissue and have both forms.

We used the method of Ellis and Alperin (*13*) to study G6PD forms in our patient. Tissues were prepared by homogenizing 4 to 5 mg in 0.2 ml of cold normal saline and then sonicating the mixture for 20 to 25 seconds. The clear supernatant was then applied to cellulose acetate strips for standard electrophoresis of G6PD. For each run, A and B standards were used as well as normal tissue and tumor tissue from the patient.

The patient for study was a 30-year-old black female from a family with an established pattern of Sipple's syndrome (Fig. 1). Preoperatively, she met diagnostic criteria for medullary thyroid carcinoma including abnormal plasma levels of calcitonin (the base value was 2.4 ng/ml, and after calcium infusion the calcitonin was 4.4 ng/ml as compared to less than 0.3 ng/ml for normal individuals) (*14*) and histaminase (11 unit/ml compared to less than 4.5 unit/ml for normals) (*15*). The patient also had abnormal catecholamine metabolites in the urine, diagnostic of pheochromocytoma. In separate surgical procedures a large left adrenal pheochromocytoma (9 by 10 cm) and bilateral medullary thyroid carcinomas with lymph node metastases were removed. The right adrenal appeared normal.

The G6PD phenotypes of the patient's tissues are shown in Fig. 2. Her red blood cells contained both the A and B forms of G6PD as did normal adrenal tissue removed with her pheochromocytoma and normal thyroid tissue. However, multiple sections of the large pheochromocytoma, taken at sites widely distant to one another, all contained only the B form of G6PD. The widely distant sites investigated in this lesion made it extremely unlikely that these results may be attributed to sampling of too small a patch size. The medullary thyroid carcinoma tissue proved more difficult to examine because of extensive calcification and heavy deposits of acellular stromal material. However, suitable tumor tissue obtained from the left thyroid, like the pheochromocytoma, contained only B enzyme. Multiple attempts to identify G6PD in other deposits of medullary carcinoma revealed no bands for either form, and again in one case a single faint band of G6PD could be seen; the form of G6PD could not be determined for this band.

The above results suggest a single clone cell origin for the particular tumors under study and may have important ramifications for study of Sipple's syndrome or type II multiple endocrine neoplasia. This syndrome has been postulated to result from a single defect in neural crest tissue (*16*); neural crest is thought to be the embryonic origin for both the parafollicular cells which give rise to medullary thyroid carcinoma, and the adrenal medullary cells which yield the pheochromocytoma (*17*). Such an inherited defect might be thought to result in a generalized susceptibility to tumor formation in certain regions of neural crest and to produce a multicellular origin for the resulting neoplasms. However, if in the two-mutational theory of Knudson (*8*) tumor formation were the result of a second mutational event superimposed on a single neural crest cell from a population of cells genetically at risk for tumor formation, or if the first mutational event produced a susceptible cell population of monoclonal origin, a tumor of single clone origin might be expected. Our current data now suggest that one of these combinations of events may occur in Sipple's syndrome, and would constitute the first such direct evidence for a genetic tumor in man. For other genetic tumors studied, neurofibromas and trichoepitheliomas, both A and B forms of G6PD have been found in tumor tissue from heterozygotes; these data suggest that the genetic defect results directly in a more generalized tendency for the target cells to become neoplastic (*1*, *7*).

Further studies of G6PD in black fe-

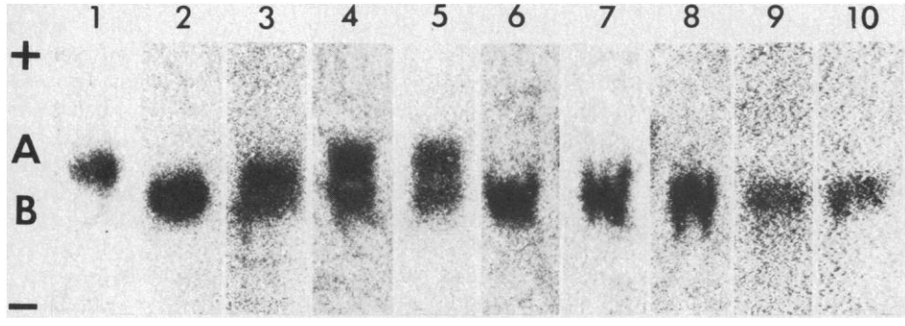

Fig. 2. The electrophoretic patterns of G6PD in normal and tumor tissues of the patient. 1, A control; 2, B control; 3, patient's red blood cells; 4, adrenal tissue; 5, thyroid tissue; 6 to 8, separate cuts of pheochromocytoma; 9 and 10, duplicate runs of left thyroid medullary carcinoma.

male patients with Sipple's syndrome could prove a potent tool for understanding several aspects of the pathogenesis of this disease. First, the time point for development of the defect in neural crest tissue might be further elucidated. There is evidence that both the thyroid and adrenal tumors are preceded by a phase of hyperplasia bilaterally in the thyroid (*18*) and the adrenal medulla (*19*); this hyperplasia may appear or persist quite late into development since C cell hyperplasia in the thyroid has been recognized in patients up to 23 years of age (*18*) and bilateral adrenal medullary hyperplasia has been found in a 12-year-old patient (*19*). It seems unlikely that the somatic mutations suggested from our data would have occurred simultaneously at each of the separate sites of hyperplasia, but rather that the susceptible cells in these regions may have derived from stem cells that were already defective. If a population of black heterozygote females with Sipple's syndrome could be examined, and the thyroid and adrenal tumors proved to be not only monoclonal but also to contain the same G6PD isoenzyme in each tumor from the same patient, the evidence that the same mutated parent cells contribute to both lesions would be strong. Thus the defect could be pinpointed to a time prior to migration of neural crest elements to the thyroid and adrenal medulla. Obviously, since chance alone could account for the same G6PD form in the thyroid and adrenal tumors 50 percent of the time, the population of patients examined would have to be quite large.

It is intriguing that in our patient both the medullary carcinoma, a malignant lesion, and the pheochromocytoma, a benign lesion in Sipple's syndrome (*16, 20*), appear to be of monoclonal derivation. This finding indicates that the factors controlling malignancy and benignity for the tumors in Sipple's syndrome may be separate from the basic inherited defect; possibly factors in the thyroid and adrenal gland influence the behavior of the neoplastic cells, or differences evolve as the stem cells giving rise to the lesions mature and differentiate.

Finally, the mechanism for the third component of the syndrome, parathyroid hyperplasia or adenoma formation (*20*), might be clarified by performing studies such as those undertaken in our patient. The parathyroid lesions have been postulated by some to be part of the primary defect in this disease and by others to arise as a compensatory response to calcitonin excess (*21*). The former situation might be expected to show monoclonal origin in view of our findings, and the latter might show a multiclonal pattern. Unfortunately, although two hyperplastic parathyroid glands were seen in pathologic sections from our patient, fresh tissue was unavailable for study.

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Morphine Analgesic Tolerance: Its Situation Specificity Supports a Pavlovian Conditioning Model

Abstract. *Rats were made tolerant to morphine in either of two environments and then assessed for morphine-induced alteration of pain sensitivity in both environments. Analgesic tolerance was displayed when rats were tested in that environment in which they previously received morphine, but not in the alternative environment. The results indicate than an association between environmental cues and the systemic effects of morphine is crucial to tolerance development.*

Many interpretations of opiate analgesic tolerance have been proposed, most postulating some systemic change which either decreases the population or sensitivity of effective opiate receptors within the organism as a result of the initial drug administrations (*1*) or prevents the drug from gaining access to these central receptors (*2*). In marked contrast with such theories is an interpretation of tolerance which emphasizes the principles of Pavlovian conditioning (*3*). As suggested by Pavlov (*4*), the administration of a drug can be viewed as a conditioning trial, with environmental cues uniquely present at the time of drug administration constituting the conditional stimulus, and the actual pharmacological stimulation constituting the unconditional stimulus. According to the conditioning interpretation of tolerance, tolerance is a manifestation of the acquisition of an association between the systemic effects of the drug and those environmental cues which reliably precede these systemic effects. Such an association may be revealed if the subject, after a history of administration of the drug, is presented with the drug administration procedure not followed by the systemic effects of the drug—that is, if a placebo is administered.

It has frequently been reported that conditional drug responses are opposite in direction to the unconditional effects of the drug (*3, 5*). Thus, in the case of a subject with a history of drug administration, the administration ritual may elicit responses antagonistic to those elicited by the drug, and these anticipatory drug responses should serve to attenuate the effects of the drug. As is generally the case with conditional responses, the compensatory conditional drug responses are expected to become more pronounced as conditional and unconditional stimuli are paired more and more often (that is, the drug administration