The values are consistent with the opinion that the syndrome is inherited as an autosomal recessive mutant.

Whereas it has previously been assumed that Kartagener's syndrome is due to some gene with an incomplete penetrance (*26*), I suggest that the gene may be the one responsible for synthesis of the dynein protein or of a protein which binds dynein to the microtubules, and that the gene has an all-or-none action with respect to manufacture or attachment of normal dynein arms. It is further assumed that chance alone will determine whether the viscera will take up the normal or the reversed position during embryogenesis, when normal dynein arms are missing.

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Lymphocyte-Differentiating Hormone of Bursa of Fabricius

Abstract. *Induction of early lymphocyte differentiation was studied in vitro in fractionated bone marrow cells of newly hatched chickens, with alloantiserums to identify newly differentiated B cells (Bu-1$^+$) and T cells (Th-1$^+$). Thymus extract induced selective T cell differentiation; the activity of the extract corresponds to that of thymopoietin. Bursal extract induced both B cell and T cell differentiation, but at lower concentrations B cell differentiation was always greater. This activity is ascribed to a lymphocyte-differentiating hormone of the bursa of Fabricius, for which the name bursopoietin is suggested.*

Two main classes of lymphocytes occur in the immune systems of vertebrates: (i) T cells, which differentiate in the thymus, and (ii) B cells, which differentiate in the bursa of Fabricius of birds and presumably in some homologous organ in vertebrates that have no bursa. The immediate precursors of T cells and B cells are found in the bone marrow. The T cell precursor (prothymocyte) is induced by the polypeptide hormone or "inducer" thymopoietin to differentiate into a thymic lymphocyte (thymocyte) which is identified serologically in the mouse by expression of distinctive surface components TL, Thy-1, Ly-1, Ly-2/3, and Ly-5 (*1*). This prothymocyte-to-thymocyte transition occurs without cell division (*2*), and it can be assayed in vitro. For this purpose a subpopulation rich in prothymocytes is prepared from bone marrow (or spleen in mice) and is incubated for 2 hours with thymopoietin. Complement-dependent cytotoxicity assay then reveals that 20 to 30 percent of the cells have acquired TL, Thy-1, and Ly antigens.

Evidently thymopoietin induces thymocyte differentiation via an adenosine 3′,5′-monophosphate (cyclic AMP) "second message" (*3*), which would account for induction by various agents that have no special relevance to the thymus (*4*) but elevate intracellular cyclic AMP. In this category of nonspecific inducers ubiquitin is of particular interest because this polypeptide is highly conserved in evolution and has been found in all tissues and all species tested (*5, 6*). In the in vitro assay ubiquitin is probably responsible for thymocyte induction by crude extracts of tissues other than thymus (*4*).

Steps in B cell differentiation also appear to be mediated by cyclic AMP (*3, 5, 7*), and this permits distinction between specific and nonspecific induction in a dual assay. Thus thymopoietin induces only T cell differentiation while all other inducing agents so far tested trigger both T cell and B cell differentiation (*3, 5*).

Both birds and mammals have a thymus but birds also have a discrete organ, the bursa of Fabricius, in which B cells differentiate. Early removal of the bursa, which is a dorsal diverticulum of the cloaca, results in failure of B cell development and agammaglobulinemia (*8*). Thus birds are especially suitable for studies of a possible B cell inducer (*9, 10*) that would correspond to the T cell inducer thymopoietin and would be ex-

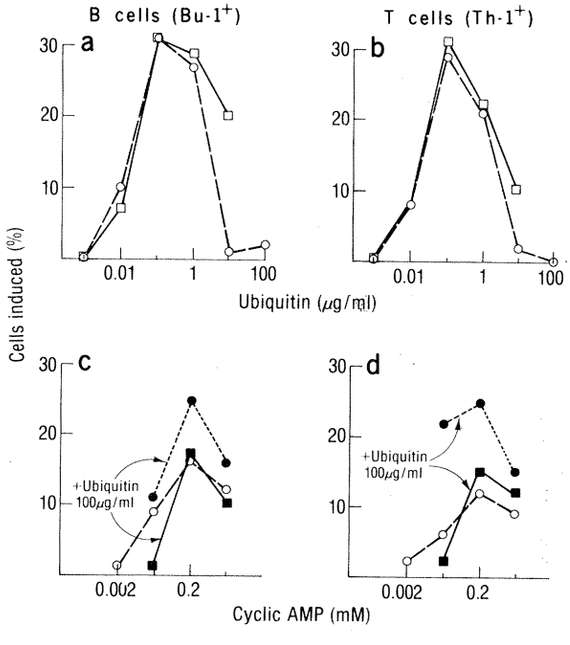

Fig. 1. The relation between concentration of ubiquitin or cyclic AMP (*15*) and induction of Bu-1$^+$ or Th-1$^+$ cells in fractionated bone marrow from newborn chickens. Ubiquitin produced maximal induction at a concentration of 0.1 μg/ml with complete inhibition at 100 μg/ml (a and b). Cyclic AMP produced maximal induction at 0.2 m*M*, and this was not affected by the presence of ubiquitin (100 μg/ml) (c and d). Each symbol represents a separate experiment; each point is the mean of duplicate or triplicate tubes.

Table 1. Distribution of neonatal chicken bone marrow cells in different layers after discontinuous density gradient centrifugation. Cells inducible by ubiquitin to express Bu-1 or Th-1 antigens were present in the lighter layers. The calculation of cells induced is given in the text. Each figure represents the mean of duplicate or triplicate tubes in a single experiment (Exp.).

Layer	Total cells in each layer (%)		Cells induced (%)			
			B cells (Bu-1$^+$)		T cells (Th-1$^+$)	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
A	16	25	32	21	31	21
B	22	42	0	26	14	32
C	33	16	0	0	1	0
D	29	16	0	0	0	0

pected to be secreted by stromal cells of the bursa.

Our studies were based upon the in vitro induction assays developed by Boyse and colleagues (*1–5*). An invaluable prelude to these avian studies was the production of chicken alloantiserums with which distinctive lymphocyte surface antigens of the chicken could be identified. These antiserums were developed by reciprocal immunizations with bursa or thymus cell suspensions between two inbred lines of chickens identical at the *B* major histocompatibility locus (*11*). Each serum was specific for donor cell type and line without absorption. Genetic studies with these antiserums established two independent autosomal loci, each with two alleles. The *Bu-1* locus determines antigens found on bursocytes and peripheral B cells, while similarly the *Th-1* locus determines antigens found on thymocytes and peripheral T cells (*11*). Using Bu-1 and Th-1 as markers we developed a dual induction assay for B cells and T cells in the chicken and used this to detect inductive activity in extracts of chicken thymus and chicken bursa.

Bone marrow from newly hatched chickens was selected as a source of inducible cells because it lacks an appreciable number of Bu-1$^+$ or Th-1$^+$ cells. We chose ubiquitin as one inducing agent because the high degree of evolutionary conservation of this polypeptide made it likely that it would be an effective nonspecific inducer of avian cells as it is of mouse cells. Pooled cells from femur and tibiotarsus of five newly hatched chicks of strain SC (Hy-Line) were fractionated by ultracentrifugation on a five-layer discontinuous bovine serum albumin (BSA) gradient (*11*). Cells from each interface were washed and suspended for incubation at a concentration of 5×10^6 cells per milliliter with ubiquitin (0.1 μg/ml) in RPMI 1630 medium supplemented with 15 m*M* Hepes, 5 percent γ-globulin-free fetal calf serum, deoxyribonuclease (14 to 18 unit/ml), heparin (5 unit/ml), penicillin (100 unit/ml), and streptomycin (100 μg/ml). Controls were incubated with BSA (1 μg/ml) or medium alone. After incubation, the cells were tested in the cytotoxicity assay using chicken C1 and guinea pig C2 to C9 complement fractions as described (*11, 12*). The proportion of Bu-1$^+$ or Th-1$^+$ cells in each layer was calculated as a cytotoxicity index, $100\ (a - b)/a$, where a and b are the percentages of viable cells in the complement control and test preparation, respectively. The percentage of cells induced was obtained by subtracting the mean values in the control incubations without inducing agents (usually 1 to 3 percent) from those of the test inductions. Cells inducible for the Bu-1 and Th-1 antigens were present in the lighter layers of the gradient (Table 1), as is the case with prothymocytes and pro-B cells in the mouse. For subsequent experiments cells of the A and B layers were pooled as the source of inducible cells.

Table 2. Induction of Bu-1 and Th-1 antigens on neonatal chicken bone marrow cells by chicken tissue extracts in the presence of a concentration of ubiquitin (100 μg/ml) that would selectively inhibit possible ubiquitin activity in extracts. Bursal extracts induced both Bu-1$^+$ and Th-1$^+$ cells but showed specificity for induction of Bu-1$^+$ cells on dilution. Thymus extracts induced Th-1$^+$ cells only. Other control tissue extracts did not induce Bu-1$^+$ or Th-1$^+$ cells. Each figure represents the mean of duplicate or triplicate tubes in a single experiment. For each extract the corresponding columns for Bu-1 and Th-1 induction represent the same experiment.

Dilution	Cells induced (%)	
	B cells (Bu-1$^+$)	T cells (Th-1$^+$)
	Bursa	
10^{-1}	27, 23, 12, 20	13, 29, 17, 23
10^{-2}	6, 19, 23	3, 10, 2
10^{-3}	0, 0, 17	0, 1, 0
	Neonatal bursa	
10^{-1}	16, 21, 17	8, 21, 16
10^{-2}	4, 16	4, 1
10^{-3}	2	0
	Thymus	
10^{-1}	2, 0, 0, 0	18, 8, 8, 0
10^{-2}	1, 0, 0, 0	13, 10, 0, 12
10^{-3}	0, 0, 0, 0	1, 0, 0, 0
	Spleen	
10^{-1}	0, 1	0, 0
10^{-2}	0, 0	0, 0
	Muscle	
10^{-1}	0, 0	0, 0
10^{-2}	0, 0	0, 0
	Liver	
10^{-1}	0, 0	0, 0
10^{-2}	0, 0	0, 0
	Kidney	
10^{-1}	0, 0	3, 0
10^{-2}	0, 0	0, 0

Induction with ubiquitin was limited to a relatively narrow range of concentrations, 0.01 to 10.0 μg/ml, and was maximal at 0.1 μg/ml. This parallels data for the mouse, in which induction by ubiquitin is inhibited by high concentrations of this inducer (*13, 14*). Since ubiquitin is present in extracts of all tissues, and could induce nonspecifically and thus mask the presence of a tissue-specific inducing agent, we used this dose-response relationship of ubiquitin to devise a method for selectively blocking its action in tissue extracts. In mammalian systems the failure of high doses of ubiquitin to cause induction appears to be related to inactivation of ubiquitin receptors; the cells can still be induced by other agents that do not act through the ubiquitin receptors (*14*). We determined that ubiquitin at 100 μg/ml did not inhibit cyclic AMP–induced differentiation of T cells or B cells in the chicken (*15*) (Fig. 1) and, therefore, added ubiquitin at this concentration to each tissue extract being tested to eliminate the possibility of nonspecific induction by this polypeptide.

Bursa and thymus from chickens aged 8 to 10 weeks were obtained frozen (Pel-Freez Biologicals, Inc.). Other organs were dissected from adult or newborn birds and frozen for storage. Thawed tissues were homogenized (10 g per 100 ml of RPMI 1630 medium supplemented as above) in a Waring blender and centrifuged. Supernatants were stored at −70°C.

The results of our experiments with extracts of chicken tissues are summarized in Table 2. Only extracts of bursa or thymus produced significant induction of Bu-1 or Th-1 antigens (or both) on fractionated bone marrow cells from newly hatched chickens. Control extracts prepared from spleen, muscle, liver, and kidney did not induce differentiation. Thymus extracts selectively induced Th-1$^+$ cells and not Bu-1$^+$ cells. This is in keeping with the findings in mammals wherein thymopoietin selectively induces T cell differentiation but

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-