flight data (Table 1) are undersampled and not fully supported by pre- and postflight ground testing observations of adequate precision.

Two principal impediments occur when characterizing trends. First, there is a spectrum of variations on shorter time scales whose root-mean-square amplitudes are comparable to the magnitude of the 5-year trend. The variance of the data is not Gaussian and therefore normal statistical inferences cannot be made. Second, the pointed-mode and spin-mode data have different systematic and random uncertainties, so a common, in-depth statistical analysis for the combined set is of questionable value. The most straightforward approach to abstracting the trend is to find the best straight-line fit to the data. We have done this for the ACRIM I experiment, balloon and sounding rocket flights, and Nimbus-7/ERB results, as summarized in Table 2.

The general agreement among the slopes of the three data sets supports a solar rather than instrumental explanation for the trend. Although failure of the ACRIM I and Nimbus-7/ERB slopes to agree within the bounds of their mutual standard errors, and the lower slope value of the rocket and balloon results, do not add credibility to the actual value of the slope derived from the ACRIM I experiment, the statistical uncertainties in Table 2 do not precisely describe the quality of agreement. The six rocket and balloon results are insufficient to support a meaningful statistical argument. Although the statistics for the ACRIM I and ERB results are based on sufficient samples, the simple statistics do not address the more fundamental issue of long-term accuracy of these databases. The ACRIM I observations of 1980 and 1984 onward can be related with a precision equal to the results of intercomparisons of ACRIM I sensors (10 ppm, root-mean-square). No comparable data are available for the ERB database during this period since no internal comparisons are possible with its single sensor.

The ACRIM I data now extend over nearly one-half of the 11-year solar cycle. These data give an accurate and precise view of the variations of total solar irradiance. Over the 5 years of daily mean values analyzed, the total irradiance systematically decreased, and its average value was reduced by about 0.1%.

The trend may be a luminosity dependence on the solar magnetic cycle of activity: either the 11-year sunspot cycle, the 22-year full magnetic cycle (*17*), or longer variations related to solar magnetic effects. The rate of variation observed thus far is not inconsistent with the currently accepted theory of solar interior structure.

Past climate variability would seem to preclude a lengthy continuation of the present downward trend of solar luminosity. Even for solar cycle time scales, however, the present database is so short that one can only speculate on the nature of the variation. But if the trend is related to the solar cycle, the positive relation between total flux and sunspot activity appears to agree with that inferred from the maunder minimum (*1*). Sunspots also exhibit a strong inverse correlation with the total irradiance on short time scales (*2, 3*), and if this effect were incorporated into our analysis, it would increase the magnitude of the observed trend.

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Pertussis Toxin–Sensitive Pathway in the Stimulation of c-*myc* Expression and DNA Synthesis by Bombesin

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The bombesin-like peptides are potent mitogens for Swiss 3T3 fibroblasts, human bronchial epithelial cells, and cells isolated from small cell carcinoma of the lung. The mechanism of signal transduction in the proliferative response to bombesin was investigated by studying the effect of *Bordetella pertussis* toxin on bombesin-stimulated mitogenesis. At nanomolar concentrations, bombesin increased levels of c-*myc* messenger RNA and stimulated DNA synthesis in Swiss 3T3 cells. Treatment of the cells with pertussis toxin (5 nanograms per milliliter) completely blocked bombesin-enhanced c-*myc* expression and eliminated bombesin-stimulated DNA synthesis. This treatment had essentially no effect on the mitogenic responses to either platelet-derived growth factor or phorbol 12,13-dibutyrate. These results suggest that the mitogenic actions of bombesin-like growth factors are mediated through a pertussis toxin–sensitive guanine nucleotide–binding protein. Furthermore they indicate that bombesin-like growth factors act through pathways that are different from those activated by platelet-derived growth factor.

IN RECENT YEARS, SEVERAL GROWTH factors for mesenchymal and epithelial cells have been identified. Included among these factors are bombesin and related peptides that are potent mitogens for cultured Swiss 3T3 cells (*1*), human bronchial epithelial cells (*2*), and several cell lines derived from human small cell lung carcinomas (SCLC) (*3–5*). These peptides, which are normally found only in neuroendocrine cells of the central nervous system and the gastrointestinal tract (*6–10*) and in the mucosal endocrine cells of fetal and neonatal lung (*11*), have been found in a number of human tumors including SCLC (*12–15*). Antibodies to bombesin inhibit the growth of these transformed cells in cell culture and block tumor progression in whole animals (*5*). Taken together, these data suggest that bombesin-like peptides participate in the regulation of cell proliferation and act as autocrine growth factors for human SCLC.

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The mechanisms by which bombesin and other growth factors stimulate DNA synthesis are not known. One possible mechanism of signal transduction that has not been previously associated with the mitogenic action of growth factors involves the guanine nucleotide–binding proteins (G proteins). These proteins are important components in the response to many neurotransmitters and peptide hormones (*16*). Their action has been well characterized in the adenylate cyclase system, where they mediate the effects of both stimulatory and inhibitory receptors on the catalytic subunit of the cyclase enzyme (*17*). Recently G proteins have also been implicated in the stimulation of neutrophil chemotaxis by *N*-formylated peptides (*18–23*). An important feature of a subgroup of G proteins is their sensitivity to the inhibitory effects of *Bordetella pertussis* toxin, which inhibits activity by stimulating the covalent binding of adenosine diphosphate (ADP)–ribose to defined sites on these proteins. Thus in a number of hormone-stimulated responses, the sensitivity to pertussis toxin has been used to indicate whether the response involves the action of a G protein. We have now examined the ability of pertussis toxin to inhibit the mitogenic effects of bombesin in Swiss 3T3 cells. These cells are known to have an adenylate cyclase–coupled G protein that is ADP-ribosylated following exposure to pertussis toxin (*24*). Our results suggest that the binding of bombesin to its receptor results in the activation of a pertussis toxin–sensitive G protein and that this interaction is an important step in the production of the proliferative response to this growth factor.

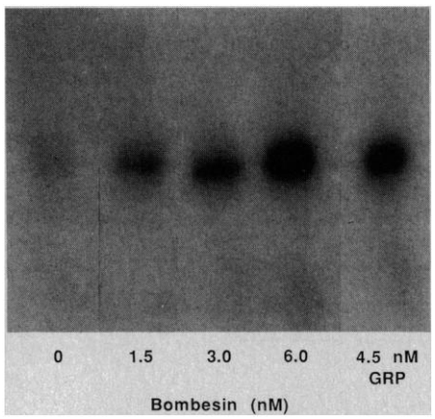

Fig. 1. Induction of c-*myc* expression by bombesin. Swiss 3T3 mouse fibroblasts were grown to confluence in 150-cm^2 flasks in medium supplemented with 10% fetal bovine serum. Six days after the last change of medium, the quiescent cultures were incubated with either vehicle (medium with 20 m*M* Hepes and bovine serum albumin at 0.5 mg/ml) or various concentrations of bombesin or gastrin-releasing peptide (GRP), for 2 hours at 37°C. Cells were then lysed in situ, and total cellular RNA was prepared by standard centrifugation techniques (*31*). To measure c-*myc* mRNA levels, we hybridized 15 μg of total cellular RNA to an excess (5×10^5 cpm) of ^{32}P-labeled RNA probe complementary to mouse c-*myc* exon 2 (a 420-base fragment). The RNA probe was prepared with an SP64 plasmid and SP6 polymerase (New England Nuclear) in the presence of [α-^{32}P]UTP (Amersham) as described earlier (*32*). Hybridization took place in 10 μl of 75% formamide, 0.1% SDS, 20 m*M* tris (*p*H 7.0), and 1 m*M* EDTA at 55°C for 18 hours. Ribonuclease T_1 (200 U) and ribonuclease A (1.5 μg) in 150 μl of 300 m*M* NaCl, 10 m*M* tris (*p*H 7.5), and 5 m*M* EDTA were then added for 1 hour at room temperature, and then 10 μl of 10% SDS and 5 μl of proteinase K (100 mg/ml) were added for 20 minutes at 37°C. The nucleic acids were then precipitated by the addition of ethanol (final concentration, 70%), redissolved in formamide loading buffer (95% formamide, 10 m*M* tris, and 1 m*M* EDTA, with bromphenol and xylene cyanol). Samples were then analyzed by electrophoresis on 4% polyacrylamide–8*M* urea gels and visualized by autoradiography. The protected fragments migrated at the predicted size of 420 bases. Gel areas corresponding to autoradiographic bands were excised, and ^{32}P content was determined by scintillation counting. The bombesin-stimulated level of c-*myc* mRNA was two (1.5 n*M* bombesin) to six times (6 n*M* bombesin) the basal level (vehicle.)

For this study we measured DNA synthesis and the expression of the c-*myc* gene in response to bombesin. This proto-oncogene is of particular interest since its expression has been correlated with the mitogenic activity of a number of other growth factors and with cell transformation. More specifically, enhanced expression of the c-*myc* gene has been reported in human SCLC cell lines, including several that are known to produce bombesin-like peptides (*25, 26*). Thus we postulated that c-*myc* expression is associated with the mitogenic activity of bombesin.

As shown in Fig. 1, when Swiss 3T3 cells were incubated with bombesin there was a concentration-dependent increase in c-*myc* messenger (mRNA). The c-*myc* mRNA response was half-maximal at a bombesin concentration of 1 n*M*. Maximal expression (six times the basal level) was stimulated by 6 n*M* bombesin and occurred approximately 2 hours after the addition of bombesin.

To investigate the possibility that G proteins are involved in the proliferative response to bombesin, we incubated Swiss 3T3 cells with pertussis toxin (5 ng/ml) for 2 hours. After incubation with the toxin, the stimulatory effect of bombesin on c-*myc* expression was totally abolished (Fig. 2). Inhibition of bombesin-stimulated c-*myc* expression depended on both the concentration of pertussis toxin and the length of toxin exposure. A partial effect was observed when cells were exposed to toxin at 0.5 ng/ml for 2 to 3 hours, and a maximal effect was observed at approximately 5 ng/ml. By contrast the enhancement of c-*myc* expression by platelet-derived growth factor (PDGF) and the tumor-promoting phorbol ester, phorbol 12,13-dibutyrate (PDB), were not affected by pertussis toxin. In parallel experiments, pertussis toxin blocked bombesin-stimulated DNA synthesis, measured by incorporation of [^{3}H]thymidine (Fig. 3A), but did not affect DNA synthesis stimulated by PDGF or PDB (Fig. 3B). The selective effect of pertussis toxin on bombesin-stimulated cell proliferation was also observed when cell counts were used as a measure of mitogenesis (Table 1). The pertussis effect was essentially irreversible and could not be removed by washing the cells for 48 hours in toxin-free medium.

We further investigated the effects of pertussis toxin treatment by measuring the binding of ^{125}I-labeled bombesin to its receptors on Swiss 3T3 cells. Exposure to pertussis toxin had no effect on the number or affinity of ^{125}I-labeled bombesin binding sites measured by previously described methods (*27*). Thus the effects of toxin pretreatment on the bombesin mitogenic response are not secondary to changes in the ability of bombesin receptors to bind bombesin.

These data show that a pertussis toxin–sensitive step mediates bombesin-stimulated mitogenesis. By inference this mitogenic pathway probably involves a G protein. In addition, these experiments provide direct evidence that different pathways mediate

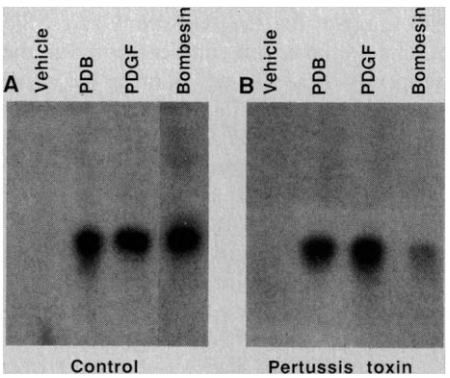

Fig. 2. Effect of pertussis toxin on bombesin-induced c-*myc* expression. Confluent, quiescent cultures of Swiss 3T3 mouse fibroblasts were incubated for 2 hours, at 37°C, in the absence (A) or in the presence (B) of pertussis toxin, 5 ng/ml, and then for 2 hours with vehicle [Dulbecco's modified Eagle's medium (DMEM) with 20 m*M* Hepes and bovine serum albumin, 0.5 mg/ml], PDB (100 ng/ml), partially purified PDGF (100 ng/ml), or 6 n*M* bombesin as indicated. Total cellular RNA (15 μg) from each group was then assayed for c-*myc* mRNA levels with the mouse c-*myc* exon 2 probe, as described in the legend to Fig. 1. In three separate experiments, pertussis toxin treatment diminished bombesin-induced c-*myc* expression by 80% to 90%, but had no effect on PDB or PDGF stimulation of c-*myc* expression.

Table 1. Effect of pertussis toxin on bombesin-stimulated Swiss 3T3 cell proliferation. The cells were seeded at a density of 2×10^5 per well in six-well plates containing 2 ml of DMEM with 3.5% fetal bovine serum and insulin at 1 μg/ml. After 24 hours, the medium was replaced by fresh DMEM with 3.5% fetal bovine serum and insulin at 1 μg/ml. Some cultures received pertussis toxin (10 ng/ml) for a period of 3 hours, followed by the addition of either vehicle [DMEM containing 20 m*M* Hepes (*p*H 7.4), insulin (1 μg/ml), transferrin (5 μg/ml), and bovine serum albumin (0.5 mg/ml)], bombesin (6 n*M*), or PDGF (100 ng/ml). Control cultures received the same additions but were not exposed to pertussis toxin. After another 48 hours, cells were counted by removing them from the plates with a trypsin solution and counting a portion of the resulting suspension in a hemocytometer.

Addition	Control (cell/ml × 10^{-5})	Pertussis toxin–treated (cell/ml × 10^{-5})
Vehicle	3.1 ± 0.1	3.1 ± 0.1
PDGF	6.4 ± 0.1	6.5 ± 0.3
Bombesin	5.1 ± 0.2	3.1 ± 0.2

bombesin- and PDGF-stimulated mitogenesis, even though both of these factors enhance c-*myc* expression and stimulate DNA synthesis. This is consistent with data showing that the effects of PDGF and bombesin on cell proliferation are additive (*1*). The pathways activated by bombesin presumably do not involve tyrosine kinase activity since there is no apparent increase in tyrosine-phosphorylated proteins after exposure of the cells to bombesin.

The finding that bombesin stimulates the expression of the c-*myc* gene was anticipated since a number of polypeptide growth factors and neurotransmitters are known inducers of c-*myc* expression (*28, 29*). Bombesin, like many of the other agents that enhance c-*myc* expression, activates the turnover of membrane phosphatidylinositol (*30*). Thus it is possible that the pertussis toxin–sensitive step in the responses to bombesin is the coupling between the bombesin receptor and the enzymes involved in the turnover of phosphatidylinositol. In some cells these enzymes are known to be regulated by peptides such as chemotactic peptides, which also act through G proteins that are sensitive to pertussis toxin (*18–23*).

One outcome of these findings is the prediction that pertussis toxin may inhibit the proliferation of SCLC cell lines that are dependent on an autocrine response to bombesin. Recent reports have shown that variant SCLC cell lines constitutively express high levels of c-*myc* and have a more tumorigenic phenotype (*26*). These cell lines may escape their dependence on bombesin, presumably by expressing pathways that bypass the bombesin receptor and thus would be expected to be resistant to the effects of pertussis toxin.

Little is known about the transduction of growth factor signals from the cell surface to the interior of the cell. The results of this study indicate that, for at least one class of growth-stimulating peptides, there is a mechanism that utilizes a G protein. Although PDGF and bombesin both stimulate c-*myc* expression and DNA synthesis, only the bombesin responses involve a pertussis toxin–sensitive step. Whether the responses to PDGF or other growth factors are mediated by a G protein that plays a role equivalent to the G protein involved in the response to bombesin, but is insensitive to pertussis toxin, remains to be determined.

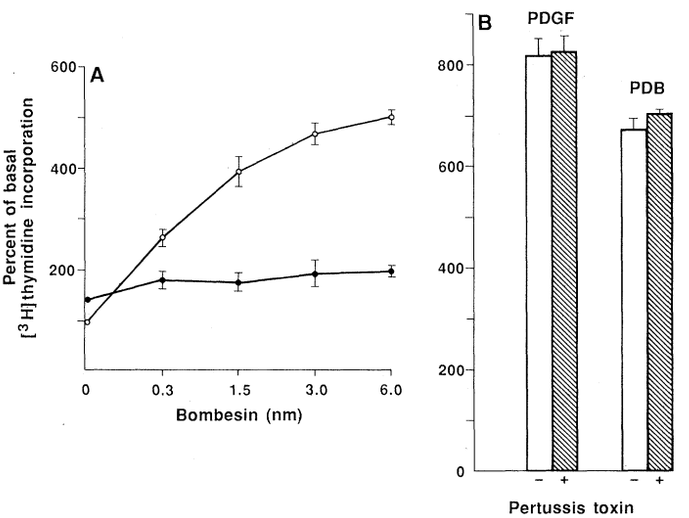

Fig. 3. Inhibition of bombesin-induced DNA synthesis in cells treated with pertussis toxin. Swiss 3T3 mouse fibroblasts were grown to confluence in Linbro 96-well plates, in DMEM H21 containing 10% fetal bovine serum. Six days after the last change of medium the cells were washed to remove residual serum and were incubated for 2 hours in DMEM containing 20 m*M* Hepes (*p*H 7.4), insulin (1 μg/ml), transferrin (5 μg/ml), and bovine serum albumin (0.5 mg/ml) with or without pertussis toxin (5 ng/ml). The cells were stimulated with vehicle (as in Fig. 2.), with various concentrations of bombesin (A), or with partially purified PDGF (100 ng/ml) or PDB (100 ng/ml) (B), as indicated, and incubated with 1 μCi of [^{3}H]thymidine per well for 24 hours at 37°C. Cells were then fixed with trichloroacetic acid (TCA), and TCA-insoluble radioactivity was measured. Data are expressed as percent of basal [^{3}H]thymidine incorporation, which refers to the level of incorporation by cells that received no growth factor and were not exposed to islet-activating protein. Basal incorporation ranged from 2.0×10^4 to 3.5×10^4 cpm. Each point represents the mean ± SEM of triplicate determinations from six experiments.

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