References and Notes

1. H. Gainer, *Brain Res.* **39**, 369 (1972).
2. ———, *ibid.*, p. 387.
3. J. L. Barker and ———, *Nature (Lond.)* **245**, 462 (1973). Dormant snails remained dormant with their calcareous epiphragms intact, while active snails moved about foraging for food. Semidormant snails were snails which had been activated from dormancy for less than 1 week.
4. Snail saline consisted of (in m*M*): 80 NaCl, 4 KCl, 10 $CaCl_2$, 5 $MgCl_2$, and 10 tris(hydroxymethyl)aminomethane hydrochloride (*p*H 7.8).
5. Lysine-vasopressin, arginine-vasopressin, and oxytocin were obtained from Sigma Chemical Co. (St. Louis, Mo.). Homolysine-vasopressin and 8-L-homonorleucine-vasopressin were generous gifts of M. Bodanszky (Case Western Reserve University, Cleveland, Ohio). Pressinamide was obtained from V. du Vigneaud (Cornell University, Ithaca, N.Y.). Lysine-vasopressin without its glycinamide terminal was a gift of D. Fisher (National Institutes of Health).
6. Cell 11's obtained from active snails typically exhibit BPP activity and nonlinear membrane current-voltage relations (*1*, *2*). In such active cells, LVP and related peptides also cause increases in both the BPP amplitude and the nonlinearity of membrane properties. The BPP is thought to be due to a K^+ conductance that is dependent on time and voltage [D. Junge and C. Stephens, *J. Physiol. (Lond.)* **235**, 155 (1973)] coupled to a high resting Na^+ conductance [D. O. Carpenter, in *Neurobiology of Invertebrates* (Plenum, New York, 1973), p. 38; H. Gainer, *Brain Res.* **39**, 403 (1972)]. The BPP is also directly associated with the presence of nonlinear current-voltage relations over a restricted range of membrane potentials (−35 to −70 mv) [D. S. Faber and M. R. Klee, *Nat. New Biol.* **240**, 29 (1972); M. R. Klee, D. S. Faber, W.-D. Heiss, *Science* **179**, 1133 (1973); H. Wachtel and W. A. Wilson, in *Neurobiology of Invertebrates* (Plenum, New York, 1973), p. 59]. Although BPP activity can be induced in these cells by manipulation of the external concentration of divalent cations (*3*), we think it highly unlikely that similar events underlie the LVP effect, which can be obtained at $10^{-9}M$.
7. The peptides that were effective in inducing BPP activity in cell 11 were without effect on identified neurons 9, 10, and 12 (*1*) in the *Otala* ganglion and cells R_2, R_{14}, R_{3-13}, L_{2-6}, L_7, L_{11}, and LPG in the ganglia of *Aplysia californica*. Only R_{15} (*Aplysia*), which is similar in its physiological and biochemical properties to cell 11 [F. Strumwasser, *Physiologist* **16**, 9 (1973)], was affected by the active peptides [in a manner similar to that described for the active cell 11 (*5*)]. For *Aplysia* nomenclature see W. T. Frazier, E. R. Kandell, I. Kupfermann, R. Waziri, R. E. Coggeshall, *J. Neurophysiol.* **30**, 1288 (1967).
8. Other substances tested (at a concentration of 1 m*M*) include: angiotensin II, bradykinin triacetate, adrenocorticotrophic hormone, growth hormone–releasing factor, melanocyte-stimulating hormone releasing factor, luteinizing hormone releasing factor, and physalaemin. In addition, neither pressinamide, the cyclic disulfide pentapeptide ring of LVP, nor LVP without the terminal glycinamide moiety could induce BPP activity in cell 11. (These agents also did not antagonize the effects of the active peptides.)
9. B. Katz, *Nerve, Muscle and Synapse* (McGraw-Hill, New York, 1966).
10. Other putative transmitters (glutamate, dopamine, octopamine, serotonin, and norepinephrine) all produced transient changes in membrane properties.
11. For a review, see B. Berde, Ed. *Handbook of Experimental Pharmacology*, vol. 23, *Neurohypophysial Hormones and Similar Peptides* (Springer-Verlag, New York, 1968).
12. W. Doepfner, in *ibid.*, p. 625.
13. R. A. Nicoll and J. L. Barker, *Brain Res.* **35**, 501 (1971).
14. In recent experiments we have isolated a peptide fraction from the snail brain which is similar to LVP in its effect on cell 11, and is eluted in the same fraction as LVP after chromatography on Sephadex G-25 (M. Ifshin, H. Gainer, J. L. Barker, in preparation).

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Growth Hormone Responses to Melatonin in Man

Abstract. *Oral administration of 1 gram of melatonin caused rapid and significant elevations of serum human growth hormone in eight out of nine normal male subjects. The mean (± standard error of the mean) of the peak response by growth hormone was 22.9 ± 4.6 microunits per milliliter. The increased secretion of growth hormone may be due to interaction of melatonin with hypothalamic serotoninergic receptors.*

Attempts have been made to show that melatonin, a pineal substance, is responsible, at least in part, for the antigonadotrophic and antigrowth effects exerted by the pineal gland. Evidence obtained in rats suggests that melatonin acts via the hypothalamus to block luteinizing hormone (LH) secretion and ovulation at critical periods (*1*). We have shown that melatonin blocks the increase in growth hormone (GH) in rats stimulated by 5-hydroxy-L-tryptophan (*2*) and also suppresses the human GH responses to insulin-induced hypoglycemia (*3*). In the latter studies (*3*) we noted a significant elevation in human serum GH before the induction of hypoglycemia. We thus investigated the question of whether melatonin could exert an early stimulatory effect on GH secretion. The serum GH response to orally administered melatonin was measured in ten healthy male volunteers from whom informed consent had been obtained. All tests on the subjects were begun at 8:30 a.m., after the subjects had fasted overnight. The melatonin was administered orally in two doses of 500 mg (in capsule form) each, 30 minutes apart, in order to duplicate conditions of the GH inhibition studies (*3*). Blood samples were collected every 15 minutes for 3 hours via an indwelling catheter in an antecubital vein. Serum GH was measured by means of the double antibody radioimmunoassay technique of Mollinatti *et al.* (*4*), and the results are expressed in microunits of the World Health Organization international reference for human GH radioimmunoassay.

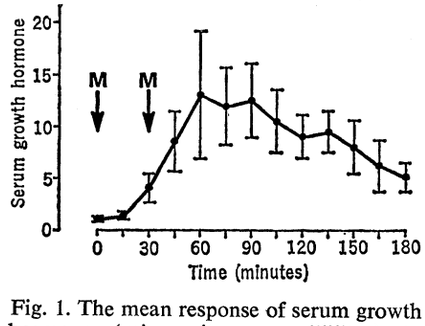

Fig. 1. The mean response of serum growth hormone (microunits per milliliter) to oral melatonin (2 × 500 mg at points indicated by *M*) in nine normal male subjects. The vertical lines represent the standard error of the mean.

The mean serum GH responses after melatonin administration are shown in Fig. 1. One subject in whom the serum GH was elevated at the start of the test was eliminated from the study. Of the other subjects only one failed to exhibit a GH rise above 10 μunit/ml in response to the melatonin. The mean (± the standard error of the mean) of the individual peak GH responses was 22.9 ± 4.6 μunit/ml.

The effects of melatonin administration on the serum levels of human GH are in the direction opposite to that which might have been expected in the light of its ability to block GH stimulation (*2*, *3*). We postulate that the ability of melatonin to increase serum GH levels in man is due to interaction with hypothalamic serotonin receptor sites mediating in the release of GH from the pituitary gland. It is suggested that once melatonin has occupied the serotonin receptors (displacing any serotonin present) it acts as a competitive inhibitor of serotonin and consequently blocks GH stimulation via serotoninergic pathways (*2*, *3*). This proposal is in accord with the observation of Anton-Tay *et al.* (*5*) that administration of melatonin to rats causes an increase in serotonin in the midbrain and hypothalamus which these workers suggested may possibly follow inhibition of serotonin release or metabolism by melatonin.

The mode of action that we have proposed for melatonin has some precedent in the known actions of D-lysergic acid diethylamide (LSD). LSD has a high affinity for serotonin receptors and enhances the actions of serotonin on uterine contractions at low doses but inhibits serotonin action at higher doses. The ability of melatonin to increase GH release in man would thus be analogous to the former (stimulatory) effect of LSD.

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References

1. R. J. Reiter and S. Sorrentino, Jr., *Contraception* **4**, 385 (1971); R. F. Collu, F. Fraschini, L. Martini, *Experientia* **15**, 844 (1971); S. Y. Ying and R. O. Greep, *Endocrinology* **92**, 333 (1973).
2. G. A. Smythe and L. Lazarus, *Horm. Metab. Res.* **5**, 227 (1973); *Nature* **244**, 230 (1973).
3. G. A. Smythe and L. Lazarus, *J. Clin. Invest.*, in press.
4. G. M. Mollinatti, F. Massara, F. Pennisi, G. A. Scassellati, E. Strumia, L. Vancheri, *J. Nucl. Biol. Med.* **13**, 26 (1969).
5. F. Anton-Tay, C. Chou, S. Anton, R. J. Wurtman, *Science* **162**, 277 (1968).

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Inhibition of Tumor-Induced Lymphocyte Blastogenesis by a Factor or Factors Associated with Peripheral Leukocytes

Abstract. *A factor or factors concentrated on leukocytes of cancer patients depress the proliferative response of lymphocytes to stimulation with autologous tumor. Inhibitory activity with material eluted from cells resides in neither high nor lower molecular weight fractions but in a combination of the two. The finding suggests that in vitro inhibition of lymphocyte proliferation to autochthonous tumor occurs because of antigen-antibody complexes.*

A factor or factors which block the proliferative response of lymphocytes to stimulation with autologous tumor can be detected in patients with primary malignancies (*1*). In our experiments inhibitory factors are concentrated on leukocytes and are removed from these cells by elution through washing with a subsequent increase in the cells' proliferative response. Crude fractionation of the material recovered in the eluate suggests that antigen-antibody complexes may be responsible for this blocking.

Many animal and human neoplasms elicit in the primary host a response against the tumor which can be measured by various serological and cell-mediated immune reactions (*2*). One such method is the mixed lymphocyte–tumor cell interaction (MLTI), which is a variant of the mixed lymphocyte culture, but recognizes tumor-specific antigenic differences (*3*). Of the various mechanisms that may effect cellular immunity, serum blocking factors have been extensively studied for their ability to inhibit the cytotoxicity of immune lymphocytes in vitro. Blocking components have been examined in tumor-bearing animals and most likely represent antigen-antibody complexes or soluble tumor antigen whose mechanism of action involves both an interaction with tumor cells and with effector lymphocytes (*1*, *4*).

Tumor specimens and normal tissues from the tumor-containing organ were obtained at the time of operation from patients who had received no other therapy for their neoplasms (seven carcinomas of the lung and one cancer of the stomach). The tissues were mechanically dispersed under sterile conditions after passage through 500- and 140-μm stainless steel sieves. Cellular material was then suspended in culture medium RPMI 1640 and layered on top of Ficoll-Hypaque gradients (*5*) which were centrifuged at 175*g* for 20 minutes (*6*). Cells were washed three to five times in RPMI 1640 at 24°C and then incubated overnight in the same medium at 37°C in a 5 percent CO_2 and air mixture prior to final washing and irradiation with 1500 r. Normal tissue from the cancerous organ was treated in a similar fashion (*7*). Peripheral blood drawn before surgery was separated on Ficoll-Hypaque gradients. Cells recovered at the interphase consisted of more than 90 percent lymphocytes, the remainder being classified as macrophages and granulocytes. Any contaminating red cells were removed by exposure to tris-buffered isotonic ammonium chloride (*8*, *9*). Various ratios of stimulating to responding cells were tried, but most cultures were performed with 2×10^5 responding lymphocytes and 1×10^5 irradiated stimulating cells in 0.2 ml of medium containing either autologous or normal plasma. Cultures were incubated for 3, 5, or 7 days. The harvesting and counting methodology has been described (*10*).

Table 1. Incorporation of tritiated thymidine in peripheral lymphocytes of cancer patients after autologous tumor stimulation, comparing a one-wash (W1) versus a five-wash (W5) procedure. The values are the means of five cultures ± the standard errors of the means.

Patients *	Peripheral lymphocyte responses † (count/min)			
	W1 lymphocytes		W5 lymphocytes	
	+ Tumor	+ Normal tissue ‡	+ Tumor	+ Normal tissue ‡
N.B.	16,869 ± 3,071	931 ± 74	58,721 ± 1,754	1,783 ± 452
H.S.	1,083 ± 97	690 ± 43	3,570 ± 186	404 ± 45
I.R.	3,271 ± 764	1,522 ± 101	15,074 ± 3,004	3,276 ± 200
B.E.	783 ± 53	990 ± 80	3,041 ± 178	597 ± 38
R.A.	2,910 ± 128	1,077 ± 194	5,938 ± 369	576 ± 66
S.S.	4,806 ± 301	1,331 ± 129	10,563 ± 1,243	1,648 ± 137
E.D.	945 ± 86	878 ± 116	2,810 ± 103	935 ± 45
J.B.	6,122 ± 505	1,899 ± 178	13,488 ± 2,061	2,111 ± 206

* Patient N.B. had an adenocarcinoma of the stomach; the others had squamous cell carcinoma of the lung. † Five-day cultures, values for W5 lymphocytes greater than those for W1 lymphocytes at all time intervals, that is, 3 and 7 days in culture. ‡ Normal tissue counterpart after 1500-r irradiation.

Table 2. Inhibition of autologous tumor-induced lymphocyte proliferation by ultrafiltrates from material eluted from a cancer patient's peripheral leukocytes. The patient (J.B.) had squamous cell carcinoma of the lung. Cultures were set with 2×10^5 responding lymphocytes stimulated with 1×10^5 irradiated tumor cells. The values are tritiated thymidine uptake (in counts per minute) for 5-day cultures: mean values for five cultures ± standard errors of the means.

Material tested for blocking activity		Patient J.B. lymphocytes + patient J.B. tumor	
Eluate	Fraction	W1 lymphocytes	W5 lymphocytes
None (control)	Control	2,971 ± 478	11,018 ± 1,062
Eluate from patient J.B. leukocytes	CW *	2,416 ± 300	3,141 ± 294
	CW_{10}	3,603 ± 249	9,003 ± 806
	CW_{100}		10,181 ± 1,817
	CW_{10+100}	2,195 ± 91	2,001 ± 94
Eluate from normal (control) leukocytes	CW *	3,694 ± 600	14,108 ± 549
	CW_{10}		
	CW_{100}		8,996 ± 900
	CW_{10+100}		11,087 ± 989

* Added at equivalent protein concentrations.