nism was recently proposed for *S*-adenosylmethionine and 5-hydroxytryptamine, where several products were suggested but none was identified (*17*). Or, an enzymatic oxidation of 5-MTHF to 5-formyl-THF might precede the release of free formaldehyde, followed by nonenzymatic condensation (*18*). A third sequence might involve the transfer of a formyl group directly from the enzyme, or coenzyme, to the substrate, with subsequent cyclization. Further experiments are required to elucidate the mechanism of the overall reaction sequence, to examine pharmacological actions of the products, and to determine whether they are formed endogenously.

Note added in proof: While in press similar enzymatic products have been described from tryptamines (*19*) and catecholamines (*20*).

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12. E. Späth and E. Lederer, *Chem. Ber.* **63**, 2102 (1930). All three compounds were pure by thin-layer chromatography (Brinkman silica gel 254, MeOH, 1*N* NH_4OH,H_2O [100 : 20 : 10]). The observed melting point for tryptoline was 205° to 206°C [the reported melting point is 202° to 203°C (Z. Vejdelek, V. Tröka, M. Protira, *J. Med. Chem.* **3**, 427 1961)]. 1-Methyltryptoline had a melting point of 209.5° to 210.5°C. 5-Hydroxytryptoline melted at 290° to 291°C. The compounds had the correct elemental analysis. Mass spectral analysis of tryptoline shows a molecular ion at 172 amu, the base peak at 143 amu (loss of alicyclic CH_2NH), and the fragment ion at 115 amu (indole nucleus). 1-Methyltryptoline shows a similar fragmentation pattern, with a molecular ion at 186 amu, base peak at 143 amu (loss of CH_2NCH_3), and fragment ion at 115 amu (indole nucleus).
13. Successive cocrystallizations of the product from tryptamine with tryptoline from ethyl acetate, toluene, and acetone gave specific activities of 11,177, 10,966, and 10,370 disintegrations per minute per milligram. Cocrystallization of the product from *N*-methyltryptamine with authentic 1-methyltryptoline from the same solvents gave specific activities of 3468; 3358; and 3363 dpm/mg. Cocrystallization of the product from 5-hydroxytryptamine with authentic 5-hydroxytryptoline from methanol, a mixture of methanol and water (4 : 1), and 95 percent ethanol, yielded specific activities of 10,338, 8,809, and 10,533 dpm/mg.
14. We thank R. Skinner, J. Cornelius, J. Knight, and R. Finnigan for their help with the mass spectrometry.
15. The gas-liquid chromatography was performed on a U-shaped column (1.5 m by 2 mm) with 2 percent OV 17 on 80 to 100 mesh Gas Chrom 2; helium was the carrier gas, with a flow of 22 ml/min. The temperatures were 185°C for the column, and 250°C at the inlet and interface.
16. The gas-liquid chromatography was performed as in (*15*), except that the U-shaped column contained 3 percent OV 17 and the column temperature was 220°C.
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21. R_F values in other solvent systems examined: (i) In isopropanol, ammonium hydroxide (10 percent), and water (200 : 10 : 20), the R_F of tryptamine was 0.26; of *N*-methyltryptamine, 0.16; of DMT, 0.42; of tryptoline, 0.30; and of 1-methyltryptoline, 0.49. (ii) In *n*-Butanol, acetic acid and water (12 : 3 : 5), the R_F of tryptamine was 0.66; of *N*-methyltryptamine, 0.59; of DMT, 0.53; of tryptoline, 0.63; and of 5-methoxydimethyltryptamine, 0.45. (iii) In toluene, acetic acid, ethyl acetate, and water (80 : 40 : 20 : 5), the R_F of tryptamine was 0.18; of *N*-methyltryptamine, 0.17; of DMT, 0.10; and of tryptoline, 0.20. (iv) In methanol, tetrahydrofuran, and formic acid (50 : 50 : 1), the R_F of tryptamine was 0.52; of *N*-methyltryptamine, 0.42; of DMT, 0.30; of tryptoline, 0.44; and of 1-methyltryptoline, 0.21. (v) In acetone and ammonium hydroxide (99 : 1), the R_F of tryptamine was 0.47; of *N*-methyltryptamine, 0.13; of DMT, 0.18; of tryptoline, 0.14; and of 1-methyltryptoline, 0.44.
22. We thank S. O'Connor, P. Angwin, and E. Blanz, Jr., for assistance. Supported by NIH program-project grant MH 23861, NIH biosciences training grant fellowship MH 8304 (G.R.E.), and a research scientist development award MH 24161 (J.D.B.). R.J.W. has been a visiting faculty member, Department of Psychiatry, Stanford University. Send reprint requests to R. J. Wyatt, Laboratory of Clinical Psychopharmacology, National Institute of Mental Health, WAW Building, St. Elizabeths Hospital, Washington, D.C. 20032.

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Primate Type C Virus p30 Antigen in Cells from Humans with Acute Leukemia

Abstract. *Antigens related to the major structural protein (p30) of type C viruses isolated from a woolly monkey and a gibbon ape were found in peripheral white blood cells from five patients with acute leukemia.*

Several lines of evidence have suggested that certain forms of acute leukemia (AL) in man may be associated with type C viruses. Cells from patients with acute leukemia contain an enzyme with biochemical properties related to those of known type C viruses and with antigenic properties similar to those of the polymerases of the woolly monkey type C virus (SSAV) and the gibbon ape leukemia virus (GALV) (*1*). The DNA products of endogenous reactions from particulate fractions of AL cells hybridize preferentially to viral RNA from SSAV and GALV (*2*). While the woolly monkey type C virus represents a single isolate from a pet with fibrosarcoma, there are several independent isolates of type C viruses from gibbons (*3*). The woolly monkey sarcoma virus produces sarcomas and brain tumors in marmosets, and GALV has produced acute myelogenous leukemia in a normal gibbon (*4*).

Radioimmunoassays can detect antigens related to the major structural proteins (p30) of type C viruses in tissues of primates (*5*) and man (*6*, *7*). The data presented here show that cells from patients with AL contain p30 antigens specifically related to viruses of the SSAV-GALV group. Peripheral blood leukocytes (provided by R. Gallo and R. Gallagher) which had been obtained from patients by leukophoresis were stored at −20°C prior to use. The cells (1 to 5 g, wet weight, packed) were thawed, extracted, and partially purified by gel filtration (*5*, *6*). These extracts were used as competing antigens in radio-

immunoassays (*8*) for the p30 proteins of SSAV, GALV, feline leukemia virus (FeLV), Rauscher murine leukemia virus (MuLV), and an endogenous baboon type C virus (M7/M28 group). The assay system used for the p30 proteins of SSAV and GALV indicates little or no difference between the two viruses. The system is sufficiently specific so that the p30 proteins of other type C viral groups could not be detected.

Figure 1 shows the competition curves obtained in four different assays with purified extracts of human AL cells as competing antigens. Of five extracts tested, all competed for the ^{125}I-labeled p30 protein of SSAV (Fig. 1A). No competition was observed with these same extracts in assays for the p30 proteins of MuLV (Fig. 1B) and FeLV (Fig. 1C). We conclude that the competition reactions observed in the SSAV assay are immunologically specific and do not reflect the nonspecific displacement of test antigens occasionally seen with certain tissue extracts (*6*). Control studies with other assays for the p30 proteins of SSAV and GALV show that the competition reactions observed in the SSAV assay are independent of the source or manner of purification of the labeled test antigens, the particular antiserums employed, or the sensitivities of the assays used (Table 1). All five assays detected comparable levels of antigen at amounts ranging between approximately 2 to 20 ng per milligram of tissue protein. In addition, a cultured AL cell line, HL-23, at the tenth passage when type C viruses were readily detected (*9*) contained considerably higher levels of SSAV-related p30 antigen (1200 ng per milligram of cell protein).

Primates, including man, contain endogenous type C viral sequences in their genome which are related to those found in endogenous baboon viruses (*10*). Antigens related to the p30 proteins of these viruses have also been demonstrated in human tumors, indicating that endogenous virogenes may be at least partially expressed (*6*). In an assay for the p30 protein of the M7 baboon virus (Fig. 1D) one AL cell extract (patient HL-60) partially competed for the labeled test antigen while the remainder of the extracts appeared antigen-negative. Since the p30 proteins of baboon type C viruses and viruses of the SSAV-GALV group do not cross react in these assays, the results indicate that endogenous virogene expression can also be detected in cells from one of the patients.

The demonstration of antigens related to the p30 proteins of SSAV and GALV in peripheral white cells from five leukemic patients suggest that viruses of this group, known to be infectious and tumorigenic in other primates, may also be associated with acute leukemia in man. The isolation of complete type C viruses from human leukemic cells should greatly assist in evaluating their etiologic role in the disease.

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Table 1. Assays for antigens related to those of SSAV and GALV in leukocytes from patients with acute leukemia. The SSAV and GALV viruses were grown in either rat K-NRK (transformed rat kidney) cells (*11*) or human A204 cells (*12*). The endogenous porcine virus (PK-15) was concentrated from pig kidney cells that spontaneously release this virus (*13*). The purification of p30 proteins, preparation of rabbit antiserums, and conditions for radioimmunoassay have been described (*5, 6*). Extracts of AL cells were partially purified on Sephadex G-100 (*6*). The absorbance at 280 nm was monitored, and material eluting in the region usual for proteins with molecular weights of 15,000 to 50,000 was pooled and concentrated. The degree of purification of proteins eluting in this region compared to the total protein applied was estimated from absorbance measurements and was generally about tenfold. The amount of p30 antigen in leukemic cells was estimated by dividing the amount of antigen detected in purified extracts by the factor of protein purification obtained by gel filtration. Parentheses indicate cell lines in which SSAV was grown, and brackets indicate the amounts in nanograms of purified p30 proteins required for 20 percent displacement in each assay system.

Patient	Diagnosis	Radioimmunoassay (nanograms of p30 antigen per milligram of tissue protein)				
		Species specific*			Interspecies	
		Anti-SSAV: SSAV (K-NRK) [0.6]	Anti-SSAV: SSAV (A204) [0.3]	Anti-GALV: GALV [2.3]	Anti-SSAV: GALV [0.3]	Anti-PK-15: SSAV [2.7]
HL-60†	Unknown type	16	18	22	16	19
HL-43	Myelogenous	8.4	13	13	9.0	14
HL-25	Myelogenous	3.3	4.3	5.7	4.2	5.4
HL-45	Histiocytic	2.6	2.4	3.3	2.7	4.3
HL-8	Myelomonocytic	1.9	2.0	1.5	1.3	2.2

* Homologous antigen-antibody systems. † Cells from one patient (HL-60) contained p30 antigen (13 ng/mg) related to endogenous baboon type C virus.

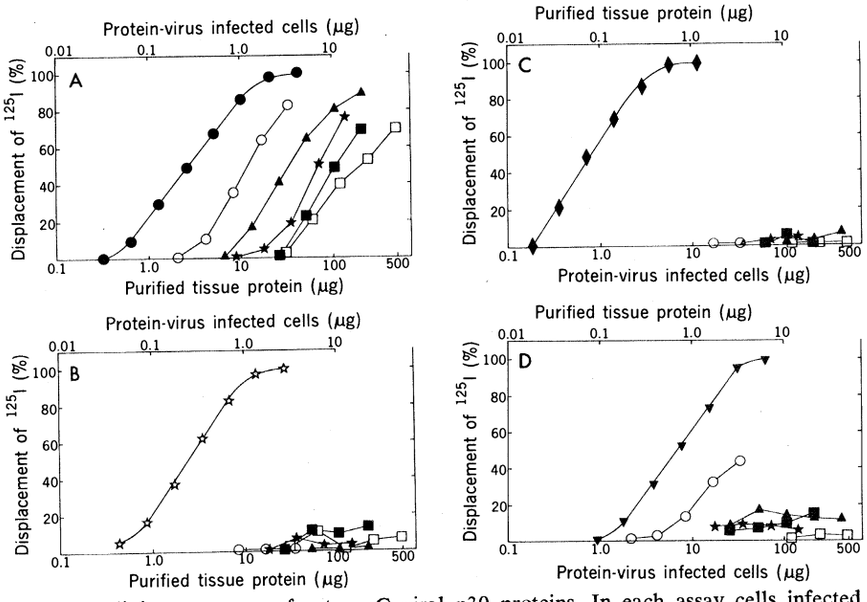

Fig. 1. Radioimmunoassays for type C viral p30 proteins. In each assay cells infected with the homologous virus were used as the positive controls (top scale). (A) ●, Human cells infected with SSAV; (B) ☆, mouse cells infected with MuLV (Rauscher strain); (C) ◆, canine cells infected with FeLV (Gardner-Arnstein strain); (D) ▼, canine cells infected with endogenous baboon virus (M7). The five AL extracts were as follows. ○, Patient HL-60; ▲, patient HL-43; ★, patient HL-25; ■, patient HL-45; □, patient HL-8.

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Potassium Chloride–Induced Lordosis Behavior in Rats Is Mediated by the Adrenal Glands

Abstract. *Potassium chloride applied topically to the neocortex facilitates lordosis behavior in estrogen-primed ovariectomized rats. This effect is absent in rats that are also adrenalectomized. Potassium chloride fails to facilitate lordosis in estrogen-primed rats, with intact adrenals, which are pretreated with dexamethasone, an inhibitor of the release of adrenocorticotropic hormone. Adrenalectomized rats that have been treated with dexamethasone are capable of responding to progesterone with the display of lordosis. The results suggest that application of potassium chloride to the neocortex acts as a stressor that causes the release of adrenal progestin.*

During the past few years the hypothesis has been developed that the lordosis posture that characterizes sexual receptivity in the rat is normally under tonic neocortical inhibition and that gonadal hormones, progesterone in particular, function to reduce that inhibition (*1*). Consistent with this hypothesis are observations that the application of potassium chloride to the cerebral cortex and electrical stimulation of the cerebral cortex facilitate lordosis behavior in rats stimulated with estrogen (*2*). The present report gives evidence that the effects on lordosis behavior of the application of KCl are mediated by the adrenal gland and do not represent the effects of a functional neodecortication. Rather, the present studies suggest that the application of KCl to the neocortex results in the secretion of adrenocorticotropic hormone (ACTH) and adrenal hormones, presumably including progesterone.

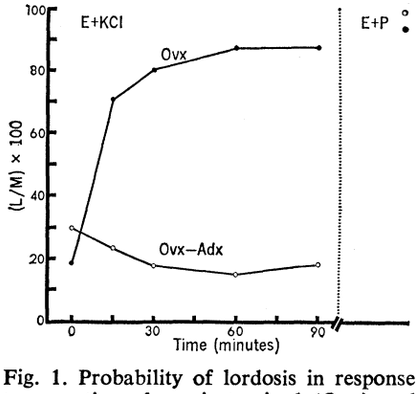

Fig. 1. Probability of lordosis in response to mounting of ovariectomized (Ovx) and ovariectomized-adrenalectomized (Ovx-Adx) female rats prior to time 0 and 15, 30, 60, and 90 minutes after the application of potassium chloride to the neocortex. The rats were estrogen primed at the time of treatment. The E + P (estrogen plus progesterone) test occurred 1 week after the KCl test and was preceded by estrogen and progesterone treatment.

In our studies mature Sprague-Dawley female rats were maintained in individual cages on a reversed light-dark cycle of 12 hours each with food and water freely available. Adrenalectomized rats were provided with 0.9 percent saline. All animals were ovariectomized and were implanted with bilateral 15-gauge aluminum cannulas fixed to the skull over the parietal-occipital cortex in the manner described by Russel and Ochs (*3*). The tip of each cannula rested on the dura.

Behavior tests consisted of placing each female with a sexually vigorous male. Testing began 2 to 3 hours after the onset of the dark phase of the lighting cycle. The male was allowed to mount the female ten times. The occurrence of each lordotic response was recorded and a lordosis quotient (L.Q.) was determined [L.Q. is (No. of lordosis responses/mounts with thrusting) × 100]. All females were screened for their response to estrogen alone by administering 5 μg of estradiol benzoate subcutaneously daily for 2 days, followed by behavioral testing on day 3. Rats that obtained an L.Q. score of 40 or higher on the screening test were eliminated.

In experiment 1 nine rats were ovariectomized and eight were ovariectomized and adrenalectomized. Testing began 2 to 3 weeks after surgery. Each rat was administered 5 μg of estradiol benzoate subcutaneously daily for 2 days. On day 3 they were given 500 μg of progesterone and were tested 3 hours later. One week later these animals were given estrogen daily on days 1 and 2; on day 3 they were tested for the display of lordosis behavior. Following this test the cannulas were filled with 15 percent KCl, and the animals were retested 15, 30, 60, and 90 minutes later. The next week the animals were again given estrogen and progesterone and were tested for lordosis behavior.

On the first test with estrogen and progesterone the L.Q. scores were 61.1

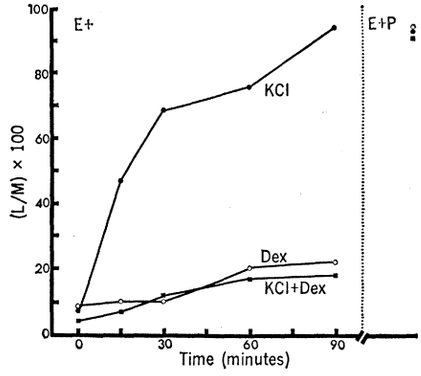

Fig. 2. Probability of lordosis in response to mounting of estrogen-primed ovariectomized rats. All animals were tested prior to treatment (time = 0). The KCl animals then had potassium chloride applied topically to the neocortex. The KCl + Dex animals received cortical application of KCl 1 hour after a subcutaneous injection of dexamethasone (Dex). The Dex animals received cortical application of saline 1 hour after a subcutaneous injection of dexamethasone. All animals were tested 15, 30, 60, and 90 minutes after either KCl or saline treatment. Following the final test all animals received progesterone and were retested 3 hours later.