suggest that brain uptake studies of systemically administered drugs should, in most cases, use intravenous injection with brain tissue concentration measurements beginning at 30 seconds.

To be entirely representative of brain uptake of clinical doses of drugs, the intravenous studies described here would have to be repeated in the presence of therapeutic blood concentrations of unlabeled drug. The tracer concentrations used in our studies would probably be bound more completely to plasma protein than when present in therapeutic concentrations. If penetration of the blood-brain barrier by the drug was, in part, carrier-mediated, significant saturation effects could be seen with therapeutic concentrations but not with trace amounts.

The observation that most of the heroin entering the brain after carotid injection is taken up by the brain suggests that delivery to any region of brain tissue is largely determined by the regional flow of blood in the brain. Accordingly, after rapid intravenous injection the amount of heroin deposited regionally in the brain is probably approximately in proportion to the blood flow (*12*). Even though heroin is rapidly hydrolyzed in blood to 6-monoacetylmorphine and to morphine (*13*), it probably largely survives as heroin the 10 to 15 seconds required to be delivered peripherally after intravenous injection in humans. Once in the brain it is rapidly hydrolyzed to 6-monoacetylmorphine and to morphine (*13*). Redistribution of this morphine back through the blood-brain barrier to circulating blood probably is retarded both by tissue binding and by the low permeability of the blood-brain barrier to morphine. These kinetics may, in part, account for the greater pharmacological effectiveness of heroin.

The observation that both codeine and morphine disappear from blood plasma substantially more rapidly than mannitol (Fig. 2) is consistent with the observation that morphine has a high affinity for many general tissue binding sites (*14*).

The high uptake of heroin reported here after carotid injection indicates that an abrupt entrance of heroin into brain tissue probably occurs 10 to 20 seconds after the usual intravenous injection by addicts. This rapid entry relative to morphine may reinforce the addict's relating the act of drug administration to central nervous system response and thereby be a factor in the more intractable addiction to heroin. This rapid entry into brain may also be a factor in nicotine dependence since its brain uptake when studied by the present carotid technique is more than 100 percent (*15*).

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References and Notes

1. L-[1-^{3}H]Methadone hydrobromide (92 mc/mmole) and D-[1-^{3}H]mannitol (nominally labeled) (3140 mc/mmole) were from New England Nuclear. [*N*-methyl-^{14}C]Morphine hydrochloride (58 mc/mmole) and [*N*-methyl-^{14}C]codeine hydrochloride (48 mc/mmole) were from Amersham/Searle. [^{14}C]Morphine from Amersham/Searle was acetylated by Dhom Products to produce diacetyl morphine (heroin).
2. Rats that received carotid injections were Wistars of either sex (300 g) that were anesthetized with pentobarbital intraperitoneally.
3. Ringer solution for carotid injections contained 4 or 10 mmole of HEPES buffer (Calbiochem) per liter and was injected at a *p*H of 7.55.
4. W. H. Oldendorf, *Brain Res.* **24**, 372 (1970); *Amer. J. Physiol.* **221**, 1629 (1971).
5. It was assumed that the chemical structure of the labeled compound was unaltered between the time of injection and penetration of the blood-brain barrier and that the radionuclide which had penetrated the blood-brain barrier remained in the brain during the 15-second vascular compartment washout. Biotransformation after penetration of the blood-brain barrier was assumed to be unimportant since it would not result in a change in the amount of radionuclide remaining in brain tissue. For mean value, $n = 6$.
6. Fisher 344 rats (Charles River) weighed approximately 200 g and were not nephrectomized.
7. Mannitol is a small molecule (molecular weight, 182) not significantly bound to plasma protein, and it served as a lipid insoluble, metabolically inert reference substance that would remain largely extracellular.
8. The calculation of percent mean body concentration is: [(count min^{-1} injected per gram of tissue)/(count min^{-1} per gram of total body weight)] × 100. Brain tissue concentrations are corrected for labeled compounds present in residual brain blood plasma, assuming 0.8 percent of brain tissue is plasma.
9. If the test substance remained confined to plasma, the plasma concentration throughout the interval studied would be about 2500 percent of the mean body concentration because the plasma volume is about 4 percent of the total body weight.
10. L. B. Mellett, *Bulletin on Drug Addiction and Narcotics* (National Academy of Sciences–National Research Council, Washington, D.C., 1964), appendix 12, 3822.
11. C. D. Olsen, *Science* **176**, 525 (1972).
12. L. A. Sapirstein, *Amer. J. Physiol.* **193**, 161 (1958).
13. E. L. Way, in *The Addictive States*, A. Wickler, Ed. (Williams & Wilkins, Baltimore, 1968), p. 13; *Arch. Biol. Med. Exp.* **4**, 92 (1967); ———, J. M. Young, J. W. Kemp, *Bull. Narcotics* **17**, 25 (1965).
14. D. N. Teller, T. De Guzman, A. Lajtha, *Neurology* **22**, 414 (1972).
15. The brain uptakes of a number of other drugs have been reported by means of the carotid injection with a tritiated water diffusible internal standard [W. H. Oldendorf, *Trans. Amer. Neurol. Ass.* **96**, 46 (1971)]. These uptakes (percent) are: nicotine, 119; ethanol, 117; imipramine, 107; procaine, 91; caffeine, 86; antipyrine, 65; cyanide, 47; diphenylhydantoin, 28; phenobarbital, 18.7; mescaline, 5.6; L-ascorbic acid, 3.8; aspirin, 2.65; and benzylpenicillin, 2.1. Brain uptakes greater than 100 percent probably represent initially complete clearance of the test substance with less washout than the tritiated water diffusible reference during the 15-second washout interval.

3 July 1972; revised 11 August 1972

Antigens Specific for Human Lymphocytic and Myeloid Leukemia Cells: Detection by Nonhuman Primate Antiserums

Abstract. *Primate antiserums to human leukemia cells can detect antigens specific for lymphocytic leukemia cells or antigens present on certain myeloid leukemia cells. The antigen specific for lymphocytic leukemia cells is destroyed by treatment with neuraminidase or trypsin. Tryptic digests of lymphocytic leukemia cells contain the antigen, which has a high molecular weight.*

Immunization of monkeys with peripheral blood cells from individual patients with chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML), or chronic granulocytic leukemia (CGL) leads to the production of high-titered antibodies which, after appropriate absorption techniques, appear to be detecting antigens specific for either lymphocytic leukemia or certain myeloid leukemia cells.

For immunization of primates, leukocytes were obtained from buffy coat cells from four different leukemia patients placed on an Aminco cell separator. Most of the erythrocytes were removed from the buffy coat suspension by sedimentation with Plasmagel (*1*). Leukemia cells used for the initial immunization of primates were thrice washed with Hanks balanced salt solution (> 90 percent viability) and injected on the same day. Some of the cells to be used later for booster immunizations and testing were suspended in RPMI (Roswell Park Memorial Institute) 1640 medium with 10 percent autologous serum and 10 percent dimethyl sulfoxide, frozen, and stored in liquid nitrogen. Cells for the initial injection (10^9) were emulsified in Freund's complete adjuvant (*2*) and injected intradermally and subcutaneously into various species of monkeys. Cells from one patient with CLL (HL-A1, 8, 12) were injected into a

Macaca speciosa, and cells from another CLL patient (HL-A2, 3, 5) were injected into a *Macaca mulatta*. Another *Macaca speciosa* received cells from a CGL patient whose cells could not be reliably typed for HL-A antigens, and a *Cercocebus atys* received cells from an AML patient (HL-A2, 5). Booster injections of 10^9 washed frozen cells were injected without adjuvants into the edges of the lesions produced by the initial immunization with adjuvants at approximately 2-week intervals until leukemia-specific antibodies were detected. Leukemia-specific antibodies could be detected after two booster injections.

The antiserums were tested by three different techniques—cytotoxicity (*3*), agglutination (*4*), and mixed agglutination (*5*)—for antibodies to membrane antigens of human cells. The specificity of the antibodies detected by all three techniques was the same although higher titers were obtained by mixed agglutination. The titers obtained by cytotoxicity and agglutination were essentially the same. We now present our data, which were obtained by cytotoxicity testing, as follows. Antiserum (1 μl) was mixed with normal peripheral blood lymphocytes (½ μl; 4000 cell/mm^3) or leukemia cells and incubated at room temperature for 35 minutes. Five microliters of rabbit complement were then added and the mixture was incubated at 37°C for 35 minutes. Five percent eosin (5 μl) and 30 percent buffered formalin (2 μl) were subsequently added, and the reaction was read on an inverted microscope. Cytotoxicity titers are reported as the highest dilution giving at least 50 percent killed cells. Lymphocytes were prepared from normal peripheral blood by sedimentation with Plasmagel (*1*) and incubation on a nylon column. Cells from leukemia patients for serological testing were also subjected to purification on a nylon column.

For the initial testing, the monkey antiserums were inactivated at 56°C for 30 minutes and absorbed with equal volumes of packed washed human erythrocytes (RBC) from blood group A and B donors until they no longer agglutinated these cells. The antiserums were subsequently absorbed at room temperature for 1 hour with normal peripheral blood leukocytes (WBC) (2×10^8 cell/ml) from several donors possessing one or more of the HL-A phenotypes of the specific leukemia donor until the antiserums no longer reacted with normal peripheral blood leukocytes by cytotoxicity, agglutination, or mixed agglutination. Some portions of the antiserums absorbed with normal erythrocytes and leukocytes were also absorbed with 4×10^8 cell/ml from individual leukemic donors.

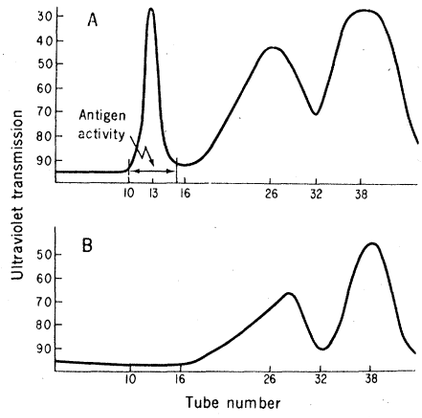

Fig. 1. (A) Sephadex G-150 fractionation of tryptic digest of CLL cells. Tube volumes were 5 ml and tubes 1 to 15 are excluded. Antigen activity was detected in tubes 11 through 15. (B) Sephadex G-150 fractionation of trypsin-trypsin inhibitor control.

The results of the cytotoxicity testing of the four antiserums (after the absorptions just described) with normal and leukemic cells are given in Table 1. The antiserums after absorption with human erythrocytes reacted with cells from all normal and leukemia donors tested. The same antiserums after being additionally absorbed with normal peripheral blood leukocytes no longer reacted with lymphocytes from normal donors, but did react with cells from certain leukemic patients. The cytotoxic activity after this absorption was usually sufficient to kill more than 80 percent of the cells at a dilution of 1 : 80 or greater. Monkey antiserums 1 and 2 to CLL cells reacted with cells from all CLL and acute lymphatic leukemia (ALL) patients tested. The antiserums to CLL did not react with cells from any of the AML or CGL patients. The antiserum to AML (monkey No. 3) or to CGL (monkey No. 4) cells after absorption with normal peripheral blood leukocytes did not react with cells from CLL or ALL patients, but did react with cells from some but not all AML and CGL patients. Cells from some patients with AML or CGL were refractory to agglutination or lysis with these two antiserums despite the presence of circulating leukemic cells.

The four antiserums after absorption with normal peripheral blood leukocytes also failed to react with cells from three patients with lymphosarcoma, and from one patient each with lymphoma, leukemoid reaction, erythroid leukemia, and refractory anemia. The antiserums to AML and CGL, however, did react with all six of six patients tested with myeloproliferative syndrome.

The primate antiserums after being additionally absorbed with cells from CLL, AML, or CGL patients who were not the immunizing cell donors, were also tested (Table 2). The two CLL antiserums after absorption with AML or CGL cells from a single donor gave

Table 1. Cytotoxicity reactions of primate antiserums to human leukemia cells. Results are shown as the ratio of the number of donors giving a positive reaction to the total number of donors tested.

Antiserum absorbed with	Cytotoxic reactions of antiserums with				
	Normal donors	CLL patients	ALL patients	CGL patients	AML patients
	Antiserum to CLL				
RBC	17/17	24/24	6/6	13/13	14/14
RBC + WBC	0/17	24/24	6/6	0/13	0/14
RBC + WBC + CGL	0/6	20/20	5/5	0/10	0/8
RBC + WBC + AML	0/6	0/20	0/5	0/10	0/8
RBC + WBC + CLL	0/6	0/20	0/5	0/10	0/8
	Antiserum to CLL				
RBC	17/17	24/24	6/6	13/13	14/14
RBC + WBC	0/17	24/24	6/6	0/13	0/14
RBC + WBC + CGL	0/6	20/20	5/5	0/10	0/8
RBC + WBC + AML	0/6	20/20	5/5	0/10	0/8
RBC + WBC + CLL	0/6	0/20	0/5	0/10	0/8
	Antiserum to AML				
RBC	17/17	24/24	6/6	13/13	14/14
RBC + WBC	0/17	0/24	0/6	10/13	4/14
RBC + WBC + CGL	0/6	0/21	0/5	5/10	2/8
RBC + WBC + AML	0/6	0/21	0/5	2/10	0/8
RBC + WBC + CLL	0/6	0/21	0/5	10/10	4/4
	Antiserum to CGL				
RBC	17/17	24/24	6/6	13/13	14/14
RBC + WBC	0/17	0/24	0/6	12/13	2/14
RBC + WBC + CGL	0/6	0/21	0/5	0/10	0/8
RBC + WBC + AML	0/6	0/21	0/5	5/10	0/8
RBC + WBC + CLL	0/6	0/21	0/5	10/10	2/2

the same reaction patterns as the antiserums absorbed with human RBC and human WBC. Absorption of the two CLL antiserums with cells from a CLL patient removed the cytotoxic activity for cells from all CLL and ALL patients tested.

The AML and CGL antiserums after absorption with cells from a CLL patient still reacted with certain CGL and AML donors. The pattern of reactivity was the same as after absorption with human RBC and WBC. Absorption of the AML antiserum with cells from a CGL donor removed the reactivity for the absorbing cell and for cells of certain individual AML and CGL donors but left good reactivity for others. Absorption of the AML antiserum with cells from an AML patient removed the reactivity for cells from all AML donors but left activity for cells from some CGL patients.

Absorption of the CGL antiserum with cells from an AML donor removed the reactivity for cells from all AML patients, but left reactivity for cells from some CGL patients. Absorption of the same antiserums with cells from a patient with CGL removed the reactivity for cells of all CGL and AML patients tested. When the antiserums to AML and CGL were absorbed with cells from different individual AML and CGL donors, different patterns of reactivity could be noted when these antiserums were tested with cells from other AML and CGL patients. Activity was always removed for the absorbing cell.

The antiserums to cells from two different CLL donors appear to be detecting a single antigen common to all ALL and CLL patients although absorptions with cells from different individual CLL and ALL patients must be performed. It appears, however, that the AML and CGL antiserums are detecting more than one antigen and that some AML or CGL patients lack all of the antigens being detected by these antiserums, or that antigens are expressed on these cells in a way that is refractory to agglutination and lysis. Absorption of monkey antiserums 3 and 4 with cells from additional CGL and AML patients will help to resolve the number of different antigen-antibody systems being detected by these reagents. Production of additional antiserums to cells from the nonreactive AML and CGL patients will also help to define additional specificities.

The four antiserums after absorption with peripheral blood leukocytes failed to react by all three methods with thymus cells from five different donors and additional absorption of the antiserums with 10^9 thymus cells per milliliter from one donor failed to alter the reactivity with leukemia cells. Suspension cultures of human lymphocytes derived from normal peripheral blood leukocytes from two different donors (*6*) also failed to react with the absorbed antiserums.

When reactive CLL, AML, or CGL cells are treated with trypsin (0.25 percent) or neuraminidase (0.2 unit/10^6 cells), the cells no longer react with their respective antiserums. Leukemic cells are still more than 90 percent viable after either enzymic treatment. Normal peripheral blood lymphocytes treated with trypsin or neuraminidase fail to react with the antiserums to leukemic cells. CLL cells (1.5×10^8) were incubated in 0.25 percent trypsin for 20 minutes at 37°C. Soya bean trypsin inhibitor was then added, and 12 mg of the digest was fractionated on Sephadex G-150 with 0.15*M* phosphate-buffered saline. Leukemic antigen activity was determined by inhibition of cytotoxicity. All antigen activity was recovered in the first protein peak, which was excluded from Sephadex G-150 (Fig. 1A). This peak was not seen in the trypsin-trypsin inhibitor material that was incubated and fractionated as a control (Fig. 1B).

The results indicate that primate antiserums to cells from individual leukemic donors can detect antigens that distinguish CLL or ALL cells from AML or CGL cells and thus appear to be leukemia type-specific. There seems to be a common antigen on ALL and CLL cells detectable by the antiserums to CLL. The antiserums to AML and CGL are detecting more than one antigen. The antigen (or antigens) detected on the CLL, AML, and CGL cells is lost by digestion with trypsin or neuraminidase, and tryptic digests of CLL cells contain antigen that has a high molecular weight. The antiserums may be important reagents for the diagnosis and immunotherapy of leukemia.

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References and Notes

1. Plasmagel was obtained from Laboratoire Roger Bellon, Neuilly-Paris, France.
2. Bacto-Adjuvant Complete H37 Ra was obtained from Difco Laboratories, Detroit, Mich.
3. R. S. Metzgar and J. L. Miller, *Transplantation* **13**, 467 (1972).
4. R. S. Metzgar, H. F. Seigler, F. W. Ward, D. T. Rowlands, Jr., *Transplantation* **13**, 131 (1972).
5. R. S. Metzgar and S. R. Oleinick, *Cancer Res.* **28**, 1366 (1968).
6. Human lymphocyte suspension cultures RPMI 4098 and 6237 were obtained from Associated Biomedic Systems, Inc., Buffalo, N.Y. Both of these cell lines are reported to be free of Epstein Barr virus.
7. Supported by NIH grant CA 08975 to Duke University and by grant FR 00165 to Yerkes Primate Research Center.

17 July 1972

Sweet Taste of Water Induced by Artichoke (Cynara scolymus)

Abstract. *Exposure of the tongue to artichoke can make water taste sweet. Two major active components of artichoke are the salts of chlorogenic acid and cynarin. The sweetening of substances by temporarily modifying the tongue, rather than by adding a substance sweet in itself, may provide an alternative to currently used nonnutritive sweeteners.*

The artichoke (*Cynara scolymus*), known to man since 700 B.C. (*1*), was originally popular partly because of the belief that it could be used as a diuretic and as an aphrodisiac (*2*); however, no unusual effects on the sense of taste were noted (*3*). To the best of our knowledge, the earliest published report of a taste-modifying property of the artichoke was Blakeslee's account of the 1934 AAAS biologists' dinner. After eating globe artichokes as the salad course, 60 percent of the nearly 250 people present reported that water tasted different—in most cases, it tasted sweet (*4*). Others have also noted anecdotally that beverages such as milk and wine, as well as water, taste sweet after the subjects have eaten artichokes, but this effect has not been studied systematically, and the artichoke constituents responsible have not been identified (*5*).

In initial tests, we compared the effects of artichoke on water with its effects on solutions of different taste qualities (that is, sucrose, citric acid, quinine hydrochloride, and sodium chloride). All solutions were sweetened to some degree by the artichoke, a suggestion that the common solvent, water, was primarily affected (*6*). We quanti-