This conclusion is supported by recent x-ray diffraction studies of HbCO Kansas (*17*).

In Fig. 2 we show the results of a similar experiment with HbO_2. The conditions were the same as before, except that atmospheric air rather than CO was present. Again we see an induced absorption with rapid rise, but in this case the response decays rapidly with a time constant of about 2.5 psec. Additional experiments with deoxygenated Hb gave the same result, indicating that this response is independent of O_2 pressure. Thus, our results indicate that the low quantum yield for photodissociation of HbO_2 is not due to rapid recombination of initially photolyzed molecules but rather to a dissociation rate that is much slower than the observed 2.5-psec nondissociative recovery. Apparently the observed response is due to a short-lived excited state whose absorption is greater than that of the ground state. At present we cannot identify the state, but its measurable lifetime has an important corollary. If this state has the same spin as the ground state, fluorescence should be observable in both Hb and HbO_2, albeit with very low quantum yield, ~ 0.001.

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References and Notes

1. E. P. Ippen and C. V. Shank, *Appl. Phys. Lett.* **27**, 488 (1975).
2. _____, A. Bergman, *Chem. Phys. Lett.* **38**, 611 (1976).
3. D. M. Surgenor, Ed., *The Red Blood Cell* (Academic Press, New York, ed. 2, 1974), vol. 2.
4. J. L. Hoard, in *Structural Chemistry and Molecular Biology*, A. Rich and N. Davidson, Eds. (Freeman, San Francisco, 1968), p. 573.
5. M. F. Perutz, *Nature (London)* **228**, 726 (1970).
6. M. Zerner, M. Gouterman, H. Kobayashi, *Theor. Chim. Acta* **6**, 363 (1966).
7. J. Haldane and J. L. Smith, *J. Physiol. (London)* **20**, 497 (1896).
8. Q. H. Gibson and S. Ainsworth, *Nature (London)* **180**, 1416 (1957).
9. R. W. Noble, M. Brunori, J. Wyman, E. Antonini, *Biochemistry* **6**, 1216 (1967).
10. T. Bucher and L. Kaspers, *Biochim. Biophys. Acta* **1**, 21 (1947).
11. M. H. Keyes, M. Falley, R. Lumry, *J. Am. Chem. Soc.* **93**, 2035 (1971).
12. G. Lang and W. Marshall, *J. Mol. Biol.* **18**, 385 (1966).
13. Human hemoglobin solutions were kindly provided to us by S. Beychok; they were passed through a Millipore ultrafilter in order to minimize sources of light scattering.
14. C. V. Shank and D. H. Auston, *Phys. Rev. Lett.* **34**, 479 (1975).
15. E. P. Ippen and C. V. Shank, in *Picosecond Pulses*, S. L. Shapiro, Ed. (Springer-Verlag, New York, in press).
16. R. H. Austin, K. W. Beeson, L. Eisenstein, H. Frauenfelder, I. C. Gonsalus, *Biochemistry* **14**, 5355 (1975).
17. L. Anderson, *J. Mol. Biol.* **94**, 33 (1975).
18. We thank J. Wittenberg for help in a preliminary experiment. R.B. was aided by NIH grant GM-19019.

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Valid Climatological Data from Historical Sources by Content Analysis

Abstract. *Content analysis is used to derive dates of freeze-up and break-up from historical descriptions of river estuaries on Hudson Bay between 1714 and 1871. Validity testing of these dates indicates that they are comparable with modern data. It is thus proposed that the method affords potential for the systematic retrieval of a broad array of environmental data from the historical past.*

Current uncertainties about short-term climatic changes are exacerbated not only by the difficulties of projecting present trends into the future, but also by the problem of securing valid information about the past. Among the types of evidence traditionally employed to elucidate past climates, that contained in historical documents has been extensively studied. Manley (*1*) has called certain sources of this nature "non-instrumental diaries" and has classified them into one of five principal categories of evidence for climatic change. More recently, Ladurie (*2*) has appealed for the development of systematic procedures for the acquisition of valid climatic measures from documentary sources. A general procedure of this nature called content analysis has been developed in the social sciences, and has been identified as one of the basic innovations of the social sciences in this century (*3*). We have employed content analysis to derive dates of break-up and freeze-up of river estuaries on Hudson Bay from 18th- and 19th-century descriptions of these estuaries. Testing of these historical dates indicates that they are not inferior in quality to those obtained within the modern Canadian observing network. Our purpose here is to report selected results demonstrating their validity, and to suggest that the method affords the potential to yield a wide array of valid environmental information about the historical past.

The documents subjected to content analysis comprise daily journals kept by personnel of the Hudson's Bay Company in the period 1715 to 1871 at forts lo-

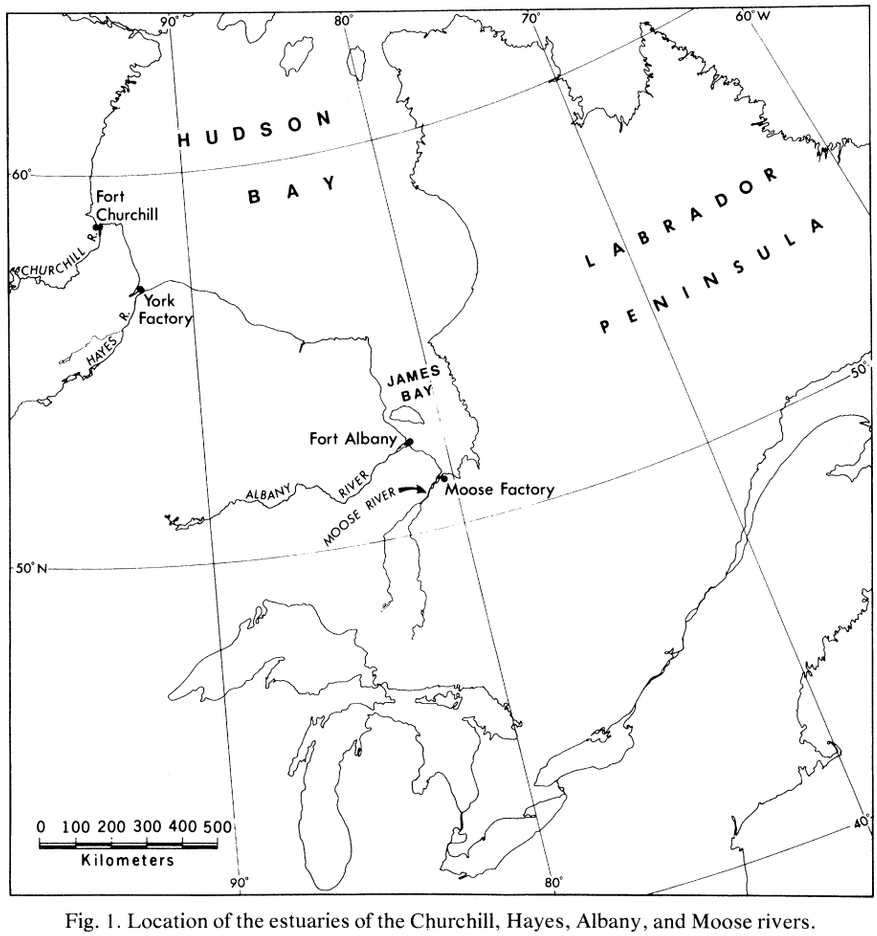

Fig. 1. Location of the estuaries of the Churchill, Hayes, Albany, and Moose rivers.

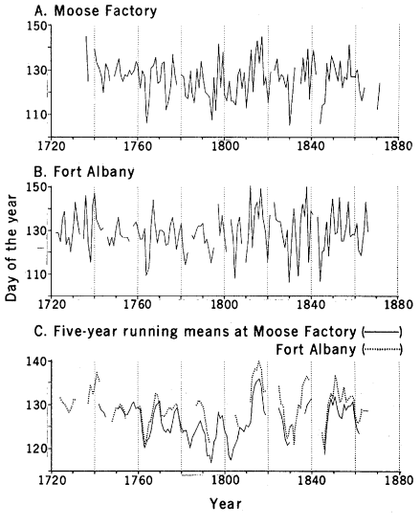

Fig. 2. Dates of first partial breaking at Moose Factory and at Fort Albany. Dates are expressed as numbers of days since 31 December and are corrected for leap years and the Gregorian calendar reform. (A and B) Actual dates; (C) 5-year running means of dates.

cated on the estuaries of the Churchill, Hayes, Albany, and Moose rivers (Fig. 1). The company's instructions ensured a degree of uniformity among these journals insofar as daily records on certain themes were faithfully recorded. Nevertheless, they differ greatly in style and content, reflecting the events of the day and the predilections of the individual journalists. Thus, although the journals provide a plethora of historical descriptions of ice and water conditions, they do not contain dates of freezing and breaking in any direct form.

In resorting to evidence of this nature, the analyst is attempting to extract scientific information from ostensibly unscientific sources. He must use the observations of predecessors whose perceptions and descriptions of the phenomenon under investigation cannot easily be measured. Unless these can be measured and tested, the nature of the documentary observations relative to their contemporary counterparts cannot be assessed and their value in ascertaining past environmental conditions remains speculative. Such conditions, however, can be readily satisfied using techniques developed in content analysis. This is possible mainly because content analysis permits the conversion of lexical and other qualitative information into numerical form. On the one hand, this permits gauging the perceptions of past observers with much more precision than classical methods of historical analysis allow. And on the other hand, the conversion of the historical observations into numbers facilitates ready and precise comparison with their contemporary counterparts.

The most widespread technique whereby textual material is given quantitative expression in content analysis is the frequency count. In this procedure, inferences are derived strictly from the frequency with which specified symbols or themes appear within text. Associated with frequency counts are measures of intensity and direction of assertions within text. Contingency analysis is employed when researchers shift from single-variable analysis to multivariate analysis within text. It "asks not how often a given symbolic form appears in text, but how often it appears in conjunction with other symbolic units" (*4*). Using these procedures, there are few documents that will not submit to quantification, and the method thereby permits the application of mathematics and statistics to virtually the entire range of source materials that might be employed in historical reconstructions of climate.

In this study, frequency counts were employed to analyze the ways in which the journalists observed and described ice and water conditions in the four river estuaries in the period 1714 to 1871. The counts revealed that the journalists most frequently recorded, and were most impressed by, the early manifestations of freezing and breaking, rather than the terminal stages of these two processes. It was also clear that they were overwhelmingly concerned with recording direct observations of the physical appearance of ice and water conditions, as opposed to indirect references to their effects on transport and other human activities. Among the direct descriptions, those identifying the effects of mechanical forces on ice and water surfaces were most prominent, accounting for 74 percent of the descriptions during the break-up period and 45 percent of freeze-up descriptions. By contrast, descriptions of the thermal processes of melting and freezing of surfaces comprised less than 2 percent of break-up comments and 13 percent of freeze-up comments. In this manner, a variety of consistent biases in the perception and recording of ice and water conditions were identified. These were used to reconstruct dates comparable to those recorded in the contemporary observing network in Canada. Thus, the results presented here are dates of first partial breaking which have been derived from journal descriptions only where the observational criteria measured in these descriptions are similar to those published in 1959 (*5*) and 1964 (*6*) by the Canadian Meteorological

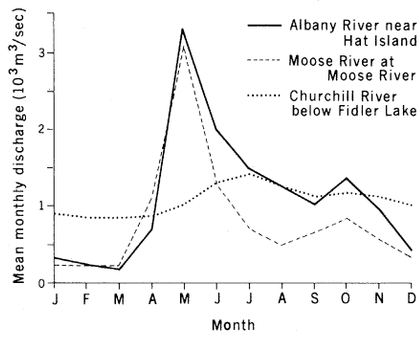

Fig. 3. Monthly mean discharges of Albany River (1965 to 1970), Moose River (1959 to 1970), and Churchill River (1959 to 1970). Each station is located in the lower reaches of the river.

Branch for isolating the commencement date of break-up.

Figure 2, A and B, shows the dates of first partial breaking at Moose Factory and Fort Albany, and Fig. 2C contains 5-year running means of these dates. Despite the complex variations in these data, a careful comparison of the curves in Fig. 2, A and B, will detect a close parallelism between them. This is reflected in a Pearson product-moment correlation coefficient of +.84 between the date of first partial breaking at Fort Albany and that at Moose Factory in the period 1742 to 1862. This parallelism, and the general nature of the long-term trends, are evident in Fig. 2C.

The two sets of data from the Moose and Albany estuaries derive from sources located more than 150 km apart and, in view of their independence, this parallelism is indicative of their validity. Validity testing in climatology is conceived in terms of the spatial homogeneity of data and is based on the assumption that the data observed in small climatological regions will respond in unison to large-scale weather processes. This is directly analogous to the procedure of concurrent validation in content analysis, wherein the validity of one set of measurements is established by its ability to confirm external ones. The correlation coefficient of +.84 obtained between 111 pairs of dates of first partial breaking at Moose Factory and Fort Albany both reflects a remarkable hydroclimatic similarity between the two river basins (Fig. 3) and is a measure of the validity of the dates extracted from the journals. The coefficient can be compared with similar coefficients derived from dates of first breaking observed within the modern Canadian network at stations paired on the basis of hydroclimatic similarities. Of 27 such pairs of stations, 24 yielded correlation coefficients less than that obtained

between Moose Factory and Fort Albany (*7*).

The content analysis procedures developed to objectively extract the dates described above are fully elaborated elsewhere (*8*) and can, with modification, be employed in any environmental research into descriptive, historical accounts. Thus, the method can be extended to a variety of environmental phenomena and to periods and regions for which instrumental data are lacking.

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References and Notes

1. G. Manley, *Q. J. R. Meteorol. Soc.* **79**, 188 (1953).
2. E. le R. Ladurie, *Times of Feast, Times of Famine* (Allen & Unwin, London, 1971), p. 268.
3. K. W. Deutsch, J. Platt, Senghaas, *Science* **171**, 252 (1971).
4. I. de S. Pool, *Trends in Content Analysis* (Univ. of Illinois Press, Urbana, 1959), p. 196.
5. Canada, Department of Transport, Meteorological Branch, *Break-up and Freeze-up Dates of Rivers and Lakes in Canada* (Ottawa, 1959), pp. 2 and 50.
6. W. R. T. Allen, in *Break-up and Freeze-up Dates in Canada* (Canada, Department of Transport, Meteorological Branch. Ottawa, 1964), p. 2.
7. B. Singh, thesis, University of Manitoba (1973).
8. D. W. Moodie and A. J. W. Catchpole, *Environmental Data from Historical Accounts by Content Analysis* (Department of Geography, University of Manitoba, Winnipeg, 1975).

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Expression of C4 on Human Lymphoid Cells and Possible Involvement in Immune Recognition Phenomena

Abstract. *Immunochemical studies revealed the presence of the fourth component of complement (C4) on surfaces of human lymphoid cells. Antiserums to C4 inhibited the mixed lymphocyte reaction and the mitogenic response to phytohemagglutinin, suggesting a role for membrane-associated C4 in the afferent phase of immune recognition phenomena.*

Genes located in the major histocompatibility complex (MHC) specify antigens and control functions concerned with immune recognition. In man, the genes coding for antigens involved in allograft rejection reside in three regions termed locus A, locus B, and locus D (formerly denoted as the LA, FOUR, and MLR regions, respectively). Antigens determined by locus D regulate allogeneic recognition in the mixed lymphocyte reaction (MLR) and are now characterized by quantitating the incorporation of [^{3}H]thymidine in mixtures of allogeneic lymphocytes after 5 days of culture at 37°C. The products of the locus A and locus B regions of the HLA system are target antigens for cell mediated lymphocytotoxic reactions (*1*), and are readily detected on cells and in serum with monospecific antiserums in a cytotoxic test dependent on complement (C). Genes regulating the lytic activity of serum complement in mice (*2*) and the expression of several complement components, including the third component (C3) in mice (*3*), factor B in monkeys (*4*), and the second component (C2) (*5*) and factor B (*6*) in man, are linked to genes governing the expression of MHC antigens. These observations led to our investigation of whether complement components might be present on the cell surface, as are other MHC coded antigens, and whether they play a role in cellular immune recognition phenomena. We report here that the fourth component (C4) is expressed on the surfaces of human lymphoid cells and that antiserums to C4 interfere with allogeneic recognition in the MLR.

Human lymphoid cell lines Raji, RPMI 1301, RPMI 1788, and WI-L2 were grown in suspension cultures in RPMI 1640 medium supplemented with 10 percent fetal calf serum (*7*). Exponentially growing cells were used for most studies.

Human peripheral lymphocytes were isolated from heparinized blood by centrifugation on Ficoll-Hypaque after plasmagel sedimentation of erythrocytes (*8*). The cells were washed with Hanks balanced salt solution (BSS) before use.

Antiserums to isolated human C4 (*9*) were raised in rabbits and goats. Antiserums were also produced in rabbits to human C4 attached to autologous rabbit erythrocytes by the action of activated C1s (*10*).

Operationally monospecific HLA alloantiserums were obtained from the serum bank at the National Institute of Allergy and Infectious Diseases.

The four goat antiserums and four of the five rabbit antiserums used in these studies were monospecific for C4 without absorption, while the fifth rabbit antiserum was monospecific after absorption with isolated immunoglobulin G (IgG).

We used two techniques to demonstrate C4 on the lymphoid cell surfaces. First, varying numbers of washed, packed, cultured lymphoid cells were incubated with undiluted rabbit or goat antiserums to C4 (anti-C4). Depletion of anti-C4 reactivity was assessed by reduced ability of the antiserum to precipitate C4 after immunoelectrophoretic separation in agar. Cultured human lymphoid cells RPMI 1788 produced a dose-dependent absorption of rabbit antibody to C4 with the largest number of cells used (1.2×10^7 per microliter) leading to virtually complete absorption. Similar results were obtained with WI-L2 cells and with two other rabbit antiserums and one goat antiserum to C4. Since the absorption procedure produced only a twofold dilution of the antiserum as evidenced by dilution of ^{125}I-labeled human albumin incorporated in the antiserum, the absorption of anti-C4 reactivity cannot be explained by dilution of the antibody.

Second, we quantitatively determined the expression of C4 on surfaces of cultured human lymphoid cells by a slight modification of the method of Ohanian and Borsos (*11*). Varying numbers of packed, washed lymphoid cells, typically 3×10^8, 1.5×10^8, 7.5×10^7, and 3.7×10^7, were incubated with 150 μl of an appropriate concentration of the IgG fraction of antiserum to C4. After 90 minutes at 37°C, the lymphoid cells were removed by centrifugation, and 100 μl of the supernatant was assayed for remaining anti-C4 reactivity by measuring its ability to sensitize sheep erythrocytes coated with C4 (EC4) (*10*), for lysis by guinea pig complement. The concentration of the IgG fraction of the anti-C4 used in our studies produced 90 percent lysis of EC4. A reduced amount of lysis was obtained after incubation of the antiserum with a source of C4. An inhibition curve with isolated C4 was included in all experiments that permitted conversion of the amount of lysis observed after incubation of the antiserum with lymphoid cells into nanograms of C4. These values were then converted to C4 molecules per lymphoid cells; we used a molecular weight of 204,000 for C4 (*9*). The numbers of C4 molecules per cell were 487 for Raji cells, 1150 for RPMI 1301 cells, 700 for RPMI 1788 cells, and 680 for WI-L2 cells. Two different antiserums to C4 and two C4 preparations produced comparable results. We used two controls. First, 5×10^9 human erythrocytes and sheep red blood cells were used to absorb the anti-C4. No absorption could be detected. Second, since cultured human lymphoid cells were perpetuated in medium containing 10 percent fetal calf serum, the ability of fetal calf