These experiments show that the difference in adaptation rates between the two types of stretch receptor cells should be attributed to differences in the properties of their electrically excitable membrane components rather than to differences in the mechanisms of their generator potentials. However, the interplay of changes in the electrically excitable membrane with the changes observed in the generator potentials during a sustained stretch probably determine the rates of adaptation among individual neurons within each class of receptor cells.

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Replacement Rates for Human Tissue from Atmospheric Radiocarbon

Abstract. *Carbon-14, derived from the testing of thermonuclear weapons in the atmosphere of the Northern Hemisphere during 1961–62, has been found in human tissues including the brain in amounts which reflect the atmospheric concentration of carbon-14 as of several months earlier. In collagen of cartilage, the rate of uptake of carbon-14 is much slower than in other tissues; essentially no radioactive carbon was found in the collagen of 70-year-old adults that had been exposed to the comparatively high concentrations of carbon-14 in the atmosphere during the years 1954 to 1964. Individuals from the Southern Hemisphere show little increase in the carbon-14 content of their tissues at present, and detailed tests with individuals traveling to the Northern Hemisphere from the Southern allow closer scrutiny of the tissue replacement rates.*

Information on the metabolic turnover of the constituents of human tissue is of considerable importance and interest to all students of the life sciences. However, such information has been difficult to obtain because of the reluctance to use radioactive tracers in considerable dosages in normal human beings. Experiments with radioactive tracers in animals usually necessitate the administration of isotopes in micro- to millicurie quantities (3.7×10^4 to 3.7×10^7 disintegrations per second). Such quantities permit the isolation of desired compounds while maintaining a sufficient amount of radioactivity to be measured within a reasonable time and at a satisfactory statistical level in solid or liquid scintillation counters. It has been accepted generally that carbon-14 is more satisfactory as a tracer than tritium or deuterium, because of the smaller isotope fractionation effects encountered with radiocarbon.

The techniques of radiocarbon dating permit the very accurate and sensitive assessment of C^{14} in organic materials at only picocurie intensities (3.7×10^{-2} disintegrations per second). Since the amount of C^{14} in atmospheric carbon dioxide has risen suddenly in the Northern Hemisphere to almost double the reference value of 1890 as a consequence of the testing of atomic and hydrogen bombs in the atmosphere (mainly during 1961 and 1962), and because this new C^{14} has not had time to equilibrate with the biosphere, the radiocarbon can be utilized as a tracer in living things including humans.

That such studies with humans are feasible has been shown in an experiment by Broecker *et al.* (*1*), who analyzed the C^{14} content of the blood and breath of one individual, and the lung tissue of another. Their major conclusions were, first, that the interval between fixation of carbon in the average foodstuffs and their consumption is somewhat less than a year and, second, that the maximum time during which the C^{14} content of the blood lags behind that of the food is about 6 months. At present it is not possible to estimate directly the turnover rate of C^{14} in man from such measurements. However, continued measurements over a longer time interval plus supportive investigations with individuals traveling to the Northern Hemisphere from the Southern, where the increase has not occurred yet to any appreciable degree, may make such an estimate possible. Thus we have an opportunity to detail the replacement rates for tissues in humans and, thus, to better assess the possible hazards in medical use of C^{14} and the dangers of atmospheric contamination from the testing of nuclear weapons. We have investigated the C^{14} content of various human tissues, including the brain, and have studied the rates of turnover of some of the tissue components.

All tissues other than blood were obtained at autopsy from unembalmed male subjects of a single age group who had resided in Los Angeles for longer than 20 years, and who had died during January 1964 (*2*). The nature of their illnesses and causes of death are given in Table 1.

The tissues to be analyzed were dissected free from connective tissue, fat, meninges, and blood vessels, and then were frozen and lyophilized. The dried tissues were extracted with 10 volumes of hot ethanol. This was accomplished

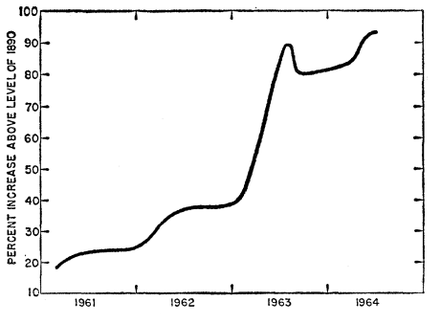

Fig. 1. Radiocarbon activity in carbon dioxide in surface air at China Lake (117°41′W, 35°37′N) (data by G. J. Fergusson, updated by R. Berger).

by grinding with ethanol in a Waring blendor, boiling the slurry for 5 minutes, and then filtering. The filter cake was further extracted with 10 volumes of a mixture of alcohol and ether (1 : 1) and finally with 10 volumes of diethyl ether. The resulting proteinaceous material left undissolved by the three extractions was dried thoroughly in a vacuum. In the case of the brain tissues the three extracts were pooled and boiled free from solvent to yield the "lipid" sample for comparison with the protein fraction.

The blood samples were withdrawn from healthy adult residents of Los Angeles, Calif., and Melbourne, Australia, and were collected in 500-ml containers containing approximately 110 ml of anticoagulant solution (*3*). The red cells were separated by sedimentation, the plasma was removed by aspiration, and the cells were washed three times with 0.85-percent saline solution (*4*). Both cells and plasma were dried by lyophilization. These samples were extracted in the same manner as the tissue samples.

All samples were converted to carbon dioxide in a stream of oxygen and argon in a combination combustion and vacuum line. The carbon dioxide was absorbed in 4*N* NaOH. For additional purification, the carbon dioxide was liberated with 6*N* HCl and passed through wash towers containing 0.1*N* $AgNO_3$ solution and was then passed over copper oxide at 500°C. The pure carbon dioxide was admitted to the 8-liter proportional counter used for radiocarbon dating and measured overnight for 1000 minutes (*5*). The operation of the counter was intermittently checked with samples of carbon dioxide prepared from the oxalic acid used (with marble as background) by most radiocarbon dating laboratories as a modern uncontaminated standard.

Table 1. Clinical data on subjects used at autopsy for radiocarbon analyses.

No. of subject	Age (yr)	Date of death	Diagnoses
Hu- 9	73	1/ 9/64	Acquired hemolytic anemia; diabetes mellitus; terminal bronchopneumonia
Hu-10	74	1/10/64	Primary refractory anemia; pyelonephritis; septicemia
Hu-11	74	1/17/64	Myocardial infarction; arteriosclerosis; diabetes mellitus
Hu-12	73	1/21/64	Carcinoma of bladder with extensive metastases

The results are summarized in Tables 2 and 3. All data refer to the percentage increase in radiocarbon activity above the reference value of 1890, which is equivalent to 95 percent of the count rate of the National Bureau of Standards oxalic acid standard.

The data in Table 2 may be compared with those in Fig. 1, which show the rise in C^{14} activity in atmospheric carbon dioxide during recent years at a rural site in southern California (China Lake). Presumably, the values in this graph may be loosely applied to the whole adjoining United States.

Schoenheimer in his monograph emphasized the dynamic state of body constituents (*6*). Thompson and Ballou (*7*), on the other hand, using tritium as a tracer, found that in most tissues of the rat the components had turnover rates of over 100 days; only a few components had turnover rates of 22 days or 4 days. The most notable long-lived component was collagen which showed very little turnover.

Our results confirm the metabolically "inert" character of collagen. The cartilage samples analyzed had incorporated essentially no C^{14} during the 10-year period in which the concentration of C^{14} in the atmosphere increased. Presumably, C^{14} will be found in the cartilage of younger subjects in whom active collagen synthesis is proceeding. This suggests that a study of the C^{14} content of collagen of humans of different ages would provide information on metabolic activities of this tissue.

Experiments with rats, conducted by Nicholas and Thomas (*8*) with C^{14}-labeled acetic acid, showed that cholesterol and fatty acids in the brain were labeled negligibly when the acetate was injected intraperitoneally. However, the so-called blood-brain barrier could be bypassed by injecting labeled acetate directly into the brain, a procedure which was followed by an immediate and significant incorporation of C^{14}-labeled acetate into the brain lipids. When Bloch *et al.* (*9*) administered deuterated cholesterol intravenously, they found labeled cholesterol in all organs except the brain or spinal cord. These experiments indicate that the rate of incorporation of organic carbon from the plasma into the fatty acids and cholesterol of the adult brain and spinal cord is negligible.

Table 2. The percentage increase in the C^{14} content of the body tissues over the reference value for 1890, in four subjects, Hu-9 through Hu-12.

Fraction	Hu-9	Hu-10	Hu-11	Hu-12
	Brain			
Total protein	41.0			
Total lipids	41.0			
	White matter			
Protein		32.3	35.0	35.1
Lipids		31.9		25.3
	Heart			
Protein	40.7		36.4	
	Liver			
Protein	40.2		41.1	
	Cartilage (collagen)			
	− 0.6		+ 0.3	

Our experiments indicate that in the adult human being there is a definite, significant incorporation of C^{14}-labeled material into the brain proteins and lipids, and that this incorporation has caused the activity of C^{14} in these substances to approach the same magnitude as that observed in the liver, heart, plasma protein, and erythrocyte proteins. It is possible that, in the subjects we studied, a major part of the accumulated C^{14} arose from the fixation of C^{14}-labeled carbon dioxide, by means of a rapid and efficient process participating in the formation of aspartic and glutamic acids in the brain (*10*). In fact, Siesjo and Thompson (*11*), using rats exposed to an atmosphere containing $C^{14}O_2$ found that in

Table 3. The percentage increase in the C^{14} content of the blood over the reference value for 1890.

Date of collection	No. of subject	Protein fraction	
		Plasma	Erythrocytes
8/9/62	Hu-13	23.4	
8/10/62	Hu-14	23.8	
9/11/63	Hu-15	33.4	
1/8/64	Hu-16	55.9	35.4
1/8/64	Hu-17	46.4	26.2
5/28/64	Hu-21	54.2	53.5
6/18/64	Melbourne*	57.0	13.7

* Blood sample obtained from an Australian subject who had been in the U.S. for 14 days.

120 minutes the protein of the brain was labeled to the extent of 10 percent of the concentration of $C^{14}O_2$ in the atmosphere. The lipid and nucleic acid fractions, however, were not significantly labeled during this time. It appears, therefore, that, in agreement with many other studies, the brain proteins incorporate C^{14} very rapidly (*11*). The turnover of lipids in the white matter of the brain is not so readily explained, however, and this observation must be clarified by further experimentation.

Some correlation appears to exist between the concentration of C^{14} in the atmosphere and that observed in human blood. The erythrocyte protein of the blood donated in January 1964 reflects the amount of C^{14} in the atmosphere during 1962, a difference of about 1½ years, which is in agreement with the conclusions of Broecker *et al.* (*1*). Since plants, during their periods of growth, would be expected to possess essentially the same amount of C^{14} as that in the surrounding atmosphere, radiocarbon would be incorporated into primary foodstuffs such as grain and hay mainly during the spring. Therefore, the erythrocyte and plasma samples appear to reflect the atmospheric radioactivity at the time of maximum photosynthesis in the spring preceeding the corresponding harvest. Presumably, the grain of the 1962 harvest, and the domestic animals fed with it, contained the same amount of radioactivity as was present in the atmosphere during spring 1962. At the time the new harvest of 1963 was being used as human food, its radioactivity had risen to the values found in the blood plasma obtained during January 1964. As more and more 1963 foodstuffs replaced those of 1962, the radioactivity of the erythrocyte protein rose to the same value as that in the plasma obtained from subject HU-21. The radioactivity of the erythrocyte protein has not increased further because that of the bulk of foodstuffs still remains at the spring-1963 value. Similarly, the radioactivity of the plasma sample of September 1963 was at a level comparable to that of the erythrocytes of January 1964. Presumably, erythrocytes during the fall of 1963 may have shown about the same radioactivity as the respective plasma.

Since the radiocarbon activity of erythrocytes and several other body tissues are grossly similar, it may be possible to make reasonable deductions from these data about other body components when only blood data are known. It will be necessary to amass additional data for greater understanding of this problem.

Several other questions arise as a result of this work. For example, what is the significance of the incorporation of appreciable amounts of C^{14} into the white matter (and presumably myelin), proteins, and lipids of the brain? Could analyses of blood samples obtained serially during changes in the C^{14} content of the atmosphere yield information concerning equilibration rates of various tissues? Or could such information be obtained by examining blood samples from individuals arriving in the Northern Hemisphere after they have resided for long periods in the Southern Hemisphere, where the concentration of atmospheric C^{14} lags behind the concentration in the Northern Hemisphere by at least 1 year?

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13. We are indebted to Prof. G. J. Fergusson for his advice and assistance and for his giving us permission to use Fig. 1. This research was supported by NSF grant GP-1893, a continuation of grant G-14287; and by contract AT(04-1) GEN-12 between the AEC and the University of California.

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Hydroxyurea: Inhibitory Effect on DNA Metabolism

Abstract. *Hydroxyurea, hydroxyurethane, and dihydroxyurea inhibit incorporation of thymidine into the DNA of monolayers of HeLa cells. They do not affect incorporation of uridine into RNA or of leucine into protein. In contrast, hydroxylamine inhibits cellular incorporation of all three precursors: thymidine, uridine, and leucine. Hydroxyurea does not affect thymidine kinase, thymidylate kinase, or DNA polymerase reactions, but it does inhibit incorporation of cytidylic and guanylic acids into DNA in cell-free supernatants.*

Hydroxyurea is under study as a cancer chemotherapeutic agent. It has antileukemic activity in mouse (*1*) and man (*2*). The mechanism by which it produces biological effects is unknown, but two proposals have been advanced. (i) Hydroxyurea is hydrolyzed in vivo and yields free hydroxylamine. This then cleaves thioesters, in particular acetyl-coenzyme A, disrupting oxidative phosphorylation and reducing concentrations of cellular and mitochondrial adenosine triphosphate (ATP) (*3*). (ii) Hydroxyurea and a number of related derivatives of hydroxylamine cause fragmentation of isolated DNA and induce chromosomal abnormalities in mammalian cells, cultured in vitro (*4*). The postulated common mechanism is that oxidative transformation of these compounds occurs, forming the nitroxyl radical (HON=) which becomes dimerized as hyponitrous acid (HON=NOH). This induces cleavage of the main chain of cellular DNA (*4*). As already reported, hydroxyurea inhibits incorporation of radioactive thymidine into the DNA of monolayers of HeLa cells (*5*). We now examine effects of hydroxyurea and a number of related compounds on the incorporation of thymidine into DNA, of uridine into RNA, and of leucine into protein of HeLa cells. We also report our efforts to identify a site of hydroxyurea-induced inhibition in DNA metabolism.

A sequential isotope technique was used to study acute effects of drugs on protein and nucleic acid metabolism of monolayers of HeLa cells grown on glass coverslips (*6*). The technique involved 15- to 30-minute prior incubation of the cells, without drugs, in