2. G. G. Avtandilov, Morphometry and Pathology [in Russian], Moscow (1973).
3. I. P. Anokhina and B. M. Kogan, Progress in Science and Technology. Series: Toxicology [in Russian], Vol. 13, Moscow (1984), pp. 151-178.
4. K. Yu. Astashenkova, Lab. Delo, No. 3, 186 (1973).
5. I. G. Zabirova and V. P. Nuzhnyi, Patol. Fiziol., No. 2, 25 (1982).
6. F. B. Levin and I. M. Yakobson, Lab. Delo, No. 4, 223 (1978).
7. V. P. Nuzhnyi, E. B. Tezikov, E. V. Savitskaya, A. I. Ugryumov, Byull. Éksp. Biol. Med., 101, No. 5, 575 (1986).
8. S. Axelsson, A. Bjorklund, B. Falck, et al., Acta Physiol. Scand., 87, No. 1, 57 (1973).
9. H. Ikeda, Folia Psychiat. Neurol. Jpn., 31, No. 1, 3 (1977).
10. S. Mallov, Stress and Alcohol Use, New York (1983), pp. 369-386.
11. L. A. Pohoresky, Fed. Proc., 41, No. 8, 2452 (1982).
12. E. C. Rich, C. Siebold, and B. Compion, Arch. Intern. Med., 145, No. 5, 830 (1985).
13. R. Sipilä, Lancet, 1, 391 (1985).

CARBON ISOTOPE FRACTIONATION IN ATHEROSCLEROTIC HUMAN TISSUE

V. P. Kaznacheev, E. F. Doil'nitsyn, S. P. Gabuda, A. F. Rzhavin, and N. G. Malikov

UDC 616.13-004.6-073.916:546.26.02.
13/-033

KEY WORDS: fractionation of isotopes; carbon; atherosclerosis; prostaglandin E; cAMP.

Biological fractionation of isotopes [4] implies that in living organisms there is a higher proportion of the ^{12}C isotope than in the carbon dioxide of the air. This phenomenon is connected with what is called the normal kinetic isotope effect, in accordance with which the velocity of chemical reactions increases with the participation of lighter isotopes. In biochemical enzymic reactions thermodynamic isotope effects also may arise [1], in connection with the accumulation of heavy isotopes during an increase in energy of the chemical bond. It can be postulated that analysis of isotope distributions in the tissues under normal and pathological conditions may shed some light on the molecular mechanisms of onset of pathological states.

Sensitivity of biological fractionation of carbon isotopes during aging and also in atherosclerosis and senile cataract, was found for the first time in the investigation described below. According to the generally accepted view, the sclerotic process is connected with general slowing of biochemical processes, and we accordingly postulated that during its development a tendency will be observed for the excess of the ^{12}C isotope in sclerotic tissues to be below normal.

EXPERIMENTAL METHOD

Autopsy material was taken from the abdominal aorta, both unchanged areas and also areas showing various stages of atherosclerotic changes in the arterial wall. An adipose strip — a region of the aortic wall with lipoidosis measuring 1 × 2 mm — was excised with all layers of the aortic wall. Fibrous plaques — the aortic wall corresponding to the site of the lesion — also was excised with all its layers. Atheromatous plaques with ulcers — a fragment of the aortic wall — were excised at the center of atheromatous ulceration, with involvement of the aortic wall to different depths. Thus the aortic material for testing could be subdivided in accordance with the generally accepted classification of the atherosclerotic process. A native specimen weighing about 0.1 g was placed in a quartz cuvette and dried in vacuo at room temperature, then introduced into a circulation reactor for oxidation in pure oxygen. The carbon dioxide thus obtained was purified cryogenically to remove impurities and introduced

Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR. Institute of Geology and Geophysics, Siberian Branch, Academy of Sciences of the USSR, Novosibirsk. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 9, pp. 295-296, September, 1987. Original article submitted November 21, 1986.

 0007-4888/87/0009-1218$12.50

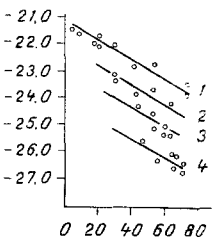

Fig. 1. Dependence of carbon isotope composition in aortic wall on age and degree of involvement with atherosclerosis. Abscissa, age (in years); ordinate, $\delta^{13}C$ (in $^0/_{00}$. 1) Microscopically unchanged region of aorta, 2) adipose strip, 3) fibrous plaques, 4) atheromatous plaques with ulceration.

into a mass spectrometer for analysis. If the specimen had to be kept for analysis later, the gas was sealed into a glass ampul. Altogether 28 specimens of the aorta (aged from 1 month to 70 years) were investigated; 10 of them consisted of microscopically unchanged regions of the aorta, and 18 were from subjects aged from 26 to 68 years with various stages of involvement with atherosclerosis. Isotopic measurements also were made on 27 specimens of the lens of the human eye (11 normal eyes, 16 affected by cataracts). The isotopic composition was determined by the usual method on a modernized MI 1201 mass spectrometer [2]. The ^{13}C content was determined against a laboratory standard $D - 1\delta^{13}C_{PDB}$–25.0%, the accuracy of determination being 0.2%.

EXPERIMENTAL RESULTS

With an increase of age and as the stages of atherosclerosis became more advanced, there was a systematic decrease in the content of the ^{13}C isotope in the specimens of arterial wall (Fig. 1). For the normal aorta (10 specimens aged from 1 month to 70 years) the value of $\delta^{13}C$ varied from −21.3 to −23.9% (average − 22.3%).

Comparison with the data in Fig. 1 shows that age changes in isotope composition were distinctly less than variations in the ^{13}C content in different regions of the same aorta, with different degrees of involvement in atherosclerosis.

Similar results were obtained in a study of specimens of the lens. For 11 specimens of normal lens from subjects of different ages values of −22.0 to −23.3$^0/_{00}$ (average −22.7$^0/_{00}$) were obtained for $\delta^{13}C$. For lenses affected with cataract (aged from 23 to 84 years) values of $\delta^{13}C$ varied from −22.8 to −25.6$^0/_{00}$ (mean −24.2$^0/_{00}$). Thus a decrease in the content of the ^{13}C isotope compared with normal tissue was found in the atherosclerotic aortic wall and in the lens affected with cataract.

It follows from these results that in tissues involved with the sclerotic process no slowing of biochemical conversions in the tissues takes place. To explain the effect of a decrease in the content of the ^{13}C isotope observed in atherosclerotic tissues of the aorta in terms of the normal kinetic isotope effect it has to be postulated that the velocities of the biochemical conversions increase significantly both during aging and in tissues involved with sclerosis. This conclusion can be regarded as the experimental basis for the hypothesis recently developed, according to which the development of sclerotic changes is associated with decreased production of prostaglandin E (PGE) in the tissues.

In fact, it is considered to be well established [3] that PGE prevents cAMP accumulation in tissue cells. Hence it follows that lowering of the PGE level leads to elevation of the level of cAMP, a catalyst for several biochemical conversions. We also know that cAMP is an intermediate hormone in the activation of adipose tissue lipase. The observed shift in isotope equilibrium in sclerotic tissues may therefore be regarded as being directly connected with acceleration of biochemical processes due to an increase in the cAMP concentration in these tissues as the result of a fall in PGE activity.

From the thermodynamic aspect, the acceleration of biochemical conversions which we observed in sclerotic tissues and which we interpreted as a reaction to slowing of PGE production in the tissues, can be regarded as one manifestation of Le Chatellier's principle. In

fact, in the region of tissue in which the regulator concentration falls, a reaction arises which attempts to compensate the unfavorable consequences of this action (since the prostaglandin regulators are one of the commonest found in the body).

LITERATURE CITED

1. É. M. Galimov, The Nature of Biological Isotope Fractionation [in Russian], Moscow (1981), p. 246.
2. E. F. Doil'nitsyn, V. I. Zyuzin, and A. P. Pertseva, Physiochemical Methods of Investigation in Geology [in Russian], Novosibirsk (1981), pp. 134-138.
3. A. S. Panosyan, Khim. Prir. Soedin., No. 6, 783 (1983).
4. A. O. Nier and E. A. Gulbransen, J. Am. Chem. Soc., 61, 697 (1939).

ANTIOXIDANT-INDUCED CALCIUM TRANSPORT IN BIOLOGICAL MEMBRANES

K. O. Muranov, N. V. Buldygerova, E. S. Kovaleva, A. N. Erin, L. L. Prilipko, A. A. Shvedova, and V. E. Kagan

UDC 615.272.4.014.425.065:616-008.924.1

KEY WORDS: biological membranes; antioxidant; calcium.

The process of lipid peroxidation plays an important role in the development of several pathological states [1]. Data have recently been published on the side effects of synthetic antioxidants, especially when used in high doses [7, 11]. Since molecules of synthetic antioxidants, which as a rule are arotomatic compounds, do not correspond structurally to the packing of the fatty-acid residues of phospholipids, the formation of defects in the lipid bilayer of the membrane can be expected on their insertion. This, in turn, is bound to affect ionic homeostasis, as has been demonstrated for artificial bilayer membranes [3].

Calcium ions (Ca^{++}) are one of the universal cellular regulators. It can therefore be understood why damage to Ca^{++}-transporting systems may be a cause of various diseases and, in some cases also, of death of the cell [12]. The aim of the present investigation was accordingly to study the effect of antioxidants on the distribution of Ca^{++} and its transport through biological membranes.

EXPERIMENTAL METHOD

Platelets were isolated from the blood of noninbred rats by the method in [9]. Aggregation was induced in medium of the following composition (in mM): NaCl — 134, KCl — 5, $MgSO_4$ — 1, Na_2HPO_4 — 0.5, HEPES — 10, glucose — 5 (pH 7.4, at 37°C). Aggregation was induced with arachidonic acid (50 μM) and recorded as the change in scattering of light (using an aggregometer from "Chronolog Corp.," USA). The rate of aggregation was estimated as the tangent of the angle of slope of the tangent drawn to the curve corresponding to the rapid phase of aggregation.

Crude and purified fractions of synaptosomes from rat cerebral cortex were obtained by the method in [8]. Release of ^{3}H-serotonin (13.2 mCi/mmole, "Amersham International," England) by the crude synaptosomal fraction was stimulated by 50 mM KCl. Incubation and washing were carried out in medium of the following composition (in mM): NaCl — 120, KCl — 5, NaH_2PO_4 — 1.2, $MgCl_2$ — 1.3, $CaCl_2$ — 0.73, Tris-HCl — 20, glucose — 10, pargyline — 10 μM (pH 7.4 at 37°C). The samples were filtered on GF/B filters ("Whatman," England), and washed 3 times with cold Tris buffer. Radioactivity was measured on a "RackBeta" liquid scintillation counter (LKB, Sweden).

Institute of Chemical Physics, Academy of Sciences of the USSR. All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. Institute of Physiology, Bulgarian Academy of Sciences, Sofia.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 9, pp. 296-299, September, 1987. Original article submitted January 30, 1987.

0007-4888/87/0009-1220$12.50