EFFECT OF TEMPERATURE ON KINETICS OF PARAMAGNETIC-CENTER FORMATION IN COMPLEXES OF CARCINOGENIC HYDROCARBONS WITH HALOGEN ELECTRON ACCEPTORS

M. M. Petyaev, V. S. Skotnikov, and V. V. Denisov

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It is shown by the electronic paramagnetic resonance (EPR) method that carcinogens have a well-marked ability to form paramagnetic complexes with charge transfer with an electron acceptor, of the bulky radical or semiconductor catalyst type. The resulting kinetic curves indicate that processes of this type are possible at the body temperature of man and animals. The intensity of the EPR signals and the corresponding concentration of the complex with charge transfer correlate with the known carcinogenic activity of the substances tested. This suggests the specific catalytic role of these magnetic complexes in the induction of free-radical branching chain (degenerate) nonenzymic liquid-phase oxidation reactions.

It has been shown by the electron paramagnetic resonance (EPR) method [1-3] that carcinogenic hydrocarbons, unlike their noncarcinogenic analogs, can form paramagnetic centers in model experiments with halogens. For example, the powerful carcinogen 3,4-benz[a]pyrene, on heating with crystalline iodine, forms a complex which gives a strong EPR signal (varying with the degree of heating). Noncarcinogenic 1, 2-benzpyrene does not give an EPR signal under these conditions. This important physical feature of the carcinogenic compounds may perhaps be connected with their specific activity and with their etiological biophysical mechanism.

Because of the high probability that carcinogenic hydrocarbons may encounter and form complexes with halogens (F, Cl, Br, I) or other electron acceptors in the body, the study of the effect of temperature conditions on this complex formation is an important task. If the paramagnetic properties of these complexes are sufficiently well expressed within the range of body temperatures of man and animals (while those of noncarcinogenic substances are not), it can evidently be assumed that these magnetic properties of the complexes are of biological carcinogenic importance.

In the investigation described below the EPR method was used to study the paramagnetic properties of artificial complexes of carcinogenic substances at different temperatures and, in particular, at temperatures close to that of the human body.

EXPERIMENTAL METHOD

The materials used were 3,4-benzpyrene (BP; Lawson, England), 1,2-benzpyrene (Schuchardt, West Germany), 20-methylcholanthrene (MC; puriss., Fluka AG, West Germany), 7,12-dimethylbenz[a]anthracene (DMBA; Fluka AG, West Germany), 1,2,5,6-dibenzanthracene (DBA; Lawson, England), and orthoaminoazotoluene (OAAT), presented by I. P. Tereshchenko (P. A. Gertsen Moscow Cancer Research Institute).

Metallic iodine in crystalline form was used as the electron acceptor. Complexes of the carcinogens with iodine were obtained by various methods: 1) by dissolving a weighed sample in benzene, mixing, and

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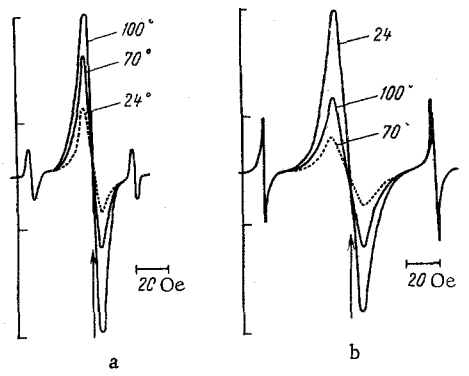

Fig. 1

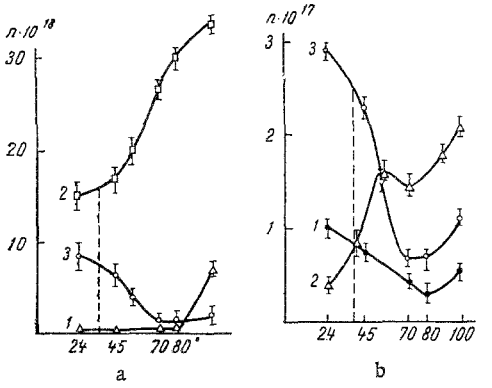

Fig. 2

Fig. 1. EPR spectra of artificial complexes of some carcinogens with iodine at different temperatures. Ratio between measurements of concentration of paramagnetic centers in sample corresponds to ratio between amplitudes of EPR signals if shape and width of spectral line remain unchanged; g-factor of signal center 2.00; a) 3,4-BP + I ($n = 3 \cdot 10^{19}$ spins); b) OAAT + I ($n = 3 \cdot 10^{17}$ spins).

Fig. 2. Change in concentration of paramagnetic centers of complexes of carcinogens with iodine in relation to changes in temperature. Correlation between known carcinogenic activity and amplitude of EPR signal (with absolute concentration of paramagnetic particles in the complex) will be evident. a: 1) 1,2-BP + I; 2) 3,4-BP + I; 3) 20-MC + I; b: 1) DMBA + I; 2) DBA + I; 3) OAAT + I. Ordinate, absolute number of spins in sample; abscissa, temperature of sample (in degrees C).

subsequent evaporation (by Sidorik's method); 2) by mixing highly dispersed powders of the carcinogen and iodine in the dry form and heating on a water bath at temperatures of 24, 30, 45, 55, 70, 80, and 100°C. The resulting samples were incubated for times ranging from a few seconds to several days, after which the newly formed paramagnetic centers were recorded by the EPR method on a type EPA-2M radiospectrometer. The sensitivity of the instrument was $5 \cdot 10^{14}$ spins. The absolute number of paramagnetic centers (spins) in the specimen was determined by comparison with a diphenylpicrylhydrazine standard, which gave an EPR signal in the region of g-factor 2.00 of approximately the same singlet form as the signal from the complexes studied. The EPR spectra were recorded at 20°C simultaneously from the second internal standard of bivalent manganese in a crystalline MgO lattice. The position of the g-factor was determined by Knäbel's method. The width of the EPR spectra thus obtained was determined by means of the phenoxyl radical, giving a triplet EPR signal with a distance of 15 Oe between the components of the superfine structure. In addition, an attempt was made to obtain analogous paramagnetic complexes with gaseous chlorine and liquid bromine. The specimens were cooled with liquid nitrogen and, after combination with the carcinogen, they were sealed in ampules made of molybdenum glass and kept at a constant temperature.

EXPERIMENTAL RESULTS

The EPR spectra are shown in Fig. 1. The process of complex formation depended on the character of carcinogen and the temperature at which the reaction took place.

Figure 2 shows the results of the recording of the temperature dependence of the concentration of paramagnetic particles. The relative amplitudes of the EPR signals have been separated, so that when the form and width of the spectral lines are unvarying, they correspond to the kinetics of changes in the concentration of the paramagnetic CTC.

It is interesting to note that during incubation of 3, 4-BPwith crystalline iodine even at room temperature and, in particular, at a temperature of 40°C, close to the human body temperature, a powerful paramagnetic complex is formed with a very large singlet with a g-factor of 2.00 and a signal width $\Delta H^1/_2$ of approximately 10 Oe. On raising the incubation temperature to 80°C the intensity of the EPR signal was more than doubled. The EPR signal in 1-mg samples of 3,4-BP and iodine with minimal amplification (and with the third shunt on the radiospectrometer) had very large amplitude for those conditions, over 20 cm.

Meanwhile, at the same temperatures 1,2-BP gave a completely different kinetic curve. It is clear from Fig. 2 that at temperatures comparable with the body temperature of animals and man this noncarcinogenic isomer formed virtually no paramagnetic charge-transfer complexes.

Tests of different methods showed that the use of 0.1 M iodine solution in benzene to obtain artificial paramagnetic complexes with the carcinogens meets the purpose completely. At room temperature such a solution yields charge-transfer complexes with a large EPR signal almost instantaneously with most carcinogenic hydrocarbons: 20-MC, 3,4-BP, 7,12-DMBA, DBA, and also OAAT. After evaporation of the solvent, interaction between these substances leads to the appearance of charge-transfer complexes with stable paramagnetism of high intensity (as during the formation of a stable π-radical).

It can thus be seen that the width of the EPR signal greater than 40 Oe (in the case of OAAT up to 42 Oe) indicates the formation of a paramagnetic charge-transfer complex of complex structure (the semiquinone monomer gives a width of 8 Oe, the dimer a width of 16 Oe). The marked dependence of a complex formation on temperature and the high yield of charge-transfer complexes at temperatures close to the body temperature of man and animals are particularly important.

The formation of analogous complexes in the organs and tissues of animals in vivo is perfectly probable. Presumably the prolonged existence of these magnetic complexes in the tissues may initiate unusual 3-radical nonenzymic oxidative chain processes.

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