# Mössbauer Effect in Biomedical Research: Variations of Quadrupole Splitting in Relation to the Qualitative Changes of Biomolecules *

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This review deals with studies of the variations of quadrupole splitting, electronic structure and stereochemistry of iron associated with qualitative changes of biomolecules. The possibility to determine various iron containing species resulting from the destruction of biomolecules using Mössbauer parameters is shown. A small change of iron stereochemistry leads to a small change of the iron electronic structure which could be detected by small changes of quadrupole splitting. It is expected that quadrupole splitting of iron gives new information for biomedical research on a molecular level.

*Key words:* Mössbauer effect, Biomedical research, Quadrupole splitting, Iron electronic structure, Hemoglobin, Molecular diseases.

## 1. Introduction

Molecular diseases are caused or accompanied by the synthesis of anomalous biomolecules. On the other hand, some pathological states of the human body are caused by environmental influences on the vitally important biomolecules. Therefore changes of biomolecules are of interest.

Iron is the most important Mössbauer element in biological systems. There are many iron containing proteins, such as hemoglobins, myoglobins, cytochromes, catalase, ferritin, and transferrin. Therefore the Mössbauer effect is a useful tool for the study of various biological materials [1, 2]. Applications of the Mössbauer effect in biochemical research demonstrated that this technique gives useful new information [3, 4].

This review deals with Mössbauer studies of iron containing proteins which revealed variations of the quadrupole splitting, electronic structure, and stereochemistry of iron in biomolecules. We consider both "drastic" and "small" changes of biomolecules. "Drastic" changes imply a change of the valence and/or spin state of iron resulting from the transformation or destruction of proteins, while "small" changes imply a change of the electronic structure of iron without any change of the valence and spin state resulting from structural modifications of protein during pathological processes or diseases. For comparison, the change of quadrupole splitting of iron associated with "drastic" and "small" changes of normal proteins is also reviewed.

## 2. Variations of the Quadrupole Splitting and Qualitative Changes of Normal Hemoproteins

Mössbauer parameters are very sensitive to the spin state and valence of iron. This is clearly understood with the Mössbauer results obtained for hemoproteins containing heme iron (iron porphyrin) as the active site (see Figure 1). Maeda [5] showed that the valence and spin states of hemoproteins could be easily deduced and classified with quadrupole splitting and isomer shift (Figure 2). Using these data we could determine the "drastic" changes of hemoproteins such as oxygenation and deoxygenation of hemoglobin accompanying changes of the iron spin state ($S=2 \rightarrow S=0$ and $S=0 \rightarrow S=2$, respectively), oxidation of hemoglobin and methemoglobin formation accompanying changes of the iron valence and spin states (Fe(II), $S=2 \rightarrow$ Fe(III), $S=3/2$), and formation of hemochromes and hemichromes, etc. Quadrupole

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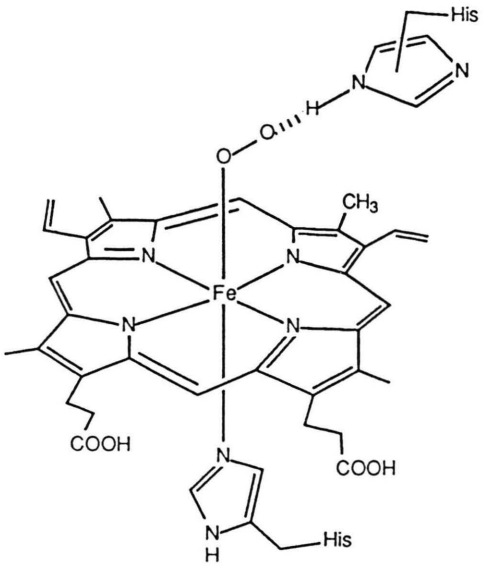


Fig. 1. The iron porphyrin (heme) structure of oxyhemoglobin and oxymyoglobin.

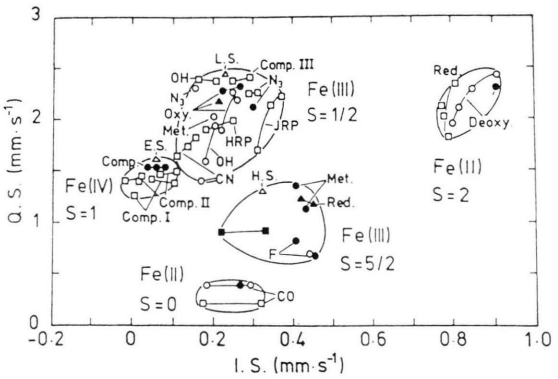


Fig. 2. Quadrupole splittings (QS) and isomer shifts (IS) of several hemoproteins in their native and derivative states. ● – myoglobin, ○ – hemoglobin, ■ – catalase, □ – peroxidase, ▲ – cytochrome c, △ – cytochrome c peroxidase. The isomer shifts are relative to metallic iron at room temperature [5].

splittings and isomer shifts of various hemoproteins and their derivatives and of other iron containing proteins were available from the literature [1–3]. Scheidt and Reed [6] reviewed the spin state/stereochemical relationships in various iron porphyrins. Therefore, observation of "new" quadrupole doublet(s) appearing in the Mössbauer spectra of hemoproteins caused by "drastic" changes of biomolecules enables us to analyze the unknown products.

However, the "small" changes of biomolecular seem to be more important because information about the relationship between the iron electronic structure and slight stereochemical modifications could be obtained. In this case, for example, for hemoproteins we could obtain information about the influence of slight stereochemical changes of the heme iron on the protein functions using the quadrupole splitting. Small differences in quadrupole splitting were found for myoglobin and hemoglobin in both deoxy- and oxyforms and for some hemoglobins from various animals [7] and humans [8]. Calculations of the quadrupole splitting for oxyheme models also demonstrated some variations of the quadrupole splitting in relation to the heme iron stereochemical modifications [9–11]. Moreover, it was shown by calculations [12–14] that the iron electron structure and quadrupole splitting are slightly different for the heme models accounting of the stereochemical variations in $\alpha$- and $\beta$-subunits of tetrameric hemoglobin and monomeric myoglobin (see Figure 3). Also slight variations of the quadrupole splitting of some artificial modifications of hemoglobin were shown by Mössbauer measurements [15–18]. These results show that the quadrupole splitting as a result of the interaction of the nuclear quadrupole moment and the electric field gradient tensor produced by the electron and ligand distribution around the heme iron nucleus could reflect slight variations of the point symmetry of the iron site, the population of the iron valence orbitals, and the energies of the

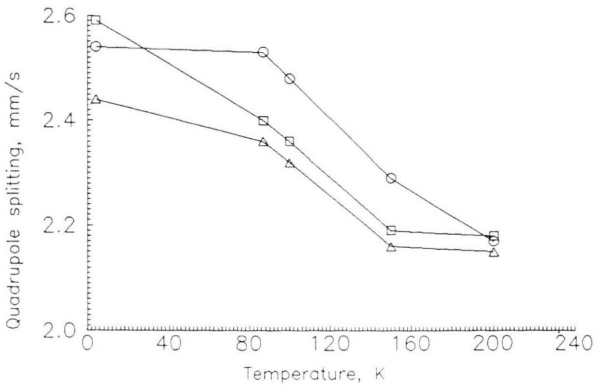


Fig. 3. Calculated quadrupole splitting vs. temperature for ion porphyrins modeling stereochemical variations of the heme iron in $\alpha$- (○) and $\beta$- (□) subunits of tetrameric deoxyhemoglobin and in deoxymyoglobin (△). Data were taken from the results of calculations by the iterative extended Hückel method [13].

molecular orbitals and electronic terms and, therefore, it could reflect the "small" changes in heme iron stereochemistry.

## 3. Variations of the Quadrupole Splitting and Qualitative Changes of Hemoproteins During Blood Diseases

*Hemoglobinopathies*

Hemoglobinopathies are the most widely known molecular diseases accompanied by hemoglobin biosynthesis disturbance. Patients with hemoglobinopathies have various kinds of anomalous hemoglobins with changed functions and protein unstability resulting from amino acid substitutions in the protein chains. Mössbauer studies of red blood cells from patients with β-thalassemia, hemoglobins E, Bart's, H, G Coushatta, Guangzhou and some other variants [19–24] revealed several new quadrupole doublet peaks (see Figure 4). Similar new components were observed in red blood cells from patients with high altitude polycythemia [25]. The values of the isomer shifts and quadrupole splitting for several new components are given in Table 1. These new components corresponded mainly to the high and low spin ferric compounds and possibly to the low spin ferrous compounds and could be related with the presence of ferritin-like iron, hemichromes, hemochromes and some other heme derivatives and nonheme compounds resulting from the unstability of anomalous hemoglobins.

Some interesting results were obtained during the studies of the "small" changes in anomalous hemoglobins. Bill et al. [26] found a differences of the temperature dependence of quadrupole splitting for

Table 1. Mössbauer parameters of the new components obtained for red blood cell samples from patients with hemoglobinopathies (~80 K).

| No. | Quadrupole splitting, mm/s | Isomer shift, mm/s | Disease | Reference |
|---|---|---|---|---|
| 1. | 0.80 | 0.57 | β-thalassemia | [22] |
| 2. | 1.57 | 0.27 | Hemoglobin Bart's | [22] |
| 3. | 1.27 | 0.38 | Hemoglobin H | [22] |
| | 0.60 | 0.35 | | |
| 4. | 0.19 | 0.31 | β-Thalassemia | [23] |
| 5. | 0.42 | 0.16 | Hemoglobin E | [23] |
| 6. | 0.19 | 0.25 | Hemoglobin E/β-thalassemia | [23] |
| | 0.3 | 0.45 | | |

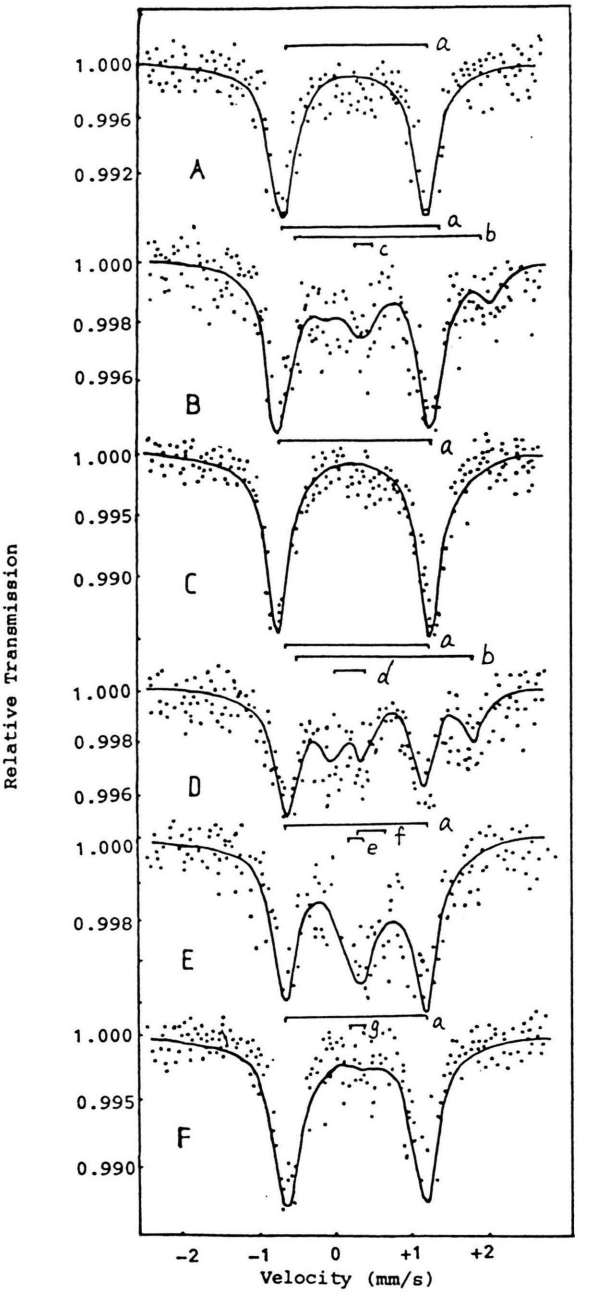


Fig. 4. Mössbauer spectra of red blood cells from a normal person (A) and patients with β-thalassemia (B), hemoglobin G (C), hemoglobin E (D), hemoglobin E/β-thalassemia (E), hemoglobin G Coushatta/β-thalassemia (F) (a – oxyhemoglobin, b – deoxyhemoglobin, c, d, e, f, g – new components). $T = 77$ K [23].

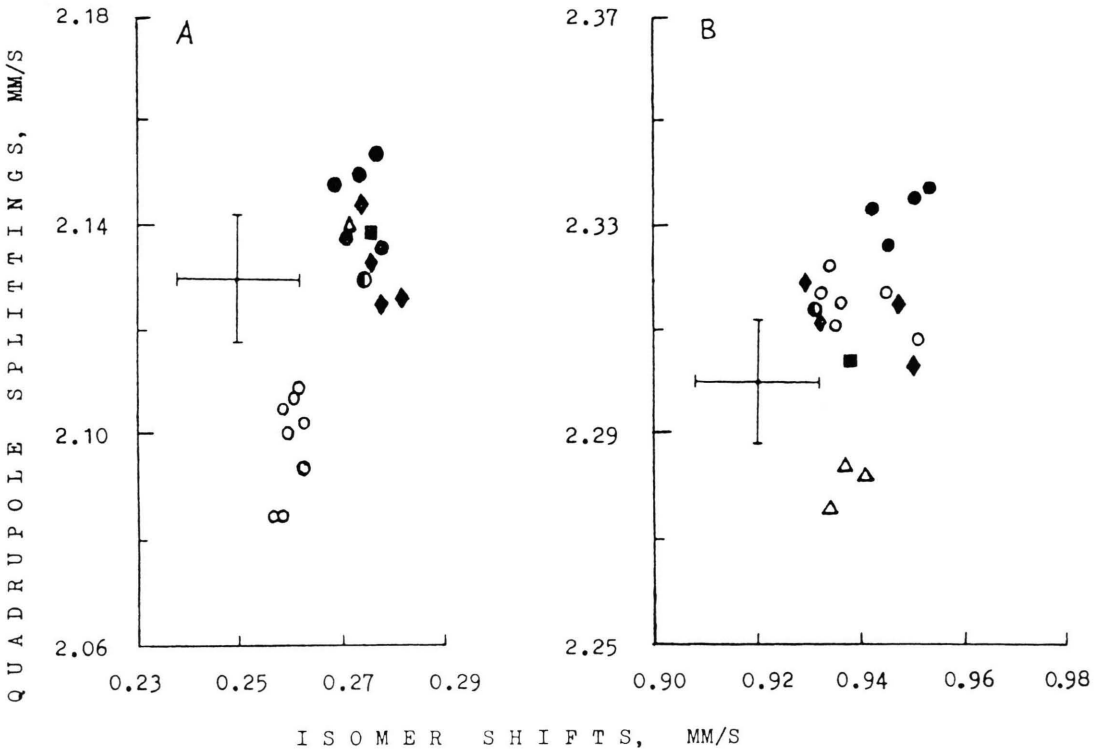


Fig. 5. Quadrupole splitting and isomer shift for hemoglobins in oxy- (A) and deoxy- (B) forms from: normal adult (○), normal fetal (△), from patients with acute myeloblastic leukemia (●), acute myelomonoblastic leukemia (◐), chronic myeloleukemia (■), erythremia (◆). Horizontal and vertical bars indicate experimental errors for data points. The isomer shifts are relative to metallic iron at room temperatue [32].

normal hemoglobin and hemoglobin Zürich in oxyform, while those for deoxyform were not found. This result could reflect slight stereochemical changes of the Fe(II)-$O_2$ bond resulting from the substitution His(63)→Arg in $\beta$ chains, which is situated in the distal heme region near the oxygen molecule (see Figure 1). Zeng et al. [21, 27, 28] studied several abnormal hemoglobins in China, such as G Coushatta, Guangzhou, Queens, G X1 and G X2. The authors found slight variations of the quadrupole splitting for some proteins in deoxy- and oxyforms (Table 2) and related it with the changes of the heme iron stereochemistry and the energies of the iron electronic terms.

*Blood System Malignant Diseases*

To learn about the "small" changes in hemoglobin during malignant blood system diseases, samples of red blood cells from patients with various forms of leukemia and erythremia were studied [29–32]. It was found that quadrupole splitting and isomer shift of patient's oxyhemoglobin were slightly higher than those of normal adult oxyhemoglobin and coincided in part with parameters of normal fetal oxyhemoglobin. In contrast, quadrupole splitting and isomer shift of patient's deoxyhemoglobin were not different from those of normal adult deoxyhemoglobin and slightly higher than parameters of normal fetal deoxyhemoglobin (see Figure 5). This result seems to be similar with that obtained for hemoglobin Zürich and could be explained as a result of slight variations of the distal heme region in patient's hemoglobin which led to fine changes of the iron electronic structure and Fe(II)-$O_2$ bond. On the other hand, slight variations of the heme iron stereochemistry in both distal and proximal regions occurred in fetal hemoglobin. Furthermore, in several cases of erythremia an additional quadrupole splitted doublet was in the Mössbauer spectra [31, 32]. This component was attributed to the ferritin-like iron. It should be noted that Ortally et al. [33, 34] did not find any changes of quadrupole splitting for oxyhemoglobin

Table 2. Values of quadrupole splitting for some anomalous hemoglobins (~80 K).

| Hemoglobin | Form | Quadrupole splitting, mm/s | Reference |
|---|---|---|---|
| Normal adult | Deoxy | 2.22 | [20] |
| G Coushatta | Deoxy | 2.17 | [20] |
| Guangzhou | Deoxy | 2.15 | [20] |
| G-X1 | Deoxy | 2.27 | [20] |
| G-X2 | Deoxy | 2.07 | [20] |
| Zürich | Deoxy | 2.28 | [26] |
| Normal adult | Oxy | 2.16 | [20] |
| G Coushatta | Oxy | 1.83 | [20] |
| Guangzhou | Oxy | 1.82 | [20] |
| G-X1 | Oxy | 2.17 | [20] |
| G-X2 | Oxy | 2.16 | [20] |
| Zürich | Oxy | 2.12 | [26] |

from leukemic patients. This disagreement may be the result of different experimental procedures in this study (low velocity resolution). However, the authors observed an additional quadrupole splitting doublet in Mössbauer spectra of red blood cells from patients with leukemia and breast cancer with parameters that corresponded to hemochromes [35]. This disagreement with the studies [29–32] could be a result of the non-Lorentzian line shape of the oxyhemoglobin Mössbauer spectra at liquid nitrogen temperature, which are better fitted by two quadrupole doublets. This feature of the oxyhemoglobin Mössbauer spectra was attributed to the slight stereochemical differences for the heme iron in $\alpha$- and $\beta$-subunits of tetrameric hemoglobin, which could be related with two quadrupole splitted doublets [8, 17]. The fitting of the Mössbauer spectra of oxyhemoglobin from leukemic patients using two doublets showed that quadrupole splittings related to the $\alpha$- and $\beta$-subunits were slightly high for leukemic oxyhemoglobin [29]. It should be noted that a similar approach was used for the analysis of Mössbauer spectra of hemoglobin E, Guangzhou and Queens in deoxyform [28]. It is interesting that the variation of quadrupole splitting for oxyhemoglobin from patients with leukemia and erythremia correlated with the change of the protein oxygen affinity.

## 4. Qualitative Changes of Hemoproteins by Environmental Factors

*Chemical effect on hemoglobin*

Garibov et al. [36, 37] studied the processes of hemoglobin destruction by hydrazine and its derivatives. They observed strong changes in Mössbauer spectra of oxyhemoglobin with hydrazine, nonsymmetric dimethylhydrazine and phenylhydrazine (see Figure 6). Using Mössbauer quadrupole splitting and the isomer shift of spectral components the possible products of oxyhemoglobin transformation by chemicals were found. Formation of the high spin ferrous compounds and then formation of the high spin ferric compounds was shown.

*Radiation effect on hemoglobin*

A number of studies were made in order to investigate the hemoglobin radiolysis by Mössbauer spectroscopy. Kellershohn and co-workers [38, 39] studied hemoglobin affected by X-rays in doses ranging from ~3 to ~126 kGy. They observed new quadrupole doublets in Mössbauer spectra of irradiated hemoglobins and determined deoxygenation of oxyhemoglobin with the heme iron spin change, and formation of hemochromes and a nonheme high spin ferric compound resulting from protein destruction. Other results were obtained in the study of $\gamma$-irradiated oxyhemoglobin. The values of quadrupole splitting and isomer shift of new components in Mössbauer spectra of oxyhemoglobin irradiated with $\gamma$-rays of ~100, ~300, and ~600 kGy with average energy of 15.5–16 MeV were attributed to the high and low spin ferric compounds. Deoxygenation of oxyhemoglobin was also found, and formation of the high and low spin methemoglobin was assumed [40, 41]. In contrast, a further study of oxyhemoglobin irradiated with $\gamma$-rays of ~16, ~26.5, and ~33 kGy with an average energy of 9 MeV showed only deoxygenation of oxyhemoglobin [42, 43]. On the other hand, the Mössbauer spectrum of oxyhemoglobin exposed to electrons of 5 kGy with average energy of 10 MeV (Fig. 7) demonstrated the deoxygenation of oxyhemoglobin and a new low spin ferrous compound formation with quadrupole splitting and isomer shift corresponding to hemochromes [44].

*Malaria*

A Mössbauer study of hemoglobin degradation by malarial parasites was given by Yayon et al. [45, 46]. A new component with quadrupole splitting and isomer shift corresponding to high spin ferric iron was found and was related to malarial pigment. Moreover, the hyperfine parameters of the malarial pigment were different for human and rat red blood cells infected with human and murine parasites.

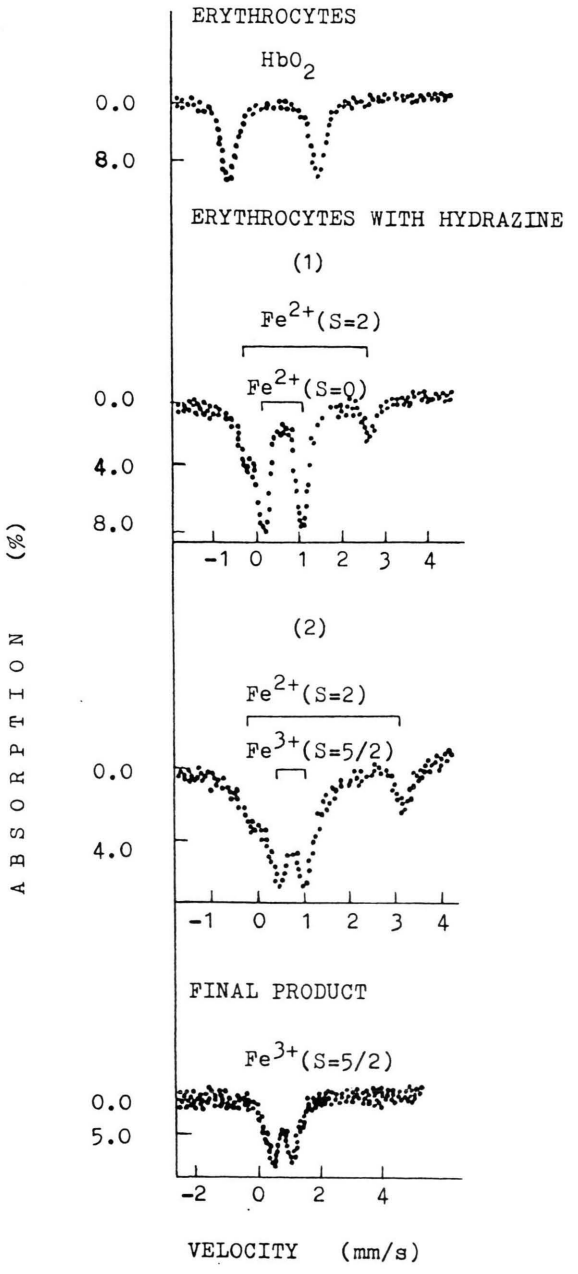


Fig. 6. Transformation of Mössbauer spectra of oxyhemoglobin during incubation with hydrazine at 80 K. Isomer shifts are relative to sodium nitroprusside [36]. (1) and (2) are two ferrous high spin compounds with different quadrupole splitting resulting from protein degradation.

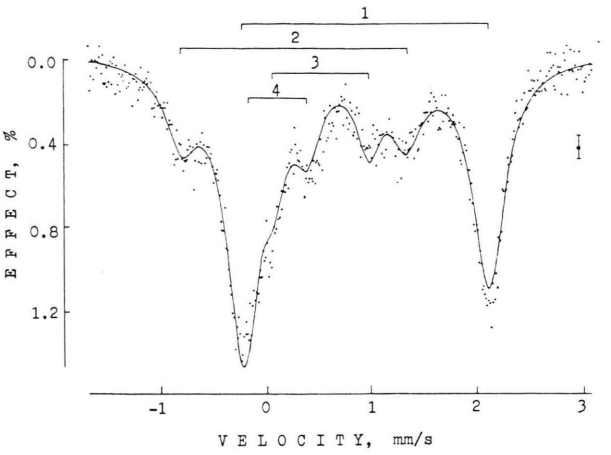


Fig. 7. Mössbauer spectrum at 87 K of oxyhemoglobin irradiated with electrons. 1: deoxyhemoglobin, 2: oxyhemoglobin, 3: hemochromes, 4: $^{57}Fe$ nuclei in the beryllium window of the scintillator detector. The isomer shifts are relative to metallic iron at room temperature [44].

## 5. Study of Quadrupole Splitting in Some Other Biomedical Research

Mössbauer study of blood substitutes (artificial oxygen carriers) based on glutaraldehyde cross-linked hemoglobin showed that the values of quadrupole splitting of the cross-linked oxyhemoglobin were slightly higher than those of normal ones [47, 48]. Guillochon et al. [47] and Chevalier et al. [48] explained this as a result of the heme iron stereochemical modifications caused by cross-linking of protein molecules and, therefore, the changes of the Fe(II)-$O_2$ bond and hemoglobin functions.

Mørup and Johansen [49] used Mössbauer spectroscopy for the study of the degradation of blood in the human digestive tract of normal persons and pathologic patients. They found strong changes in the Mössbauer spectra in which new components appeared with quadrupole splittings and isomer shifts corresponding to the high spin ferric and ferrous compounds resulting from hemoglobin degradation.

Studies of pharmaceutically important iron-dextran complexes, which are synthetic models of ferritin, should be noted [50–52]. The small variations of quadrupole splitting for different samples of iron-dextrans may due to the slight changes of the symmetry of the iron core structure during sample preparation. Moreover, the better fitting of two peaks of the Mössbauer spectra of iron-dextrans using more then

one quadrupole doublet reflects structural modifications of the iron core or different quadrupole splitting for surface and interior $^{57}Fe$ nuclei in the core.

## 6. Conclusions

Changes of biomolecules during pathological processes can be revealed by the analysis of quadrupole splitting. "Drastic" valence/spin state variations resulting from protein transformation or destruction can easily be observed by new peak(s) in Mössbauer spectra. The hyperfine parameters of these components suggest the formation of new compounds. The observation of the "small" changes of biomolecules is more difficult because there are no well resolved new components in the spectra. However, measurements of the Mössbauer spectra with high resolution yield variations of quadrupole splitting due to modifications of the electronic structure and stereochemistry of iron.

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