# EFFECT OF X-RAY CONTRAST MEDIA ON HUMAN RED CELL DEFORMATION

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KEY WORDS: x-ray contrast media; red blood cells

X-ray contrast media (XCM) containing iodine are widely used at the present time in diagnostic radiology. Most XCM are injected directly into the blood stream. It was shown previously that XCM which are 2,4,6-tri-iodinated aromatic compounds, on interacting with red blood cells, modify their morphology and rigidity [1, 3, 5], their aggregation properties, and the limit of blood flowability [2]. It is these effects of XCM that may cause the development of serious side effects such as disturbance of the microcirculation and others connected with it such as pulmonary edema and renal failure [14].

The aim of this investigation was to study the effect of various XCM on the ability of red cells to change their shape, an essential condition for their passage through capillaries.

## EXPERIMENTAL METHOD

A method widely used to assess deformability of red cells is filtration through pores of an assigned diameter. However, despite its simplicity, this method is not without many shortcomings which reduce its objectivity. We therefore use the method of ectacytometry [3], which is based on diffraction of a laser beam by a suspension of red cells in shear flow and recording the ratio between the axes of ellipsoids, into which the red cells are converted, in the form of diffraction patterns. The measure of deformation is the index of deformability of the erythrocytes (IDE), calculated by the formula $(L - H)/(L + H)$, where L and H are the length length of the major and minor axes of an ellipse, respectively.

Blood was obtained from healthy donors of both sexes, and diluted 1:300 with suspension medium. The suspension medium was physiological saline (0.9% NaCl) containing 0.7% of polyethylene-oxide (5 kD). To ensure better preservation of the shape of the cells in the solution containing the red cell suspension, human serum albumin ("Serva," West Germany) was added up to a concentration of 2 mg/ml.

The curve reflecting changes in deformability was studied within the range of shear velocities from 40 to 4500 $sec^{-1}$, the viscosity of the suspension medium being 10-13 cP.

To verify the reversibility of the effect of XCM on the deformation properties of the red cells, the cell suspension with assigned concentration of XCM was centrifuged to sediment the cells for 10 min at 2000g. The red cells were resuspended in medium not containing XCM, after which the suspension was transferred into the working cell of the ectacytometer.

XCM used in cholecystography (bilimin, 20% bilignost, 50% bilignost), in angiourography; iodamide-380, 76% triombrast (USSR), and nonionogenic XCM for angiourography and myeolography (metrizamide, from "Serva," West Germany) were studied. Solutions of XCM were added directly to the working cell of the ectacytometer in the volume necessary to create concentrations with the range from $5 \cdot 10^{-4}$ to $5 \cdot 10^{-2}$ M for XCM to be given by intravenous injection, and from $10^{-5}$ to $10^{-3}$ M for those intended to be given per os. Because of its instability, the solution of metrizamide was made up before use.

The osmotic pressure of the solution of XCM was determined as the freezing point by means of a Beckman thermometer. The experimental data were subjected to statistical analysis by the Wilcoxon–Mann–Whitney nonparametric test.

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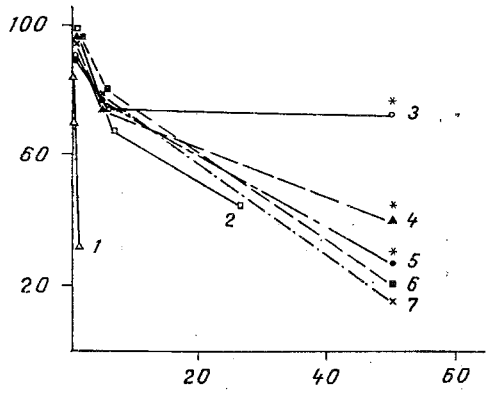


Fig. 1

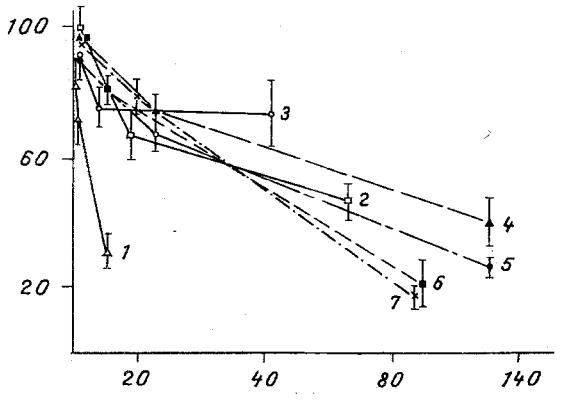


Fig. 2

Fig. 1. Dependence of IDE on XCM concentration. Abscissa, concentration of XCM (in mM); ordinate, IDE (in percent of control). 1) Bilimin; 2) NaCl; 3) metrizamide; 4) 50% bilignost; 5) 20% bilignost; 6) iodamide-380; 7) 76% triombrast. *$p < 0.05$. Values of IDE given for a shear velocity of 3498.2 $sec^{-1}$.

Fig. 2. Dependence of IDE on osmolarity of XCM solutions. Abscissa, osmolarity of XCM solutions (in milliosmoles); ordinate, IDE (in percent of control). Legend as to Fig. 1.

## EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that XCM of different structure and different organ affinity caused a varied degree of concentration-dependent lowering of IDE. Metrizamide, which reduced IDE by 30%, had the weakest effect; 50% bilignost lowered this parameter by 57%, and 20% bilignost, iodamide, triombrast, and bilimin reduced it by 60-80% compared with the corresponding control.

To discover the role of osmotic activity of XCM in the change in the value of IDE, the osmotic pressure of the XCM solutions for investigation was measured and compared with values of IDE in the presence of contrast media (Fig. 2). Good correlation was found beteen the intensity of the action of XCM on IDE and their osmotic pressure only in the case of angiourographic XCM: metrizamide, iodamide, and triombrast ($r = 0.84$), they virtually do not bind with proteins and membranes [1] and their effect on the red cells is evidently due to their osmotic activity. In that case, reduction of IDE by the action of these substancs may be attributable to the increase in viscosity of the internal contents due to escape of water from the cells. The results point to a much greater danger of nonionic XCM than ionic from the point of view of their ability to affect deformation properties of red cells, and consequently the microcirculation.

Meanwhile the observed effect of XCM used in cholecystography on IDE cannot be explained purely by their osmotic activity. Analysis of the results (Fig. 2) reveals the following relationships: 1) the higher the protein-binding capacity of XCM anions (it is higher for bilimin than for bilignost), the more strongly IDE is changed; 2) an important role in the effect of cholecystographic XCM on IDE is played by the cationic part of their molecules (methylglucamine is less active than sodium).

In our opinion the changes recorded in IDE in the presence of cholecystographic XCM can be explained both by their interaction with membranes and by the osmotic activity of their solutions (which is reduced in the presence of biomolecules which bind XCM).

Among the XCM studied, bilimin has the greatest ability to reduce red cell deformability. However, in clinical practice its maximal blood concentration is two orders of magnitude lower than that of the other XCM, since, of all the preparations studied, only bilimin is administered perorally.

To determine the reversibility of these effects of XCM, the value of IDE was obtained before and after addition of the foreign agents. A single immersion of red cells in the suspension medium was found to restore completely their ability to change their shape. Consequently, to reduce the risk of microcirculatory disturbances in diagnostic roentgenology

with XCM, the schedule of administration of the preparation should ensure the shortest duration of their contact with the blood cells.

It can be concluded from the results that XCM reversibly reduced the ability of red cells to undergo deformation and that this reduction is concentration-dependent and can be arranged in the following order: bilimin > 76% triombrast = iodamide-380 > 20% bilignost > 50% bilignost > metrizamide. The action of triombrast, iodamide, and metrizamide on red cell deformability is attributable to the osmotic activity of their solutions and is linked with an increase in viscosity of the internal centers of the red cells. For angiourography it is therefore preferable to use the new nonionic XCM of metrizamide type, with low osmotic activity.

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# EFFECT OF ANTISERUM TO BRAIN γγ-ENOLASE (PROTEIN 14-3-2) ON ETHANOL CONSUMPTION BY RATS


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Much information has now been obtained on the disturbance of function of individual enzymes and enzyme systems of the brain during the formation of alcohol addiction [1, 4]. It is not yet clear, however, whether changes observed in enzyme activity in the brain are primary factors in the pathogenesis of alcoholism or the result of a general disturbance of metabolism caused by chronic ethanol poisoning. One possible experimental approach to the solution of these problems is by selective inhibition of activity of individual enzymes (or isozymes) in the brain with the aid of specific immune sera, followed by investigation of the dynamics of ethanol consumption by the animals.

The aim of this investigation was to study the role of isozymes of brain enolase in the mechanisms of formation of addiction to ethanol. The glycolytic enzyme enolase is represented in nerve tissue by three isozymes with a dimer structure (αα, αγ, and γγ), of which isozymes containing the γ-subunit are specific for nerve tissue [3]. The isozyme γγ-enolase is contained mainly in neurons, and it is generally considered to be a marker of neuronal cells [9].

## EXPERIMENTAL METHOD

The γγ-isozyme of enolase was isolated from bovine brain by the method in [8] without modifications [2]. The sample of γγ-enolase thus obtained was electrophoretically homogeneous. It was later used to immunize rabbits [6]. The immune serum was kept in the freeze-dried form at 4°C and dissolved in sterile distilled water immediately before the experiments with intracisternal injections. Immune activity of the serum was verified in Ouchterlony's test

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