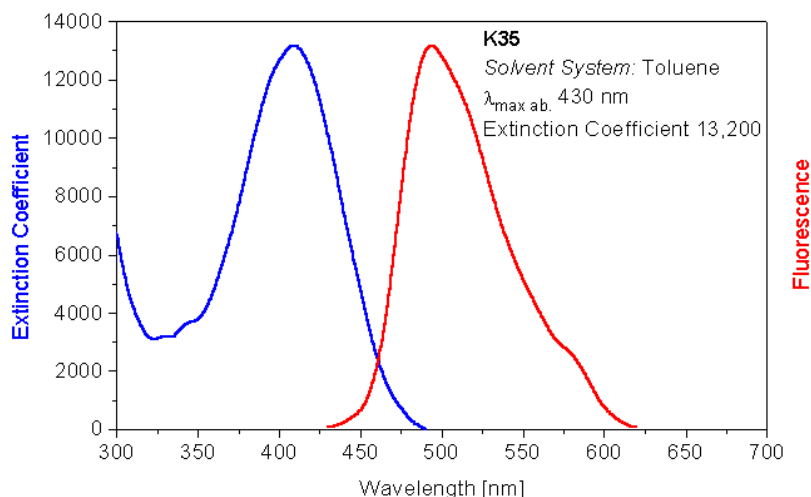


## Fluorescent Probe K-35

**K-35** is a fluorescent probe for albumin binding sites in plasma and serum [*Serum Albumin in Clinical Medicine. Book 2 (Editors: Gryzunov Yu.A. and Dobretsov G.E.), Moscow, Geotar, 1998; Miller Yu.I., Gryzunov Yu.A., Dobretsov G.E., Aidyraliev R.K., Krasovitski B.M., Kormilova L.I., Ermolenko I.G. A New fluorescent probe for albumin binding sites in plasma and serum. EURO-TOX' 95, Toxicology Letters Supplement 1/78, 1-88 (1995); USSR Pat. 1,681,266 (1989)*].

**K-35** is excitable by a 405 nm laser and has fluorescence at 490 nm in toluene. The fluorescence intensity of **K35** increases many times after it's binding with serum albumin in aqueous solutions.



Subsequent clinical investigations shows that by using **K-35** it is possible to correlate the albumin binding site properties detected by **K-35** to several human disorders and diseases including coronary heart disease and myocardial infarction [*Gryzunov Yu.A., Pestova A.B., Kozaimany E.N. et al. Clin. Lab. Diagnostics (in Russ.), 1994, No.5, P.23–25*], liver disorders including hepatitis [*Andreeva O.L., Shmeleva L.T., Dobretsov G.E. Changes in the albumin transport function and their control by use of a fluorescent method in hepatitis and hepatic cirrhosis. Efferent. Therapy (Sanct-Peterburg, in Russ.), 1995, No.3, P.35–39*], critical states in surgery practice [*Gryzunov Yu.A., Dobretsov G.E., Zaks I.O., Komarova M.N. Blood albumin: properties, functions and their estimation at critical states (a review). In Reanimatology and Intensive Therapy (in Russ.). Moscow: VINITI, 1997, No.3, P.3–13; Ivleva V.V., Zaks I.O., Mescheriakov G.N., et al. Lung metabolic function in critical states of patients. In First Russian Congress on Pathophysiology. Moscow, 1996, P.297*], schizophrenia [*Gryzunov Yu.A., Misionznik E.Yu., Uzbekov M.G., Molodetskich A.V. Influence of haloperidol treatment on the time course of serum albumin binding capacity in schizophrenia patients. Toxicol. Lett. 1995, Suppl.1/78, P.38*] and other situations.

Several applications of fluorescent probe **K-35** are described in the book [*Serum Albumin in Clinical Medicine. Book 2. (in Russ). Eds. by Yu.A.Gryzunov and G.E.Dobretsov. Moscow: Geotar, 1998*]. Whole this book is mostly devoted to investigations and applications of **K-35** probe. Some abstracts of articles from this book are listed [here](#).

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## Summary

A FLUORESCENT METHOD TO STUDY ALBUMIN PROPERTIES  
IN THE AQUEOUS HUMOR AND IN THE TEAR

Gryzunov Yu.A., Deyev A.I., Kourysheva N.I., Komarova M.N.

A fluorescent method with probe K-35 to study albumin properties in the aqueous humor (AH) and in the tear (T) has been proposed. Parameter TA in AH has significant correlation with albumin content determined by electrophoresis ( $r=0.93$ ,  $n=14$ ). Parameters EA and EA/TA in AH and T introduced by analogy with serum were lower in patients with eye diseases (cataract, glaucoma) than in men without ones. The method can be useful to evaluate EA in AH using the tear ( $r=0.75$ ,  $n=18$ ) and to study hemato-ophthalmic barrier permeability by comparing EA, TA, and EA/TA in serum-tear pair samples.

p.161.

## Summary

DRUG INFLUENCE ON THE FLUORESCENCE  
OF K-35 ALBUMIN COMPLEXES IN NORMA

Miller Yu.I., Dobretsov G.E.

The fluorescence intensity of two fluorescent probes, ANS and K-35, increases many times after their binding with human serum albumin in water solutions. Addition of some drugs – specific markers of the site I and site II in albumin molecules – influenced slightly the ANS fluorescence decreasing it by 10–30 % at drug/albumin molar ratio close to 1.5. The same drugs dramatically decreased K-35 fluorescence intensity – by 40–80 %, both site I and site II markers were active. The data show that K-35 is much more sensitive to the occupation of the albumin binding sites by organic ligands in comparison with the well-known fluorescent probe ANS. It may be assumed K-35 is sensitive to both site I and site II markers.

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## Summary

INTERACTION BETWEEN BOVINE SERUM ALBUMIN WITH STEARIC ACID,  
SPIN-LABELED STEARIC ACID, AND FLUORESCENT PROBE K-35

Ryjikov S.B., Bogachjeva A.A., Safonova S.V.

The interaction between bovine serum albumin (BSA) with stearic acid (SA) and spin-labeled stearic acid (16-doxil-stearat, SL) was studied with fluorescent probe K-35. Both SA and SL-dependences of K-35 fluorescence intensity have a maximum at fatty acid/BSA near 5. The effect of SL was stronger than one of SA. The BSA of different firms were very similar in the form of the fatty acid dependence, but there was the difference in the value of the effect. For BSA of every manufacture the form of the curve (K-35 fluorescence intensity) vs (fatty acid/BSA ratio) was independent of the SA/SL ratio. The results suggest the SA and SL have the same binding site in the BSA molecule.

p.170.

## Summary

BINDING PARAMETERS FOR K-35-ALBUMIN INTERACTION  
IN SERUM IN NORMA  
Dobretsov G.E.

The interaction of the fluorescent probe K-35 with albumin binding sites in a standard human serum was studied by use of a new method described earlier (Dobretsov, Biophysics 1996, 41, 1073-1077). In the serum diluted by 300-600 times there are about 2 sites of strong K-35 binding in each albumin molecule. Mean binding constant is close to  $4,2 \cdot 10^5$  (litre/mol). The mean quantum yield of K-35 fluorescence is 0.10 at the occupation up to 1 site per albumin molecule but the occupation of more than 1 site leads to significant decrease of the yield, and at 2 occupied sites the mean yield is 0.06. In more concentrated serum (dilution 1:40) the quantum yield was increased to 0.12. It may be assumed the main cause of K-35 fluorescence decrease is quenching by water molecules. Probe molecules hidden into the first binding site are partially accessible (or inaccessible) to water molecules but are more accessible in the second one.

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## Summary

PARAMETERS OF THE INTERACTION OF THE PROBE K-35  
WITH ALBUMIN SITES AND THEIR CHANGES  
AT PATHOLOGICAL STATES OF THE BODY  
Andreeva O.L., Dobretsov G.E., Osadchaya N.A., Zvirenko S.V.

Interaction of the fluorescent probe K-35 with albumin molecules in diluted serum samples was studied in healthy donors and 6 groups of patients with *angina pectoris*, *hepatitis*, *hepatocirrhosis*, *pancreatitis*, *pyelonephritis* and *duodenal ulcer*.

Fluorescent titration in the simplest mode was used to estimate the mean number of binding sites per one albumin molecule ( $n$ ), binding constant ( $K$ ) and fluorescence quantum yield of bound probe molecules ( $Q$ ).

The mean  $n$  was estimated as 1,3 in all groups studied.  $K$  was close to  $(1,8 \pm 0,3) \cdot 10^5 M^{-1}$  in all groups excluding hepatitis where  $K$  was decreased by 40 %.  $Q$  was the same in all groups besides of cirrhosis where  $Q$  was decreased by 23 %.

Thus, all three parameters ( $n$ ,  $K$  and  $Q$ ) could be responsible for the decrease of so-called ratio "effective/total albumin concentrations" ( $EA/TA$ ) but more detailed study needs to obtain the quantitative dependence of the ratio on these parameters.

p.183.

## Summary

THE INFLUENCE OF LASER IRRADIATION IN VITRO  
ON THE ALBUMIN BINDING SITES  
Ambartsumjan R.V., Voinova V.M., Dobretsov G.E.,  
Gryzunov Yu.A., Yurieva E.A., Bryljova V.V., Kazantseva L.Z.

Helium-neon low-intensity laser and infrared laser were used to illuminate diluted (1:200) samples of capillary plasma in vitro. The lasers are used in paediatric practice for patients with hereditary diseases. Total dose was 0.6 J/5 min for helium-neon and 0.015 J/4 min for IR lasers per each plasma sample. That illumination significantly changed fluorescent parameters of albumin-bound molecules of the probe K-35 added after illumination. The effect was expected in 30 % plasma samples of 170 children with hereditary pathology. Thus, albumin molecules in diluted plasma can be sensitive to low-level laser illumination.

### Summary

#### THE CHANGES OF ALBUMIN BINDING SITES PROPERTIES IN BALB MICE AFTER THE TOTAL SUBLETHAL GAMMA EXPOSURE DOSE 3.5 Gr

Aseychev A.V., Tesjelkin Yu.O., Deyev A.I., Babenkova I.V.,  
Tjukavkina I.A., Kabachenko A.N.

The changes of serum albumin binding sites properties of mice (Balb) were studied within 5 months after total gamma exposure dose 3.5 Gr using the fluorescence probe K-35. The effective and total albumin concentrations (EC and TC, respectively) were measured, and the parameter EC/TC independent of albumin content was used to characterise binding sites properties. The average value of EC/TC was equal to  $0,68 \pm 0,07$  (n=13) in control group and  $0,63 \pm 0,061$  (n=18) in the group of irradiated mice. The variance of EC/TC in the group of irradiated mice was 13% less than in control group. The treatment with the antioxidant dihydroquercetin after irradiation lead to growing in EC/TC variance in this experimental group (n=29): 40 % of mice had the EC/TC close to average value of non-irradiated mice, 60 % of mice had the EC/TC close to average value of irradiated mice.

**K-35** probe has also been presented at the EUROTOX'95, Toxicology Letters Supplement 1/78 (1995) 1–88:

#### A Fluorescent Assay for Serum Albumin Binding Capacity

Yu. I. Miller, Yu. A. Gryzunov, G. E. Dobretsov.

*Research Institute for Physical Chemical Medicine, Moscow, Russia*

A fluorescent assay for the binding capacity of albumin (ABC) in human serum is suggested. The method is based on the use of a new fluorescent probe **K-35**. The probe is sensitive to metabolites and drugs bound to albumin sites of I and II types. Competition of the probe and toxic metabolites or drugs leads to decrease in **K-35** fluorescence in whole human serum or plasma. The greater blood level of the toxic ligands the smaller **K-35** fluorescence in plasma. The present assay presented includes two tests. The first allows to measure free unoccupied capacity of plasma albumin which was called "effective albumin concentration" (EAC). The second test allows to measure total albumin concentration (TAC) as a biochemical parameter insensitive to the metabolite of serum or plasma. The assay can be used in clinical laboratories for toxicosis control and prognosis.

*Key words*, albumin binding capacity, fluorescent assay

#### **K-35, A New Fluorescent Probe for Albumin Binding Sites in Plasma and Serum**

Yu. I. Miller<sup>1</sup>, Yu. A. Gryzunov<sup>1</sup>, G. E. Dobretsov<sup>1</sup>, R. K. Aidyraliev<sup>1</sup>,  
B. M. Krasovitski<sup>2</sup>, L. I. Kormilova<sup>2</sup>, I. G. Ermolenko<sup>2</sup>.

<sup>1</sup>*Research Institute for Physical Chemical Medicine, Moscow, Russia.*

<sup>2</sup>*Research Institute of Monocrystals, Kharkov, Ukraine*

A new fluorescent probe **K-35** (N-carboxyphenylimide of 4-dimethylamino-1,8-naphthalenedicarboxylic acid) is presented as a substance for investigation of albumin binding sites in whole human serum. The weak fluorescence of **K-35** in water solutions greatly increases at serum albumin addition. Albumin is the only serum protein that causes such significant fluorescence increase. The albumin molecule has about 1.7 binding sites for **K-35** with binding constant  $(4.0 \pm 0.6) \times 10^5 \text{ M}^{-1}$ . **K-35** is very sensitive to various substances including bilirubin and many drugs. Its fluorescence significantly decreases when the level of these substances in blood elevates as a result of competition of the probe. Comparative study has shown that **K-35** is much more sensitive to these substances than well-known fluorescent probe ANS.

*Key words*, albumin, binding capacity, specific probe, serum