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ACTIVATING AND INHIBITING EFFECTS OF SOME ORGANIC SUBSTANCES ON LUMINOL REACTION

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The effect of glucose, glutathione, thiourea, cysteinic acid and dithiourazol on the chemical luminiscence of luminol in the presence of haemin has been investigated by luminophotometric method. It was found that glucose and glutathione had the activating while the other studied substances the inhibitory effect on luminol reaction.

The maximum intensity and the time of the chemical luminescence of luminol are influenced also by substances which do not affect the reaction itself but increase considerably the emission maximum and, simultaneously, prolong the reaction time in the presence of accelerators (haemin). These activators (e. g. benzaldehyde and glucose) together with some other compounds, as presented here, produce a very intense effect upon the reaction when the following components take part:

1) 1.4×10^{-4}M luminol; 5.0×10^{-2}M NaOH
2) glucose, and
3) haemin 50 μg/100 ml

The solutions should be mixed in the order (1 + 2) + 3. No oxidizing agent need be present. The activating effect does not occur when glucose is added to the mixture 1 + 3. Kinetic luminescence curves (I – t) corresponding to various glucose concentrations are shown in Fig. 1. Curves 0–5 relate to the following amounts of glucose: 0%; 0.04%; 0.1%; 0.5% and 1.0%. Maximum intensity corresponds to a solution containing 0.5% glucose. The time necessary for the complete quenching of luminescence is about 20 hours. The curves were recorded by a luminometer (1).

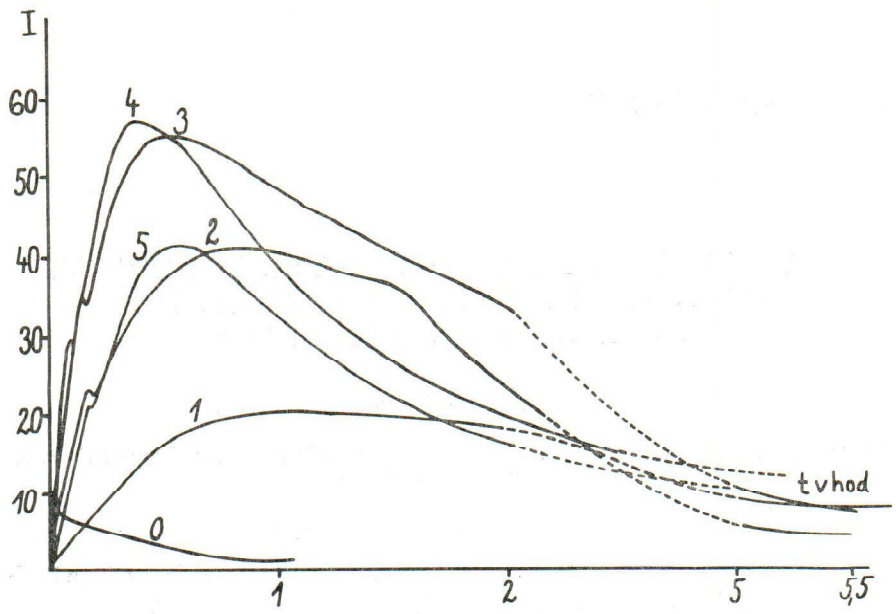

Fig. 1. *Effect of the addition of glucose upon the luminescence of luminol: curve No. 1: no glucose added, No. 2: 0.04% glucose, No. 3: 0.1%, No. 4: 0.5%, No. 5: 1.0% glucose in the reaction mixture*

The effect of the addition of glutathion is somewhat different from that of glucose, as seen in Fig. 2. The emission maxima are considerably sharper and the emission time is much shorter. The maximum effect is reached at a concentration as low as 1.6×10^{-4}M (curve 3). The other concentrations of glutathion were: 0.0; 3.3×10^{-5}; 1.6×10^{-4}; 2.5×10^{-4}, and 3.3×10^{-4}M. In this case also luminescence occurs without any admixture of oxidizing substance and only if haemin is added to a mixture of luminol and glutathion. Considerable activating effects were observed also with urazol (NH.NH.CO.CO) and phenylhydrazine hydrochloride.

The second group of substances called inhibitors decelerates the reaction and decreases in this way the luminous effect of luminescence. Some new inhibitors have been found which are considerably efficient when present in small amounts. Kinetic curves of cysteic acid and thiourea are shown in Figs. 3 and 4, respectively; the curves marked 1 refer to the solutions containing no inhibitor, while those marked 4 correspond to concentrations 2.1×10^{-3}M (cysteic acid) and 1.1×10^{-2}M (thiourea). Besides a series of aminoacids, luminescence is also strongly reduced by benzotriazol and still more by dithiourazol (NH.NH.CS. NH.CS) which decreases the maximum of luminescence by as much as 98.5% at a concentration of 1.5×10^{-5}M.

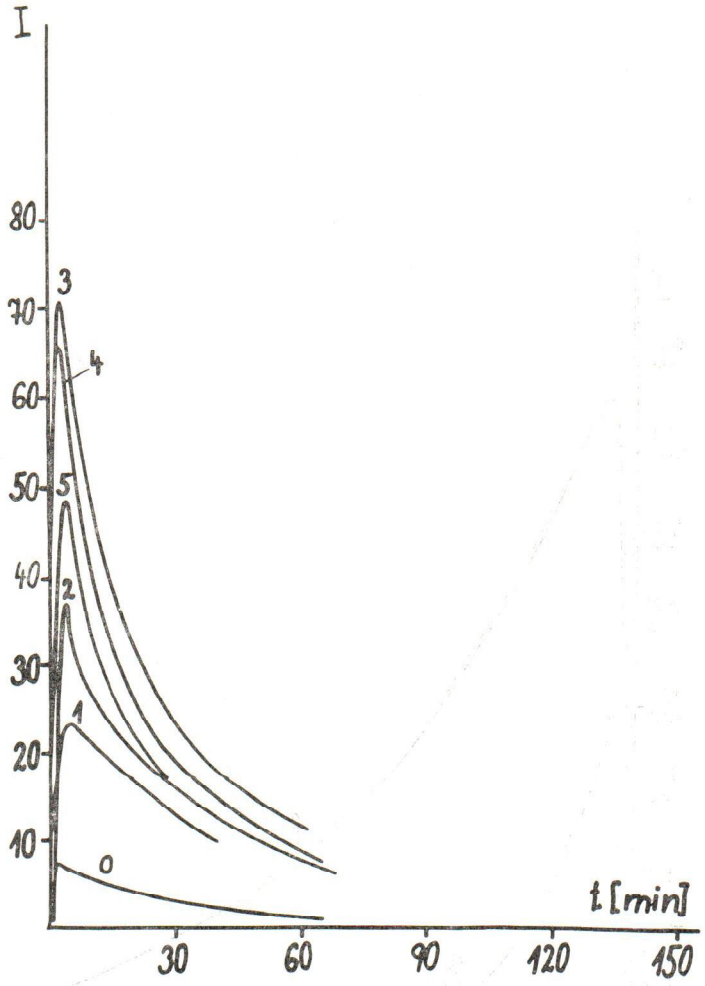

Fig. 2. *Effect of glutathion upon the luminescence of luminol. Curves O through 4: 3.3 x 10—5, 6.6 x 10—5, 1.6 x 10—4, 2.5 x 10—4, 3.3 x 10—4 M glutathion in the reaction mixture respectively*

These preliminary results obtained with the above inhibitors can be used to determine the efficiency of radioactive substances; they suggest the basis to a quantitative method for the determination of the protective efficiency of substances which can serve as a substitute for tedious biological methods.

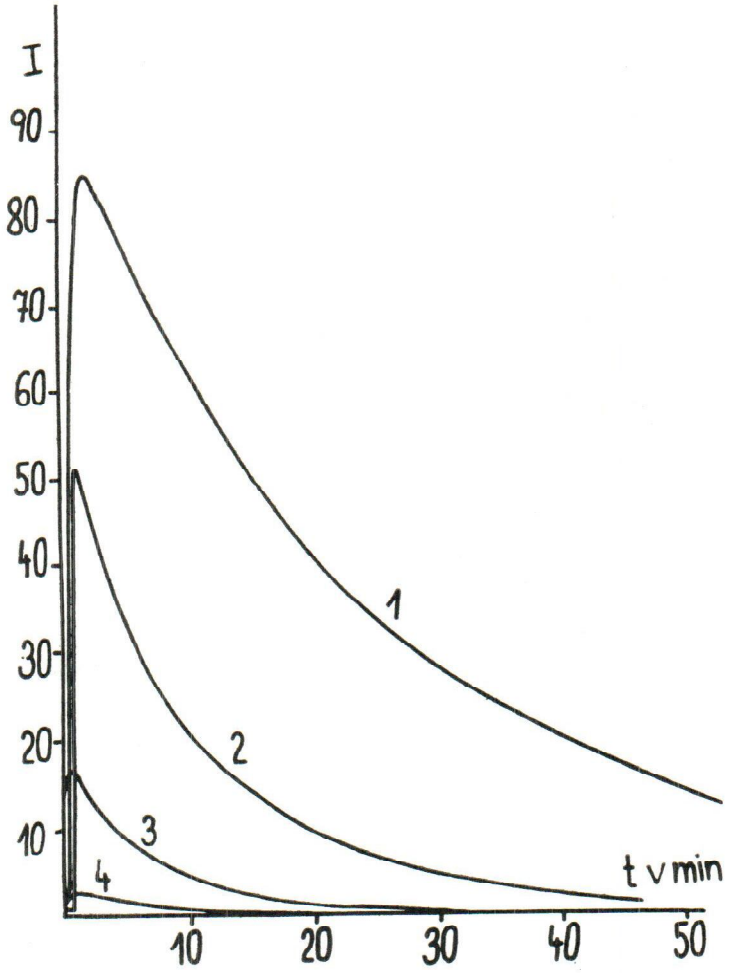

Fig. 3. *Effect of cysteic acid upon the luminescence of luminol (for explanation see text)*

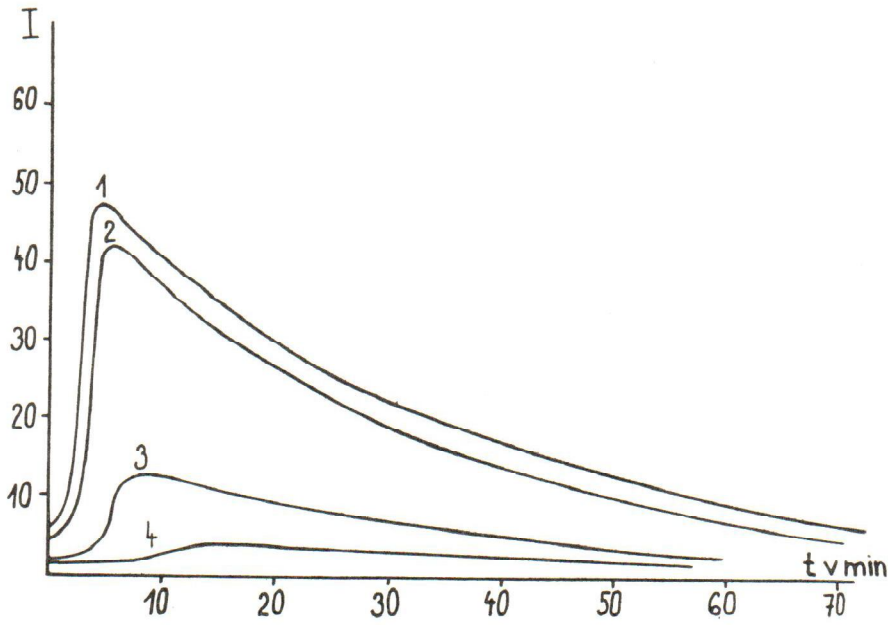

Fig. 4. *Effect of thiourea upon the luminescence of luminol (for explanation see text)*

Literature

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Sažetak

AKTIVATORNI I INHIBITORNI UČINAK NEKIH ORGANSKIH TVARI NA LUMINOLSKU REAKCIJU

Luminofotometrijskom metodom proučavan je učinak glukoze, glutationa, tiomokraćevine, cisteinske kiseline i ditiourazola na kemiluminescenciju luminola u prisutnosti hemina.

Glukoza i glutation djeluju kao aktivatori, dok ostale proučavane tvari inhibiraju luminolsku reakciju.

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