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# DETERMINATION OF DOUBLE BONDS OF THE BLOOD PLASMA LIPID FRACTION BY THE ADS-4M APPARATUS IN BURNED PATIENTS

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Double bonds (DB) between two carbon atoms in blood lipids are characteristic of unsaturated fatty acids (USFA), whose biological significance has been inadequately studied. Research in the last 10 years has shown the important role of USFA in the formation of highly active substances in the body, among which arachidonic acid, a precursor of the prostaglandins, and linoleic and linolenic acids deserve particular attention. As polyenic compounds, these acids have very high ability to participate in addition reactions or to split at the double bond.

We know that in burns the fatty-acid spectrum and concentration of USFA in the plasma change, and these changes apply both to free fatty acids and to acids of a lipid complex [3, 4]. Correlation has been found between the severity of the condition of a burned patient and the character and degree of changes in the spectrum and concentration of USFA, and the latter have been shown to be influenced by therapeutic measures. The importance of the study of the concentration and spectrum of USFA to the evaluation of the state of patients and effectiveness of their treatment has thus been demonstrated and the high sensitivity of an investigation of this type has also been observed. However, determination of the fatty acid spectrum by a chromatographic method is hardly suitable for regular monitoring of the patient's state because of the long time taken by the investigation and its complexity. The present writers have used a method of quantitative determination of DB in the lipid fraction of blood on an instrument known as a double bond analyzer (the ADS-4M), designed by the Institute of Chemical Physics, Academy of Sciences of the USSR [1].

The velocity of interaction of ozone with DB is several orders of magnitude greater than the velocity of its interaction with other functional groups, so that the analysis is highly selective [2]. This method has not been used hitherto in clinical practice.

In this investigation the diagnostic scope of the method of determination of DB in the blood lipid fraction in order to assess the state of burned patients and to monitor the effectiveness of their treatment, when used as an express method of detecting changes in the above parameters of USFA, was studied.

## EXPERIMENTAL METHOD

Repeated tests were carried out on 25 patients aged from 18 to 50 years with thermal burns of the IIIA + B to IV degree, affecting from 7 to 80% of the body surface. Blood plasma

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samples measuring 0.5 ml, taken from the fasting patient, were analyzed. The lipid fraction was extracted from the plasma by Folch's method. The aqueous part of the solution was removed, the remaining extract was filtered through a filter, defatted in hexane, and anhydrous sodium thiosulfate (to dehydrate the extract), after which the chloroform was vaporized to dryness on a rotary evaporator at 65°C. The blood lipids thus obtained were dissolved in 5 ml of ozonified hexane. For analysis, 0.5 ml of the solution was introduced into the ADS-4M apparatus. The ozone absorption curves thus obtained were recorded on paper, and to calculate DB in the sample, numerical parameters of the number of impulses were used, by the equation:

$$B = \bar{d}\frac{S}{n},$$

where B is the number of DB in 1 ml of blood (in moles DB/ml), S the area of the peak of the curve (in impulses) according to readings on the panel of the integrator, $\bar{n}$ the quantity of test substance in the sample (in ml), $\bar{d}$ the number of DB in the control substance, corresponding to 1 $cm^2$ of area of the peak, or per impulse (in moles DB/$cm^2$ or/impulse). The value of $\bar{d}$ was determined after analysis of a standard solution. In this investigation scintillation stilbene, with 1 DB in its molecule ($C_6H_5$—CH=CH—$C_6H_5$), was used as the standard substance [1]. To establish normal values, 15 healthy subjects aged from 22 to 47 years were investigated. The results were subjected to statistical analysis by Student's method.

## EXPERIMENTAL RESULTS

Under normal conditions the number of DB in lipid fractions of blood plasma varied from $190 \cdot 10^{-8}$ to $311 \cdot 10^{-8}$ moles DB/ml, with a mean value of $244 \cdot 10^{-8}$ moles DB/ml. For this group of subjects the ozone absorption curves had similar characteristics and, as a rule, they repeated one another (Fig. 1). No differences were discovered depending on the sex and age of the subjects.

In patients with burns the number of DB varied from $34 \cdot 10^{-8}$ to $287 \cdot 10^{-8}$ moles DB/ml. A considerable difference was found in the number of DB depending on the time after trauma. In the acute periods of burns, there was a sharp decrease in their number compared with normal (to $94 \cdot 10^{-8}$ moles DB/ml).

By the 25th-30th days, with stabilization of the patient's state, an increase was observed in the number of DB to $119 \cdot 10^{-8}$ moles DB/ml compared with the previous period ($P < 0.01$). By the 40th-60th days, their number had increased on average to $165 \cdot 10^{-8}$ moles DB/ml, and reached normal values in some patients. When these results were compared with the USFA concentrations, it was discovered that the time course of the changes in DB repeated fluctuations in the level of USFA such as arachidonic, oleic, and linoleic acids (Table 1).

On analysis of the values of these parameters in patients over a period of time, changes in the number of DB were found to coincide with changes in the patient's state. As an example, the results of 8-day monitoring of patient G., aged 26 years with a chemical burn affecting 40% of the body surface and of the IIIA + B degree, starting from the first few hours after trauma and until discharge from the clinic, are given in Fig. 2.

The number of DB in the blood plasma lipid fraction of the patients 5 h after burning was $183 \cdot 10^{-8}$ moles DB/ml, and on the 2nd day after burning it was $88 \cdot 10^{-8}$ moles DB/ml. At this time massive suppuration had begun in the patient's wounds, the nitrogen balance was showing negative features, and marked toxemia was present.

From the 18th day after burning the number of DB gradually began to increase, and as the burn healed and the patient's condition improved, the number increased steadily until the patient's discharge from hospital ($165 \cdot 10^{-8}$ moles DB/ml).

The results of investigation of patient F., aged 21 years, with thermal burns of the III A + B degree affecting 70% of the body surface, admitted to the All-Union Burns Center on the 12th day after burning, are shown in Fig. 3. Her state on admission was extremely grave because of toxemia and extensive burns: the number of DB on admission was $56 \cdot 10^{-8}$ moles DB/ml. On the 18th day of her stay in the burns unit, treated by the open method on a "Clinitron" bed (France), the number increased to $116 \cdot 10^{-8}$ moles DB/ml, and as the wounds healed gradually, the number of DB continued to rise.

However, after 40 days the patient's condition deteriorated, healing became sluggish, and signs of burn exhaustion appeared: loss of body weight, loss of appetite, inability to assimilate food (vomiting, retching, meteorism). The DB level on the 42nd day had fallen from

TABLE 1. Changes in USFA Concentrations (in millimoles/liter) and in Number of DB (in millimoles DB/ml) in Blood Plasma of Burned Patients Over a Period of Time

| Name of USFA | Time after burning, days | | | |
|---|---|---|---|---|
| | 5 | 10 | 30 | 37 |
| $C_{18:1}$ | 1,47 | 0,10 | 5,09 | 5,97 |
| $C_{18:2}$ | 0,68 | 0,49 | 3,82 | 3,96 |
| $C_{20:4}$ | 0,09 | 0,04 | 0,96 | 3,29 |
| DB | $94 \cdot 10^{-8}$ | — | $119 \cdot 10^{-8}$ | $165 \cdot 10^{-8}$ |

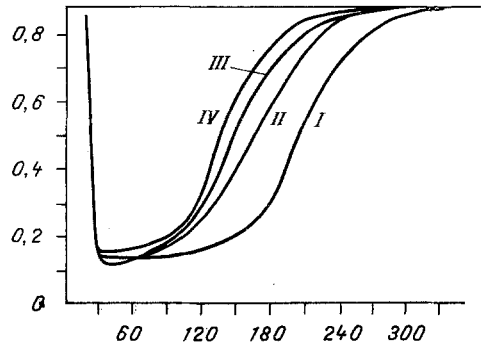


Fig. 1

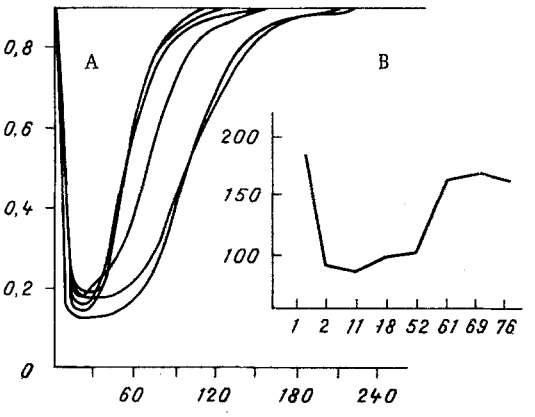


Fig. 2

Fig. 1. Curves of ozone absorption by blood plasma lipid fraction of healthy subjects. Abscissa, time (in sec); ordinate, ozone absorption (in moles/liter $\times 10^{-8}$). I, IV) Extreme values, II, III) intermediate values.

Fig. 2. Results of investigation of blood plasma lipid fraction of patient G. A) Curves of ozone absorption by lipid fraction. Abscissa and ordinate — as in Fig. 1; B) time course of number of DB in blood plasma lipid fraction depending on time after trauma. Abscissa, time after trauma (in days); ordinate, ozone absorption (in moles/liter $\times 10^{-8}$).

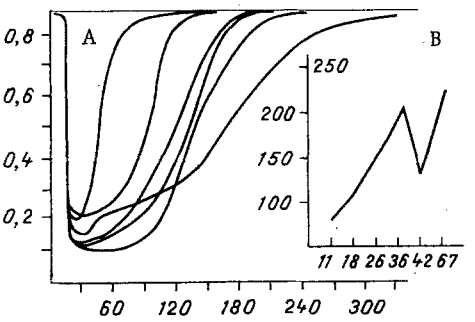


Fig. 3. Results of investigation of blood plasma lipid fraction of patient F. Legend as to Fig. 2.

$197 \cdot 10^{-8}$ to $133 \cdot 10^{-8}$ moles DB/ml. From that moment the patient began to receive additional feeding through nasal gastric tube, and after 3 weeks the number of DB became normal ($236 \cdot 10^{-8}$ moles DB/ml). This coincided with a considerable improvement in the patient's condition: during this period she gained 7 kg in weight, her appetite improved, pink granulations appeared, and marginal epithelization of the wounds took place.

A fall in the number of DB was found in these patients also on the appearance of any kind of complications and, in particular, of lysis of skin autografts after transplantation. As a rule, the fall in the number of DB preceded clinical manifestations of the complications. Disappearance of the latter as a result of appropriate measures led to an increase in the number of DB also.

The method of determination of DB in blood lipid fractions of burned patients thus is an important addition to the diagnostic possibilities available when assessing the patient's condition. A fall in the DB level below normal coincided with a severe course of the underlying burns, and intensive and successful treatment was accompanied by an increase in their number.

The response of a change in the number of DB, it will be noted, preceded clinical manifestation of the corresponding change in the patient's condition. The time course of the change in the number of DB in the blood plasma lipids is evidence that the method of determination of DB can be rightly used to assess disturbances in the USFA concentration, and justify its use as a rapid diagnostic method in clinical practice.

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