MICROWAVE METHOD OF DETERMINING CEREBRAL BLOOD FLOW

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Clearance methods of determining the cerebral blood flow are widely used in experimental Physiology. They are based on calculation of the blood flow from the clearance curve of an indicator — a radioactive isotope or hydrogen [2] — introduced into the brain.

The possibility of using heat as a clearance indicator was based on an investigation [8] as a modification of another known method, namely the thermoelectric method [2]. The methods mentioned above are to some degree traumatic. This is connected either with the introduction of electrodes into the brain or puncture of an artery to inject the isotopes into the blood stream.

In this paper a noninvsaive method of determination of the cerebral blood flow by means of microwaves is described.

EXPERIMENTAL METHOD

The method also is based on the phenomenon of thermal clearance. If a certain part of the brain is heated for a short time, the excess heat will be mainly removed due to the inflow of colder blood. Consequently, the rate of the temperature drop of the heated area is a function of the blood flow, and the latter can be calculated from it. In the method described the test area is heated noninvasively, from an external source, by means of microwave technology. Microwaves [the superhigh frequency (SHF) range] have the property of penetrating into dielectric and semiconducting media, and when absorbed, give out heat. This phenomenon has been used for a long time in physiotherapy [6]. Under the given conditions heating takes place, not by transmission of heat from the outer to the inner layers, but simultaneously throughout the irradiated volume. The depth to which microwaves penetrate into biological tissue is about equal to their wavelength in that medium [3].

In the present experiments a transistorized generator (1400 MHz) was used for heating. Consequently, the wavelength in the brain tissue and the corresponding depth of penetration of the microwaves were about 3.5 cm. The microwaves were transmitted to the brain by means of a contact applicator-antenna, applied to the skull. The antenna was similar to one used previously [4]. The maximal power of the generator was 2 W. The cranial bone is sufficiently thin, so that losses of microwaves due to absorption by it are comparatively small [3]. Most absorption thus takes place in the brain tissue, in a region limited below by the depth of penetration of the wave, and at the sides by the aperture dimensions of the antenna.

The method of recording temperature is based on the use of intrinsic thermal electromagnetic radiation, whose intensity is proportional to the body temperature [5]. Infrared thermographs working on this principle are already in use in medicine. However, they give information only about the surface temperature. By using microwaves, however, which have higher penetrating power, it is possible to measure the depth temperature. In the microwave range the antenna receives thermal radiation arising not only from the surface, but also from the internal layers. Methods of measuring the internal temperature of biological objects by means of microwave receivers of thermal radiation, or SHF-radiometers [4, 7], previously used only in radioastronomy, have recently been developed.

In this investigation, temperature was measured by the ARAKS-4 radiometer [1]. The frequency range of the radiometer is 1350-1650 MHz, its fluctuation sensitivity 0.05 K, and its integration time 1 sec. Because of reflection at skin-bone and bone-brain boundaries, abso-

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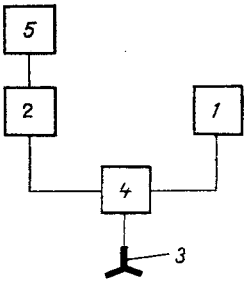

Fig. 1

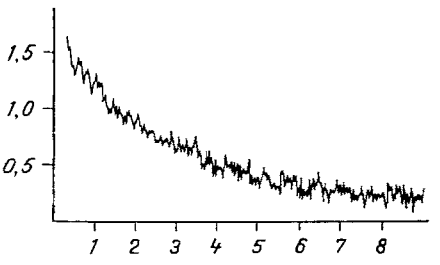

Fig. 2

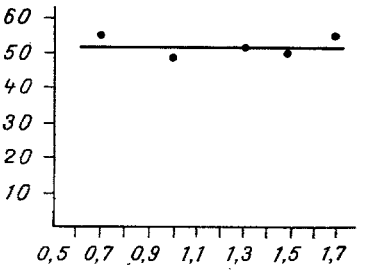

Fig. 3

Fig. 1. Block-diagram of microwave apparatus for measuring the cerebral blood flow. 1) Generator; 2) radiometer; 3) applicator-antenna; 4) switch; 5) automatic writer.

Fig. 2. Experimentsl temperature drop curve. Abscissa, time (in min); ordinate, heating (ΔT, °C).

Fig. 3. Cerebral blood flow of a cat measured with different heating powers. Abscissa, power (in W); ordinate, cerebral blood flow (in ml/min/100 g). Maximal power of 1.7 W corresponds to heating of 1.73°C.

lute measurements of temperature are difficult to make. In this case, however, it is unnecessary, because we are concerned only in relative changes of temperature. The same applicator-antenna is used for heating and for measuring temperature. Since the frequency of heating lies within the frequency range of the radiometer, it can be accepted that regions of heating and of integral measurement of temperature will coincide. A block-diagram of the apparatus is shown in Fig. 1. The measuring process consists of three stages: 1) recording the initial temperature level; 2) heating the test object (the time was chosen empirically in order to raise the temperature by 1.5-1.7°C); 3) recording the temperature drop. The cerebral blood flow was calculated from the temperature drop curve, which is exponential in character. The experimental curves were analyzed by the standard clearance method [2]. One typical example of temperature drop is given in Fig. 2.

Experiments were carried out on anesthetized cats. The applicator-antenna was fixed to the animal's heat in the parietal region. Parallel recordings were made of parameters of the acid-base balance of arterial blood (pH, pCO_2, pO_2).

EXPERIMENTAL RESULTS

The aim of the experimental was to verify the objectivity of the method. Parallel measurements were therefore made of the cerebral blood flow of the cat by the ^{133}Xe clearance method. The results obtained were virtually identical. The cerebral blood flow under normal conditions, calculated from measurements on more than 20 animals, was 48 ± 10 mg/min/100 g tissue. The method also was tested by intracarotid injection of known vasoactive preparations (noradrenalin and papaverine), and by inhalation of a mixture of 5% CO_2 + air. After injection of noradrenalin a reduction of the cerebral blood flow was recorded, whereas in the other two cases an increase was recorded.

For instance, with a duration of inhalation of 1 min and a value of pCO_2 of 36.4 mm Hg, the cerebral blood flow was 46 ml/min/100 g tissue, and the corresponding value for 2 min and 39.5 mm Hg was 53 ml/min, for 3 min and 52.3 mm Hg it was 59.2 ml/min, for 4 min and 59 mm Hg — 64.5 ml/min, and for 5 min and 64 mm Hg — 58 ml/min.

The accuracy of the method was also studied with regard to the vasodilator action of heat. This could lead to a change in the blood flow during measurement, which is unacceptable. Small deviations of blood flow at different powers of irradiation (Fig. 3) are evidence that heating was below the limit when vasodilatation is observed.

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INDICATION OF VIRUSES AND VIRUS-SPECIFIC ANTIBODIES BY ELISA USING CONJUGATES BASED ON β-LACTAMASE OBTAINED BY GENETIC ENGINEERING

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The method of enzyme-linked immunosorbent assay (ELISA), by means of which antigens and antibodies of different origin can be detected with high sensitivity and specificity in biological samples, has achieved widespread popularity in recent years. Virtually all varieties of immunoenzyme techniques are based on the use of conjugates, which are macromolecular complexes formed by covalent "attachment" of enzyme molecules to antigen or antibody molecules. Enzyme-labeled macromolecules, immobilized during the assay, can be detected by adding chromophore, fluorocheome, or radioactive substrates, modified by the chosen enzyme, to the system [1].

Conjugates based on peroxidase, alkaline phosphatase, and β-galactosidase are most frequently used at the present time to construct immunoenzyme test systems [10]. However, the use of these enzymes (in particular, peroxidase, which is most widely used) in ELISA is complicated by the fact that they are often present in the free or bound form in the biological material to be studied [2], and also by the fact that substrates of these enzymes in most cases either possess low stability, or are difficult to synthesize, or are toxic to the operator [1]. Accordingly, the search for new enzyme-substrate pairs for use in ELISA techniques still remains an urgent task.

Most recently conjugates containing β-lactamase (penicillinase, penicillin-amido-β-lactan hydrolase, EC 3.5.2.6) have begun to be used in the construction of new immunoenzyme test systems [3, 14, 15]. This enzyme, whose amino-acid sequence and principal physicochemical properties are known [8, 13], hydrolyzes the β-lactam ring of penicillin and its derivatives, and converts them into penicillonic acids, which is detectable by a relatively simple iodometric method [6]. The chief advantage of using lactamase conjugates is the simplicity and convenience of the substrates used for its detection — mixtures of penicillin, starch, iodine, and potassium iodide [15] or cadmium iodide [3].

The source of the enzyme used to synthesize lactamase conjugates is usually penicillin-resistant strains of bacteria [3, 14, 15]. In the present investigation we used for this purpose β-lactamase, synthesized by a genetic engineering method, which has many advantages over the traditional methods of obtaining the enzyme. We used the lactamase conjugate in immunoenzyme test systems intended for the detection of viruses and virus-specific antibodies in biological specimens (influenza virus was used as the model). It was also natural to compare the sensitivity of immunoenzyme methods based on β-lactamase conjugates and those

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