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16. Abstracts

The report contains information on aerospace medicine, agrotechnology, bionics and bioacoustics, biochemistry, biophysics, environmental and ecological problems, food technology, microbiology, epidemiology and immunology, marine biology, military medicine, physiology, public health, toxicology, radiobiology, veterinary medicine, behavioral science, human engineering, psychology, psychiatry and related fields, and scientists and scientific organizations in biomedical fields.

17. Key Words and Document Analysis. 17a. Descriptors

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# TRANSLATIONS ON USSR SCIENCE AND TECHNOLOGY

## Biomedical and Behavioral Sciences

### No. 33

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**TOXICOLOGY**
Providing free, universally accessible, and skilled medical care to our country's citizens is an embodiment of the principle of communism in public health. This principle was laid at the basis of Soviet public health following the victory of Great October. About 100 decrees signed by V. I. Lenin were aimed at improving the state of the country's public health and creating conditions promoting improvement of the health of Soviet people.

Not one capitalist country is able to create anything similar to the Soviet system for protecting the health of the public. In the entire history of capitalist society, not a single progressive official who had become the head of state has been able to provide therapeutic and preventive care to all strata of the society; the reason for this lies in the social principles of public health. "The socialist state," the CPSU Program states, "is the only state which accepts the responsibility of protecting and constantly improving the health of the entire population. This is being achieved through a system of socioeconomic and medical measures."*

The advantages of the Soviet public health system have been embodied in the law. The "Fundamental Principles of USSR and Union Republic Public Health Legislation" which went into effect on 1 July 1970 clearly spell out the basic obligations of medical workers in relation to providing medical care to the public. Legal documentation of the successes achieved in the Soviet Union in protecting public health and further legalization of social relations in this area are unique features of Soviet public health legislation.

**"Programma Kommunisticheskoy partii Sovetskogo Soyuza" (Program of the Communist Party of the Soviet Union), Moscow, Politizdat, 1976, p 96.
The party is now increasing its requirements on the activity of public health agencies. In his report to the 25th CPSU Congress L. I. Brezhnev emphasized: "Among the social tasks, none are more important than concern for the health of Soviet people. Our successes in this area are known to everyone. But we must also be aware of the problems in this area. They are associated with improving public health organization, broadening the network of hospitals and polyclinics, and increasing the production of medical equipment and highly effective medicines. They are also associated with developing medical science and initiating an extensive struggle against the most dangerous diseases. There is much to do in improving protection of the health of mothers and children and in expanding the network of health resorts, rest homes, and holiday hotels."*

The Constitution of the Union of the Soviet Socialist Republics adopted on 7 October 1977 is a new clear manifestation of the party's concern for the health of Soviet people. The fundamental law of the Soviet Union guarantees the right of health protection to all of its citizens. "This right," states Article 42 in the Constitution, "is supported by free skilled medical care rendered by state public health institutions; by expansion of the network of institutions treating citizens and improving their health; by development and improvement of industrial safety and industrial sanitation; by implementation of extensive preventive measures; by measures to improve the condition of the environment; by special concern for the health of the growing generation, to include prohibition of child labor not associated with school and vocational education; by initiation of scientific research aimed at preventing and decreasing morbidity and at insuring a long active life for citizens."

The constitutional guarantee of the rights of Soviet people for health protection as well as for court protection against transgressions of honor and merit, life, and health, and protection of personal freedom and property must be supported by all state agencies, public organizations, and officials, including the workers of forensic medical expert commissions.

Creation and improvement of our country's forensic medical service is inescapably associated with development of Soviet public health. A forensic medical expert examination section, which is the administrative center of forensic medical expert examination in our country, was organized within the Peoples Commissariat of Public Health in October 1918. A well-organized forensic medical service was created, making it possible to conduct expert examinations and monitor their process. The "Statute on Forensic Medical Experts" published in 1920 and the "Statute on the Conduct of Forensic Medical Expert Examinations" published in 1934 were official documents regulating the activity of forensic medical expert examination in the USSR.

*Brezhnev, L. I., "Report of the CPSU Central Committee and the Party's Immediate Tasks in Domestic and Foreign Policy," in "Materialy XXV s"yezda KPSS" (Proceedings of the 25th CPSU Congress), Moscow, Politizdat, 1976, p 41.
Our country's forensic medical service was created and improved in accordance with the same principles at the basis of Soviet public health. This is why the tasks, organization, and activities of forensic medical expert examination in the USSR differ significantly from the way forensic medical expert examination is organized in bourgeois countries.

In capitalist countries, forensic medical expert commissions provide services only to investigative and court agencies, providing no assistance to public health agencies. Expert examinations are conducted by private court-appointed physicians, by physicians working for police administrations, insurance companies, and so on, since a state forensic medical service does not exist. Nor is there a state system for paying for expert examination services, as a result of which the work of experts is paid for by private persons. The private enterprise nature of forensic medical expert examination in capitalist countries cannot insure objectivity of expert examination and in a number of cases even promotes falsification of examination results. This can be seen from data concerning forensic medical expert examinations pertaining to a number of landmark cases (the (Beylis) case, the Sacco and Vanzetti case, the (Katynskoye) case, the expert examination connected with the assassina-
tion of President Kennedy in the USA, and so on).

In contrast to bourgeois expert examination, Soviet forensic medical expert examination provides full support not only to justice agencies but also to public health agencies in improving the quality of therapeutic care for the public, in decreasing morbidity and mortality, in improving working and living conditions, and so on.

An order of the USSR Ministry of Public Health dated 10 April 1962 demands that workers of the forensic medical service systematically discuss forensic medical expert examinations at clinical-anatomical conferences and promptly inform public health agencies concerning cases of gross discrepancies in clinical and anatomical diagnoses and other defects in therapeutic care. This order obligates forensic medical experts to analyze cases of sudden death, transportation injuries, and poisonings in the home and in industry, and to reveal shortcomings in therapeutic and preventive care with the goal of developing and implementing measures aimed at preventing them. Consequently as with all other sectors of medicine, Soviet forensic medical expert examination is oriented toward prevention.

A well-organized state forensic medical service exists in the Soviet Union; as with other sectors of medicine, it is structured in accordance with the territorial principle. Efficient organization of forensic medical service in our country, independence of experts from justice agencies, and absence of material interests of the expert in the outcome of a case promote objectivity in Soviet forensic medical expert examination.

The country's forensic medical service consists of 156 republic, kray, and oblast offices of forensic medical expert examination employing 14,831 colleagues, 5,388 of whom are experts. Much work has been done in recent years
to upgrade the qualifications of the experts through specialization and advanced training, and to train experts through clinical residency. Just in 1971-1976 2,718 specialists—that is, more than half of all forensic physicians and chemists—underwent specialization and advanced training.

Improvement of the material-technical base of a number of forensic medical expert examination offices, primarily provision of the latest equipment and apparatus, has promoted improvement in the work of the forensic medical service. This has led to greater possibilities for physicotechnical, forensic biological, and forensic chemical research, and to a reduction in the time required for expert examination.

The successes attained by our country's forensic medical service are in many ways the product of the inseparable ties between forensic medical science and expert practice. Scientific research is not only permitting us to solve the complex problems posed by expert practice, but it is also indicating the perspectives of new directions in expert activity.

Today, 64 doctors of medical sciences, about 250 candidates of sciences, and a significant number of specialists are working on the scientific problems of forensic medicine in schools of forensic medicine and toxicological chemistry, at the Institute of Forensic Medicine, and in many institutions of practical forensic medical expert examination. During the last 5-6 years 38 doctorate and 170 candidate dissertations on forensic medicine have been defended, and 47 collections of scientific works, 31 monographs, and many journal articles and training and teaching aids have been published.

It was emphasized in recommendations of the First All-Union Congress of Forensic Physicians held in September 1976 that introduction of new objective analysis methods developed in recent years into the practice of analysis of material evidence and forensic chemical expert examination, and use of physicotechnical methods have broadened the possibilities of forensic medical expert examination and increased the value of such analyses as evidence, which has significance to the fight against crimes against human life and health.

The congress recommended expansion of scientific research pertaining to determining time of death, establishing the implement and mechanism of injury on the basis of the damage evident, revealing the thanatogenesis of sudden death caused by cardiovascular diseases, and improving methods affording a possibility for identifying individuals from traces of blood and other objects of biological origin detected in microquantities.

Considering the great volume of scientific work being done in forensic medicine and toxicological chemistry, and the need for centralized planning and coordination of research being conducted in the country, in April 1977 the Presidium of the USSR Academy of Medical Sciences created an all-union problematic commission to study the problems of forensic medicine and toxicological chemistry. The commission's tasks include writing summary plans and reports on research pertaining to specific problems and topics, and reviewing the
research plans for 1978-1980 with the goal of eliminating possible duplications and canceling topics in the plans that are not sufficiently important in scientific and practical respects. The problematic commission must prepare reviews on the state of scientific research in the main directions of Soviet medicine and forward these reviews to local agencies, check the results of major projects that have been completed, and recommend them for practical introduction.

Comparing the level of development of forensic medical science and practice in our country and abroad, and considering personal impressions, I can say that in relation to the principal subdivisions (expert examination of living individuals and corpses), forensic medicine in the Soviet Union is ahead of that in highly developed capitalist countries (USA, France, Italy, and so on). As far as forensic chemical and biological expert examination is concerned, scientific research in these areas is not lagging behind similar research abroad.

However, utilization of the results of scientific achievements often elicits significant difficulties in view of the problems in supplying new apparatus, reagents, and diagnostic serums to forensic medical laboratories. The Soviet experience of organizing state forensic medical expert examinations and of providing training and advanced training to forensic medical personnel is assessed quite positively by specialists in foreign countries. This experience is being utilized in the work of forensic medical agencies of a number of fraternal socialist countries.

However, the level of scientific research and especially of practical introduction of scientific achievements still fails to satisfy the higher requirements posed today before forensic medical expert examination by police agencies. This was reflected in recommendations of the All-Union Scientific-Practical Conference organized in November 1977 by the USSR Ministry of Justice together with the USSR Procurator's Office, the USSR Ministry of Public Health, and the USSR Ministry of Foreign Affairs; this conference was devoted to introduction of scientific-technical resources and scientific recommendations into the practice of investigation and court examination of terminal cases.

Public health agencies are posing many complex problems before the forensic medical service concerning improvement of therapeutic and preventive care to the public. The decree "On Measures for Further Improvement of Public Health" adopted by the CPSU Central Committee and the USSR Council of Ministers on 22 September 1977 suggests a multifaceted program for strengthening and developing the material-technical base of public health, upgrading the quality of specialist training, developing specialized services, and broadening scientific research to support protection of the health of the Soviet people. This decree imposes new, higher requirements on all medical workers, including those involved in forensic medical expert examination.

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The practice of organizing the fight against crime has shown that successful discovery and investigation of crimes and efficient court investigation depend in many ways on coordination of the actions of law enforcement agencies, the organization of their work, and the ability to make full and competent use of modern scientific-technical resources and scientific recommendations.

These problems were the topic of thorough examination at the All-Union Scientific-Practical Conference "Introduction of Scientific-Technical Resources and Scientific Recommendations Into the Practice of Investigation and Court Examination of Criminal Cases," organized by the USSR Ministry of Justice jointly with the USSR Procurator's Office, the USSR Supreme Court, the USSR Ministry of Foreign Affairs, and the USSR Ministry of Public Health and held in Moscow in November 1977.

The following reports were given at the conference: "Introduction of Scientific-Technical Resources and the Scientific Recommendations Into the Work of Expert Institutions and Courts as a Means to Heighten the Quality and Effectiveness of Criminal Court Proceedings in Light of Decisions of the 25th CPSU Congress" by USSR Deputy Minister of Justice N. A. Osetrov, "The Growing Significance of Science and Technology in the Work of Investigatory Agencies in Discovering and Investigating Crimes" by USSR Assistant Procurator-General S. A. Gusev,* "The Role of Forensic Medical Science in Expert Practice" by USSR Deputy Minister of Public Health D. D. Venediktov, and "Some Problems in Using Modern Methods and Resources of Criminalistic Techniques in Preliminary Investigation" by S. V. Murashov, chief of the investigatory administration of the USSR Ministry of Internal Affairs.

*S. A. Gusev is presently Deputy Chairman of the USSR Supreme Court.
The conference participants, who included practical workers and scientists, noted that law enforcement agencies have done a significant amount of work to introduce the achievements of science and technology into operational-investigatory, court, and expert practice in compliance with the CPSU Central Committee and USSR Council of Ministers decrees "On Measures for Improving the Work of Investigatory Staff of Procurator and Police Agencies" adopted on 10 December 1965 and "On Measures for Improving the Work of Court and Procurator Agencies" adopted 30 July 1970.

Operational-technical services and investigatory agencies are outfitted with equipment permitting them to discover, document, and subject material evidence to preliminary analysis.

Technical resources are being applied more extensively in court: More-frequent use is being made of tape recorders, motion pictures, photographs, and other demonstrative materials facilitating assessment of the evidence presented not only to the participants of the process but also to citizens present in court.

Introduction of these methods for analyzing material evidence into expert practice has improved the scientific grounds and heightened the value of expert conclusions as evidence. The following achievements are included among the most significant results of criminalistic expert practice: The possibility for establishing intentionally altered handwriting and the identity of the writer using mathematical and cybernetic methods, and the methods of statistical probability for identifying an author from the linguistic clues of written material; integrated analysis of document materials—ink, paste, paper, and glues using apparatus methods; the scientific grounds and methods of expert examination of transportation-related clues; development of the methods of profiling and of optical and mathematical modeling heightening the possibilities for analyzing various objects subjected to forensic expert examination. Expert examination of fibrous materials, expert forensic soil examination, and analysis of paints, varnishes, and coatings making use of standardized quantitative techniques with the goal of accumulating data on the composition and structure of substances and their computer processing have completed their development. Such expert examination has become widespread in investigatory and court practice in recent years; with its assistance, elements of the surrounding material situation are being identified, interaction of objects is being established, and so on on the basis of micro-particle analysis. Analog modeling has been developed with the goal of solving important problems arising during investigation of traffic accidents. Methods have been developed for performing analyses associated with cases of embezzlement at industrial enterprises and enterprises of state and cooperative trade employing mechanized bookkeeping techniques. Teaching aids have been written for court and investigatory workers and experts on the important problems of expert bookkeeping examination.

Representatives of forensic medicine related their successes. In forensic traumatology, criteria have been developed for determining the direction of a blow and the position of the victim at the moment of injury, differentiating...
head injuries in relation to whether they resulted from a blow or a fall, establishing the implement causing the injury, and so on. The laws associating the mechanism of injury and the specific features of the arising injuries are being established with the help of modeling methods. The arsenal of methods for determining whether or not an injury was caused while the victim was still alive and the time since injury on the basis of changes in enzymatic activity and histochemical, spectral, and other signs has grown. Establishment of the time of death continues to be one of the difficult problems of forensic medical practice. The efforts of forensic physicians are aimed at solving this problem through the use of a broadened complex of various analysis methods. A special stimulator of climatic influences making it possible to model the conditions in which a corpse may have existed will be of help in this regard. Methods have been introduced for diagnosing sex from traces of blood, saliva, and particles of tissues and organs. It has become possible to answer the question as to the groups of blood and saliva from negligibly small quantities. Methods are being developed to establish the species, organic and tissue origin, and sex of deposited cells, and to diagnose the origin of cells in traces on material evidence. The possibilities have been expanded for establishing the sex of skeletal bones and determining age from cranial bones, rib cartilage, and so on. Successes have been attained in forensic stomatological and toxicological expert examination.

A special forensic medical expert examination vehicle outfitted with apparatus and instruments intended for examination of corpses and conduct of expert examination at the site has been developed and recommended for series production.

In addition to the successes enjoyed in introducing scientific-technical resources and scientific recommendations into investigatory and court examination practice, there are a number of unsolved problems, difficulties, and shortcomings. The equipment available in operational-technical services, forensic expert institutions, and criminological offices employing new techniques does not always produce the desired results. Specialists to which this equipment is entrusted are not always typified by sufficient occupational skills and habits. Duplication and parallelism still exist in the activities of operational-technical subdivisions and forensic expert institutions. All of this is resulting in omissions in the work and the inability to efficiently find and correctly document material evidence, fill out forms properly and submit the necessary materials for expert examination, and make full use of the possibilities for analysis of these materials. Introduction of the achievements of science into investigatory, court, and expert practice is being retarded to a certain extent by the absence of adequate coordination of interested departments in the development of new criminalistic equipment and in centralized disposition of orders for such equipment.

Complaints were also directed at forensic medical experts who do not always make full use of analysis methods that have been tested and recommended for
introduction. This failure is sometimes the reason why questions such as
time of death, the mechanism and sequence of injuries, the nature of the
weapons, and so on, which are important to investigatory agencies and courts,
remain unanswered. Cases have occurred in which offices of forensic medical
expert examination have not made sure that specialists participate in inspec-
tion of corpses at the scene.

One of the obstacles to broad application of scientific-technical resources
is absence of the necessary equipment and recommendations pertaining to
documentation and demonstration of various articles of evidence in many
courts. Local fear and mistrust of new methods stem to a certain extent from
the failure of legal regulation to remain up-to-date with development of
science and technology and the needs of investigatory staffs and courts.

Having as their goal greater effectiveness in the fight against crime with
the help of scientific-technical resources and implementation of scientific
recommendations, the conference participants resolved that it was necessary
to devote more attention to coordinating the actions of law enforcement agen-
cies, to jointly solve important problems pertaining to operational-investi-
gatory and expert techniques, to conduct integrated inspections, to discuss
their results at interdepartmental conferences, to submit appropriate recom-
mendations to local agencies, and so on.

It was recommended that interested departments implement measures to stand-
ardize regulation of expert activities; determine the principles and forms
of scientific-methodological management of standardized expert practice;
develop interdepartmental instructions on organizing and conducting inte-
grated criminalistic, radical-criminalistic, and other expert examinations;
establish concrete forms of constant scientific-technical and methodological
interaction in training specialists to use new equipment; provide fuller
information on criminalistic technology and develop the criteria of the
effectiveness of its application.

The problems of ordering and conducting forensic expert examinations and
assessing the scientific level and significance, as evidence, of the con-
clusions of forensic experts require greater coordination in scientific-
methodological work with examining magistrates, procurators, and judges.
A suggestion has been made to conduct joint scientific-practical seminars,
to study and generalize the status of the work of recruiting specialists to
examine various categories of cases and develop concrete recommendations on
their participation in preliminary investigations, and to disseminate the
experience of the best examining magistrates in relation to application of
scientific-technical resources and scientific recommendations, and the use
of the possibilities of forensic expert examinations.

The conference appealed to the USSR Ministry of Justice, the USSR Procurator's
Office, the USSR Ministry of Internal Affairs, and the USSR Supreme Court
with a request to examine the issue of preparing proposals concerning amend-
ments and additions to the union republic criminal codes in relation to the
use of scientific-technical resources and scientific recommendations in the
investigation of crimes and court examination of criminal cases, with emphasis primarily on developing general principles of the permissibility of using criminalistic equipment.

The proposals suggested at the conference were the foundation for recommendations adopted by participants of this forum of criminalists and forensic physicians.

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DAMAGE TO CLOTHING MATERIAL, SKIN, AND CADAVERIC BONES BY DISCHARGES FROM A 5.45-mm CALIBER AKS-74 ASSAULT RIFLE

Moscow SUDEBNO-MEDITSINSKAYA EKSPERTIZA in Russian No 2, 1978 pp 17-20

[Article by M. I. Marchenko, Y.-V. Y. Naynis, A. P. Zakaras, G. S. Bartenov, and V. P. Shardakov, School of Anatomy and Forensic Medicine, Kaunass Medical Institute]

The AKS-74 assault rifle is supplied with a muzzle brake, the design features of which define the nature of damage to fabrics and deposition of discharge soot. Three AKS-74 rifles supplied with muzzle brakes were experimentally fired at targets made of broadcloth (22 percent viscous fiber), gabardine (50 percent lavsan), impregnated and nonimpregnated camouflage-colored cotton fabric, rubberized cotton fabric, cotton fabric saturated with water repellent, 82007 camouflage-colored fabric (60 percent lavsan, 40 percent cotton fiber), white coarse calico, close-woven cotton fabric, staple, satin, wool, knitted fabric, varnished fabric, Gobelin fabric containing synthetic fiber, dermatin, (krimplen), capron, nylon, synthetic fur, viscous fabric, triacetate, and (ortalon) (so-called (bolon'ya)) in order to study damage to textile fabrics. In all we studied 505 cases of gunshot damage caused by discharge at point-blank range, at ranges from 1 to 27 cm at centimeter intervals, and at 30, 35, 40, 50, and 100 cm. In relation to point-blank range we analyzed damage arising with the barrel at angles of 30, 45, and 90° with respect to the target. To study damage caused to textile fabrics by an AKS-74 assault rifle without a muzzle brake we fired 56 rounds into targets made of white coarse calico and various other fabrics.

The nature of damage to human tissues was studied using 44 entrance and exit skin wounds and 52 cases of damage to cadaveric bones and bone preparations, as well as the materials of two practical expert examinations.

The nature and intensity of deposition of discharge soot was studied with color impressions detecting presence of copper, iron, and lead (170 contactograms using photographic paper) and by the X-ray method (21 roentgenograms).
A deposit of discharge soot having the form of a two-sided "butterfly" (insert Figure 1a, b [the figures not reproduced]) was evident on white coarse calico targets at ranges from point-blank to 5 cm, covering an area of 14×19 and 17×29 cm. The distance between the segments of the "butterfly" for point-blank range was 1.4-2 cm for the upper plates of the muzzle brake and 5.5-6.5 cm for the lower plate. Tearing of the fabric occurred regularly at point-blank and 1 cm range and was encountered in sporadic cases at ranges of 2-4 cm. An intense central discharge soot mark was observed at ranges up to 7-8 cm, and a uniform deposit was observed at up to 27 cm.

A (poyasok obtiraniye) up to 0.05 cm wide was noted at 8-9 cm ranges. Damage to the target was rounded in shape with a diameter of 0.4-0.5 cm, and the fabric defect had 0.1×0.1 cm dimensions. Copper metallization was detected by color impressions at ranges up to 30 cm. We were unable to detect iron and lead in the soot (25 percent acetic acid was used as the solvent). The deposit of discharge soot produced by point-blank discharges at 30 and 45° angles, the shape of which depends on the structural features of the muzzle brake, makes it possible to determine the side of the muzzle brake that is next to the target and thus the position of the rifle at the time of discharge (Figure 1c, d, e, f).

A deposit of discharge soot having a "butterfly" shape and visible to the naked eye is typical at ranges to 1-2 cm and a clearly visible deposit of soot is observed at up to 10 cm on a number of fabrics (broadcloth, gabardine, impregnated and nonimpregnated camouflage-colored cotton fabric, cotton fabric saturated with water repellent, rubberized cotton fabric, 82007 fabric), while such deposits are evident at 15-20 cm ranges on cotton fabric saturated with water repellent and 82007 fabric. Tearing of the target is typical of all fabric at point-blank range as well as at 1-2 cm ranges (except for broadcloth and gabardine). A (poyasok obtiraniye) is readily evident at 10-15 cm ranges. A thermal action manifests itself at ranges of up to 7 cm on broadcloth, cotton fabric saturated with water repellent, and 82007 fabric.

A soot deposit of "butterfly" shape is observed at ranges up to 1-6 cm on targets made of staple, satin, knitted fabric, and wool; a uniform discharge soot deposit is observed at ranges from 10 to 20 cm (depending on the color of the fabric), tearing of the target is observed at ranges up to 5 cm (staple), at point-blank range (satin), and at ranges up to 16 cm (knitted fabric) and 1 cm (wool), and visible singeing of wool is observed at ranges up to 10 cm.

Tearing of targets was noted at ranges up to 25 cm when varnished fabrics were used.

"Butterfly" tears are typical at point-blank range for capron, viscous fiber, nylon, and triacetate. Tearing of these fabrics as well as krimplen and ortalon is encountered at ranges up to 10-15 cm (except for viscous fabric). Pronounced thermal action of the discharge resulting in melting is observed at ranges up to 20 cm for krimplen, up to 15 cm for capron, up to 6 cm for nylon, and up to 3 cm for Gobelin fabric, the melting occurring beneath the "butterfly" wings. When subjected to point-blank fire, targets made from synthetic fur tear, and the nap undergoes intense burning and melting in the area of the central soot mark.
Tearing does not occur on dermatin targets subjected to point-blank fire, and the nature of damage corresponds to that of white coarse calico.

In the experiments without the muzzle brake soot was almost entirely absent from targets made from white coarse calico and the fabrics listed above when subjected to point-blank fire; tearing of the target occurred at ranges up to 5 cm, producing damage with dimensions from 1.0×1.0 to 2.0×2.0 cm. The extent of fabric tearing grew proportionately as the range was increased from point-blank to 5 cm. Discharge soot marks had a rounded shape and could be seen readily at ranges up to 20-23 cm. A (poyasok obtiraniye) appeared beginning with ranges of 15-20 cm. Beginning with a range of 6 cm the hole in the fabric was rounded in shape with a diameter of 0.5 cm, and the defect had dimensions up to 0.2×0.2 cm. Except for in the (poyasok obtiraniye), copper metalization was detected by color impressions at ranges up to 25 cm.

A 4.5×5.5 cm "butterfly"-shaped soot deposit was noted on skin at point-blank range, and presence of powder burns taking the form of two concentric circles with 1.5-1.6 cm and 2.5-2.6 cm diameters were noted (Figure 2a, b; see insert [figure not reproduced]). The damage was rounded in shape with the diameter of 0.4-0.5 cm, the (poyasok obtiraniya) and (osadneniya) was 0.1 cm wide, and the tissue defect was 0.15 cm. The edges of the damaged area contained small radial tears 0.1 cm long. When a bone (the shin) is present beneath the skin, a linear or cross-shaped tear up to 1.5 cm in size forms (Figure 2c). Cross-shaped tears with 2.5×3.0 cm dimensions arose on the hair-covered part of the head at point-blank range. At greater ranges the damage was rounded in shape with a diameter of 0.4 cm, the tissue defect was 0.1-0.2 cm, and the (poyasok obtiraniya) and (osadneniya) was 0.05-0.1 cm broad (Figure 2d).

Skin exit wounds were cross-shaped gaping wounds with dimensions up to 3.0×4.0 cm (when the bullet does not hit a bone). When damage to bone occurred, the gaping exit wound had dimensions up to 10.0×10.0 cm as a result of the action of secondary wounding projectiles--bone fragments. The zone of damage to soft tissues increased by five to six times after penetration of bone by the bullet.

When the rifle was fired perpendicular to the cranial vault, the entrance hole in flat bones took the form of a perforated fracture with a 0.5 cm diameter. A tapered hole opening to the outside and with insignificant superficial radial cracks appeared in the area of the outer bony plate (Figure 3a, see insert [figure not reproduced]). Correspondingly a tapered hole opening into the skull which was not any smaller than that on the outer plate also formed on the inner bony plate (Figure 3b, see insert).

Exit holes in flat bone of the cranial vault took the form of perforated fractures with a diameter of 0.5 cm. For practical purposes a tapered hole was not formed on the inner plate, assuming we ignore insignificant uniform "chipping" of the plate along the edge of the fracture. A tapered hole opening inward with radial superficial cracks and chips directed outward appeared on the outer plate. The nature of fracture of the inner plate may be of assistance in differentiating between the entrance and exit holes, since
distinct differences in damage to the outer bony plate near the holes are not evident.

Fracture of the diaphysis is typified by damage to not less than one-third of the length of the diaphysis coupled with formation of a large number of fragments. Perforated fractures of the diaphysis occurred only with isolated bones (Figure 3c, d, see insert). In the vicinity of the entrance hole the cracks were radially oriented, while there were many more such cracks in the vicinity of the exit hole and the cracks were oriented irregularly, often intersecting. In attempts at restoring damaged bones, we were almost always able to restore the entrance hole while it was almost impossible to completely restore the exit hole. Marginal circular uniform "chipping" of the outer part of compact tissue occurred in the vicinity of the entrance hole; if such chipping occurred in the vicinity of the exit hole, it was eccentric as a rule. The possibility cannot be excluded that the entrance hole in bone can exceed the size of the exit hole in different amounts (see Figure 3c, d; see insert).

Epiphyseal fractures were perforated with a diameter of 0.5 cm and more; insignificant uniform circular "chipping" of contact tissue was observed on the entrance side, and "chipping" of greater irregularity was observed on the exit side, sometimes in the form of a cone open in the direction of movement of the bullet.

In all cases damage to bones acquired in experiments with a cadaver is similar in extent and diversity to damage to bones removed from the body.

In addition to the color impression method we employed soft X-rays to detect discharge soot marks. Photographs were taken with FT-31 photographic film at 10 kv and 10 mamps; the focal length was 60 cm, and exposure time was 40-60 seconds. Metallization was observed in this case on closely-woven fabrics such as broadcloth. Similar results were obtained with skin bearing point-blank discharge traces (Figure 4, see insert [figure not reproduced]).

Conclusions

Combined with high muzzle velocity, the structural features of the muzzle brake of the 5.45-mm caliber AKS-74 assault rifle predetermine the nature of damage to clothing fabric and the degree to which the traces of close discharge are expressed. Specific deposition of soot differing from deposition accompanying the use of similar models of the rifle, pronounced action of powder gases at point-blank range and, in relation to synthetic and artificial fabric, at greater range, a uniquely shaped impression of the rifle muzzle on unclothed skin at point-blank range, significant damage to long hollow bones, and unique entrance and exit holes on bones of the cranial vault are noted in this case. X-ray analysis can be used to detect discharge soot on clothing fabric and human skin when damage is caused by jacketed bullets fired from a 5.45-mm caliber AKS-74 assault rifle.

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Recent achievements in microcirculation (A. M. Chernukh et al., 1975; V. V. Kupriyanov et al., 1975, etc.) and morphometry (G. G. Avtandilov, 1973; Weibel, 1970) have indicated the basic possibility for a new approach for studying impairment of intracerebral circulation in various extreme states (B. N. Klosovskiy, 1951; G. I. Mchedlishvili, 1968, 1977; I. V. Gannushkina, 1973, etc.), particularly in fatal strangulation; these impairments can be used to diagnose whether or not the victim was still alive at the time of strangulation (V. G. Naumenko and N. A. Mityayeva, 1977).

Changes in terminal vascular channels resulting from death by hanging were first described in forensic medical literature in a work by Ya. Ya. Rumyantsev (1974). Employing morphometry, in his experiments the author acquired new data for diagnosis of intravital asphyxiation using film preparations impregnated with silver nitrate (V. V. Kupriyanov's method). However, film preparations do not provide information permitting assessment of intracerebral circulation impairment.

I studied the volumetric density of arterial and venous vessels and capillaries in different divisions of the brain and spinal cord with the goal of acquiring new quantitative criteria characterizing circulation in intracerebral vessels at the time of fatal strangulation.

The research method is based on the principles of histometry, and it involves application of G. G. Avtandilov's ocular measuring grid (1972), computation of the volumetric density of vessels (G. G. Avtandilov et al., 1977), and use of the sectioning method to study the brain (V. G. Naumenko and V. V. Grekhov, 1967).

The brain and spinal cord of 16 cadavers of persons dying due to strangulation were studied; four cases of death resulting from decapitation were studied as well ("normal" conditions). This choice of the "norm" was based on the
fact that the possibility for movement of blood in cerebral vessels is absent in decapitation owing to the absence of an agonal period in this form of death. "The brain remains in the conditions of asphyxiation for 5-6 minutes between the moment of decapitation and the time of nerve cell death, a consequence of which is insignificant dilation of the entire vascular-capillary network. This dilation of the vascular channel results in retention of a significant proportion of the blood in intracerebral vessels."

Thirteen out of 16 of the hanging victims were in a state of alcoholic intoxication. Gas chromatography revealed alcohol concentrations in blood and urine from 0.6 to 5.2 °/00, the elimination stage dominating at this time. The group with alcohol present contained 12 men from 24 to 50 years old and one woman 24 years old; three men from 22 to 45 years old had not consumed alcohol prior to death.

Histometrical analysis was performed with the frontal cortex, the temporal lobes, Ammon's horn, the medulla oblongata, and the first cervical and first thoracic segments of the spinal cord.

Chunks of brain tissue were embedded in celloidin and paraffin, and sections of "standard" thickness—10 μ—were stained with hemotoxylin-eosin and according to (Van-Gizon's) method to count arterial and venous vessels, and with (Shiff's) reagent (as modified by me) to count capillaries.

The number of points coinciding separately with small arteries and arterioles, with small veins and venules, and with capillaries were counted at a ×220 magnification. The counting was performed with a mechanical keyboard differential counter. All numerical data were treated by the methods of variational statistics; Student's coefficient of significant differences was computed and used to determine the probable error (P).

The research results established the following (see table).

In "normal" conditions the volumetric density of intracerebral vessels is characterized by regional stability of the indices for each area of the brain and spinal cord analyzed. At the same time there are some topographical differences in indices for similar vessels in different divisions of the brain. Thus the volumetric densities of capillaries in the hemispheres and in the medulla oblongata do not exhibit differences that are statistically significant, while the indices for capillaries in the spinal cord are lower than those for the cerebral hemispheres (P<0.2). The volumetric densities of arterial channels in all of the divisions analyzed do not differ significantly either, with the exception of the cortex of Ammon's horn, where a tendency toward reduction of indices as compared to other divisions can be noted. As compared to that of the medulla oblongata and the spinal cord, the volumetric density of venous vessels in the cerebral hemispheres is higher.

*Klosovskiy, B. N., "Tsirkulyatsiya krovi v mozgu" (Circulation of Blood in the Brain), Moscow, Medgiz, 1951.
### Indices of Volumetric Density \((V_0 \pm m_0)\) of Intracerebral Vessels

<table>
<thead>
<tr>
<th>Сосуды (1)</th>
<th>Участок мозга (2)</th>
<th>Отделение головы (3)</th>
<th>Стравглиацию (4) без алкоголя (5)</th>
<th>(P)</th>
<th>С алькогольс (6)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0,035423±0,000672</td>
<td>0,065519±0,014893</td>
<td>0,2</td>
<td>0,0854296±0,002847</td>
<td>0,001</td>
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<tr>
<td>2</td>
<td>0,033561±0,001112</td>
<td>0,063466±0,010192</td>
<td>0,05</td>
<td>0,051257±0,003190</td>
<td>0,001</td>
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</tr>
<tr>
<td>3</td>
<td>0,031222±0,000896</td>
<td>0,065666±0,006533</td>
<td>0,02</td>
<td>0,043206±0,003145</td>
<td>0,01</td>
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<tr>
<td>4</td>
<td>0,033709±0,003850</td>
<td>0,061700±0,015158</td>
<td>0,2</td>
<td>0,044002±0,002575</td>
<td>0,05</td>
<td></td>
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<tr>
<td>5</td>
<td>0,021583±0,002968</td>
<td>0,034110±0,008189</td>
<td>—</td>
<td>0,025981±0,001482</td>
<td>—</td>
<td></td>
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<tr>
<td>6</td>
<td>0,028233±0,002740</td>
<td>0,028249±0,011907</td>
<td>—</td>
<td>0,022959±0,001726</td>
<td>0,2</td>
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<tr>
<td>7</td>
<td>Капилляры</td>
<td>0,003175±0,000171</td>
<td>0,002994±0,000563</td>
<td>—</td>
<td>0,003766±0,000383</td>
<td>0,2</td>
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<tr>
<td>8</td>
<td>Мелкие артерии, артериолы</td>
<td>0,003085±0,000343</td>
<td>0,002636±0,000713</td>
<td>—</td>
<td>0,003336±0,000303</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>Мелкие вены, венулы</td>
<td>0,003293±0,000329</td>
<td>0,002950±0,000521</td>
<td>—</td>
<td>0,003154±0,000249</td>
<td>0,1</td>
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<td>10</td>
<td>Мелкие артерии, артериолы</td>
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<td>0,002813±0,000347</td>
<td>—</td>
<td>0,003674±0,000416</td>
<td>0,2</td>
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<tr>
<td>11</td>
<td>Мелкие вены, венулы</td>
<td>0,003158±0,000516</td>
<td>0,002559±0,000555</td>
<td>—</td>
<td>0,004014±0,000371</td>
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<tr>
<td>12</td>
<td>Мелкие артерии, артериолы</td>
<td>0,003504±0,000413</td>
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<td>—</td>
<td>0,003858±0,000328</td>
<td>—</td>
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<td>13</td>
<td>Мелкие вены, венулы</td>
<td>0,001017±0,000167</td>
<td>0,000775±0,000111</td>
<td>—</td>
<td>0,001152±0,000190</td>
<td>—</td>
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<tr>
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<td>0,000735±0,000054</td>
<td>0,000957±0,000140</td>
<td>—</td>
<td>0,000975±0,000182</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>Мелкие вены, венулы</td>
<td>0,001078±0,000118</td>
<td>0,001281±0,000533</td>
<td>—</td>
<td>0,001324±0,000245</td>
<td>—</td>
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<tr>
<td>16</td>
<td>Мелкие артерии, артериолы</td>
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<td>0,000506±0,000135</td>
<td>0,2</td>
<td>0,001081±0,000256</td>
<td>0,05</td>
</tr>
<tr>
<td>17</td>
<td>Мелкие вены, венулы</td>
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<td>0,000356±0,000340</td>
<td>—</td>
<td>0,000976±0,000295</td>
<td>—</td>
</tr>
<tr>
<td>18</td>
<td>Мелкие артерии, артериолы</td>
<td>0,000407±0,000086</td>
<td>0,000554±0,000186</td>
<td>—</td>
<td>0,000721±0,000136</td>
<td>0,05</td>
</tr>
</tbody>
</table>

Note: Brain areas are designated as follows: 1--Frontal lobe, 2--temporal lobe, 3--Ammon's horn, 4--medulla oblongata, 5--first cervical segment, 6--first thoracic segment of the spinal cord.

Key:

1. Vessels
2. Brain area
3. Decapitation
4. Strangulation
5. Without alcohol
6. With alcohol
7. Capillaries
8. Small arteries, arterioles
9. Small veins, venules
When fatal strangulation occurs in a state of alcoholic intoxication, the volumetric density of capillaries is significantly higher in the frontal cortex, the temporal lobe, Ammon's horn, and the medulla oblongata, and it is lower in the first thoracic segment. In the absence of alcohol the volumetric density is once again significantly greater in the cortex of the temporal lobe and Ammon's horn and, at a lower level of significance, in the frontal cortex and the medulla oblongata. The volumetric densities of capillaries do not differ significantly from the "norm" at the level of the first cervical segment of the spinal cord, irrespective of the presence of alcohol, and at the level of the first thoracic segment.

In the absence of alcohol, the volumetric densities of arterial vessels in all areas of the brain and spinal cord studied do not differ significantly from the "norm," though a tendency toward decline of indices for the frontal cortex, the temporal lobe, and the first cervical segment of the spinal cord can be seen. On the background of alcoholic intoxication the indices for these vessels are greater in the cortex of Ammon's horn and, at a somewhat lower level of significance, in the medulla oblongata and the frontal cortex. As is the case with asphyxiation in the absence of alcohol, the volumetric density of vessels does not differ from the "norm" in the other areas of the brain and spinal cord.

When alcohol is present the volumetric density of venous vessels is significantly greater only in the medulla oblongata and in the first thoracic segment of the spinal cord, while in the absence of alcohol it is higher only in the medulla oblongata, though at a lower level of significance. In other areas the indices of volumetric density do not differ significantly from the "norm."

Depending on the concentration of alcohol in blood and urine, the indices of volumetric density of intracerebral vessels have a tendency to increase with higher concentrations (greater than 4%0). However, this question requires further study.

Comparative histometric analysis of both hemispheres, performed in six out of 16 observations, revealed that the volumetric density of capillaries is significantly greater on the side of greatest compression of the neck by the noose (P<0.05). The indices of volumetric density of arterial and venous vessels in both hemispheres are more variable, though they predominantly tend to decline for arterial vessels in the hemisphere on the side of greatest compression of the neck by the noose.

Thus quantitative criteria based on assessment of the volumetric density of the terminal portion of the microcirculatory channel in the frontal cortex, the parietal lobe, and Ammon's horn have the greatest diagnostic significance.
in regard to establishing intravital strangulation. In these areas of the brain, the indices of capillary volumetric density are significantly higher in the hemisphere on the side of greatest compression of neck organs by the noose.

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Most authors believe that shock and complications on the part of kidneys and the liver play the dominant role in the thanatogenesis of acetic acid intoxication. However, pneumonia which can have dominant significance in the pathogenesis of intoxication often develops in the presence of such intoxication. Research by V. D. Tsinzerling (1956, 1958, 1961), O. I. Bazan (1957), V. D. Tsinzerling and A. V. Tsinzerling (1963), A. V. Tsinzerling (1970), and others has shown that pneumonias of different etiologies are different diseases, each of which has its own agents and its own morphological features. At the same time research on pneumonias accompanying acetic acid intoxication had been conducted earlier without a consideration for their etiologies and the influence of the agent on the morphology of the inflammatory process. This encouraged me to study the morphology of pneumonias of different etiologies accompanying acetic acid intoxication with the goal of clarifying some questions concerning the pathogenesis of these pneumonias and their role in thanatogenesis.

Morphological research was conducted on the lungs, epiglottis, and the trachea in 48 cases of fatal intoxication by acetic acid. In all observations 50-250 ml of the poison were taken in the form of acetic essence for suicidal purposes or due to carelessness. The victims included 20 men and 28 women from 22 to 80 years old.

Medical aid was rendered in the first 1-3 hours following intake of the poison, and it consisted of gastric lavage and antishock and detoxification therapy.

On being admitted to the hospital, in addition to the dominant symptoms (shock, chemical burns in the gastrointestinal tract, acute hepatorenal insufficiency) the victims exhibited complications on the part of respiratory organs, and beginning with the second and third days pneumonia was diagnosed in 20 of the patients. No pathological changes of any sort were revealed
in the respiratory organs of 28 victims in the clinic (with the exception of acute edema of the larynx in two patients).

Autopsy revealed minor hemorrhaging in the mucous membrane of the larynx and trachea, necrotic foci in the mucous membrane of the epiglottis, and purulent tracheobronchitis and focal or confluent pneumonia.

In the histological research I employed Gram-Weigert staining with hematoxylin-eosin and Loeffler's methylene blue, and the sections were also processed by (Levaditi's) method. Bacterioscopic research was conducted with sections and smears. The smears were stained by Gram's method and methylene blue. The lungs were also subjected to bacteriological examination.

Histological research revealed intense plethora, hemorrhaging, and leukocyte infiltration of the mucous membrane of the epiglottis and upper respiratory tract, and foci of superficial necrosis were sometimes observed in the epiglottis.

We can subdivide the analyzed material into three groups depending on the nature of pathological changes in the lungs: Group 1— aspirational bronchitis elicited by mixed flora (seven observations); group 2— aspirational pneumonia elicited by mixed bacillar and coccal flora (35 observations); group 3— monomicrobial pneumonia elicited by staphylococcus (six observations).

Death occurred in group 1 in 7 to 47 hours. Histological research revealed massive bronchitis coupled with serous-leukocytic and leukocytic exudate. Foreign particles of various types and colonies of Gram-positive cocci and Gram-negative bacilli were revealed in exudate from the bronchi. However, no pathological changes were revealed in respiratory organs in the clinic and by the autopsy.

Death of the victims in group 2 occurred from 8 hours to 17 days after intoxication. Microscopic analysis of inflammation foci in the lumen of the bronchi and alveoli revealed leukocytic or leukocytic-fibrinous exudate containing foreign particles of various types (plant cells, bile pigment, squamous epithelial cells, and so on) and microbial colonies (Figure 1 [figure not reproduced]). Gram-negative bacilli (E. coli) and Gram-positive cocci dominated. Microbial phagocytosis was observed in all cases in the smears and sections. Pneumonia was not clinically identified in 15 cases of this group (35 observations). Pneumonia was not diagnosed in 12 observations following autopsy.

Death of patients in group 3 (monomicrobial staphylococcal pneumonia) occurred after 1-5 days. Histological research revealed widespread purulent or purulent-necrotic bronchitis and pneumonic foci coupled with leukocytic exudate (Figure 2 [figure not reproduced]). Necrosis of exudate and pulmonary tissue cells was noted in the central areas of the foci. Clusters of Gram-positive cocci were present in the necrotic areas, and a zone of toxic fibrinous exudate containing no microbes was on the periphery of the inflammatory foci.
Pneumonia was not clinically diagnosed in three cases and, following forensic medical analysis, in one case.

The fact that most cases of bronchitis and pneumonia were aspirational indicates impairment of the protective and cleaning function of respiratory organs and its significance to the genesis of this pathology.

Thus pneumonia was not clinically diagnosed in 23 out of 41 cases of acetic acid intoxication, not only when the patients died early (8-12 hours), at which time it is difficult to clinically diagnose pneumonia on the background of shock or burns in the gastrointestinal tract, but also later. Bronchitis was never diagnosed in the clinic.

Nor was pneumonia always diagnosed following forensic medical examination of the cadavers (it was diagnosed in 18 of our cases). This can obviously be explained by difficulties in macroscopic diagnosis of pneumonia (especially that characterized by small foci) on the background of intense plethora, edema, and hemorrhaging.

The data presented here indicate shortcomings both in clinical examination of victims and in forensic medical analysis of the cadavers of persons dying due to acetic acid intoxication.

As we can see from these data the role of affliction of respiratory organs (by bronchitis and pneumonia) is not always considered in the pathogenesis and thanatogenesis of acetic acid intoxication in the clinic and in sectioned material, and as a rule hepatorenal insufficiency is believed to be the dominant cause of death. And yet pneumonia was often the principal complication leading to a lethal outcome.

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Despite extensive study of the toxic action of ethanol, many problems of the thanatogenesis of alcoholic intoxication are still inadequately explained and require an integrated approach to their solution. Just the concentration of alcohol in blood and urine alone obviously cannot serve as a sufficiently dependable criterion for thanatological diagnosis of acute alcoholic intoxication.

The goal of our research was to establish dependable criteria for thanatological diagnosis of acute alcoholic intoxication.

With this goal in mind we studied and compared ADH activity, the intensity of alcoholic intoxication, and the extent of morphological changes in the liver tissues of persons who died owing to or on the background of alcoholic intoxication.

Research Methods

Liver tissue taken from 64 cadavers during autopsies was immediately frozen and analyzed 24-48 hours later. ADH activity was determined spectrophotometrically (Bonnichsen and Brink, 1955), and the degree of this activity was expressed in micromoles of NADH per minute, corrected for 1 mg of liver tissue protein. Blood alcohol concentration was determined by gas chromatography. Structural changes in liver tissue were revealed through histological analysis.

Results

The observations were subdivided into the following groups.
1. Persons dying as a result of injury, hypertonic illness, and chronic insufficiency of coronary circulation (13 cases) in the blood of whom alcohol was not detected post mortem (the control group).

2. Persons who had died as a result of injury revealing alcohol in blood post mortem (22 cases).

3. Persons who had died owing to alcoholic intoxication (16 cases).

4. Persons who had died from accidental causes, whose medical histories indicated prolonged abuse of alcohol, but who did not reveal alcohol in their blood post mortem (six cases).

5. Persons who had died owing to chronic cardiac ischemia on the background of alcoholic intoxication (seven cases).

Among persons who had died with alcohol present, we distinguished a group exhibiting phenomena of acute intoxication, where the blood alcohol concentration exceeded $2.5 \%_0$, and a group of persons for whom blood alcohol concentration did not exceed $2.5 \%_0$ (30 and 15 cases respectively). This subdivision was made in accordance with recommendations in the "Metodicheskoye ukazaniye o sudebno-meditsinskoy diagnostike smertel'nykh otravleniy etilovym alkogolem" (Methodological Instructions on Forensic Medical Diagnosis of Fatal Ethanol Intoxication) (Moscow, 1974).

Morphological analysis of the liver of persons who had died exhibiting phenomena of alcoholic intoxication revealed dystrophy of fatty liver tissue of varying intensity in 29 cases; the histological pattern of the liver was normal in the rest of the cases.

Data on ADH activity are shown in Table 1.

Analysis of these data revealed that ADH activity in liver tissue is low as compared to control in all cases where death occurred on the background of alcoholic intoxication. A statistically significant decline in liver ADH as compared to control was revealed in all victims exhibiting alcoholic intoxication phenomena ($0.032\pm0.005$ and $0.043\pm0.005; P<0.05$) as well as in persons who had died directly owing to alcoholic intoxication ($0.031\pm0.0035$ and $0.043\pm0.005; P<0.05$). ADH activity is also low in persons who had abused alcohol in the past for a long period of time but who had died in a state of abstinence of unknown duration. The absence of a statistically significant difference in this case can be explained by the small size of the group.

Liver ADH activity and alcoholic intoxication intensity are shown in Table 2, from which it follows that ADH activity in liver tissue of persons who had died exhibiting phenomena of severe alcoholic intoxication is lower ($0.029\pm0.003$) than that of persons who had died exhibiting moderate alcoholic intoxication phenomena ($0.037\pm0.004; P<0.1$), and that it is dramatically lower as compared to the control group ($P<0.02$).
### Table 1. ADH Activity in Liver Tissue of Persons Who Had Died Exhibiting Phenomena of Acute Alcoholic Intoxication (M±m).

<table>
<thead>
<tr>
<th>Observation Group</th>
<th>Number of Observations</th>
<th>ADH Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>0.043±0.005</td>
</tr>
<tr>
<td>All cases of death with phenomena of acute alcoholic intoxication</td>
<td>45</td>
<td>0.032±0.005</td>
</tr>
<tr>
<td>Persons dying due to acute alcoholic intoxication</td>
<td>16</td>
<td>0.031±0.0035</td>
</tr>
<tr>
<td>Persons dying on the background of acute alcoholic intoxication as a result of injury</td>
<td>22</td>
<td>0.034±0.003</td>
</tr>
<tr>
<td>Persons dying on the background of acute alcoholic intoxication due to chronic cardiac ischemia</td>
<td>7</td>
<td>0.026±0.0044</td>
</tr>
<tr>
<td>Persons abusing alcohol for a long period of time and dying following an unknown time of abstinence</td>
<td>6</td>
<td>0.033±0.005</td>
</tr>
</tbody>
</table>

### Table 2. ADH Activity in the Liver and Intensity of Alcoholic Intoxication (M±m).

<table>
<thead>
<tr>
<th>Observation Group</th>
<th>Number of Observations</th>
<th>ADH Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>0.043±0.005</td>
</tr>
<tr>
<td>Severe alcoholic intoxication</td>
<td>30</td>
<td>0.029±0.003</td>
</tr>
<tr>
<td>Moderate alcoholic intoxication</td>
<td>15</td>
<td>0.037±0.004</td>
</tr>
</tbody>
</table>

### Table 3. ADH Activity and Morphological Changes in the Liver (M±m).

<table>
<thead>
<tr>
<th>Observation Group</th>
<th>Number of Observations</th>
<th>ADH Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>0.043±0.005</td>
</tr>
<tr>
<td>Alcoholic intoxication and liver tissue with a normal histological pattern</td>
<td>9</td>
<td>0.039±0.006</td>
</tr>
<tr>
<td>Alcoholic intoxication and dystrophy of fatty liver tissue</td>
<td>29</td>
<td>0.029±0.002</td>
</tr>
</tbody>
</table>

Comparison of ADH activity in liver tissue and the degree of morphological changes in the liver (Table 3) showed that when death occurred on the background of alcoholic intoxication in the absence of morphological changes on the part of the liver, the ADH activity did not differ significantly.
from that of persons in the control group (0.039±0.006 and 0.043±0.005; P>0.1). When dystrophy of fatty liver tissue is present, however, ADH activity is significantly lower than in cases in which morphological changes were not revealed in liver tissue (0.029±0.002 and 0.039±0.006; P<0.05), and in comparison with the control group (0.029±0.002 and 0.043±0.005; P<0.02).

Thus the activity of ADH in the liver was found to be significantly lower as compared to control in all cases where death occurred owing to or on the background of acute alcoholic intoxication.

The decline in ADH activity is more intensive in the presence of severe intoxication and in cases of fatty liver tissue dystrophy.

These data appear to indicate that ADH activity in liver tissue declines in response to acute ethanol intoxication. However, it would be difficult to conclude that acute massive ethanol intoxication has a direct influence on liver ADH activity, inasmuch as we studied material from autopsies, and in most cases we did not possess catamnestic information as to the nature and duration of previous consumption of alcohol, presence of clinical signs of alcoholism, and information as to whether acute alcoholic intoxication occurred on the background of preceding chronic intoxication or following prolonged abstinence from alcohol. Citation of the presence of the signs of chronic alcoholism has little significance since it had been demonstrated earlier that liver ADH activity decreases in accordance with the duration and severity of chronic alcoholism (A. S. Mukhin et al., 1975). At the same time, despite absence of this information, we cannot exclude the possibility that the decline in liver ADH stems directly from acute alcoholic intoxication. An argument in favor of this can be found in the fact that the liver ADH activity of persons who had died exhibiting phenomena of severe alcoholic intoxication is significantly lower than among persons who had died in the presence of moderate alcoholic intoxication or persons in the control group. The statistical significance of differences between these groups permits the hypothesis that the intensity of acute alcoholic intoxication has a direct influence on ADH activity in liver tissue. This encouraged us to compare ADH activity with blood alcohol concentration in each separate case (ranked correlation coefficients were computed). However, we were unable to reveal a dependence between these values (the ranked correlation coefficient was +0.29; P>0.1). Apparently individual sensitivity to alcohol, which might be dependent on change in the initial level of ADH, is typical of each case, and therefore the toxicity of the same quantities of ethanol may be different in different cases. This is precisely why information concerning the concentration of alcohol in cadaveric material could have only relative significance to an assessment of the degree of intoxication.

The data also indicate that the decline in ADH activity in liver tissue is associated with dystrophy of fatty liver tissue, but it would be difficult to judge from our data which of these processes is dominant.
Conclusions

1. The activity of the enzyme ADH can be measured in autopsy samples of liver tissue from persons who had died exhibiting phenomena of acute alcoholic intoxication.

2. ADH activity in liver tissue is low in the presence of acute alcoholic intoxication as compared to control. This decline is combined with dystrophy of fatty liver tissue and it is associated with the intensity of alcoholic intoxication.

3. Data acquired from determining ADH activity in autopsy material, utilized as an index of the state of protective mechanisms in relation to a standard, can be used as additional tests in assessment of the intensity of alcoholic intoxication.

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Oligophrenia is one of the most frequently encountered mental disorders: According to data of different authors its frequency of occurrence is 2-2.4 cases per 1,000 people. Acute forms of oligophrenia (idiocy and embicility) are noted among only 20-25 percent of the patients. Most oligophrenics are persons suffering debilities. Many of them are able to acquire some education and work, and visits to psychiatric hospitals due to temporary worsening of condition occur rarely. At the same time owing to their mental deficiency such persons are often helpless in unusual situations, and they cannot correctly assess complex interpersonal and social relationships and requirements of the law. They frequently become victims of crimes, especially sexual. Among sex crime victims subjected to forensic psychiatric expert examination, about 40 percent are oligophrenics.

Little research has been performed on forensic psychiatric expert examination of witnesses and victims suffering oligophrenia. O. Ye. Freyero (1957) noted that oligophrenics suffering mild intellectual deficiency may provide adequate testimony, but weakness of judgment, greater suggestibility, and possible insufficiency of critical attitude make great caution in expert assessments of the mental state of such persons necessary. Leferenz (1972) believed that witnesses and victims exhibiting mental retardation "are more prone to various external influences and their own instinctive drives, which should be given special attention in expert examination."

I analyzed 40 female oligophrenics 14 to 20 years old who were victims in cases of sex crimes and who underwent outpatient forensic psychiatric expert examination at the Institute imeni V. P. Serbskiy and other institutions. Most of the examined patients had been diagnosed to be oligophrenics prior to the forensic psychiatric examination. The subjects included students at schools for the handicapped, workers with low skill levels, and some with pronounced mental disorders who did not work and did not attend school, being certified as disabled. There were no patients suffering idiocy and embicility; only a state of debility coupled with psychopathological disorders expressed to varying degrees was revealed.
Two series of psychopathological signs were considered in examination of the clinical structure of debility: 1) Intellectual deficiency, which was dominant in relation to both the clinical pattern and the diagnosis of the pathological state; 2) accompanying disturbances in the emotional-volitional sphere. The subjects were subdivided into three groups depending on clinical features defining the degree of intellectual deficiency and the extent to which emotional-volitional disturbances were pronounced: Group 1—persons with relatively minor intellectual deficiency, without pronounced emotional-volitional disturbances; group 2—persons with relatively minor intellectual deficiency and pronounced emotional-volitional disturbances; group 3—patients with profound intellectual deficiency and pronounced affective-volitional disturbances.

Patients in group 1 (16 persons) revealed the "simple" variant of debility, in which the clinical pattern consisted only of the main disturbance accompanying oligophrenia—intellectual insufficiency. Intellectual deficiency was relatively minor. In addition to the capability for sound abstraction and dominance of concrete-situational forms of thinking, these patients retained the capability for forming concepts, making simple conclusions, and generalizing personal experience. Sufficiently differentiated emotional reactions, adequate sympathies and antipathies, and the capability of active volition and purposeful activity were revealed on the background of a certain general primitiveness and rigidity of emotional-volitional manifestations. The subjects were usually able to acquire some knowledge and work skills; in their day-to-day lives they were sufficiently active and diligent, and their behavior was typified by order and an ability to orient themselves within concrete personal situations. As a rule their academic success was not bad in schools for the handicapped, and they were subsequently able to perform simple jobs (housekeeper, orderly).

Their testimony concerning the circumstances of the case was coherent, consistent, and constant in detail. A sufficient understanding of the incident and the situation that evolved, and of the requirements imposed on them in the court process was typical of them. Expert commissions concluded that such subjects could correctly perceive the circumstances of the case and provide testimony on them.

The subjects in group 2 (14 persons) can be divided into two subgroups depending on clinical manifestations of emotional-volitional disturbances.

One of them consisted of subjects for whom formally minor intellectual deficiency was accompanied by significant emotional-volitional disturbances of the "hyperdynamic" type. Since childhood they had been highly excitable and exhibited insufficient differentiation of affective reactions coupled with weak will, disinhibition, and impulsiveness in behavior. At adolescent age these disturbances were usually compounded by tendencies toward vagrancy, abuse of alcohol, sexual promiscuity, primitive thoughts, and falsehood. Owing to emotional-volitional instability they were incapable of prolonged purposeful activity. Despite formally minor intellectual deficiency the
subjects were poorer learners and could not work. Their testimony in different stages of the investigation was often contradictory and inconsistent due to instability of emotions and motives and an inadequate understanding of their role and responsibility in the investigation. Sometimes the victims discussed facts but assessed them incorrectly and inadequately, revealing inadequate conceptualization of the essence of the incident. "Unintentional slander" was encountered—that is, accusations of compulsion, which had not in fact occurred, associated with an uncritical attitude toward their own behavior and the situation combined with primitiveness and excessiveness of affective reactions. This is why expert commissions decided on some occasions that owing to their mental disturbances such persons could not adequately perceive and affect the events and provide truthful testimony concerning them. Here is an example.

Subject A., 15 years old, suffered mental retardation since childhood and was excessively excitable and capricious. She assimilated information satisfactorily in a school for the handicapped, but she was perpetually distracted, she exhibited motor disinhibition, she found herself in conflict situations, and she performed aggressive actions. Beginning at 12 years old she missed classes, led a vagrant life, consumed alcoholic beverages, and became familiar with men. She responded to attempts at influencing her behavior with threats of suicide, and she would run away from home. She engaged in sexual intercourse several times with Citizen Ya. After several months following a gynecological examination she revealed that Citizen Ya. had raped her while she was drunk and asleep. Examination at the institute revealed minor intellectual deficiency coupled with concrete thinking and a decline in the level of generalization and abstraction. Affective reactions were extremely unstable and excessive. The patient was uncritical of the situation that had evolved and her behavior. She stated that Citizen Ya. should be convicted of rape because he had "insulted" her, but immediately thereafter she said with a smile that she herself had agreed to intercourse because she liked Citizen Ya.. Her words concerning the same facts differed, and when the contradictions were pointed out to her she became angry, declaring: "I say what I want to say." The commission concluded that A. suffered oligophrenia in a stage of debility coupled with the psychopathoid syndrome, and that her testimony should be treated as that of a mental patient.

The clinical features of this case are typified by relatively minor intellectual insufficiency and distinctly pronounced phenomena of heightened excitability and disinhibition, which encourage development of the psychopathoid syndrome coupled with a negative attitude toward surrounding individuals, affective and hysterical reactions, and disinhibition of drives at puberty. Combination of intellectual insufficiency and psychopathoid manifestations results in a significant decline in the ability to critically assess surrounding individuals and the subject's own personality, and in loss of the capability for controlling behavior. The subject was not able to correctly assess the incident and the situation that evolved, or understand the contradictions in her statements and testimony.
The other subgroup consisted of subjects with distinctly pronounced heightened suggestibility, which was often accompanied by lack of initiative, subservience, sluggishness, and a certain degree of adynamia. Because such persons did what they were told, they often studied satisfactorily in schools for the handicapped and acquired some knowledge and work skills. However, they found themselves helpless in cases when they had to display a certain amount of independence in judgments and acts. This is manifested especially clearly among adolescents, who experience new, more-complex demands upon the personality. Yielding to the influence of unfavorable surroundings, such girls often engage readily and thoughtlessly in chance sexual encounters. The testimony of subjects in this subgroup was quite often contradictory and inconsistent, and the content of replies depended on the nature of the questions posed and the tone of the discussion. Accusations or, on the other hand, denials of previous testimony under the influence of interested persons were often noted. Examination made it clear that patients in this subgroup could not fully conceptualize the evolved situation, make correct conclusions, and produce an independent critical assessment of the incident. Obviously oligophrenic patients exhibiting a significant decline in volitional qualities coupled with heightened suggestibility cannot participate in court proceedings or recreate events correctly and objectively. Here is an example.

Subject P., 15 years old, suffered mental retardation since childhood, and in second grade she was transferred from public school to a school for the handicapped, in which she was in the 8th grade at the time of examination. Her academic success was mediocre; the teachers described her as obedient and excessively suggestible ("she easily yields to persuasion and does not think about the consequences of her acts"). A few adolescents took her down to a basement several times, where they engaged in sex acts with her, including deviant practices. During the investigation P. gave contradictory testimony, accused various people, and slandered her stepfather. Examination revealed intellectual insufficiency coupled with difficulties in generalization and abstraction, meager knowledge, and primitive judgments. Sometimes she responded to questions with rehearsed phrases, oblivious to their content. She could not describe the events consistently, she readily altered the content of her story under the influence of the questions asked, and she was extremely suggestible. She did not deny that she had slandered her stepfather, explaining her action by the fact that his relationship to her and her mother was poor ("I wanted him to be arrested"). According to the commission's conclusions P. suffers oligophrenia in a stage of pronounced debility, and her testimony should be treated as that of a mental patient.

In this case intellectual insufficiency combined with volitional weakness, heightened suggestibility, and subservience made the subject both incapable of understanding and defending her interests and unable to purposefully control her behavior and statements in the situations she had faced.

Significant intellectual insufficiency was expressed in persons of group 3 (10 persons) as purely concrete thinking and an inability to make generalizations, formulate concepts, and make conclusions, and their speech was primitive, relying on a meager vocabulary. Pronounced intellectual deficiency
was compounded by underdevelopment of the emotional-volitional sphere coupled with extreme primitiveness and lack of differentiation of reactions, passiveness, subservience and, in addition, unmotivated stubbornness, negativism, and disinhibition of lower drives. Such disorders made these subjects unable to assimilate simple general knowledge and acquire work skills, or to independently orient themselves in simple living situations. Having graduated with difficulty from first or second grade in a school for the handicapped, most of them subsequently stayed at home, they were certified disabled, and sometimes they did simple housework, though only with constant encouragement and observation.

Even in long discussions employing leading questions, these subjects were unable to coherently describe the incident or separate the main from secondary facts, they gave extremely contradictory answers, they could not understand the essence of the incidents, and they did not have an adequate emotional relationship to the event. Expert commissions came to the conclusion that the testimony of these persons would have to be treated as that of mental patients.

In accordance with requirements of the RSFSR Criminal Code (Article 79, Paragraph 3), during forensic psychiatric certification of a witness (victim) the experts must determine whether or not the given person is capable of "correctly perceiving the circumstances having a bearing on the case and providing correct testimony about them." The subject's capability for conceptualizing and remembering the incident, describing it, and understanding the requirements imposed on her as a participant of legal proceedings and, in correspondence with this, controlling her own behavior, to include her statements, must be evaluated. The last premise is not directly implied by the Criminal Code, but it is important to expert assessment, especially of people suffering oligophrenia. Satisfactory memory, sufficiently developed speech, and the ability to present the basic facts are often noted among the latter, but they are incapable of providing consistent testimony since they cannot orient themselves in the more-complex situation of court investigation. This is why the expert conclusion must be based on clinical assessment of the general degree of mental soundness—that is, not only the formal possibilities for memorizing and relating but also the capabilities for a higher level of mental activity—integrated understanding of the situation.

This is confirmed by the results of my research, which indicate that in each concrete case, the decision as to capability or incapability of providing testimony depended in the end on whether or not the given person was able to assess the incident and the present situation critically and correctly.

It should be emphasized that in analyses of the chronic signs of debility, the degree of mental deficiency preventing presentation of testimony was determined on the basis of the depth of intellectual insufficiency predominantly in some cases and the expressiveness and nature of concurrent emotional-volitional disturbances in others.
The degree of intellectual insufficiency was the principal clinical diagnostic sign in groups 1 and 3. The expert decision pertaining to each of these groups was the same, and it was based on an assessment of the depth of intellectual deficiency.

Group 2 was the most difficult in relation to expert assessment; the reason for this lay in the more-complex clinical structure of the patient's state, in which minor intellectual deficiency combined with pronounced emotional-volitional disturbances. O. Ye. Freyerov (1957, 1964) and Yu. A. Novikov (1974) emphasized in relation to the features of the emotional-volitional sphere of deficient persons committing crimes that pronounced affective-volitional disorders aggravate the clinical pattern of oligophrenia, and therefore they have important significance to the question of responsibility. Affective excitability, impulsiveness, disinhibition, and heightened suggestibility and subservience was accompanied in deficient personalities by a significant decline in the capability for critically assessing the surrounding situation and controlling personal behavior, which was manifested in particular in their relationship to the event and to behavior and statements in the situation of the court investigation. This is why experts decided in most cases that subjects in this group cannot adequately evaluate past events and provide correct testimony about them.

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11004
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A MODIFIED DEVICE FOR INJECTING A SAMPLE IN A GAS CHROMATOGRAPH

Moscow SUDEBNO-MEDITSINSKAYA EKSPERTIZA in Russian No 2, 1978 pp 43-44

[Article by Yu. V. Yemolkin, Smolenskaya Oblast Forensic Medical Expert Examination Office]

We use an LKhM-8MD (model 1) gas chromatograph to determine ethyl alcohol and higher alcohols. The device used to inject samples into this chromatograph has certain shortcomings making the work difficult. The neck of the sample injection device is very thin (3 mm), and with time it undergoes deformation and makes introduction of the needle difficult. The sample injection hole is small (1 mm), and it is quickly clogged by fragments of the rubber gasket and reaction products. The column must be disconnected each time to permit cleaning of the channel. The gas injection point is low, requiring the use of a long, thick needle. There is a "dead zone" between the gas injection point and the rubber gasket; when the sample is injected with a short needle, that part of the sample in this "dead zone" is bypassed by the carrier gas.

I have manufactured a more convenient device. A capillary is inserted into the channel on the sample injection side; the upper end of the capillary has a screwdriver slot, and the other end is covered loosely by a glass wool tampon. This capillary is used to collect waste products and rubber gasket fragments. The neck of the sample injection device is thicker and stronger. The carrier gas is fed upward from the device to the rubber gasket, and then to the screwdriver slot into the capillary, after which it passes into the channel of the sample injection device in the column (see figure). The device and the capillary are made of stainless steel, and the gas inlet tube is soldered with silver alloy or simple metal with the help of argon.

The proposed modification has the following advantages.

At the rubber gasket the sample is captured completed by the carrier gas. This avoids possible loss of the sample. A needle of any length and diameter can be used. To clean the channel, one simply unscrews and cleans the capillary.
Modified injection device: 1—Device, 2—capillary, 3—nut.

Enlargement of the depth of the nut makes it possible to use a stronger thicker (vacuum) rubber gasket.

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11004
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A RESECTION METHOD FOR AUTOPSY OF THE SPINAL CANAL USING CUTTING PLIERS

Moscow SUDEBNO-MEDITINSKAYA EKSPERTIZA in Russian No 2, 1978 p 45

[Article by V. M. Balanchuk]

I have developed a model of bone cutting pliers with which to cut the vertebral arch, and a procedure for autopsy of the spinal canal using these pliers. The bone cutting pliers are a single-unit single-joint system with long straight lance-shaped cutting jaws that are smooth and well-sharpened. Two paired springs are mounted between the long handles to facilitate opening of the instrument's jaws during work (see figure [figure not reproduced]). The structure of the pliers permits their introduction into anatomical openings and spaces between bones, and easy cutting of the vertebral arch down to its root. Expert practice has shown that such pliers facilitate, simplify, and accelerate manipulations required to achieve easy access to the contents of the spinal canal both from the dorsal and ventral directions without damaging membranes and the spinal cord itself.

In order to dissect the spinal canal from the dorsal direction, soft tissues of the back are separated from the vertebral costal processes and the vertebral arches, and the dorsal muscle group and the ligaments of the atlantooccipital joint are severed. In this case the head drops maximally downward in response to its own weight. The cutting jaws of the pliers are inserted into the spinal canal through the slit between the atlas and the foramen magnum; they are oriented in such a way that the line of section of the arches is close as possible to the transverse processes of the vertebrae. Together with remaining soft tissues, the vertebral arches successively separated in this manner are drawn caudad by hooks, and then analysis of the contents of the spinal canal can begin.

Autopsy of the spinal canal from the ventral direction begins in the thoracic division with sectioning of the pedicles of the vertebral arches on the side of the intervertebral foramina, the dimensions of which do not hinder introduction of the cutting jaws of the pliers into them. After the intervertebral cartilages are cut the bodies of the vertebrae are easily removed from the spinal column with a hook. Resection of the anterior wall in other divisions of the vertebral column is performed by inserting the cutting jaws into the...
canal and cutting the pedicles of the arches from both sides. Owing to total resection of the anterior wall of the spinal canal achieved in this way, easy access to its contents is created.

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It is not always possible to determine presence or absence of soot in the lumen of the respiratory tract macroscopically. In expert examination of charred corpses, the recommendation is to make impressions of the mucous membrane of the respiratory tract on glass slides and study them with a microscope or an electron-optic converter (V. N. Ovsyannikov, 1969; M. A. Fayn, 1971).

We have been employing a simpler and more-graphical method for detecting and fixing soot, in which impressions are made of the mucous membrane of the larynx, trachea, and bronchi on white cardboard, paper, or fabric. Soot particles are easily revealed visually or with a stereomicroscope on the white background. To make a permanent preparation, the prepared impressions are glued to a sheet of paper and covered with a polystyrene solution or covered with colorless cellophane glued to the paper to secure the soot.

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Citizen F. was wounded in the rib cage with a small-caliber rifle. There was a circular wound with dimensions 0.3×0.3 cm and (osadnennyye) margins and a circular soot mark in the fourth intercostal space along the left midclavicular line. There was an oval wound with dimensions 0.3×0.2 cm and uneven margins at the level of the fourth rib to the left of the postaxillary line; it was initially described by the surgeons as the bullet's exit hole. However, X-ray analysis revealed splintered fracture of the fourth rib on the left midclavicular line and the bullet was found there. Organs of the rib cage were normal, and there were no indications of a penetrating wound.

Reexamination of the exit wound revealed that the wound canal traveled from front to rear to the fourth rib, where there was a splintered fracture and where the bullet was located. The wound canal did not penetrate into the pleural cavity, instead traveling laterally and backward into subcutaneous cellular tissue, girdling the rib cage from the left, and terminating as a hole at the level of the fourth rib on the postaxillary line. On the 21st day the patient was released in good conditions.

Circular wounding of soft thoracic tissues was caused by a secondary projectile—a rib fragment.

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Many contradictory observations concerning the possibility of establishing spermatozoa in the genital ducts of women a long time after death have been described in the special literature. Our observations confirm the possibility of morphologically determining spermatozoa not only in the vagina but also in the oral cavity, even of corpses altered by decay.

1. The corpse of M., murdered on 21 December 1976, was discovered in a state of sharply expressed decomposition on 9 March 1977 in the sewer of a public toilet, where it had been located since the murder.

2. The corpse of Citizen Zh. was discovered at home with a noose around its neck in a state of highly expressed decomposition 40 days after death.

3. The corpse of a girl with multiple knife wounds at the neck was discovered at sea near the coast on 13 February. The body had been in the water for about 16 hours after death.

4. The corpse of an unidentified woman was recovered from the water near a pier on 12 February after remaining in the water for 5-10 hours.

Forensic biological analysis of gauze tampons and smears revealed spermatozoa in the contents of the oral cavity and the vagina (observations 1 and 2), in the oral cavity (observation 3), and in the oral cavity and in foam in the lower third of the tracheae (observation 4).
CHARACTERISTICS OF R6K PLASMID DELETION MUTANT

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian
No 11, 1977 pp 111-115

[Article by S. N. Gnedoy, L. M. Babushkina and V. S. Levashev, Second Moscow
Medical Institute imeni Pirogov, submitted 22 Dec 76]

[Text] The R6K plasmid occupies a unique place among the known factors of
drug resistance in enterobacteria. The plasmid replicates in E. coli
strains under relaxed control, and the pool of its DNA reaches 12-50 copies
per cell, depending on the stage of culture growth [10]; it also increases
significantly in the presence of ampicillin [4]. In addition, R6K has a
rather broad range of host bacteria [2, 10], which broadens the opportunities
for using the plasmid in basic and applied research, including construction
of recombinant DNA in vitro [1]. However, it is difficult to use plasmid
R6K as a genetic vector in such research, since the plasmid contains two
restriction sites that are specific to EcoRI endonucleases [11, 17].

In view of the foregoing, we undertook the task of isolating a variant of
R6K plasmid with one restriction site specific to EcoRI endonuclease, as
well as of studying the phenotypic and physical properties of such a variant,
as compared to the original plasmid. In our previous work [2], we described
the conditions that influence the competence of E. coli C600 for R6K DNA,
transforming activity of the plasmid, as well as recipient properties of
some enterobacterial strains. In our opinion, the obtained information is
essential to the study of vector properties of plasmid R6K, as related to
specific systems of host bacteria.

Material and Methods: In our study we used the following bacterial strains:
E. coli 15-3 (R6K) pro- met- (strain of R. Hodges, England, was obtained
from the laboratory of A. F. Moroz, Institute of Epidemiology and Micro-
biology imeni Gamaleya); E. coli χ925 thr- leu- lac Y" minA gal- minB thi- strF; E. coli C600 thr- leu- thi- lac- (strains obtained from the collection
of the Institute of General Genetics, USSR Academy of Sciences); E. coli
C600nal, a spontaneous mutant resistant to nalidixic acid (obtained in our
laboratory). The R6K plasmid was transferred to strain χ925 by conjugation.
To isolate plasmid DNA, we used the method of gel filtration on sepharose 4B
[2], as well as centrifugation in a cesium chloride gradient [9].
Transformation of plasmid DNA was performed by the method of Cohen [5]; recipient cultures were treated with calcium chloride in a concentration of 75 mM [2]. Selection of transformants was conducted on peptone agar with ampicillin (100 µg/ml) or streptomycin (25 µg/ml).

Conjugative transfer of the plasmid, as well as determination of extent of antibiotic resistance in the strains, was conducted as described by Macrina et al. [13].

We used the techniques described by Clowes [3] and Miller [14] to treat the strains with acridine orange, ethidium bromide and sodium dodecylsulfate. The method of Perret [15] was used for demonstration of penicillinase-negative colonies. We used the instrument of the Reanal Company (Hungary) for electrophoresis in agarose gel of plasmid DNA treated with restriction endonucleases* [17]. DNA was treated with EcoRI endonuclease [7] under the following conditions: 0.5-1.0 µg DNA, 100 mM tris-HCl buffer pH 7.5, 10 mM MgCl₂, 1.0 µl EcoRI; the following conditions applied for treatment of DNA with BamHI endonuclease [18]: 1.0 µg DNA, 100 mM tris-HCl pH 7.6, 6.6 mM MgCl₂, 6.6 mM 2-β-mercaptoethanol, 2 µl BamHI. Restriction was performed at 37°C for 15 min.

We used, as marker DNA, EcoRI restrictor of λ phage DNA [16], as well as EcoRI restrictor [11] and BamHI restrictor of plasmid R6K DNA. A calibration curve of electrophoretic mobility of linear DNA as function of molecular weight, plotted according to marker DNA to determine the molecular weights of DNA restrictors. Electron microscopy of DNA was performed according to Davis et al. [8]. We searched for spontaneous plasmid segregants, which could have been formed in the host strain, leading to a change in its phenotypic properties, to isolate plasmid R6K with one restriction site. We chose E. coli strain χ925, which produces mini-cells, as the host strain.

Results and Discussion: Analysis of transforming activity of plasmid DNA isolated from strain χ925 (R6K) by the method of gel filtration on sepharose revealed that the population of E. coli C600 transformants is represented primarily by clones that are resistant to ampicillin but sensitive to streptomycin; transformants with the phenotype amp<sup>+</sup> str<sup>+</sup> constituted about 0.03% of the total number. Subsequent studies of the plasmid were conducted on a preparation of DNA isolated from transformants C600 amp<sup>+</sup> str<sup>+</sup> by the method of centrifugation in a gradient of cesium chloride with ethidium bromide. As was the case for the original plasmid, the transforming activity of such DNA in strain C600 constituted 2·10<sup>4</sup>/cell/µg DNA.

All of the tested transformants (5000 clones) were sensitive to streptomycin.

Electron microscopy** of DNA revealed that the contour length of circular molecules was shorter than the contour length of DNA of the original plasmid, and

*The restrictase preparations were kindly provided by V. I. Tonyashin (Institute of Physiology and Biochemistry of Microorganisms, Pushchino-na-Oke).

**S. M. Klimenko and A. A. Manykin (laboratory of electron microscopy, Institute of Virology imeni Ivanovskiy) conducted the electron microscopy analysis.
it constituted 8.61±0.50 and 12.55±0.44 μm, respectively. Consequently, the isolated plasmid (designated as R6KΔ) lost about one-third of the R6K genome.

One fragment of R6K, and two fragments of R6K (Figure, a) are demonstrable upon electrophoresis of DNA of both plasmids treated with EcoRI restrictase. This shows that the deletion in the R6KΔ genome involves one of the two EcoRI-specific sites present in the R6K genome. Electrophoresis of BamHI restrictors of DNA (Figure, b) revealed that both plasmids have a single site that is attacked by BamHI endonuclease. The molecular weight of R6KΔ DNA, determined according to electrophoretic mobility of EcoRI and BamHI restrictors, constituted 17.2±0.4·10^6 dalton, which corresponds, rather accurately, to the figure obtained by electron microscopy.

Electrophoresis of DNA in 0.7% agarose gel after treatment with restrictases. Direction of electrophoresis is from top to bottom.

a) after treatment with EcoRI
b) after treatment with BamHI
1, 4) R6K DNA
2, 5) R6KΔ DNA
3, 6) λ DNA (top fragment in tube 6 corresponds to intact λ DNA)

We determined the transforming activity of DNA of both plasmids, treated with EcoRI and BamHI restrictases to confirm the data obtained from electrophoresis of DNA restrictors (Table 1). In both cases, R6KΔ DNA restrictors presented activity that was 20 times lower than in intact DNA; at the same time, the DNA of R6K retained activity only when treated with BamHI restrictase; there was complete loss of activity in the R6K EcoRI restrictors.

In order to determine the phenotypic and genetic properties attributable to plasmid R6KΔ, we studied C600 (R6KΔ) transformants with regard to the following parameters: 1) extent of resistance to ampicillin and streptomycin; 2) transmissibility according to the amp marker; 3) reaction to agents that eliminate plasmids (acridine orange, ethidium bromide, sodium dodecylsulfate). The obtained data are submitted in Table 2.
Table 1. Transforming activity of DNA of R6KΔ and R6K plasmids treated with EcoRI and BamHI restrictases

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Frequency of transformation of intact DNA (per recipient cell, per µg DNA)</th>
<th>Frequency of transformation after restrictase treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EcoRI</td>
</tr>
<tr>
<td>R6KΔ</td>
<td>2·10⁻⁴</td>
<td>1·10⁻⁵</td>
</tr>
<tr>
<td>R6K</td>
<td>2·10⁻⁴</td>
<td>&lt;1·10⁻⁸</td>
</tr>
</tbody>
</table>

Table 2. Phenotypic and genetic properties of R6KΔ and R6K plasmids in E. coli transformants

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Antibiotic resistance (µg/ml)</th>
<th>Transmissibility (frequency of transfer to donor cell)</th>
<th>Plasmid elimination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ampicillin</td>
<td>streptomycin</td>
<td></td>
</tr>
<tr>
<td>R6KΔ</td>
<td>1000</td>
<td>&lt;5.0</td>
<td>5·10⁻⁴</td>
</tr>
<tr>
<td>R6K</td>
<td>1000</td>
<td>75</td>
<td>5·10⁻⁴</td>
</tr>
</tbody>
</table>

The level of ampicillin resistance, as well as frequency of transfer of plasmid markers after crossing C600 R⁺×C600 R⁻-nal, was found to be the same for both plasmids. Identical results were also obtained in the elimination test. Plasmids R6KΔ and R6K presented resistance to each of the agents used. There was not a single penicillinase-negative variant among the 2500 selected clones of both strains.

Analysis of EcoRI restrictors of R6KΔ DNA in agarose gel, as well as electron microscopy of the plasmids, warrants the conclusion that the genome of R6KΔ, which constituted 17.2·10⁶ dalton (26·10⁶ dalton in R6K) had lost a fragment, 8.8·10⁶ dalton in size, containing one of the two EcoRI-specific sites present in R6K DNA. Treatment of R6KΔ with EcoRI endonuclease leads to 20-fold decline in biological activity of DNA in the transformation, as a result of the change from circular to linear DNA [6]. While it retains the properties of the replicon, linear DNA closes into a ring when it enters a competent cell due to adhesion of the sticky ends of the molecule and activity of cellular DNA ligase; the plasmid maintains its independent state in this form [6]. Conversely, R6K DNA loses transforming activity completely after treatment with EcoRI, since each of the two DNA fragments is either incapable of replication, or its presence cannot be phenotypically demonstrated in the cell. Cotransformation by both fragments has a very low probability (1·10⁻¹⁸), which virtually precludes reassociation thereof in vivo under these experimental conditions. Use for restriction of the second endonuclease, BamHI, in the experiments dealing with electrophoretic separation of DNA in agarose demonstrated that the DNA of both plasmids contained a single site attacked by BamHI, which is consequently not
of cardiological departments in hospitals and of cardiological offices in
polyclinics is to be expanded and the number of cardiological brigades of
first medical aid is to be increased. The decision to allocate budget
passes to sanatoria to public health bodies for the further treatment of
patients suffering from acute myocardial infarction is of great importance
for the stage treatment of such patients. Patients in sanatorium treatment
are to be given medical certificates for the entire period of their stay in
a sanatorium.

For the purpose of improving patient care, in 1979-1981 plans are made to
expand the network of specialized educational institutions for the training
of medium-level medical personnel at the base of large multispecialization
hospitals. By 1981, as compared with 1975, the admission of students to
medical schools will increase 1.2-fold.

The decree assigns a special place to the protection of mother and child.
The number of children's sanatoria, as well as sanatoria and boarding houses
for the treatment of parents with children, specialized all-year-round san-
atorium-type pioneer camps and specialized sanatoria (departments) for the
treatment of patients suffering from gynecological diseases will increase
considerably. Specialized children's sanatoria of a total capacity of 40,000
beds will be built during the Eleventh Five-Year Plan. The possibilities of
treating children under sanatorium conditions will expand considerably and
the production of special baby food products will increase. The organiza-
tion in 1979 of the All-Union Scientific Research Center for the Protection
of Maternal and Child Health at the base of the All-Union Scientific Research
Institute of Obstetrics and Gynecology of the USSR Ministry of Health will be
of great importance for intensifying and coordinating scientific research and
the organizational and methodological guidance of the service for the pro-
tection of mother and child.

The decree envisages the further development of the preventive trend in pub-
lic health and medical science. Provision has been made to increase the vol-
ume of mass preventive examinations and dispensary services and to improve
the efficiency and quality of this work. For the purpose of expanding sani-
tary measures, increasing prevention and lowering morbidity among workers in
industry, construction, transport and agriculture, the network of preventive
sanatoria will be further developed. Improvement in the sanitary and hygi-
enic education of the population and rise in the level of popularization of
a healthy way of life, physical culture and sports are envisaged.

Exceptionally important tasks have been set for medical science. The USSR
Ministry of Health and the USSR Academy of Medical Sciences together with
the councils of ministers of the Union republics and the USSR Academy of Sci-
ences will have to determine the basic trends in scientific research in the
field of fundamental and applied clinical and hygienic problems of medicine
for the period up to 1985. An outstripping development of fundamental trends
in theoretical, experimental and clinical medicine, as well as the elabora-
tion of effective methods and means of preventing, diagnosing and treating
widespread diseases and of the medical aspects of environmental protection, rational nutrition and research on physiologically active substances, are envisaged in medicobiological sciences. Special attention is drawn to improving the prophylaxis and treatment of heart, vascular and virus diseases and to preventing morbidity among children and mothers.

The decree attaches great importance to the further improvement in the training and utilization of personnel and to their education and improvement of skills. The organization of institutes for advanced training of physicians and of faculties for advanced training and specialization of physicians and pharmacists at medical and pharmaceutical institutes will begin during the Tenth Five-Year Plan. The following task is set: No less frequently than once in 5 years physicians and pharmacists should improve their skills in institutes for advanced training of physicians and at faculties for advanced training and specialization at medical and pharmaceutical institutes. Special attention is drawn to the creation of conditions for improving the skills of physicians of primary public health units—polyclinics, first-aid and emergency medical care institutions and rural district hospitals and ambulatory clinics. The training of pediatricians and pharmacists with higher education will expand considerably. The organization of pharmaceutical institutes and pharmaceutical faculties at medical institutes in a number of republics is envisaged.

A number of privileges are granted to physicians, pharmacists, medium-level medical personnel and druggists working in rural public health institutions. Privileges have been established for continuous work for physicians of district hospitals and ambulatory clinics located in rural areas, district internists and pediatricians of territorial districts of city polyclinics, physicians of traveling brigades of first-aid and emergency medical care stations and departments, sanitary aviation stations and departments of planning and urgent consultative aid.

The institution by the ukase dated 25 December 1977 of the Presidium of the USSR Supreme Soviet of the title of honor "USSR People's Physician" stresses the creative nature and great importance of the medical profession and the great respect in which the people hold the Soviet physician.

The decree places great demands on the organizers of public health, directors of medical institutions and every medical worker.

The tasks following from the decree were examined at the board of the USSR Ministry of Health and discussed at a conference of the ministers of health of the Union republics. The order of the USSR minister of health determined the specific tasks for the fulfillment of the decree No 870 of the CPSU Central Committee and the USSR Council of Ministers.

Specific plans for the development and efficient distribution of the network of ambulatory polyclinic institutions, reorganization of the available beds in accordance with the population's need and development of the network of institutions for the protection of mother and child and other services must be worked out in the Union republics, krayas and oblasts.
It is necessary to carry out extensive work on cooperating the resources of enterprises in industry, construction, transport, sovkhozes and organizations for the construction of new medicosanitary units, polyclinics, medical ambulatory clinics and district and central rayon hospitals and for the reconstruction and expansion of existing ones, as well as for the implementation of major and current repairs and the purchase of medical equipment and apparatus.

The ministries of health of the Union republics must ensure the inclusion of the fulfillment of assignments for putting into operation ambulatory polyclinic institutions and hospitals into the draft plans for the economic and social development of the republics.

Public health bodies and institutions must pay special attention to, first of all, improving the work of primary units, expanding preventive work and further developing specialized aid and primarily the cardiological service.

It is necessary to improve the work of the admission departments of hospitals and registries and to more widely use advanced forms and methods of admitting patients to polyclinics and of providing medical care for patients at home. The work of polyclinics should be expanded during evening hours and on Saturdays and patients must be provided with the necessary volume of therapeutic and diagnostic procedures. This will make it possible to examine patients under conditions of polyclinics more completely and in a short period, to increase the effectiveness of treatment and to expand dispensary services for the public, which in the end will improve the quality of medical aid in ambulatory polyclinic institutions. The work regime of medical institutions, primarily of medicosanitary units and polyclinics, should be brought into line with the work regime of industrial enterprises (organizations).

The problems of improving the quality and completeness of patient prehospital examination, of ensuring a more efficient interconnection and continuity in the work of hospitals and polyclinics and of expanding the use of methods of restorative treatment in ambulatory polyclinic institutions acquire great importance. Local public health bodies must determine the specific measures for the organization of departments for restorative treatment at large polyclinics.

Subdividing therapeutic and pediatric districts and ensuring the preferential appointment of young specialists and graduates of higher medical educational institutions as district and shop physicians in first medical aid institutions, central rayon and rayon hospitals and rural district hospitals and ambulatory clinics set important tasks for republic and local public health bodies. Their successful fulfillment also requires the preparation of annual specific plans for subdividing medical districts and increasing the positions of district physicians, which will make it possible to fully complete this work in accordance with the period set by the decree.
Public health bodies must more systematically implement measures for the further improvement in the forms and methods of providing preferential medical aid to workers in industry, construction, transport and agriculture, intensification of the prevention of occupational morbidity and reduction in temporary incapacity and disability. For the preferential provision of medical care for workers in industrial enterprises who do not have their own medico-sanitary units it is necessary to increase the number of shop districts in territorial polyclinics. It is necessary to actively participate in the work on the development of a network of preventive sanatoria, which are of great importance for improving workers' health.

The problems of further refining the forms and improving the quality of medical care for the rural population also must be constantly in the field of vision of public health bodies. First of all, it will be necessary to ensure the development of central rayon hospitals of a capacity of up to 250 or 400 beds and more, organization of interrayon specialized departments and expansion of the network of rural medical ambulatory clinics, as well as to systematically implement measures to retain medical personnel in rural public health institutions.

The expansion of the network of maternity homes, children's hospitals and polyclinics, women's consultation offices and children's sanatoria, organization of departments for anesthesiology and reanimation in maternity hospitals and of departments for reanimation and intensive therapy in children's hospitals, improvement in the quality and accessibility of all types of specialized care for children and pregnant women, in the observation of pregnant women and in public health services for children from the first year of life, prompt hospitalization of sick children and expansion and increase in the effectiveness of preventive work among healthy children--this is the central task of public health bodies of all levels in the cause of protection of mother and child.

The further development of cardiology, stomatology, first aid and other types of specialized medical care requires special attention on the part of public health bodies. The ministries of the Union republics and local public health bodies, proceeding from the periods set by the decree, must ensure the training of cardiologists, determine the place and time of establishment of cardiological dispensaries and the periods of organization of cardiological departments at republic, kray, oblast and large city hospitals and of cardiological offices at polyclinics and medico-sanitary units and continue the development of the network of rheumatological offices which provide medical care for arthrological patients. The problem of establishing departments of cardiology at all institutes for advanced training of physicians is now being discussed. It is necessary to constantly see to it that the network of stomatological polyclinics and offices is expanded, that they are staffed with personnel whose training will be increased considerably, that the material and technical base of first-aid and emergency medical care institutions is reinforced and that their work is improved. It is necessary to carry out
extensive work on the further organization of first medical aid hospitals united with first medical aid stations, development of urgent specialized medical care and, primarily, establishment of cardiological brigades, intensive therapy brigades and pediatric, toxicological and other brigades.

The bodies and institutions of the sanitary epidemiological service should strive for a further rise in the level and efficiency of state sanitary supervision of the observance of sanitary hygienic and sanitary entiepidemic rules and norms by enterprises, institutions and organizations for the prevention of environmental pollution by enterprises and organizations producing and selling food products, including special baby food products. Special attention should be drawn to an unconditional fulfillment of the sanitary entiepidemic regime by medical institutions, as well as children's preschool institutions and schools. It is necessary to intensify the control over the implementation of all measures for the prevention and further reduction of infectious morbidity.

In the field of medical science the task is to improve the planning and coordination of scientific research, to eliminate the unjustified duplication in science, to regulate the network of medical scientific research institutions, to increase the efficiency of scientific research and to expand the introduction of the achievements of medical science into the activity of public health institutions.

It is necessary to increase the role of head scientific research institutions in the scientific and methodological guidance of research on problems of Union importance. A unified overall system of planning and coordinating scientific research in the field of medicine with the use of computer equipment will have to be developed and introduced before 1982.

Refining the training of physicians, systematically improving their skills, developing high moral qualities in them and creating in every collective a creative atmosphere ruling out any manifestations of a formal and careless attitude toward one's duties are tasks of primary importance. Fulfillment of these tasks requires an improvement in the selection and training of managerial personnel and increase in their responsibility for the management of public health bodies and institutions and organization of medical care for the population. "The modern manager," L. I. Brezhnev stressed in his report to the 25th CPSU Congress, "should organically combine party mindedness with profound competence and discipline with initiative and a creative approach to work. At the same time, at any section the manager must also take social and political educational aspects into account, be sensitive to people and to their needs and requests and serve as an example in his work and way of life." 2

At present the USSR Ministry of Health has worked out measures for the further improvement in the system of training medical specialists, as well as improvement in the training and skills of managerial personnel in public health. The managers of public health bodies and institutions should intensify their attention to the education of medical personnel in accordance with the demands of the oath of the physician of the Soviet Union. It is necessary to ensure the further development and improvement among the collectives of public health institutions of socialist competition, of the movement for a communist attitude toward labor, of tutorship and of review contests for the best in one's profession, because this is a powerful means of improving the quality of medical care and developing in workers the Marxist-Leninist world outlook, high moral qualities, conscious state discipline and high civic responsibility for the fulfillment of their duty to the people and state.

The Communist Party teaches us to soberly evaluate our work and not to close our eyes to the shortcomings that still exist in our country. L. I. Brezhnev, general secretary of the CPSU Central Committee, chairman of the Presidium of the USSR Supreme Soviet, indicated in the accountability report to the 25th Congress "... that, unfortunately, the level of work of some medical institutions still gives rise to valid criticism by workers." This is connected primarily with the inefficient organization of work, incomplete use of existing reserves and errors in management. Improvement in planning and management, rise in the level of organizational activity and introduction into the activity of hospitals and polyclinics of progressive forms and methods of work, advanced experience and scientific organization of the labor of medical personnel, especially of the labor of physicians, are urgent tasks. They should be actively solved both on a scientific and practical level. Public health bodies and scientific research collectives should concentrate their attention on these tasks.

A successful fulfillment of the tasks set by the party and government requires persistent and purposeful work on the part of the entire medical community and a creative search for a modern scientific approach to the solution of the problems of public health and medical science. There is no doubt that physicians, scientists and all medical workers will direct their efforts, knowledge and experience to a further improvement in the quality and standard of medical care for the population and in public health.


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OBTAINING HOMOLOGOUS HYPERIMMUNE PREPARATIONS FROM THE BLOOD OF IMMUNIZED DONORS—ANTIGOLY (ANTIESCHERICTIA) PLASMA AND ANTITOXIC ANTIPYOCYANIC PLASMA

The use of homologous blood preparations obtained from immunized donors is one of the effective methods of immunotherapy of infections diseases of varying etiology (S. V. Skurkovich and co-workers). According to the data in the literature, experimental and clinical investigations connected with the development of preparations for the specific prevention and therapy of escherichioses are basically directed to the problem of active immunization (Koch et al.; Nauss et al.; Kulin et al.). We did not find data on the use of direct-action blood preparations for the purpose of the immunotherapy of infections caused by Escherichia.

Recently, investigators have also directed their efforts toward obtaining hyperimmune specific preparations against Ps. aeruginosa. The homologous hyperimmune preparations against some serotypes of Ps. aeruginosa obtained abroad are antimicrobial and possess limited effectiveness in the immunotherapy of pyocyanic infection in connection with the presence of a large number of serotypes of Ps. aeruginosa (Peller and Kamei; Alexander and Fisher). Therefore, obtaining human plasma hyperimmune to the toxin of Ps. aeruginosa is a very urgent task.

In this work we investigated the possibilities of obtaining hyperimmune antiescherichia plasma from donors immunized with live vaccines from Escherichia and antitoxic antipyocyanic plasma (immunoglobulin) from donors immunized with the anatoxin of Ps. aeruginosa.
Hyperimmune anticoli (antiescherichia) plasma. To obtain immune plasma, donors were immunized perorally with live Escherichia vaccines from Str-d mutants of E. coli. Str-d mutants of Escherichia used as live vaccines were obtained from E. coli isolated from patients suffering from various diseases of Escherichia etiology (Mel and Paro; Koch et al.). Live Escherichia monovaccines 01, 02, 015 and 020 were prepared from the washings of daily cultures of Str-d mutants of the corresponding strains of E. coli.

In the experiment immunogenicity was studied in the test of active protection of white mice against an intraperitoneal infection with virulent cultures of the initial strain (determination of \( E_{50} \)) and in the test of passive protection of mice with immune serums (determination of \( P_{50} \)). Triple peroral immunization of white mice with live vaccines from Str-d mutants protected the animals against infection with virulent cultures of the initial homologous strains.

During a comparative study of the immunogenicity of vaccines from Str-d mutants of E. coli in rabbits with various methods of antigen administration to them an increase in the protective activity of rabbit serums was revealed.

The results of experimental investigations indicating the harmlessness, avirulence and pronounced immunogenicity of vaccines obtained from Str-d mutants of E. coli made it possible to use them for the immunization of donors. For the purpose of selecting the optimal scheme donors were immunized with five vaccine strains of serotypes 01, 02, 015, 020 and 0111 according to three schemes: first scheme—three cycles of immunization, each cycle consisting of a daily administration of \( 100 \times 10^9 \) cells during 3 days (interval between cycles, 4 days); second scheme—single administration of \( 300 \times 10^9 \) cells; third scheme—daily vaccine administration during 3 days (single dose of \( 300 \times 10^9 \) cells).

The \( P_{50} \) (in the test of passive protection of mice) of the serums of donors was determined before immunization and on the 7th, 14th, 21st and 35th days after the end of immunization. The harmlessness and reactogenicity of vaccines were judged from the general state of the donors and the change in body temperature. Furthermore, a clinicolaboratory investigation of peripheral blood and of functional liver tests was conducted. As the results of investigations conducted on 80 donors showed, immunization with live vaccines from Str-d mutants of E. coli did not affect the general state of the donors and, essentially, did not change the indexes of peripheral blood and of the functional state of the liver.

The third scheme of immunization proved to be optimal (see figure). During immunization according to this scheme the protective activity, judging from the decrease in \( P_{50} \), increased tenfold, on the average. This increase was most pronounced 7 days after the end of immunization. After 14 days the protective activity of the serums was lowered.

For the purpose of further industrial production of immune plasma donors were immunized according to the third scheme. Blood was taken by the method of plasmapheresis with an extraction of 250 to 500 ml of plasma on the 7th day after the end of immunization. The preparation was stored in a frozen (\(-30^\circ C\)) and lyophilic dry state.
Protective activity of the serums of donors (average PD$_{50}$ index) in the process of peroral immunization with live escherichia vaccines prepared from Str-d mutants of E. coli of serotypes 01, 02, 015 and 020.

Abscissal axis—vaccine serotypes. 
a—before immunization; 6—after 7 days; b—14 days after the end of immunization.

The average indexes of PD$_{50}$ of the plasma of donors after immunization increased more than tenfold.

It should be noted that the serum and plasma of immune donors protected white mice only from infection with a virulent homologous strain and did not protect them from infection with the virulent strains of other O-groups.

Immune 020 serums were also serologically active with ultrasonic erythrocytic diagnosticus from Escherichia in the passive hemagglutination reaction. The antibody titer before immunization was 1:4.9±0.28 (M±m), increasing to 1:120±0.18 after immunization.

We successfully used the anticoli (antischerichia) plasma obtained by us for the treatment of diseases of Escherichia etiology (peritonitis, sepsis, colitis, meningitis and so forth). A single dose of the preparation during intravenous administration was 3 to 5 ml/kg, and in children under 1 year old, 10 ml/kg. During the course of treatment three to four transfusions with an interval of 1 or 2 days were given. In addition to this, according to indications immune plasma was used intralumbarly and locally.

It should be noted that in the experiments of active and passive protection of white mice the immunogenicity of live vaccines from E. coli was characterized by pronounced O-specificity. During a study under clinical conditions it was established that hyperimmune plasma gives a pronounced immunotherapeutic effect not only in diseases caused by strains of E. coli with
homologous antigens, but also in cases when strains of E. coli of other groups or other representatives of intestinal bacteria (protei) are isolated from patients. Apparently, during a coli-infection in man the specific antibodies of immune plasma affect not only homologous antigens, but also related antigen determinants.

Recently, immunoglobulin has been isolated from hyperimmune anticoli (anti-escherichia) plasma.

Hyperimmune antitoxic antipyocyanic plasma. To obtain immune plasma, donors were immunized subcutaneously with the anatoxin of Ps. aeruginosa prepared by G. P. Cherkas, L. G. Podgornaya and A. A. Shinkarenko. The anatoxin of Ps. aeruginosa was obtained by detoxicating with 0.4% Formalin at 37°C the toxin of Ps. aeruginosa produced by aeruginosa strain PA-66-16, serotype 05 (according to Habs's scheme) with its cultivation in Martin's broth and a subsequent concentration of the active principle with ammonium sulfate or other precipitators (S. V. Skurkovich et al., 1976), author's certificate No 548275, 1976.

The harmlessness of the obtained preparation was controlled in a biological experiment on guinea pigs. During a subcutaneous administration of 5 ml of the anatoxin to the animals intoxication and a local reaction were not observed. Control of the immunogenic properties of the anatoxin in experiments on mongrel mice showed that an intraperitoneal administration of 0.5 ml of the preparation to these animals protected them from a subsequent infection with 1 DLM of the toxin of Ps. aeruginosa.

The experimental results enabled us to immunize the donors according to various schemes of anatoxin administration: 0.5-0.5-1.0 ml (first scheme) and 1.0-1.0-1.5 ml (second scheme). Immunization envisaged a subcutaneous administration of the anatoxin of Ps. aeruginosa with an interval of 5 to 7 days between injections.

The harmlessness and reactogenicity of the anatoxin of Ps. aeruginosa were judged from the general state of the donors, clinicolaboratory indexes of peripheral blood and functional liver tests. As the results of investigations conducted on 20 donors showed, immunization with the anatoxin of Ps. aeruginosa did not have a negative effect on the general state of the donors and did not produce deviations from the physiological norm of the indexes of peripheral blood and functional liver tests.

The titer of specific antibodies for the anatoxin of Ps. aeruginosa was determined in the passive hemagglutination reaction according to Faulk's and Hauba's method.

In the process of immunization of the donors with the anatoxin of Ps. aeruginosa the titer of specific antibodies increased. During a comparison of the two schemes of immunization the advantage of the second scheme was disclosed, because the average geometrical titer of antibodies in the serum of
donors immunized according to this scheme greatly exceeded the one in the serum of donors immunized according to the first scheme. Immune serums gave distinctive lines of precipitation in agar gel both with the toxin and anatoxin of Ps. aeruginosa.

The study of the preventive properties of the immune serum showed that the serums obtained possessed pronounced protective properties in the experiment on white mice. The plasma of donors containing specific antibodies in the passive hemagglutination reaction in a titer of no less than 1:128 and possessing a distinctive preventive action was prepared by the method of plasmapheresis and stored in plastic bags at -30°C. Part of the plasma was subjected to lyophilic drying.

Hyperimmune antipyocyanic plasma was used in diseases caused by Ps. aeruginosa (sepsis, osteomyelitis, mediastinitis, pyelonephritis, suppurative infections of open fractures and so forth). The preparation was administered intravenously in a single dose of 3 to 5 ml per kg of the patient's weight (in a course of treatment of up to four or five transfusions) and locally in the form of applications to the wound surface.

A preliminary clinical study of immune plasma showed that, as a result of the use of this preparation, there was a distinctive improvement in the state, the temperature dropped and was normalized, intoxication phenomena stopped and the pathological secretion from infection foci disappeared. During a repeated bacteriological investigation growth of Ps. aeruginosa was not revealed. A serological identification of the cultures of Ps. aeruginosa isolated from patients to whom immune plasma was administered showed that antitoxic antipyocyanic plasma had an immunotherapeutic effect on a range of serotypes of Ps. aeruginosa causing an infectious process (O2, O17, O23 and O5).

At present we have obtained immunoglobulin from hyperimmune antitoxic antipyocyanic plasma.

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TRANSFUSION OF WHOLE FIBRINOLYSED BLOOD AND ITS THAWNERYTHROCYTES IN SURGERY OF THE MAIN VESSELS

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Reconstructive operations on the main vessels often are accompanied by blood loss requiring large quantities of blood for replacement. In the process of operation and during the immediate postoperative period 1.5 to 2 liters of blood and more from several donors must be transfused into the patient's body. During the postoperative period with an adequate replacement of blood loss complications can develop as a result of the transfusion of large volumes of homologous blood. These complications are manifested by a number of symptoms: arterial hypotension resulting from the deposition of transfused blood, congestive phenomena in organs and tissues, especially in the lungs and liver, anemia, disturbance in microcirculation, focal nephroses and so forth. A direct relationship between massive transfusions and thromboembolic complications was disclosed.

Investigations showed that, in addition to the complications connected with citrate and potassium intoxications, complications during massive transfusions of donor blood can be due to the immunological reaction of the body (V. I. Burakovskiy et al.; B. V. Petrovskyi and Ch. S. Guseynov; Gadboys et al.; Litvak et al.).

In connection with this in the last few years investigators have sought to limit the dose of transfused blood, as well as to search for new transfusion media.
From our point of view, whole fibrinolysed blood and its components can be such a medium. In contrast to donor blood, fibrinolysed blood can be prepared instantly in amounts of 2 to 3 liters from one donor—corpse. This makes it possible to carry out hemotransfusion with the use of the blood of one donor, which lowers the number of complications of an immune nature.

As is well known, fibrinolysed blood can be stored without anticoagulants, which eliminates the danger of citrate intoxication in cases of transfusion of massive blood doses. Fibrinolysed blood is devoid of fibrinogen and its coagulological potential is lower than in donor blood. Furthermore, in a number of cases (about 15%) the fibrinolytic activity of this blood is much higher than that of donor blood.

Owing to these properties fibrinolysed blood can be purposefully used for transfusion to patients with a hypercoagulation tendency. In particular, this occurs in case of vascular pathology in patients with occlusions of the main vessels, in whose blood the content of fibrinogen is higher and the fibrinolytic activity is lower. If transfusion is needed, it is advisable to use fibrinolysed blood and its components in these patients.

This work studied the effect of whole fibrinolysed blood and its thawed erythrocytes on patients with occlusions of the main vessels with a hypercoagulation tendency.

A total of 37 male patients aged 40 to 62 were under observation.

Whole fibrinolysed blood prepared from one donor was transfused to 20 patients during the operation and the immediate postoperative period. The single volume of transfused blood ranged from 500 to 2,500 ml. Fibrinolysed blood with a storage period of 5 to 7 days prepared according to the saccharose and phosphate prescription in the Institute of First Aid imeni N. V. Sklifosovskiy was used.

Before transfusion the content of hemoglobin in whole fibrinolysed blood was 14.1 to 15.2 g%, hematocrit index, 40 to 50% and level of free hemoglobin, up to 9.8 mg%. The concentration of potassium in plasma ranged from 14.1 to 15.2 meq/l, and of lactic acid, 15.4 to 131 mg% (on the average, 62.2 mg%). The content of pyruvic acid ranged within 0.2 to 1.22 mg%, averaging 0.40 mg%. The blood pH was 5.8 to 6.7. The level of general protein in plasma was within 7.2 to 9.8 g%, of bilirubin, 0.41 to 1.0 mg% and of residual nitrogen, 31 to 50 mg%.

1. Synonyms: corpse blood, postmortem blood and cadaver blood.
Washed thawed erythrocytes of fibrinolysed blood from one donor in amounts from 300 to 700 ml resuspended in the TsOLIPK 8v solution were transfused to seven patients. The cryoconservation of freshly prepared erythrocytes of fibrinolysed blood was carried out in the Department of Blood Conservation and Transfusionology of the Central Institute of Hematology and Blood Transfusion (Prof. V. A. Agranenko, head). The period of storage of erythrocytes was 1 to 16 months at -196°C.

In the suspension of thawed erythrocytes of fibrinolysed blood during the first days after washing the level of free hemoglobin ranged from 0 to 160 mg% (on the average, 72 mg%), which was slightly lower than in thawed erythrocytes of donor blood (146±18.8 mg%). The content of potassium in the infusion was 3.2 to 9.9 meq/l, and of sodium, from 10.8 to 17 meq/l. The level of hemoglobin was 12.5 to 20 g% and the hematocrit index, 40 to 78 percent. The content of lactic acid ranged within 26 to 36.4 mg%, and of pyruvic acid, from 0.4 to 0.95 mg%, and the pH was 5.6 to 6.57.

The qualitative composition of thawed erythrocytes of fibrinolysed blood after washing was studied by the method of acid erythrograms, and individual samples, by the conductometric method of automatic analysis on the KMFKh apparatus (2,000 cells per minute). The data of acid erythrograms and the results of dispersion analysis showed that young cells with a high stability predominated in the population of the investigated erythrocytes. This was due to the fact that spherocytes and weakly resistant erythrocytes were destroyed in the process of freezing and thawing and removed during washing. The predominance of young stable erythrocytes advantageously distinguishes the suspension of thawed erythrocytes of fibrinolysed blood from fibrinolysed blood.

Whole donor blood in amounts of 500 to 2,000 ml prepared from several donors on the TsOLIPK 7v preservative with a period of storage from 3 to 5 days was instantly transfused to 10 patients (control group).

The patient's general state, hemodynamic indexes, body temperature, hemogram indexes, hematocrit and the level of free hemoglobin, general protein, bilirubin, residual nitrogen, electrolytes and lactic and pyruvic acids were evaluated during the transfusion and 3 and 24 hours after it. The state of the coagulating system of the recipient's blood was judged from the number of thrombocytes and the data of the coagulogram (time of blood coagulation, time of plasma recalcification, thrombin and heparin time, fibrinogen and fibrinolytic activity) and of the thromboelastogram. The kidney function was evaluated on the basis of the results of clinical urine analysis. The obtained data were compared with similar indexes in the patients of the control group, which were gathered at the same time.

The length of life of the erythrocytes of fibrinolysed blood and that of thawed erythrocytes of fibrinolysed blood was studied in 10 observations by means of radioactive indication. Erythrocytes were labeled with Cr^51 by
the accepted method (G. A. Malov and Ye. N. Ryumina; Ye. N. Ryumina et al.).
The period of biological half-decay of the labeled erythrocytes in the recipient's body was determined (50-percent reduction in the volume of introduced cells).

The patients tolerated the transfusions of fibrinolysed blood and of thawn erythrocytes of fibrinolysed blood quite satisfactorily. The temperature reaction 3 hours after the transfusion of fibrinolysed blood was not pronounced significantly. In only two patients the body temperature rose to 38.2°C without a disturbance in hemodynamics or the development of some other side effects. In five patients of the control group, especially when blood in an amount exceeding 30% of the volume of circulating blood was transfused to them, hemotransfusion reactions—chills, headaches and a rise of 2 to 3°C in the body temperature—were noted during the first hours.

In the group of patients to whom thawn erythrocytes of fibrinolysed blood were transfused the temperature reaction was weakly pronounced and did not exceed 37.8°C and side effects were not observed.

After massive transfusions of both donor and fibrinolysed blood and its erythrocyte mass changes in the composition of peripheral blood were revealed. Despite the replacement of the blood loss, moderately pronounced anemia was observed in almost all cases. This was expressed in a drop of 1.5 to 2.5 g% in the level of hemoglobin, of 1.0 to 1.5 million in the number of erythrocytes and of 30% of the initial number of reticulocytes in the recipients' peripheral blood. During the first days after the operation additional hemotransfusions in a volume of 250 to 500 ml were given to three patients of the control group and to two patients to whom fibrinolysed blood was transfused in connection with a drop in their hemoglobin level to below 9 g%.

Lymphocytopenia and eosinopenia, including a complete absence of eosinophils, were noted in the blood after transfusions. These changes in the indexes of the morphological blood composition in the patients of the control group were slightly more pronounced. When the blood replacement was adequate, 3 hours after transfusions and during the next few days there were no shifts in the content of general protein, electrolytes or residual nitrogen as compared with the initial data and between the groups. Bilirubin, whose level rose in all the patients and slightly to a greater extent in the control group (on the average, 1.1 mg% at the expense of its indirect fraction), constituted an exception. Changes in the urine (moderate proteinuria, hematuria and isolated hyaline casts) were observed in all the patients during the first days, but a significant difference between the groups was not revealed.

An analysis of the indexes of the coagulating blood system also disclosed a number of differences between the groups in stages. Whereas the initial content of fibrinogen before the operation in all the patients was above the norm—from 550 to 1,200 mg%—3 hours after the replacement hemotransfusion a considerable drop in it, more pronounced in patients to whom fibrinolysed blood and thawn erythrocytes of fibrinolysed blood were transfused, was observed.
Fig. 1. Change in the concentration of fibrinogen in recipients after transfusion of fibrinolysed blood (a), thawed erythrocytes of fibrinolysed blood (b) and donor blood (c).

Abscissa—period of investigation after transfusion (in hours); ordinate axis—content of fibrinogen (in mg%). Crosshatched field—norm. The moment of transfusion is indicated here and in fig. 2.

Twenty-four hours after the transfusion the content of fibrinogen increased, but the degree of this increase was more significant in patients to whom donor blood was transfused (see fig. 1).

An inverse relationship was observed with regard to the fibrinolytic activity. In patients to whom fibrinolysed blood and thawed erythrocytes of fibrinolysed blood were transfused during the immediate hours after hemotransfusion and the next days the fibrinolytic activity of the blood increased as compared with the initial activity and was higher than in patients to whom donor blood was transfused (fig. 2).

Other indexes of the coagulogram in the patients of both groups changed in one direction and deviated from the norm insignificantly.

As a rule, the data of the thromboelastogram corresponded to the indexes of the coagulogram. Lengthening of the time of constants R and K and reduction in the maximum amplitude MA indicated the presence of heparin.
The length of life of erythrocytes of fibrinolysed blood expressed in the period of biological half-decay was from 16.2 to 24 days (absolute figures), and for thawed erythrocytes of fibrinolysed blood, from 21.5 to 28.5 days (fig. 3).

**Fig. 3.** Length of life of erythrocytes of fibrinolysed blood.

Abscissal axis—period of investigation (in days): ordinate axis—number of Cr$^{51}$-labeled erythrocytes (in % of the initial number).

The research results indicate the biological value of the studied transfusion media. In all cases an adequate transfusion of fibrinolysed blood and thawed erythrocytes of fibrinolysed blood ensured the replacement of surgical blood loss.

During massive blood loss transfusions of fibrinolysed blood and thawed erythrocytes of fibrinolysed blood prepared from one donor have a number of advantages as compared with transfusions of donor blood prepared from several donors, that is, good tolerance of transfusions and an absence of post-transfusion reactions and of a negative effect on the blood system, liver and kidneys. The rapid restoration of the composition of peripheral blood with minimal changes in some indexes of the hemogram and with an absence of hypoproteinemia and pronounced changes in the urine composition attests to the above.

The effect of fibrinolysed blood and donor blood on the recipient's system of hemocoagulation was different. These differences lay in the fact that the high level of fibrinogen in the patients of both groups during the first
hours after transfusions of fibrinolysed blood was lowered to a greater extent than after transfusions of donor blood. With regard to the fibrinolytic activity, conversely, it increased, more significantly after transfusions of fibrinolysed blood.

During a differentiated use of fibrinolysed blood and thawed erythrocytes of fibrinolysed blood it is necessary to take into consideration the characteristics of these transfusion media. A higher content of general protein and a higher concentration of potassium are noted in fibrinolysed blood and the level of general protein in the suspension of thawed erythrocytes of fibrinolysed blood is lowered. Furthermore, washed thawed erythrocytes of fibrinolysed blood are essentially devoid of products of metabolism and can be recommended for reactive patients (immunized and allergized) with affection of the liver and kidneys. In cases of massive blood loss it is advisable to combine transfusions of thawed erythrocytes of fibrinolysed blood with the transfusion of plasma or protein preparations.

On the basis of the latest data in the literature (F. R. Vinograd-Finkel et al., S. V. Cidurova and V. N. Lubskiy; Huggins) and the results of this investigation it should be noted that thawed erythrocytes of fibrinolysed blood used as a transfusion medium are most biologically valuable, whereas erythrocytes of fibrinolysed blood and donor blood with a period of storage of more than 5 to 7 days contain a negligible number of organophosphorus compounds, which play an essential role in the respiratory function of erythrocytes. With due regard for the technical complexity of obtaining fresh fibrinolysed blood for transfusion and our slight experience in a comparative evaluation of transfusions of fibrinolysed blood and thawed erythrocytes of fibrinolysed blood we consider the use of the latter advisable.

The combination of thawed erythrocytes of fibrinolysed blood prepared from one donor jointly with blood components (albumin, protein, thromboconcentrate and so forth) can be recommended during the hemotherapy of patients in whom hemotransfusions represent a certain danger owing to sensibilization, allergization, hypercoagulation and other phenomena causing complications, especially in cases of use of massive blood doses.

BIBLIOGRAPHY


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CSO: 1870
The problem of the advantages and shortcomings of dextran and gelatin preparations as antishock agents is now being discussed. Some authors give preference to gelatin.

For example, Lundsgaard-Hansen placed blood substitutes according to the degree of restoration of the volume of circulating blood in the following order: Rheomacrodex, Macrodex, gelatin and saline solutions. However, this author gives preference to gelatin on the basis that it can be infused in large doses without the danger of affecting the kidneys and the coagulating blood system and of disturbing the water and salt balance between the intravascular and intercellular space. Such a conclusion was drawn mainly on the basis of clinical observations. The same author in experiments on 180 rabbits under conditions of a standard hemorrhagic shock studied the therapeutic effectiveness of Macrodex and three gelatin preparations (Physiogel, Haemaccel and Gelifundol). In the index of the survival rate, arterial pressure and acid-base equilibrium there was no significant difference in the effect of the indicated methods of blood replacement (Lundsgaard-Hansen and Riedwyl). Later the results of investigation of 11 colloid preparations conducted on dogs with acute blood loss were published and it was concluded that the French gelatin preparation is safer than dextran and hydroxyethyl starch (Gollub).

In the Soviet Union there is no such comparison between the domestic gelatinol preparation and polyglucin. The weaker antishock effect of gelatinol was reflected in the indications for its use in first- and second-degree shock and moderate blood loss (I. R. Petrov et al.). This conclusion was
also expressed later (N. A. Fedorov et al.). In experiments on dogs with acute lethal blood loss it was shown that in effectiveness gelatinol corresponds to similar foreign preparations, but is inferior to polyglucin (V. B. Koziner and V. B. Troitskiy).

At the same time, L. G. Bogomolova and T. V. Znamenskaya in the famous monograph "Krovezameniteli /Blood Substitutes/ write that an intravenous injection of gelatinol to exsanguinated dogs leads to a rapid restoration of the volume of circulating blood and arterial pressure and that in clinical practice gelatinol was widely and successfully used for the prevention and treatment of surgical and traumatic shock.

The task of this investigation is to give a comparative evaluation of the effect of polyglucin, gelatinol and plasma in the treatment of dogs with standard blood loss—40 ml/kg. According to long-term observations in our laboratory such a degree of blood loss in dogs is the limit of their possible survival rate without treatment and treatment even with agents that are not very effective (physiological solution) preserves the life of animals. Knowing the great tolerance of dogs for blood loss and their rapid positive reaction to infusion, we classify this degree of blood loss more with moderate than with severe, although A. G. Shifrin classifies it with severe. Since during the transfusion of blood substitutes it is necessary to take into consideration not only the degree of restoration of the blood volume, but also the equilibrium between the intravascular and intercellular water space, in addition to determining the usual hemodynamic indexes, we investigated the volume of intravascular, extracellular and extravascular fluid and the volume of extracellular fluid.

The experiments were conducted on 26 dogs under Nembutal narcosis (30 mg/kg). Blood loss (40 ml/kg) was effected through a cannula introduced into the femoral artery. Blood substitutes in the volume of blood loss were infused into the femoral vein—one third of the volume by injection and the rest by drip. Plasma was prepared from donor dogs and stored in plastic bags in a frozen state. The content of protein in plasma was 5 to 6%.

The arterial and central venous pressure, ECG in the second standard lead and the volume of circulating blood and its components according to the dilution of Evans' blue and the hematocrit index were studied in experiments. The volume of extracellular fluid was determined by means of sodium thiocyanate solution (thiocyanate space) according to Grandall and Anderson's method in Molehaar and Roller's modification. The volume of intravascular, extracellular and extravascular fluid was calculated from the volume of extracellular fluid and the volume of circulating plasma. The urine was collected by a catheter 2 hours before the beginning of blood loss and 4 hours after blood loss and transfusion. The concentration of sodium in the urine and plasma was determined by the method of flame photometry every 2 hours. Hemodynamic indexes were recorded before the experiment, at the end of blood loss (except for the volume of circulating blood) and 10 to 15 minutes and 2, 4 and 24 hours after transfusion. The statistical processing of the results was carried out by the difference method.
The infusion of polyglucin and plasma produced a stable restoration of arterial pressure to the initial values in experimental animals (table 1). After the infusion of gelatinol arterial pressure reached the initial value, but after 1 hour it was lowered and after 2 hours dropped even more. Changes in the central venous pressure and ECG were significant and the same in all the experiments, which indicated a moderate degree of blood loss. Venous pressure was slightly lowered during blood loss, was rapidly restored during the infusion of gelatinol and did not change subsequently. A reduction in the voltage of the R wave, a negligible increase in the P-Q interval and moderate bradycardia with a preservation of a fixed sinus rhythm were observed on the ECG at the end of blood loss. These changes were easily reversible (see figure, A, B).

Table 1. Results of Determination of Arterial Pressure in Dogs After Replacement of Blood Loss With Polyglucin, Gelatinol and Plasma (M±m)

<table>
<thead>
<tr>
<th>Препарат</th>
<th>до кровопотери</th>
<th>в конце кровопотери</th>
<th>после замещения кровопотери</th>
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<td></td>
<td>(3)</td>
<td>(4)</td>
<td>(6) через 15 мин</td>
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<tr>
<td>Полиглюкн (9)</td>
<td>153</td>
<td>19±2*</td>
<td>155±3</td>
</tr>
<tr>
<td>Желатиноль (10)</td>
<td>168</td>
<td>36±3*</td>
<td>170±4</td>
</tr>
<tr>
<td>Плазма (11)</td>
<td>156</td>
<td>36±1</td>
<td>155±6</td>
</tr>
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</table>

*P>0.05.

Remark. Here and in tables 2 and 3 the number of observations is in parentheses.

Key:
1. Preparation
2. Arterial pressure, millimeters of mercury column
3. Before blood loss
4. At the end of blood loss
5. After replacement of blood loss
6. After 15 minutes
7. After 1 hour
8. After 2 hours
9. Polyglucin
10. Gelatinol

The infusion of polyglucin and plasma stably normalized the volume of circulating blood, whereas after the infusion of gelatinol a significant normalization of the volume of circulating blood was observed only during the first minutes after infusion and after 24 hours (table 2).
Changes in arterial and venous pressure and ECG after replacement of blood loss with polyglucin (A) and gelatinol (B).

Curves from top to bottom: arterial pressure (curve with a small amplitude of oscillations—averaged), zero line to it; venous pressure, zero line to it; ECG. 1—initial data, 2—end of blood loss, 3—15 minutes, 4—1 hour, 5—2 hours after the administration of blood substitutes.

Key:
1. Millimeters of mercury column
Table 2. Results of Determination of the Volume of Circulating Blood (in ml/kg) After Replacement of Blood Loss With Polyglucin, Gelatinol and Plasma (Mm)

<table>
<thead>
<tr>
<th>(1) Препарат</th>
<th>(2) ОЦК</th>
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<th>(3) ОЦП</th>
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<th>(4) ОЦЭ</th>
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<td>(10)</td>
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<td></td>
<td>10 мин</td>
<td>2 ч</td>
<td>4 ч</td>
<td>24 ч</td>
<td>10 мин</td>
<td>2 ч</td>
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<tr>
<td>Полиглюкин (11)</td>
<td>88</td>
<td>84±3,8</td>
<td>83±2,4</td>
<td>80±3,7</td>
<td>83±2,1</td>
<td>63±3,3*</td>
</tr>
<tr>
<td>Желатинол (12)</td>
<td>96</td>
<td>90±3,5</td>
<td>82±2,4*</td>
<td>89±2,1*</td>
<td>91±4,3</td>
<td>73±2,4*</td>
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<tr>
<td>Плаазма (13)</td>
<td>85</td>
<td>80±3,2</td>
<td>80±2,3</td>
<td>80±3,1</td>
<td>79±1,8</td>
<td>57±2,0*</td>
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</table>

*P<0.05.

Key:
1. Preparation
2. Volume of circulating blood
3. Volume of circulating plasma
4. Volume of circulating erythrocytes
5. Before blood loss
6. After infusion
7. 10 minutes
8. 2 hours
9. 4 hours
10. 24 hours
11. Polyglucin
12. Gelatinol
13. Plasma
When evaluating the therapeutic effect of blood substitutes, it is necessary to take into consideration not only the replacement of the volume of circulating blood, but also the equilibrium between the intravascular and intercellular space.

Two hours after the replacement of blood loss with polyglucin the volume of circulating plasma was higher than the initial, while the volume of intravascular, extracellular and extravascular fluid was slightly reduced, but not as a result of its transfer to vessels, because the increase in the volume of plasma corresponded to the volume of administered polyglucin (table 3). After 4 and 24 hours the volume of extracellular fluid was restored to the initial and the volume of plasma remained increased. A similar picture was observed after the replacement of blood loss with plasma. The lack of significant changes in the volume of intravascular, extracellular and extravascular fluid after the replacement of blood loss with polyglucin seems unexpected, because the colloid-osmotic pressure of polyglucin is twice as great as that of plasma and it was natural to assume that polyglucin would cause a significant flow of fluid from tissues to vessels. Apparently, this did not happen, because the low molecular fractions of polyglucin, on which the excess colloid-osmotic pressure mainly depends, are eliminated from the body very rapidly and, as established previously, 2 hours after the replacement of blood loss of 40 ml/kg the colloid-osmotic pressure of plasma is leveled out (V. B. Koziner). The volume of intravascular, extracellular and extravascular fluid is also normalized during this period.

In experiments with gelatinol the volume of circulating plasma in animals increased immediately after infusion. After 2 hours it decreased and nearly all the administered fluid left the vascular bed. The volume of extracellular fluid and the volume of intravascular, extracellular and extravascular fluid began to decrease by that time. These volumes were reduced even more after 4 hours. After 1 day the volume of extracellular fluid reached the initial level.

The replacement of blood loss with blood substitutes led to an increase in diuresis. During the first 2 hours after the administration of polyglucin diuresis in dogs doubled and after the infusion of plasma and gelatinol it increased sevenfold. During the first 2 hours in experiments with polyglucin diuresis returned to the initial, in experiments with plasma it was four times as great as the initial and in experiments with gelatinol, 1.5 times. According to the data by Yu. N. Shamlin et al., gelatinol is hyperosmolar, that is, it contains many low molecular components, which, apparently, determines its distinct diuretic action. The osmolarity of polyglucin approximately corresponds to the limit of a normal serum and its diuretic action is lower. It does not seem possible to explain the changes in the volume of extracellular fluid and in the volume of intravascular, extracellular and extravascular fluid by an increased elimination of sodium with the urine and reduction in the osmolarity of extracellular fluid, because during all the periods of observation after the transfusion of all the studied preparations the concentration of sodium in the blood of dogs did not change.
Nor is it possible to explain the reduction in the volume of extracellular fluid by an increase in diuresis. Four hours after the administration of polyglucin, gelatinol and plasma 4.4, 13.6 and 8.8 ml/kg of urine were eliminated respectively, that is, much less than the administered fluid (40 ml/kg), and the volume of extracellular fluid changed. For example, in experiments with gelatinol after 4 hours the volume of extracellular fluid was lowered to 57 ml/kg and only 13.6 ml/kg of urine were eliminated. Although gelatinol has an oncotic pressure depending on protein colloids and close to plasma, it greatly differs from plasma in the protein composition. With a relatively moderate blood loss gelatinol contributes to the restoration of the volume of circulating blood and arterial pressure to a lesser extent than plasma and polyglucin. Furthermore, in contrast to gelatinol polyglucin hardly disturbs the physiological relationships with the water sectors of the body.
Table 3. Results of Determination of the Volume of Extracellular Fluid in Dogs After Replacement of Blood Loss With Polyglucin, Gelatinol and Plasma (M±m)

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<td>43,1</td>
<td>63,1±3,8</td>
<td>61,4±2,7</td>
<td>53,7±2,2</td>
<td>56,5±1,9</td>
<td>242</td>
<td>234±9</td>
<td>240±11</td>
<td>256±6</td>
<td>198</td>
<td>169±10</td>
<td>185±13</td>
<td>198±8</td>
<td></td>
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<tr>
<td>Желатино (14)</td>
<td>53,6</td>
<td>72,6±2,4</td>
<td>55,1±2,4</td>
<td>55,4±2,0</td>
<td>58,3±3,0</td>
<td>243</td>
<td>233±4</td>
<td>176±10</td>
<td>251±4</td>
<td>189</td>
<td>177±2</td>
<td>120±9</td>
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<td>Плааза (15)</td>
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<td>57,2±2,0</td>
<td>54,4±1,6</td>
<td>54,4±1,7</td>
<td>53,3±4,5</td>
<td>251</td>
<td>223±6</td>
<td>239±13</td>
<td>254±10</td>
<td>205</td>
<td>171±6</td>
<td>185±13</td>
<td>206±12</td>
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</table>

*P<0.05

Key:
1. Preparation
2. Volume of circulating plasma
3. Volume of extracellular fluid
4. Volume of intravascular, extracellular and extravascular fluid
5. Before blood loss
6. After blood loss
7. 10 minutes
8. 2 hours
9. 4 hours
10. 24 hours
11. After replacement of blood loss
12. After replacement
13. Polyglucin
14. Gelatinol
15. Plasma


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FUNCTIONAL ACTIVITY OF RETICULO-ENDOTHELIAL SYSTEM IN SEVERE BURNS TREATED WITH ANTIBURN \( \gamma \)-GLOBULIN

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Text: We investigated the therapeutic effectiveness of directed-action monospecific \( \gamma \)-globulin and its effect on the phagocytic activity of the reticuloendothelial system after severe thermal burns.

Experiments were conducted on 40 mongrel rats weighing 180 to 200 g. The animals were divided into three groups: first group—burns without treatment (control); second group—animals with burns similar in degree and length treated with normal \( \gamma \)-globulin; third group—animals treated with immune \( \gamma \)-globulin.

Burns were inflicted on a body surface free of hair with an alcohol flame during exposition for 45 to 50 seconds (fourth-degree burns). Normal \( \gamma \)-globulin (2 ml) was administered intraperitoneally to rats of the second group 1 hour after the burns. Immune \( \gamma \)-globulin isolated by the method of Baumstark et al. from antiserums of rabbits subjected to lengthy hyperimmunization with the toxin of the burned skin of rats (B. Ye. Movshev) was administered to rats of the third group. Investigations were conducted on the first, second and fifth day after the infliction of burns.

The functional state of the reticuloendothelial system was judged from its phagocytic activity, which was determined according to the clearance of the blood of the intravenously administered solution of radioactive colloid gold Au\(^{198}\) selectively absorbed by Kupffer or stellate liver cells (Berndt et al.). Radioactive Au\(^{198}\) was administered into V. jugularis in a dose of 2.5 \( \mu \)Ci per rat. After 1, 5, 10 and 15 minutes blood was taken from these animals (1 ml) for the calculation of the radioactivity of a sample. In especially conducted experiments it was established that in intact rats a complete purification of blood from Au\(^{198}\) occurs 12 to 15 minutes after the administration of the preparation. The radioactivity of a blood sample taken
at this time comprised 1% of the administered amount. Radioactivity of a blood sample taken 1 to 1½ minutes after the administration of Au\(^{198}\) into the blood was taken as 100%. The USD data unit with well crystal was used for the calculation of radioactivity. The intensity of the phagocytic function of the reticuloendothelial system was evaluated by means of the phagocytic index \(K\) according to the following formula:

\[
K = \frac{\lg C_1 - \lg C_2}{t_2 - t_1}.
\]

where \(C_1\) and \(C_2\) are the colloid concentration in the blood present in time \(t_1\) and \(t_2\). The phagocytic index shows the numerical value of the phagocytic activity of all the cells of the reticuloendothelial system of any live organism with regard to a certain test substance. Thus, we judged the functional activity of reticuloendothelial liver cells from the percent of retention of radioactive Au\(^{198}\). This index reflects the absorption function of reticuloendothelial liver cells, as well as the activity of phagocytosis calculated according to the above-cited formula.

The results of experiments showed that under the effect of a burn trauma (first group, fourth-degree burn) there was a significant depression in the phagocytic activity of reticuloendothelial liver cells (see figure). The rate of elimination of the radioactive Au\(^{198}\) introduced into the blood flow was lowered and its retention in the blood after 15 minutes comprised 61% on the first day with a norm of 1%. On the second day the depression of the phagocytic activity of the reticuloendothelial system increased, the rate of elimination of Au\(^{198}\) from the blood flow decreased progressively and its retention in the blood comprised 71%. On the fifth day the phagocytic activity of the reticuloendothelial system increased negligibly, the rate of absorption of Au\(^{198}\) by liver macrophages increased and the percent of its retention in the blood comprised 54%.

The index of phagocytosis characterizing the numerical value of intensity of Kupffer cells was lowered to catastrophically low figures—0.02 with a norm of 0.12. Blockade of the reticuloendothelial system occurred on the first day after the burns. This term assumes that the phagocytic cell is either incapable of absorbing more of the administered exogenous material, or the rate at which additional particles can be absorbed is slowed down considerably. On the second and fifth days after the infliction of a burn trauma the index of phagocytosis still remained within minimal values—0.02. Depresssion of the reticuloendothelial system was regularly accompanied by a general depression of animals, development of weak granulations and wound infection.

The effect of toxemia depends on the functional state of the cells of the reticuloendothelial system. The high toxic effect of the serums of burned rats was shown in our previous investigations against the background of blockade of the reticuloendothelial system. Toxins continuing to come from
the focus of infection could no longer be metabolized by macrophagal elements. A high degree of toxicity was detected in the blood serum during these periods (first, second and fifth days) after the burns. The lethality of the rats serving as the criterion of the toxigenicity of the investigated postburn serum comprised 80, 40 and 65% respectively.

Change in the index of phagocytosis (K).

Abscissal axis—period of observation (in days); ordinate axis—index of phagocytosis K.

Our data coincide with the results of other investigators, who also found an early depression of the reticuloendothelial system after severe burns (Hanback; Lemperle; Rittenbury). The degree of depression of the phagocytic function increased with a deterioration in the state of animals after a burn trauma right up to their death. Obviously, depression of the phagocytic activity of the reticuloendothelial system precedes the intoxication of the body, leading to an accumulation of toxic products in the blood. In turn, the appearance of toxigenic properties of the serum contributes to the blockade of the reticuloendothelial system—a vicious circle is created. In cases of improvement in the course of burn disease often there is a brief stimulation of the reticuloendothelial system following its depression. All this makes it possible to believe that the determination of the phagocytic activity of the reticuloendothelial system can be used as an evaluation of the severity of a burn trauma and the effectiveness of the conducted therapy.

In rats of the second group (treatment of burn disease with normal γ-globulin) changes in the phagocytic function were less pronounced than in the animals of the control group. On the first day after the infliction of a burn trauma and the beginning of treatment the purification of blood from \( \text{Au}^{198} \) was lowered, its retention in the blood comprised 21% of the administered amount (control 61%; \( P<0.001 \)) and on the fifth day the intensity of absorption of \( \text{Au}^{198} \) was 7.7%. The index of phagocytosis decreased to more than one half as compared with the norm—0.05—and on the fifth day it comprised 0.08.
The administration of immune γ-globulin to rats of the third group contributed to their protection against postburn toxemia owing to the activity of the reticuloendothelial system. The phagocytic activity of liver macrophages was lowered insignificantly. On the first day after the administration of the preparation the rate of blood purification from Au^{198} comprised only 11% of the total amount of the administered radioactive preparation, and in control, 61%. The numerical value of the phagocytic activity of liver cells decreased (P<0.001) to less than one half—the index of phagocytosis was 0.07. On the fifth day after the trauma and treatment with immune γ-globulin all the indexes of the phagocytic activity of the reticuloendothelial system reached the initial level in contrast to those in the control group (burn without treatment). In animals with a burn trauma the indexes of phagocytic activity of the reticuloendothelial system were still at low figures during these periods.

Thus, a burn trauma causes a considerable weakening of the cellular factors of nonspecific resistance expressed in the depression of the phagocytic activity of the reticuloendothelial system. When immune γ-globulin is administered to burned rats, a more rapid restoration of the phagocytic activity of the reticuloendothelial system is observed than when normal γ-globulin is administered. We assume that a specific interaction of antibodies with the toxin leads to the neutralization of the latter, ensures deblockade of the reticuloendothelial system in burns and intensifies the phagocytic activity of liver macrophages, increasing the natural resistance of the body.

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The Introduction of chemistry into agriculture is producing a large economic effect, but uncontrolled utilization of chemical poisons results in environmental pollution and increases the amount of residues of these substances in agricultural and animal raising products. Several of the chemicals have toxic and allergenic properties [1-5].

The effect on the organism of magnesium chlorate pesticide, which is extensively used as a defoliant and desiccant on cotton and vegetable farms, has not been studied, nor has its toxic action been elucidated. We studied possible pathogenic mechanisms of the effect of magnesium chlorate pesticide. The biological effects of magnesium chlorate were studied in both acute and chronic forms in experiments on the effect of various doses (50, 100, 400, 750, 1500 and 4000 mg per kilogram of weight; 1.5, 12 and 40 percent solutions) entering the organism in food, through the skin and through the respiratory passages.

Hematological, immunological, biochemical, allergic and morphological indicators were studied on the 1st, 3d, 5th, 10th and 15th days in the acute case and on the 3d, 7th, 14th, 21st and 30th days of the periods of exposure in the chronic case, and also at various times after the conclusion of exposure (10-12 months).

The results of the study indicated that progressive hypoalbuminemia and hyperglobulinemia began to develop beginning on the third day after administration of magnesium chlorate to experimental animals (Fig. 1).

The decrease in albumins apparently resulted from the disruption of synthetic processes in the liver. Experiments using radioactive methionine-S³⁵ revealed a sharp decrease in the rate of its incorporation into blood albumen and liver tissue proteins. The latter result apparently stemmed from activation of specific defenses of the organism against the effects of the toxic substance.
The radioactivity level of protein fractions depends upon the dose of chlorate. Larger doses of the poison (1,500 mg/kg) depressed synthetic processes starting on the first day, while smaller doses initially stimulated them, but began to depress them toward the end of the period of administration. The rate of incorporation of tagged atoms into liver protein also increased at the beginning of administration of the compound but toward the end of the period had either reached the control level or fallen below it, depending on the dose. The disruption of synthetic processes in the liver of experimental animals is also indicated by changes in transaminases, in particular alanine-glutamic transaminase (ALT) and aspartic-glutamic transaminase (AST). Changes in the enzyme activity of blood serum and liver tissue depended upon the dose and the duration of exposure to the magnesium chlorate. Toxic doses (1,500 mg/kg, 40% and 12 solutions) led to a sharp depression of the activity of transaminases, especially alanine-glutamic transferase. Smaller doses led to the development of a moderate hyperelevation of blood enzyme content in which an increase of aspartic-glutamic transaminase activity in liver tissue was predominant. A concomitant marked increase in blood residual nitrogen was observed, indicating increased protein decomposition.

A significant impoverishment of glycogen in the liver, with a simultaneous increase in blood glucose concentration, was noted even in the first days of exposure. The glycogen content of liver tissue decreased over three weeks. At the same time, pyruvic acid concentration in the blood increased. The decrease in glycogen was accompanied by an increase in total lipids in the blood and liver tissue and of nonesterified fatty acids in the blood serum. The concentration of beta-lipoproteids in blood serum and liver tissue initially increased, but fell below the control level after three weeks from the beginning of exposure.
With exposure to the pesticide in a dose of 50-100 mg/kg, cholesterol concentration increased in the blood and decreased in liver tissue, while it decreased in both blood and liver with a dose of 1,000 mg/kg. A decrease in the level of cholesterol esters and phospholipids was also noted.

Metabolic changes were accompanied by disruption of liver structure, with the formation of autoantigens, as indicated by immunological indicators. As the organism became increasingly sensitized, the titre of antitissue antibodies increases steadily and was accompanied by simultaneous increases in serum gamma-globulins and plasma cells in the bone marrow (Fig. 2).

Fig. 2. Changes in Specific Immune Reaction Indicators in White Mice. Key: 1. Wayne reaction; 2. Passive hemagglutination reaction; 3. Gamma-globulins; 4. Number of plasma cells

There was a sharp increase in mast cells in the mesentery of the small intestine. The degree of degranulation increased significantly both during exposure and afterward. The same pattern was observed for heparin in blood plasma (Fig. 3).

Fig. 3. Changes in Functional State of Mast Cells. Key: 1. Percentage of degranulation; 2. Heparin; 3. Number of mast cells in 10 fields of view
At the same time, depression of the nonspecific immunological reaction of the organism was noted, as indicated by suppression of phagocytic activity of polymorphonuclear cells and monocytes in the reticuloendothelial system, changes in acidic and basic phosphatase activity and the phospholipid and glycogen content of granulocytes. It should be stressed that immunoallergic tests were positive before the appearance of clinical symptoms of intoxication (premorbid stage), which is especially important since it makes it possible to avert the pathological process at the very beginning of its development.

In spite of the increased number of megacaryocytes in bone marrow and the activation of thrombocytopoiesis, the number of thrombocytes in peripheral blood decreased sharply and remained at a low level throughout the observation period. The serotonin content increased at the same time, while cholinesterase activity sharply decreased and the activity of hyaluronidase and the capillary filtration factor increased.

Maintenance of the liquid state of the blood and its coagulation depend on two material systems of the organism, the coagulants and the anticoagulants, and disruption of their equilibrium leads to increased or decreased blood coagulability. During chronic exposure of experimental animals a decrease in blood fibrinogen concentration was noted in the very first days. In subsequent days the fibrinogen level continued to decrease, reaching a minimum by the 21st day (26.7 percent of the initial value). The recalcification and thrombin times were increased by a factor of 1.5-2 or more. These changes increased throughout the period of exposure. The most pronounced changes were those in indicators of the anticoagulation system. The free heparin content, plasma heparin tolerance and antithrombin activity increased and did not return to normal during the period of exposure.

The disruption of the integrity of cell structure and the hemostatic properties of the blood and the liberation of biogenic amines, which resulted from increased permeability of the histohematic barriers, were manifested clinically by an increased tendency to hemorrhage.

Changes in the morphological composition of peripheral blood were accompanied by slight temporary activation of hematopoiesis in bone marrow, with subsequent development of hyporegenerative anemia resulting from depression of hematopoiesis.

Study of the endocrine reaction mechanisms of the organism, in particular suprarenal function, identified an initial activation of it. The content of 17-oxycorticosterone in the blood increased sharply in the first days of exposure, but by the 7th day a decreasing tendency was already evident, and by the 30th day the level of 17-oxycorticosterone was lower than the control figures. The content of 11-oxycorticosterone during the exposure period varied significantly, but by the 30th day was lower than the control figure. A pattern analogous to that for 17-oxycorticosterone was found for cholesterol, the source of corticosteroid hormone precursors, in the suprarenal gland. To a certain degree this is an indication of disruption of hormone synthesis. In addition there was a sharp, pronounced rise in blood eosinophils, with the increase continuing until the 21st day of exposure. Thereafter the quantity of eosinophils gradually decreased,
but the normal values were not restored even after conclusion of exposure. The physiopathological shifts in metabolic processes can be explained by histomorphological changes in the organs. On the 10th day of exposure, circulatory disorders, along with profound morphological changes in various organs, for example in the form of productive meningoencephalitis of the brain and chronic interstitial pneumonia with consolidation of the primary matter of the cartilaginous plated of the bronchi and other symptoms, were observed. The gonads, especially male, were profoundly affected, with complete disruption of spermatogenesis and destruction of the seminiferous tubules. The toxic effects of magnesium chlorate were manifested in irreversible changes in the organs of sight, as a result of which the animal became blind as early as the second ten days from the beginning of administration of large doses of the substance.

The results of the studies that were conducted 10-12 months after the beginning of administration of the substance to the animals indicated the exhaustion of the organism's adaptive and compensative capabilities, as shown by the depression of the functions that were studied. A significant lowering of the defensive and compensative capabilities of the organism creates favorable conditions for the additional occurrence of purulent infection, which was observed in most of the experimental animals.

Conclusions

1. Magnesium chlorate pesticide frequently administered to an organism, even in small quantities, results in disruption of metabolism and harm to tissue structures, with the formation of autoantigens.

2. The action of small doses of magnesium chlorate results in sensitization and that of large doses in toxic effects. Sensitization decreases the natural resistance of the macroorganism to the effects of various pathogenic microbes.

3. Immunoallergic tests may be used in diagnosis of exposure to magnesium chlorate, making possible the application of desensitizing treatment.

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eliminated from plasmid R6KA. In this case, the DNA restrictors of both plasmids have transforming activity that corresponds to the activity of linear DNA.

The presence of a unique restriction site imparts several potential properties to the plasmid genome, including the possibility of using it as a genetic vector in constructing hybrid DNA molecules in vitro [6]. Without dwelling specially on a discussion of criteria that vector plasmids must meet, let us recall that the main ones are referable to the topology of restriction sites in the plasmid genome. If the site is in a region controlling functions that are not essential to replication (for example, R determinants, tra operon, etc.), insertion of a fragment of foreign DNA should not affect the replicative properties of the plasmid. Ultimately, this will make it possible to use the R6KA plasmid as a genetic vector.

In 1975, plasmid RSF1040 [7], obtained as a spontaneous str\textsuperscript{8} segregant from R6K, was described in the laboratory of Falkow. This plasmid contains one site for restriction by EcoRI endonuclease; it has a molecular weight of \(17.3 \times 10^6\) dalton, retains transmissibility and replicates under relaxed control. It is quite probable that the deletion mutant R6KA, which we have described, is identical to RSF1040. In this regard, the nature of deletion of a specific fragment of the R6K genome in different E. coli K12 strains is of definite interest. The genetic instability of the fragment that is \(8.8 \times 10^6\) dalton in size may reflect the phylogenetic distinctions of plasmid R6K. This is confirmed by the results of studies of replication of plasmids R6K and RSF1040. While R6K has one point of initiation of replication [12], RSF1040 has two, and DNA synthesis may begin in each of them or in both at the same time [7]. This is a unique nature of replication for plasmid replicons of prokaryotes.

A comparison of the main properties of plasmids R6K and RSF1040 also shows that, in the latter event, the number of copies per chromosomal genome is doubled, whereas the indirect data of our study indicate that there was no change in number of R6KA copies, as compared to the original plasmid. Still unclear is the question of whether this difference is important and the extent to which it can be attributed to the use of different strains of E. coli K12.

Conclusions

1. A deletion mutant of the R6K plasmid has been isolated, which lost (or failed to express phenotypically) resistance to streptomycin.

2. It was shown that the deletion, constituting \(8.8 \times 10^6\) dalton, involves one of the two DNA sites that are attacked by EcoRI restriction endonuclease.

3. It was found that both plasmids, R6K and R6KA, contain only one site that is attacked by BamHI endonuclease.
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The Communist Party at all the stages of development of the Soviet State has systematically implemented socioeconomic measures aimed at protecting the health of the Soviet people and has done everything to implement the principle "everything in the name of man, everything for the good of man." During the 60 years of Soviet rule public health has made major advances. The development of the Leninist principles of organization of public health protection and their implementation have shown the vast advantages of the socialist public health system to the whole world.

Problems connected with the establishment of a powerful modern material and technical base of public health and medical science and of the medical industry, training of medical and scientific personnel, elaboration, development and constant improvement in the organization of therapeutic-preventive and sanitary-epidemiological care for the country's population, development of all trends of medical science and introduction of its achievements into the practice of public health have been solved successfully in the USSR during a short historical period. In the country a developed network of therapeutic-preventive and sanitary-epidemiological institutions functions and a system for the protection of mother and child operates. Extensive sanitary measures have been carried out, many previously widespread infectious diseases have been liquidated and occupational morbidity and traumatism have been lowered systematically.

In our country the right of citizens to health protection is now legally consolidated in the USSR Constitution. This right is safeguarded by free competent medical care provided by state public health institutions; expansion of the network of institutions for the treatment and strengthening of citizens' health; development and improvement of safety techniques and industrial sanitation; implementation of extensive preventive measures; measures for the sanitation of the environment; special care for the health of the growing generation; development of scientific research aimed at preventing and lowering morbidity and at ensuring a long and active life of citizens.
At present Soviet public health has available a powerful material and technical base. Medical care is provided in 23,900 hospitals with 3,076,000 beds and in 35,700 ambulatory polyclinic institutions, which include 4,051 first-aid and emergency medical care stations and 3,029 dispensaries. About 6 million people, including 865,000 physicians, work in the public health system, which makes up almost one-third of the physicians throughout the world. The high rates of development and improvement of public health are ensured by the ever increasing allocations from the state budget. For example, during the Eighth Five-Year Plan alone they totaled 40.5 billion rubles and during the Ninth Five-Year Plan, 52.6 billion rubles. As compared with 1940 the expenditures on public health and physical culture increased more than thirteenfold, reaching 11.8 billion rubles in 1976.

Under the party guidance our country is successfully fulfilling the great plans of the 25th CPSU Congress. The socioeconomic program for the advance of the people's well-being adopted by the 25th CPSU Congress also marks a new stage in the development of public health.

Stressing the importance of protecting the health of the Soviet people, L. I. Brezhnev, general secretary of the CPSU Central Committee, chairman of the Presidium of the USSR Supreme Soviet, said the following in the accountability report to the 25th Party Congress: "Among social problems there is no more important a problem than the concern for the health of the Soviet people."1 Measures for protecting the health of the Soviet people are connected with the development and improvement of the material and technical base of public health and medical science, further rise in the level of medical care, development of medical science, increase in the production of medical equipment and highly effective drugs and expansion of an extensive fight against dangerous diseases.

During the Tenth Five-Year Plan the number of hospital beds will reach 3,316,000 and modern polyclinic institutions for almost 400,000 visits per shift will be built. By the end of the five-year plan the number of physicians in the country will reach more than 973,000. Measures aimed at further improving the quality of medical care for the public will be implemented. Medicinal aid and the equipment of medical institutions with therapeutic and diagnostic apparatus will improve. The output of the medical industry will increase by 44 to 46 percent. The production of more than 200 highly effective, new medicinal preparations will be mastered.

The decree No 870 dated 22 September 1977 of the CPSU Central Committee and the USSR Council of Ministers "On Measures for the Further Improvement of Public Health" is a striking, new example of the party's constant concern for protecting the health of the Soviet people. The decree is the most important

program document determining the ways for the further development of Soviet public health and medical science. "Quality, efficiency, improvement"—these words determine the spirit of this decree.

The USSR Ministry of Health, councils of ministers of the Union republics, ministries and departments and local public health bodies will have to implement a set of measures ensuring the further development of the material and technical base, rise in the level of work organization, in the first place, of primary public health units, improvement in the quality of preventive and therapeutic-diagnostic work, development of specialized medical care, refinement of the system for protecting maternal and child health and improvement in the training, utilization, skills and education of medical personnel.

The decree determines a wide program for the construction of polyclinics, large multispecialization, specialized and maternity hospitals, children's polyclinics and sanatoria and other medical institutions. As of 1979 the assignments for the construction of hospitals will be envisaged in the plans for national economic development of both ministries and departments—customers—and of the ministries and departments engaged in construction and installation work. Hospitals for 364,000 beds and polyclinic institutions for almost 630,000 per shift will be built during the Eleventh Five-Year Plan. As of 1981 assignments for the development of a network of ambulatory polyclinic institutions will be set in the plans for the social and economic development of the Union republics, ministries and departments.

USSR ministries and departments and the councils of ministers of the Union republics are permitted to build new medicosanitary units and polyclinics and to expand and reconstruct the existing ones from the capital investments allocated for the construction of projects for production purposes, and in existing enterprises, from the capital of the fund for social and cultural measures, to give managers of enterprises and organizations the right to allocate part of the capital for major and current repairs and for the purchase of medical equipment and apparatus for medicosanitary units, as well as for the hospitals and polyclinics that provide medical care for the workers of these enterprises (organizations), and to pool resources for the construction of sanatoria and preventive clinics.

The decree calls for a wider utilization of the cooperation of the resources of enterprises, sovkhozes and organizations for strengthening the material and technical base of central rayon and district hospitals and ambulatory clinics. Measures for strengthening the material base of scientific research institutions have been determined.

Annual ceilings on the purchase of medical furniture replacing worn-out furniture are envisaged. Much attention is given to a fuller satisfaction of the need of medical institutions and the public for medical equipment and drugs. Assignments have been set for the development and production of new
medical equipment used primarily in cardiology, oncology, reanimation and anesthesiological and laboratory services for conducting preventive mass examinations. In 1980, as compared with 1977, the output of medical equipment will increase 1.7-fold and by 1985, 2.5-fold. By 1985, as compared with 1975, the volume of production of highly effective medicinal substances for the treatment of cardiovascular diseases will increase no less than 2.8-fold and of preparations used in oncological diseases, in diseases of the endocrine system and in pediatric practice will double. The volume of production of semisynthetic antibiotics will quintuple and of prolonged-action sulfanilamide preparations and of contrast and other diagnostic media will double. Industrial production of rifampycin and of new synthetic anti-diabetic and other medicinal substances will be organized.

The increase as of 1 January 1978 in the norms of expenditures on the purchase of medicaments and dressings in maternity hospitals, oblast, kray and republic children's and clinical hospitals (departments) and clinics of scientific research institutes and higher educational institutions, therapeutic, cardiological, pulmonological and allergological departments of city, central rayon and other rayon hospitals, cardiological departments and departments for the treatment of patients suffering from myocardial infarction of republic and kray hospitals and of cardiological dispensaries and first-aid and emergency medical care stations (departments) is of great importance for improving medicinal supplies for public health institutions. The norms of expenditures on nutrition in maternity and children's hospitals, clinics of scientific research institutes and higher educational institutions, oncological hospitals and dispensaries (departments) and a number of other institutions have also been increased.

The decree assigns an especially important place to the further improvement in the work of primary public health units—polyclinics, first-aid and emergency medical care institutions and rural district hospitals and ambulatory clinics. The subdivision of therapeutic and pediatric medical districts of territorial polyclinics has begun in 1978. By 1982 the number of adults serviced by one district internist is to be increased to 2,000, on the average, and by 1985, to 1,700. By 1980-1982 the number of those serviced by one district pediatrician will be 800, on the average. A considerable reduction in the physician's load in a rural medical district is envisaged.

The task of widely using methods of restorative treatment in ambulatory polyclinic institutions and of organizing appropriate departments at large polyclinics follows from the decree. Measures to improve the work of first-aid and emergency medical care institutions and to develop a network of first medical aid hospitals united with first medical aid stations have been mapped out. The assignment envisaging a full satisfaction of the needs of public health for sanitary motor transport will be of great significance for an improvement in the activity of first medical aid.

Specialized services will be further developed in the next few years. The cardiological service will be developed and improved. In 1979-1985 republic, kray and oblast cardiological dispensaries are to be organized, the network