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CONTENTS

Comparative Analysis of Relational and Network Approaches to Creation of Databases on Animal Systematics, Ecology and Geographic Distribution

937C0214 Moscow ZHURNAL OBSHCHEY BIOLOGII in Russian Vol 54 No 1, Jan 93 (manuscript received 30 Jul 91) pp 96-103

[Article by A. F. Alimov, A. L. Lobanov and O. N. Pugachev, Zoological Institute, Russian Academy of Sciences, St. Petersburg; UDC 591.5:681.3]

[Abstract] A generalized overview is presented on the creation of databases (defined as a specialized data file) and data banks (combination of interrelated databases and software) using relational and network approaches. The merits of both methodologies are discussed as applied to animal systematics, ecology, and geographical distribution. In the present formulation to the problem a species is regarded as an information storage unit, and the hierarchical organization of the species provides a structural framework. Current trends demonstrate that, although relational databases predominate (eg., dBASE, FoxBase, FoxPro, Clipper), pogrammers are gradually switching to network systems such as db-Vista and MDBS in view of memory management advantages and mathematical efficiency. Creation of such databases and data banks at the Zoological Institute is hampered by inadequate financial support and emphasis on acquiring hardware rather than on software development. Unless the slide backward is halted, Russian zoological research may fall irreversibly behind the advanced countries. References 6: Russian.

Fluorescence Polarization Immunoassay for 2,4-Dichlorophenoxyacetic Acid

937C0222 Moscow AGROKHIMIYA in Russian No 2, Feb 93 (manuscript received 15 Sep 92) pp 113-118

[Article by I. M. Lunskaya, S. A. Yeremin, A. M. Yegorov, V. Kolar and M. Franek, Chemical Faculty, Moscow State University; Veterinary Institute, Brno, Czechoslovakia; UDC 615.212.24:612.46.174678.048.212:543.426]

[Abstract] Utilization of monoclonal antibodies directed against 2,4-D (2,4-dichlorophenoxyacetic acid) in fluorescence polarization immunoassay (FPI) for 2,4-D provided an assay with a sensitivity of 0.1 μg/ml (5 ng/50 μL) with 10 nM of 2,4-D-fluoroscein tracer. The sensitivity of monoclonal antibody-based FPI—requiring only 1-5 min for completion—is thus on the order of sensitivity seen in radioimmunoassays and liquid chromatography. In addition, the present FPI was shown to have a specificity for 2,4-D on the order of 95.0-97.2 percent vis-a-vis common congeners, a marked improvement over specificities in the 43-92.3 percent range of FPIs relying on polyclonal antibodies against 2,4-D. Figures 4; references 10: 4 Russian, 6 Western.

Effects of He-Ne Laser Irradiation on Leukocytes

937C0211B Moscow KLINICHESKAYA LABORATORNAYA DIAGNOSTIKA in Russian No 11-12, Dec 92 (manuscript received 10 Jul 91) pp 50-52

[Article by S. G. Khomerin, Scientific Research Institute of Experimental and Clinical Therapy, Tbilisi; UDC 616.155.3-02.:615.849.19/-076]

[Abstract] Venous blood samples from 56 patients with ischemic heart disease and 33 healthy donors were irradiated in vitro with He-Ne laser (0.633 nm wavelength, 1 mW output) to assess the effects on leukocytes. The results showed that 45 min of exposure resulted in a statistically significant ($p < 0.05$) reduction in total, granulocyte and segmenter counts, and significant elevations in mononuclear and stab populations. The changes, which were more pronounced in patient blood, indicate that the therapeutic effects of laser therapy are due to direct laser action on leukocytes. References 10: Russian.

Transplantation of Embryonal Brain Tissue

937C0208B Moscow ARKHIV PATOLOGII in Russian Vol 54 No 11, Nov 92 [manuscript submitted 29 Mar 91] pp 43-46

[Article by S. V. Savelyev, Laboratory of Nervous System Development, Scientific Research Institute of Human Morphology, Russian Academy of Sciences, Moscow; UDC 616.831-053.1-089.843]

[Text] Transplantation of entire organs or parts of them is a logical method of compensating for lost or damaged functions. That tenet is reinforced by the successful operations involving the transplantation of kidneys, hearts, and other organs. Among the recipient tissues, the brain is of the greatest interest. The presence of an immunological barrier and the good adaptability shown by transplantates have prompted basic and applied research. The most widespread are transplantations of chromaffine cells from the adrenal cortex and the brain of individuals with Parkinson's disease. Those operations are being performed by 20 groups of researchers, primarily in the United States and Sweden. As of today, the total number of such operations is approaching 200. As a result, the required dose of L-(dihydroxyphenyl)alanine (L-dopa) administered to the patient is reduced, but no significant improvement in condition is observed,[29, 38] and transplantation in elderly individuals has led to memory disorders.[11] Generally speaking, this type of autotransplantation has not led to the expected results, and the post-op complications have engendered doubts about the sense of such transplantations.[32] The poor therapeutic effect associated with allogenic transplantations of chromaffine cells has prompted a search for new sources of donor cells. The use of embryonal nerve tissue is preferable, and for that reason, experiments involving the transplantation of various of its sections into nerve trunks, into ventricles of the brain, or right into the brain structures of mature animals are the most widespread.

Transplantation of neuroblasts isolated from 17-day-old embryos of rats into the earlier-damaged spinal cords of young rats demonstrated that the neuroblasts not only survived for four months, but also differentiated into catecholaminergic neurons and formed axons up to 15 mm in length, which was accompanied by restoration of movement at the level of the thoracic segments.[19] Similar transplantations of sections of embryonal spinal cord in rats have, within 12 months, led to extensive innervation of damaged host spinal cord by donor neurons. The fibers formed have replaced the damaged serotoninergic neurons of the recipient and have restored functions.[21] Similar results have been obtained in the transplantation of the olfactory bulb or the substantia nigra into the cavities that come about after removal of the olfactory bulb of the recipient.[12, 44] The transplantate established a bond with the host brain, although it retarded the regeneration of its own neurons. The transplantation of embryonal nerve tissue of the brain into a regenerating lower nerve has been accompanied by differentiation of the transplantate.[4, 15] Bonds have been formed with interneurons and projection neurons. However, after 12 months, the transplantate cells were observed to degenerate, which was accompanied by the formation of Hirano bodies [teltsa Khirano].[15] Thus, in the transplantation of embryonal nerve tissue, the donor cells survive and differentiate, and the transplantate-host bonds bear a functional character.

Transplants of cells into the cavities of the ventricles of the brain of Wistar rats have revealed not only differentiation of the transplantate, but also the growth of host vessels into the transplantate.[27] Similar experiments involving the transplantation of the suprachiasmatic nucleus of the hypothalamus of 15-day-old embryos of the IYC:LAK golden hamster into the cavity of the third ventricle of the brain have yielded similar results. If the transplantation was done after electrolytic destruction of the host nucleus, circadian activity is restored after some period of time, although the sexual behavior remains disturbed.[28]

In transplantations between embryonal and mature brains, there are several types of operations. The work most frequently encountered involved transplantation of cortical germs into homologous structures of the brain of mature animals.[2, 3, 5, 8] In all transplants of the type cortical germ/cortex, a host capillary network forms in the section of transplanted tissue, the cells differentiate, and bonds are formed with recipient structures.[34, 42] It should be noted that removal of a section of tissue from the recipient before the transplantation disrupts the bonds that have already been formed. Comparison of the results of the restoration of functions in control animals and in animals with transplantates of nerve tissue has shown that such operations improve spatial memory disrupted by injury and accelerate the restoration of acquired skills.[8, 42] When an embryonal germ of the hippocampus is introduced into the hippocampal region of the host, the neuroblasts differentiate and form remote bonds.[33, 41] A six-month observation of rats with transplanted sections of the hippocampus showed that the donor neurons restore the damaged neuronal chains and form branching dendritic systems. The use of cells of an embryonal suture in transplants to the hippocampus diminish disturbance of spatial memory in rats with serotonin-cholinergic deficiency.[35] However, transplantation of embryonal cells from a tonsil to a homologous section of the brain in rats does not eliminate memory defects within two months.[10] Transplantation of a suspension of noradrenergic neurons to the brain of rats with unilateral disturbance of the nigrostriatal tract does not restore the dopa level.[25] Results that are more promising have been obtained in the transplantation of embryonal oligodendrocytes of newborn mice. Myelinization of brain fibers has been increased with such transplantation.[16] It should be noted that transplantation of embryonal nerve tissue can slow the death of ganglion cells of the retina if, after the optical nerve is cut, donor cells are introduced into its proximal section.[37]

Favorable results in experiments on mice, hamsters, and rats have led to the need for setting up experiments that model human disease on highly organized mammals.[9, 43] Monkeys have been used in which Parkinson's disease was modelled by damaging the striatum with 1-methyl-4-phenyl-1,2,3,5-tetrahydropyridine. Substantia nigra cells were transplanted into the animals. The survival of the cells and their integration with the recipient brain resulted in reduced tremor and bradykinesia. Transplantation of medial basal nuclei into the monkeys caused innervation of the cortex of the major hemispheres of the brain and formation of synaptic contacts after destruction of the cholinergic afferents with ibotenic acid. Also noted was an improvement of conditioned reflexes.[43]

In animal modelling of Parkinson's and Alzheimer's diseases, hormone-producing neurons can be replaced successfully, as can mediators (when cholinergic hippocampal and cortical adituses are damaged). Numerous experiments have shown that transplantation of substantia nigra is considerably more effective than transplantation of the adrenal cortex.[17, 23, 36] Moreover, analysis of the sequelae of transplants in animals has made it possible to identify five basic mechanisms of action on the brain of the recipient[20]: the strong influence of isolated neurotrophic and growth-stimulating factors on an injured brain; chronic diffuse isolation of neurohormones or neurotransmitters into the recipient neuropile; reinnervation of the recipient brain with stable isolation of mediators on the physiological level; use of transplantate tissue as a matrix for germination of the neurites connecting separated sections of a damaged brain; reciprocate innervation and integration of the transplantate with the recipient brain.

Based on the enumerated facts, transplantations of tissue of the ventral midbrain of an 8- to 10-week-old human embryo have been made into the striatum of two individuals with Parkinson's disease.[30] Those operations led to a moderate improvement in motor function, although the patients were receiving cyclosporin, azathioprine, and steroids to prevent rejection of the transplantate, along with the preparations used for treating Parkinson's disease. A similar operation was performed on a patient with pronounced schizophrenia,[26] in whom embryonal nerve tissue was transplanted to the septal region, which had a favorable effect on memory.

In animal experiments and in operations performed on humans, embryonal tissue of a donor belonging to the same species as the recipient is used for transplants. Attempts at interspecies transplantations have been few.[14, 18, 22] Transplantation of brain fragments of a rabbit embryo into a mouse brain does not result in catastrophic consequences. The transplanted cells differentiate into astrocytes that migrate to the host brain structures as host cells migrate to the transplantate.[40] Subpial, pericapillary, fibrous, and ameboid astrocytes are formed. It has been established that after several weeks, the astrocytes of the recipient penetrate to the transplantate, and astrocytes of the transplantate differentiate in brain structure that are far from the transplantation site. Three months after the transplantation, however, donor astrocytes cannot be identified in the recipient brain. Transplantation of embryonal nerve tissue of 15- to 17-day-old rat embryos to the vascular bed of the anterior thalamus of mature rats with various haplotypes of histocompatibility has demonstrated survival of the transplantate for three months.[31] It should be noted that transplants between rats of different lines has been accompanied by the appearance in the transplantates of lymphocytes, mast cells, and eosinophils. For that reason, lengthy survival of the transplantate when there is an immune response in the recipient requires additional study. Considerably worse results were obtained in xenogenic transplantations between similar species. For example, transplantation of embryonal nerve tissue of a mouse to the striate body of a rat produces rapid rejection of the foreign tissue.[18] At the same time, transplantation of human embryonal nerve tissue to the anterior chamber of the eye of a rat[24] has led to vascularization of the transplantate by vessels of iris of the host and its differentiation. Those experiments, however, used rats with no thymus in whom the embryonal transplantate of a human brain was also adapted to the striatum. After 3-6 months, neurons were found in the host brain that stored human-specialized gene products.[39] The possibility of interspecies transplantations of nerve tissue have also been demonstrated for amphibians.[1]

The xenoplastic transplantations examined here cover groups of animals that are relatively close in terms of systematic classification, although the fundamental possibility of operations involving the transplantation of tissue between remote species has been demonstrated.[13, 14, 22] At first glance, the possibility of neurotransplantation between remote species does not have any appreciable advantages over intraspecies transplants. In all cases of transplantation of embryonal nerve tissue, however, there are two yet unresolved problems. First, it is impossible to absolutely precisely isolate the necessary section of the embryonal brain that is required as the donor tissue. It is possible that that difficulty would fall away if cell cultures were used, although such an approach could create additional problems.[32] Second, even if the isolation of the section of embryonal nerve tissue were precise, it is impossible to predict the fate of the transplanted cells. Guaranteeing a strictly defined means of differentiation is difficult, since the donor cells are affected by a multitude of uncontrollable factors. For that reason, achievement of the goal of the operation—compensating for the functions with the differentiating neurons of the transplantate—cannot be guaranteed until the fate of the transplantate is strictly determined. Another means of solving the problem, however, is possible. An organism has to be chosen whose neural cells are strictly determined in their development. Use of such animals would make it possible to predict the fate of the differentiation of neuroblasts and to more precisely accentuate the effect produced. Understandably,

insects that have a well- determined neurogenesis are the best for that. Drosophila is the most convenient to use, since it is the best studied, with a multitude of specific mutations. The first experiments involving the transplantation of drosophila embryonal germs to an amphibian nervous system showed that the donor cells survive, differentiate, and enter into functional bonds with the host brain.[6,7] Identification of transplantate cells with in situ hybridization on sections with DNA-mobile genetic elements of drosophila has made it possible to establish that drosophila cells penetrate structures of the amphibian brain and enter into contact with host neurons. The interaction between donor and recipient neurons has a variable character. Host brain cells penetrate the transplantate localized in the cavities of the ventricles of the brain. Mixed ganglia are produced that contain donor and recipient cells. A response migration of the insect neuroblast to the amphibian brain walls is observed. The introduction of the insect neural germs into the brain of vertebrates stimulates growth of axons and dendrites, with the neural network of the insects determining the direction of the growth. Under the influence of donor cells, the growth of experimental animals is accelerated, motor activity is reduced, and success of the search for a way out of the vertical labyrinth grows. Preliminary data have been obtained on the transplantation of neural germs of insects and amphibians into mammal brains. Donor neuroblasts transplanted into the mouse brain survive and differentiate. The transplantate vascularizes, and its cells form bonds with host neurons.

Conclusion

The results of basic and clinical research on the study of the consequences of the transplantation of nerve tissue do not provide hope for their rapid transfer into practical health care. Indicating that are the controversial nature of the effects produced in clinical conditions and the incomplete integration of the transplanted nerve tissue with the recipient tissue. Apparently, the expected restoration of functions, even in successful transplants, is very problematic.

Transplants of embryonal nerve cells between remote species could provide additional possibilities in transplantations in experiment. On the one hand, the use of amphibians and insects as donors will enable the transplantation of entire nervous systems as morphofunctional units. On the other hand, the transplantation of cells taken from embryonal insects will make it possible to predict more precisely the type of differentiation of the transplantate, and the use of neural germs of amphibians enable the lengthy, autonomous development of transplanted cells.

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Clinical Hemo- and Plasmasorption Trials With Hemosorbent Actilen

937C0221 St. Petersburg VESTNIK KHIRURGII IMENI I.I. GREKOVA in Russian No 1, Jan 93 (manuscript received 11 Apr 91) pp 379-384

[Article by K. Ya. Gurevich, A. A. Sokolov, A. K. Shvedov, Ye. L. Kalin and A. R. Osmak, Clinical Center for Extracorporeal Detoxication, St. Petersburg; UDC 615.38-036.8:66.08]

[Abstract] Clinical trials were conducted on extracorporeal hemo- and plasmasorption with Actilen [sic] hemosorbent on 43 patients representing a broad spectrum of clinical cases. A total of 50 procedures were carried out, involving perfusion of 1X to 1.5X of the total blood volume in 1-1.5 h. Blood chemistries showed an immediate effect in reduction of a number of cellular and chemical hematologic indicators, as well as in a number of metabolic products such as bilirubin, (by 59.6 percent), urea (by 21.3 percent) and creatinine (by 18.7 percent). In general, within five days most factors returned to approximate baseline values, while IgG, for example, showed a rebound phenomenon to 18.2 g/L. Assessment of the clinical outcome demonstrated that both procedures are indicated in crush syndrome, peritonitis, infectious, asthmatic, rheumatoid arthritis, and acute poisoning. Clinical benefits were not seen in schizophrenia, thermal burns, or acute encephalomyelitis. On balance, Actilen-based hemosorption was more effective than plasmasorption with efficacy attributed to detoxication, improved hemodynamics, and stimulation of the immune and other systems. References 13: Russian.

Nontraditional Psychoregulation and Supportive Devices in Sports

937C0223 Moscow TEORIYA I PRAKTIKA FIZICHESKOY KULTURY in Russian No 2, Feb 93 (manuscript received 24 Jan 92) pp 36-37

[Article by Yu. A. Khachaturyan, cand. pedagogical sci., docent, Moscow Institute of Radiotechnology, Electronics and Automation]

[Abstract] The concept of a nontraditional form of psychoregulation (autogenic training) is advanced in which performance is used to influence psychophysiological status. The concept, applicable to sports or work situations, rests on a schematic for a feedback device. The latter incorporates sensors for detecting deviations in baseline or optimum performance which trigger laser stimulation of appropriate acupuncture points, also designated as biologically active points, to alleviate mental fatigue and improve perfomance. Figures 1; references 17: 16 Russian, 1 Western.

Methylobacterium Extorquens: Novel Facultative Poly-β-Hydroxybutyrate-Producing Methylotroph

937C0205A Moscow in Russian MIKROBIOLOGIYA Vol 61 No 4, Jul-Aug 92 (manuscript received 14 Aug 91) pp 678-682

[Article by N. V. Doronina, M. Ostafin and Yu. A. Trotsenko, Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino; Institute of Catalysis and Surface Physicochemistry, Polish Academy of Sciences, Krakow; UDC 579.841.4.017.7]

[Abstract] A methylotrophic isolate—14P—from oil deposits in Sanok, Poland, with a DNA GC content 65.5 mole percent, has been identified as *Methylobacterium extorquens* on the basis of DNA homology. The isolate is a gram-negative unencapsulated rod with a polar flagellum, asporogenic, and contains a carotenoid pigment. Optimum growth requires a temperature of 28-32°C and pH 6.8-7.2. 14P is unique in that 80 percent of the cell weight is represented by poly-β-hydroxybutyrate. Chemical analysis also revealed that cis-vaccenic (cis18:1w7) is the predominant fatty acid, while the phospholipid profile is characterized by phosphatidylcholine (45 percent), phosphatidylethanolamine (27 percent) and phosphatidylserine (17 percent). In addition, the major ubiquinone in 14P has been identified as Q-10 (95 percent), with Q-8, Q-9 and Q-11 present as minor components. Finally, in the absence of isocitrate lyase, 14P evidently employs the icl⁻variant of the serine pathway. Figures 2; references 12: 6 Russian, 6 Western.

Plasmid Transfer by Conjugation in Chernozem Soil

937C0205B Moscow in Russian MIKROBIOLOGIYA Vol 61 No 4, Jul-Aug 92 (manuscript received 21 Feb 91) pp 692-696

[Article by S. A. Lukin and A. A. Prozorov, Institute of General Genetics imeni N. I. Vavilov, Russian Academy of Sciences, Moscow; UDC 631.46:577.113]

[Abstract] Unsterilized chernozem (60 percent moisture) was used a medium for assessing plasmid RP4 transfer by intraspecies (*Azospirillum brasilense* 94-3) and interspecies (*Escherichia coli* c600 to *Azospirillum brasilense* 94-3) conjugation. Assessment was based on recipient's acquisition of the Sm^r trait encoded by RP4. The intraspecies experiments yielded transformed cells within six hours of mixing Sm^r and Sm^s *Azospirillum brasilense* 94-3 cultures in the soil, with transformed cells reaching a maximum in 24 hours and decreasing thereafter for 30 days. In the case of the interspecies experiments transfer of RP4 from *E. coli* c600 to the *Azospirillum brasilense* 94-3 recipient yielded a maximum number of transformed cells in 2 hours with a frequency of 10^{-5}, falling thereafter to undetectable level in 20 days. Accordingly, these preliminary findings demonstrated that under certain conditions intra- and interspecies conjugation may occur in unsterilized soil. Figures 3; references 12: 5 Russian, 7 Western.

Preservation of Gold Solubilizing Bacteria on Solid Substrates

937C0205C Moscow in Russian MIKROBIOLOGIYA Vol 61 No 4, Jul-Aug 92 (manuscript received 31 Jan 90) pp 705-708

[Article by M. G. Sagdiyeva and S. I. Kukanova, Institute of Microbiology, Tashkent; UDC 57.082.56:579.8.017.7:546.59]

[Abstract] A study was conducted on the best means of preservation of *Bacillus megaterium, B. subtilis* and *B. cereus* isolated from various gold ores (sulfide, quartz, arsenic) and shown to be efficient in gold solubilization. Best retention of viability and gold solubilization after three years was obtained when the cells were stored on ores from which they had been originally isolated. The successful method of preservation consisted of mixing a 2 ml bacterial suspension (10E9 cells/ml) with 2 g of the sterilized ore for 30 min and storage at room temperature at pH 7.5-8.0. After three years viability was on the order of 10E5 cells/ml and solubilizing activity represented 83-91 percent of original activity. References 8: 7 Russian, 1 Western.

Organics-to-Methane Degradation Pathways of Adapted and Unadapted Sludge Derived From Methacrylate Facility Waste Waters

937C0205D Moscow in Russian MIKROBIOLOGIYA Vol 61 No 4, Jul-Aug 92 (manuscript received 15 Apr 91) pp 709-716

[Article by N. B. Shtarkina, K. S. Laurinavichyus and V. K. Akimenko, Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino; UDC 579.695]

[Abstract] A comparative assessment was made of the potential of adapted (obtained from contact reactor) and unadapted (from UASB-type reactor) sludge for degradation of organics to methane at a methacrylate facility. The results demonstrated that both types of granulated sludge were equally effective in the degradation of fatty acids and alcohols, as well as reversible conversion of acetone to isopropanol and isomeric conversions between butyrate and isobutyrate. However, degradation of methacrylic acid, acrylic acid and acetone occurred only in adapted sludge. On balance, the data demonstrated that specific components prevalent in the waste waters of a methacrylate facility are not converted to lower fatty acids by the microbial associations in unadapted sludge, presumably due to low concentration of microorganisms capable of metabolic transformations of methacrylic and acrylic acids. Figures 1; references 12: 2 Russian, 10 Western.

pDs Plasmid of *Desulfovibrio Desulfuricans*

937C0205E Moscow in Russian MIKROBIOLOGIYA Vol 61 No 4, Jul-Aug 92 (manuscript received 9 Sep 91) pp 717-718

[Article by N. B. Tarasova, M. N. Davydova and M. I. Belyayeva, Kazan Institute of Biology; UDC 579.25.5+579 844.91]

[Abstract] Conventional restriction analysis was used to identify a 17 kbp low copy-number (< 10) plasmid—pDs—in *Desulfovibrio desulfuricans* B-1388. The use of pDs for transformation of *Escherichia coli* HB101 imparted to the latter the ability to express CO-dehydrogenase activity. Figures 1; references 7: 2 Russian, 5 Western.

Preservation of Reference Cultures of Legionella by Lyophilization

937C0211D Moscow KLINICHESKAYA LABORATORNAYA DIAGNOSTIKA in Russian No 11-12, Dec 92 (manuscript received 20 Sep 91) pp 73-74

[Article by V. P. Dobrynin, A. S. Artemyev, B. A. Shabalin, A. V. Kibirev and I. V. Zhivov, Kirov; UDC 579.887.9.082.542]

[Abstract] Lyophilization was evaluated as a means of preserving reference strains of Legionella, using a protective medium consisting of 10 percent sucrose, 2 percent polyglucin, 2 percent thiourea, 1.5 percent ascorbic acid and 1 percent glycerol. After two years of in-vacuo storage at -18 to -22°C, viability of the various strains was as follows: 19.8 percent Bloomington-2, 96.0 percent Dallas-17, 52.3 percent Togus-1 and 12.9 percent Philadelphia-1. References 8: 5 Russian, 3 Western.

Accelerated Assessment of *Vibrio Cholerae* 01 Virulence

937C0218 Moscow KLINICHESKAYA LABORATORNAYA DIAGNOSTIKA in Russian No 1, Jan-Feb 93 (manuscript received 20 Aug 91) pp 65-66

[Article by V. N. Savelyev, T. A. Abolina, I. I. Onatskiy, S. N. Degtyareva and A. F. Bryukhanov, Scientific Research Antiplague Institute of the Caucasus and Transcaucasia, Stavropol; UDC 579.013.1.083.1]

[Abstract] Standard bacteriological and biochemical technique for virulence assessment of *V. cholerae* 01 were modified to allow earlier results. The studies were conducted with 144 01 and non-01 specimens collected in Stavropol during a cholera outbreak in 1990. The results showed that indications of virulence could be obtained at least a day or more earlier than by conventional methods. Suspect colonies (oxidase positive; agglutination with anti-O sera) were suspended in 2-3 ml of meat-peptone broth for phage typing and the results read after 4-6 hours of growth at 37°C. Parallel suspensions were used for determination of hemolytic activity after two hours of incubation at 37°C. This appoach made possible preliminary determinations of virulence within 18-20 hours without pure culture isolation. References 2: Russian.

Pathologic Anatomy of Acute Radiation Sickness Caused by a Relatively Uniform Combined Radiation Injury

937C0208A Moscow ARKHIV PATOLOGII in Russian Vol 54 No 11, Nov 92 [manuscript submitted 18 Jul 91] pp 10-15

[Article by A. Ye. Ivanov, Moscow; UDC 617-001.28-036.11]

[Text] Information on the pathologic anatomy of acute radiation sickness that has appeared in peacetime in humans is based on a relatively small number of observations.[2-4, 9, 12, 13, 15-19, 21, 22] For that reason, the United Nations Science Committee on the Effects of Atomic Radiation[11] feels that the materials pertaining to whole-body irradiation of humans continue to be pertinent at present because of the constant probability of accidents in the nuclear industry.

As a result of the sociopolitical transformations that are taking place in our country, various events of the past are coming out into the open. Specifically, information has been published about the effects produced by an accident that occurred on a nuclear submarine on 4 July 1961.[6] The cooling system for the nuclear power plant ruptured, and eight crew members suffered fatal radiation injuries. Six of the injured died in the Moscow Special Clinic. Thus, the possibility has appeared to report new information on the radiation injuries of a whole group of people in the same accident situation. Such materials will add to what we know about the pathologic anatomy and the pathogenesis and thanatogenesis of acute radiation sickness in humans and, above all, of the sickness that resulted in Chernobyl accident of 1986. In both cases, there was relatively short-term exposure to β- and γ-radiation, application of β-active radionuclides to the skin, and inhalation of radioactive gases and aerosols; according to the data of isotope analysis, in the zone of the Chernobyl Nuclear Power Station, for example, isotopes of radioactive iodine could have constituted 30 percent of all the activity associated with radioactive fallout.

Based on clinical data, all the seamen who died had been diagnosed when they were still alive as having a toxemic form of acute radiation sickness. The illness proceeded quickly, without any pronounced changeover in the periods of its development, but with clear-cut phenomena of bone-marrow and intestinal syndromes and hemorrhagic diathesis and with severe focal lesions of the skin resulting from β-radiation and contact with β-particles. Moreover, in the very first days of the illness, signs of hypofunction of the thyroid gland were noted.

Fatal outcomes came within two weeks after exposure—between days 7 and 13 (Table 1).

Table 1. Total and Separate Doses of Absorbed Energy

Patient	Age, in years	Total dose, in Gy	Tissue dose from incorporated nuclides, in Gy	Contribution of tissue dose from incorporated nuclides, in percent	Length of survival after exposure, in days
K-v	24	58	8.26	14.6	7
K.	26	16	1.62	10.1	7
O.	23	17	1.88	11.1	8
S.	22	8.7	0.32	3.7	9
Kh.	20	3.6	0.66	18.3	11
P.	21	7.5	1.24	16.5	13

There is obviously at present no need to describe in detail the morphological changes that take place, since they have already been illuminated in detail in the work of many domestic and foreign researchers.[7, 8, 12-14, 17, 19, 20] We will note only certain distinguishing features of the effects produced by the exposure in this specific case. They include primarily the injury to the skin on various parts of the body that were not covered with clothing during the accident. In the places where there was direct contact with radionuclides, noteworthy were a cyanotic coloring and acute edema of the skin, expansive bubbles of exfoliated epidermis and necrotic ulcers that involved primarily the epidermis and the upper layers of the dermis. Under the microscope, visible in the area of the ulcers were necrotic masses with broad clusters of microorganisms and fungi and homogenized tufts of swollen collagen fibers, and there was no cellular inflammatory response. In the deep layers of the dermis, destructive changes were considerably less pronounced. Here, in the forefront were phenomena of acute venous congestion and edema. Beyond the necrotized tissue, the epidermis was extremely thin, and there was no clear-cut differentiation of epitheliocytes. In places, between the basal layer and the adjacent connective tissue were slits filled with edematous fluid. Noted in the dermis were metachromasia of swollen collagen fibers, a small quantity of cell elements, necrobiotically dystrophic changes in the cells of the skin processes, and moderate edema phenomena (Fig. 1). Thus, a distinctive feature of this skin injury was the predominance of destructive changes in the epidermis and the upper layers of the dermis on the parts of the body that were directly exposed to radiation. That corresponded to a deep distribution of doses of absorbed energy in the skin.

We should note the obvious lack of correspondence between the levels of the biological doses of absorbed energy that

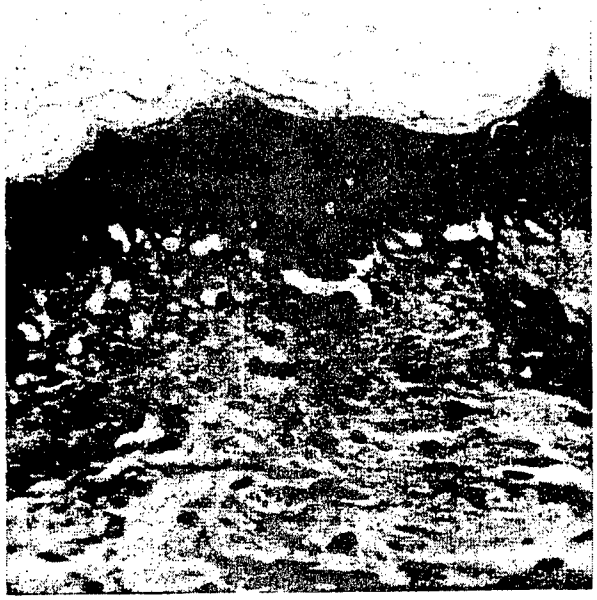

Fig. 1. Skin. Formation of Slits Between the Basal Layer of Cells of the Epidermis and the Papillary Layer of the Dermis, and Edema of the Dermis. Patient K-v. Hematoxylin and Eosin Dyes. Magnification X40.

were determined from local skin response, on the one hand, and from standard dose curves for the change in the number of neutrophil leukocytes in the peripheral blood, on the other, when the individual was exposed to relatively uniform whole-body γ-radiation.[5, 10] Judging from the severity of the skin changes, the local doses of absorbed energy among all those who died (8-16-25 Gy) were considerably larger than those that were determined the individuals were alive on the basis of hematological tests (6 Gy). In due time, in the clinical-morphological analysis of the autopsy materials, attention was brought to that circumstance, and the assumption was made that such a lack of correspondence is typical for external β-γ-radiation that is not uniform, when the application of a large quantity of radionuclides is possible on individual parts of the body. As a result of the lack of uniformity of the irradiation of the body in accidents, the total absorbed dose may not correspond to the clinical and pathologic-anatomy picture of the injury. Such a situation is confirmed by observations of a great many people irradiated during the Chernobyl accident. In that situation, there was also uneven distribution of absorbed energy in which the dose to the skin exceeded by 10- to 20-fold the radiation dose to the bone marrow because β-radiation got on the surface of the body.[5]

In almost all the seamen who died, massive hemorrhaging occurred in the soft tissue of the mouth, throat, and anterior surface of the neck (Fig. 2), and under the microscope, wholesale destructive changes to the point of necrosis were identified in the vicinity of that hemorrhaging. In four of the victims (K-v, K., O., and S.), that was combined with apoplexy of the most of the thyroid gland. It's important to note that such hemorrhaging was seen in those who died 7-8 days after being irradiated when hemorrhagic diathesis in the other organs (except the lungs) was less pronounced. For that reason, the massive hemorrhaging in the thyroid gland and the tissue surrounding it was difficult to link to merely the general breakdown of the permeability of the vascular walls that is typical of acute radiation sickness. In addition, radiometric studies showed that the tissue dose of radiation of the thyroid gland, because of the pooling of ^{131}I in that gland in all those who died, was 1189-4408 Gy, i.e., it exceeded by several tenfold the tissue doses from internal irradiation in other tissue and organs. Under the microscope, almost total necrosis of the parenchyma was found in the area of the hemorrhaging (Fig. 3); in areas with more or less preserved structural elements, there were large dystrophic changes in the follicular epithelium, atrophy of some of the follicles with dramatic diminution of colloid in them, a lack of interfollicular cells, and a severe edema of the interstitial tissue. Such changes correspond to the data of the literature, according to which the concentration of radioactive iodine in the thyroid gland in a quantity creating a local absorbed dose of more than 500 Gy kills the organ tissue.[1] That fact merits especial attention since it once again indicates the important role played by incorporated short-lived products of nuclear fission in the development of acute radiation sickness as a whole and in critical organs in the combined action of various forms and types of radiation. As we know, depending on the physicochemical state of radioactive iodine, 10-90 percent of the resorbed nuclide can be confined in the thyroid gland. Unfortunately, that circumstance is not discussed in the description of the clinical picture of acute radiation sickness among Chernobyl victims.[5] Information on the content of radioactive iodine in the thyroid gland of the victims is limited to information on the performance of 4-6 radiometric studies in each patient, beginning with the second day after the accident and on the fact that the radiation dose to the gland in most of those studied was below a damaging dose level (more than 3.67 Si).[5] However, it is not indicated how long after the injury the measurements were made or in what groups of victims the measurements were made. Meanwhile, it is fully apparent that the quantity of incorporated radioactive matter in the critical organ and the corresponding local absorbed radiation dose can be a function of a number of factors. Suffice it to mention, for example, such factors as the concentration of radionuclides in the air as a function of the distance of the victim from the source of radioactive contamination or as a function of wind direction; the length of time spend by the victim in the radioactive zone; the time elapsed from the moment of radionuclide incorporation to the radiometric study in a given subject; the use of antidotes and other therapies, etc. Without such information, it is hard to judge the dose-effect dependence, especially with regard to the damaging action of the short-lived ^{131}I (half-life eight days), which constitutes 80 +/- 20 percent of the total activity of all incorporated isotopes of radioactive iodine in Chernobyl victims.[5]

Of particular interest is the predominate localization and nature of the manifestation of local infectious complications. As we know, the most frequent manifestations of such

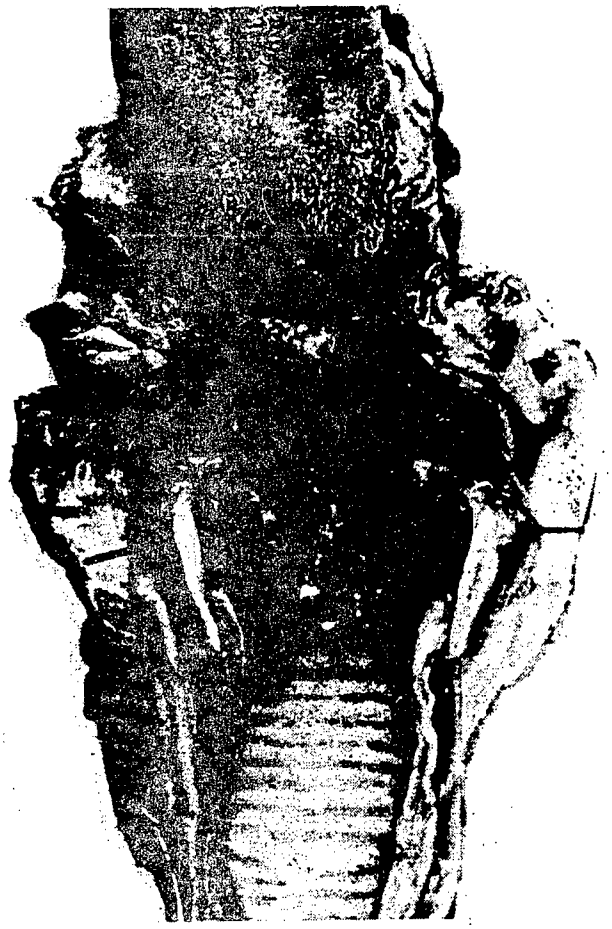

Fig. 2. Massive Hemorrhaging in the Soft Tissue of the Anterior Surface of the Neck. Patient Kh. Day 11 After Irradiation With a Dose of 7.5 Gy.

infections in acute radiation sickness are stomatitis, tonsillitis, pneumonia, and enteritis.[4, 7, 8, 12, 14, 19] Nevertheless, in our observations, what was noteworthy was the fact that the most severe inflammatory changes occurred primarily in the mouth cavity, the throat, the upper part of the esophagus, and the large bronchi of both lungs, i.e., in the places that came in direct contact with aspirated radionuclides, as well as in the skin on the parts of the body that were accessible to the direct external action of the radionuclides when the victims were in the core area. The mucosa of the soft palate, the epiglottis, the throat, and the upper part of the trachea and esophagus had broad grayish-blue superpositions loosely linked to edematous and dramatically plethoric adjacent tissue. Under the microscope, such places evidenced necrosis of the mucosa and fibrinous inflammation that was distinguished by the presence in the exudate of a

Fig. 3. Thyroid Gland of Patient O. Destruction of Tissue of the Gland in the Area of Massive Hemorrhaging. Day 8 After Irradiation and Incorporation of Radioactive Iodine. The Total Absorbed Dose in the Thyroid Gland, 145 Si. Hematoxylin and Eosin Dyes, Magnification X200.

large amount of microorganisms and fungal micellae, as well as a meager content of leukocytes and lymphocytes with polymorphic nuclei. In the parts of the mucosa that were free of the fibrinous superpositions, there was extensive desquamation of the covering epithelium, a large amount of cylindrical cells with signs of enhanced production of mucus, cystically expanded excretion ducts and mucous gland vesicles. There were clusters of microorganisms in some glands. In the forefront in the submucosal layer were phenomena of massive edema and congested plethora. Some of the victims (O., in particular) showed signs of catarrhal bronchitis, in which case there was a large amount of desquamated bronchial-epithelium cells, mucus, microbes and fungi in the lumina of the bronchi, which indicated bronchogenic development of the infectious process. Leukocytes with polymorphic nuclei, however, were virtually absent.

Macroscopically, against a backdrop of dramatic edema and plethora in the lungs, there were multiple small hemorrhage sites, the size and quantity of which grew larger the later the onset of the fatal outcome. Moreover, there were foci of hemorrhagic saturation of substantial areas of lung tissue, similar in appearance to an infarct or hemorrhagic pneumonia. Under the microscope, in the forefront were also acute congested plethora, edema of interstitial tissue, and the presence in the lumina of small bronchi, bronchioles and alveoli of edematous fluid, fibrin, erythrocytes and desquamated alveolar-epithelium cells, and microorganisms without any pronounced cellular inflammation response on the part of the surrounding tissue (Fig. 4, top and bottom). An especially large number of microbes were in areas with extensive hemorrhaging and destruction of alveolar parenchyma.

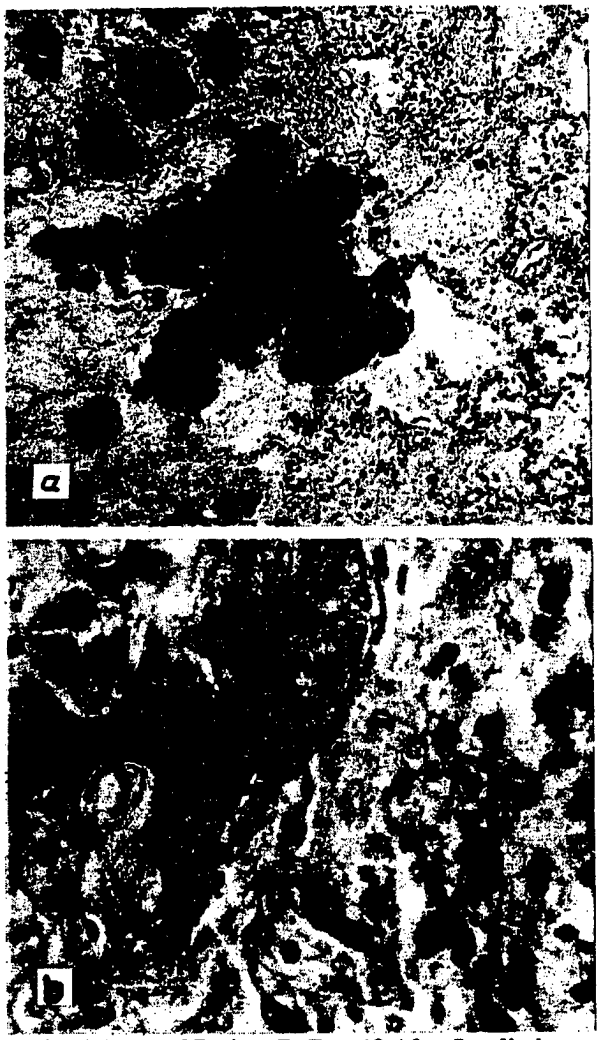

Fig. 4. Lung of Patient P. Day 13 After Irradiation With a Dose of 13 Gy. Hematoxylin and Eosin Dyes. Top, Focus of Pneumonia. Dramatic Plethora, Clustering of Edematous Fluid and Hemolyzed Erythrocytes in the Alveolar Lumina, Clustering of Microorganisms, Absence of Signs of Cellular Inflammation Response; Magnification, X200. Bottom, Inflammatory Exudate Consisting of Mass of Fibrin, Serous Fluid, and a Few Local Cells; Magnification, X400.

The mucosa of the mouth cavity, the throat, and the upper part of the esophagus were also covered with a greenish-gray film consisting of fibrin and fungal growth.

The substantial effect that radioactive fallout plays on the response properties of the body was evidenced also by the unusual tissue response in the region of ulcerative defects of the skin and adjacent soft tissue. That showed up as the absence of a pronounced separative tissue response along the edges of necrotized tissue and the progressing expansion of such areas. In that connection, it is pertinent to recall the work of V. G. Garshin (1938), which convincingly shows that the inflammatory response in preliminarily irradiated sections of the skin is always distinguished by the absence of or small amount of leukocytes with polymorphic nuclei, the intense decay of local cellular elements, the diminution of emigration and the lowering of the phagocytic function of mononuclear cells, the elevated permeability of capillaries and small blood vessels, massive edema of the tissue, the presence of a large amount of fibrin in the exudate, and retarded formation of granulation tissue. Similar deviations from the typical inflammatory response also show up in acute radiation sickness,[7, 12, 19] especially when the illness is in its full-blown phase. For that reason, pathologic-anatomy diagnosis of infectious processes and other inflammatory processes in acute radiation sickness is very complex. That is precisely why we were unable to identify pneumonia, macroscopically, in any of the six observations described; whereas, microscopically, signs of infectious inflammation were found in the lungs of all those who died. Proceeding from that is a conclusion that we regard of practical importance—that the absence of clear macroscopic signs of the inflammation process is not a sufficient basis for denying the existence of infectious complications or for confirming a positive effect in the treatment of such complications in acute radiation sickness, as some writers of articles today are doing.[5]

When speaking of the features of the manifestation in the lungs of inflammatory changes in acute radiation sickness, one can't help but touch on the question of the correctness of the present use of the term "pneumonitis."[5, 11] If such a description of the pathologic phenomena observed in x-ray and physical studies of patients treated with massive irradiation of the chest was largely justifiable 40-50 years ago, because it fitted in with the level of radiobiological knowledge at the time, today it is inadequate. Rather long ago, it was already known that such changes are more often than not a manifestation of elevated permeability of vessel walls or of so-called neutropenic pneumonia.[7] Thus, the term "pneumonitis" does not reflect the essence of the phenomena that occur usually in the lungs in severe acute radiation sickness and, consequently, does not facilitate a proper understanding of the etiology and pathogenesis of the pathologic process.

It should be noted especially that in the analysis of pathologic- anatomy changes found in the six irradiated submariners, we were essentially unable to identify a clear-cut relationship among the total absorbed-energy dose, the length of time the victims survived, and the nature of the tissue changes found in them. Conversely, manifestations of changes that were identical in nature and severity were found in individuals irradiated with different dose levels. If we were to depend on the criteria in the literature, then of the six fatal cases of radiation injury explained in this article, only one—whose dose was 58 Gy (K-v)—should have developed the toxemic form of acute radiation sickness; only two—K. and

O.—should have developed the intestinal form; and only three—Kh., 3.6 Gy; P., 7.8; S., 8.7—should have developed the bone-marrow form. As already noted, however, autopsy revealed in all the victims the same changes without any predominate injury of the bone marrow, the gastrointestinal tract, or other organs. Nor did the length of survival of some of the victims fit into the notions that had come about earlier with regard to the dose-effect dependence. Specifically, the fatal outcome for patient Kh., who had been irradiated with a total dose of 3.6 Gy, came almost as soon as did the deaths of patients P. and S., who had been irradiated with much larger doses (7.5 Gy and 8.7 Gy). It is essential that the pathologic-anatomy observations be in good agreement with clinical data. For example, patient O., who died on day 8 after irradiation with a dose of 17 Gy, did not have pronounced signs of anemia. In patient P., however, who died on day 13 after irradiation with a dose of 7.5 Gy, an almost total absence in the peripheral blood of reticulocytes and a substantial reduction in erythrocyte count were noted as early as day 2 after irradiation.

Apparently, the identicalness of the pathologic-anatomy changes in all those who died can, to some extent, be linked to severe injury to the skin cover, the thyroid gland, and early infection augmentation. Moreover, it is noteworthy that in those cases, the contribution of external γ-radiation to the total dose of radiation was one-tenth the contribution of the external remote and direct-contact effects of β-radiation (Table 2).

Table 2. Dose of Radiation Exposure on Day 1 After Irradiation

Patient	External -radiation, in Gy	External -radiation, in Gy	Activity of ^{131}I in thyroid gland, in KBq	Survival time after irradiation, in days
K-v	9.45	95	9.25×10^4	7
K.	8.45	83	0.24×10^4	7
O.	9.9	100	1.00×10^4	8
S.	9.5	90	1.19×10^4	9
Kh.	9.35	93.5	0.28×10^4	11
P.	8.9	89	0.19×10^4	13

Of great importance, undoubtedly, was the correlation of the contribution to total dose of absorbed energy of ionizing action of radionuclides applied to the skin and radionuclides aspirated, as well as the fact that the iodine isotope predominated among the radionuclides. For that reason, the total response of the body in all cases was due to the relatively equal absorbed doses of external and internal radiation, since the incorporated radioactive iodine had a damaging effect primarily on the thyroid gland. "With radioactive contamination of the environment by the fission products, radioactive isotopes can be substantial factors of radiation hazard" (Ilin L. A., Moskalev Yu. I. "Raspredeleniye, kinetika obmena i biologicheskoe deystviye radioaktivnykh izotopov yoda" ["Distribution, kinetics of metabolism, and biological action of radioactive iodine isotopes"]. Moscow, 1970, p 3).

As calculations have shown, the doses of external radiation on day 1 of the illness in all cases were rather similar, but ^{131}I content in the thyroid gland differed substantially (0.19×10^4-9.25×10^4 KBq), which later affected the severity of the illness and the nature of morphological changes at the time of death. It was in the cases in which radioactive iodine content was high in the thyroid gland that the earlier fatal outcomes came.

Conclusion

The new factual data cited in this article and the analysis of those data indicate once again the importance of objective determination of calculated and actual doses of whole-body and critical-organ irradiation in each specific case of radiation injury when irradiation is a combination of external and incorporated radiation sources. In such cases, the quantitative and qualitative dependence of the total effect on total absorbed dose can change, as can the damaging effect of any of the radiation factors. Moreover, deviations from the standard response of the irradiated body or critical organ may be linked to specific conditions of radiation injury and its treatment. Practical experience indicates that the explanation of pathogenesis and thanatogenesis on the basis of morphological changes that are of the same nature may vary with the completeness and reliability of radiometric and clinical data.

We hope that the ideas put forth in this article and based on our experience with the study of pathologic anatomy and various aspects of the pathogenesis of acute radiation injuries to animals and man will in some measure help the interpretation of the materials existing in the literature on the pathologic anatomy of acute radiation sickness in victims of nuclear-reactor accidents.

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Cytochemistry of Nonspecific Esterase in Peripheral Blood Lymphocytes of Children. Part 2. Expert Studies on Lymphocyte Populations in Blood Smears of 101 Children in Ukrainian Polissya in June 1990

937C0211A Moscow KLINICHESKAYA LABORATORNAYA DIAGNOSTIKA in Russian No 11-12, Dec 92 (manuscript received 07 May 91) pp 47-49

[Article by R. V. Lenskaya, O. A. Ikonnikova, A. M. Dzerzhinskaya, Ye. Yu. Shvedova, V. D. Drozdova and A. G. Rumyantseva, 2d Moscow Medical Institute; Kiev Institute of Hematology; UDC 616.155.32-008.931:577.152.311]-074]

[Abstract] Percentages of peripheral blood lymphocytes positive for nonspecific (α-naphthylacetate) esterase (NE) were determined in 101 children 6-14 years old in the villages of Dibrova, Shkneva, Radynka, Martynovychi and Vilcha in the Polissya Rayon of Ukraine. The cytochemical data were collected as part of a health survey in areas affected by Chernobyl fallout since NE serves as a marker of mature T cells, in particular helper cells. Data on eight cases had to be eliminated for technical reasons. Although the average values for the cohort fell within the lower range of normal, the staining patterns (1-3 distinct granules; numerous large granules; diffuse cytoplasmic staining) revealed three different subgroups. In general, relative depletion of helper T-cells was evident in two groups, suggesting—in conjunction with other hematologic findings—that these groups were at an increased risk of lymphoproliferative disorders. References 5: 1 Russian, 4 Western.

Early Diagnosis of Tick-Borne Encephalitis (TBE)

937C0211C Moscow KLINICHESKAYA LABORATORNAYA DIAGNOSTIKA in Russian No 11-12, Dec 92 (manuscript received 30 Jul 91) pp 62-64

[Article by A. Kh. Mamunts and U. T. Gileva, Perm Medical Institute; UDC 616.831-002-022:578.833.26]-07:616.155.1]

[Abstract] A formula is described for obtaining a specific binding index (SBI) for the early diagnosis of TBE (Tick-Borne Encephalitis) based on the binding of tick-borne virus hemagglutinin by human erythrocytes. Trials with 227 sera in the early stages (1-3 days) of TBE yielded an overall positive incidence of 51.5 percent. In addition, of the 44 sera that were negative on conventional serologies, 25 (56.8 percent) yielded diagnostically significant SBI values. References 7: Russian.

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