ABSTRACTS

THEODORE H. SPAET, M.D., Editor

ABSTRACTS OF SPECIAL INTEREST


A lethal dose of sodium nitrite was injected into rats. All died. The average methemoglobin content of blood collected just before death was 72 per cent. Another group of rats received a simultaneous injection of sodium glutamate. They were all free of symptoms, and their average blood methemoglobin was 43 per cent. The methemoglobin content of blood to which sodium nitrite was added in vitro was 69 per cent. Addition of sodium glutamate as well resulted in a methemoglobin content of 20 per cent. The effects observed could be due either to the inhibition of methemoglobin formation or to its subsequent conversion into hemoglobin.—J. J. B.


Intact red cells are inactive in the “stypven time” and other clotting tests, but red cell ghosts suspended in water and red cell membranes disrupted by various means have activity similar to that of platelet lipoid. Red cell ghosts lose this activity when suspended in saline. HeLa cells, human leukocytes and sperm all showed no activity when intact, but platelet-like activity when fragmented. Extracts of various guinea pig tissues also showed platelet-like activity when added to human plasma. It is suggested that dead tissue cells may play a part in the extravascular coagulation associated with inflammation. An association is postulated between loss of osmotic activity and the development of stickiness and coagulant activity of the cell membrane, and it is suggested that these properties may be related to the orientation of cephalin molecules at the surface.—R. M. H.


The enlargement of this month’s abstract section was made possible by a kind contribution from Gloger and Osserman, Estate Planners, New York, N.Y.
GOT activity was examined in 20 healthy persons and in 9 patients with hemolytic anemia, 4 patients with Addison-Biermer anemia, 7 patients with lymphogranulomatosis, 19 with leukemias, 5 with aplastic and hypoplastic anemia, 7 with various blood diseases and 6 patients with infectious hepatitis. Only in patients with hemolytic anemia and with hemolytic syndromes was a significant increase of GOT activity observed. No correlation between red cells and plasma GOT activity was found. In liver disease plasma GOT activity was increased, but red cell GOT activity was normal. Increase of red cell GOT activity is considered to be a new biochemical sign of the defective red cell in hemolytic disorders.—E. K.

CERTAIN PROPERTIES OF HUMAN “LEUKEMIC FACTOR” CULTURED ON CHICK’S EMBRYONIC MEMBRANE. V. M. Bergoltz. From the Virological Laboratory of the Guertzen State Oncological Institute, Moscow. Bulletin of Experimental Biology and Medicine, USSR. 47:71, 1959.

It had been shown before that extract from leukemic tissues cultured on chick’s membranes can reproduce the disease in about 20 per cent of mice and that it possesses antigenic properties. The culture filtrate was subjected to treatment with formalin, to heating or to freezing and drying. After several subsequent passages it was injected into mice. The formalin-treated or autoclaved “leukemic factor” did not reproduce leukemia. The “factor” subjected to heating to 60 to 80 C. produced leukemia in one mouse of six. Following freezing and lyophilization, several mice presented with the disease. In another experiment the “leukemic factor” adsorbed onto erythrocytes was recovered and produced leukemia in mice as well as anaphylactic shock in guinea pigs. The electron microscopy of the allantoid fluid containing the “leukemic factor” revealed a great number of particles, 100 to 125 μ in diameter, which were much less numerous in the controls.—J. J. B.


RNA extracted from leukemic human lymph nodes produced local mesotheliomas 3 weeks after intraperitoneal inoculation into newborn mice. The tumors produced were transplantable; and RNA extracted from these tumors induced highly invasive and extensively metastasizing malignancies in recipient mice.—G. M.

ERYTHROCYTE IMMUNOLOGY


Employing three different rabbit anti-A-like and three different chicken anti-B-like immune sera for dilution inhibition studies of human saliva, marked variations in secretor status were observed, and all appeared to be ABO-gene determined.—R. E. R.


Mild acid hydrolysis (pH 1.5 to 2.0 at 100 C. for 2 hours) reduces the usual hemagglutination inhibition activity of soluble human ABO blood group substances, increases its
ability to react with anti-SXIV, and discloses a new antigenicity. These P1 fractions are antigenic to persons of the same blood group from which the original substance was obtained, and yet the resulting precipitins react specifically with all P1 fractions of the same blood group. Thus, soluble human blood group substance contains, in addition to the familiar surface antigen structure, an internal structure to which the antibody-forming mechanism of the body is not exposed during synthesis or degradation of the blood group substance. In addition, and not stated by the authors, each ABO blood group gene is responsible for at least two rather than just one ABO blood group antigen structure. BP1 specificity is partially determined by terminal alpha-galactosyl residues probably not in 1→6 linkage. N-acetylglucosamine is related to AP1 specificity.—R. E. R.


Three different types of galactosyl residues in B substances are each associated with a different immunochemical property. α-Galactosyl residues, probably in 1→6 linkage, determine blood group B specificity. α-Galactosyl residues probably not in 1→6 linkage and exposed by mild acid hydrolysis confer BP1 specificity. β-Galactosyl residues in untreated and degraded materials, present in 1→4 and possibly 1→3 linkage, confer type XIV cross reactivity.—R. E. R.


A method is described whereby the fluorescent antibody technique may be used for the specific staining of A, B, AB and D positive red cells. The experiments were performed on cells kept in suspension. All reagents were made specific by means of absorption before use, and no fixatives were applied.—O. P. J.


In a study of the blood groups of 300 siblings of 99 Parsi families, two unrelated persons with Rh chromosomes were detected. Both these subjects were negative for D antigen.—J. B. C.


Apparently Rhpositive persons who are iso- but not auto-immunized to what seems to be the Rh blood factor have been a puzzle for a number of years. Presented is a schematic comparison of three cases, one of which was described in 1956. In addition to Rh, seven other "Rh" antigens (distinguished from "Rh-Hr" antigens) are postulated. Antigens Rh∗, Rh and Rh surveys are alleged to be defined by the antibodies of the three cases, and antigens Rh, Rh and Rh are postulated as combinations of the first three. Analogy is sought from "Rh-Hr" antigens, hr (f or ce) and rh (Ce), and new Rh-Hr antigens, rh (CE) and rh (CE), predicted. By assuming that both the red cell antigens and the isoantibody specificities of these individuals are unusual, the authors are free to state that Rh antisera agglutinate only Rh positive erythrocytes. But Rh blood typing has led to errors in regard to these patients, and a more direct explanation would admit that Rh antisera vary considerably (which they do) and that these patients are actually Rh negative (and immunized to Rh) but positive for an antigen which is either similar to usual Rh, or part of usual Rh.—R. E. R.

Using the red cells and serum antibodies of three patients found to be Rh positive but isoimmunized to the Rh factor, Unger and Wiener attribute the individual differences to subdivisions of the Rh factor, Rh^A, Rh^B and Rh^C, on the assumption that all three patients are Rh^0 positive and are isoimmunized not to Rh^0 but to Rh^A, Rh^B and Rh^C respectively. Proof is not given that the isoantibodies of these patients differ significantly from the specificity of unselected examples of Rh isoimmunity encountered in the serum of ordinary Rh negative persons, and proof is also lacking that the unusual Rh antigens of these patients are not part of the long-standing problem of weak Rh variants (D^*). Despite the large literature that has accumulated concerning the complexities of the problem of weak Rh variants (some of the reports coming from these same authors), this study treats the problem with extreme superficiality: a weak variant exists or does not exist solely upon the agglutination result obtained with a single example of saline agglutinating anti-Rh^0. With this serum, and the serum of one of the patients, anti-Rh^A, the authors encountered 5 phenotypes, Rh^A, Rh^B, Rh^C, Rh^D and Rh^E, and they predict a sixth, Rh^F. Had the sixth phenotype been encountered, Unger and Wiener would have had a stronger argument for the independence of the strength of expression of the Rh^A factor from the strength of expression of Rh^B. In another experiment they show that Rh^B red cells fail to absorb all Rh antibodies from an incomplete (univalent) Rh^B antiserum, a result to be expected from much earlier work reported by others. Just as fl has been used by Wiener to indicate a weak variant Rh factor, a, b, and c superscripts are suggested to indicate the unproved independent occurrence of weak Rh^A, Rh^B and Rh^C variants. The need for an unbiased international committee on blood group notation is indeed apparent.—R. E. R.


Nine Venezuelan family were studied for evidence of linkage between the inheritance of the Di^a (Diego) antigen and the inheritance of other blood group antigens and sex. The results show that Di^a is inherited as a simple dominant character independent of the inheritance of ABO, MNs, P Rh, Kell, Duffy, Kidd and Js blood group antigens, and also independent of sex. Information now is lacking only for Lutheran blood group antigens and the salivary secretor status of ABO and Lewis. The Di^a antigen has also been shown to be independent of Le,Wr^*, Rm, B^e and By, although a possible third allele for each has not been excluded. Similarly, Vel, I and Yt^a blood group antigens do not appear to be related to Di^a.—R. E. R.


An excess of MN children in back and inter crosses is highly significant and consistent with heterozygote advantage, apparently limited to MN mothers. Selection intensities estimated from the data would produce the observed genotype frequencies at equilibrium. The data are not consistent with hypotheses of illegitimacy, false positive reactions, ambivalent alleles, nondisjunction, preferential fertilization, somatic mutation, or meiotic drive to explain the MN excess. Requirements for direct demonstration or refutation of heterozygote advantage are discussed.—R. E. R.

This report is a fine example of important blood group detective work. A serum specimen that appeared to be "anti-Tj" on the basis of tests with a panel of rare bloods was found to have a different significance when the red cells of the propositus were tested. Previously, anti-Tj had been shown to be related to the P blood group system because the red cells of all persons found to have anti-Tj (anti-P) in their serum were P (P1) negative and anti-P (anti-P1) could be demonstrated in the Tj' antisera after suitable absorptions. The red cells of the new case, however, appeared to be both P (P1) and Tj' (P) positive. Because of the very unlikely possibility that the new antibody was unrelated to the P-Tj system, several examples of both anti-P and anti-Tj sera were absorbed with the red cells of the new case: in all instances, anti-P and anti-Tj were unaffected and a new antibody could be recovered in eluates, anti-Pk. These eluates reacted with the red cells of the new case and her sister but no others.

With this report, the present status of the P system is as follows: Ordinary anti-P sera, now appear to be anti-P1 + anti-Pk (separable serologically); the old "anti-Tj" sera now appear to be anti-P + anti-P1 + anti-Pk (separable serologically); cross reaction has been encountered. The P blood types are: P1 (P1 positive, P positive, Pk negative), P2 (P1 negative, P positive, Pk negative), P (negative with anti-P1, anti-P and anti-Pk), and Pk (positive only with anti-Pk). The notations used were patterned after the A1A2BO blood group system, although k has no analogy in the ABO system. The mechanism for the inheritance of Pk must yet be elucidated.—R. E. R.


Cellulose columns (DEAE cellulose for anion exchange and CM cellulose for cation exchange) have permitted separation of A and B isohemagglutinins into three components: (1) enhanced by proteins and resistant to inhibition; (2) heavy (15.2) α-2 globulin which is a saline agglutinin not enhanced by protein and easily inhibited; and (3) γ-1 globulin of high anionic binding capacity which is a saline agglutinin not enhanced by protein, easily inhibited, and lytic.—R. E. R.


Cellulose columns (DEAE cellulose for anion exchange and CM cellulose for cation exchange) have permitted separation of saline-agglutinating Rh antibody (γ-1 globulins) from incomplete Rh antibodies (γ-2 globulins), from the bulk of A and B isohemagglutinins, and from inert globulins.—R. E. R.


Addition of one volume of 0.5 per cent bromelin in pH 5.5 buffered saline to two volumes of antiserum and one volume of 4 per cent cells in saline, followed by centrifugation either immediately or after 15 minutes incubation, has been suggested as a new and almost all-inclusive test for human erythrocyte antibodies. This test is valuable because of (1) speed, (2) unusual sensitivity for some isohemagglutinins, and (3) apparent superiority to the earlier and similar Löw technic with activated papain. This study is too narrow, however, to support the author’s enthusiasm, and this test should not be used to the exclusion of the indirect antiglobulin technic. Furthermore, incubation beyond even 10 minutes may result in a falsely negative result.—R. E. R.
THE USE OF BROMELIN IN ESTABLISHING A STANDARD CROSS-MATCH. Bernard Pirofsky, M.D.

A second report on the use of bromelin as a proteolytic enzyme technique for detection of all human isoagglutinins again is dangerously encouraging. Despite the wide variety of isoagglutinins studied, the author fails to give many quantitative data and none that are inconsistent with his proposals. The bromelin technic is apparently valuable, but certainly not the final answer to all compatibility testing. In the reviewer's experience, serum from a fatal transfusion accident due to anti-k had a titer of only 4 U. by bromelin test, but a titer over 200 by indirect antiglobulin test with incubation restricted to 5 minutes. The author perhaps is not sufficiently familiar with transfusion accidents such as this, or the possible medicolegal consequences.—R. E. R.


A test is described for demonstration of immune antibodies in ABO erythroblastosis fetalis which uses antiglobulin neutralization by gamma globulin (Dacie), but only one single dilution of gamma globulin is used. First, the complete antibodies are precipitated from the maternal serum by use of AB blood group substance. In the second step A1 or B erythrocytes (according to the blood group of the child or father) are incubated in this serum in different dilutions. These erythrocytes are tested after washing, against (a) Coombs serum + 0.9 per cent NaCl, (b) Coombs serum + diluted y globulin and (c) 0.9 per cent NaCl alone. The test is positive (for incomplete antibodies) if there is complete neutralization by gamma globulin (agglutination only in the mixture a). The test seems to be simple, and commercial reagents are used.—H. M.


An anti-O agglutinin caused a serious hemolytic reaction in the course of a blood transfusion given because of hemorrhage resulting from abortion. Further study demonstrated a correlation between abortions of the patient and the antibody titer in her serum.—S. R. H.


This is another in a series of reports by two of the authors concerning Rh positive persons iso- but not auto-immunized to Rh. The patient suffered obvious hemolytic transfusion reactions despite negative antiglobulin matching tests. The authors' suggestion for minor as well as major cross matching is puzzling, as is the summary warning that physicians should watch for these incompletely Rh positive persons. Minor compatibility tests have nothing to do with the difficulties reported, and physicians can hardly look for these rare bloods when even the authors cannot recognize them routinely without iso-immunization.—R. E. R.


Studies were performed on 36 cats. It was found that splenectomy performed 15 minutes before the injection of a shock dose of heterologous blood had no protective influence. Splenectomy performed with simultaneous intravenous administration of trypan blue 3
hours before the induction of shock, was only partially protective. Splenectomy performed 14 days before the experiment was very effective and sometimes led to a total inhibition of symptoms of heterologous hemolytic shock.—E. K.


LEUKOCYTES


The influence of ionizing radiation as studied biochemically on the whole tissues underrates variable effects on DNA synthesis. Generally, it can be stated that a depression of DNA synthesis is usually observed by means of isotopic tracer studies. However, one cannot ascertain whether or not the DNA synthesis in the individual cells is changed after irradiation. A direct assessment of the DNA content of single cells is possible with Feulgen microspectrophotometry, which permits the analysis of DNA in cells, preserving their morphology and location within the marrow. Limiting the studies to the cells capable of undergoing mitosis, the major portion of nonirradiated marrow cells contain amounts of DNA in the range of 6 and 12 $\times 10^{-9}$ mg. The main consequence of x-irradiation to exposed femurs has been to reduce the number of synthesizing cells.—O. P. J.

A Method for the Cytochemical Demonstration of Succinic Dehydrogenase in Human Leucocytes. E. J. deSouza and S. N. Kothare. From Topiwala National Medical College, Bombay, India. J.Histochem.& Cytochem. 7:77, 1959.—T. E. B.


A tryptophane-deficient diet induced leukopenia in albino rats. Following parenteral treatment with 10 mg. tryptophane/100 Gm. body weight leukocyte counts returned to the initial levels. Tryptophane derivates act similarly, but other amino acids proved to be ineffective.—S. R. H.


Using cultures of rat lymph node cells the author observed an apparent specific toxic effect of barbiturates on lymphocytes. He studied the relative toxicity of several different barbiturates, compared their activity with that of known lymphocytocidal agents (e.g., colchicine, cortisone), and he examined the ability of some proposed barbiturate antagonists to protect against the cell damage caused by barbiturates. Toxic action of the drugs studied was measured solely by the percentage of lymphocytes with pyknotic nuclei found. It is of interest that lymphopenia has not been reported in acute barbiturate poisoning in man.—T. E. B.

The Effects of Thymectomy on the Lymphocyte Count in Patients with Myasthenia Gravis. R. A. Joske. From Department of Medicine, University of Western Australia. Med.J.Australia. 2:859–861, 1958.
This paper records the absolute lymphocyte counts made before and after operation on 24 patients who underwent thymectomy. In 16 cases the postoperative counts were lower than the preoperative, in 5 they were higher, and in 3 the results were equivocal. When the lymphocyte count failed to fall after operation, some other factor was present. In patients suffering from myasthenia gravis, the fall in lymphocyte count correlated well with the clinical result, suggesting that lymphocyte levels may be of possible prognostic value in these circumstances.—G. C. de G.


Opinion is divided as to whether the lymph leaving a region can reach the blood stream without passing through a lymph node. This problem has been investigated by using an x-ray contrast substance, metallic mercury, which was injected into the animals after death. Radiographs were made of the animals and the findings controlled by dissection under the microscope. It has been shown that the lymph in 19 of 65 rats from three different strains may pass from the testis to the thoracic duct without passing through any lymph nodes. Direct drainage was also demonstrated in 2 of 4 dogs, and it is possible that the same phenomenon may take place in other mammals and in humans.—O. P. J.

RELATION OF SPLENIC AND LYMPH NODE CHANGES TO HYPERGAMMAGLOBULINEMIA IN CIRRHOSIS. S. Glagov, G. Kent and H. Popper. From Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Ill. A.M.A. Arch. Path. 67:9-18, 1959.

Elevation of serum gamma globulin accompanies hepatic disease, particularly cirrhosis, reaching the highest levels in the postnecrotic variety. The mechanism of this elevation and the site of gamma globulin formation under these circumstances are poorly understood. It has been suggested that hypergammaglobulinemia is related to increased Kupffer cell or liver cell activity. However, there is no evidence that the liver forms gamma globulin, at least under normal conditions. Recent observations based on a comparative study of serum globulin levels and cytoplasmic pyroninophilia implicate the spleen and abdominal lymph nodes in the gamma globulin elevation in ethionine-induced rat cirrhosis. The present article deals with a study of small pieces of liver, spleen, bone marrow and lymph nodes obtained from necropsies of 30 patients with diffuse septal or postnecrotic cirrhosis and 8 patients dying shortly after gunshot wounds. The distribution and morphology of the cells with cytoplasmic pyroninophilia removed by ribonuclease, and therefore presumably active in protein synthesis, were studied in the spleen and lymph nodes. The splenic red pulp and medullary cords of lymph nodes revealed relatively few, usually isolated pyroninophilic cells in normals and a marked increase of such cells, usually arranged in clusters, in cirrhosis. The destructive pattern of the pyroninophilia and its correlation with serum gamma globulin levels suggests that the spleen and lymph nodes represent sites of gamma globulin production in cirrhosis.—O. P. J.

PROGRESSIVE CELLULAR ALTERATIONS OF LYMPH NODES. R. D. Moore, G. D. Sorenson and M. D. Schoenberg. From Institute of Pathology, Western Reserve University, Cleveland, Ohio. A.M.A. Arch. Path. 67:274, 1959.

A progressive alteration in the histologic pattern of lymph nodes is frequently associated with a variety of unrelated diseases. The initial change consists of enlargement of follicles with prominent germinal centers, increase in number and immaturity of lymphocytes throughout the cortex and medulla, prominent sinusoids with numerous lining cells, and more frequent plasma cells in the medulla. The increased number and location of plasma cells, along with the transition from basophilic to acidophilic and PAS-positive cytoplasm, seem to reflect the severity of the disease and its duration. The marked plasma cell response associated with the variety of disease processes reported in this article makes an assignment of a specific stimulus uncertain although the response appears to be similar in all cases. This would indicate that the plasma cell is a multifunctioning cell which responds to a variety of stimuli.—O. P. J.

The pattern of incorporation of amino acids into the proteins of the rat connective tissue cell has been described. The present paper deals with a similar study on macrophages from peritoneal exudates in the rabbit, and HeLa cells in the tissue cultures of epithelial origin. All of the radioactive compounds, except methionine and cystine, were labeled with C14. The others were synthesized and labeled with S35. Each labeled amino acid was added to the medium to give a final concentration of 2 to 4 μc./ml. The differences in the pattern of amino acid incorporation are quantitative only in the 3 cell types covering a wide range of characteristics. It may be supposed that the pattern of amino acid incorporation common to them all is of wide distribution among animal cells.—O. P. J.


L-Antibodies were examined by an original complement consumption test in 90 patients with hematologic disorders. Positive results were obtained in 21 patients, of whom 17 were leukopenic. Marked leukopenia was found also in another 24 patients who had negative L-antibodies. Accordingly, the immunologic nature of leukopenia remains uncertain. The authors investigated factors influencing L-antibodies. After steroid therapy a drop of L-antibodies occurred only in a small number of cases. Chemotherapy for leukemia in most instances did not affect L-antibodies, although in two instances a drop was noted coincident with the preterminal phase of acute leukemia, in which a similar drop of L-antibodies was noted in two patients not receiving any chemotherapeutic treatment. In six patients L-antibodies were repeatedly found, although no blood transfusions had been given: it is thus justified to assume that these were autoantibodies. In another seven patients L-antibodies were found after a small number of transfusions, and it is thus also rather unlikely that these were iso-L-antibodies. These 13 patients had practically no febrile responses with subsequent blood transfusion. Iso-L-antibodies were probably present only in six patients of the positive group. Evidence of the rarity of iso-L-antibodies is provided by the finding that in 12 patients, who had received more than 10 transfusions, no L-antibodies were detected. Practically all patients with presumed iso-L-antibodies had repeated febrile reactions after the transfusions, whereas patients with L-autoantibodies had practically no reactions to transfusion. In several instances L-antibodies were examined repeatedly, up to 19 months. In some patients they were consistently positive; in others there were alternate positive and negative results. For technical reasons tests were performed with leukocytes from a single donor and a panel of leukocytes from several donors was not used. Thus it is possible that with the leukocytes from one donor the test was positive but with the leukocytes from another donor it was negative.—L. D.


Rabbits were subjected to injections of peptone, to sensitization by means of horse serum, to immunization by injections of intestinal bacilli, or to anaphylactic shock. In each experiment serum was tested for leukocytolytic and opsonic properties, the leukocyte count was done, and the phagocytic power of leukocytes was assessed. Similar determinations were carried out on patients suffering from typhoid fever and cancer. It was found that immediately after the injection of peptone in small doses, there occurred a leukopenia associated with an increase in the leukocytolytic and opsonic properties of serum. Eight hours later, however, there was leukocytosis together with increased phagocytosis but the leukocytolytic level dropped considerably. Similar results were obtained following immunization and sensitization in the rabbits and during the recovery period of typhoid.
fever. Following large doses of peptone as well as in anaphylactic shock and in cancer patients, the leukocytolysis and phagocytosis were low. It is concluded that the high leukocytolytic reaction is associated with leukocytosis and an increased phagocytic power and that it occurs as a result of the immunologic reactivity of the organism.—J. J. B.


RETICULOENDOTHELIAL RESPONSE TO CARBON TETRACHLORIDE. Z. T. Wirtschafter and M. G. DeMeritt. From Research Laboratory, Veterans’ Administration Hospital, Portland, Ore. A.M.A.Arch.Path. 67:146–148, 1959.

These studies were designed to show the influence of a single subcutaneous injection of carbon tetrachloride on the hematopoietic system of rats. Imprint preparations stained with Osgood’s modification of Wright’s stain were made of thymus, liver, lymph nodes, spleen and femoral marrow. Liver imprints did not deviate from the normal cytologic characteristics. The reticuloendothelial cells were not increased in the thymus, but a plasmacytosis was observed at 48 hours which reached a peak at 72 hours. A marked R. E. cell response was found in the lymph nodes, spleen and bone marrow. This profound reaction of the reticuloendothelial system indicates an important need to evaluate the impact of a particular poison or chemical not only on the kidneys and liver, but on the hematopoietic system in general and cells of reticuloendothelial origin in particular. —O. P. J.


The author has investigated the influence of carbon tetrachloride on rabbit marrow in the course of acute and chronic intoxication. In all, 50 rabbits were used: 10 for acute and 40 for chronic studies. (1) Acute poisoning with carbon tetrachloride provoked only insignificant changes. (2) Chronic poisoning with carbon tetrachloride produced marked general marrow hypoplasia.—E. K.

A STAIN FOR MAST CELLS AND ITS APPLICATION IN VARIOUS VERTEBRATES AND IN A MASTOCYTOMA. J. Padawer. From Albert Einstein College of Medicine, New York 61, N. Y. J.Histochem.& Cytochem. 7:352, 1959.

There are several disadvantages to the present methods of staining mast cells. Among these are the damage caused by aqueous media and the reversion to the orthochromatic color. The use of tetramethylene glycol ether obviates some of these difficulties, even though the rationale of the method is not understood. It seems strange that basophilic leukocytes of man and rabbit did not stain.—O. P. J.


Considering the increasing interest in diseases of the connective tissues, it is not surprising that the mast cell today is subject to greater attention than ever before. The remarkable granularity and inhomogenicity of mast cells has deterred them from being subjected to spectral analysis for quantitative appraisal of metachromasia. The scanning procedures developed by Caspersson have been useful in this respect. Spectrophotometric analysis of 1200 showed that metachromasia in many cells attained a level characteristic of extremely concentrated solution of toluidine blue. Calculated amounts of dye bound per cell are on the order of $3 \times 10^{-14}$ more or less. The synthesis of the metachromatic com-
ponent(s) of the mast cell has superficial characteristics of a massive secretory process. Cortisone and thyrotropic hormone produced marked changes in the mast cells. In addition to distinctive morphologic changes, the proliferation of a relatively nonmetachromatic component seems to take place in otherwise normal looking mast cells, followed later by granular changes and cellular polymorphin.—O. F. J.


The intergranular substance of mast cells becomes anisotropic in acid media following treatment with pyronine G, toluidine blue or neutral red. The anisotropic substance consists of a lipoprotein which is dissolved by the ground substance of connective tissue when the cell disintegrates.—S. R. H.


The distribution of the mast cells in the body and their functional responses to experimental procedures have proved to vary from species to species. Water and physiologic saline solution were injected, in various doses, into the rat and hamster, and the mast cells and eosinophils were counted in chambers. Smears were also prepared. The results indicate that water can irreversibly disrupt the mast cells. The regeneration observed 16 days after the injection of water coincides with the time of regeneration of mast cells in the rat mesentery following intraperitoneal injection of the histamine liberator 48/80. The eosinophil leukocyte seemed to be more resistant than the mast cell to water. Injection of physiologic saline solution was followed by degranulation and was interpreted as a sign of physiologic activity to bind fluid. An increased quantity of fluid in the cell environment may be considered a factor provoking degranulation.—O. P. J.


In the connective tissue throughout the mammalian organism the tissue mast cell is characteristically round or spindle-shaped and is filled with large cytoplasmic granules that stain metachromatically with toluidine blue. Occasionally, however, there occur apparently abnormal mast cells, which show singly, or in combination, conglomeration of metachromatic granules, colorless or metachromatic vacuoles, or alterations in shape. Such cells make up 1 to 2 per cent of the total mast cell population of normal animals. It was recently reported that fibroblasts of the connective tissue selectively take up and dispose of the cytoplasmic granules shed from mast cells disrupted by various treatments. The present article concerns the fate of cytoplasmic granules shed from mesentrcic mast cells broken up as a result of exposure to distilled water and of abnormal mast cells in several tissues from normal and treated Sprague-Dawley rats. Fibroblasts, macrophages and leukocytes were observed to take up and digest the shed mast cell granules. These phagocytes were quite similar in appearance to the “abnormal” or degenerated mast cells previously described as occurring in normal and treated animals.—O. P. J.

Phagocytosis of mast cell granule by the eosinophil leukocyte in the rat. R. A. Welsh and J. C. Greer. From Department of Pathology, Louisiana State University, New Orleans, La. Am.J.Path. 35:103-111, 1959.

The histamine liberator, 48/60, was injected intraperitoneally into rats and peritoneal fluid was aspirated at 5, 10 and 15 minute intervals. Wright-stained, air-dried smears showed that an average of 20 per cent of the eosinophils contained large, blue-stained
granules numbering 2 to 3 per cell, but an occasional eosinophil contained 12 or more. These granules were similar to those in mast cells and to the large numbers of extracellular mast cell granules encountered. Electron microscope studies of these granules revealed that the liberated mast granules were covered by a thin irregular coating of granular amorphous material. Eosinophils contained varying numbers of small ovoid bodies in their cytoplasm, lying in a clear space surrounded by a single membrane, identical in size and shape with mast cell granules. Mast cell granules are known to be a potent source of histamine, and a chemical substance with antihistaminic properties has been obtained from eosinophils. The fact that eosinophils engulf mast cell granules may indicate a common line of defense, in which eosinophils serve as a means of rapid isolation and neutralization of histamine-laden particles.—O. P. J.


There is evidence which suggests that the presence of mast cells may protect against the occurrence of atherosclerosis, while on the contrary their absence predisposes to it. The experiments reported here concern double-hooded Norwegian rats maintained on a high cholesterol regimen and another group which also received injections of compound 48/80 to cause the disruption and disappearance of mast cells. The eradication of mast cells from a group of rats fed on a cholesterol-enriched diet was associated neither with fatty infiltration nor with atheroma. Perhaps the heparin released from the distintegrating mast cells, through its effect on blood lipids, may have a role in the prevention of atherosclerosis. Within the limits of these experiments, the tissue mast cell is considered not to have a direct action in preventing atherosclerosis in the rat.—O. P. J.


Blood basophils have aroused increasing interest as evidence has accumulated indicating that they may contain histamine and heparin. One line of evidence that mast cells produce or at least contain a considerable amount of histamine is that after administering the histamine liberating compound 48/80 in the vicinity of mast cells, many of them show signs of disruption. Cockerels were selected for the study because they have a relatively large number of basophils and because they are susceptible to experimental atherogenesis. The intravenous injection of 48/80 into cockerels caused many of the same changes in the blood basophils as occur in the tissue mast cells of the rat after injection. As far as could be determined, the intravenous injection of 48/80 had no effect on the tissue mast cells which in the chicken occur in large numbers in the serous membranes, the dermis and subcutaneous tissues, lung, spleen and thymus. After injecting 48/80 intravenously in the chicken, it is not known what became of the 70 or 80 per cent of the preinjection number of basophils. Possibly they were filtered off in the spleen or liver, although no evidence of this has been seen.—O. P. J.

LEUKEMIA


Some hormones play an important part in spontaneous and experimental leukemia of mice. This fact is clearly outlined by a general survey of the literature and by the authors’ own experiments. Estrogens increase the production of spontaneous leukemia in AKR mice and of leukemias induced by methylcholanthrene in the C3HBl and NLC strains. Testosterone derivatives, particularly testosterone heptylate, strongly inhibit leukemogenesis.
ABSTRACTS

Pregnancy delays the appearance of leukemia in AkR females, whereas no change is produced by the continuous administration of progesterone. The role of the pituitary gland and that of cortisone are not yet clearly established. The usefulness of the different types of leukemia (spontaneous, provoked or grafted) in studies of hormonal therapy are briefly discussed.—G. M.


It has been shown that both the total and protein nitrogen values of lymph nodal and thymic cells of leukemic AK mice were greater than those of the corresponding cells of nonleukemic AK mice. In view of the importance of the nucleic acids, a study was made to determine whether these differences would be reflected by differences in their nucleic acid contents of these cells. The concentration of DNA was not altered significantly in the malignant cells of either the spontaneous or transmitted leukemic variety as compared to the nonleukemic AK mice, but there was significantly more RNA in them. In the spontaneous leukemic AK group, the RNA values were greater for the thymic cells than they were for the nodal cells, while the DNA concentration did not vary in the cells of the corresponding tissues.—O. P. J.

SERUM LACTIC DEHYDROGENASE ACTIVITY IN MICE WITH TRANSPPLANTED LEUKEMIA WITH RESPECT TO CALORIC RESTRICTION AND RESISTANCE. F. Hetmansky, V. Volek and V. Passenerova. From the University Hospital, Prague, Czechoslovakia. Neoplasma 6:125, 1959.

Caloric restriction was evaluated for one month before transplantation of leukemia in two experimental groups of strain C57 Black mice. The first group was fed ad libitum after transplantation and no difference in the course of leukemia was found, in comparison with controls. In the second group restriction of food was continued after transplantation. In this group a marked inhibition of the leukemic process was observed, which was similar to the inhibition found previously when caloric restriction had been started immediately after transplantation. Serum lactic dehydrogenase (LDH) activity progressively increased in controls and in the group with unrestricted food intake after transplantation. In mice with continued food restriction, the increase of LDH was similar; the maximum values, however, were reached somewhat later, and occasionally showed a terminal drop.—L. D.


The activity of riboflavin and its co-enzymes was determined microbiologically in human leukocytes. The lymphocytic cells were found to contain only 1/3 to 1/2 as much total riboflavin activity per cell as did the granulocytic cells, but no striking differences were found between normal and leukemic leukocytes.—T. E. B.


A study of the concentrations of 10 different free amino acids in normal and chronic myelocytic leukemic leukocytes has been made. Glutamic acid and taurine were found to be present in much higher concentration in both normal and leukemic leukocytes than in either plasma or erythrocytes. All of the amino acids studied were present in greater concentrations in leukemic cells than in normal leukocytes.—T. E. B.

ABSTRACTS


Both these reports deal with changes in certain body enzymes in various malignant and nonmalignant states. Of especial interest for the hematologist are the studies of the lactic dehydrogenase (LD) in serum and in body fluids in the leukemias and lymphomas. Fundamentally, the LD content of a fluid is a reflection of the rate of growth of the tissue responsible for the fluid, so that the LD activity of a malignant effusion tends to be high, while that of nonmalignant effusions is normal. In animal experiments with transplantable leukemia, carcinoma and sarcoma, for example, the serum LD level was abnormally elevated, and showed reduction towards normal following successful treatment, with re-elevation on relapse. Many, though not all, patients with chronic myelocytic leukemia, other lymphomas, disseminated carcinoma and sarcoma showed an elevated serum LD. The level of serum LD in such patients also fell with successful treatment, and rose with relapse, so that the changes in the serum LD might be used as an indication—sometimes the first indication—of remission or relapse. In such patients, it was concluded, the serum LD roughly parallels the activity and/or the rate of growth of the malignancy. These reports afford another small tool in the clinical and theoretical understanding of these disorders. Since the LD level reflects rate of growth or activity, it is not specific for leukemia or other disseminated cancer, but may also be elevated in such disorders as acute myocardial infarction, hepatitis, muscular disorders and hemolytic anemias. Its value as an additional parameter in certain leukemias, however, seems certain.—S. E.


Myelofibrosis with myeloid metaplasia is a condition characterized pathologically by gradual fibrosis and sclerosis of the bone marrow, with simultaneous proliferation of bone marrow elements in various organs, especially in the spleen and liver. Histochemical studies of the blood cells indicate that there is usually an increase of alkaline phosphatase of the mature neutrophils. The clinical and pathologic diagnosis of myeloid metaplasia do not always go hand in hand. However, the four spleens removed surgically were from patients who had the following criteria: (1) splenomegaly, splenic aspiration showing myeloid metaplasia, (2) anemia with nucleated red blood cells and immature white blood cells in the blood, and (3) repeated dry bone marrow taps and fibrosis of the bone marrow, as shown by surgical biopsy. Two spleens had high alkaline phosphatase levels, and two had low levels for the mature granulocytes. Spleens in the latter group showed greater cytologic immaturity and greater distortion of the splenic structure. From a morphologic-cytochemical point of view, some of them are similar to the cases of chronic granulocytic leukemia; but, as they have entirely different initial clinical causes, it is thought that a similar or almost identical pathologic picture may be present in two different but related processes, chronic granulocytic leukemia and myeloid metaplasia.—O. P. J.


Alkaline phosphatase levels of mature neutrophils were found to be invariably high in 22 patients with polycythemia vera, whereas in all of 22 patients with a variety of forms of secondary polycythemia the leukocyte alkaline phosphatase was essentially normal. Staining of mature granulocytes for alkaline phosphatase activity may thus be useful in the differential diagnosis of the various forms of polycythemia.—T. E. B.
ABSTRACTS


This paper reports a study of the type, age and sex incidence of leukemia in Adelaide public hospitals from 1933 to 1953, and of the numerical incidence of the disease from mortality rates in both South Australia and the Commonwealth of Australia from 1929 to 1956. The findings indicate that a considerable increase in mortality rates in both State and Commonwealth has occurred, and that this increase has been comparable to that reported from the United States of America. In South Australia, the incidence of deaths from leukemia per 100,000 rose from 1.03 in 1929 to 5.18 in 1956. In the Commonwealth as a whole, the rise over the same period was from 1.88 to 5.21. A statistical analysis of the Commonwealth figures suggests a steady rate of increase from 1940 to 1945 and from 1947 to 1956, with a sudden increase in frequency of deaths at 1947. The rate of increase from 1933 to 1940 was nearly 10 per cent per annum. The mortality rates for 1933, 1940, 1947 and 1954 divided into quinquennial age periods also suggest that the increase after 1940 has been mainly in the older age groups. The type, age and sex incidence studied from 295 hospital cases in Adelaide show a few unusual features. Chronic myeloid leukemia was the commonest type, but a greater increase in acute leukemia and the chronic lymphatic type had occurred over the final ten years of the survey. No obvious seasonal trend or pattern of presentation of acute leukemia is noted; but more patients were admitted to the hospital from the metropolitan area of Adelaide as opposed to rural areas, than would be accounted for by differences of population.—G. C. de G.


Information from the records of all persons in England and Wales certified as dying from leukemia at the age of 15 years or above between 1945 and 1957 has been used to calculate age-specific mortality rates for each of the following types of leukemia: chronic myeloid, chronic lymphatic, and acute leukemias of all types. These rates have been calculated for each sex and for three periods of time—1945–49, 1950–54 and 1955–57. The results show that between 1945–49 and 1955–57 the mortality of both sexes from leukemia has increased at all ages, but much more markedly over the age of 60 years than below. All types of leukemia contribute to the increase in the older age groups, but below 60 years, the increase is largely confined to acute leukemia, though there has also been a slight increase in mortality from chronic myeloid leukemia in males. The estimated mortality from chronic lymphatic leukemia at these ages has remained practically constant. Further examination of these findings suggests that the great increase in mortality over the age of 60 is due to improvements in diagnosis and death certification and does not reflect a true increase in the incidence of the disease. If this is so, then the data show no real change in the incidence of chronic lymphatic leukemia, but an increase in the incidence of acute leukemia at all ages. No firm conclusion is reached as regards the incidence of chronic myeloid leukemia. These conclusions, are considered in relation to possible etiologic factors, and it is pointed out that they are compatible with the concept that increased exposure to ionizing radiations plays a part in the increased incidence of the disease.—R. M. H.


After six years of anemia and 15 years of almost continuous exposure to benzol, a man developed acute leukemia. During the period of anemia, there was macroovalocytosis, leukopenia and neutropenia. Bone marrow showed 71.2 per cent nucleated red cells and an absolute hypoplasia of the leukopoietic tissue. Acute leukemia was aleukemic and thrombo-
cytopenic. Some parts of the bone marrow were identical with those of six years before; others contained many atypical myeloblasts; there were also zones with intermediate characteristics. The authors believe that both the anemia and leukemia were produced by benzol, the former representing a preleukemic state.—J. G.


Most patients with leukopenia in which leukocyte agglutinins have been reported have received blood transfusions. These agglutinins are considered iso-agglutinins and not auto-agglutinins. The author reviews 34 non-transfused cases from the literature and adds two cases of his own: one case of chronic lymphocytic leukemia with a low leukocyte count, and one case of chronic idiopathic leukopenia. A third patient suffered from acute leukemia and had received two transfusions, but the titer was high and the agglutinin is considered an autoantibody. In this case leukocyte agglutinin could be eluted from the patient's washed leukocytes.—C. W.


The lymph nodes of patients suffering from lymphogranulomatosis and treated with Degranol for prolonged periods were examined by histologic, histochemical and polarization optic methods. The cell population characteristic of lymphogranulomatosis had been entirely or partially destroyed by chemotherapy. A fibrous substance appeared arranged in bundles and connected with the reticular fibers. These bundles gave the same histochemical reactions as the reticular fibers. It was shown by polarized light that these bundles contain widely scattered small elementary fibers showing a positive birefringency like collagen fibers. The observed change seems to represent an intermediary phase of the transformation of reticular fibers to collagen fibers.—S. R. H.

**RADIATION EFFECTS**


Delta-aminolaevulinic acid dehydrase is an enzyme, converting delta-aminolaevulinic acid into porphobilinogen. The activity of delta-aminolaevulinic acid dehydrase (ALAD) was studied in liver, kidney, spleen and bone marrow homogenates of rats exposed to a single whole-body x-irradiation with doses ranging from 25 to 1000 r. Determinations were made at different intervals of time after exposure (from several hours to 60 days). With doses below 100 r ALAD activity increased during the first few days after exposure. With intermediate doses (400 r) ALAD activity decreased and then increased again in the regeneration phase of the radiation disease. With doses of 650 and 1000 r ALAD activity rapidly dropped in these organs and remained at this level until the animal's death. ALAD activity in kidney and liver under the same conditions of irradiation underwent no essential changes. Only a slight decrease was observed.—E. K.

ABSTRACTS

Rabbits of about 2 Kg. were irradiated for 30 minutes with 700 r. The erythrocyte membrane permeability to potassium was studied before and after irradiation. It was demonstrated that the permeability increased after irradiation.—P. d. N.


The intravenous injection into mice of 0.4 mc. of radioactive gold resulted in a severe hypogammaglobulinemia as well as aplasia of lymphoid tissue. The hypogammaglobulinemia was delayed several days after the lymphoid hypoplasia, presumably dependent on the turnover time of the gamma globulins.—G. M.


Pregnant rats were subjected to total body irradiation either on the 9th or the 15th or the 19th day of gestation. The adult rats suffered from radiation sickness, leukopenia and anemia. All the fetuses irradiated on the 9th day of intrauterine life were still-born; half of those irradiated on the 15th day were still-born, and the others died within three weeks of delivery; of those irradiated on the 19th day, 93 per cent were born alive, but half of them died within a month. Histologic examination of the hemopoietic organs of the offspring revealed a marked arrest of hematopoiesis in the marrow and liver, but the spleen was little affected. The peripheral blood showed leukopenia and anemia.—J. J. B.


Quantitative technics have shown that lymphocytes form an important group in bone marrow from normal young guinea pigs. Therefore, it was thought that a similar quantitative approach to a study of the various marrow cell groups at close intervals during recovery from whole body irradiation might reveal significant changes in the marrow lymphocyte population if these cells have an important role in hemopoiesis. Three conclusions were drawn from these experiments: (1) Up to the 18th day after irradiation, an increase in the bone marrow lymphocyte level occurred concurrently with their increase in the cortex of the thymus. (2) Although lymphocytes are very sensitive to irradiation, recovery of lymphopoiesis in the thymus and mesenteric lymph node was relatively rapid. Restoration of marrow lymphocyte levels preceded the recovery of granulocyte and erythroid cells in the marrow. (3) Blood lymphocyte levels occurring during recovery from irradiation were not necessarily good indexes of the underlying state of activity in the lymphoid tissues examined, particularly the thymus.—O. F. J.


The theoretical considerations behind the treatment of disease with massive irradiation followed by instillation of homologous marrow are detailed in this paper, as well as some instances of practical application. The tolerance of the host to homologous tissue, specifically marrow, depends among other things on irradiation sufficient to destroy all immunologic mechanisms of the host, so that the host can no longer distinguish between a cell which is “self” and “nonself.” In animals, lethal body irradiation (about 1000 r) stops mitosis
in the marrow and lymphopoietic tissue and results in death within a matter of days because of panctyopenia and lack of immune defenses to invading organisms. However, it is this lack of immune reaction which allows the host to accept a homograft of marrow which, once accepted, forms a new defense system for the host. In the human, however, marrow homograft can replace marrow, but cannot replace the lymphoid defense mechanisms, which reside in spleen and lymph tissue. Adult spleen and lymph node tissue have been found to be harmful to the host, and do not take. Fetal lymphoid cells, however, if sufficiently immature, may take, and, if properly filtered and prepared, are innocuous. Such tissues of course, are not readily available. In human leukemia, these principles have been followed and demonstrated. A patient is reported in whom avowedly inadequate radiation (300 r) was followed by bone marrow instillation; the leukemia improved, and death occurred 8 months later, not of leukemia, but of immunologic helplessness (yeast infection). Another patient with acute leukemia is mentioned, in whom 300 r of total body irradiation was followed by instillation of marrow from a sister, and who was carried over immunologic defenselessness by isolation from bacterial contacts plus the use of antibiotics. Her marrow became normal and the patient well, although the followup is only 3 months. The authors believe that a proper mechanical set-up may ultimately permit uniform massive irradiation, which to date has been impossible, with the expectation that the marrow homograft may then become permanent and the patient’s marrow may never regenerate. The question of restoring adequate immunologic defenses, however, still remains.—S. E.


This paper computes the dose of radiation to the bone marrow of the Australian population due to the diagnostic use of x-rays, and it assesses the possibility of the increased incidence of leukemia being due to the increased amount of radiation received by the population. It is estimated that some 10 per cent of the cases of leukemia are attributable to radiation from diagnostic x-rays. It is concluded that causes other than the diagnostic use of x-rays must be sought to explain the large increase in the incidence of leukemia in the adult population.—G. C. de G.


The paper computes the radiation dose to the gene material of the population from the medical use of ionizing radiations. It also shows that the diagnostic use accounts for the largest of the man-made contributions, with the therapeutic applications the second largest contribution; the former is some 6 times greater than the latter.—G. C. de G.