HEMOSTASIS

EFFECT OF GLASS CONTACT ON THE ELECTROPHORETIC MOBILITY OF HUMAN BLOOD PLATELETS.


Contact with a glass surface causes a reduction in the electrophoretic mobility of platelets which appears to be due to a decrease in their surface charge. Contact with glass mediates the effect and it appears to be related to reactions involving sulfhydryl groups. ADP also appears to be involved. During the preparation of platelet-rich plasma, ADP or some similar substance may be absorbed onto the platelet surface. When the platelets come into contact with unsiliconized glass, the ADP may be either removed or inactivated. The change in mobility on glass contact does not appear to be due to absorption by the platelets of Factors XI or XII.—P. d. N.

ULTRASTRUCTURAL ALTERATIONS OF PLATELETS IN UREMIC THROMBOPATHY.


Ten cases were studied. A deficiency of platelet thromboplastin factors with slight impairment of other platelet functions was observed. Electron microscopic examination revealed a reduction in platelet size and quantitative and qualitative alterations in thick granules.—P. d. N.

THROMBOCYTOPENIC PURPURAS AND DYSTHYROIDIS.


Two cases were reported in which thrombocytopenic purpura was associated with hypothyroidism and Basedow's disease, respectively. In the first case, total bone marrow impairment seemed to be related to the functional impairment of the thyroid. Substitution therapy corrected the abnormality. In the second case, hyperthyroidism triggered the onset of thrombocytopenia in a constitutionally predisposed patient.—P. d. N.

GIANT HEMANGIOMA WITH THROMBOCYTOPENIA; REPORT OF TWO CASES IN CHILDREN WITH CHROMOSOMAL STUDIES.


In a two year old male, the tumor was large and the child had thrombocytopenia and hemorrhages in the skin and mucous membranes. A chromosomal study with short-term culture of peripheral blood cells revealed an acrocentric chromosome similar to, but smaller, than those of...
the 21-22 group. The platelet count returned to normal only after roentgen-therapy of the tumor. In a 20 day old female, there were many small hemangiomas disseminated over the entire body and associated with transient thrombocytopenia. The platelet count returned to normal and the tumors diminished spontaneously. A chromosomal study of the peripheral blood cells was apparently normal. The pathogenesis of thrombocytopenia and its treatment were reviewed.—M. J.


The authors have obtained another sub-unit of prothrombin which is the immediate precursor of thrombin, and they propose to call it "prethrombin." Its amino-acid composition is similar to that of purified thrombin. Autoprothrombin C seems to be the sole substance concerned with uncovering the essential thrombin sites of prothrombin and this activation is inhibited by soybean trypsin inhibitor. Prethrombin also becomes activated in concentrated sodium citrate solution, but gives rise only to thrombin activity.—P. B.


Hemorrhages in an 8 year old girl began at age one with ecchymoses, epistaxes and gingival bleeding. The Factor V level was 7.5 per cent and there was prolongation of the reaction time in the thromboelastogram, of the whole blood clotting time and of the recalcification time. The TGT with the patient's plasma was also abnormal. —P. d. N.


The anticoagulant inhibited Factor IX. The 68 year old man had severe hemorrhages. After remission, following treatment with high doses of corticosteroids, he had a relapse and died.—P. d. N.


The determination of heparin by the method of Bassiouni and Warren has several disadvantages: instability of color and buffer solvent and the requirement of large volumes of sample. To produce the blue color, the pH of the buffer solvent was changed to 9.5, a wave length of 600 mu was used instead of 530 mu, and the amount of water to remove free Azure A was increased to 10 ml. With these modifications, consistent color development, stable color and higher sensitivity were obtained. Only 1 to 2 ml. of plasma or whole blood were required. Heparin concentrations of 0.0030 to 0.0125 mg./ml. (mean: 0.0066) in the blood of normal adults were obtained.—K. F.


The structure of the plasma clot was investigated under different experimental conditions and in cases of hemorrhagic thrombocytopenia, immunologic thrombocytopenic purpura, fibrinogenopenia, thrombocytopenic purpura and in platelet-poor and platelet-rich plasma. Significant alterations were observed and typical photomicrographs were reported and compared with other investigations of hemostasis.—P. d. N.


The change in the fibrinolytic activity in blood and urine of humans and rabbits was examined after injection of such pyrogens as TTC, Pyrexal and etiocholanolone. The influence of prednisolone, ACTH, t-aminocaproic acid or sulpyrine was investigated. Transient increases in spontaneous fibrinolytic activity and more sustained increases of fibrinolytic substances in blood were recognized just before and after pyrexia. Pyrexia and elevation of fibrinolytic activity were suppressed by the prior or simultaneous administration of prednisolone, but only fibrinolytic activity was suppressed by ACTH and t-ACA. Transient increases in spontaneous fibrinolytic activity in blood were recognized just before the pyrexia induced by Pyrexal injected into
patients suspected of latent pyelonephritis. The fibrinolytic activity in urine initially went down after the injection and then activity increased to the maximum value which coincided with the peak pyrexia. The fluctuation in urinary activity of patients with normal renal function by Fishberg's test was much greater than that of patients with disturbed renal tubular function, but the elevation of activity in blood with pyrexia was the same. Manifest fever and elevation of fibrinolysis were evoked by the injection of etiocholanolone, but occurred later and were more sustained than after the injection of endotoxins. These phenomena were suppressed by the simultaneous injection of hydrocortisone, but were not influenced by the injection of sulpyrine or ε-ACA. Antiplasmin potency of serum fractions albumin, α, β- and γ-globulin, were increased to different degrees when they were mixed with high concentrations of prednisolone in vitro. No effect of prednisolone on plasmin activity was observed in vitro with the concentration attained in blood after the injection of therapeutic doses. Fibrinolysis was increased slightly after injection of therapeutic doses of prednisolone. These findings led to the conclusion that prednisolone did not inhibit fibrinolysis directly, but suppressed all reactions to pyrogen injection.——K. F.


Four patients were treated with 25 units of ACTH and four with 100 mg. of cortisone daily for 8 days. An initial, transient diminution in fibrinolytic activity was observed. Thereafter, there was an increase, as evaluated by several tests. The increased fibrinolytic activity was thought to have resulted from inhibition of antiplasmin.——P. d. N.

LEUKOCYTES


An extensive histologic and cytologic investigation was carried out in 31 patients with acute leukemia who were in "complete remission," as judged by normal peripheral blood and bone marrow pictures. The investigations comprised the counting of blast cells in the circulating blood, examination of bone marrow from 6 sites, examination of the cytology of the C.S.F., an electroencephalogram, renal, hepatic and testicular biopsies, bone marrow histology and x-ray examination of the skeleton. Nests of leukemic cells were found in 12 patients. None of the tests gave consistently negative results. The authors, therefore, advocated an increased dosage of the therapeutic agents which induced the apparent remission in order to obliterate the nests of leukemic cells in an attempt to obtain a state of "true" remission.——P. B.


The study was based on examination of the following clinical material: acute lymphatic leukemia (51 cases), acute myeloid leukemia (63), lymphoid leukosarcoma (27), reticulum cell leukosarcoma (4), erythro-leukemia (7), reticulo-histio-monocytic leukemia (Schilling type) (6), chronic lymphatic leukemia (17), chronic myeloid leukemia (36), myelofibrosis (10) and polycythemia (6). The cytochemical reactions used were: sudan black B, peroxidase, PAS and alkaline phosphatase. The score method was used to evaluate the PAS reaction of lymphocytic cells (normal for adults: 28.4 ± 8). The scores were higher in acute lymphatic leukemia (70.9) and in lymphoid leukosarcoma (90.8) than in acute myeloid leukemia (17.0) or reticulum cell leukosarcoma (0). For alkaline phosphatase, the score of segmented neutrophils of peripheral blood was used (normal: 223.6 ± 75 for children, 116.4 ± 40 for adults). In chronic myeloid leukemia, the score was lower (11.5), as it was in several cases of chronic myeloid leukemia in remission. In one case of chronic myeloid leukemia, neutrophilic type, the scores were always normal. The scores were high in the acute terminal phase of the illness.——M. J.

TREATMENT OF LEUKAEMIA BY EXTRACORPOREAL IRRADIATION. E. D. Thomas, R. B. Epstein, J. W. Eschbach, D. Prager, C. D. Buckner and G. Marsaglia. From University of Wash-
ABSTRACTS


In most of the 10 cases, neurologic symptoms occurred during hematologic remission. Mean survival was about 6 months. Some cases experienced more than one episode. Intrathecal antimitotics, intrathecal antiteichoic acid, or both were used for treatment.


A technic for removing granulocytes from the circulating blood of anesthetized intact animals was devised. The selective adherence of granulocytes to glass in the presence of normal plasma constituents was exploited with subsequent elution of the granulocytes after chelation of bivalent cations. The procedure described was suitable for...
obtaining quantities of granulocytes adequate for chemical analyses, such as DNA labeling studies, and was shown to have a capacity for granulocyte removal adequate for leukopheresis. The cells obtained were viable, as judged by resistance to trypan blue staining and retention of phagocytic activity.—T. E. B.

**ABSTRACTS**


The present experiments were designed to test if the cytolytic activity of sensitized lymphocytes for target tissue could be due to adsorbed cytotoxic antibody. Known cytotoxic serum was treated with x-irradiation or hydrocortisone. These treatments did not impair the ability of the serum to damage susceptible cells. On the other hand, treatment of sensitized lymphocytes with x-irradiation (15,000 V) or with hydrocortisone markedly impaired their ability to produce cytotoxic damage of sensitized cells. The authors concluded that the ability of sensitized lymphocytes to damage target tissue was not due to adsorbed cytotoxic antibody.—I. G.


In labeling experiments in adult rats using tritiated thymidine, foreign-body-type multinucleated giant cells appear to have been derived from previously labeled blood mononuclear cells, either by their fusion after they had emigrated into tissues or through their phagocytosis by locally resident macrophages.—P. B.


Using autoradiographs of serial sections and imprints of tissues, a comparison was made of the localization, numbers, morphology and intensity
of nuclear labeling of cells in healing excised wounds and implanted sponges of Millipore filters in rats injected intravenously with tritiated thymidine. Blood mononuclears, labeled preoperatively, or their descendants were shown to be present in fibrosing sites up to 14 days postoperatively. The frequent existence of cells, morphologically indistinguishable from fibroblasts and fibrocytes, with labeled nuclei, many days after the last isotope injection, taken with the localization, cytology, movements and apparent transformation of mononuclears labeled preoperatively, was considered to indicate that some fibroblasts in healing wounds and chronic inflammatory processes may be derived from some blood-borne pluripotential cell.—P.R.


Can connective tissue forming cells arise from hematogenous precursors? Blood was drawn from guinea pigs by: (1) external cardiac puncture. (2) cannulation of carotid artery. (3) venepuncture of inferior vena cava and (4) direct cardiac puncture. Buffy coat cells were grown in subcutaneously placed diffusion chambers. After removal of the chambers, the cells inside were studied by light microscopy using Van Giesen staining and by electron microscopy. Chambers implanted with blood cells obtained by methods 1 and 4 almost always contained fibroblasts. Chambers containing blood cells obtained by methods 2 and 3 rarely contained fibroblasts. The authors concluded that fixed connective tissue cells can easily contaminate blood during collection and that, therefore, all previous evidence purporting to show that fibroblasts can arise from hematogenous precursors must be viewed with suspicion.—I. G.

HEMATOLOGIC EFFECTS OF THYMECTOMY IN RATS

The study was performed with 104 male and female rats, 52 of which were thymectomized 3 to 4 weeks after birth. Each thymectomized rat was paired with an unoperated control of the same sex, litter and initial weight. Thymectomy, even at a relatively late age, induced the disappearance of about 50 per cent of the blood lymphocytes and partial involution of lymph nodes. Erythrocytes, hemoglobin and neutrophils remained unchanged. Lymphocytopenia involved especially small and large lymphocytes in males and small and medium sized cells in females. In the majority of males, but not females, thymectomy was followed by a decrease in serum y-globulin levels. In thymectomized and in intact rats, prolonged deprivation of protein produced a fall in the number of lymphocytes. In operated rats, protein starvation amplified the lymphocytopenia due to thymectomy. All protein starved rats showed a reduction in the cell and nuclear diameter of lymphocytes. Conversely, protein realimentation induced an increase in lymphocyte size after a few days. Thus, protein deprivation led to a selective disappearance of large and medium sized lymphocytes, whereas small lymphocytes were the last to decrease in number. There were some differences in the patterns of morphologic changes observed in intact and thymectomized rats. The quantitative decrease in large and medium lymphocytes was more pronounced in the former than in the latter. After 50 or 70 days of protein starvation, the number of both types of cells was identical in intact and thymectomized animals. Whereas it had been much higher in the former at the beginning of the diet. Apparently, atrophy of the thymus caused by malnutrition played an important role in the selective disappearance of medium and large lymphocytes. Reduction of lymph nodes induced by the protein-free diet and their increase during refeeding involved both the thymus-conditioned and the thymus-independent weight fractions of these organs. Despite regeneration of the thymus, the first of these two lymph-node fractions had not recovered its normal weight after one month of repletion. In males, but not females, the decrease in y-globulin observed after protracted protein deprivation seemed to be correlated with the involution of the thymus. —G. M.

ERYTHROCYTES


Folic acid in the liver of man was in the form of 5 methyl-tetrahydrofolate. The amount of folate
in the liver of patients undergoing laparotomy varied from 0.7–17 μg./Gm. of liver. The urinary excretion of formiminoglutamic acid (FIGLU) was always within the normal range when the hepatic folate level was above 5 μg./Gm. and was abnormally elevated when the level of hepatic folate fell below that level. Three patients had abnormally elevated excretions of FIGLU. Despite "normal" hepatic folate levels. There was a good correlation between hepatic folate levels and serum folate (L. casei) concentrations. Two patients with megaloblastic anemia due to folic acid deficiency and excessive alcohol intake had low hepatic folate levels.—P. B.


The histologic appearance of the gastric biopsy was correlated with the assay for intrinsic factor. Acid content of gastric juice and vitamin B12 absorption. There was a good correlation between the decline of intrinsic factor output and the loss of gastric secreting cells. The secretion of intrinsic factor was reduced in 10 of 11 patients with atrophic gastritis, but most of them still absorbed vitamin B12 normally. The assay of intrinsic factor appeared to be a better index of secretory capacity of the gastric mucosa than either acid production or vitamin B12 absorption.—P. B.


Previous work had suggested that methylmalonic acid accumulating in B12-deficient patients might be neurotoxic. The authors reported an incidence of 5.8 per cent low and 9.6 per cent borderline serum B12 values in 396 mental patients aged over 30. Patients between 70 and 79 had the lowest values. Of 23 patients with low values, 3 had had their stomachs resected, 1 had a previous diagnosis of pernicious anemia and 11 had Schilling tests below 7 per cent. The pernicious anemia frequency in the area had been estimated at 9.3 × 10–5 annually. B12-treatment had some effect in 8 of 20 patients. (Abstracter’s comment: What is the frequency of low serum B12 levels in old people with poor nutrition?)—P. C. R.


Sustained release products did not produce sustained serum iron levels and the levels found usually were less than those obtained with a standard dose. Dissolution in vitro at 2 hours was only 20–75 per cent. Since transit time through the duodenum was less than 4–6 hours, these preparations appeared unsatisfactory, even on theoretical grounds. Formulation of sustained release iron preparations seems unwarranted.—R. O. W.


Daily intramuscular injections into rabbits of 0.075 M CoCl2 solution for 29 days, 1 ml./Kg. on the first day and 0.5 ml./Kg. on the other days, resulted in a sustained increase in reticulocyte count. The red cell count increased to 8–11.5 million/mm.3 on the 29th day. Utilization of Fe59 injected on the last day was accelerated. No remarkable change was observed in the urinary excretion of coproporphyrin throughout the treatment period. Erythropoietin activity was elevated in plasma obtained on the 4th day. When CoCl2 was added at a concentration of 10–8–10–4 M to an incubation mixture consisting of chicken red cells, glycine, Fe59Cl3 and phosphate buffer, the biosynthesis of heme and protoporphyrin were inhibited. In bilaterally nephrectomized rabbits, acceleration of Fe59 utilization was not observed, but in rabbits with bilaterally ligated ureters, utilization was accelerated as it was in splenectomized rabbits or hypophysectomized or adrenalectomized rats. From these results, it was concluded that cobalt stimulates erythropoiesis through erythropoietin production in the presence of the kidney.—K. F.


The effects of a single intramuscular injection of 0.075 M CoCl2 solution, 1 ml./Kg., on urinary
excretion rate of PSP, amount of urine excreted and blood pressure in the femoral artery were investigated in normal intact rabbits, right-sided nephrectomized rabbits and rabbits with both right-sided nephrectomy and left-sided denervation of the kidney. In control experiments. Mohr's salt solution or saline injections or blood letting were used. In right-sided nephrectomized rabbits, reduction of blood flow to the left kidney to about one-seventh of normal with a Goldblatt ring resulted in a marked elevation of erythropoietin activity in plasma obtained directly from the left renal vein. From these results, it was concluded that elevation of erythropoietin activity in blood plasma following intramuscular injection with CoCl₂ solution results from reduction in renal blood flow elicited by direct action of cobalt on the renal artery.—K. F.


A well-documented case of acquired moderate hemolytic anemia occurring during administration of huge doses (100,000,000 u/day) of aqueous penicillin G was presented. The direct Coombs test was positive and serum from the patient imparted a strongly positive Coombs test to normal red cells only if they had been previously sensitized with penicillin. Antibody activity resided in the gamma globulin fraction and was partially inhibited by high concentrations of benzylpenicillin in vitro. Other rather mild manifestations of allergy in this patient were leukopenia, relative eosinophilia and pruritic skin rash. Following recovery, the provocative administration of high doses of penicillin again led to significant hemolysis of red cells in which Coombs reactivity had reappeared.—H. S. J.


A case strikingly similar to that reported above was presented. Following recovery, the patient's red cells which had been preincubated with penicillin were destroyed immediately when autologously reinjected into the patient, but survived normally in the circulation of a nonimmune recipient.—H. S. J.


Examination of the hemoglobins of two West Indian women living in Birmingham revealed two minor fractions, each amounting to about 1 per cent of the total hemoglobin. On paper and on starch electrophoresis at alkaline pH, one moved in the position of hemoglobin A₂ and the other moved more slowly in the position of hemoglobin A'₂. Peptide and aminoacid analysis showed that hemoglobin A'₂ differed from hemoglobin A₂ in the δ chain and it was concluded that hemoglobin A'₂ was a₂ δ₂₁₆ Gly-Arg. These results confirm the findings of R. T. Jones et al. published earlier (Amer. J. Hum. Genet. 17: 511. 1965).—P. B.


A general review, extensive bibliography and summary of the authors' contributions are presented.—P. d. N.


A Scotch-Cree Indian family was described in which several members had hemolytic anemia associated with erythrocyte G-6-PD deficiency. Of greatest interest was the finding of significant hemolytic disease (reticulocytes = 12 per cent) in the mother of several affected male children, despite nearly normal enzyme levels in her red cells. The possibility that she harbored a population of extremely deficient cells and a population of normal cells was suggested from Cr⁵¹ survival curves which were biphasic. Primaquine, given to recipients of her labeled cells, caused increased red cell destruction only during an initial phase in which survival of red cells was already diminished. The second phase in which survival was normal was unaltered by the drug.—H. S. J.

G-6-PD activity of red cells of 1,103 normal individuals, comprising members of 289 families, and of 15 pairs of identical twins were determined by the "glutathione stability test." The level of the enzyme was expressed in terms of the postincubation concentration of GSH (mg. per 100 ml. erythrocytes). For both sexes, there was a tendency towards a slight increase in GSH with advancing age; the regression coefficient was significantly larger than zero, but the rate of increase was very small (approximately 0.06 per cent a year). A highly significant difference in GSH level between the sexes was found. Females had, on the average, about 6 per cent higher values than males, indicating that dosage compensation was not complete. The implication of this finding was discussed with reference to the Lyon hypothesis. Genetic analysis of family data revealed significant correlations for mother/son and sister/sister. The observed correlation coefficients were 0.17 and 0.31, respectively. Studies of twin data showed a significant correlation for male identical twins; the intra-class correlation coefficient was 0.73. There was an indication that genotypic variance may be larger for female twins. It was suggested that the genetic system controlling the quantitative variation in erythrocyte G-6-PD activity may be related to the X chromosome, though participation of autosomal modifiers cannot be excluded. Only one male subject was found to be "enzyme deficient," but it was not clear if he corresponded to the mutant hemizygoty found in certain ethnic groups.—K. F.


No differences in cholesterol and total phospholipid content were noted between normal and G-6-PD deficient red cells. Sphingomyelin content was lower and phosphatidyl serine content was higher in enzyme-deficient cells, but the differences were statistically only barely significant. In red cells incubated in vitro with nitrofuran or acetylphenylhydrazine, significant decreases in phosphatidyl serine and concomitant increases in phosphatidyl ethanolamine occurred only in enzyme-deficient cells. The authors suggested that this vulnerability to alterations in structural lipids may weaken the red cell membrane and increase its sensitivity to the destructive action of the reticuloendothelial system.—H. S. J.


Despite the fact that mature mammalian erythrocytes cannot synthesize lipids de novo from acetate (Marks et al., J. Biol. Chem. 235: 2579, 1960), their ability to incorporate plasma free fatty acids into membrane phospholipids is intact and is accelerated markedly by ATP and coenzyme A. The second paper presents evidence that red cell membranes have enzymatic systems for synthesizing complete phospholipids (diacyl phosphoglycerides) from lyso phosphoglycerides (monoacylphosphoglycerides) normally present in plasma. Such anabolic reactions may be important for the replacement of membrane material lost during mechanical or metabolic stress in the circulation.—H. S. J.


Electron microscopic studies of rabbit corneas, fixed in situ with osmic acid containing pyroantimonate ion, were performed to localize areas of high cellular sodium content. In untreated corneas, precipitates, presumably of sodium pyroantimonate, were localized sporadically along the intracellular surface of cell membranes. Corneas from animals in which ouabain was injected into the aqueous humor to “freeze” sodium transport demonstrated striking increases in (sodium) pre-
elusion that intracellular, rather than extracellular, precipitation. But in transport processes, including those in red cells (e.g., Whittan, Biochem. J. 84:110. 1962).
—H. S. J.


Some experiments are described in which ATP was formed in human erythrocyte ghosts through the phosphoglycerate dehydrogenase and phosphoglycerate kinase reactions.—P. B.


Cyanosis developed due to methemoglobin formation. There were no deaths. All recovered after treatment which consisted of removal of the causative agent and administration of methylene blue, ascorbic acid, transfusions and oxygen. The differential diagnosis, pathogenesis and treatment were discussed. Emphasis was placed on the importance of early diagnosis, the removal of the toxic dye and proper treatment, as well as instruction of all personnel in charge of nurseries and the hospital laundry. The authors recommended the use of marked baby linen only 12 hours after boiling or autoclaving and drying.—M. J.

MISCELLANEOUS


The electrophoretic mobility of G-6-PD from homogenates of myometrium and leiomyomata obtained from women heterozygous for the electrophoretic variants A and B were studied. Myometrium contained A and B bands in nearly equal amounts, but leiomyomas from these uteri contained either one or the other variant, never both. Both “pure” A and B tumors were found in uteri with multiple tumors. These findings provided exceptionally strong evidence for G-6-PD mosaicism in females and for the permanence of X-chromosome inactivation (Lyon hypothesis). They were also consistent with the hypothesis that these benign multiple tumors arise from single cells.—H. S. J.


A detailed analysis of the “cytophilic antibody” described originally by Boyden and Sorkin is presented. Guinea pig antisheep erythrocyte antibodies cytophilic for macrophages are 7S y2 globulins. The F, fragment of the antibody contains the receptor site for the macrophage surface. Complement, however, is not necessary for the attachment of cytophilic antibody. If cytophilic antisheep red cell antibody is not attached to the antigen, it can be rather easily eluted off the macrophage. The presence of the antigen binds the cytophilic antibody much more tightly to the macrophage surface. The authors feel that this antibody is not responsible for delayed reactions, since it is so weakly bound to macrophages. If sheep red cells are attached to macrophages with cytophilic antibodies at 22 C. and the temperature is raised to 37 C., the sheep cells are phagocytized. Thus, “cytophilic antibody” and opsonizing antibody appear to be identical.—I. G.


Haptoglobin types were determined in 445 blood donors. A characteristic frequency for the Hp 1 allele was found among the white population (38.4 per cent). but was 59.5 per cent in colored people, including mulatoes. No significant differences were observed between the distribution frequencies of the 3 phenotypes among Brazilian-born or foreign-born whites.—M. J.

The variations in the sequence of the L chains of 5 Bence-Jones proteins were investigated. The disulfide bridges were quantitatively reduced and alkylated with C14-iodoacetate, the radioactive protein was digested with trypsin or chymotrypsin and the radioactive peptides were purified by Sephadex fractionation and paper electrophoresis. The C-terminal stretch and the “variable” region were analyzed. Detailed analysis of the variable region made it possible to test hypothetical mechanisms by which variability in protein sequences could be achieved. Starting from a much simpler set of genes, several simple hypotheses which could explain the variability have been tested, but none of them accounted for all the variations observed. —P. B.