Erythrocytes


The venom obtained from Vipera aspis contains several interrelated phospholipases $A_2$ (phosphatide-acyl-hydrolases EC 3.1.1.4) that hydrolyze phosphatidylcholine and phosphatidylethanolamine. The venom has no action on washed erythrocytes or on red cells suspended in serum free from lipids and degrades specifically $\alpha$- and $\beta$-lipoproteins with liberation of highly hemolytic products. The fatty acids liberated are mainly oleic and palmitic acids. The hemolytic activity depends considerably on the nature of the original lipoprotein. This indicates that the lysoderivatives originating from the phosphatidylcholine and phosphatidylethanolamine differ in their hemolytic activity according to the length of the carbon chain and the degrees of saturation of the fatty acid bound to the lysophosphatide.—J.M.P.


Phosphofructokinase of normal human erythrocytes was purified 15000-fold by $(NH_4)_2SO_4$ precipitation, heating, and column chromatography on Sepharose-6-B. With cellulose-acetate electrophoresis, only one band was observed after detection of the enzyme activity with the fluorescent technique. The inhibition by ATP was pH dependent. Cyclic AMP was able to reverse the inhibition by ATP to some extent. With
GTP, ITP, and UTP no inhibition was observed. At saturating concentrations of GTP, ATP still inhibited phosphofructokinase. The variation of the activity of phosphofructokinase at various ATP and fructose 6-P concentrations was studied.

—K.P.


A significant frequency of low glutathione reductase activity in the erythrocytes of the Skolt Lapps in Finland was found. Investigations of the enzyme properties in subjects with low activity showed no abnormalities in the enzyme protein. In addition, the $K_m$ for GSSG and FAD was normal. The enzyme activity could be increased by FAD. It was assumed that decrease in glutathione reductase activity was due to a riboflavin deficiency. On the other hand, the activity of NADH-dependent methemoglobin reductase (also a flavoprotein with FAD as prosthetic group) was not lowered.—K.P.


The above authors reported previously that an augmented response to erythropoietin (ESF) occurs in germfree mice. Possible mechanisms for this enhanced response were proposed: (1) increased numbers of ESF-committed stem cells or increased sensitivity of these cells to ESF in germfree animals, and (2) the internal environment of the axenic animal favors an augmented activity of ESF. This paper presents evidence supporting a slower rate of disappearance of exogenous ESF in the plasma of the germfree mouse. These studies were done in male, germfree mice maintained in flexible plastic isolators. Conventional mice of the same strain were raised under ordinary laboratory conditions. Four groups of mice were established: (1) ESF-injected conventional mice, (2) ESF-injected germfree mice, (3) ESF-injected x-irradiated conventional mice, and (4) ESF-injected x-irradiated germfree mice. The x-radiation dose was 900 R. The results indicated that the rate of disappearance of ESF from the plasma of germfree mice was considerably slower than in conventional mice. Although no remaining ESF was observed in the conventional mice at about 9 hr postinjection, considerable activity was present in the germfree animals at this time. In addition, significantly greater quantities of ESF ($p < 0.05$) were noted in the plasma of the latter mice at all time intervals. The x-radiation resulted in a highly significant delay in the rate of disappearance of the intravenously administered ESF in both the conventional and germfree mice. Possible mechanisms for these findings include the reduced ability of the liver to destroy ESF in the germfree animal and the decreased capacity of the blood-forming organs in the radiated mouse to utilize ESF.—M.G.B.


Significantly greater ouabain-insensitive sodium efflux occurs from erythrocytes of patients with hereditary spherocytosis (HS) than from normal controls. Erythrocyte sodium content and ouabain-insensitive sodium efflux were significantly greater in men than in women. The rate of efflux in four parents with no other stigmata of HS was as great as in the patients, suggesting that the sodium “leak” does not have pathogenetic significance in the hemolytic process. The authors suggest that the gene for HS may express itself in several ways, one of which is as an increase in red cell
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membrane permeability without abnormal morphology or decreased lifespan.—J.B.S.


It is known that there is an increase in the affinity of hemoglobin for oxygen during storage of blood with ACD, due mainly to a decrease in the red cell 2,3,-diphosphoglycerate (2,3-DPG) level. It has also been considered that the concentrations of 2,3-DPG and of ATP are the determinants of the affinity of hemoglobin for oxygen in stored blood. It is also known that a significant increase of 2,3-DPG and ATP can be obtained by the addition of 120 moles of inosine/g of hemoglobin to blood collected in ACD solution. With this treatment, the viability of the red blood cell is greatly improved, and the useful storage period of the blood can be extended to 42 days with a post-transfusion survival of better than 70% at 24 hr. In the present work, the effect of three variables on the levels of ATP, 2,3-DPG, and the related oxygen dissociation curve (P50) was determined. These variables were: the time of addition of adenine and inosine, the concentration of inosine, and the mode of addition of inosine. The main purpose was to determine the quantity of adenine and inosine and the time and mode of addition resulting in the best maintenance or generation of ATP, 2,3-DPG, and P50 of red blood cells in blood stored at 1°C. Adenine concentration was maintained constantly at 2 μmoles/g of hemoglobin. With all concentrations of inosine and at all times of addition, the ATP was maintained at a level compatible with post-transfusion survival of 70% or better. The level of 2,3-DPG in all experiments and at all times of addition of adenine and inosine was found to be significantly higher than in the ACD controls. The level of 2,3-DPG was found to be directly related to the concentration of inosine. About 4-8 days were required for the optimal level of 2,3-DPG to be reached after addition of inosine. With a constant concentration of inosine, regeneration of 2,3-DPG became less with increasing length of storage. With 120 μmoles of inosine, added in fractional doses, the level of 2,3-DPG was maintained above 90% of the original for 42 days. Similarly, P50 with 120 μmoles of inosine remained at the level of 90% for 35 days. The apparent limitation of ATP regeneration when adenine and inosine were added simultaneously at zero time and 7 days is discussed.—M.G.B.


It is known that the use of adenine and inosine is effective in the preservation of hemoglobin function in stored red blood cells. However, it is not known if adenine and inosine also aid in the preservation of the red cell membrane. In this study, a membrane analysis procedure was applied to red cells treated with adenine and inosine by the method of Strumia (M. M. Strumia et al. Proc. Soc. Exp. Biol. Med. 135:443, 1970), in an effort to determine if hemoglobin function and membrane integrity are related or are independent phenomena. Whole human blood with and without addition of adenine-inosine was stored for 42 days at 4°C. Adenine-inosine was effective in maintaining hemoglobin function as shown by a continued high level of 2,3-diphosphoglycerate. Analysis of the erythrocyte membrane was carried out by fluorescent probe spectroscopy of the red cells. It was shown that adenine-inosine treatment did not alter the rate of membrane deterioration, indicating that maintenance of membrane integrity and hemoglobin function are separate problems and that each must be considered individually. The determining factor in long-term red cell preservation may be in membrane stabilization. Sensitivity of the analytical system used was such that membrane changes could readily be seen. Lack of membrane stabilization may turn out to be the limiting factor in long-term red cell preservation and would
have to be considered separate and different from hemoglobin function.—M.G.B.


Normal hemoglobin function depends on 2,3-diphosphoglycerate (2,3-DPG) in the human erythrocyte. Citrate-phosphate-dextrose (CPD) anticoagulant, because of its higher pH, is superior to acid-citrate-dextrose (ACD) anticoagulant for maintaining hemoglobin function (i.e., oxygen dissociation and 2,3-DPG) in stored blood. In the present study, an attempt was made to establish optimal pH of preservation for maintaining 2,3-DPG (hemoglobin function) and ATP (red blood cell viability) during liquid storage at 4°C under blood bank conditions. Ten units of blood from normal volunteers were subjected to an automated analytical system for determining concentrations of 2,3-DPG and ATP. Each unit was split during donation into five parts containing citrate-dextrose solutions of pH 5.0, 5.5, 6.0, 6.5, and 7.0. Significant differences at the 95% level were based on the paired t test. In addition, osmotic fragility and methylene blue uptake were determined to assess their possible usefulness as indicators of either red blood cell viability or ATP. With pH 5.0 preservative, 2,3-DPG fell from day 0 to day 3. With pH 5.0 and 5.5 preservatives, it fell from day 3 to day 7 and from 7 to day 14 in all pH groups. In excess of $1 \times 10^{-7}$ hydrogen ion concentration (corresponding to pH 7.0), 2,3-DPG concentration fell at a rapid rate. From the 2,3-DPG and ATP data obtained, a preservative with pH higher than 5.5 would seem to be optimal for maintaining hemoglobin function and red blood cell viability, but it is suggested that adenine may be needed to maintain adequate ATP levels.—M.G.B.


The absorption of radioactive cobalamin was measured in patients with pernicious anemia and control subjects by whole-body counting technique. In controls, food did not affect the absorption of cyanocobalamin, but with pentagastrin the absorption of cyanocobalamin was significantly greater than that of hydroxycobalamin. In patients with pernicious anemia where the mass of hog intrinsic factor is small, the amount of cyanocobalamin absorbed is directly related to the mass of intrinsic factor, but absorption approaches saturation value with increasing mass of intrinsic factor.—J.M.B.


In 28 of 33 patients with prosthetic heart valve replacement examined 2–4 yr after operation, the serum haptoglobin level was below normal, and in 25 of 28 there was a rise in serum lactic dehydrogenase. These were found to be the most accurate indicators of red cell damage. Other parameters, including the presence of fragmented red cells or of serum methemalbumin, or raised reticulocyte or serum bilirubin levels, were inconstant and were found mainly in cases of severe hemolysis. Only one case in 33 was entirely free of laboratory evidence of traumatic hemolysis, although most had no clinical signs of it.—F.W.G.


A new abnormal, heat labile hemoglobin causing marked Heinz body anemia is reported. The abnormality was found to be in the nonalphapolypeptide chain. Therefore, the nonalpha chains were purified, and
their amino acid sequence was determined. Multiple aberrations in the amino acid sequence of the abnormal chain were found. The authors suggest that Hb-Frankfurt is the result of a "crossover" in Lepore-Hb or of a deletion of one or two nucleotides on the DNA or RNA chains.—K.P.


Fulminant infections caused by *D. pneumoniae* are seen in children with sickle cell anemia (SCA), most frequently involving the meninges and occurring primarily before the age of two. Seven per cent of all severe pneumococcal infections seen in children at Cook County Hospital occurred in patients with SCA, and pneumococcal sepsis or meningitis accounted for 3% of the admissions of children with SCA. In three of the seven instances of meningitis described, CSF cultures were positive in the absence of pleocytosis or abnormal spinal fluid chemistries. On this basis, the authors favor intravenous penicillin for febrile children with SCA who appear seriously ill.—J.B.S.


In most patients with homozygous sickle cell disease, sickled erythrocytes segregate into an opaque layer at the bottom of the microhematocrit tube, ergo, the sicklecrit. This observation, described in 1962 (Kochen, J. A., Radel, E. G., and Schorr, J. B. The Sicklecrit. Proc. IX Cong. Int. Soc. Hemat. 1:171–176) was reevaluated in this study. A distinct sicklecrit was present in 73% of the Hb SS patients and was never seen in people with AA, AS, or SC hemoglobin. A positive sicklecrit correlated with the presence of more than 4% sickled red cells on peripheral smears. The height of the opaque column was proportional to the degree of sickling, with a correlation coefficient of 0.95. Although helpful in quickly identifying patients with SS disease, the frequency of false negatives, even during sickle cell crisis, limits the value of the sicklecrit. It is, however, a neat way to isolate the circulating sickled erythrocytes. —J.B.S.


Erythrocyte enzymes were investigated in 35 patients with pancytopenia. In 23 of these cases an erythrocyte enzyme deficiency was detected; six patients showed a significantly decreased glutathione reductase activity, while in 11 patients a decrease of the pyruvate kinase activity could be demonstrated. In the other six patients a combined, defect, namely a decrease of glutathione reductase as well as pyruvate kinase activity, was observed. In these cases a double heterozygosity is assumed since family studies in five cases revealed the same enzyme deficiencies in other family members, thus indicating the possibility of an inherited disorder. These results seem to support the concept that enzyme deficiencies in blood cells may represent at least one cause for certain forms of pancytopenia. —K.P.


The effect of dietary protein on the development of orally induced siderosis was investigated in guinea pigs. One group of animals was given a maize-based diet of low protein content that was similar to the diet consumed by the local Bantu population. Another group was given the same diet supplemented with protein cubes. Each of these groups was divided into three. One received no extra iron, the other was given home-brewed Bantu beer providing 5 mg iron/day, and the last one received an equivalent quantity of aqueous ferric ammonium citrate. Animals on a high protein diet supplemented with either form of oral
iron exhibited an increase in hepatic and splenic iron concentrations of about 50% after 25 wk. The degree of siderosis was materially unchanged after 50 wk. In contrast, animals on a low protein diet showed a progressive rise in iron stores; by 25 wk the concentrations of iron in liver and spleen had increased eightfold. No evidence of hepatic fibrosis or necrosis was noted. These results indicate that the degree of overload induced by excessive oral iron is profoundly influenced by the composition of the diet.—T.H.B.


A boy with onset of manifestations of polycythemia vera at age 6 yr was studied. In addition to erythrocytosis, he had thrombocythemia with giant and bizarre platelets, mild leukocytosis, considerable splenomegaly, and diminished levels of factors I, V, and VIII in association with increased levels of serum fibrinopeptides. The patient suffered from a bleeding duodenal ulcer; he also had frequent soft tissue bleeds, as well as occasional hemarthroses. He and his two siblings, both severely retarded, had low IgA levels, and he, his father, and the one sister who was adequately tested demonstrated partial deletion of the E-18 chromosome. On Melphalan therapy normal blood cell levels were maintained, and the boy's coagulation status became normal.—J.B.S.

LEUKOCYTES


An extensive review of the evidence suggests that the G-group chromosomes are concerned with the development and progression of chronic granulocytic leukemia and may have a special role in acute leukemia and malignant lymphomas and could perhaps be of importance in the development of some solid tumors.—F.W.G.


The appearance in an electron microscopic study of gonococci inducted by polymorphonuclear leukocytes (PMN) from men with gonorrhea was studied. It was found that PMN degranulate into vacuoles containing the organisms and that morphologically intact gonococci were found in vacuoles showing evidence of recent degranulation. In vitro experiments with virulent gonococci and polymorphonuclear leukocytes suggest that degeneration of the bacteria occurs in the 30–60 min of phagocytosis.—J.M.B.


In a retrospective study, the degree of lymphocytic infiltration was assessed in 23 primary neuroblastomata. A direct correlation was found to exist between duration of patients survival, both in infancy and childhood, and intensity of lymphocytic infiltration in the primary tumor. The presence of metastases did not invalidate this correlation.—J.M.B.


In a child with erythroleukemia, there was a direct correlation between the severity of anemia and the levels of circulating erythropoietin, as well as the degree of normoblastosis in peripheral blood and bone marrow. Reticulocytes did not parallel normoblastosis. No correlation between bone
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marrow myeloblastosis and peripheral blood normoblastosis was seen. Thus, although qualitatively abnormal, erythropoiesis in erythroleukemia is regulated by physiologic control mechanisms and appears to be independent of the leukemic myeloproliferation.—J.B.S.


During the terminal acute blastic phase in an adolescent with chronic myelogenous leukemia, four episodes of severe hypoglycemia accompanied by hypothermia were seen. During these episodes body temperature was between 95.5°F and 96°F, and the patient was stuporous, confused, decerebrate, or even comatose. Although the white count was low during the last episode, the authors speculate that there was increased peripheral utilization of glucose by the massive numbers of leukemic cells present in the liver and spleen.—J.B.S.


The role of infection in the deaths of 199 leukemic children was studied. Only one-fifth of the children were infection free at the time of death. In almost half of the patients, infection was the primary cause of death, with Pseudomonas, Escherichia coli, Staphylococcus aureus, and candida albicans the most frequently implicated organisms. Fever, not suppressed by steroids or chemotherapy, was a frequent presenting sign. At autopsy, disseminated infection and pneumonia were usually present, and in patients with gram-negative bacterial infection, gross ulceration of the GI tract was also seen. The tendency to mycotic infection increased with duration of disease, exceeding 30% in youngsters whose leukemia had been present for more than 15 mo. Of the four children whose fatal infection occurred during remission, three succumbed to Pneumocystis carinii pneumonia, and one succumbed to acute hepatitis.—J.B.S.

HEMOSTASIS


Rat, rabbit, and human plasma contain a prophospholipase that can be activated by an enzyme present in rat but not in human platelets. It is also present in crude trypsin preparation but is distinct from trypsin. The reaction is believed to be the source of the phospholipase that is present in serum but nearly absent in plasma and that is active on phosphatidylethanolamines and phosphatidyglycerol.—J.M.P.


The main property of Vipera venoms is to activate coagulation. The authors report the chromatographic separation of the following activities from Vipera aspis venom: (1) a fibrinolytic and fibrinogenolytic activity, (2) a procoagulating activity possibly due to activation of factor X, (3) an enzymatic activity on platelet phospholipases with liberation of hemolytic derivatives and activation of platelet aggregation. Phospholipase activity is also associated with activation of factor V. (4) Inhibition of platelet aggregation by ADP. In the intact venom this effect counteracts the aggregating agents associated with (3), and finally, (5) an anticoagulant with antifactor 3 properties.—J.M.P.


A very nice review of current knowledge concerning the development of vascular, platelet, and coagulation factor functions, including hitherto unpublished observations...
of fetuses obtained from therapeutic abortions.—J.B.S.


Increased platelet adhesiveness, platelet factor-3 and platelet factor-4 activities, and shortened ADP-induced aggregation time have been reported in platelets of diabetic patients. The authors studied the effect of diabetic plasma on aggregation of normal platelets in 87 diabetic subjects and in 62 healthy controls. The degree of platelet aggregation induced by ADP was measured in mixtures of 1:8 of diabetic plasma and normal platelet-rich plasma. The results were based on the difference in percentage change in optic transmission 4 min after the addition of ADP. A significant increase of aggregation over controls was observed in diabetic patients. The presence of complications, such as retinopathy and nephropathy, caused a significantly greater platelet aggregation. For the former, a positive correlation could be noted between the activity and severity of retinopathy as established by retinal photography and fluorescent angiography and the aggregation-enhancing activity. There was no correlation between this aggregation-enhancing activity of diabetic plasma and age, body, weight, height, therapy, insulin doses, hematocrit or fasting plasma levels of glucose, triglyceride, cholesterol, free fatty acids, albumin, or free fatty acid:albumin molar ratio. Both plasma and serum carried the aggregation-enhancing activity, which was nondialyzable, heat resistant, and not affected by aspirin treatment. It did not spontaneously aggregate platelets but enhanced the second phase of aggregation induced by either ADP or epinephrine. There was no effect of diabetic plasma on the rate of release of platelet factor 3 in normal platelets. The authors conclude that in some diabetics a plasma factor is present that enhances the second phase of platelet aggregation.—M.S.


Platelet concentrates were prepared from 480 ml whole blood collected into citrate-phosphate-dextrose (CPD) anticoagulant. In consecutive trials, an average of 0.81 ± 0.07 × 10^11 (mean ± SE) platelets were harvested per unit of platelet concentrate. This represented a recovery of 72.4 ± 7.5% of the calculated number of platelets originally present per donor unit. After labeling with 51Cr and autotransfusion, the in vivo recovery of labeled platelets was 46.3 ± 4.6%/unit. The disappearance of the labeled platelets from the circulation was linear between 6 and 144 hr after transfusion, with the mean t 1/2 at 4.36 days. A method of calculating in vivo recovery by extrapolation of the curve and of estimating blood volume is suggested to simplify comparisons among the works of various investigators. CPD at the slightly alkaline pH provides a superior in vitro recovery of platelets for transfusion. The net in vivo recovery is similar to that reported for ACD at pH 7.1 or 6.5. The suitability of CPD for preparation of platelet concentrates (considered with the longer shelf life it allows for red cells and the better preservation of 2,3-DPG) further supports the superiority of CPD anticoagulant over ACD for blood-banking practice. It is concluded that CPD is a suitable anticoagulant for the preparation of platelet concentrates and for studies of their survival in vivo.—M.G.B.


Platelet adhesiveness to glass beads and aggregation with ADP and collagen were measured in 14 patients under treatment with streptokinase after myocardial infarction and in one patient with a bleeding tendency due to fibrinolysis in disseminated carcinoma of the prostate. Both parameters were usually normal, even while high levels of fibrin degradation products (FDP) were
present. Platelet aggregation with thrombin was abnormally low, but the abnormality could be corrected by adding more thrombin. Thrombin times were concurrently prolonged. The major hemostatic effect in this group of patients with fibrinolysis seemed to result from the antithrombic action of FDP and not from platelet defects, unless fibrinogen levels were excessively low.

—F.W.G.


The diagnostic criteria were studied in 141 patients with septicemia. Thrombocytopenia was found in about 80% of patients with gram-negative and in 60% of patients with gram-positive infection. The thrombocytopenia was more marked in gram-negative cases. Decreased serum inorganic phosphate was found in 70% of gram-negative and in 20% of gram-positive infections. In cases of renal failure, the ratio P/BUN of less than 0.04 proved to be a useful criterion. The white blood picture showed the characteristic shift to the left in all cases; toxic granulations were seen in 65% and vacuolization in 43% of the patients studied. Thus, vacuoles containing leukocytes are indicative of, simultaneous thrombocytopenia is almost equivalent to, and concomitant hypophosphatemia is considered to be identical with the diagnosis of gram-negative septicemia.—H.-J.H.


Abnormalities of bleeding time, tourniquet test, and clot retraction (as well as defects in the clot-promoting properties of platelets) have been reported in patients with acute leukemia. This study evaluated platelet function in a group of adult patients with acute leukemia by measuring platelet aggregation in response to ADP, epinephrine, thrombin, and collagen and by measuring the release of endogenous adenine nucleotides in response to stimulation by thrombin. Fourteen untreated patients with acute leukemia were examined. None of the subjects had ingested drugs known to affect platelet function for 1 wk prior to the study, nor was there evidence of active bleeding or infection. Initial rate and maximal extent of aggregation induced by ADP, epinephrine, thrombin, and collagen were reduced in platelets from patients with acute leukemia, as compared with simultaneously tested normal controls. The second wave of ADP- and epinephrine-induced aggregation was absent. Subnormal amounts of adenine nucleotides were released in response to thrombin from platelets obtained from seven out of eight patients with leukemia. In view of the higher protein content of the leukemic platelets (approximately twice that of normal platelets), the abnormality of the release reaction was even more apparent when calculated on the basis of protein content. The time course of the release reaction paralleled that in normal platelets. Platelet factor 3 availability averaged 42% of normal. A complete remission in one patient was associated with partial correction of the qualitative platelet defects noted prior to treatment. The question of whether factors exist circulating in the plasma of leukemic patients that may affect the platelet function was measured by suspending normal platelets in leukemic platelet-poor plasma. In two patients in which this was studied there was a decreased response to aggregating agents. The extent of the impairment, however, was less than that observed with the leukemic subjects’ own platelets. These results suggest that qualitative platelet defects are comparatively common in leukemic patients. Substances capable of depressing platelet function may also be present in the leukemic blood.—M.S.


Two infants with factor XIII deficiency presented in the newborn period with severe umbilical cord stump hemorrhage
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that remitted following whole blood transfusion. Inheritance patterns suggested an autosomal recessive gene. All the parents and the one sibling studied had factor XIII levels of 11%–32%.—J.B.S.

IMMUNOHEMATOLOGY


Complement titers were the same in normal newborns and in newborns with laboratory or clinical evidence of ABO disease. The red cells of the ABO-erythroblastotic infants did not appear to have C3 or C4 fixed to their surfaces.—J.B.S.


If maternal fetal ABO incompatibility plays a role in causation of spontaneous abortion before 20 wk, one would expect a higher incidence of Group O subjects among such mothers. This was found in a group of 229 patients of whom 52.0% were Group O, as compared with 44.5% among the general population. Further support for the importance of ABO incompatibility as an important cause of early abortion came from determination of ABO blood types on the abortuses. Of 78 fetuses examined, 44.7% were found to be incompatible with the mother, and this is significantly higher than would be expected.—J.M.B.

MISCELLANEOUS


The IQ scores of patients with beta thalassemia were not different from the expected norms. Somewhat lower scores were seen in patients presenting the more severe stigmata of the disease or in whom transfusion therapy was less vigorous. Personality changes suggestive of any chronic illness (depressed mood, floating anxiety) were seen in most patients.—J.B.S.


Newly born infants given phenobarbitone, 5 mg every 8 hr, demonstrated a significant increase in Bromsulphalein (BSP) clearance. Analysis of the two phases of BSP removal suggested that the drug increased both hepatocellular uptake and rate of BSP excretion.—J.B.S.