ABSTRACTS

THEODORE H. SPAET, M.D., Editor

ABSTRACTERS

John Bateman, M.D., Upton, L. I., N. Y.
Ernest Beutler, M.D., Duarte, Calif.
Jerzy Jozef Biezenski, M.R.C.P.I., New York City
T. H. Bothwell, M.D., Johannesburg, South Africa
T. E. Brittingham, M.D., St. Louis
J. B. Chatterjea, M.D., Calcutta, India
Amoz Chernoff, M.D., Knoxville, Tenn.
G. C. deGruchy, M.D., Melbourne, Australia
Pietro deNicola, M.D., Pavia, Italy
Ludvik Donner, M.D., Prague, Czechoslovakia
A. J. Ersev, M.D., Philadelphia
Robert Goldstein, M.D., Boston
J. Guinach, M.D., Barcelona, Spain
Roger M. Hardisty, M.D., London, England
Victor Herbert, M.D., Boston
C. Wasastjerna, M.D., Vasa, Finland

ABSTRACTS OF SPECIAL INTEREST


Acceleration of the ESR is caused by the presence of specific plasma proteins, “agglomerins.” These are adsorbed to the erythrocyte surface, and in the presence of high molecular supplements (for example 0.6 per cent dextran, which is inactive alone at this concentration) the agglomeration of the RBC occurs. Thus, the mechanism seems to be similar to that of incomplete antibodies. The agglomerins are in the plasma globulin fractions and are never found in the albumin fraction. Fibrinogen at a concentration of under 1 per cent is not an agglomerin; but under pathologic circumstances, when agglomerins are present, fibrinogen leads to a further acceleration of the sedimentation rate. There are numerous low molecular weight compounds which are capable of blocking the agglomerins. Some of them (for example laurinic acid) have the property of inhibiting all the different agglomerins (full blockers). Others (for example Antazoline-Antistin) are active only against a few of them (partial blockers). In this way there is a characteristic spectrum for each agglomerin. The blocker substances are in balance with the agglomerins in the plasma; the blockers are not adsorbed to the surface of the red cells. Most of the antipyretic drugs are “full blockers” (for example Phenylbutazon, salicylic acid, gentisinic acid, Atophan). Chloroquine and Cortisone are partial blockers. It seems as if the different agglomerins are specific for different diseases. Long ago Fahraeus found that after incubation of plasma at 37 C. its high sedimentation rate for RBC was lost, and this was thought to be due to the formation of lysolecithin. The present studies showed that after incubation of plasma at 37 C. a high molecular weight substance develops which is not dialyzable (proinhibitor). This proinhibitor seems to be a lipoprotein, since it is converted to an active inhibitor by a specific serum lipase. It is of interest that nearly all antipyretic drugs are full blockers (for example Phenylbutazon, salicylic acid, gentisinic acid, Atophan). Chloroquine and Cortisone are partial blockers. It is concluded that the agglomerins leads to a regression of the inflammatory process. It is concluded that the agglomerins have their origin in the plasma cells. It was noted that intravenous endotoxins from E.coli produce a high ESR in rabbits. Thus it seems possible that in inflammation the breakdown of bacteria or tissue leads to the liberation of substances which stimulate the plasma cells to produce agglomerins.—H. M.
**ABSTRACTS**


Studies were performed on white inbred newborn mice (strain AB). Leukemia was induced in these animals by cell-free extracts of lymph nodes from rat leukemia, from culture medium and used to grow mouse leukemia lymph nodes and from the filtrate of mouse leukemia SOV 16. In about 40 per cent of the experimental animals virus particles were found in lymph node sections. The particles were 60 to 150 μm in diameter and were mostly extracellular, although some were seen in cytoplasm but not nuclei.—H. M.


In experiments with dogs and man it was demonstrated that pretreatment of donors with alpha-tocopherol (i.m. injection of 200 mg. on 4 successive days) resulted in a remarkable reduction of hemolysis in the heart-lung machine and in blood bank bottles. In blood stored 28 days the average reduction of hemolysis from 10 donors was 57 per cent, as compared to blood of the same donors when they were not pretreated.—H. M.

**Toxic Marrow Failure Treated by a Homograft of Fetal Haemopoietic Tissue.** J. B. Bridges, J. M. Bridges, G. J. A. Edelstyn, A. R. Lyons and M. G. Nelson. From the Queen’s University, Belfast, Northern Ireland. Lancet 1:629, 1960.

A woman who developed generalized marrow aplasia while being treated for disseminated mammary carcinoma with thio-TEPA and testosteron was given 18 ml. of a suspension of liver from a male fetus intravenously. Her clinical condition immediately improved, and the leucocyte and platelet counts rose. Studies of nuclear chromosome of the neutrophils and differential agglutination of the red cells both showed that donor cells matured and survived in the patient’s circulation for about three weeks; rejection of the graft at this time was followed by restoration of normal marrow function in the host.—R. M. H.


Isolated rabbits kidneys were perfused with 80 μ1. of autologous blood circulating in a closed circuit. Borsook extracts from this blood were tested on white mice. When the kidneys were perfused under conditions of hypoxia, the extract exhibited marked erythropoietic activity (250 per cent increase of reticulocyte number and marked increase of erythroblast percentage in bone marrow). Extracts from fully oxygenated kidney perfusates possessed only insignificant erythropoietic activity. Control experiments, in which extracts from hypoxic blood circulated in the perfusion apparatus without the kidney, were negative.—E. K.

**ERYTHROCYTES**


The incidence of the blood factors RhA, RhB and RhC in Caucasians and Negroes was determined. In “standard” Rh positive blood, all 3 factors were almost invariably found, but in weak variant Rh positive blood, about half were found to lack one or more of these factors. RhA, RhB and RhC appeared to be relatively independent of each other. The authors fail to state whether the Rh,- factor is distinct from, and/or the sum of, RhA, RhB, RhC, RhD, and other Rh factors yet to be observed.—R. E. R.


Very extensive blood groupings were performed on 801 Maya and non-Maya Indians in Mexico and Guatemala. Of interest was the finding of Lu* absence, a relatively high frequency (20 to 30 per cent) of phenotype Le (a-b-) and a very low frequency of phenotype Le (a+b-). The report is thorough, and the results are compared to other similar studies of Amerinds.—R. E. R.
A 'New' Human Blood Group Antigen, Swa.

The red cells of a donor named Swann were found to be strongly agglutinated in all media by the serum of a patient with autoimmune hemolytic anemia of the 'cold' non-gamma globulin type. The patient's serum also contained anti-A and anti-Wr, of which antibodies could be separated by absorption from the antibody against Swann's cells. No other examples of this latter antibody were found in over 1200 normal sera, but several examples were found in other cases of autoimmune hemolytic disease, all of which also had anti-Wr, and some also anti-Mi or anti-Vw. These antibodies could all be separated from each other by absorption without significant loss of avidity of titer. The 'new' antigen is designated Swa. Nearly 30,000 random blood samples from adults revealed 4 more Swa positives, 2 of whom were related. Family studies showed the antigen to be inherited as a Mendelian dominant character which segregates independently of the ABO, Rhesus, MNSs, Kidd (and ABH secretion), Duffy and Kidd blood group systems.

A "New" Antibody, Anti-Rhc, Resulting from Iso sensitization by Pregnancy with Special Reference to the Hereditary of a New Rh Agglutinogen Rhc.

A patient of type Rhc (ccD\textsuperscript{+}Ee) was found to be immunized to what appeared to be Rhc, and to have given birth to mildly erythroblastotic fraternal twins. Studies with 2 other related sera from previously reported cases revealed the woman to be Rhc\textsuperscript{+} and Rhc\textsuperscript{+} positive. She was therefore classified as type Rhc\textsuperscript{+}Ee and her antibody as anti-Rhc. Family investigation revealed 3 siblings, 2 of whom were twins, to have the same apparent genotype.

Anti-A Haemolysin in Group O Blood Donors.

A routine screening test for the detection of anti-A haemolysin in group O donors is described, using horse serum as a source of complement. In a series of over 25,000 consecutive group O donations, 8.1 per cent of serum samples gave a positive result. The frequency increased progressively with age of the donor, from 5.2 per cent of those under 20 years to 11.0 per cent of those aged 50 and over. There was no evidence that the routine inoculations received by members of the armed forces resulted in an increased frequency of anti-A hemolysins among such donors as compared with the civilian donor population.—R. M. H.


These 2 excellent articles formed the Oliver-Schirp lecture delivered to the Royal College of Physicians of London in March, 1959. The first begins with a survey of the early histo-blood transfusion, which leads up to a discussion of the discovery of human blood groups, and the characteristics of blood group antibodies in vivo. Methods of estimating the survival of transfused incompatible red cells in vivo are considered, with particular reference to the use of very small volumes (less than 1 ml.) of labeled cells, and the use of \textsuperscript{3}P\textsubscript{32} as a label for control sample of compatible cells injected simultaneously. The second lecture is devoted to the relationships between the interactions of red cells and serum in vitro and the fate of the same red cells in vivo, illustrated mainly by the author's own published and previously unpublished work. It is shown that incomplete antibodies which bind complement bring about rapid removal of injected red cells, mostly in the liver, with half-time of a few minutes, whereas those which do not bind complement result in a slower rate of removal, mainly in the spleen, with a half-time varying from minutes to days, depending on the antibody titer. Other aspects considered include the relationships between the in vivo effect of antibodies and their thermal range, and titer in vitro, the rate of transfer of Rh and from sensitized to unsensitized cells, and the use of an in vivo compatibility test with labeled red cells in cases in which in vitro testing may give misleading results, such as some cases of acquired hemolytic anemia. Finally, the occurrence of incompatibility without demonstrable antibodies is illustrated and discussed. These two papers, and particularly the second, should be read in full by all those concerned with blood grouping or the mechanisms of red cell destruction.—R. M. H.

A NEW METHOD FOR DETECTING INCOMPLETE RH ANTIBODIES BY MEANS OF METHYLENE BLUE.
ABSTRACTS


Methylene blue dye indicates the presence of incomplete Rh antibodies by precipitation and by color reaction. This method of detecting incomplete Rh antibodies differs completely from those formerly used. Further possibilities of applying the methylene blue test are discussed.—S. R. H.


Two cases of hemolytic anemia and renal failure are described, one following the ingestion of high doses of phenacetin, and the other treatment with p-aminosalicylic acid. In each case the anemia appeared to be of the autoimmune type. The direct antiglobulin test was positive, the serum contained a factor capable of causing normal red cells of the patients’ blood to agglutinate by antigen antibody serum, and incubated in the presence of the appropriate drug. The glutathione stability test was normal, and an inherent red cell defect as a cause of the hemolysis seemed unlikely. The cause of the renal failure is discussed; although this may have been a result of intravascular hemolysis, the alternative possibility of a direct toxic effect on the renal parenchyma seems likely in the case due to phenacetin.—R. M. H.


A presumed case of fetal-maternal transfusion with neonatal anemia is presented. The severe anemia in the newborn infant was unassociated with hyperbilirubinemia, positive Coombs test or spherocytosis. In addition, 3.5 per cent Hgb F was found in the maternal circulation, with a gradual reduction to normal levels within 2 months. The infant’s course was marked by uneventful recovery following exchange transfusion and iron therapy.—A. I. C.
THE USE OF HEPARINIZED BLOOD FOR EXCHANGE
TRANSFUSION IN INFANTS. Herschel P. Bentley, Jr., Newell R. Ziegler and William Krivit. From

Because of the possible implication of stored citrated blood in the pathogenesis of untoward
reactions associated with exchange transfusions, the authors have carried out 152 exchange trans-
fusion procedures using heparinized fresh blood. The donor blood was collected in siliconized sys-
tems containing 20 mg. of heparin per 500 ml. whole blood and was used immediately there-
after. Following completion of the exchange procedure, 10 mg. of protamine sulfate were given
intramuscularly. No deaths occurred during the exchange transfusion, but 2 deaths may have
been associated with the procedure. Prolonged clotting times found immediately after the ex-
change transfusion were normal or near normal 15 minutes later. The authors conclude that the
potential hazards of hypocalcemia and citrate intoxication as well as those from hyperkalemia
from the use of stored blood make the use of heparinized blood exchange transfusion less
hazardous.—I. S.

THE ADMINISTRATION OF SODIUM GLUCURONATE
TO JAUNDICED NEWBORN INFANTS. J. Henry
Dwyer and Carolyn M. McCue. From the De-
partment of Pediatrics, Medical College of Vir-
ingia, Richmond, Va. Pediatrics 24:400,
1959.

Based on reports that a reduction in the con-
centration of serum bilirubin might be achieved by the parenteral administration of sodium glu-
curonate the authors studied the effect of this
mode of therapy in 16 newborn infants with
hyperbilirubinemia, including 10 babies with he-
molytic disease due to ABO incompatibility, 1
baby with E. coli sepsis and 5 with unexplained
hyperbilirubinemia. The sodium glucuronate was
administered as a 2 to 3 per cent solution by
vein or hypodermoclysis with a total quantity of
the sodium glucuronate varying from 2.4 to 21
Gm. The rate of administration was 180 to 200
mg. per kilo per hour. One patient showed a
decrease in the concentration of indirect bilirubin,
2 cases showed a slight increase in bilirubin, and
in 13 cases no change was apparent. It was con-
cluded that the parenteral administration of so-
dium glucuronate does not enhance the forma-
tion of bilirubin glucuronide in the newborn
infant and that it is an inefficient and unreliable
method of reducing the concentration of bilirubin
in serum.—I. S.

INFANTILE PYKNOCYTOSIS. Philomena Tuffy, Audrey K. Brown and Wolf W. Zuelzer. From the
Wayne State University College of Medicine,

In the course of study of hyperbilirubinemia
in full term normal infants not giving evidence of
hemolytic disease, the authors became aware of
the occurrence of red cells which were de-
scribed as “distorted, completely irregular, densely
stained erythrocytes, usually appreciably smaller
than the undistorted cells, and having from sev-
eral to many spiny projections.” These red cells,
which were very dense, were designated as
“pyknocytes” and conforming to the erythrocytes
described by previous authors under such designa-
tions as “burr cells” and “irregularly distorted
and contracted erythrocytes.” The authors deter-
mined the frequency of occurrence of such erythro-
cytes in normal adults, full term infants and
premature infants and then were able to identify
a hemolytic disorder in young infants associated
with a significant increase in these cells. In nor-
mal adults the pyknocytes never constituted more
than 0.3 per cent. In unselected full term healthy
infants, however, the number of pyknocytes ranged
from 0.3 to 1.9 per cent in the first few
days of life. It was the authors’ impression that the
number of pyknocytes increased somewhat
at 5 to 8 weeks of life and then rapidly decreased
after the second or third month. Premature in-
fants were found to have greater numbers of
ypknocytes than full term infants, the range
being 0.3 to 5.6 per cent. Again, it was the im-
pression that the number of pyknocytes tended
to increase within the first several months of
life. There was no correlation between the num-
er of pyknocytes in the normal premature and
full term infants with reticulocytosis, bilirubinemia
or serologic incompatibility between mother and
child. The authors describe 11 cases of hemolytic
anemia associated with an increase in the num-
er of pyknocytes over the previously desciribed
normal limits. In 7 of these patients the onset
of the hemolytic anemia occurred in the im-
mediate neonatal period with jaundice as the
presenting symptom. In 2 of these patients the
icterus was so severe that an exchange trans-
fusion was considered necessary. In one patient
who presented 50 per cent pyknocytes before
the exchange procedure a majority of the erythro-
cytes again were found to be pyknocytes follow-
ing replacement transfusion thus suggesting the
possibility of an extracorpuscular mechanism af-
fcting not only the infant cells but donor cells
as well. The other 5 infants who had demon-
strated jaundice in the neonatal period were
noticed primarily because of the development of
anemia at 3 to 7 weeks of life. The anemia was

ABSTRACTS
ABSTRACTS

associated with a marked reticulocytosis and with the presence of significant numbers of pyknocytes. Two infants of the 11 had never demonstrated icterus whereas 2 others who had shown no unusual jaundice in the immediate neonatal period developed this symptom at 3 weeks of age.

In the entire group of 11 cases, hemoglobin levels ranged from 4.6 to 9.7 gm. per cent at about 3 weeks of age, and the pyknocyte count from 5 to 33 per cent. There seemed to be a good degree of correlation between the severity of the anemia and the intensity of the pyknocytosis. In only one patient were Heinz bodies demonstrated.

Studies of erythrocyte glutathione levels and glucose-6-phosphate dehydrogenase were consistently normal, as were electrophoretic studies of the hemoglobin. Spleenomegaly was found in all but 2 infants, and hepatomegaly was usual. Serum transaminase levels were found to be high in 4 of the 7 cases studied but there was no other laboratory evidence of liver disease or dysfunction. Likewise, there was no indication of nitrogen retention. No history of drug ingestion by either mother or child was elicited. Two of the patients were siblings, and 3 of the 11 cases were of Jewish origin; 1 was Negro. The high incidence of Jewish infants and the low incidence of Negro infants were in contrast to the composition of this age group in the population of the hospital from which the report was submitted. The hemolytic process in all 11 infants disappeared by the age of 4 months, and complete recovery was the rule. The authors distinguish infantile pyknocytosis from those other disorders in which similar erythrocytes have been described, namely gastric carcinoma, bleeding peptic ulcer, uremia, the uremic-hemolytic syndrome, toxic hemolytic anemia in premature infants who had been given excesses of vitamin K, hemolytic anemia of primquine sensitivity, and "acanthocytosis" in which abnormal erythrocytes are associated with celiac syndrome, atypical retinitis pigmentosa and neurologic manifestations but not with hemolytic anemia. The authors postulate that infantile pyknocytosis may be linked in some way to metabolic processes characteristic of early infancy and that there may be an additional genetically determined predisposing factor.—I. S.


The median serum levels of pantothenic acid, thiamine and "folic acid" of newborns were found to be approximately fivefold the levels of new mothers. The pantothenic acid levels of new mothers were normal, but their levels of thiamine and "folic acid" were low. The results strongly suggest parasitization of available vitamins by fetus at expense of mother.—V. H.


This paper reports a study of the urinary excretion of formiminoglutaric acid, an intermediate product in the metabolic breakdown of histidine, in patients who were clinically deficient in folic acid. Four such patients excreted considerably more formiminoglutaric acid during the 24 hours following an oral dose of histidine than did 6 patients with vitamin B12 deficiency or 7 normal subjects. Three of the folic acid deficient subjects also showed an increased excretion of formiminoglutaric acid after histidine; after treatment with folic acid, excretion before and after histidine fell to normal. Gross deficiency of folic acid, leading to frank megaloblastic anemia, is probably necessary before excretion is significantly increased. Nevertheless, the authors, after discussing the limitations of the test, suggest that it may be a valuable additional tool in the investigation of the megaloblastic anemias.—R. M. H.

Three patients with megaloblastic anemia due to folic acid deficiency responded with significant reticulocytosis to 0.4 mg. folic acid daily (intramuscularly) for 10 days. Three patients with megaloblastic anemia due to vitamin B<sub>12</sub> deficiency did not respond to 0.4 mg. folic acid daily but did respond to 15 mg. folic acid daily. Therapeutic trials should be conducted with smaller doses, which are physiologic, in order to avoid making the erroneous diagnosis of folic acid deficiency in a patient who has vitamin B<sub>12</sub> deficiency. Interestingly, one of the patients with folic acid deficiency, proved both by therapeutic trial and low serum “folic acid” level, did not have an elevated urinary excretion of formimino-glycine (FGA). This provides further evidence that urinary FGA excretion may not be as useful a parameter of nutritional folic acid deficiency as the serum “folic acid” level or therapeutic trial.—V. H.

Megaloblastic Anemia Associated with Anticonvulsant Drugs. J. M. Flexner and R. C. Hartmann. From the Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tenn. Am. J. Med. 28:386–396, 1960.

Two patients taking anticonvulsants developed megaloblastic anemia. In both the anemia was completely explainable by dietary folic acid deficiency, and responded to folic acid per os (0.5 mg. daily in one case, 15 mg. daily in the other). The authors review the literature on megaloblastic anemia associated with anticonvulsants and discuss the possibility that malnutrition may be a prerequisite for the development of megaloblastic anemia in such cases.—V. H.


Two children with pernicious anemia (with classic Schilling test results) had normal gastric structure and function. As the author states, these findings add evidence to the hypothesis that the gastric manifestations of pernicious anemia (other than the inadequate intrinsic factor secretion) are not an intrinsic part of the disease, but are secondary to chronic deficiency of vitamin B<sub>12</sub>—V. H.


The authors present evidence indicating three phases in the mechanism of intrinsic factor absorption: (1) A nonspecies related competitive binding by intrinsic factor of dietary vitamin B<sub>12</sub>; (2) adsorption of intrinsic factor-bound B<sub>12</sub> to the intestinal mucosa involving a calcium-dependent bond, and (3) release of vitamin B<sub>12</sub> at the surface of or within the intestinal wall by a species-dependent process.—V. H.


Injection of 1 mg. vitamin B<sub>12</sub> prolonged the Achilles reflex contraction time of hypothyroid patients “overtreated” with thyroid (criterion: sustained sense of clinical impairment at the lower dosage level), but had no effect on the Achilles reflex contraction time of normal or hypothyroid subjects. It is hard to lend credence to this report, which is not based on any known action of vitamin B<sub>12</sub>—V. H.


Five rabbits received 4 to 6 μC Co<sup>60</sup>B<sub>12</sub> over the period of a month. Acute hepatic necrosis was then induced by an oral dose of 1 ml./Kg. of CCl<sub>4</sub>. Serial estimations of blood radioactivity and serum transaminase showed parallel changes, with maximum values between 24 and 48 hours. This finding suggests that the rise in serum B<sub>12</sub> during toxic hepatitis is due to release of the vitamin from damaged parenchymal cells.—G. M.


After several years on a diet severely deficient in folic acid and B<sub>12</sub>, a 19 year old soldier developed a generalized malabsorption syndrome, including inability to absorb a test dose of radioactive vitamin B<sub>12</sub>. After being placed on a good diet the patient “spontaneously” recovered. This case might be considered evidence in favor of the possibility that dietary deficiency of folic acid may result in generalized malabsorption, an explanation previously suggested for the pathogenesis of “tropical” sprue.—V. H.
ABSTRACTS


Less than 8 per cent of orally administered FeSO₄ appeared in the red cells of each of 10 patients with idiopathic steatorrhea or celiac disease, but a marked improvement in absorption occurred in 6 of 7 cases after a few days' corticosteroid therapy; adrenal steroids were more effective in this respect than corticotropin. In another case, iron absorption fell to 8 per cent a week after cortisone was discontinued. Steroids had no constant effect on iron absorption in 12 control subjects without anemia or gastrointestinal disease. A Gluten-free diet was also shown to improve the absorption of FeSO₄ in 4 patients with celiac disease, but in only 1 case was the improvement as dramatic as that following the steroids.—R. M. H.


Prophylaxis of the late iron deficiency anemia of full term and premature infants through the use of an iron-fortified milk formula is reported. The iron-containing milk employed was a commercially prepared formula containing 12 mg. of elemental iron per liter. Similar milk formulas without added iron were administered to control full term and premature babies. In both control and experimental groups milk alone constituted and supplemented infants did a fall of hemoglobin concentration to this level take place. Thus, if the study had been carried further, the differences between supplemented and control groups would have been expected to be much more striking. This study demonstrates clearly that elemental iron may be absorbed adequately from milk and that prophylaxis of the iron deficiency anemia of infancy may be readily accomplished.—I. S.


The effect of various antidotes in the treatment of acute iron toxicity in dogs was studied. The animals were given 200 to 300 mg. per kilogram of elemental iron by direct injection into the jejunum. Control animals demonstrated extremely rapid and marked elevations of the serum iron concentrations, and death occurred at 5 to 6½ hours, apparently from irreversible shock. Administration of 2 per cent Edathamil calcium-disodium intravenously soon after the introduction of the iron resulted in a lowering of the serum iron concentrations and a prolongation of life, but all animals died in shock. Of 2 animals treated with combine intravenous Edathamil calcium-disodium and oral installation of 20 per cent disodium orthophosphate dihydrate, 1 animal survived and the second died of suffocation. Intravenous administration of plasma and dextran were ineffective. The acute iron poisoning produced hypotension and shock long before tissue damage occurred, and it was concluded that the death of these animals was primarily due to irreversible shock and not to tissue damage. Blood of 1 animal was tested at intervals following the iron administration for the presence of ferritin, and none was found. In the treatment of iron poisoning in humans the authors suggest a regimen employing gastric lavage with 10 per cent disodium orthophosphate dihydrate, leaving 25 to 50 ml. in the stomach, intravenous administration of EDTA supportive measures with plasma and intravenous fluids and the administration of antibiotics.—I. S.


The erythrocytes of rats were labeled in vivo with S¹⁵ or Fe²⁺ and the animals bled at various intervals. Red cells were fractionated by serial osmotic hemolysis and the very young ones shown to have extremely fragile and relatively resistant components; both these components contained high activities of glutamic oxaloacetic transaminase and lactic dehydrogenase.—R. M. H.
STUDIES OF THE DIPYRRYLMETHENE pigment has not been determined. C. biirubin, humb its exact point of origin in the normal product of the bacterial reduction of the 3 compounds varies.

Violin-mesobilirhodin and glaukobilin and n-urobilinogen group. D-Urobihn appears to be with an enterie source of all 3 members of the composition of mesobilin mainly to glaukobilin. The composition of any particumlar state of health or disease. The bilin mainly to glaukobilin. The composition of uruhibin is

\[
\text{ABSTRACTS}
\]

The relationship of the fecal dipyrromethene compounds or mesobilifuscins to hemoglobin metabolism has been investigated in 4 human subjects with the aid of N\(_{15}\)-labeled glycine. The data appeared to point to an anabolic source of mesobilifuscin because of the finding of a lack of significant concentration in N\(_{15}\) in mesobilifuscin at the time of destruction of the mature circulating cytochrome which contained N\(_{15}\) in the protoporphyrin ring and gave rise to stercobilin N\(_{12}\), which was markedly elevated. There is an early incorporation of N\(_{15}\) in mesobilifuscin which appears to precede the early incorporation of N\(_{15}\) into stercobilin, and there is a lower N\(_{15}\) value in mesobilifuscin than in stercobilin during the period of time when there is no apparent degradation of N\(_{15}\) protoporphyrin from the cytochrome.

---

G. W. J., III.


The quantities of mesobilifuscin and urobilinogen in the feces were compared in a group of normal subjects and in various cases of anemia and liver disease. Mesobilifuscin is present in normal feces from 7 to 18 mg. per day and the urobilinogen:mesobilifuscin ratio (U/Mbf) is 8 : 14. Conditions in which there were increased erythropoietic activity, i.e., hemolytic anemias and megaloblastic anemias, were generally associated with increased fecal mesobilifuscin (Mbf) and urobilinogen (U) and low or normal U/Mbf ratios. On the other hand, hypop-pregenerative anemias usually had decreased Mbf and high U/Mbf ratios. The data support the concept that mesobilifuscin is anabolic and is formed during the biosynthesis of heme in liver disease the mesobilifuscin is very scanty and the U/Mbf ratio high, but crude urinary mesobilifuscin is consistently elevated. Whether this represents a diversion of Mbf ordinarily excreted in the bile, or derivation from bilirubin or urobilinogen in the kidneys or urine, has not yet been determined.---G. W. J., III.

STUDIES OF THE DIPYRRYLMETHENE ("Fuscin") Pigments. I. The Anabolic Significance of the Fecal Mesobilifuscin. A. Sigrid Gilbert-
still direct, but that the pituitary and adrenal hormone activities seemed to facilitate this process, and however, results from other laboratories (J.Clin.Invest. 39:1001, 1959) have been quite discouraging.—A. J. E.


Decreased oxygen need has been proposed as a cause for anemia in hypophysectomized animals. In order to test this hypothesis, hypophysectomized rats were given thyroxine until their oxygen consumption had reached normal values. This was associated with a return of red cell mass and bone marrow activity towards normal.

However, the addition of growth hormone and cortisone seemed to facilitate this process, and it was concluded that the oxygen-need theory of post-hypophysectomy anemia is probably correct, but that the pituitary and adrenal hormone still must be of some significance for erythropoietic function.—A. J. E.

**Effect of Erythropoietin on the Mitotic Rate of Erythroblasts in Bone Marrow Cultures.** Y. Matonth and E. Ben-Porath. From the Rothschild-Hadassah University Hospital, Jerusalem, Israel. J.Lab.& Clin.Med. 54:722, 1959.

Mitotic activity was evaluated in rabbit bone marrow after incubation with normal or anemic serum. Colchicine was added to the serum, and the number of mitotic figures per 1000 immature erythroid cells was determined after 5 to 12 hours. The results supported the authors' earlier work (J.Lab.& Clin.Med. 51:420, 1958) by revealing a high mitotic activity in the bone marrow samples suspended in anemic serum than in the samples suspended in normal serum. Since bone marrow from anemic animals suspended in normal serum showed a high mitotic activity, the authors concluded (somewhat surprisingly) that erythropoietin is present in erythroid cells. These results kindle the hope that it may be possible to devise an in vitro test for erythropoietins. However, results from other laboratories (J.Clin.Invest. 39:1001, 1959 and Stohlman: The Kinetics of Cellular Proliferation, New York, Grune & Stratton, 1959, p. 299) have been quite discouraging.—A. J. E.


All working with the present crude in vivo systems for the assay of erythropoietin agree on the need for an accurate in vitro technic. Reports so far have been confusing since some workers claim to have developed workable in vitro techniques whereas others, employing basically the same tools, have been completely unsuccessful. In this report, the authors have tested plasma from anemic and normal rabbits and rats on bone marrow suspension from rabbits and rats respectively. The parameters measured were oxygen consumption, bone synthesis, DNA synthesis and iron uptake, and the tools employed were C14-formate, glycine-2-C14, H3-thymidine and Fe59. The results were fairly conclusive in that none of the metabolic activities so measured was influenced by the presence or absence of erythropoietin in the plasma.—A. J. E.


In order to standardize the activity of various preparations of erythropoietin, it is suggested to define 1 unit of erythropoietic activity as the activity induced by administering 5 µM of cobalt to 30 hour-starved adult rats. The activity is determined by measuring the 16 hour Fe59 red cell uptake. Fractionation of plasma from phenylhydrazine-treated sheep yielded a stable standard preparation containing 1 unit per mg. Further fractionation yielded fractions with activities as high as 50 units per mg.—A. J. E.


In a series of papers, Naets has demonstrated the presence of erythropoietic factor in plasma.
and urine of dogs and provided powerful support for the theory that this factor is produced exclusively by the kidney. Erythropoietic factor could only be demonstrated in plasma and urine when the hematocrit was lower than 15 per cent and 10 per cent, respectively, but partial inactivation through boiling (Borsook's technic) was held responsible for the poor yield. The erythropoietic response to bilateral nephrectomy and bilateral ureter ligation was examined. Nephrectomy was found to result in a rapid depletion of erythroblasts from the marrow (within 72 hours) and iron turnover and iron utilization were reduced severely. Bilateral ureter ligation on the other hand, hardly had any effect on the bone marrow, iron turnover or iron utilization. In one nephrectomized dog the erythroblastic depletion could be prevented by injections of erythropoietic factor, and it was concluded that the kidney is probably the source of an erythropoietic factor. Attempts to demonstrate this factor in extracts from kidneys obtained from anemic dogs were not too successful. Extracts from "anemic kidneys," normal kidneys, anemic livers and "anemic kidney" extract was somewhat more active than extracts from normal kidneys and "anemic livers," it was only questionably more active than extracts from "anemic" spleens. These results are completely different from results in rabbits in whom both ureter ligation and nephrectomy are associated with erythropoietic suppression and in whom red cell production continues at a slow rate even 9 days after nephrectomy. However, the results in dogs are so clear-cut that they obviously need both confirmation and extension.—A. J. E.


Serum (3 ml.) from 14 normal and 29 anemic patients was injected once into rabbits, and hematologic changes in the blood and marrow were observed. With one insignificant exception the injection of normal serum did not produce any changes. Following the injection of sera from anemic patients, however, the rabbits' reticulocyte count went almost invariably up from 2 to 5-6 per cent, and the marrow showed a corresponding increase in the erythromyeloid ratio. The changes took place from 2 to 5 days after injection and lasted for several days. They were particularly marked in cases of posthemorrhagic and vitamin B12-deficiency anemias, but much less so in iron deficiency cases. The Hb and RBC did not change at any time. There was no change in a few additional cases of polycythemia and hepatic cirrhosis. The study is offered as contributory clinical evidence of the presence of erythropoietin in serum of certain anemic patients.—J. J. B.


Another in an excellent series of papers in which this group has attempted to enumerate and evaluate the erythropoietic cells. When examined quantitatively, it was found that the erythropoietic response to hemolysis is roughly the same as to blood loss, despite the often much higher reticulocyte percentage in the former condition. Acute anemia was observed to increase the rate of erythropoiesis to about 3 times normal and was associated with a shift of late nucleated red cells and reticulocytes from the marrow to the peripheral blood.—A. J. E.


Nine male patients with various types of anemia and with hemoglobin ranging from 5.2 Gm. per cent to 9.3 Gm. per cent were studied at rest and after heavy exercise. At rest the oxygen and CO2 transport were normal. Minute ventilation and respiratory rate were increased above normal, but the alveolar ventilation was found to be normal. Cardiac output was increased, and there was an increased desaturation of venous blood. During exercise the ventilatory activities and the cardiac output increased to the same levels found in normal control subjects similarly stressed. However, these compensatory mechanisms were inadequate to prevent a marked depression of oxygen intake and CO2 production in comparison with the normal subjects. Despite the fact that the anemic subjects must have increased their rate of anaerobic metabolism to meet the demands of the exercise, there was no evidence for an increased release into the circulation of acid metabolites and no symptom which could be attributed to the anaerobic metabolism. In the anemic subjects the oxygen dissociation curve was found to be shifted to the right in its lower half, a shift which also appeared to be present in the normal subjects during exercise, and it

This extensive review (401 references) is very valuable for the understanding of the physiologic effect of a change in the hemoglobin concentration of circulating blood. The author discusses the factors which lead to a decrease in the tissue tension of oxygen and the cardiovascular compensatory devises mobilized by this decrease. The circulatory response to anemia is reviewed, but


Despite a wide-spread impression that cobalt accelerates erythropoiesis at the expense of tissue anoxia, no conclusive biochemical evidence for such an action exists. The author reviews some of the literature concerning possible toxic effects of cobalt and shows that 1 mg. doses of cobalt in rats result in an increased urinary excretion of coproporphyrin. He regards this as a toxic reaction but points out that 0.25 mg., although capable of producing polycythemia, will not increase the excretion of coproporphyrin. With both dosage schedules adrenal hypertrophy was noted, and the spleen revealed disintegration of the follicular structure, decrease in lymphatic tissue and reticular hypertrophy. The action of cobalt appears to be as mysterious as ever.—A. J. E.


Two cases of renal polycythemia are described; one was associated with unilateral hydronephrosis, the other with a unilateral polycystic kidney. In each case the polycythemia disappeared after removal of the diseased kidney; the red cell volume returned to normal for the lean body mass in one case, and very nearly to normal in the other. The plasma iron turnover, which was initially raised, reverted to normal after operation. Erythropoietin estimations were performed by bioassay, using the reticulocyteosis induced in rats by intraperitoneal injections of graded doses of plasma. Preoperative specimens showed erythropoietin levels of 8 and 43 times normal, respectively: these fell to normal after operation. In one case estimations of erythropoietin in blood obtained from the aorta and from the renal vein at operation showed less activity than blood obtained simultaneously from a peripheral vein.—R. M. H.


Nine patients with hematocrits between 51 and 62 per cent but with normal total red cell volume were studied. It was found that the plasma volumes, as measured with iodinated serum albumin, were also normal. Consequently, the relative polycythemia was not caused by a reduction in plasma volume. It is suggested that shifts of blood from high hematocrit compartments (like the spleen) to circulating blood or even to low hematocrit compartments (like the kidney) may be responsible for the polycythemia.—A. J. E.


Equimolar mixtures of adult human and canine hemoglobin were acidified to pH 4.7, to allow dissociation into subunits to occur, and then were neutralized. On electrophoresis 2 new components were present as well as the original ones, suggesting that mixed molecules had been formed and that both hemoglobins must split into subunits with similar structures at their surfaces of union. Human and canine carboxyhemoglobins A can be made to dissociate and recombine with their respective ferricdimers; the observation that the corresponding recombination cannot be brought about in the case of bovine hemoglobin suggests that it does not dissociate at pH 4.7. This technic was used to recombine hemoglobins D, E and J with hemoglobins S and C. No difference was noted on subsequent electrophoresis; this was explained on the grounds that the abnormality of each of the first three hemoglobins lies in the beta chain, the abnormal chain of hemoglobin S and C. On the other hand, hemoglobins I and Hopkinso-2 showed changes in electrophoretic composition when recombined with hemoglobins S and C, S and J, respectively, suggesting that the abnormalities are in the alpha chain in both cases.—R. M. H.

A Terminal Peptide Sequence of Human Haemoglobin? J. A. Hunt and V. M. Ingram. From
Broad-based gamma globulin, without any rise
B. C.

Electrophoresis of serum proteins revealed a peak. The gamma globulin pattern was early childhood, “the hands were the optimal sites for roentgen identification of this disease.”

A record of observations on 14 cases of thalassemia-Hb. E disease in children: In infancy and early childhood, “the hands were the optimal sites for roentgen identification of the disease.” Electrophoresis of serum proteins revealed a broad-based gamma globulin, without any rise in the peak. The gamma globulin pattern was similar to what the authors had found in infantile cirrhosis of liver.—J. B. C.

LEUKOCYTES


A slightly increased oxygen consumption was found in leukocyte homogenates from thyrotoxic persons as compared to those of euthyroid persons. It was also found that the leukocytes contained a thyroxine deiodinase, and some of the properties of this deiodinase were described. The authors suggest that since leukocytes are known to participate in metabolic abnormalities in several human diseases, they may comprise a useful test organ for a much-needed direct study of peripheral thyroid hormone metabolism in man. —T. E. B.


The lipid content and composition of rabbit polymorphonuclear leukocytes were determined. Both lipid content and composition were kept remarkably constant under different metabolic conditions (rest and phagocytosis), but during active phagocytosis the leukocytes showed an increase in the rate of turnover of lipids. It is suggested that the increased lipid turnover reflects a general metabolic stimulation of the leukocyte accompanying phagocytosis.—T. E. B.


The addition of zinc in 10⁻⁴ M concentration was found to both prevent and restore the progressive loss of alkaline phosphatase activity which human leukocytes show on storage in saline. Zinc is a known component of human leukocytes and obviously may be of significance in leukocyte alkaline-phosphatase activity.—T. E. B.


Cystine crystals in leukocyte cytoplasm were found in the Wright-stained smears of the patient’s buffy coat and could be well seen in unstained buffy coat preparations examined with phase contrast microscopy. To avoid confusing artifacts in searching for cystine crystals, it is...
ABSTRACTS

best to use blood collected with an anticoagulant such as heparin which does not result in crystal formation. The finding of cystine crystals in peripheral leukocytes suggests that study of the intermediary metabolism of leukocytes in cystine-storing disease may provide a convenient tool for the understanding of the underlying metabolic defect.—T. E. B.


“The lymphocyte is perhaps the least understood of all metazoan cells.” The authors conclude their brief article with this statement after studying the behavior of cells from a mouse ascites lymphocytic tumor within macrophages. The latter react to the presence of living lymphocytes within it by forming another membrane, which is not very different from the contact between two contiguous cells. “With what peculiar immunity is the lymphocyte endowed that permits it to wander in and out of cells and undergo mitosis within them?”—O. P. I.


Sixteen of 20 patients with disseminated lupus erythematosus demonstrated a delayed cutaneous reaction (erythema and induration greater than 10 mm. in diameter) to intradermal injection of autologous leukocytes and platelets. A similar positive reaction occurred in only 2 of 7 patients with rheumatoid arthritis and in only 1 of 52 miscellaneous control patients. The authors suggest that the delay of 18 to 24 hours in appearance of the cutaneous reaction in lupus patients may reflect the time required for leukocyte breakdown with resultant exposure of nuclear material to the circulating abnormal “anti-nuclear” gamma globulin so frequently found in patients with lupus.—T. E. B.


Skin homograft sensitivity (defined as accelerated homograft rejection) was transferred from sensitive to nonsensitive human recipients by the injection of deoxyribonuclease-treated leukocyte extracts prepared from sensitive donors. The conditions for successful transfer of homograft sensitivity were carefully defined. In animals, in sharp contrast to humans, intact viable cells are apparently necessary for passive transfer of transplantation immunity. Serum from sensitized persons was apparently incapable of transferring homograft sensitivity. The transfer of homograft sensitivity by leukocyte extracts is strikingly similar to the transfer of delayed type bacterial, fungal, viral and contact chemical hypersensitivities, which can be effected by minute quantities of peripheral blood leukocytes thus further suggesting a relationship of tuberculin type hypersensitivity to homograft sensitivity.—T. E. B.


Using the antiglobulin consumption test, a technically difficult method, the author demonstrated abnormal amounts of protein on the surface of washed leukocytes from about 50 per cent of patients with unexplained pancytopenia or granulocytopenia. The protein was described as being eluted readily from the leukocytes at 56 C., producing prolonged leukopenia on intravenous injection into rabbits, and migrating largely as a gamma globulin. An abnormal amount of protein was also found on the washed platelets of 66 per cent of patients with idiopathic thrombocytopenic purpura. This protein too was readily eluted, and on intravenous injection into rabbits it produced prolonged thrombocytopenia. Eluates from the leukocytes and platelets of control patients produced much less effect in rabbits. The author interprets his results as demonstrating the presence of autoantibodies attached to the appropriate cells of many patients with unexplained granulocytopenia or thrombocytopenia.—T. E. B.


ISOLATION OF “BIOLOGICALLY INTACT” MAST CELLS. B. Ucnás and I. L. Thon. From Karo-
When either a histamine liberator or antigen is applied to mast cells, they disrupt due to the activation of an enzymatic process located in the cell membrane and a slow reacting substance with histamine appears. However, the usual methods for isolating mast cells from rat peritoneal fluid are unsuitable because histamine leaks out, so to speak. Accordingly, the authors have obviated this pitfall by developing a technique which employs Ficoll (A B Pharmacia, Uppsala, Sweden), a highly water-soluble substance with properties similar to a polysaccharide character. The fraction used had a molecular weight around 400,000. One reason for the favorable result may be that the spinning down of the mast cells in Ficoll-salt solution and then their subsequent transfer to a buffer solution does not expose them to such osmotic strain as do the corresponding processes using concentrated sucrose solution as a centrifugation medium.—O. P. J.


Cells from 5 mast cell sarcomas of dogs were grown in tissue culture, but only cells from one tumor continued to multiply for 50 passages over a 2 year period. Apparently the cell now has the same cytologic characteristics that were observed soon after it was placed in culture. The cells contain two different structures which stain with Sudan black B. The presence of PAS positive granules may mean that this mast cell produces heparin which is not fully sulfated.—O. P. J.


Analysis of 106 cases of leukemia substantiated the recent data in the literature that the age incidence of acute leukemia has recently become similar to that of chronic leukemia. Acute leukemia is now more frequent in adults over 50 than in younger patients.—S. R. H.


Two patients with acute myeloid leukemia are described who developed fatal hepatic necrosis and intestinal hemorrhage after standard treatment with 6-mercaptopurine (2.5 mg./Kg. per day). A review of 34 cases treated at the same hospital with 6-M.P. showed that 4 had terminal jaundice and hepatic necrosis and 5 terminal blood-stained diarrhea. Except for the raised serum glutamic pyruvic transaminase in one case, liver function tests were unhelpful. It is suggested that jaundice or anorexia with tenderness in the right hypochondrium should be considered as indications for withholding 6-M.P.—R. M. H.

HEMOSTASIS


An ether-precipitation method of preparing fibrinogen from the plasma of "time-expired" blood (21 or more days old) is described. Transfusion of this fibrinogen into a young woman with congenital afibrinogenemia resulted in normal hemostasis after tooth extraction. Blood fibrinogen levels were measured at intervals after transfusion on two occasions, and showed that between a third and a half of the transfused fibrinogen disappeared rapidly from the circulation, and that the remainder was removed at 14 to 15 per cent per day. This rate of removal compares well with those found by various methods in the case of fibrinogen prepared from fresh blood, and this suggests that "time-expired" blood is a suitable source of fibrinogen for clinical use.—R. M. H.


The authors utilize starch gel electrophoresis and moving boundary electrophoresis to analyze highly purified plasminogen prepared by their own methods. The purity of the starting material is about as good as that of previous investigators, and moving boundary electrophoresis separates the plasminogen into two components, comparable to the earlier results of Shulman. Starch gel, however, effects a separation of the plasminogen into 5 components, one of which contained most of the caseinolytic and fibrinolytic activity. The
ABSTRACTS

1069


Erythrocytes were incubated with varying components of the urokinase fibrinolytic system in this report, and adsorption was found to occur with urokinase, plasma inhibitors to urokinase and possibly plasminogen. Each of these materials could be eluted off the erythrocytes. Previous in vivo fibrinolysis, in dogs infused with beef thromboplastin, affected the adsorptive properties of the erythrocytes variably, causing an increase in inhibitory activity in the eluate from some animals and frank fibrinolytic activity in the eluates from others. The adsorptive capacity of the erythrocytes for urokinase was shown to be affected by the concentration of urokinase, the temperature of the incubation mixture, previous washing of the erythrocytes, and the pH of the incubation mixture. The authors speculate on the possible thrombolytic role of erythrocytes entrapped in a fibrin clot and the further role of the erythrocytes in contributing to “spontaneous” fibrinolysis in vivo by adsorbing urokinase or urokinase inhibitor from the blood of the intact animal.—A. J. J.


Epsilon-aminoacaproic acid has been shown previously to be a potent inhibitor of plasminogen activation. In this study, it was found that epsilon-aminoacaproic acid could be utilized in a concentration which would inhibit plasminogen activation in plasma but did not appreciably inhibit activation of plasminogen which had been adsorbed on 113I-labeled fibrin clots. This finding emphasizes the potential importance of selective inhibition of the streptokinase plasminogen system. The authors also confirmed the findings of Alkjaersig by their demonstration that plasminogen is adsorbed onto a clot as it forms. The rate of fibrin clot dissolution by streptokinase was increased by the addition of exogenous plasminogen.—A. J. J.


At least 2 fibrinopeptides (A and B) are released by the proteolytic action of thrombin on fibrinogen during the coagulation of blood. These fibrinopeptides have been analyzed for their amino acid composition by electrophoresis and reverse phase partition chromatography by Bettelheim. The present studies constitute a similar analysis on high purified fibrinopeptides isolated by the technic of Blomhâck and Vestermark, hydrolyzed in HCl and the residual amino acids converted to phenyl thiolydantoin derivatives and quantitatively estimated after separation on paper chromatograms. The author concludes that there are approximately 19 residues in peptide A and 21 in peptide B, with a calculated molecular weight of 1890 and 2460, respectively. No cysteine or cystine was found. A tabular presentation of the amino acids is made.—A. J. J.

Fibrinolysis and Fibrinolytic Activity in Man. S. Sherry, A. P. Fletcher and N. Alkjaersig. From Washington University School of Medicine, St. Louis, Mo. Physiol.Rev. 39:343, 1959.

This very excellent review is required reading for those investigators working in the field of fibrinolysis and thrombolysis. The review is packed with information, and it is unusually readable, due to the authors’ literate style and superior organization. The fact that the point of view is relatively definite in a complex field will undoubtedly please some and offend others but makes for a more compact, effective presentation. Highly recommended.—A. J. J.


Enhanced fibrinolytic activity was demonstrated in the blood of patients following electroshock, pyrogens, epinephrine, acetylcholine, ischemia and exercise. The fibrinolysis was attributed to an increase in plasminogen activator as measured by: activation of purified human plasminogen, lysis of 113I-tagged human plasma clots and the lysis of euglobulin clots with and without the addition of 10^{-4} M epsilon-aminoacaproic acid. No increase in plasmin could be detected by casein proteolysis and BAME esterase activity, nor could significant changes be detected in the plasminogen, fibrinogen and antiplasmin levels of the blood, as compared with control observations. The re-
RESPONSE may have been mediated through the nervous system as described previously by Kwaan, Lo and McFadzean, since fibrinolytic activity was produced locally in one experiment, in an arm in which the arterial and venous flow had been arrested during electroshock treatment by a blood pressure cuff 50 mm. Hg greater than the systolic pressure.—A. J. J.


Fibrinolytic activity was measured by a modification of the method of Celander and Guest (Fed.Proc. 17:63, 1958) in platelet-rich and platelet-poor plasma from 10 normal subjects. The activity was significantly greater in platelet-poor than in platelet-rich plasma. The addition of washed platelets to platelet-poor plasma inhibited fibrinolytic potentiality, but the addition of cephalin was without effect. No significant effect on fibrinolytic potentiality could be demonstrated by this method in 9 healthy males 3½ to 4 hours after a fatty meal, nor was there any significant correlation between fibrinolytic potentiality and serum cholesterol in 40 normal subjects.—R. M. H.


A wide variety of blood coagulation tests was performed on a group of healthy rural Bantu men, a group of white men with overt coronary heart disease and 2 control groups of healthy white men, 1 age-matched with the Bantu and 1 with the coronary group; some control subjects were included in both groups. Dietary histories were obtained from each group, and serum cholesterol and other blood chemistry investigations performed. The only significant difference in coagulation tests between the coronary group and their controls was in the plasma fibrinogen levels, which were higher in patients with coronary disease over the age of 50 than in normal men of the same age group. The Bantu differed significantly from their controls in having lower plasma prothrombin and factor VII, higher antihemophilic globulin and better prothrombin consump-

tion in siliconed (but not in glass) tubes. Thromboplastin generation was slightly greater in the Bantu group than in their controls, and fibrinolytic activity, as measured by clot lysis times, was significantly greater. The fat:calorie ratio and serum cholesterol level were very significantly lower in the Bantu than the other groups. The Bantu had lower serum albumin and higher serum globulin, and greater thymol turbidity and zinc sulfate values than the other groups. Possible explanations of some of these findings are given, and their significance in relation to coronary heart disease is critically discussed. They provide no clear evidence that the relative immunity of the Bantu to such disease is due to a lesser coagulability of his blood.—R. M. H.


Column chromatography on silicic acid was carried out on phospholipids obtained from acetone-dried human platelets by chloroform extraction. Fractions obtained had the following components: (a) phosphatidylethanolamine (PE)—phosphatidylserine (PS) plus inositol phosphatide; (b) phosphatidylethanolamine plus phosphatidylserine; (c) lecithin plus sphingomyelin; and (d) phosphatidylserine. Fractions containing only phosphatidylserine and phosphatidylethanolamine were rechromatographed in an attempt to obtain more complete separation. The resulting fractions, which appeared to contain only one component by paper chromatography (either PE or PS), were still contaminated with small amounts of the other since on hydrolysis both bases could be identified. In high dilutions each of these mixtures could support thromboplastin formation as tested in the thromboplastin generation test and prothrombin consumption test. It was concluded that both PS and PE were active in this regard. Inhibitory activity was not observed in the concentrations tested. The presence of choline phosphatides in the fraction resulted in loss of activity. Lysolecithin was not detected in any fractions.—R. G.


Normal plasma was subjected to continuous flow electrophoresis and the fractions tested for fibrinogen, prothrombin, proconvertin, PTC, AeG,
ABSTRACTS

AHF, Hageman and Stuart factors. There was incomplete resolution of the coagulation factors with fibrinogen and Hageman in beta and gamma globulin areas; prothrombin, proconvertin and PTC in the alpha-2, alpha-1, and late albumin areas; AcG in the albumin area, and Stuart factor in the albumin and prealbumin area. However, it was possible to obtain preparations of each of the following factors devoid of other coagulation activities: Hageman, proconvertin and Stuart factor. These findings indicate that in plasma proconvertin and Stuart factor exist as distinct entities from prothrombin.—R. G.


A procedure for the preparation of purified human and bovine prothrombin fractions is described. It involves adsorption of plasma by BaSO4, elution with citrate, ammonium sulfate fractionation and acid precipitation. Fractions of high specific activity for prothrombin were obtained, although they were still contaminated with other clotting factors. Filtration of bovine plasma through a 20 per cent asbestos Seitz pad before processing yielded prothrombin fractions which could not be activated to thrombin biologically unless clotting factor(s) obtained from serum presumed to be factor VII and X were simultaneously added. In this respect it resembled prothrombin derived from a patient with factor VII deficiency. It was concluded that the Seitz filter removed the essential prothrombin entities rather than altering the prothrombin molecule, thereby rendering it biologically inert. It was also concluded that prothrombin preparations which are readily activated are contaminated with nonprothrombin factors essential for rapid conversion of prothrombin to thrombin. Some biochemical and physiologic properties of the prothrombin preparations are described. Human plasma could not be made preferentially deficient in the nonprothrombin factors by passage through a Seitz filter.—R. G.


The authors describe a technic for the preparation of a fraction rich in prothrombin, proconvertin, Stuart-factor and PTC. The blood is collected through an ion-exchange resin (Dowex 50) column since anticoagulants containing trisodium citrate prevent the adsorption of prothrombin. The plasma is adsorbed by Baker tricalcium phosphate in 0.4 per cent concentration. The supernatant is used for the preparation of a I-A fraction rich in AHF. The prothrombin, proconvertin, Stuart factor and PTC are eluted with 0.18 M trisodium citrate. Two precipitations are effected with ethanol, the first at pH 6.8 to a final concentration of 18 per cent in order to eliminate the lipoproteins, and the second at pH 5.1 to a final concentration of 25 per cent. The precipitate thus obtained is dissolved in a stabilizing solution containing antithrombin (human serum) and heparin (4 mg.) at pH 6 to 6.5. The sterilizing filtration is effected with a chamberland 5 L 5 filter. The final product has a clotting activity 100 to 400 times per mg. of protein that of the original plasma.—G. M.


The authors investigated the adsorption of thrombin by particles of silica (bentonite and celite) from a fraction rich in prothrombin, proconvertin, Stuart factor, PTC and thrombin. It was demonstrated that the particles of silica are far more efficient thrombin absorbants than the usual prothrombin absorbants (barium sulfate and tricalcium phosphate). Differential adsorption of the coagulation factors present in this fraction showed that bentonite and celite enable thrombin to be selectively adsorbed and prothrombin to be preserved at the same time; but they do not allow complex VII (proconvertin and Stuart factor) and PTC to be retained, these sharing the fate of thrombin. Attempts to elute thrombin adsorbed by bentonite and celite were unsuccessful.—G. M.


Antithrombin levels were measured following the addition of varying amounts of thrombin to defibrinated plasma and following the intravenous injection of thrombin into rats. In the in vitro studies, the decrease in antithrombin was proportional to the thrombin added. Similar results were obtained in the in vivo studies. Heparin added to blood in vitro or injected intravenously decreased the plasma antithrombin titer. No measurable difference was found between the
antithrombin level of plasma and the antithrombin level of serum from the same animal. Thrombin generation in samples of calcified plasma was measured by the method of Pitney and Dacie, and values compared with changes in antithrombin. According to the author (and this could be interpreted differently) the amount of thrombin generated when full strength plasma or diluted plasma was used was only a fraction of the amount potentially available as estimated by the 2-stage method. More thrombin was generated by diluted plasma. The antithrombin of the residual serum was decreased corresponding to the thrombin generated. The author believes that the lack of significant difference between the antithrombin levels of plasma and serum is due to the fact that only a small amount of thrombin is actually formed when blood clots. The greater part of the prothrombin becomes inert.—R. G.


Heparin-$^{35}S$ was injected into dogs, and the properties of the radioactive materials excreted in the urine were studied. The total radioactivity excreted in the urine after injection of 1.1 to 2.0 mg./Kg. of heparin-$^{35}S$ and of sodium sulfate-$^{35}S$ were similar as regards the rate of excretion and the amount of radioactivity recovered in the urine after 4 days. It was found that with doses of the heparin-$^{35}S$ magnitude, all the label is excreted as inorganic sulfate. On the other hand, after injections of larger amounts, the urine also contains an appreciable portion of $^{35}S$ associated with heparin and other mucopolysaccharide material. It is concluded that as a result of metabolic degradation the sulfate linkages of heparin are cleaved. The capability of the animal to perform this hydrolysis is limited so that when excessive amounts are given a significant portion is excreted unchanged or only partly disulfated. There is also some suggestive evidence that with higher doses some sulfate oligosaccharides are excreted, indicating that the dog may be able to cleave the glycosidic linkages of heparin.—R. G.


Negative results were obtained in the attempt of reproducing in the guinea pig a general sensitization to heparin. A localized manifestation of sensitivity to heparin was obtained in the skin, through various cutaneous reactions, which were represented by the simple infiltration as well as necrotic phenomena. Antigenic properties could be attributed to heparin, independently of the impurities contained in the commercial products, also on the basis of cross reactions, which were carried out with different types of heparin.—P. d. N.


The authors point out that most of the hemorrhagic complications with use of the artificial heart-lung machines occur when surgical teams first use them. With increased experience, hemorrhagic episodes decrease. When hemorrhage does occur it may be due to (1) severe thrombocytopenia usually induced by improperly cleaned equipment or by the administration of excessive amounts of bank blood with nonviable platelets, (2) inadequate neutralization of heparin or administration of excessive amounts of protamine, (3) rarely fibrinolysis, (4) a defect in the first stage of coagulation, usually occurring with prolonged use of the bypass, which has some characteristics of PTC deficiency. The article also describes a method for determining the amount of protamine necessary for neutralization of heparin at the end of the procedure.—R. G.


A deficiency of Christmas factor is demonstrated in the 2 daughters of a man with Christmas disease of relatively mild degree. The defect could be shown by recalcification time, prothrombin consumption and thromboplastin generation technics. Quantitative assay procedures showed that the two sisters had Christmas factor levels of 13 and 18 per cent, respectively, their father 3 per cent and their mother 84 per cent. The elder sister had not suffered from excessive bleeding, but the younger had bled for 48 hours after tooth extraction on two occasions. The data appear to support the view that the gene for Christmas factor is incompletely recessive.—R. M. H.