LEUKOCYTES


A method (Blood 17:216, 1961) for the cytochemical demonstration of aryl sulphatase activity was used on bone marrow preparations from children with acute leukaemia and from controls. Myeloid cells were negative and lymphocytes were positive. Blasts, regarded in routine films as "lymphoblasts" or "stem cells," were positive, while others were negative. This technic may be useful in the differential diagnosis of acute leukaemia.—F. W. G.


A case of acute leukaemia, preceded by persistent monocytosis with an otherwise normal blood picture for more than 9 years, was presented.—B. R.


Two cases of benzene poisoning with hematologic signs of leukemia and two cases without were studied. Marrow hypoplasia and peripheral blood pancytopenia were found. About 70 per cent heteropoloidism was noted, but without demonstrable connection with the evolution of benzene poisoning. In one case with bone marrow and peripheral blood patterns of leukemia in the late stages, a higher degree of dispersion was detected. Trisomy was found only in lymphatic cells of the peripheral blood in subjects with progressive benzene blood disease. The authors were not inclined to correlate such modifications with the severity of the disease.—P. d. N.


A 61-year-old cobbler worked for 46 years with sticking paste material which contained 20 to 60 per cent benzene. Panmyelophthisis developed first, but this clinical picture later changed into that of a typical subacute myelosis. The diagnosis was confirmed at autopsy.—L. D.

The proteins associated with the mitotic chromosomes have not been identified. Chromosomal protein, however, may be visualized in mitotic cells by means of histochemical methods, or by radioautographic localization of labeled protein precursors in chromosomes. The results indicated that incorporation of lysine-H³ into chromosomal protein occurred throughout interphase and was not restricted to any particular portion of interphase. As was incorporation of thymidine-H³ into chromosomal DNA. Grain counts made over chromosomes of dividing cells indicated that the rate of lysine-H³ incorporation into chromosomal protein differed during various periods of interphase. These experiments showed that chromosomal DNA and chromosomal protein do not segregate in like fashion and that their synthesis does not occur simultaneously.—O. P. J.


The mechanism of formation of cytoplasmic granules in rabbit heterophilic myelocytes as reflected by the intracellular flow of tritiated lysine observed by electron microscope radioautography was described. Data obtained under in vivo and in vitro conditions were similar in the following respects: there was a flow of 30 to 40 per cent of cytoplasmic label; the Golgi complex had a high quantity of label at 30 minutes, and approximately 3 hours were required for 30 to 35 per cent of the label to be evacuated from the Golgi complex and to be incorporated into cytoplasmic granules.—O. P. J.


The guinea pig lymphocyte is unique in its ability to produce large cytoplasmic mucoprotein bodies following estrogenic stimulation. The Kuloff body (KB), as this cytoplasmic inclusion is commonly called, is of unknown origin and function. This paper describes studies on 82 guinea pigs to determine the origin of the KB at the ultrastructural level. All evidence indicates that the usual KB cell is a large lymphocyte which in the guinea pig possesses a complex cytoplasmic structure. The lack of phagocytic ability and the negative acid phosphatase reaction further support the lymphocyte classification of this cell. The origin of the KB appears to be an internal synthesis not related directly to external secretion. The finding of KB substance in the Golgi complex implicates this structure. Not related directly to KB synthesis, but a new finding of possible fundamental significance is the stimulation of mitosis in lymphocytes by estrogens. These phenomena should be investigated in other species and could add to the understanding of hormonal effects upon lymphocytes and immune responses.—O. P. J.


After administration of colchicine to young rats, the morphology of free peritoneal fluid cells (eosinophils, mast cells, monocytic and lymphoid elements) became altered in a characteristic manner which involved displacement of the nucleus to the periphery and a pronounced aniso-diameter of the cytoplasm. Although all the cellular types in the peritoneal fluid were affected similarly, the response was most strikingly evident in the mast cell which, therefore, affords an excellent test object for detailed study of this reversible morphologic effect.—O. P. J.


In chickens infected with erythroblastosis virus, the magnesium, phosphorus, sugar, lactic acid, cholesterol and non-protein nitrogen concentrations and the protein composition of the serum were altered. Besides the characteristic changes in the nucleic acid content of the liver, increased aerobic glycolysis of liver, kidney and spleen were observed.—S. R. H.

Erythrocytes

Deficiency of Hexokinase in the Blood Cells of a Family with Familial Panmyelopathy (Type Fanconi). G. W. Lohr, H. D. Waller,
Two male siblings from one family and one male from an unrelated family with the clinical picture of familial, infantile, pernicious-like anemia originally described by Fanconi were investigated. The activity of hexokinase in their erythrocytes was reduced to 24 to 60 per cent of normal. The activities of other enzymes in the Embden-Meyerhof and hexose monophosphate shunt pathways, of GSSG-reductase and of ATPase were either normal or elevated in the erythrocytes of the 2 siblings investigated most completely who had reticulocyte counts of 1.5 and 2.4 per cent. The activity of hexokinase was normal in the erythrocytes of the parents and an unaffected brother. The concentration of ATP in the erythrocytes of two patients was decreased to 33 and 36 per cent of normal, but was normal in the cells of the other patient. Lactate and pyruvate formation were decreased, but the concentrations of methemoglobin and of GSH were normal. The stability of CSH upon incubation with acetylphenylhydrazine was normal, but the concentration of GSSG was increased, and the Heinz body test was abnormal in the erythrocytes of one patient. The Michaelis-Menten constants of hexokinase in hemolysate prepared from the erythrocytes of the most severely affected patient were markedly elevated for both glucose and ATP. Decreased lactate formation and no detectable hexokinase activity were observed in the leukocytes and platelets of the one patient whose cells were examined. Other investigators found multiple chromosomal abnormalities in leukocyte and bone marrow cultures prepared from these patients, but their association with the deficiency in hexokinase activity was not determined. The authors suggested that the genetic disorder involved an abnormal gene product with an altered enzyme protein and that the deficiency in the activity of hexokinase contributed to a shortened erythrocyte survival.—E. R. J.


Human erythrocytes were separated into age groups according to their specific gravity by using a two-phase centrifugation method. The correspondence between density fraction and age group of cells was determined by labeling young cells in vivo with Fe59. The activities of G-6-P D, 6-phosphogluconic dehydrogenase, hexokinase, aldolase and the ATP content were determined in 9 fractions of increasing density which corresponded to progressively older age groups of erythrocytes. The decline in enzymatic activity as a function of red cell age was found to be similar for all enzymes studied, except hexokinase. Hexokinase activity was relatively low in young cells and decreased markedly in the oldest cells. ATP levels were found to be high in very young cells, had plateau values in the mean population and underwent a steep decline in the oldest cells. It was suggested that hexokinase and ATP may be important factors in determining the life span of the normal human red blood cell.—B. R.

Effect of Iron Deficiency and Red Cell Age on Human Erythrocyte Catalase Activity. S. P. Balcerzak, J. W. Vester and A. P. Doyle
ABSTRACTS

946


Red cell catalase activity was measured in 47 iron-deficient patients and was compared to levels in normals. The method for determining catalase activity differed from those employed in other studies in that a high enzyme-substrate ratio and a short incubation time were used. Significantly decreased catalase activity was demonstrated in erythrocytes of iron-deficient subjects. Catalase activity correlated well with hemoglobin and mean catalase activity was demonstrated in increased activity differed from those employed in other subjects. Erythrocytes of iron-deficient time were used. Significantly de-

and normal subjects, sug-

similar in iron-deficient

corpuscular hemoglobin, but not with the red count. Enzyme activity per unit hemoglobin was similar in iron-deficient and normal subjects, suggesting a close relationship between hemoglobin and catalase. Presumably, heme was equally available for both.—R. O. W.


A procedure was described for determining in vivo the turnover of purine nucleotides in human erythrocytes labeled in vitro with 8-C14-purines. The rates of renewal of adenine, guanine and hypoxanthine in red cell nucleotides were determined after transfusion into human volunteers. The merits of the procedure and some aspects bearing on the metabolic pathways involved in red cell turnover and their possible physiologic significance were discussed.—B. R.


The patient, of Norwegian-Ukrainian and uncertain French or Indian stock, was admitted in shock with acute hemolytic anemia which developed during the 4 days in September after he had eaten cooked and raw freshly harvested broad beans. The beans were identified as Vicia fava. Except for a “bout of flu” there was no certainty that ingestion of these beans on other oc-
casions had been followed by illness. There was no detectable G-6-PD activity in his erythrocytes or leukocytes five months after the acute episode. The activity of G-6-PD in the erythrocytes of his mother and an older sister was reduced to intermediate levels, but no activity could be demonstrated in their leukocytes. G-6-PD activity was normal in the erythrocytes and leukocytes of his maternal grandmother and of another sister. This case probably represented the first example of favism in Canada where the fava bean plant blooms near the end of June and the pods are harvested in August and September.—E. R. J.


The blood smear obtained from a white-tailed deer who had suddenly taken ill showed a sickled appearance of almost all of the red cells. On electrophoretic analysis, there were two abnormal components with the mobility of Hb I (40 per cent) and Hb Bart’s (60 per cent). The amount of alkali-resistant hemoglobin approximated 28 per cent.—J. B. C.


Various aspects of the hemoglobinopathies due to hemoglobin E-thalassemia and their genetic interactions were investigated in the Bengalee population. Resistance to iron deficiency, malaria and other infections was studied. Studies of erythrocyte GSH and related enzymes, G-6-PD and glutathione reductase, yielded interesting data which were related to the stability of circulating red cells.—J. B. C.


The presence of hemoglobin A'2 in a white woman of Greek extraction was described. The propositus was a Turkish citizen living with her family on a small island in the Aegean Sea. She was examined during the family study of her son who was suffering from Cooley’s anemia and who was found to be heterozygous for β-thalassemia. Her son and her husband, who were the only other members of the family investigated, did not show the A'2 abnormality. This study was important from the point of view of population genetics because, except for 3 individuals of German origin, all the other persons showing this abnormality have been Negroes or of Negro extraction.—B. R.

Megaloblastic changes were found to be present in the bone marrow in 37 of 95 children with kwashiorkor at the time of hospital admission and developed in an additional 21 patients during hospitalization. On admission, serum folate levels were subnormal in 41 children, serum iron levels were subnormal in 78 and serum vitamin B₁₂ concentrations were elevated above normal in 11. Eleven of the children who presented with a megaloblastic anemia had normal serum vitamin levels; in 7 of these subjects, serum folate concentrations became subnormal within 2 weeks. Only one child exhibited hypoplasia of the bone marrow.—F. A. K.


Over 80 per cent of 51 infants with protein malnutrition were found to excrete excessive quantities of FIGlu and urocanic acid following histidine loading. Excessive excretion of urocanic acid correlated with elevated serum vitamin B₁₂ levels, but not with serum folate levels or bone marrow changes. Many infants with excessive urinary FIGlu had normal serum folate levels and normoblastic erythropoiesis. Excessive excretion of this metabolite could be eliminated by folate acid therapy in only one-third of the subjects. These observations indicated that excessive excretion of histidine derivatives in patients with protein malnutrition are dependent on a number of factors, some of which appear to be unrelated to folate deficiency.—F. A. K.


Treatment with vitamin E in 6 children with protein deficiency who presented with subnormal serum folate concentrations and a megaloblastic anemia resulted in a variable reticulocytosis response and a rise in serum folate levels in all patients. Some degree of megaloblastic change persisted in the bone marrow following this therapy.—F. A. K.


One hundred fifty-six children with iron deficiency were analyzed. In 32 per cent, the birth weight had been below 2500 Gm. Surprisingly, a marked preponderance of underweight was found for the entire group. Growth curves prior to therapy showed a slowing of weight gain as anemia developed and a return to a normal distribution curve after therapy.—R. O. W.


Wines, such as sherry, produced in Australia contain considerable quantities of iron (1.8–5.6 mg. per liter). Beer and spirits contain only 0.15–0.4 mg. per liter. Consumption of alcoholic beverages has been found to be correlated significantly with hepatic hemosiderosis and increased iron content. A mild degree of hepatic hemosiderin deposition is found in normal subjects killed accidently. If, therefore, stainable iron is found in a liver biopsy, e.g., in a relative of a patient with hemochromatosis, this fact is not enough to permit a conclusion that iron metabolism is abnormal.—F. W. G.


A 4 month old infant was found to have a megaloblastic anemia due to receiving an inadequate quantity of vitamin B₁₂ from the milk of a mother who had had undetected pernicious anemia. This case emphasized the fact that megaloblastic anemia in infancy is not always secondary to folate deficiency.—F. A. K.


Treatment with prednisolone in 5 patients with pernicious anemia resulted in reappearance of gastric glands containing parietal and chief cells, return of intrinsic factor secretion, and enhancement of vitamin B₁₂ absorption in all 5 subjects and in return of acid secretion in 3. Titer of intrinsic factor antibody decreased in 2 patients.—F. A. K.


The response to therapy with "titrated" doses of folic acid while receiving a folate-free diet was studied in 2 subjects who developed a megaloblastic anemia due to folate deficiency during the third trimester of pregnancy. Dosages of up to 200 μg. per day resulted in a hematologic remission only after parturition. These observations were a further demonstration that folate requirements are increased above normal during pregnancy.—F. A. K.


Three infants, aged 4 1/2 to 5 months and fed for 14-15 weeks on goat's milk, had severe anemia, thrombocytopenia and megaloblastic bone marrows. Two had a low serum folate level and responded to folic acid administration. One responded to vitamin B₁₂. The folate level of goat's milk was found to be only one-third to one-tenth of that of fresh cow's milk or of various commercial preparations of cow's milk. The anemia and thrombocytopenia in these children probably resulted from a low folate intake, and it was recommended that infants on goat's milk diets should receive folate supplements.—F. W. G.


Serum and erythrocyte folate levels, which were subnormal in all 9 patients with untreated adult celiac disease who were studied, rose to normal without supplemental vitamin therapy within several months after the institution of a gluten-free diet in all 5 patients in whom serial studies were carried out.—F. A. K.


Binding of streptolysin S (SLS) by human red cell membranes was investigated. Intact erythrocyte ghosts bound SLS irreversibly. No effect on binding was obtained following the treatment of red cell ghosts with phospholipase A and D. trypsin, lipase or neuraminidase, but treatment with phospholipase C from Clostridium welchii markedly reduced their binding capacity for SLS. Although about 90 per cent of the phospholipids of the ghosts were split by phospholipase C and the release of phosphorus was proportional to the decrease in binding, only about 40 per cent of the capacity of the ghosts to bind SLS was lost. Phospholipids, as well as cholesterol at concentrations similar to those in the ghosts, markedly inhibited SLS binding. The authors suggested that phospholipids and cholesterol constitute binding sites for SLS in erythrocyte membranes, but that other constituents, such as sphingomyelin, ganglioside, sialic acid and the various protein components, have no effect. The possible mechanism of lysis of red blood cells by SLS and its inhibition by lipids were discussed.—B. R.


The effect of corticosteroids on the phagocytic capability of human leukocytes for opsonized (anti-A coated) erythrocytes was studied in vitro. Glucocorticoids added directly to the phagocytizing leukocytes had minimal effect, unless present in huge doses. In contrast, intravenous administration of reasonable doses of corticosteroids to both normal and Addisonian subjects caused sequential decrease in the erythrophagocytic capacity of their leukocytes. The authors suggested that this effect might underlie the enhancement of infections and the amelioration of certain hemolytic disorders by steroids.—H. S. J.
ABSTRACTS


Fixation of Fundulus erythrocytes by silver acetate-osmium tetroxide solutions preserves marginal band microtubules while extracting the obscuring hemoglobin background. At high magnification (× 680,000) of thin sections, microtubules are found to be composed of 6 or 7 globular subunits. Marginal band microtubules may be remnants of structures which were once morphologically important. If these structures are transitory and arise in connection with the changing asymmetry of the cell, they may be similar to tobacco mosaic virus protein.—O. P. J.

HEMOSTASIS


In the first reported instance of hereditary resistance to the action of coumarin, members of a family representing three generations showed extraordinary resistance to the prothrombinomimetic effects of these compounds. The propositus required 145 mg. of warfarin daily (20 times the average dose) for maintenance of the prothrombin time within the therapeutic range. With single doses of up to 1010 mg. the dose-response curve was logarithmic and parallel to that of normal subjects. Gastrointestinal absorption of warfarin, as measured by its rate of appearance in plasma, was normal. The degree of protein binding, volume of distribution and rate of disappearance of warfarin from the plasma were normal in the propositus and no unchanged drug was found in the urine or stool. The rate of disappearance of Factors II, VII, IX and X from the plasma after sufficient warfarin was administered also was normal. The propositus showed equal resistance to bishydroxycoumarin and phenindione; his response to heparin was normal. The level of the clotting factors dependent upon vitamin K in the kindred was not abnormally high and, in some instances, was low normal or slightly less. During a period when he was not receiving anticoagulant drugs, the propositus showed no significant rise in the K-dependent coagulation factors when given vitamin K. He was extremely sensitive, however, to the antithrombotic action of vitamin K. It was postulated that a pair of allelic genes controlled the structure or amount of an enzyme which was concerned with the synthesis of the clotting factors dependent on vitamin K and that resistance to the coumarin anticoagulant drugs was a polymorphic characteristic. This resistance appeared to be transmitted as an autosomal dominant characteristic and to be caused by the presence of an abnormal enzyme or receptor site which had either a decreased affinity for the coumarin drugs or an increased affinity for vitamin K.—R. G. (Editor’s Comment: The authors later corrected this last conclusion by stating that the inheritance could be either as an autosomal or a sex-linked characteristic [New Eng. J. Med. 272: 108, 1965].)


In 73.8 per cent of 51 cases of chronic azotemia, various thromboelastographic alterations were found, chiefly represented by signs of hyperfibrinogenemia, but in some cases due to thrombocytopenia and deficiency of some coagulation factors. In 32.3 per cent of cases, irregular thromboelastograms were obtained after partial or complete disruption of the clot.—P. d. N.

HEMOBHYDIC DIATHESIS WITH PROLONGED BLEEDING TIME WITHOUT THROMBOCYTOPENIA. I. Bonechi and G. Pasero. From the University, Pisa, Italy. Medicina 14:197–219, 1964.

Special features in the case of a 14 year old girl were: abnormal TGT with intact platelets, but not with lysed platelets; abnormal clot retraction with platelet-poor plasma, but not with platelet-rich plasma; ultrastructural alterations of platelets; no correction of prolonged bleeding time after transfusion of Cohn’s fraction I or of antihemophilic, hypophylized plasma (800 ml.).—P. d. N.


Patients splenectomized for various conditions developed persistent thrombocytosis, if anemia persisted after operation. Platelet adhesiveness, measured in vitro, increased after splenectomy, whether or not thrombocytosis or anemia was pre-

From www.bloodjournal.org by guest on October 22, 2017. For personal use only.
sent. Thrombo-embolic complications occurred in 6 of 25 patients with persistent post-splenectomy thrombocytosis and anemia. Three of these patients also had increased platelet adhesiveness. The authors postulated a complex mechanism for post-splenectomy thrombo-embolism with thrombocytosis, raised platelet adhesiveness and possibly other factors, such as thromboplastin released from hemolyzed red cells, all acting together.—F. W. G.


Acute thrombocytopenic purpura during treatment with phenylbutazone occurred in a 59 year old patient. Severe hemorrhagic manifestations were controlled successfully with prednisone. On repeated administration of phenylbutazone and with daily studies of the number of platelets, the patient was found to be allergic to the drug.—L. D.


Although this abstract should have been published a year ago, the observations were important enough to warrant publication at this late date. The authors studied analogies between the reactions of zymogen granules in the mouse pancreas and the dense granules in human blood platelets with respect to digestion by proteolytic enzymes with various techniques of section staining for electron microscopy. They concluded that the thrombocytic dense granules were essentially protein in nature and that it was unlikely that platelet factor 3 was localized in these organelles.—O. P. J.


Lead-containing media are not suitable for visualizing fine structural localization of enzymatic activities in glutaraldehyde-fixed rat blood platelets.—O. P. J.


It has been shown that diisopropyl fluorophosphatase (DFP) labeled with tritium binds covalently with a 1:1 molar ratio to a number of different enzymes, including acetylcholinesterase (AChase). The uptake of labeled DFP is reduced by one-third by eserine and by the AChase inhibitor, 284C51, and by reactivation with pyridine-2-aldoxime. The authors conclude that one-third of the DFP taken up by megakaryocytes represents binding to true AChase.—O. P. J.


Platelet alterations included anisocytosis, atypical thick granules and the presence of lipid droplets.—P. d. N.

MISCELLANEOUS


No association was found between the degree of baldness, rated from photographs in their 25th Class Reports, and the number or sex ratio of the 2,775 children born to the 1,008 fathers among the 1,297 men who entered Harvard College between 1880 and 1912 and who subsequently married.—E. R. J.


This group of investigators previously reported the results of their studies with a modified ACD medium and have now described their clinical experiences. Whole blood was collected into one plastic bag of a triple pack system which contained standard ACD solution, NIH formula A, and was stored at 4 C for 24 hours. The super
Guanosine dissolved in 200 ml. in the closed system and the IAG solution bag cells. After storage at 4 C, it was possible to buffer, pH 7.0) in a third bag was mixed with the plasma or to administer packed erythrocytes. Erythrocyte survival was determined with a double isotope technic with 32P and Cr51. IAG-preserved erythrocytes had a 24 hour survival of 80 ± 2.6 per cent (11 units) after 42 days of storage, while ACD-preserved erythrocytes had a 41.3 ± 2.9 per cent survival (6 units) after 42 days and a 72.0 ± 3.5 per cent survival (6 units) after 21 days of storage. The concentration of K+ in the erythrocytes stored in IAG solution declined somewhat more slowly than did the concentration in ACD-stored cells during 40 days. A 50 per cent restoration of intracellular K+ occurred during incubation at 37 C for 3 hours of IAG-stored cells, but not of ACD-stored cells. The concentration of ATP in IAG-stored erythrocytes declined about 20 per cent during 40 days while the concentration decreased about 80 per cent in ACD-stored cells. Clinical experience was obtained with transfusions of 3,028 IAG-stored units; 2,445 were given with the IAG solution, 426 as packed erythrocytes and 157 after restoration with the onigii.

ABSTRACTS


In addition to the usual methods, studies were performed with 3-, 5- and 10-fold dilutions of blood in physiologic NaCl solution. The results were recorded after one and 24 hours. The sedimentation rate curves of diluted bloods after 24 hours were different in pathologic conditions. Patients with paraproteinemia had characteristic curves.—S. R. H.


The present study was undertaken to clarify by electron microscopy some of the obscure features of splenic development. The problems investigated included the development of the peculiar circulatory pattern, the time and nature of establishment of communications between endothelium lined blood vessels and the reticuloc or sinususes, the development of the white pulp and the occurrence and extent of erythropoiesis.—O. P. J.


Rabbits were immunized with antibody-sensitized human and rabbit erythrocytes and antisera were obtained which specifically precipitated human immune globulins. Immunization with incomplete anti-D hemagglutinins bound to human D-positive erythrocytes and with warm autohemagglutinins resulted in precipitins affecting primarily gamma globulin. In the majority of cases, injection of human leukocytes sensitized with autoantibodies resulted in the formation of precipitins affecting gamma and beta-2M globulins. After the
injection into rabbits of rabbit erythrocytes sensitized with the heterohemagglutinins of human milk. Precipitins reacting only with the three immune globulins were formed. Administration of rabbit erythrocytes sensitized with heterohemagglutinins obtained from human sera was followed by the appearance of 4 to 10 precipitins. The antibodies were effective against immune globulins, complement factors or proteins specifically absorbed to erythrocytes. If immunization was carried out with non-sensitized erythrocytes or leukocytes, precipitins affecting serum proteins were not formed in most rabbits. In some cases, antibodies affecting specifically bound proteins (mainly alphalipoprotein and alpha-2-globulin) could be detected. Precipitins affecting immune globulins could be used also for antiglobulin reactions, immunoelectrophoresis and immunodiffusion studies.—S. R. H.


Natural tolerance of rats to NK/Lv ascites mouse tumor persists until the tenth day of life in newborn animals. By repeated introduction of splenic tissue of mice, newborn rats can be rendered tolerant to the tumor for more than one month. Tolerance can be obtained in a higher percentage in this heteroimmune system if intraperitoneal antigen injection is supplemented by intrathympic administration.—S. R. H.


In 21 of 22 patients with verified hilar or disseminated forms of pulmonary sarcoidosis, the Kveim reaction was positive. The authors described the procedures used in preparing their own antigen from sarcoidosis-affected lymph nodes.—L. D.


The hepatomegaly of radiation chimeras results from an increase in all functional elements of the liver. The infiltrates arise from a host response to graft vs. host activity of donor cells. A numerical tally of microscopic foci of leukocytic infiltrates provides a sensitive means of detecting early graft-host interaction in radiation chimeras.—H. H. F.