SAMA-AΩA Student Honors Day: Abstracts of Scientific Presentations

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A Technique for the Measurement of Reagenic Antibody: Technical Development and Future Studies

PETER F. HOFFMAN (M–70)

The purpose of this study was to establish in our laboratory a method for the measurement of reagenic antibody, now known as Immunoglobulin E (IgE). Immunoglobulin E specifically contains within it the antibody moiety that is responsible for reactions of the immediate hypersensitivity type in man. The reaction of IgE with specific allergens has been shown to lead to the release of histamine *in vitro*. *In vivo* it appears to be the immunoprotein involved in extrinsic asthma, hayfever, atopic dermatitis and anaphylactoid drug reactions. Because the normal level of IgE in the serum is only approximately 250 ± 100 nanograms/ml (ng/ml), more conventional techniques for measuring immunoglobulin levels such as the single radial diffusion method are not sensitive enough to determine the concentration of IgE. In order to quantitatively detect IgE a modification of the solid phase bromacetyl cellulose radioimmunoassay technique (Mann, et al. J. Imm. 102: 618, 1969) was undertaken. The principle of the technique is the inhibition of the binding of a trace (less than 0.1 ng) of "hot" or I^{125}-labelled IgE to an insoluble polymer of anti-IgE coupled with bromacetyl cellulose (BAC:anti-IgE) by unlabelled or "cold" IgE. By first establishing a standard curve it is possible to detect the presence of IgE down to approximately 1 ng/ml. The methodology necessary to instrument this procedure in our laboratory will be discussed. Elevated levels, up to 100 times normal, have been reported in patients with asthma, atopic dermatitis, and anaphylactoid drug reactions. Unfortunately there has been little clinical correlation between elevated IgE levels and specific symptomatology. Over the next year we will be attempting to measure both total IgE levels as well as specific IgE antibody levels in patients with drug reactions, particularly penicillin reactions and we will attempt to better define the relationship between IgE and adverse anaphylactoid drug reactions. Hopefully this will lead to a more precise ability to prejudge whether or not a particular patient will or will not experience a potentially devasting reaction when given a drug such as penicillin.

Preceptor: W. Kenneth Blaylock. *Division of Dermatology, Medical College of Virginia.*

Suppression of Homograft Immunity with Thiamphenicol

J. D. LINEHAN (M–70)

Thiamphenicol, the methylsulfonyl analogue of chloramphenicol, is believed to suppress newly induced antibody production by inhibiting messenger—RNA at the cellular level. Accordingly, thiamphenicol was evaluated in prolonging canine renal homograft and canine cardiac homograft survivals. METHODS—18 dogs underwent bilateral nephrectomy and renal homotransplantation, receiving thiamphenicol as sole immunosuppressant. 14 control dogs—same operation but no immunosuppression. 8 dogs underwent orthotopic cardiac homotransplantation, receiving thiamphenicol immunosuppression (prednisone added for acute rejection episodes). 27 control dogs from a previous series underwent the same operation but received no immunosuppression. Rejection was monitored by daily blood urea nitrogen (BUN) and serum creatine (S-Cr) determinations in renal homografts and daily electrocardiograms in cardiac homografts. RESULTS—Renal—Median survival of dogs receiving thiamphenicol was *17 days* (range 8–49 days), significantly greater ($p < .01$) than control group median survival of *9 days* (range 7–14 days). Only 6–18 dogs

receiving thiamphenicol died primarily of rejection; other dogs died of infection and pneumonia. Cardiac—Median survival of dogs receiving thiamphenicol was *29 days* (range 5–250 days) versus *7 days* median control survival (range 4–21 days). One dog receiving thiamphenicol is currently alive 250 days post transplant. Histopathologic changes in the renal and cardiac homografts will be presented, as well as side effects of thiamphenicol therapy. SUMMARY—Thiamphenicol is a potent immunosuppressive agent, producing significantly prolonged canine renal and cardiac homograft survival quite similar to that produced by azathioprine and 6-mercaptopurine, which have different mechanisms of action. Because of its immunosuppressive properties, thiamphenicol may be a useful adjunct in clinical immunosuppression.

Preceptors: David M. Hume and H. M. Lee. *Department of Surgery, Medical College of Virginia.*

Blood Volume Regulation Following Cardiac Transplantation*

MARC D. THAMES (M–70)

The changes in blood volume and the peripheral plasma renin responses to hemorrhage were studied in dogs following cardiac transplantation. Each dog was anesthetized with pentobarbital, blood volume was determined by dye dilution technique (Evans blue), and control samples for plasma renin were obtained. Then the animal was bled 15 ml/kg and samples for plasma renin were drawn 20 min. and 30 min. after hemorrhage and 15 min. after reinfusion of the blood. The blood volume of dogs following cardiac transplantation before reinnervation of the heart was significantly greater than that of normal dogs. Plasma renin of normal dogs rose to 340% of control following hemorrhage, while that of animals with cardiac transplantation rose to only 180% of control value. This difference was significant. To determine if this difference was due solely to the expanded blood volume found in the denervated animals, 6 normal dogs were transfused with 25 ml/kg of whole blood the day prior to study. The renin response to hemorrhage in these dogs was similar to that of dogs with cardiac transplantation. However, 4 dogs with cardiac transplantation diuresed with i.v. ethacrynic acid 1 mg/kg the day prior to study so that their blood volumes were near that of normal dogs showed no increase in plasma renin following hemorrhage. These results indicate that dogs with cardiac denervation have increased blood volumes and show a subnormal renin response to hemorrhage which cannot be attributed entirely to expanded blood volume. These data are consistent with the view that there are afferent receptors in the heart which participate in the reflex regulation of blood volume and that this mechanism is interrupted by denervation of the heart subsequent to cardiac transplantation.

Preceptor: Hermes A. Kontos. *Department of Medicine, Division of Cardiovascular Disease, Medical College of Virginia.*

* Abstract submitted for publication to *American Journal of Physiology.*

The Roentgenographic Diagnosis of Aneurysms of the Superior Mesenteric Artery with Two Case Reports

PARHAM R. FOX (M–71)

Superior mesenteric artery aneurysms are rare. The majority of the cases are diagnosed at autopsy, and the incidence is approximately one in 12,000 consecutive unselective autopsies. In a review of the world literature, De Bakey and Cooley found a total of 63 cases of aneurysms of the superior mesenteric artery reported up to 1953, and there have been at least 8 additional cases reported to date. The majority of these aneurysms are mycotic and occur as a complication of bacterial endocarditis. The clinical picture of aneurysms of the superior mesenteric artery is not characteristic. The occurrence of epigastric pain associated with an epigastric mass in a patient with bacterial endocarditis should arouse suspicion of a mycotic aneurysm of the superior mesenteric artery. On physical examination, expansile pulsation and a systolic bruit are additional evidence for this diagnosis. The ability to displace the pulsatile mass laterally is also very important. Epigastric pain associated with back pain which may be clinically indistinguishable from cholecystitis or pancreatitis has also been described. The first patient, a 70 year old white male, presented with obstructive jaundice, an unusual clinical manifestation of aneurysms of the superior mesenteric artery, occurring more frequently with aneurysms of the hepatic and celiac artery. Gastrointestinal hemorrhage has been described as another symptom of hepatic, celiac and pancreaticoduodenal artery aneurysms, but not before with superior mesenteric artery aneurysms. Roentgenograms

of the abdomen may only reveal a soft tissue mass in the epigastrium. This is a non-specific finding and will not permit the diagnosis of aneurysm of the superior mesenteric artery. However, if there is calcification in the wall of the aneurysm, the location and characteristics of this calcification are very important. The calcification is curvilinear and is usually located in the midline or just slightly lateral to the midline, approximately one-half lumbar vertebral body width anterior to the bodies of the upper lumbar vertebra. In our cases, and in the published cases with roentgenographic illustrations, there is a posterior defect in the continuity of the circumferential calcification shown in the lateral projection which represents the origin of the superior mesenteric artery or its major proximal branches. This is a distinctive finding and permits a presumptive diagnosis. However, aneurysms of the celiac artery which are less common, could theoretically have similar plain film findings. The definitive diagnosis can be made with angiography, preferably with selective visceral angiography. It is important not only to make the diagnosis of aneurysm, but also to demonstrate whether or not occlusion of the superior mesenteric artery has occurred and whether or not collateral circulation is present. Selective angiography also permits the demonstration of the relationship of the celiac axis to the aneurysm and patency of the superior mesenteric vein. In the second case, a 72 year old Negro female, the celiac axis was displaced superiorly and draped anteriorly over the aneurysm. In view of the high mortality rate associated with this lesion, preoperative mapping of the vascular tree is very important in order to plan surgical correction.

Preceptor: William A. Weidner. *Department of Radiology, Medical College of Virginia.*

Studies on the Clearance of Circulating Leucocytic Pyrogen*

M. TENENBAUM (M–71) AND
D. LORBER (M–72)

Fever is believed to be caused by action on the hypothalamus of a protein pyrogen released by leucocytes. The magnitude of the fever appears to be dependent on the amount of pyrogen circulating in the blood. The present studies were designed to determine the site of removal of leucocytic pyrogen (L. P.) from the circulation. Rabbits were given endotoxin fevers and their urine collected during the time that they were febrile. As much as 200 ml urine from febrile rabbits was nonpyrogenic when injected intravenously. Crude rabbit leucocytic pyrogen was prepared from rabbit peritoneal exudate cells which were incubated for 8–12 hours in phosphate buffed saline solution. When urine from afebrile rabbits was incubated with crude leucocytic pyrogen, no significant decrease of pyrogen activity was observed. Similarly no decrease in pyrogenic activity was observed after incubating L. P. with rabbit plasma. However, when rabbit livers were perfused with fluid containing leucocytic pyrogen, all pyrogen disappeared within 15 to 45 minutes from the perfusate in 7 out of 9 perfusion experiments. The BSP test after perfusion was normal in those 7 perfusions. In the two experiments in which some L. P. remained after 45 minutes, the BSP tests indicated liver damage. The data indicate that leucocytic pyrogen is not directly excreted in the urine or inactivated in the plasma or urine. The liver appears to be the site of destruction of circulating pyrogen.

Preceptor: George W. Gander, *Department of Pathology, Medical College of Virginia.*

* Abstract submitted for publication to *Proceedings of the Society for Experimental Biology & Medicine.*

Study of the Combined Effect of Pyran Copolymer with Actinomycin D or Cytoxan on Some Mouse Tumors

STEVEN H. GROSSMAN (M–73)

Through the use of Pyran Copolymer, a reticuloendothelial system stimulant, some cancers have been retarded. Many other drugs have been used effectively as carcinotoxic agents. This series of experiments has been undertaken to explore the possible synergistic effects of combining these drugs with Pyran, representing increased host resistance. Three tumor systems were used: Exp. I. solid Ehrlich tumor placed sub-cutaneously; Exp. II. Ehrlich ascites tumor; and Exp. III. Friend Virus Leukemia. Pyran causes depressed function of the RES for seven to eight days beginning at 24 hours and stimulation thereafter. Experiment I used six groups of Swiss mice: (1) Tumor control (2) Tumor plus Cytoxan (3) Tumor plus Pyran (RES stimulated) (4) Tumor plus stimulation plus Cytoxan (5) Tumor plus RES depressed (6) Tumor plus RES depressed plus Cytoxan. Cytoxan (cyclophosphamide) therapy produced 51.9% tumor inhibition, Pyran therapy produced 80.5% tumor inhibition when the tumor was received during RES stimulation, and 85.8% when depressed.

Groups four and six produced weaker inhibition (79.1% and 70.2%, respectively) than with Pyran alone. Colloidal carbon clearances had shorter T½'s than in controls or in untreated tumors. Individual organ weights were greater in all cases compared to controls but not necessarily to untreated tumor mice. Experiment II utilized cummulative survival time for evaluation. Mean survival time was not increased significantly in relation to the total duration of the study, in any group. Experiment III used individual organ weights in evaluating the combined therapy against the Friend Virus Leukemia. Spleens, livers, and lungs were generally enlarged and no tumor inhibition was demonstrated. These three experiments have failed to demonstrate an additive antitumor effect as postulated, however, this methodology has enabled further studies to demonstrate positive results using methotrexate, 5-flurouracil, hydroxyurea, and other drugs in conjunction with Pryan.

Preceptor: William Regelson. *Department of Medicine, Medical College of Virginia.*

Trypanosome Infection and Tumor Growth

GARY HOFFMAN (M–72)

The effects of *Trypanosoma duttoni* and *T. lewisi* infections were studied in mice bearing either Friend Virus Leukemia or Leukemia L-1210 and appropriate controls. Both species of trypanosomes are non-pathogenic and have been previously shown to be potent stimuli of the reticuloendothelial system. Mice were either immunized to trypanosome infection, or infected with trypanosomes at the time of tumor inoculation. In neither case was diminished tumor growth noted in reference to tumored controls.

Preceptor: William Regelson. *Division of Medical Oncology, Medical College of Virginia.*

Plasma-Pressor Activity in Metabolic Acidosis

F. J. MARTORANO (M–71)

Arterial blood from twenty-one patients was analyzed for pO2, pCO2, pH, and plasma-pressor activity (PPA) to assess the effect of hypoxemia, hypercapnia, and acidosis on catecholamine levels in the plasma. PPA was measured using a semimicro (0.2 ml.) bioassay utilizing an isolated rabbit ear artery. Pressor response was expressed in norepinephrine equivalents (nanograms per ml.). Specificity studies in our laboratory indicated that PPA measured in this bioassay reflects primarily the plasma concentration of active catecholamines. Fifteen patients had an arterial pH greater than 7.3 associated with a PPA of less than 2 ng/ml. The remaining six patients were in metabolic acidosis with pH ranging from 7.08 to 7.28 and demonstrated PPA levels from 2.5 to 25 ng/ml. There was no direct correlation between arterial pO2 and pCO2 with PPA. In five of the six acidotic patients, further monitoring during medical management showed the PPA to vary inversely with arterial pH. This work confirms previous animal studies by other investigators which demonstrated acidosis to be an important primary stimulus for catecholamine release rather than hypoxemia or hypercapnia *per se*.

Preceptor: Reuben H. Young. *Department of Pediatrics, Medical College of Virginia.*

Specificity of Ribonuclease Activity as Related to Homograft Rejection

DAVID WALDMAN (M–72)

Many methods to detect or confirm homograft rejection have been devised utilizing change in hemodynamics, immunological reactions, enzymatic change, and biopsies. Utilization of many different approaches as well as poor sensitivity and specificity denotes the complexity of the rejection mechanism. Jolley and others have suggested studying rejection processes with an enzymatic approach and showed the change in ribonuclease activity relating to skin graft rejection. This project was undertaken to study the correlation of ribonuclease activity with renal homograft rejection with regard to its specificity and

sensitivity and possible use of this approach to help elucidate the rejection mechanism. METHOD—Mongrel dogs weighing 10 to 15 kgm were used as an experimental animal and kidney transplants were done to the pelvic vessels according to the established technique in this lab. Blood urea nitrogen and creatinine levels were followed in all animals measured by the autoanalyzer. Dogs were autopsied and histology sections made of the kidney, lymph node, liver and lungs. Serum ribonuclease activity was assayed by the Roth method at pH 5.8 and pH 7.8. Control samples were obtained on all animals prior to experimentation and all samples were measured in triplicate ribonuclease activity at intervals during the experiments. Animals were grouped into four categories: (1) autotransplants, (2) hydronephrosis, (3) renal infarct and (4) homotransplant. Homotransplant was subdivided into two groups, (1) with uremia, having homotransplanted kidney as sole kidney, (2) without uremia, having one of his own kidneys left in to exclude uremia as a variable factor. RESULT —We have defined one unit of RNAse activity as a change of .001 O. D. over 30 minutes at 260 mu. Using this scale we have established a range of 625–710 units at pH 5.8 and 710–810 units at pH 7.8 as a normal control value in mongrel dog population. Our preliminary study showed no significant change in ribonuclease activity relating to sex and there were no significant diurnal variations or daily fluctuations. Immunosuppressive therapy with imuran and prednisone produced very slight but general decrease in activity at both pH 7.8 and pH 5.8. Homotransplant group showed a significant increase in ribonuclease activity at pH 7.8 while showing no change at pH 5.8. Absence of uremia did not modify this increase of ribonuclease activity. Uremic homotransplant group ranged to 1150–1700 units at 7.8 pH and nonuremic group 1150–2300 units at pH 7.8. Both hydronephrosis and renal infarct group showed slight increase in activity immediately after surgery at both pH 7.8 and pH 5.8. However they decreased to the normal control levels by the third postoperative day. This initial rise never reached the level of the homotransplant group increase. SUMMARY—There is a measurable increase in the serum ribonuclease activity at pH 7.8 in kidney homotransplant dogs regardless of the presence or absence of uremia. This seems to be related to homograft rejection itself rather than nonspecific kidney damage. Autotransplantation, renal infarct, or hydronephrosis failed to show any change in the ribonuclease activity. This result may suggest use of ribonuclease activity as detection or confirmation test of renal homograft rejection and also one approach to the elucidation of the rejection mechanism.

Preceptor: H. M. Lee. *Department of Surgery, Medical College of Virginia.*

An Increase in Brain Serotonin in Experimental Porphyria

WILLIAM R. REAMY (M–72)

Acute intermittent porphyria is a rare inherited disease. Biochemically it is characterized by an increase in the urinary excretion of δ-aminolevulinic acid (ALA) and porphobilinogen. The primary molecular lesion is an elevation in hepatic ALA synthetase (Tschudy *et al.*, 1965, Proc. Nat. Acad. Sci. *53,* 841), the rate-limiting enzyme in heme biosynthesis (Granick and Urata, 1963, J. Biol. Chem. *238,* 821). Clinically, acute intermittent porphyria is manifested by neurological disorders and is sometimes accompanied by depression, confusion, and visual hallucination (Wetterberg, 1967, *A Neuropsychiatric and Genetical Investigation of Acute Intermittent Porphyria,* Svenska Bokforlaget, Stockholm). The relationship between the aberration in heme synthesis in the liver and the nervous system is enigmatic. ALA synthetase can be induced in embryonic chick liver in vitro by a variety of compounds including barbiturates, collidines, and steroids (Granick, 1966, J. Biol. Chem. *241,* 1359). 3, 5-dicarbethoxy-1, 4-dihydrocollidine (DDC) is a potent inducer of ALA synthetase in embryonic chick liver in vivo (Simons and Boell, 1967, Am. Zool. *7,* 48). At thirteen days of development, when the enzyme is normally not yet present in detectable amount, DDC produces a massive increase in the enzyme—forty times the adult value. The production of experimental porphyria provides a useful approach to the study of the biochemical aspects of this disease. Experimental porphyria was induced in 13 day chick embryos by DDC and the concentration of serotonin measured in different regions of the brain by the fluorometric method of Snyder *et al.* (1965, Biochem. Pharmac. *14,* 831). The concentration of serotonin in the cerebral hemispheres of both normal and treated animals is less than that in the remainder of the brain. A significant increase in the concentration of serotonin occurs in porphyric animals in the noncerebral portion of the brain but not in the hemispheres.

A growing body of evidence implicates disturbances in serotonin metabolism in mental disorders. The elevation of brain serotonin accompanying the induction of hepatic ALA synthetase may be a concomitant of faulty transmission in serotonergic neurons, thereby eliciting through neurochemical events as yet unknown the neurologic symptoms which occur in acute intermittent porphyria. The induction of ALA synthetase in the liver involves genetic regulatory mechanisms (Granick, 1966). Heme apparently acts as a corepressor of the synthesis of messenger RNA

for ALA synthetase. The inducer competes with heme for the hypothetical repressor and renders it inactive, thereby allowing the synthesis of messenger RNA to proceed. Whether the increase in brain serotonin is a consequence of enhanced heme synthesis or whether it is an independent effect of DDC remains to be seen.

Preceptor: Jarid A. Simons. *Department of Biology, College of William and Mary.*

Inhibition of the Lupus Erythematosus Cell Phenomenon in Uremia

HAROLD L. REKATE (M–70)

Immunologic reactions may be deranged in uremic patients. We present clinical and laboratory evidence indicating that the L. E. cell test may be suppressed in such patients. Two patients at the Medical College of Virginia Hospitals have shown inhibition of the L. E. cell test due to their uremia. The first of these, T. Y., a 21-year-old male, was admitted for peritoneal dialysis and was found to be uremic with an elevated BUN and serum K of 9.61. The presumptive diagnosis was subacute glomerulonephritis. An L. E. cell test on admission was negative. After dialysis his BUN fell to 64 and a repeat L. E. cell preparation was found to be positive. Autopsy revealed evidence of lupus nephritis. The second patient, G. S., was a 36-year-old female known to have systemic lupus erythematosus with progressive renal insufficiency. On admission her BUN was 219 mg% and the L. E. cell test was negative. Following peritoneal dialysis her BUN fell to 68 mg% and a positive L. E. cell test was obtained. The mechanism of inhibition has been studied with tests designed to distinguish sensitization and phagocytosis phases of the L. E. cell phenomenon. There are two stages in the formation of an L. E. cell, and these can be separated in the laboratory. The first phase is the sensitization phase which is presumably an antigen-antibody reaction between a 7S globulin (the L. E. factor) and nucleoprotein. The second phase is the phagocytosis of the sensitized nucleoprotein, resulting in formation of the L. E. cell. In the first phase mouse liver nuclei are sensitized with a known positive L. E. serum to form "loose bodies" which correspond with hematoxylin bodies. The formation of these "loose bodies" is inhibited by the presence of uremic serum. The "loose bodies" obtained from stage 1 are then presented to competent phagocytes and L. E. Cells are formed (stage 2). The second stage is not inhibited by uremic serum. Fluorescent antibody studies (determinations of anti-nuclear factors) indicate that urea itself is responsible for the inhibition. Conclusions: (1) the L. E. cell test may be suppressed in patients with uremia. (2) This suppression has been diagnostically confusing. (3) Inhibition is relieved by peritoneal dialysis. (4) Inhibition is attributable to a dialyzable serum factor. (5) Inhibition affects the primary reaction of nucleoprotein with L. E. globulin. (6) The dialyzable factor is urea.

Assessment of the Role of Pancreatoduodenectomy in the Treatment of Chronic Relapsing Pancreatitis—Analysis of 16 Cases

DAVID E. MULLINS (M–70)

A review of 35 pancreatoduodenectomies (Whipple procedure) performed at McGuire V. A. Hospital between 1961–68, showed that 16 were done for chronic relapsing pancreatitis and 19 for carcinoma of either the pancreas, ampulla of Vater, or common bile duct. Because of continuing interest in the assessment of the results of surgery for chronic relapsing pancreatitis, these 16 cases were reviewed to evaluate the pre- and post-operative clinical course and to further evaluate the role of the Whipple procedure in the surgical management of chronic pancreatitis. Pre-operative evaluation included: history of pain, alcoholism, narcotic addiction, diabetes, and jaundice, and the presence of biliary tract disease, and pancreatic calcification and cysts. A history of alcoholism was present in 14 of the 16 patients and diabetes and pancreatic calcification was found in 10 patients. Seven patients had undergone 21 previous surgical procedure, prior to the Whipple operation, for the management of their pancreatitis, ie., biliary tract surgery, sphincterotomy, caudal pancreatic drainage, and vagotomy and antrectomy with Billroth II anastomosis. These operations failed to control the patient's recurrent pancreatitis if they continued to consume alcohol or if there was an enlarged, indurated mass in the head of the pancreas at the time of surgery. Post-operatively, three patients developed mild diabetes not present before surgery, 13 required supplemental oral pancreatic enzyme replacement because of weight loss and mild steatorrhea, three became Vitamin A deficient, and two developed osteomalacia. There was one hospital operative death from acute hemorrhagic pancreatitis of the remaining pancreas. Two patients died three and one-half years post-operatively from liver failure

and its sequellae secondary to continued alcohol consumption. The 13 surviving patients have to date demonstrated no further clinical progression of their pancreatitis. *Conclusions*: If the patient has an enlarged, indurated mass in the head of the pancreas at the time of surgery, the Whipple operation has adequately controlled further progression of their pancreatitis. However, only those patients who have a reasonable chance of eliminating alcohol from their diet and who are able to care for their pancreatic endocrine and exocrine deficiencies are candidates for the Whipple operation for control of their symptoms of chronic pancreatitis.