

MEDICAL BULLETIN



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Special Report

A Medical Journey through the Orient

Wesley W. Spink, M.D.*

"- - - the two greatest things in life are
sailing into a strange port and thinking"

Hawaii—James A. Michener

*A*s National Consultant in Internal Medicine to Major General O. K. Niess, Surgeon General of the U.S. Air Force, I made a tour of the Pacific Air Force Command during October 1961. This included visits to Hawaii, Japan, Korea, Okinawa and the Philippines. The purpose of the trip was to provide consultative services in the form of lectures, conferences and ward rounds at the various installations. Visits were also made to U.S. Naval and Army bases, and to military and civilian facilities of the different countries. I participated in the Annual Pacific Air Force Medical Conference held at Baguio, Philippine Islands, October 23-25.

This was an exciting and unique experience, affording an opportunity not only to evaluate military medicine, but to view at first hand the customs and culture of the Far East. I saw some common diseases of the Orient for the first time. Visits were made to Japanese and Korean Universities. The editors of the *MEDICAL BULLETIN* asked me to record some of my observations and impressions, and this is being done in more or less chronological order of travel.

My first stop in the Pacific was Hickam Air Force Base at Honolulu, Hawaii, where I was briefed for the entire trip by Brig. Gen. Robert S. Brua, Command Surgeon of the Pacific Air Force. I visited Tripler General Hospital, a first-class Army installation, and also the Hickam Air Force Dispensary. The latter structure still bore the marks on the outside of the building from the Japanese attack of December 7, 1941. No American can fail to be moved when looking upon the rows of headstones in Punch Bowl Cemetery, marking the graves of our men who died in the Pacific, or upon the American flag flying above the submerged battleship *Arizona* in Pearl Harbor. These first few days of my journey served as a grim reminder that I was to

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travel over a tremendous area of water and land where thousands of Americans had died during the second World War and the Korean War.

JAPAN

After arrival in Japan at Tachikawa, the large USAF Base just outside of Tokyo, I was billeted at the Sanno Hotel for two weeks. This is a modern hotel in the heart of Tokyo operated by Japanese for American military officers. Lt. Col. Robert Smith accompanied me on my tour in Japan. He is a fine officer and physician with a delightful Japanese wife. It was largely because of them that I was to learn so much about Japan in such a short period of time. Tokyo is the largest city in the world, comprising 9 million people, and the traffic hazards while making my way on foot or by car left their lasting impression on me. The city boils with activity. Japanese hustle about at all hours of the day. Rarely does one encounter an obese person. There is a mixture of the old and the new. Japanese women dressed in classic kimonos with children on their backs run through the streets, while young women can be seen in the latest of Western apparel and hair style.

Japan is a nation of almost one hundred million people occupying an area about the size of California and with only 15 per cent of the land that can be tilled for food. Although food is not in abundance, the people appear healthy, and the children are exceptionally well nourished. They are an alert people. The bookstores are crowded with old and young. Throngs of school children, well dressed and clean, are seen on the streets. Japan possesses the only heavy industry in the Far East, and production is considerable, if not excessive, as reflected in the stores and sales rooms. The people appear friendly toward the United States, largely because of our exemplary post-war conduct in a conquered nation.

I visited two Universities and their associated Medical Schools in Tokyo. The first was Tokyo University, the leading state University, and always the pride of the Emperor. The second was Keio University, a privately endowed institution, and the oldest University in Japan. The latter had been badly bombed during the war. These two medical schools represented the best of almost 50 in Japan.

Professor Hideo Ueda, Chief of the Department of Medicine, and a cardiologist, was my host at Tokyo University. As I walked through the University Hospital with its wide halls and high ceilings I was reminded of the Krankenhaus in Munich. As a matter of fact, prior to World War II, and even up to the pres-

ent time, Japanese medicine was markedly influenced by the German system. I saw ample evidence of the persistence of dogmatic and didactic teaching, although Professor Ueda reflected the growing American tradition of bed-side discussion. Tuberculosis and dysentery constitute two major diseases in Japan, but chemotherapy and better hygiene and nutrition are reducing the incidence. There is a high incidence of viral hepatitis. Enteric disorders are probably abetted by the persistent practice of employing human feces for fertilizer. This was the most repelling sight that I witnessed all through the Orient. It certainly curbed my wish for a fresh salad on several occasions. Professor Ueda stated that the major medical illness in Japan was hypertension, with cerebral hemorrhage as the main complication. Coronary occlusion with myocardial infarction occurred much less frequently. I thoroughly enjoyed visiting with Professor Ueda and his staff, all of whom spoke English. My visit at Keio was restricted because of time to a pleasant and instructive visit in the Department of Bacteriology headed by Professor Daizo Ushiba, who was trained at Harvard Medical School. They are doing excellent genetic studies on antibiotic-resistant dysentery bacilli.

The first military installation that I visited was the U.S. Naval Hospital at Yokosuka, which is just south of Yokohama. This is the headquarters of the 7th U.S. fleet, and was formerly the base of Japan's Imperial Navy. The medical staff of this hospital is highly competent. A conference on staphylococcal sepsis was just as informative and impressive as at the University of Minnesota Hospitals. I encountered a new clinical entity—"Kanto Plain Asthma". I was to see this in other sections of this plain that stretches away from Tokyo. Young military personnel after being in the area for several months, acquire asthma, which is debilitating in its severity and requires evacuation of some of the patients from Japan. These individuals are without any allergic history. The asthma is possibly related to the smog and contamination of the atmosphere by fumes from heavy industry.

I encountered a rather unusual experiment in medical education at the Naval Base at Yokosuka, and I was also to see it in operation at U.S. Army and Air Force Hospitals throughout Japan. Outstanding graduates of Japanese medical schools were selected for one-year rotating internships in the hospitals of the three military services on the basis of class standing, personal interview, and the results of a written examination. These young people were an impressive group, speaking excellent English, eager to learn, well disciplined, and willing to work hard. They

were contributing to the excellent care of our military personnel, and at the same time they were acquiring training that would contribute to better medicine in all of Japan. These people would obviously be among the future leaders of medicine in their country. I felt that this effort of the U.S. military service was an excellent exhibition of international good will.

Since I returned to Tokyo from Yokosuka late at night by train, I must comment on this aspect of Japan's transportation. The trains are excellent in every way. In fact, they run precisely on time; the cars are clean; and they are fast, being powered by electricity. The efficiency of the Japanese railways is similar to that found in Switzerland.

In observing U.S. Air Force and Army Hospital facilities I was impressed by the competent leadership and the excellent care that was being given to our military people. Particularly outstanding was the inter-service cooperation. Furthermore, conferences and scientific meetings were held in which Japanese educators participated. A facility known all over Japan is the 406 Medical General Laboratory of the U.S. Army at Zama, under the direction of Col. Tesmer. This laboratory is engaged in basic and clinical research, and also acts in a consultative capacity for the area. I enjoyed seeing the work being carried out on schistosomiasis.

Before leaving Japan, I had occasion to discuss at length the general health problems of the 5th Air Force with Brig. Gen. Charles H. Morehouse, Command Surgeon. Gen. Morehouse is concluding a remarkable career in the service. As a young Army surgeon he was selected on Bataan to accompany Gen. Douglas MacArthur on his escape to Australia via PT boat and airplane. Gen. Morehouse was one of those individuals who was not to see his young wife for almost four years during World War II.

KOREA

To see Japan and Korea in October 1961 is to observe two sharply contrasting nations in the Orient, separated only a few hours by air travel. South Korea is a pathetic victim of war, where a state of truce exists today between two powerful war machines. Three flags fly over every U.S. military installation—the American, Korean and the United Nations. Travelling through the country one sees the squalor of small villages; the absence of heavy industry; the free use of manual labor, both men and women, for large-scale building and road projects; clusters of children along the highways; a profusion of orphanages; and, fortunately, evidence of an excellent rice crop.

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Twenty-five million people live in an area the size of Indiana under a government run by the Army. Wherever people congregate a pungent odor permeates the air, due to Kimchi, their national food. This is a concoction of fermented cabbage, garlic and chili peppers, to which may be added fish and meat.

Directly related to squalor, poverty and inadequate public health facilities, the predominant diseases are tuberculosis, dysentery, typhoid fever, hepatitis and parasitic disorders. Carcinoma of the stomach in the male, and carcinoma of the uterus in the female, are the leading forms of malignancy. Carcinoma of the liver is common in both sexes.

Perhaps the most precious memory of my entire trip occurred at the National University and Medical School in Seoul, Korea, a city of two million people. The College of Medicine



The author (center) was welcomed to National University College of Medicine at Seoul, Korea, by a faculty group including Dr. Saejin Rha (left, front), Dean of the College, and Dr. Sung Hwan Kim (right, front), Superintendent of SNU's Attached Hospital. Also shown (rear rows, left to right) are Dr. Huong Kee Min, an unidentified Korean military aide, Dr. Chong Hwee Chun, Miss Yoon Hi Kang, Dr. Sung Ho Lee, and Dr. Seung Hoon Lee.

has been a special project of the University of Minnesota (see *Med. Bull.* Vol. XXXIII, Nov. 1961—"Korea—A New Venture in International Medical Education" by Dr. N. L. Gault, Jr.). The faculty, the Dean, and the Superintendent of the Hospital greeted me and my military escorts and proudly took us through their hospital and medical school. The good work, as well as the needs, were clearly visible. Never have I encountered a more friendly and a more grateful people. The expenditure of time and effort by the University of Minnesota at Seoul University Medical School has been very worthwhile. South Korea is an armed camp, but the people are extremely friendly to the United States.

OKINAWA

Kadena Air Force Base, Okinawa, was reached after only a few hours' flight south from Tokyo. My visit to Okinawa proved to be the most pleasant surprise of the trip. Okinawa, one of the largest of the group of Ryukyu Islands, is 62 miles long and has a population of 800,000. Just previous to my visit, Okinawa had been struck by a typhoon, which resulted in considerable damage. In view of this and the devastating battles that had occurred there during the war, I had anticipated a wind-swept and barren area. Instead, Okinawa stood out like a pearl in the Pacific. The coral shoreline with the white sands and deep blue-green sea was magnificent. The hilly country was covered by vegetation, including sugar cane and rice. The sides of the hills were dotted with stone structures, which I had presumed to be battle shelters. In reality, they contained the ancestral remains of the islanders, but the Japanese actually did use them for shelters in the defense of Okinawa.

The U.S. Air Force has two dispensaries on Okinawa, one at Kadena, and the other at Naha, the capital city. The Army has an excellent general hospital at Fort Buckner, which is near Naha. It was at this hospital that I saw my first cases of scrub typhus. The illness responds rapidly to therapy with chloramphenicol. About 5 per cent of the population on Okinawa has tuberculosis. Although streptococcal pharyngitis occurs commonly in this tropical and humid area, rheumatic fever and glomerular nephritis are said to be uncommon. There are 7000 known lepers on Okinawa, who are taken care of by the government of Okinawa.

No one can visit Okinawa without seeing the large market areas in Naha, many of which are in the open air, and practically all run by women. Here one can see and buy excellent fresh ocean fish, such as red snapper and lobster. Mothers trot about



A market place in Naha, Okinawa

with infants asleep on their backs as they pick up their daily supplies.

THE PHILIPPINES

Clark Air Force Hospital, 60 miles north of Manila, is an unusual installation because it provides services, not only for military personnel, but on occasion for civilians throughout Southeast Asia. My host at Clark was Col. John Rizzolo, the Command Surgeon of the 13th Air Force, who had started his medical career in the Philippines, was captured at Bataan, and was one of the 10,000 men who survived the march from Bataan to Camp O'Donnell. Amoebic dysentery, tuberculosis, hepatitis, schistosomiasis and the bites of venomous snakes are among the disorders that are encountered in this tropical area. Filipino interns are selected for this hospital. I was surprised by the constant threat of rabies that exists there, although precautions are being taken to control the rabid animal population.

As one travels through the countryside one gains the impression that the Philippines are emerging from centuries of political subjugation with its poverty, ignorance and disease. The caribou is the work animal of the peasant, and sugar cane, rice and bananas among the chief products. There is little or no heavy industry. The Filipinos are trying their hand at democracy, and while I was there a wild political campaign, not without violence and several deaths, was being carried on to elect a President. The people are extremely friendly toward the United States.

I did not have an opportunity to visit the medical schools in Manila, because I concentrated on observing cholera patients, the results of which I shall relate shortly. Manila is a large bustling city and the large number of "jeepies" that swarm through the streets and suburbs is more than distracting. These are surplus American military jeeps that have been converted into buses, and decorated and painted. They provide the principal means of transportation, and are usually loaded down with humanity.

CHOLERA

Just 100 years ago the first International Sanitary Conference met in Paris for the purpose of controlling pestilential diseases, but it was not until 1892 that 14 European countries agreed to abide by strict regulations in the control of cholera. This initial step of international health cooperation was the forerunner of the World Health Organization of the United Nations. This effort to control cholera has resulted in restricting the disease to a few areas in Southeast Asia, principally along the Ganges in India. During the past few years epidemics have broken out in Southeast Asia, including Thailand and Hong Kong. Increasing numbers of cases of dysentery erupted around Manila in 1961, which were identified as instances of cholera, due to the E1 Tor strain of *Vibrio vibrio*, the same type that had precipitated the epidemic in Hong Kong.

In company with Col. Rizzolo I visited the San Lazarus Hospital for communicable diseases in Manila. This institution of 900 beds is maintained by the national government. One of the buildings was devoted to the care and treatment of the cholera cases. On October 20, 1961 I saw 65 cases in this hospital, mostly adults, representing all stages of the disease. The majority of the patients exhibited an associated state of malnutrition.

The outstanding features of cholera are chills, fever, severe diarrhea with vomiting, and circulatory collapse due to *de-*



This unit of the 900-bed San Lazarus Hospital, Manila, Philippines, is devoted to the care and treatment of cholera patients.

hydration. As a result, cyanosis and oliguria are evident. The patients are acidotic. The loss of liters of fluid from the intestinal tract, along with sodium, often caused severe muscle cramps. A most impressive observation was the thick, dark venous blood that was withdrawn with difficulty through a needle. Specific therapy consisted of administering normal saline solution intravenously, and a solution of 2 per cent sodium bicarbonate in distilled water. Potassium contained in meat broth was administered by mouth. Up to 8-10 liters of intravenous infusions were frequently necessary to correct the dehydration, which was done within a few hours. No antibiotics were employed. Recovery was the rule after a few days. Death occurs because of circulatory collapse and renal failure, although the mortality rate in Manila was low.

These patients could not have been treated properly without the direction and aid of American military physicians during the cholera outbreak of 1961. In addition, supplies were provided through this source. Qualified U.S. Naval personnel from Subic Bay, Philippines, and from the Naval Research Unit in Taipei (NAMRU No. 2) quickly had matters under control, setting up a temporary laboratory, and instructing the Filipino doctors and nurses in the treatment of the patients. One member of each patient's family was in constant attendance, having been taught about the necessary nursing care of the patient. Personnel and

supplies from Clark Air Force Base were also provided. The Filipinos were most grateful for this help, which served to increase our friendship and good relations with this people.

Although the epidemiology of cholera has been intensively studied over the years there still remain many unexplored features. Without much question, water contaminated with fecal material can serve as a source of an epidemic. This was dramatically illustrated over 100 years ago when an epidemic abated after John Snow removed the pump handle on Broad Street in London, prohibiting the use of drinking water from this source. The carrier state endures for a very brief period of time, but these individuals along with those having a mild infection, must be the source of the disease. The disease is kept going by the infection of susceptible individuals in areas where poor sanitation and hygiene exist.

THE PACIFIC AIR FORCE (PACAF) MEDICAL CONFERENCE

When Maj. Gen. Niess was Surgeon of PACAF he initiated a medical conference in the Pacific area for U.S. Armed Forces medical personnel, and military and civilian physicians from other nations in the Far East. On October 23-25, 1961, the Eleventh PACAF Medical Conference was held at Baguio, Philippines with over 300 physicians in attendance representing 10 Far East nations and the United States. Although military medicine was widely discussed, the theme of the conference was "International Fellowship through Medicine." I was particularly interested to see the respect and attention paid to Dr. Kazuo Miyamoto, a scholarly Hawaiian physician who spoke on the subject, "To Live More Fully." My own contribution to the conference was a summary of my observations in the Far East, and a presentation of our research on "Shock."

Maj. Gen. Niess is particularly interested in aero-space medicine, and it was fascinating to listen to the past experiences of the Air Force in this field, and about some of the engineering plans for the future. I manifested such enthusiasm that I am now a member of the "Far East Aero-Space Medical Society."

Baguio is one of the most beautiful areas that I have ever seen. John Hay Air Force Base is located there. It is also the summer capital of the Philippines, located in northern Luzon, 5000 feet above sea level. During the conference I stayed in the beautiful summer home of the U.S. Ambassador to the Philippines, Mr. John D. Hickerson. It was in the living room of this residence that the officers of the Japanese Army surrendered their command to the Americans. It was not all work at Baguio, but a chance to relax and talk with people from Aus-

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tralia, Singapore, Burma, Thailand, Taiwan, Philippines, Korea and other nations. Some used the marvelous golf course. During this part of my visit I was reminded of those lines of Plutarch:

"For the noblest deeds do not always show men's virtues and vices; but often times a light occasion, a word, or some sport makes men's natural disposition and manners appear more plain than the most famous battles won."

SOME GENERAL IMPRESSIONS

The most significant impression that I gained in this rather extensive tour of the military installations of the Far East was the excellent cooperation between the Army, Air Force and



Maj. Gen. O. K. Niess, Surgeon General of the U. S. Air Force, and Dr. W. W. Spink, the author, conferred in Baguio, Philippine Islands, during the 11th annual Pacific Air Force Medical Conference. Dr. Spink is a national consultant in internal medicine to the Air Force.

Navy, which was apparent at all levels. This collective effort was reflected in the high standard of medical care being given to American personnel. Furthermore, this cooperation extended to civilian physicians of the various nations.

I was also very favorably impressed by the leadership that we have in the Armed Forces. Young men away from home for the first time, and physicians just out of their internships, need wise counsel at times, and this is being provided. Particularly impressive were some of the officers who had been prisoners of war and had seen the horrors of the war. In addition, a competent nursing service is well established in the three services.

One of the finest gestures that I have seen toward better international relationships involved the use of Japanese and Filipino interns in U.S. military hospitals and dispensaries. These carefully selected young people will make a good contribution to their own native land as a result of this advanced training. Some have even expressed a desire to further their postgraduate training in the United States.

The Far East is important not only for the United States, but also for the rest of the western world and for peace. Our culture and heritage stem largely from Western Europe, which continue to bind us closely to those nations. But with the avenues of communication being opened up more widely, and travel made easier, our attention must be turned more and more to the Orient. We must learn more of the culture and history of these ancient civilizations. This will bring about a better understanding of these people, and better serve international good will and peace.

Staff Meeting Report

Histochemical Studies of Induced Enzymes Metabolizing Carcinogenic and Non-Carcinogenic Polycyclic Hydrocarbons*

Lee W. Wattenberg, M.D.†

The induction of malignant tumors in man and experimental animals by contact with polycyclic hydrocarbons represents one of the oldest significant findings in cancer research. Such malignant tumors may occur at the initial site of contact with the carcinogen or at a distance from this site. The direct effect, in particular that on the skin, is the most familiar one.¹ However, the production of malignant tumors at a site distant from that of administration of the carcinogen can readily be demonstrated experimentally. An excellent example of this is the occurrence of mammary carcinoma in the rat which follows feeding of certain polycyclic hydrocarbons.²

Of possible relationship to the carcinogenic potential of polycyclic hydrocarbons at a site distant from that of administration is the recent observation that a variety of these compounds are capable of inducing the synthesis of a group of closely related microsomal enzymes which metabolize compounds not normally occurring in the animal body.³⁻⁶ An early indication of this effect was the observation that azo dye carcinogenesis in the liver was prevented by the simultaneous administration of methylcholanthrene. It was later shown that the administration of any one of a number of polycyclic hydrocarbons caused a marked enhancement of two specific enzymes in liver which convert the azo dyes to inactive metabolites.⁶ In addition to the azo dye metabolizing enzymes,



Lee Wattenberg

*This paper was presented at the staff meeting of the University Hospitals on Dec. 1, 1961

This study was supported by research grant C-9599 from the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service

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an increase in activity of enzymes causing hydroxylation and perhydroxylation of polycyclic hydrocarbons, cleavage of aromatic ethers, and a number of other reactions have been observed.³

In the microsomal systems whose activities are enhanced by administration of a polycyclic hydrocarbon, maximum reaction rates are characteristically obtained when both reduced triphosphopyridine nucleotide (TPNH) and reduced diphosphopyridine nucleotide (DPNH), or systems which will generate the reduced forms of these coenzymes, are present in the reaction mixture.⁴ Omission of TPNH causes almost complete loss of activity, whereas only a slight loss of activity results from omission of DPNH. In normal animals some activity of these enzyme systems is found. When any one of a large number of polycyclic hydrocarbons, both carcinogenic and noncarcinogenic, has been injected into animals, marked increases in enzyme activity have been shown to occur in hepatic cells.⁵ According to available evidence, the diverse catalytic reactions whose increased activities are induced by polycyclic hydrocarbons are not the result of a single enzyme. The exact number of distinct enzyme moieties which function in these reactions has not been established.³

A knowledge of the precise localization of enzyme systems is desirable since it may furnish information bearing on their function and interrelationships with other systems. In addition, under pathologic conditions selective changes in activity occurring at one site and not another may be overlooked if the tissue as a whole is analyzed. Accordingly, it was decided to devise a histochemical procedure for demonstrating reduced nucleotide-dependent hydrocarbon metabolizing systems. As a possible substrate for this type of procedure 3,4-benzpyrene was chosen because it had been shown to be converted rapidly to hydroxy derivatives and quinones by an inducible microsomal system in rat liver.⁴ This agent (3,4-benzpyrene) is intensely fluorescent. Alterations of its characteristic fluorescence as a result of enzyme activity^{7,8} suggested a potential method of histochemically demonstrating this metabolic system so that its localization might be observed. This type of histochemical procedure was readily worked out, and it was found to be applicable to a number of additional polycyclic hydrocarbon substrates.⁹

MATERIALS AND METHODS

The distribution of reduced nucleotide-dependent polycyclic hydrocarbon metabolizing systems was studied in normal Sprague Dawley rats and also in rats in which an increase in activity of these systems was induced by the intraperitoneal admin-

istration of 2 ml. sesame oil containing 10 mg. methylcholanthracene. Studies of the activities of these systems in the livers of normal animals of several other species (mouse, hamster, monkey, and man) were also carried out. In the studies to be reported two substrates were employed: 3,4-benzopyrene, which is a potent carcinogen; and perylene, which is noncarcinogenic. The details of the histochemical procedures employed are described elsewhere.⁹

RESULTS

The distribution of reduced pyridine nucleotide-dependent polycyclic hydrocarbon metabolizing systems in normal rats. In the normal animals these systems have been found in the liver, adrenal, and kidney. In the liver the centrilobular cells show a positive reaction, whereas the cells at the periphery of the hepatic lobules are negative. In the adrenal the positive reaction is found in the zona fasciculata and zona reticularis. The zona glomerulosa and medulla are negative. The kidney yields a positive reaction, which is limited to the distal portion of the proximal convoluted tubules. The reaction in all three tissues is quite weak, being strongest in the adrenal, and least intense in the kidney.

The distribution of reduced pyridine nucleotide-dependent polycyclic hydrocarbon metabolizing systems in rats injected with methylcholanthrene. In these experimental rats the activity of the hydrocarbon metabolizing systems is strongly enhanced in the liver and the kidney. The centrilobular cells of the liver show an intense reaction, and the cells at the periphery of the lobules show a weaker, but nevertheless quite appreciable, reaction. In the kidney the cells in the distal portion of the proximal convoluted tubules are still the only ones that show a positive reaction. The intensity of this reaction, however, is greatly increased over that found in the normal kidney. In the adrenals of these rats there appears to be no change in either the distribution or the intensity of the reaction as compared to normal animals. In addition to the three tissues which show a positive reaction in both groups of rats, three additional tissues show a positive reaction only in animals injected with methylcholanthrene. These are the lung, the thyroid, and the testis. In the lung the alveolar walls show activity, while other structures are negative; in the thyroid the follicular epithelium shows a positive reaction; and in the testis a positive reaction is observed in the interstitial cells. Both normal animals and those given methylcholanthrene injections exhibit parallel levels of activity with the two polycyclic hydrocarbon substrates, 3,4-benz-

pyrene and perylene. A summary of these findings is given in Table 1.

Liver studies in species other than the rat. Sections of liver from normal mouse, hamster, monkey, and man have been studied for the presence of polycyclic hydrocarbon metabolizing systems. In each species a positive reaction has been obtained. As in the normal rat, the activity is observed only in the centrilobular cells.

TABLE I
TYPICAL ACTIVITIES OF REDUCED PYRIDINE NUCLEOTIDE-DEPENDENT
POLYCYCLIC HYDROCARBON METABOLIZING SYSTEMS IN NORMAL
AND METHYLCHOLANTHRENE-INJECTED RATS

Tissue ¹	Enzyme Activity ²	
	Control Rats	Test Rats ³
Liver	2+	8+
Adrenal	3+	3+
Kidney	1+	4+
Thyroid	0	2+
Lung	0	2+
Testis	0	1+

¹The activities recorded refer to the following: liver, centrilobular cells; adrenal, zona fasciculata and zona reticularis; kidney, distal portion of the proximal convoluted tubules; thyroid, follicular epithelium; lung, alveolar walls; testis, interstitial cells.

²Enzyme activity is estimated by visual microscopic study. Activities with perylene and benzpyrene as substrates are similar.

³Each test rat was given an intraperitoneal injection of 10 mg. methylcholanthrene 48 hours before being killed.

DISCUSSION

The distribution of reduced pyridine nucleotide-dependent systems metabolizing polycyclic hydrocarbons which has been found in the present study is of some interest with regard to consideration of the function of these systems. These reactions metabolize foreign compounds and are generally considered as being related to their detoxification and excretion. This concept is based on the ability of an enhanced activity of these systems to protect against azo dye carcinogenesis, to inactivate certain drugs, and likewise on the fact that the hydroxylated polycyclic hydrocarbons which result from their activity have either little or no carcinogenicity.^{3,6,7} An alternate possibility has been suggested by *in vitro* studies which have shown that the hydroxylating system also may result in binding of hydrocarbons to protein, which is considered a probable prerequisite to carcinogenicity.¹⁰

The occurrence of these hydrocarbon metabolizing systems in liver, kidney, and lung would be in accord with the concept that these systems are related to detoxification and excretion. But their presence in the adrenal cortex, the interstitial cells of the testis, and the thyroid suggests a function specifically having to do with the endocrine system. What this function is and how it may be related to carcinogenesis, particularly that which takes place at a site remote from the site of administration of these compounds, remain to be determined.

SUMMARY

1. Histochemical procedures have been developed for the demonstration of reduced pyridine nucleotide-dependent polycyclic hydrocarbon metabolizing systems.

2. Positive reactions have been obtained in the hepatic cells of rat, mouse, hamster, monkey, and man.

3. A survey of rat tissues has shown positive reactions also in the distal portions of the proximal convoluted tubules of the kidney, the zona fasciculata and zona reticularis of the adrenal, the interstitial cells of the testis, the follicular epithelium of the thyroid and the alveolar walls of the lung.

4. The activity is considerably higher in sections of liver, kidney, thyroid and lung from rats injected with methylcholanthrene than in control animals. In the adrenal no such increase is found.

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Staff Meeting Report

Laboratory Control of Anticoagulant Treatment with Coumarin Derivatives*†

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Since the isolation and synthesis of dicumarol by Link and his colleagues in 1940,^{1,2} extensive use has been made of coumarin and indanedione derivatives in the treatment and prophylaxis of thromboembolic disease. Clinical experiences with these anticoagulant agents³⁻¹⁶ suggest that the incidence both of recurrence of thrombosis and of mortality is decreased significantly in patients receiving such treatment on a long-term program. But even when the hypocoagulability of the treated patients has been kept within presumably safe limits for both coagulation and bleeding, the recurrence rate of thrombosis and of hemorrhage has not been reduced to zero.



LORRAINE GONYEA

Since the limits of hypocoagulability are ideally those of a limited reduction in the synthesis of certain clotting factors, the desired disturbance in coagulation can be achieved with these drugs only if an adequate quantitative description of the defect is available. The lower limit may be considered as that concentration of these factors that is associated with a negligible incidence of hemorrhagic complications. The upper limit implies a significant reduction in the concentration of these factors as compared with their concentration in the normal population. The degree of reduction necessary, however, must be defined by the incidence of recurrent thromboembolic disease. In the management of individual patients for whom the rapidity, degree, and duration of action of the drugs are all variable, quantitative estimations of clotting factors become essential.

Coumarin and indanedione derivatives affect the coagulation

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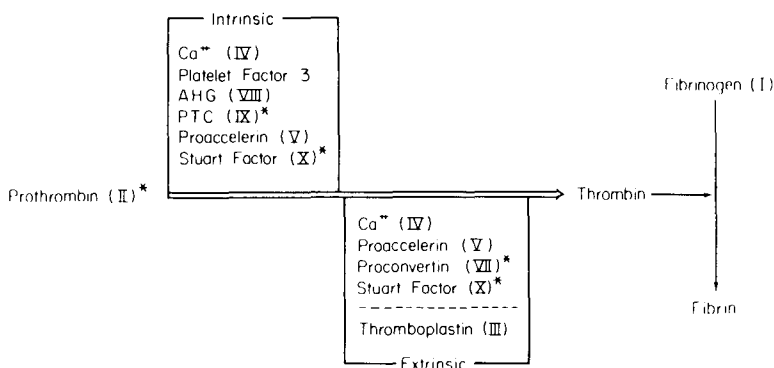
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mechanism through the group of factors now recognized as being dependent upon vitamin K for their synthesis. The four factors affected are prothrombin, proconvertin, plasma thromboplastin component (PTC), and Stuart factor^{7,17-27}. Proconvertin functions in what has been termed the extrinsic system of coagulation, and PTC functions in the intrinsic system, while prothrombin and Stuart factor are common to both activation systems. The intrinsic system involves factors found wholly in the circulating blood. The extrinsic system involves plasma factors, most of which also operate in the intrinsic system, plus a thromboplastic agent from the tissues. In both systems, a complex process of interaction occurs, with the subsequent generation of an active enzymatic system that results in the conversion of prothrombin to thrombin. Figure 1 diagrammatically represents those factors recognized by the International Nomenclature Committee, 1960^{28,29} which operate in each of these systems. Calcium is required in both systems for the formation of the prothrombin converting agents and for the conversion of prothrombin to thrombin. Besides prothrombin and Stuart factor, proaccelerin can be shown to participate in both the intrinsic and extrinsic systems. The amount of thrombin formed is limited pri-

FIG. 1



* Decreased during Treatment with Coumarin Derivatives (Vitamin K- Dependent Factors)

Fig. 1. Factors required for optimal conversion of prothrombin to thrombin in the intrinsic and extrinsic systems. Only the factors assigned a numerical designation by the International Nomenclature Committee as of 1960 have been listed.

marily by the amount of prothrombin present, and the rate of its formation is in turn limited by the amount of thromboplastic and accelerating factors present.

The vitamin K-dependent factors comprise a group of relatively stable proteins. Except for prothrombin, they can be demonstrated in serum after coagulation has occurred. Separation of these factors has been effected by means of selective adsorption and electrophoretic and chromatographic techniques.^{18,30-38} During the administration of the anticoagulant drugs, the vitamin K-dependent factors probably decrease at similar rates, although there may be some variation with individual drugs and individual patients; and the rates of depression are probably related to the turnover rates of the various factors.²⁴

It has been emphasized^{7,39} that an adequate quantitative description of the effect of these drugs requires measurement of all the factors affected. This position implies that the factors must be assayed individually or, if jointly, must be measured by some technique that, as a minimum, will reflect deficiencies in these factors. In this paper we will discuss the applications and limitations of some available laboratory methods and will present the comparative results obtained when these methods were applied to patients undergoing appropriate anticoagulant treatment.

MATERIAL AND METHODS

One hundred and forty-four blood samples were obtained from 48 patients. All these patients were under treatment with dicumarol or coumadin in the outpatient clinic at the University of Minnesota Hospitals.

The *Quick prothrombin* time determination was performed as described by Quick,⁴⁰ except that Difco rabbit brain thromboplastin and 0.015 molar CaCl₂ were used. All determinations were performed in duplicate. In order to permit comparison with the activities observed with the other methods, the results have been expressed in percentages of normal values. The per cent activity was read from a standard curve obtained from a series of dilutions, from 10 to 100 per cent, of a pool of normal plasma in fibrinogen diluent (0.3 per cent clottable protein, Armour, in Tris-HCl buffered NaCl, pH 7.4, ionic strength 0.154⁴¹). The range based on determinations from 70 normal adults was 11.2 to 15.0 seconds (\pm S.D.).

The *thrombotest* was performed as described by Owren³⁹ for citrated plasma, using a reagent obtained from Nyegaard and Co., Oslo, Norway.

The *prothrombin-proconvertin* assay was modified from the

method described by Owren and Aas.¹⁸ Citrated plasma samples were frozen on the day they were drawn, and were tested later. One-tenth ml. thromboplastin (Difco), 0.1 ml. BaSO₄ adsorbed bovine plasma, 0.1 ml. test plasma diluted 1:20 in buffered NaCl (as used in the Quick method), and 0.1 ml. 0.02M CaCl₂ were mixed at 37° C. Clotting times were converted to per cent activity from a correlation graph based on plasma dilutions, 10 to 100% in buffered NaCl, each dilution treated as a test plasma.

The *prothrombin* assay, adapted from the method of Owren and Aas,¹⁸ was made in the same way as the prothrombin-proconvertin determination, except that 0.1 ml. of a serum reagent^{30,42} was added, to provide the activities of proconvertin and Stuart factor.

The *proaccelerin* assay was modified from the method of Stormorken.⁴³ Freshly drawn citrated plasma was used. One-tenth ml. thromboplastin (Difco), 0.1 ml. proaccelerin-free plasma, 0.1 ml. test plasma diluted 1:20 in buffered NaCl, and 0.1 ml. 0.02 M CaCl₂ were mixed at 37° C. Clotting times were converted to per cent activity from a correlation graph based on a series of plasma dilutions, 10 to 100% in buffered NaCl, each dilution being treated as a test plasma.

The *partial thromboplastin* time was performed on freshly drawn citrated plasma, using the method of Langdell et al.⁴⁴ Inosithin, a phospholipid soybean extract obtained from Associated Concentrated, Inc., Woodside, N.Y., was used. Calcium chloride concentration was 0.03 molar. The range based on determinations from 35 normal adults was 51 to 75 seconds (\pm 2 S.D. from the mean).

The *whole-blood clotting* time was modified from the method of Lee and White.⁴⁵ One ml. blood was put into each of three glass test tubes, 10 x 75 mm., and left undisturbed for ten minutes at room temperature. The first two tubes were then tipped gently, in succession, at one minute intervals until a solid clot was formed. The third tube was tipped at 30 second intervals until a clot was formed. The end point was taken as the clotting time of the third tube. The range based on determinations from 38 normal adults was 18 to 25 minutes (\pm 2 S.D.).

RESULTS

Since the majority of clinical laboratories have used the Quick test as the only laboratory control method for the administration of coumarin derivatives, it seems appropriate to compare the other control tests with the Quick method. Fig. 2, a and b, presents these comparisons, for the Quick test and the prothrom-

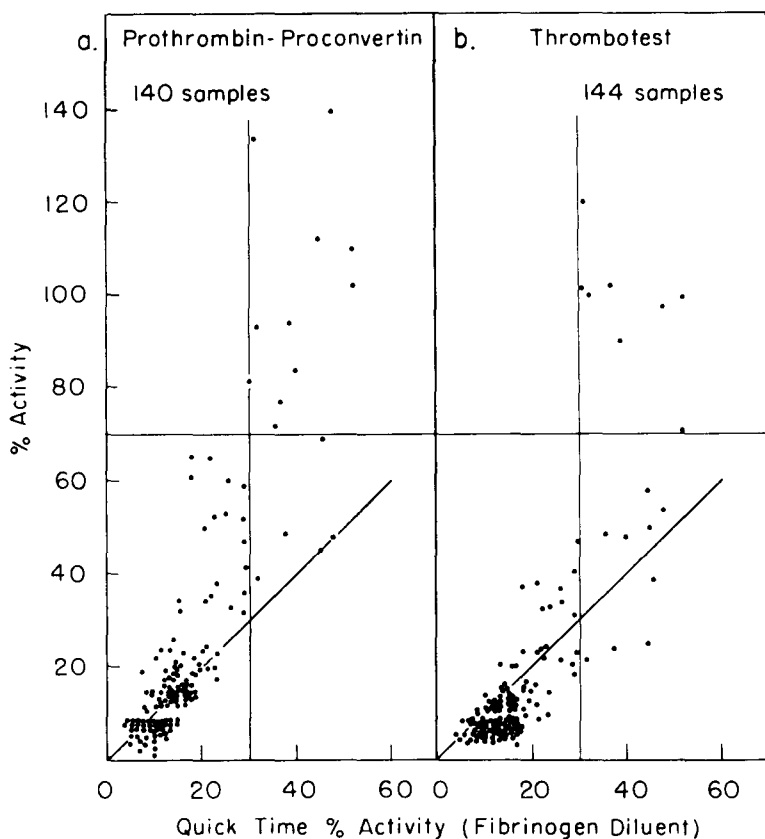


Fig. 2a. Scatter diagram comparing results with the Quick method (abscissa) and the prothrombin-proconvertin method (ordinate).

Fig. 2b. Comparison of results with the Quick method (abscissa) and the thrombotest method (ordinate). The horizontal line indicates the lower limit of normal (70%) for the prothrombin-proconvertin and thrombotest methods. The vertical line indicates the lower limit of normal (15 seconds, or 30%) for the Quick method.

bin-proconvertin and thrombotest methods, in the form of scatter diagrams. Comparison of the Quick method with the prothrombin assay showed similar correlation. In general there appears to be a reasonably good correlation between the Quick test and each of the three other methods at the low levels of activity.

Those values falling in the middle range provide another basis for a comparison of the methods. Since the number of tests in this range is small, the lower limit of normal for each test has been taken as a point for a comparison of the values. For the Quick test the normal values obtained in this laboratory have been used to set the lower limit, since this test does not provide values that allow direct comparison of the results from various laboratories. This lack of comparability is due primarily to the wide variations encountered among different thromboplastins⁴⁶⁻⁴⁸ and to the various ways of expressing activity. With the fibrinogen dilution curve used in this study to convert results with the Quick test to per cent activity, the lower limit of the normal range may be taken as 30 per cent, which corresponded to 15 seconds (2 S.D. below the mean). The prothrombin-proconvertin, thrombotest, and prothrombin methods are more rigidly controlled, and therefore should yield results that are comparable among various laboratories. Studies indicate that 70% can be considered as the approximate lower limit of normal.⁴⁹ Most of the specimens examined were found to be abnormal by all methods. With all four of these tests, however, seven patients fell within the normal range even though they were under therapy with a specific anticoagulant agent. Two patients who had abnormal Quick times were within the normal range with one or more of the other methods. Three samples, however, gave abnormal values for all three of the more specific tests but yielded Quick times that fell within the normal range.

Although the Quick time can be shown to measure proaccelerin and to be sensitive to gross deficiencies of fibrinogen (in addition to measuring three of the four factors affected by the coumarin derivatives), adequate levels of those factors not affected by these drugs do not greatly decrease the validity of the test when it is used as an index of the anticoagulant effect. No significant correlation was evident when the proaccelerin values were compared with those of the Quick method, although a small number of patients did have abnormal values in the proaccelerin assay. These can probably be explained by the presence of barely optimal or suboptimal levels of the vitamin K-dependent factors present in this test system. Some of these patients may also have had liver disease of sufficient severity to have produced minor deficiencies of proaccelerin. In none of these patients did the values suggest a severe deficiency; all were within the range that has been found in asymptomatic carriers of the congenital deficiency of proaccelerin.⁵⁰

As might be expected from the comparisons made with the Quick method, those assays which measure only the factors de-

EVALUATION OF TESTS IN ANTICOAGULANT THERAPY

		PROTHROMBIN- PROCONVERTIN	PROTHROMBIN	QUICK	PARTIAL THROMBOPLASTIN	CLOTTING TIME	
THROMBOTEST	Both tests	< normal normal	129/140 = 92%	126/140 = 90%	128/144 = 89%	61/83 = 73%	40/66 = 61%
			7	7	7	4	4
	Discordant	T Low	4 P = 0.125	7 P = 0.016	9 P = 0.004	18 P = <0.001	22 P = <0.001
		T High	0	0	0	0	0
Both tests	< 50%	118/140 = 84%	112/140 = 80%				
	> 50%	9	10				
Discordant	T Low	12 P = 0.006	18 P = <0.001				
	T High	1	0				
PROTHROMBIN- PROCONVERTIN	Both tests	< normal normal	126/140 = 90%	124/140 = 89%	61/83 = 73%	40/66 = 61%	
			11	10	6	5	
	Discordant	PP Low	3 P = 0.25	5 P = 0.44	16 P = <0.001	21 P = <0.001	
		PP High	0	1	0	0	
Both tests	< 50%		111/140 = 79%				
	> 50%		21				
Discordant	PP Low		8 P = 0.008				
	PP High		0				
PROTHROMBIN	Both tests	< normal normal		123/140 = 88%	61/83 = 73%	40/66 = 61%	
				12	6	5	
Discordant	P Low			3 P = 1.0	16 P = <0.001	21 P = <0.001	
	P High			2	0	0	
QUICK	Both tests	< normal normal			60/83 = 72%	40/66 = 61%	
					8	7	
Discordant	Q Low				14 P = <0.001	19 P = <0.001	
	Q High				1	0	
PARTIAL THROMBOPLASTIN	Both tests	< normal normal				35/66 = 53%	
						13	
Discordant	PT Low					14 P = 0.038	
	PT High					4	

Normal: 70% and higher, thrombotest, prothrombin-proconvertin and prothrombin methods; 15 seconds and less, Quick method; 75 seconds and less, partial thromboplastin time; 25 minutes and less, clotting time. Note: P values were determined with the Sign test.

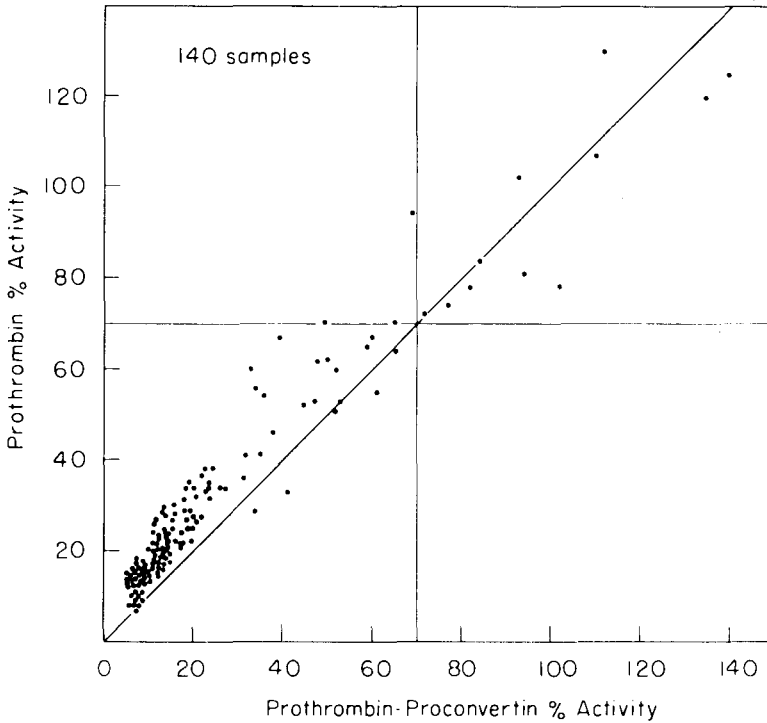


Fig. 3. Comparison of results with the prothrombin-proconvertin method (abscissa) and prothrombin assay (ordinate). The horizontal and vertical lines indicate the lower limit of normal activity (70%) for each method.

pressed by the administration of the coumarin derivatives showed good intercorrelation. Fig. 3 shows the excellent correlation between the prothrombin assay and the prothrombin-proconvertin assay. Comparison of the results obtained with the thrombotest and the prothrombin-proconvertin method is shown in Fig. 4. Seven patients can be seen to fall within the normal limits for all of the tests. All patients who had normal values in the thrombotest also had normal values in both the prothrombin-proconvertin and prothrombin assays. Four patients, however, did have values in the normal range for the prothrombin assay and the prothrombin-proconvertin assay, although the same samples were below the normal range within the thrombotest. This at

least suggests that the thrombotest may be a more sensitive index of anticoagulant therapy of this nature.

A more precise representation of the correlation among the various methods is presented in the Table. The abnormal results expected during anticoagulant therapy occurred with significantly less frequency in determinations of partial thromboplastin time and whole-blood clotting time than in the other four methods. But comparison of the thrombotest, prothrombin-proconvertin, prothrombin, and Quick methods, showed a large majority of values below the normal range, as well as a significant degree of consistency in frequency of paired results falling within or below the normal range. A small number of values were within the normal range of activity with some methods, but below normal with others. The thrombotest consistently showed the lower values when compared with any of the other methods. These discordant results were examined by means of the Sign test⁵¹

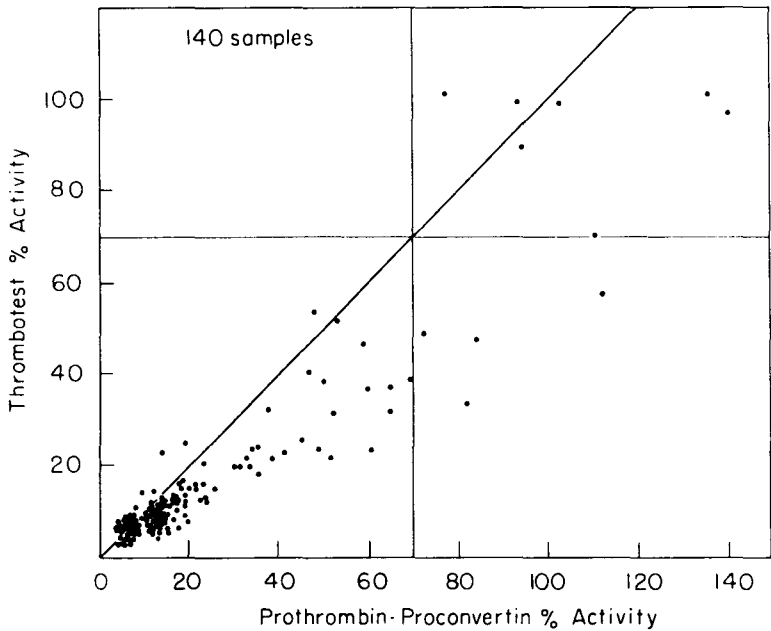


Fig. 4. Comparison of results with the prothrombin-proconvertin method (abscissa) and the thrombotest method (ordinate). The horizontal and vertical lines indicate the lower limit of normal activity (70%) for each method.

to determine the significance of the incompatibilities. When values falling within and below the normal limits were considered, the thrombotest method, while only slightly more sensitive than the prothrombin-proconvertin method, was noted to be a significantly better indicator of hypocoagulability than was either the prothrombin or the Quick method. No significant differences in sensitivity were observed between the prothrombin-proconvertin and the prothrombin or the Quick methods. When the 50 per cent level of activity was used as a point of discrimination for the thrombotest, prothrombin-proconvertin, and prothrombin methods, the differences were more pronounced. In order of increasing sensitivity, the tests can be classified 1) the Quick method, 2) the prothrombin assay, 3) the prothrombin-proconvertin method, and 4) the thrombotest method.

DISCUSSION

Of the various assay procedures employed in this study, all but the proaccelerin determination measure the clotting factors affected by the coumarin derivatives. They are not, however,

	Whole Blood Clotting Time	Partial Thromboplastin Time	Quick Prothrombin Time	Thrombotest	Prothrombin-Proconvertin	Prothrombin Assay	Proaccelerin Assay
Thromboplastin		Phospholipid (Inosithin)		Saline Brain Ext + Phospholipid + BaSO ₄ ADS. Plasma	Saline brain extract		
AHG (VIII)							
PTC (IX)*							
Fibrinogen (I)				BaSO ₄ Adsorbed Plasma		Proaccelerin Free Plasma	
Proaccelerin (V)							
Stuart Factor (X)*						Serum (Eluted BaSO ₄ Adsorbate)	Proaccelerin Free Plasma
Proconvertin (XII)*							
Prothrombin (II)*							
* Decreased with Coumarin Derivatives (Vitamin K-Dependent)							

Fig. 5. Diagrammatic representation of the methods used in the study. The source of the controlled factors for each method is indicated in the boxes. The shaded areas indicate the factors to be accounted for in the test material. Calcium chloride, in optimal amounts, is added in all but the whole-blood clotting time method.

equally sensitive indices. The extent to which these tests reflect anticoagulant therapy parallels the specificity of the tests in terms both of the number of factors that may affect the *assay* and of the number of factors measured in the assay that are affected by the *therapy* (Fig. 5).

Two of the tests—the whole-blood clotting time and the partial thromboplastin time—were poor indices of anticoagulation. Although all the samples studied were obtained during therapy, thirteen yielded values that were still within the normal range. Neither test reflected the rather extreme coagulation defects present in some of these patients. Although these two tests have been included in certain comparative studies,^{24,52,53} they are not generally considered adequate guides to therapy.

Four methods, the prothrombin-proconvertin, prothrombin, thrombotest assays, and the Quick time, have shown a good correlation with anticoagulant therapy. Three of these techniques—the Quick, prothrombin-proconvertin, and prothrombin methods—are based on the use of a thromboplastin which will substitute for the activities of antihemophilic globulin (AHG), PTC, platelet factor 3, and such other factors as are concerned in the very early stages of the intrinsic system of coagulation. The thromboplastin used is a lipoprotein of very high molecular weight,⁵⁴ extracted with saline solution from a suitable tissue such as brain or lung. This thromboplastin is not a direct activator of prothrombin, since it requires the presence of calcium, proaccelerin, proconvertin, and Stuart factor for optimal conversion of prothrombin to thrombin. The reaction is very rapid in the Quick method, provided all factors are optimally present. Reduction of any one of the plasma factors, however, will prolong the reaction.⁴² In the prothrombin-proconvertin method, an excess of proaccelerin and fibrinogen is provided by bovine plasma from which prothrombin, proconvertin, and Stuart factor have been removed with barium sulfate. The activities of the latter three factors in the test plasma will, therefore, determine the clotting time with this method. The specific prothrombin assay uses, in addition to adsorbed plasma as the source of proaccelerin and fibrinogen, a prothrombin-free serum reagent to provide the activities of proconvertin and Stuart factor.

The assay for proaccelerin was included in this study because variations in this factor are known to have a marked effect on the Quick method.^{42,50} No correlation was observed between the results of the Quick method and of the proaccelerin assay on plasma from patients receiving coumarin anticoagulants.

Although the Quick time, prothrombin-proconvertin, and prothrombin assays differ in factors that may influence the re-

sults, they did not show a statistically significant difference when used as an index of anticoagulant therapy. The best agreement was that noted between the Quick time and the prothrombin assay. This degree of correlation is of some interest, since the Quick method is considered by some to be a poor index of prothrombin deficiency and to depend in large part on the level of proconvertin.^{55,56} The degree of correlation that we have observed between the Quick time and the prothrombin-proconvertin assay does not agree with the reports of several authors.⁵⁷⁻⁶⁰ Although no statistical evaluations have been reported, it would appear from the data presented that in some laboratories the prothrombin-proconvertin method is a better index of anticoagulation than our findings indicate. Matthews and Walker⁵⁶ have discussed the reasons for the differences in results obtained in different laboratories by means of this technique.

Because of the nature of the thromboplastin used, the effect of anticoagulant therapy on PTC is not reflected in the results obtained with the Quick time, prothrombin-proconvertin assay, or prothrombin assay. The thrombotest method was, however, designed to provide a measure of PTC by the use of a weak tissue thromboplastin (ox or horse brain) together with a brain phospholipid; in this technique barium sulfate-adsorbed plasma is used to provide an excess of the other coagulation factors not affected by the coumarin derivatives. The thrombotest method has usually indicated lower activities than have any of the other methods studied, at all levels of values observed. Many authors agree that the thrombotest is a more sensitive index of anticoagulant therapy than is the Quick time.^{56,57,61,62} The practical value of such a sensitive index has been alluded to by these authors, although there is not yet available the clinical data from which the proper therapeutic range may be determined.

In comparisons of the thrombotest with the prothrombin-proconvertin assay, the degree of correlation has varied widely among different laboratories. In the present study, when the two methods were compared statistically using the lower limits of normal as a reference point, no significant difference was obtained. But when the same statistical comparison was made using a 50% level of activity as a reference point, the difference found was significant ($p = 0.006$). No other statistical comparisons have been reported previously; inspection of the data of various authors, however, reveals the correlation between the two methods to range from nearly perfect (Owren³⁹) to a rather wide scattering of results (Seaman⁵³). These differences presumably reflect again the varying nature of the prothrombin-

proconvertin assay from one laboratory to another.

In evaluating the results that have been presented, it should be remembered that all of these studies reported have used a commercial reagent to perform the tests. This problem has concerned several authors,^{56,62,63} who have tested the validity of the dilution curves provided with the reagent and have found close agreement, as we have. The reproducibility and stability of various lots of reagents present further problems. Although the complete details of preparation have not been published, the technical complexity of preparation has been commented upon by Owren.³⁹ Because of this lack of complete information, as well as the high cost of the commercial reagent, Frohn, Haanen and Morselt⁶⁴ have published the details of preparation of a thrombotest reagent which followed the general outline provided by Owren. In their hands the two reagents agreed closely. Such agreement, however, will not be obtained consistently if various laboratories prepare their own reagents or if multiple commercial thrombotest reagents become available. If generally applicable therapeutic limits are to be defined, this consistency of results becomes a problem of extreme importance.

SUMMARY

The results of seven coagulation methods were evaluated with respect to anticoagulant therapy. Results of four of these—the thrombotest, prothrombin-proconvertin, prothrombin and Quick methods—showed a good degree of correlation. These four methods are limited, or nearly limited, to a measure of the factors dependent on Vitamin K. The whole-blood clotting time and the partial thromboplastin time methods lacked adequate sensitivity to anticoagulation. The thrombotest method appeared to be somewhat more sensitive to the administration of anticoagulants than did the prothrombin-proconvertin, prothrombin, and Quick methods. Certain problems revolving about the use of a commercial reagent have been discussed as they relate to the use of the thrombotest for the control of anticoagulant therapy.

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Staff Meeting Report

Electrochemical and Colloidal Phenomena in Pulmonary Edema*

Maurice B. Visscher, M.D., Ph.D.,† Karel Absolon, M.D.,‡
and Henry Ballin, B.S.§

The fact that several polybasic macromolecular substances are employed in clinical therapeutics has prompted us to report some observations upon the types and effects of such substances upon physiologic processes. Our current studies were actually prompted by recent reports on the edematigenous action of certain dyestuffs which carry an electric charge in solution.

Shelley, Hodgkins, and Visscher¹ reported that the protamine, salmine sulfate, when added to blood perfusing the liver or the lung, greatly increased resistance to the flow of the blood. They noted that salmine had no such drastic effect if the organ; were perfused with physiological saline solution or plasma. Their results suggest that the protamine might act by promoting the aggregation of red blood cells into masses which embolized small blood vessels. More recently Nevo et al.² have provided experimental proof that polybasic molecules do actually cause agglutination of red cells. Employing a synthetic polybasic polypeptide, poly DL-lysine hydrobromide, they were able to show by microscopic electrophoresis that a reversal in sign of the charge occurs. The erythrocyte is normally negatively charged. Suspension in solutions in polylysine at 10 to 20 micrograms per ml. not only reversed the charge but increased the charge density on the surface of the cell, indicating that many dissociated basic groups on the branched chain polypeptide molecule remained unattach-



MAURICE B. VISSCHER

*This report was given at the Staff Meeting of the University of Minnesota Hospitals on December 15, 1961.

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ed to the cell membrane—a condition that promotes agglutination. Electron microscopic studies by Katchalsky et al.² upon erythrocyte ghosts treated with polylysine show clearly that close attachment of the membrane occurs. Treatment of agglutinated cells or ghosts with suitable anionic agents reverses the agglutination.

Lagrange and co-workers^{4,5} showed that intravenous injection of methylene blue or methyl violet produced pulmonary edema. Since these are electropositive or basic dyes, it seemed to us that this property might be related to the dyes' edema-producing effect. Consequently, we have studied the action of a number of basic and acid dyes in order to ascertain whether or not any correlation exists between electrochemistry and edematogenous action.

If basic polyelectrolytes produce pulmonary edema by arteriolar embolization, it should be possible to simulate their action experimentally by the intravenous introduction of glass microspheres into perfusion fluids free from red-blood cells; therefore, we performed such studies.⁹ In addition, since embolization in the lung with clumped red cells should be visible microscopically, we determined to make photomicrographs.†

METHODS AND MATERIALS

Mongrel dogs anesthetized with sodium pentobarbital 25 mg/kg were used. The edematogenous properties of 14 dyes were studied in open chest preparations in which pressures in the carotid artery, pulmonary artery, and left and right atria were measured and recorded using Statham strain gauges led into a Sanborn recorder. In another series of experiments, the completely isolated lung was employed, perfused at constant flow rate with heparinized whole blood, heparinized plasma, serum, low molecular weight dextran (10 per cent Macrodex®) or suspensions of red cells in dextran. In these experiments the lung was weighed continuously by the counterpoised bar strain gauge method of Stish et al.⁶ The pulmonary arterial pressure and occasionally other pressures were monitored. Observations were made of the effects of various dyes, of glass microspheres, and of protamines and polybrene given via the pulmonary artery on the isolated lung. Substances whose effects were to be tested were administered in various dilutions. Edema was assessed by comparing experimental lung weight/body weight ratios with the normal, and by gross examination of sectioned lungs.

⁹The microspheres employed were kindly donated by the Minnesota Mining and Manufacturing Co.

†We are indebted to Dr. Maurice W. Meyer for the photomicrographic studies.

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RESULTS

Table 1 summarizes the effects on the lung of various dyes in doses of 20 mg/Kg body weight in saline solution administered intravenously in dogs at the time of thoracotomy. Note that only basic dyes produced both an elevation in pulmonary artery pressure and pulmonary edema. Six of the basic dyes studied, however, did not cause pulmonary edema. No acid dye caused lung edema, and only two caused a transient elevation in pulmonary artery pressure.

An example of the effects of the basic dye, Janus Green, is shown in Figure 1. It will be noted that the mean pulmonary arterial pressure rose from about 18 mm. Hg to a value around 35 mm. Hg. About five minutes after injection of the dye, the carotid arterial pressure began to fall, but neither the left atrial nor the jugular vein pressure rose, indicating that ventricular failure was not responsible for the pulmonary edema which developed.

TABLE 1
EFFECTS OF SEVERAL DYES IN THE OPEN-CHEST DOG

Name of Dye	Class	Pulmonary Arterial Pressure	Pulmonary Edema
Methylene Blue	Basic	+	+
Toluidin Blue	Basic	+	+
Nigrosine	Basic	+	+
Crystal Violet	Basic	+	+
Janus Green	Basic	+	+
Brilliant Cresyl Blue	Basic	+	+
Saphramin 0	Basic	(+)	0
Neutral Red	Basic	0	0
Thionin	Basic	(+)	0
Methyl Blue	Acid	0	0
Methyl Red	Acid	0	0
Fuchsin Acid	Acid	0	0
Phenol-sulphophtalein	Acid	0	0
Azocarmine	Acid	(+)	0

Notations: Under pressure + represents a sustained rise,
(+) represents a transient rise,
0 represents no change.

Dyes dissolved in isotonic saline solution were injected at 20 mg. per Kg. body weight, into the femoral vein.

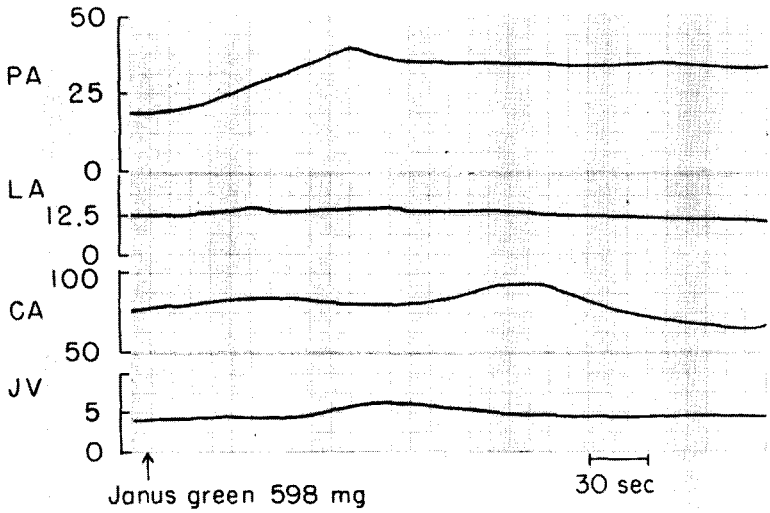


Fig. 1

Effects of injection of 20 mg/Kg body weight of Janus Green into femoral vein of dog with open chest under artificial respiration. Measurements were made of the pressures in the pulmonary artery (PA), left atrium (LA), carotid artery (CA), and jugular vein (JV).

Our more critical studies were made on the isolated dog lung perfused with various fluids. When polybrene (hexadimethrine bromide) or any of the electropositive dyes which produced edema in the open chest dog is added to whole blood perfusing the lung at constant flow rate, pulmonary arterial pressure is elevated and the weight of the lung is increased. On the other hand, when dextran in isotonic NaCl or glucose was employed as the perfusate, none of these agents produced either a rise in pulmonary arterial blood pressure or pulmonary edema. A summary of the effects of methylene blue with various perfusates is given in Table 2. This agent produced moderate pulmonary weight gains when added to either heparinized dog plasma or dog serum, but its edematogenous effect was more pronounced when red blood cells were also present. Although pulmonary arterial pressure rose when the dye was administered to a red blood cell suspension in dextran, no edema developed.

With respect to protamine and polybrene, these agents produce pulmonary pressure elevations and lung edema in blood

perfused lungs except when serum, plasma, or dextran is the perfusate. Unlike the edematigenous electropositive dyestuffs, polybrene does produce massive edema and large pulmonary arterial pressure elevations in the lung perfused with a red cell suspension in dextran. (See Fig. 2.) Thus there are distinct differences between the actions of the dyes and of polybrene. Since the relative difference in action of protamine has been less fully studied, we cannot make positive statements about it at this time. The microscopic studies of Dr. Maurice W. Meyer, however, show positively that when whole blood is employed as a perfusate, pulmonary arterial or arteriolar embolization occurs with protamine addition into the blood reservoir.

When suspensions of glass microspheres (5-50 microns diameter) in isotonic dextran-saline solution are added in sufficient number to dextran-saline solution perfusing the lung, an elevation of pulmonary artery pressure occurs. Most significant, however, is the fact that massive pulmonary edema also occurs. Microscopic studies show that the beads embolize the small arterial and the arteriolar branches of the arterial tree. Approximately one million beads are necessary to produce acute massive edema.

DISCUSSION

The fact that strongly electropositive ions of large molecular weight are able to cause clumping of erythrocytes is not

TABLE 2
EFFECTS OF METHYLENE BLUE UPON
PERFUSED ISOLATED DOG LUNGS WITH SEVERAL PERFUSATES

Perfusion Fluid	Pulmonary Artery Pressure	Pulmonary Edema
Whole Blood	+++	+++
Dextran	0	0
Plasma	+	++
Serum	+	++
RBC Dextran	(+)	0
Dextran → Added Blood	0 → +++	0 → +++
RBC Dextran → Added Plasma	(+) → +++	0 → +++
RBC Dextran → Added Serum	(+) → +++	0 → +++

Notations: Number of pluses represents approximate magnitude.

(+) represents transient change.

→ indicates an addition or the change after addition.

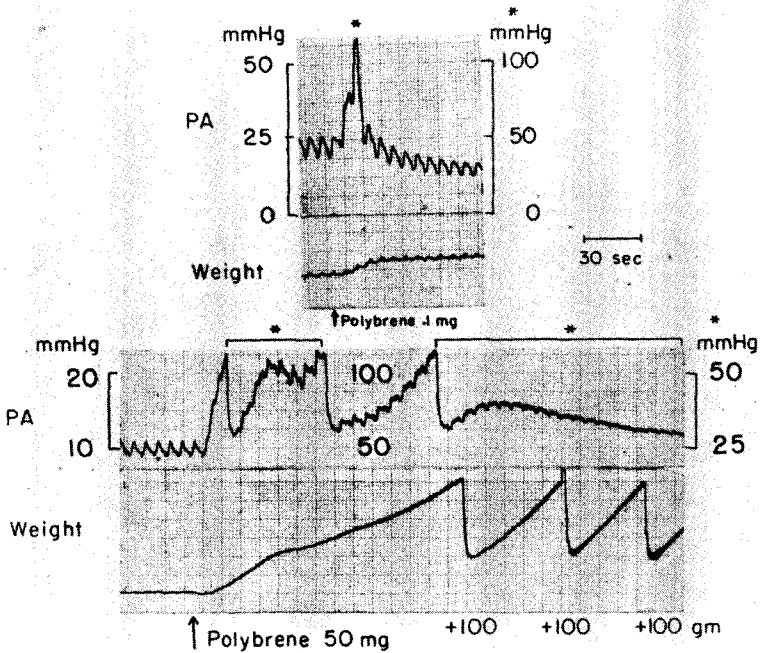


Fig. 2

Pulmonary arterial pressure and weight response to addition of polybrene. The perfusate was a dog red blood cell suspension in dextran. In the upper segment 0.1 mg of polybrene was injected into the pulmonary artery perfusate. In the lower segment 50 mg. was administered. At the points marked with stars, changes in attenuation were made. At the points marked + 100 gm. on the lower tracing, additions of 100 gm. to the counterpoise of the strain gauge weighing device were made. It will be noted that the smaller dose produced a transient blood pressure rise and that the larger dose produced massive edema.

surprising, since erythrocytes are known to bear a negative surface charge. Abramson, Moyer, and Gorin⁷ have reviewed their own and other studies relating to this property of red blood cells. The suspension stability of erythrocytes is dependent upon the maintenance of this electrical charge; when polybasic electrolytes neutralize it, and at the same time provide loci for electrochemical bonds with negative groups on other cells, definite agglutination becomes possible. (Specific agglutinins may

operate by virtue of similar actions controlled by steric factors in the molecular configuration of membrane surfaces.)

Clearly, agglutination of erythrocytes by electrochemical bonding is an important factor in the production of pulmonary edema following intravenous administration of solutions of polybasic large molecules. This fact has both theoretical and clinical significance. First, it may be noted that the arteriolar occlusion produced can cause edema. The reason is presumably that the resistance rises, and when the total perfusion rate is held constant, the pressure gradient from artery to vein also rises. Inevitably, the capillary pressure in patent channels must also rise because there is a significant capillary to large vein resistance. This rise in capillary pressure has been measured in our laboratory⁸ previously. When the capillary transmural pressure exceeds the effective colloid osmotic pressure, edema ensues.

This line of reasoning is supported by the microsphere study. Glass microspheres were employed because glass is nearly inert and did not in our microscopic studies agglutinate red cells. The glass microspheres, furthermore, are not known to cause liberation of vasoactive substances in blood as toxic organic molecules might. Therefore, the pulmonary edema produced by glass microspheres would seem to be due entirely to mechanical obstruction. By analogy, the edema produced by organic polybases which are now shown to produce similar obstruction can have the same origin. It seems unnecessary to invoke any other mechanism for erythrocyte agglutination. A problem remains concerning the edema following injection of methylene blue in plasma- or serum-perfused lungs. Two equally plausible possibilities exist: 1) These agents might "dissolve" intercellular cement substance, thus increasing permissiveness to plasma protein, or 2) conversely, they might cause polymerization of plasma protein, thus raising the average particle weight and reducing the colloid osmotic pressure.

Clinically, the observations reported in this paper are of interest because some of the substances studied are currently being administered intravenously under conditions in which the adverse effects may be of some consequence. Protamine and polybrene, being anti-heparins by virtue of the electronegative character of heparin in solution at blood pH, are widely employed. The dose levels employed clinically are much lower than those used to produce passive pulmonary edema—but not lower than those which have detectable effects. Further, in studies not yet completed, we have noted that the shock-inducing effects of these agents are accentuated in animals previously subjected to hemorrhage. Great caution should be exercised therefore in

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the intravenous administration of any polybasic electrolyte. Under circumstances of incipient shock the intravenous use of polybasic macromolecular substances may very possibly accelerate the induction of shock.

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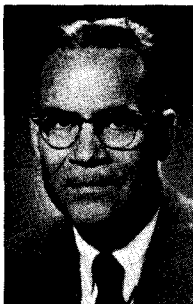
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Medical School News

DR. JOHN J. BITTNER DIES

Dr. John J. Bittner, Professor and Head of the Division of Cancer Biology, University of Minnesota Medical School, collapsed and died of a heart attack December 14, 1961. He was 57 years old, and was en route to his office when he was stricken.

Dr. Bittner was one of the nation's outstanding cancer scientists, having spent most of his life studying its causes. He joined the University of Minnesota faculty in 1942. His research, particularly with the breeding and studying of cancerous mice, earned him high honors in his chosen field. He was president of the American Association for Cancer Research in 1947-48; won the first British Comfort Crookshank award for cancer research in 1951; won the Bertner foundation award for cancer research achievement in 1957; and the 1950 medal of the Minnesota Division, American Cancer Society.



DR. JOHN J. BITTNER

He was a graduate of St. Stephen's (New York) college, and received M.S. and Ph.D. degrees from the University of Michigan. He was holder of the George Chase Christian Professorship in Cancer Research at the University of Minnesota.

Dr. Bittner lived with his wife, Esther, at 112 Seymour Avenue S.E., Minneapolis 14, Minn.

Faculty News

SCHOOL OF PUBLIC HEALTH

Dr. Ruth Gront, Professor, is now in the Congo on special assignment as a consultant to the World Health Organization. She is on a three-months leave of absence from the University.

SCHOOL OF NURSING

Isabel Harris, lecturer and assistant to the director, has been appointed a special consultant to the U.S. Public Health Service for the Professional Nurse Traineeship program. Her appointment extends through June, 1964.

SCHOOL OF PUBLIC HEALTH

Prof. Herbert M. Bosch, public health engineer, has been named to a three-year term on the National Advisory Health council, a unit which assists Surgeon General Luther L. Terry of the U.S. Public Health Service, on matters relating to health activities and functions of the Service. Prof. Bosch has been a faculty member at Minnesota since 1952.

SURGERY

Dr. C. Walton Lillehei lectured and conducted teaching clinics at several universities and medical schools in the Far East during October, 1961. He was a member of a team of American specialists who represented the American College of Cardiology, with support from the U.S. State Department. Purpose of the tour was to acquaint foreign physicians and surgeons in detail with the newest American theory and techniques in managing heart and circulatory diseases.

OTOLARYNGOLOGY

Dr. L. R. Boies, Professor and Head of the Department, has been elected President of the American Academy of Ophthalmology and Otolaryngology. He took office Jan. 1 for a one-year term at the head of the 7,000-member organization. Dr. Boies is also serving a concurrent term as President of the American Otological Society.

PSYCHIATRY AND NEUROLOGY

Dr. Irving C. Bernstein, Assistant Clinical Professor of Psychiatry and Obstetrics and Gynecology, presented four papers on psychiatric problems at a postgraduate medical course held November 25, 1961 at the University of Utah. He spoke on Management of Hyperemesis Gravidarum, Natural Childbirth and Hypnosis in Labor, Pelvic Pain and the Management of Gynecologic Hypochondriasis, and Surgery in Neurotic Women.

MEDICAL RESEARCH GIFT

The Minnesota Medical Foundation received a gift of \$1,500.00 from the Schwan Ice Cream Company of Marshall, Minn., to be used for support of research in heart disease and cancer. The firm makes the contribution annually in lieu of courtesy gifts to its customers at Christmas time.

Medical Alumni News



DOCTORS MIRIAM AND CHARLES Mc CREARY
They're holding Daniel, 1, and Margaret, 2

ALUMNI COUPLE BEGINS INDIA MISSIONARY CAREER

A husband and wife team of Minneapolis doctors have arrived in India where they will spend five years as medical missionaries of the Lutheran church.

Dr. Charles McCreary and Dr. Miriam Naumann McCreary, both graduates of the University of Minnesota Medical School, plan to practice medicine at Wandoor, Kerala state, where the Lutheran church is building a new hospital. Their two small children are with them in India.

The McCrearys, both children of clergymen, met while in Medical School. Charles, 29, graduated in 1959. He interned and spent a year as a resident in surgery at St. Louis County Hospital, St. Louis, Mo. Miriam was a member of the Class of 1958. She practiced as a public health physician in St. Louis during her husband's residency.

Their first stop in India is at Bangalore, for a six-months course in Indian language study. Next September, they'll move to the School of Tropical Medicine in Calcutta. Later, at the new hospital, Charles and Miriam will divide the medical workload, since Moslem custom forbids male doctors from seeing married women. Miriam will handles cases in obstetrics, gynecology, and pediatrics, while Charles will see adult males and do surgery.

Miriam McCreary, 26, was born in India, and has returned there as a fourth generation missionary in her family. Correspondence will reach the McCrearys if mailed in care of Rev. John Naumann, Ambur, N. Arcot District, South India.

'U' ESTABLISHES CENTER TO EVALUATE DRUGS USED IN TREATING MENTALLY ILL

The establishment at the University of Minnesota of a clinical drug evaluation center for the intensive study of compounds affecting abnormal human behavior was accomplished by a \$427,804, four-year grant from the National Institute of Mental Health announced June 27, 1961 by the U. S. Department of Health, Education and Welfare.

Co-principal investigators for the project, in which patients in Minnesota mental institutions are participating, are Dr. Burtrum C. Schiele, professor of psychiatry, and Dr. Gordon T. Heistad, associate professor of clinical psychology.

The grant, made from funds appropriated by Congress for an all-out, nationwide investigation of the full possibilities of various compounds in treating mental illness, has been allocated on the basis of \$100,000 for the first year, \$104,483 for the second, \$109,190 for the third, and \$114,131 for the fourth. The project probably will be continued on a permanent basis.

Working with the principal investigators are Drs. Titus Bellville, Aberlardo Mena, and Patricia Sharpley, of the University's staff in psychiatry, and the staff of the Anoka State Hospital, headed by Dr. Donald Peterson, director, and Dr. Gordon Olson, psychologist. The program is now underway at Anoka and the University Hospitals.

After the drugs have been studied extensively and proved effective under carefully controlled hospital conditions, Dr. Schiele indicated, the program may be extended to benefit patients in other state mental hospitals.

Alumni Notes



MOSES BARRON

◆ 1911

Moses Barron was honored before the 1961 annual meeting of the Hennepin County Medical Society in Minneapolis for his lifetime accomplishments as a physician. He was presented with the St. Barnabas Hospital Bowl by Mr. S. H. Rogers, President of the hospital's Board of Trustees.

◆ 1923

Kenneth H. Sutherland has been appointed County Health Officer for Los Angeles County, California.

◆ 1924

Arnold O. Swenson, Duluth, was appointed to the Minnesota State Board of Health.

◆ 1927

Carl E. Norberg is chief of the medical staff of the Community Memorial Hospital, Cloquet, Minn.

Arthur C. Kerkhof, Minneapolis cardiologist, was elected to the Board of Directors of the American Heart Association.

◆ 1932

Herbert W. Schmidt was elected vice chairman of the Board of Regents of St. Olaf College, Northfield, Minn. He received the College's Outstanding Achievement award in 1960. He is head of a section of medicine in the Mayo Clinic, Rochester, Minn.

◆ 1933

Roy H. Nyquist, chief of the physical medicine and rehabilitation service, spinal cord injury section, at the Veterans Administration Hospital, Long Beach, Calif., has received the 1961 John Eisele Davis Award, denoting distinguished leadership and outstanding service in the field of physical medicine and rehabilitation.

◆ 1937

Nere J. Sundet was elected to a six year term on the Board of Regents of Concordia College, Kadota, South Dakota. He practices in that city.

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◆ 1937

Arnold H. Dysterheft is administrator of the McNary Hospital, McNary, Arizona. He was recently appointed to the Arizona Board of Medical Examiners.

◆ 1939

Robert B. Potter was elected president of the Minnesota Academy of General Practice at the 1,000-member organization's 15th annual Fall Refresher held Sept. 27-28 in Minneapolis. Named president-elect was Dr. James A. Cosgriff, Jr., (Med. '46), Olivia, Minn. Dr. Matthew K. Plasha, (Med. '45), Coon Rapids, Minn., was named vice president, and Dr. Walter E. Krafft (Med. '51), Minneapolis, was elected secretary-treasurer.



ROBERT B. POTTER

◆ 1940

William B. Stromme, Minneapolis, attended the Third World Congress of Gynecology and Obstetrics in Vienna, Austria, in 1961.

◆ 1941

Julien V. Petit, Minneapolis internist, was elected chief of the medical staff at Fairview (Minneapolis) Hospital.

◆ 1942

Grant R. Diessner of the Mayo Clinic, Rochester, has been elected President of the Southern Minnesota Medical Association.

◆ 1943

Robert G. Tinkham has begun a residency in physical medicine and rehabilitation in the Mayo Foundation, Rochester, Minn.

◆ 1944

A. J. Schroeder was elected president of the medical staff at Northwestern (Minneapolis) Hospital. A pediatrician, he succeeds Dr. Paul N. Larson (Med. '30), who served the past two years.

◆ 1945

Edward M. Litin, a member of the Section of Psychiatry at the Mayo Clinic, was a guest lecturer at the University of Missouri School of Medicine, and before members of the Missouri Division, American Cancer Society, during October, 1961.

◆ 1956

John Milton Brown was married late in 1961 to Karen Ann Olson of Baldwin, Wis. They are now at home at 965 N. Avon St., St. Paul, Minn.

◆ 1957

LaVonne Bergstrom, former associate medical director of the Presbyterian Hospital, Embudo, New Mexico, has joined the medical staff of the Sangre de Cristo Medical Unit, San Luis, Colorado.

Richard D. Cunningham has been appointed a resident in ophthalmology in the Mayo Foundation, Rochester, Minn. **Paul A. Jensen** was appointed a resident in obstetrics and gynecology.

◆ 1958

Gerald J. Anderson is a resident in anesthesiology in the Mayo Foundation, Rochester, Minn. **Alan A. Peterson**, a classmate, is a resident in internal medicine.

◆ 1959

Dale L. Anderson was appointed a resident in plastic surgery in the Mayo Foundation, Rochester, Minn.

Lt. Thomas F. Mulrooney, Jr. is now on duty as a medical officer with the Administrative Command, Naval Training Center, San Diego, Calif.

◆ 1960

John C. Henry has begun practice in Owatonna, Minn. in association with his brother, Dr. Kenneth Henry. John was named "Intern of the Year" while at Miller Hospital, St. Paul.

Roger H. Princell is on duty with the U.S. Navy Medical Corps for two years, and is attached to the First Marine Division, Camp Pendleton, Calif. His address is 1215 Tylee Street, Vista, Calif.

Capt. Ronald V. Vilella is a resident in pathology at the Tripler General Hospital, Honolulu, Hawaii, with the U.S. Army.

ALUMNI DEATHS

◆ 1911

Dr. Thomas Ziskin, a Minneapolis heart specialist for more than 40 years, died November 18, 1961 at his home. He was 72 years old. Dr. Ziskin was an associate professor of medicine at the University of Minnesota and chief cardiologist at the Minneapolis Veterans Administration hospital from 1920 to 1946. He was an associate editor of *Modern Medicine* magazine for 26 years and editorial secretary of *Journal Lancet* since 1930. Dr. Ziskin was a native of Grand Forks, N.D. He served in the medical corps in both World War I and World War II. He was on the medical staff at Mt. Sinai hospital, Minneapolis.

◆ 1924

Dr. George L. King, St. Paul, Minn., died October 31, 1961 at the age of 66. He had retired in 1950 following 25 years of practice in St. Paul. He was former chief of the medical staff at St. Joseph's Hospital.

◆ 1934

Dr. Samuel Ashby Grantham, Joplin, Missouri, died September 13, 1961 of a myocardial infarction at the age of 54 years. He was a member of the American Academy of General Practice, was certified by the National Board of Medical Examiners, and was a veteran of World War II.

Memorial Gifts

Memorial gifts to the Minnesota Medical Foundation have been received recently in memory of:

Joey Andrews
Sayre, Oklahoma

Dr. Gordon R. Kamman
St. Paul, Minn.

Memorial contributions are a practical means of honoring the memory of a friend or loved one, while helping the Minnesota Medical Foundation in the advancement of medical education and research. Appropriate acknowledgements are promptly sent to both donor and family of the deceased.

Coming Events

University of Minnesota Medical School

List of Continuation Courses for Physicians

University of Minnesota
Center for Continuation Study

1962

- All Year Cancer Detection for General Physicians
- January 2-6 Intermediate Electrocardiography for
General Physicians and Specialists
- February 12-14 Pediatric Neurology
- March 5-7 Anesthesia for General Physicians
- March 16-17 Treatment of Traumatic Injuries
- April 12-14 Otolaryngology for General Physicians
- April 16-18 Internal Medicine for Internists
- April 26-28 Surgery for Surgeons
- April 30-May 2 Gynecology for General Physicians
- May 7-9 Ophthalmology for Specialists
- May 14-18 Proctology for General Physicians
- May 31-June 2 Psychiatry for General Physicians

The University of Minnesota reserves the right to change this schedule without notification.

Courses are held at the Center for Continuation Study or the Mayo Memorial Auditorium on the campus of the University of Minnesota. Usual tuition fees are \$30 for a two-day course, \$50 for a three-day course, and \$75 for a one-week course.

Specific announcements are sent out about two months prior to each course to all members of the Minnesota State Medical Association and to any physicians who request information for a specific course. For further information write to:

DIRECTOR
DEPT. OF CONTINUATION MEDICAL EDUCATION
THE MEDICAL CENTER
UNIVERSITY OF MINNESOTA
MINNEAPOLIS 14, MINNESOTA

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A Word About Memorial Gifts

The **Minnesota Medical Foundation** welcomes your memorial contributions when an appropriate occasion arises. Memorial gifts serve the living and pay thoughtful tribute to the memory of a friend or relative.

The Foundation will promptly acknowledge your gifts to both the donor and the family of the deceased. The gift will help finance the Foundation's program for the advancement of medical education and research. The Medical School at the University of Minnesota will be the direct beneficiary.

Gifts should be sent to the **Minnesota Medical Foundation, 1342 Mayo Memorial, University of Minnesota, Minneapolis 14, Minn.**