VOLUME XXXI, NUMBER 15 • JUNE 1, 1960

MEDICAL BULLETIN

IN THIS ISSUE

Aminonucleoside Nephrosis

Phospholipids

Subacute Bacterial Endocarditis

University of Minnesota Medical Bulletin

UNIVERSITY OF MINNESOTA

Medical Bulletin

Official Publication of
UNIVERSITY OF MINNESOTA HOSPITALS
MINNESOTA MEDICAL FOUNDATION
MINNESOTA MEDICAL ALUMNI ASSOCIATION
Circulation this issue: 2,350

VOLUME XXXI *June 1, 1960* NUMBER 15

CONTENTS

Published semi-monthly from October 15 to June 15 at Minneapolis, Minnesota

Staff Meeting Report

Immunologic and Metabolic Aspects of Aminonucleoside Nephrosis*†

Vincent R. Hunt, M.D.‡ and Carl S. Alexander, M.D.§

Puromycin is an antibiotic derived from *Streptomyces albboniger*[1] which has also been used as a cancerocidal and trypanocidal agent in man.[2,3] Removal of the tyrosine moiety results in aminonucleoside¶ (6-dimethyl-aminopurine-3-amino-d-ribose) which causes the nephrotic syndrome in rats.[4] The structure of this compound is important in a specific way because seemingly small alterations in the side group inactivate the molecule,[5] as will be dscussed more fully later.

VINCENT R. HUNT

This experimental disease—with its proteinuria, hypoalbuminemia, hyperlipemia, ascites, and pathological characteristics—is strikingly similar to the nephrotic syndrome in man.[6,7,8] It may thus furnish a valuable tool in understanding the cause of human nephrosis. The structure of P-A is uniquely related to its toxicity. Its similarity to adenine, adenosine, and ATP—well known ribonucleic acid building blocks—poses the question of whether or not P-A acts as an antimetabolite in blocking RNA synthesis. It may also block or inhibit essential enzymes concerned in nucleic acid synthesis. Another possible mode of action is that of provoking an immune response in the recipient animal leading to the production of nephrotoxic antibodies, as seems to be the case with some types of antikidney serum disease.[9] The following experiments were therefore undertaken to explore the mechanism of action of P-A. They may be divided into three categories:

1. A search for an immune mechanism.

*This report was given at the Staff Meeting of the University of Minnesota Hospitals on April 22, 1960.

†A complete transcript of this abridged report is available from the author.

‡Performed as a Senior Medical Student under the supervision of Carl S. Alexander, M.D.

§Clinical Investigator, Department of Medicine, University of Minnesota, and Veterans Administration Hospital, Minneapolis, Minnesota.

¶Henceforth referred to as P-A

2. Investigation of possible antienzyme activity.

3. A study of the competitive aspects of RNA precusors in blocking the action of P-A.

I. Investigations of Immune Mechanisms in Aminonucleoside Nephrosis:

Introduction

In 1955, Frenk and co-workers described the induction of aminonucleoside* (P-A) nephrosis in the rat.[4] The nephrotic syndrome had previously been induced by various immune mechanisms such as administration of rabbit antirat kidney serum to rats[10] and duck antirabbit kidney serum to rabbits.[9] The strik- clinical, morphologic, and laboratory similarity of P-A nephrosis to these antibody induced diseases has stimulated speculation as to similar etiologic mechanisms.[6,11]

Evidence for an immune factor was further supported by the finding of Wilson *et al.*[11] that a latent period of at least five days occurred in all P-A nephrotic rats no matter how high the dose of P-A. This is similar to the minimum latent period of three to four days found by various observers in rabbits injected with duck antirabbit kidney serum.[12,9] Lange has expressed the belief that duck antirabbit kidney serum contains a localizing factor which serves to coat the glomeruli; these altered glomeruli then stimulate antibody formation, and progressive renal damage results as antigen and antibody unite.[9,13] It is possible that P-A serves as a similar localizing factor which may also in turn stimulate host antibody formation. The following experiments were therefore undertaken:

1) Investigation of the effects on normal rats of the injection of serum, gamma globulin fraction, and beta globulin fraction from P-A nephrotic rats.

2) Precipitin and complement fixation studies with serum obtained from P-A nephrotic rats.

3) Effect of cortisone and nitrogen mustard on the prevention and/or course of P-A nephrosis.

Methods

All animals were housed in individual metabolism cages in a temperature controlled environment (78° F.) and were given free access to fox chow and water. Twenty-four hour urine specimens were collected daily, and urine protein was determined by the Shevky and Stafford sedimentation method as modified by McKay.[14] Thirteen female rats of the Holtzman strain weighing approximately 200 (± 20) gm. were given 6 to 7 consecutive

*Aminonucleoside of Puromycin-6-dimetylamino-purine-3-amino-d-ribose. Generouslv furnished by Dr. Stanton Hardy of the Lederle Laboratories.

daily subcutaneous injections of P-A in a 0.5 per cent aqueous solution (1.5 mg/100 gm). Control rats received injections of saline solution. Serum was collected on the twelfth to seventeenth days, when ascites, edema, and proteinuria were prominent. Part of the pooled frozen serum was fractionated into beta and gamma globulin components by starch block electrophoresis.* The control serum was handled in a similar manner.

Serum from Rats with P-A Induced Nephrotic Syndrome

Twenty-six rats, divided into six groups (A-F) were given injections† of whole serum or serum fractions intravenously or by the intracardiac route (Table 1). The intracardiac injections were made in order to bypass the liver, lungs, and other reticuloendothelial tissue which may trap or bind nephrotoxic antibodies.[15,16] To compare the effect of P-A serum with that of a known nephrotoxic agent, four rats were given rabbit anti-rat kidney serum‡ of varying immunologic potency (NTS 1 + NTS 2).§

Precipitin and Complement Fixation Tests¶

For preparation of the precipitin test, a 50 per cent saline extract was made from eight normal kidneys which had been homogenized in a Waring blendor.® Saline extract and the gamma globulin fraction of serum from rats with P-A induced nephrotic syndrome were mixed in equal proportions by volume, incubated for two hours at 37° C., and then refrigerated. Readings were made 24 hours later. For the complement fixation test, whole serum from P-A nephrotic rats was used with kidney extract prepared as for the precipitin test.

Cortisone + P-A Treatment

Six rats received daily subcutaneous injections of cortisone (2.5 mg.) and P-A (1.5 mg/100 gm) simultaneously for 14 days. Two groups of three controls each received P-A alone or cortisone alone.

Eight rats were divided into two more groups. Group A, consisting of four rats, received P-A for five days, while Group B received P-A plus 5 mg. cortisone for five days and then cortisone alone for an additional nine days, making a total of fourteen days.

*We are indebted to Dr. Horace Zinneman, Associate Professor of Medicine, for the serum electrophoresis.

†Injections were performed under light ether anesthesia.

‡Serum was prepared according to the method of Heymann.[2]

§One serum preparation was kindly supplied by Dr. Robert Vernier, Assistant Professor of Pediatrics.

¶We are grateful for the assistance and advice given by Mr. Melvin Neren, Supervisor of Clinical Bacteriology, Veterans Administration Hospital.

Nitrogen Mustard

A single dose of 0.05 mg. of HN_2 was injected into the tail vein of each of 24 rats. P-A was then given subcutaneously daily for fourteen days. Only four rats survived the injection of HN_2 long enough to permit evaluation of the occurrence of proteinuria.

Results

Passive Transfer of Serum from P-A Nephrotic Rats

Regardless of the route of administration, the injection of whole serum or its fractions obtained from P-A nephrotic rats did not produce abnormal proteinuria. On the other hand, proteinuria occurred immediately after the injection of potent rabbit antikidney serum.

Precipitin Test

Precipitin tests were negative except for a slight precipitate observed in the 1:512 dilution. This was believed to have been due to extraneous foreign particulate matter, for no precipitate was formed when additional antigen dilutions of 1:500, 1:600, and 1:700 were run against serial dilutions of gamma globulin.

Complement Fixation

All determinations using an antigen dilution of 1:600 were negative. This dilution was chosen because higher concentrations of antigen were so viscous and turbid that the test could not be properly evaluated.

Cortisone

Cortisone administration did not prevent or ameliorate the proteinuria of P-A nephrotic rats (Figure 1). In fact, proteinuria appeared earlier and more severely in rats which received cortisone. Furthermore, even in larger doses cortisone failed to protect rats which received only a five-day course of P-A.

Nitrogen Mustard

Administration of nitrogen mustard failed to prevent nephrosis in surviving rats treated with P-A.

Discussion

Intravenous or intracardic injections of serum from P-A nephrotic rats did not produce proteinuria in normal rats, although similar amounts of rabbit nephrotoxic serum readily caused proteinuria. This failure to produce proteinuria by passive transfer of P-A nephrotic serum may not completely exclude the possibility of an antibody induced mechanism. It may be necessary, as in the case of avian nephrotoxic serum nephrosis in the rabbit, to sensitize the normal kidney with P-A before injecting

TABLE 1

PASSIVE TRANSFER OF SERUM OR SERUM FRACTIONS FROM RATS WITH P-A INDUCED NEPHROTIC SYNDROME*

Group	No.	Material	Amount Injected†	Daily No. of Doses	Route
A	6	Nephrotic serum	0.4-0.5 ‡	3	IV
B	8	Nephrotic Serum	0.4-0.8 cc.	2-3	Intracardiac
C	3	Nephrotic Gamma Globulin	6 mg.	2	IV
D	3	Control Gamma Globulin	6 mg.	2	IV
E	3	Nephrotic Beta Globulin	15 mg.	2	IV
F	3	Control Beta Globulin	15 mg.	2	IV

*Nephrosis induced by daily subcutaneous injections of P-A 3 mg/200 gm. x 6-7
†Ml. of whole serum or mg. serum protein in each injection
‡One rat received one dose of only 0.2 cc.

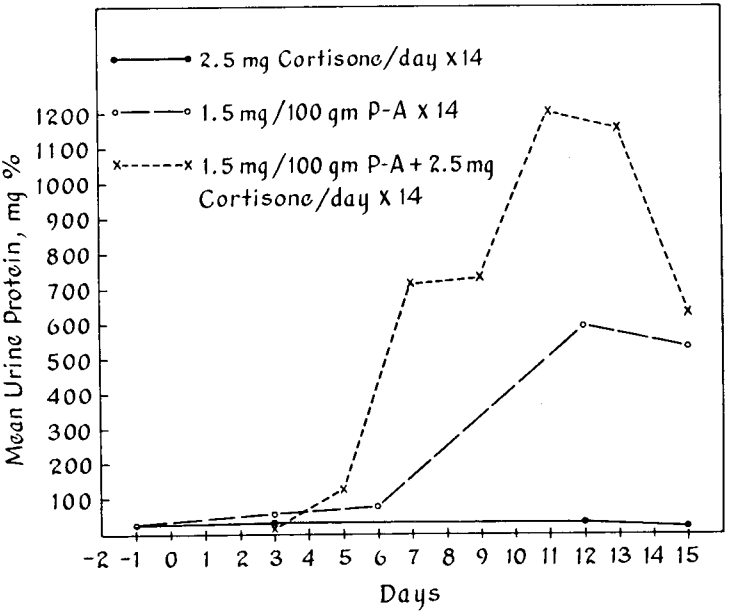


Fig. 1
Failure of cortisone to ameliorate P-A induced nephrosis as reflected in urine protein excretion

P-A nephrotic serum, as suggested by Lange.[17] Such an experiment is now in progress.

Precipitin and complement fixation tests also failed to provide evidence for an antibody mechanism. Conceivably, however, the use of a more potent kidney antigen—such as glomerular basement membrane obtained by sonic oscillation—might have yielded different results.

Our finding that cortisone did not prevent or ameliorate the proteinuria of P-A treated rats corresponds to the observations of Fiegelson and co-workers[6] and Wilson *et al.*[11] This failure of cortisone to influence P-A induced nephrosis is in contrast to its beneficial effect in antikidney serum induced nephrosis, in which cortisone administration is followed by a decrease in proteinuria, edema, and severity of basement membrane alterations.[18,19] Das Gupta and Giroud[20] noted a decrease in rate of aldosterone production and marked diminution of ascites in P-A nephrotic rats treated with cortisone; they did not, however, mention the effect of cortisone on proteinuria.

Nitrogen mustard administration also did not alter the occurrence of proteinuria in P-A treated rats. We are unable to account for the death of 20 of the 24 rats resulting from the administration of 0.05 mg/100 gm, the dose recommended by Boone *et al.* as insuring 100 per cent survival.[21] It is interesting that total body irradiation, which is believed to act in a manner similar to HN_2, does not ameliorate P-A induced disease,[11] although it is effective in preventing the nephrotic syndrome in rats receiving duck antirat kidney serum.[12]

Summary and Conclusion

Various *in vivo* and *in vitro* attempts to demonstrate an immunologic basis for the action of aminonucleoside were all negative. These consisted of passive transfer of whole serum, beta globulin, and gamma globulin from aminonucleoside treated rats to normal rats. Complement fixation and precipitation studies were also negative. Cortisone and nitrogen mustard failed to prevent or ameliorate the proteinuria of aminonucleoside treated rats. The results of these experiments suggest that P-A does not produce nephrosis in the rat by means of an immune mechanism.

II. The Effect of Decreasing Doses of P-A: Indirect Evidence that P-A Does Not Act as an Antienzyme

Certain investigators have suggested that one possible explanation for the action of P-A is that it blocks nucleic acid synthesis through enzyme inhibition.[6,22,23] We therefore decided to study the effect of decreasing doses of P-A, administered each week or every other week instead of daily. We reasoned that

observation of the presence or absence of proteinuria under these circumstances might provide an insight into the mechanism of action of P-A. Thus, for example, if proteinuria occurred after the injection of a small amount of P-A every two weeks this would constitute strong evidence that direct enzymatic inhibition was not involved. It is unlikely that this dosage would raise blood levels sufficiently to block a postulated enzymatic system over such a prolonged period. Support for this view is provided by the findings of Agosin and Von Brand[24] that P-A does not circulate in the blood of rats for longer than 14 hours after intraperitoneal injections of 5 mg/100 gm.

Methods

Fourteen female rats weighing 200 (± 20) gm. were divided into four groups consisting of three to four rats each. Doses of P-A ranging from 1.5-0.5 mg/100 gm were administered subcutaneously to each group once each week or every other week (Table 2). Urine was collected weekly and 24 hour protein excretion determined.

TABLE 2

Dosage Schedule of P-A Administration

Group	No.	Dosage	Interval
A	3	1.5 mg/100 gm	Every week
B	3	1.5 mg/100 gm	Alternate weeks
C	4	1.0 mg/100 gm	Alternate weeks
D	4	0.5 mg/100 gm	Alternate weeks

Results

In all rats, regardless of the dose or interval between doses, a significant degree of proteinuria developed by the sixth week (Figure 2). Thus, proteinuria occurred following only three injections in those rats which had received small doses on alternate weeks. Rats in groups C and D which received the smaller doses (0.5-1.0 mg/100 gm on alternate weeks) are still alive and under observation.

Discussion

An increase in protein excretion occurred in all groups of P-A treated rats even with the smallest dose given once every two weeks. (This dose is only 1/3 the usual daily dose). As previously stated, this response, *i.e.*, the appearance of proteinuria following the administration of such a small amount of P-A over relatively long intervals, is *not* typical of *direct* enzyme inhibition.

The minimum latency period of five to eight days between the first daily administration of P-A and the appearance of proteinuria[6,11,25,26] also provides evidence against enzymatic blockade. In the event of direct enzymatic inhibition proteinuria would be expected to appear almost immediately.

Summary

The administration of small doses of P-A subcutaneously to normal rats at intervals of one to two weeks caused significant proteinuria. These results were discussed from the standpoint of a possible enzymatic block. It is concluded that P-A probably does not produce nephrosis in the rat by means of *direct* enzymatic inhibition.

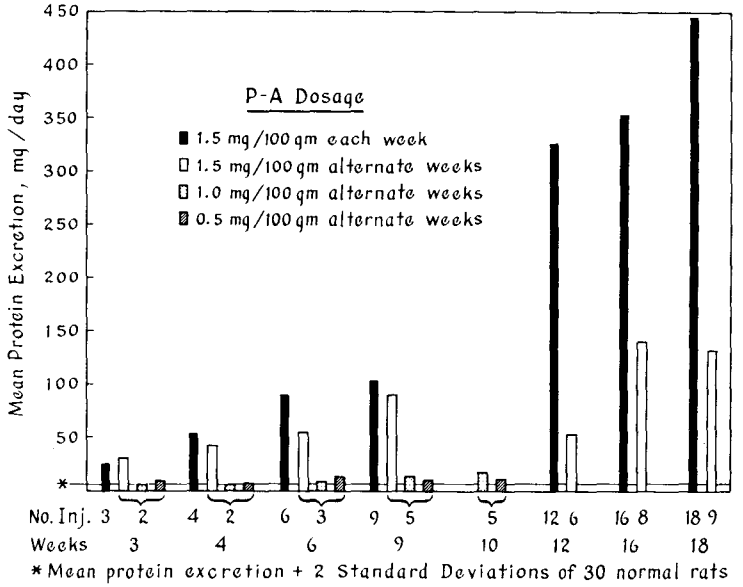


Fig. 2

Twenty-four-hour protein excretion determined once weekly in rats receiving decreasing doses of P-A at intervals of one to two weeks. (The usual dose is 1.5 mg/100 gm daily.)

III. The Effect of RNA Precursors on P-A Nephrosis

Introduction

The striking specificity of chemical structure required for P-A toxicity is unique, since even analogues with seemingly minor alterations of the molecule are ineffective in causing the neph-

rotic syndrome.[5] Such specificity suggests a third possible mechanism of P-A action, *i.e.*, successful competition with an essential nucleic acid precursor which in the rat may be represented by adenine[24,27–30] or by other purines or pyrimidines or both.[31] Hartman et al.[32] have shown that adenine but not adenosine partially protected rats from developing the nephrotic syndrome. We have confirmed these results, but by extending the period of observation we found that this protection was not lasting and that the animals eventually displayed the usual acute syndrome. Six of these rats were followed for three and one-half months in order to determine the long-term effects of adenine in ameliorating P-A induced nephrosis. We also investigated the effectiveness of known nucleic acid intermediaries[30] in affording protection against the P-A induced nephrotic syndrome. These precursors included adenosine, ATP, guanine, 4-amino-5-imidazole carboxamide, D(-) ribose, 2,6-diaminopurine, orotic acid, and inosine.

Materials and Methods

Female rats of the Holtzman strain weighing approximately 200 ($\pm$ 20) gm. were again used under conditions similar to those previously described. Since P-A causes nephrosis in 100 per cent of treated rats, relatively few animals were required for any experiments employing P-A treated animals as controls. Drugs that were given once daily were administered subcutaneously in the evening, and in some cases injections were given both morning and evening; the drugs were dissolved or suspended in distilled water in the concentrations shown in Table 3. The dose of these substances, expressed as x molar or x moles relative to P-A, was usually limited by the volume of the specific suspension which could be given conveniently subcutaneously. The schedule of administration is also summarized in Table 3. In order to insure and maintain stability of the injected drug or to prevent sloughing at the site of injection, the pH of the solution or suspension was adjusted to pH 7 with 1.0 N NaOH when indicated. These preparations were kept refrigerated until ready for use and were warmed to body temperature prior to injection.

Maximum urine concentrating ability was evaluated by collecting nocturnal urine under oil during food and water deprivation. At the time of sacrifice animals were anesthetized with ether and weighed, and the abdominal cavity was entered through a midline incision extending from the xiphoid to the pubis. Any ascitic fluid present was removed by sponging, and the animal was reweighed. Blood pressure was measured directly at the bifurcation of the aorta with a 20-gauge needle attached to a mercury manometer by way of a 3-way stopcock and 90 mm. of polyethylene tubing of 3 mm. inside diameter.

TABLE 3

Dosage Schedule of P-A and Intermediaries

Group	No. Rats	Molar Ratio (I/P-A*)	Concentration of Preparation	Days of Administration
Control (Saline inj.)				
A	30	–	–	9-14
P-A				
B	6	0/1	0.5%	9
P-A + Adenine				
C_1	7	7.5x/1	2.5%	9-10
C_2	3	10x/1	2.5%	10
C_3	3	15x/1	2.5%	9
C_4	3	22x/1	2.5%	9
C_5	4	20xBID/1	5.0%	28
Adenine				
D_1	3	7.5x/1	2.5%	10
D_2	2	20xBID/1	5.0%	20
P-A + Adenosine				
E_1	3	1x/1	2%	12
E_2	3	5x/1	2%	15
E_3	3	10x/1	2%	12
P-A + ATP				
F_1	4	10x/1	10%	11
P-A + Specific RNA Precursors:				
P-A + Guanine:HCl				
G_1	4	7x/1	2%	10
P-A + 4-amino-5-imidazole carboxamide				
G_2	4	12x/1	2%	10
P-A + D(–) Ribose				
G_3	4	13x/1	2%	10
P-A + 2,6-diaminopurine: sulfate				
G_4	4	8.5x/1	2 or 1%	10
P-A + Orotic Acid				
G_5	4	10x/1	1%	10
P-A + Inosine				
G_6	4	5x/1†	2%	10

*Standard P-A dose = 1.5 mg/100 gm body wt/day.
I/P-A = Molar ratio of intermediaries as compared to standard P-A dose.
†This dosage was reduced by one-half because of an error on day three.

The animal was then exsanguinated from the aorta into a heparinized springe attached to one end of the stopcock.

Urea nitrogen,[33] total protein,[34] cholesterol,[35] and triglyceride[36] were determined on plasma previously frozen. Urine protein was determined in the same way as previously mentioned, and urine concentration was determined using the Fiske osmometer.

Results

Short Term Observations

P-A + Adenine. Control rats (Group B) which were treated with 3 mg P-A/day developed a significant degree of proteinuria by the seventh to eighth day.* The smaller doses of adenine (Groups C_1 and C_2) failed to prevent proteinuria in P-A treated rats. Larger doses (Groups C_3, C_4, C_5) however appeared to prolong the onset and decrease the severity of proteinuria on days 9-11. Nevertheless, the proteinuria which soon developed in these rats was comparable in severity to that of the P-A treated control group (*e.g.*, on day 14 the mean protein excretion of Group C_5 was 230 mg/day, while on day 17 that of three rats from Group B was 170 mg/day).

Animals in Groups B (3 rats), C_1, C_2 and D_1 were killed after 10 to 14 daily injections. At the time of sacrifice the adenine treated rats had somewhat less ascites than P-A controls. Group C_1 was the only P-A adenine group in the acute stage to show normal plasma urea nitrogen with less hyperlipemia and hypercholesterolemia than the group of rats treated with P-A alone. The weights and gross appearance of P-A and P-A adenine rat kidneys were similar. The microscopic appearance of the P-A treated rats, however, revealed occasional dilated tubules containing protein casts, and the basement membrane of an occasional glomerulus appeared thickened. In contrast, the histology of the adenine P-A treated animals, which were killed on days 10-11, appeared normal.

Rats in Groups C_5 and D_2 were sacrificed on days 28 and 20 respectively, and the animals of Group C_5 showed evidence of severe renal damage. Many tubules were dilated and contained casts. There was infiltration by neutrophils and round cells into the interstitial area. The glomeruli and Bowman's capsules were thickened with deposits of hyaline material, sometimes present in the shape of crescents. Furthermore, plasma urea nitrogen, cholesterol, and triglyceride were elevated above the P-A control values.

Surprisingly, adenine itself in large doses was toxic to the kidney (Group D_2). The kidneys were heavy, soft, and flabby

*Mean protein excretion for 30 normal rats was 2.2±1.7 mg/day.

with a peculiar yellow material deposited in streaks paralleling the collecting ducts, especially in the papilla and the outer medullary area. Microscopically, these deposits appeared in the form of crystalline rosettes. There was an intense infiltration of inflammatory cells in the interstitial tissue, and clumps of neutrophils were seen in the convoluted tubules and collecting ducts. The laboratory data revealed mild azotemia and slight proteinuria in two of the four rats, unaccompanied by lipid, cholesterol, or serum protein changes. Rats in Group C_3 and C_4 were spared for long-term observations.

P-A + Adenosine

Adenosine in molar doses one to ten times the P-A dose failed to alter the usual pattern of the disease, as reflected by protein excretion, plasma chemistries, and kidney pathology.

P-A + ATP

This combination also failed to prevent P-A nephrosis using the same parameters as above.

P-A + Specific Nucleic Acid Precursors

The degree of proteinuria was not influenced by any of the specific nucleic acid precursors as observed in short-term experiments.

Long-Term Observations (Figs. 3 & 4, Table 4):

P-A Controls

Four animals which survived a ten-day course of P-A were killed at 140 to 150 days after treatment. Blood pressure and blood chemistry data are available for only three of these survivors. None showed the presence of ascites, but two of the three exhibited moderate hypertension (Table 4). These two rats also had severe impairment of urine concentrating ability, and all three exhibited considerable proteinuria (Figure 3). One of the four survivors had normal plasma urea nitrogen and blood pressure. Cholesterol and triglycerides were elevated in all (Table 2). Figure 4 compares a severely damaged kidney of one such animal to kidneys from normal and P-A adenine treated rats. Most glomeruli are hyalinized and show thickened basement membranes. Some show endothelial and epithelial cell proliferation and round cell infiltration into the interstitial areas. Proximal and distal tubules are dilated and lined with flattened epithelial cells, some of which have been desquamated into the lumen. Protein casts are numerous. While these extensive degenerative changes were not observed in all long-term survivors, all showed some degree of degeneration.

TABLE 4

LONG-TERM OBSERVATIONS ON RATS WITH P-A NEPHROSIS

Group	Day of Sacrifice	Treatment	Ascites	B.P. (Mean)
P-A	146-150	P-A x 10	0	160 (112-187)
P-A + Adenine				
C_3	102-103	15 x /1	0	127 (121-137)
C_4	102-103	22 x /1	0	132 (130-134)

Group	Urea N (gm/100 ml)	Cholesterol (gm/100 ml)	Triglyceride (gm/100 ml)	Protein (gm/100 ml)
P-A	35 (18-46)	217 (183-235)	68 (44-80)	5.8 (5.6-6.0)
P-A + Adenine				
C_3	16 (14-17)	81 (64-107)	11 (8-16)	6.0 (6.0-6.0)
C_4	15 (14-16)	96 (72-135)	9 (2-17)	6.2 (6.0-6.3)

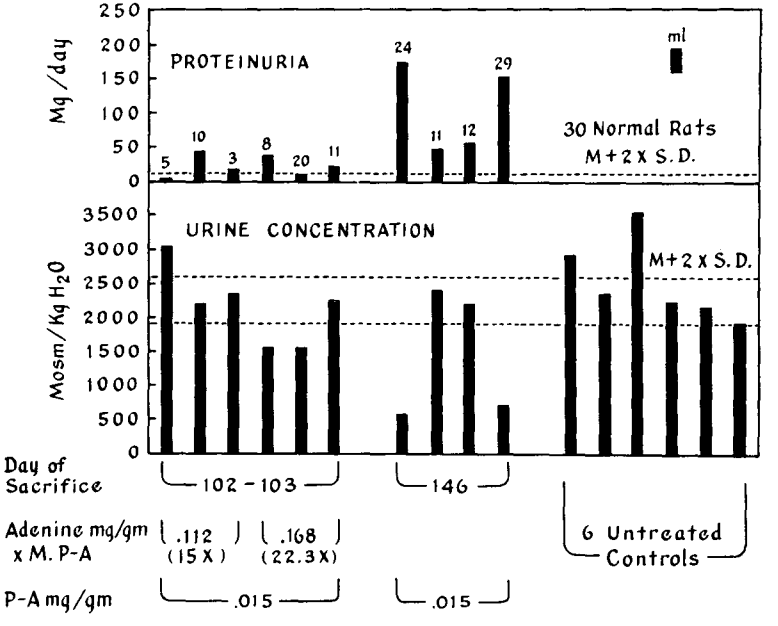


Fig. 3

Long-term observations of the influence of adenine on P-A nephrosis as reflected by proteinuria and urine concentrating ability

PA + Adenine

The animals in Groups C_3 and C_4 were still in good health when killed. Ascites was absent, and plasma urea nitrogen, cholesterol, triglyceride, and total protein were all normal (Table 4). This picture contrasts strikingly with that presented by the P-A control group. Furthermore, the kidneys were considerably smaller in size and appeared grossly normal (Figure 4). The histologic appearance of the adenine P-A kidney was normal, or nearly so (Fig. 4).

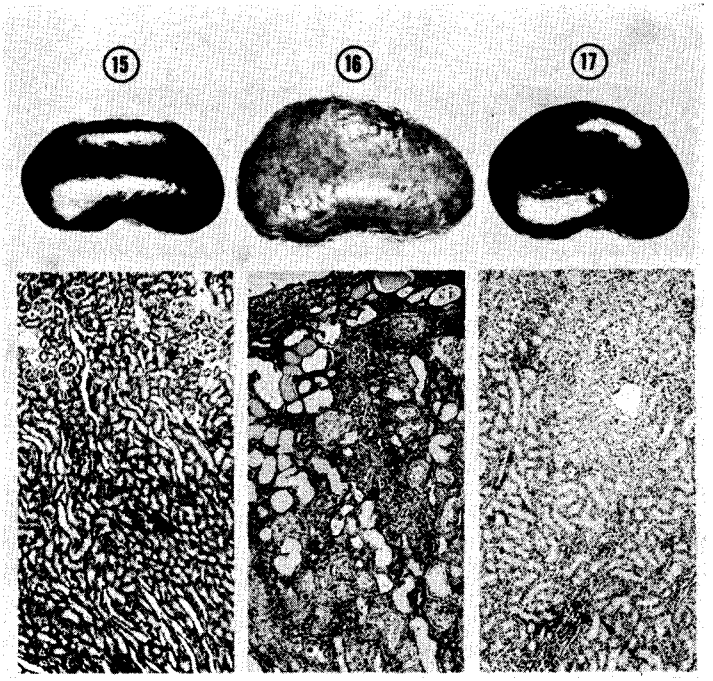


Fig. 4

Chronic nephrosis in rat kidneys several months after treatment. Hematoxylin and eosin. *Rat No. 15*—Normal rat kidney, gross and microscopic × 35. *Rat No. 16*—P-A nephrotic kidney four and one-half months after ten days initial treatment, gross and microscopic × 50; note extensive tubular dilatation, casts, and glomeruli in varying stages of hyalinization, and interstitial thickening. *Rat No. 17*—P-A + adenine (15 x molar) nephrotic kidney three and one-half months after nine days initial treatment, gross and microscopic; residual changes are mild, some infiltration with leucocytes.

Discussion

Of the nucleic acid precursors tested in this study, only adenine reduced the severity of P-A nephrosis. The partial protection afforded by adenine against the acute phase of nephrosis confirms the work of Hartman and co-workers.[32] Our animals, however, were not protected beyond the first week. From the data of Hartman *et al.* it is not possible to determine the degree of proteinuria that would have occurred had they followed their animals for a longer period. By extending the period of treatment with the adenine-P-A combination, we have shown in our studies that the protection afforded is only temporary and that treatment carried beyond the second week results in the usual nephrotic syndrome. It is likely, however, that the relatively slight residual kidney damage observed three and one-half months after P-A-adenine treatment was due to the adenine's initial lessening of the damage during the acute phase.

The partial success of adenine on the one hand, and the failure of adenosine and ATP on the other, in ameliorating P-A induced nephrosis may be attributed to the unique importance of adenine as a nucleic acid precursor in the rat.[29] Furthermore, Brown *et al.*[30] found that adenosine was incorporated into rat RNA and DNA only about half as well as adenine. Also adenosine is rapidly converted in the rat to inosine,[32] which is a poor nucleic acid precursor. The relative deficiency of adenase in the rat, and the slow action of xanthine oxidase on adenine may be two important factors which make adenine suitable for utilization in nucleic acid synthesis.[30]

It is interesting that the rat kidney, despite its relatively slow rate of cell turnover, has been found to have the highest PNA/DNA ratio of any organ as judged by the incorporation of radioactive formate.[37] The observed stability of labeled DNA in the rat kidney suggested that the basement membrane had a high PNA turnover rate—a hypothesis compatible with the regulation and transport of multiple substances passing into and out of the cell. It is possible that P-A may be incorporated into the PNA of rapidly regenerating basement membrane and by thus altering its physical structure, permit increased protein filtration.

Additional evidence that P-A may interfere with nucleic acid synthesis is provided by the fact that the trypanocidal and bactericidal action of P-A can be blocked by: 1) simultaneous administration of various purines and pyrimidines, especially adenine,[24,27,31] and 2) even by simple nucleic acid building blocks such as d(-)ribose, glycine, glutamine, and sodium formate.[31]

Nephrotoxicity from adenine itself has been described previously. The insoluble yellow crystals deposited in the kidney

have been shown to be 2, 8-dioxyadenine and to be associated with progressive renal damage.[38] Nephrotoxicity has also been described in a patient suffering from pernicious anemia in relapse.[39] The patient was given an oral dose of 32.5 gm. of adenine hydrochloride over a six-day period. Following this treatment, urine examination showed decreased specific gravity and white cells in the sediment. Ten months later, residual impairment of urine concentration to a minor degree was still evident.

The chronically diseased P-A kidney in the rat is unlike the typical "small scarred kidney" of chronic glomerulonephritis seen in the adult human (Figure 4). We have never observed the "small, scarred kidney" even in rats dying of renal insufficiency of several months duration. The appearance of the chronically diseased P-A kidney does, however, resemble the large, pale kidney of children and some adults dying after a protracted course of the nephrotic syndrome.

Summary

Attempts were made to reverse the course of P-A nephrosis by RNA precursors such as adenine, adenosine, adenosine triphosphate, guanine, 4-amino-5-imidazole carboxamide, d(-) ribose, 2, 6 diaminopurine, orotic acid and inosine. Of this group, only adenine was capable of ameliorating the acute stage of the nephrotic syndrome. Adenine administration also appeared to have a beneficial effect upon the long-term course of P-A nephrosis.

The significance of these results was discussed from the viewpoint of rat nucleic acid metabolism, and it was concluded that Puromycin-aminonucleoside very probably exerts its effect by successful competition with an essential nucleic acid precursor in the rat.

References

1. Porter, J. N.; Hewitt, R. I.; Hesseltine, C. W.; Krupa, G.; Lowery, J. A.; Wallace, W. S.; Bohonos, N.; and Williams, I. H.: Acromycin: A New Antibiotic Having Trypanocidal Properties, Antibiotics & Chemother. 2:409, 1952.
2. Taylor, D. J.; Bond, H.W.; and Sherman, K. J.: Puromycin; I. Activity Against Experimental Amebiasis, in *Antibiotics Annual*, 1954-1955, Ed. by H. Welch and F. Marti-Ibanez, New York, Medical Encyclopedia, Inc., 1955, pp. 745-750.
3. Farber, S.; Toch, R.; Sears, E.M.; and Pinkel, D.: Advances in Chemotherapy of Cancer in Man, Advances Cancer Res. 4:46, 1956.

4. Frenk, S.; Antonowicz, I.; Craig, J. M.; and Metcoff, J.: Experimental Nephrotic Syndrome Induced in Rats by Aminonucleoside: Renal Electrolytes and Body Electrolyte Composition, Proc. Soc. Exper. Biol. & Med. 89:424, 1955.
5. Borowsky, B. A.; Kessner, D. M.; and Recant, L.: Structural Analogues of Puromycin in Production of Experimental Nephrosis in Rats, Proc. Soc. Exper. Biol. & Med. 97:857, 1958.
6. Fiegelson, E. B.; Drale, J. W.; and Recant, L.: Experimental Aminonucleoside Nephrosis in Rats, J. Lab. & Clin. Med. 50:437, 1957.
7. Vernier, R. L.; Papermaster, B. W.; and Good, R. A.: Aminonucleoside Nephrosis; I. Electron Microscopic Study of the Renal Lesion in Rats, J. Exper. Med. 109:115, 1959.
8. Feldman, J. D. and Fisher, E. R.: Renal Lesions of Aminonucleoside Nephrosis as Revealed by Electron Microscope, Lab. Invest. 8:371, 1959.
9. Lange, K.: Delayed Nephritis Due to Avian Antiserum, Proc. Ninth Annual Conf. on the Nephrotic Syndrome, 1958.
10. Heymann, W.: Nephrotic Syndrome Induced by Injection of Anti Kidney Serum, in *Methods in Medical Research*, Vol. V, Chicago, The New York Publishers Inc., 1952.
11. Wilson, S. G. F.; Hackel, D. B.; Harwood, S.; Nash, G.; and Heymann, W.: Aminonucleoside Nephrosis in Rats, Pediatrics 21:963, 1958.
12. Kay, C. F.: The Mechanism by Which Experimental Nephritis Is Produced in Rabbits Injected with Nephrotoxic Duck Serum, J. Exper. Med. 72:559, 1940.
13. Lange, K.: Proc. Tenth Ann. Conf. on Nephrotic Syndrome, Ed. J. Metcoff, 1959, p. 65.
14. Peters, J. P.; and Van Slyke, D. D.: *Quantitative Clinical Chemistry*, Vol. II, Methods, Baltimore, Williams & Wilkins, 1932, p. 682.
15. Bale, W. F.; Irwing, L. S.; Goodland, R. L.; and Wolfe, D. E.: In Vivo and In Vitro Studies of Labeled Antibodies Against Rat Kidney and Walker Carcinoma, Proc. Soc. Exp. Biol. & Med. 89: 564, 1955.
16. Cruickshank, C. A. and Greenspan, S. A.: The Histochemical Identification of a Connective Tissue Antigen in the Rat, J. Path. & Bact. 65:283, 1953.
17. Lange, K.: Proc. Tenth Ann. Conf. on Nephrotic Syndrome, Ed. J. Metcoff, 1959, p. 215.
18. Heymann, W. and Lund, H. Z.: Nephrotic Syndrome in Rats, Pediatrics 7:691, 1951.
19. Elrich, W. E.; Forman, C. W.; and Seifer, J.: Diffuse Glomerular Nephritis and Lipid Nephrosis, A.M.A. Arch. Path. 54: 463, 1952.
20. Das Gupta, D. and Giroud, C. J. P.: Experimental Aminonucleoside Nephrosis; (1) Action of Cortisone on Aldosterone and Cor-

ticosterone Production, Proc. Soc. Exper. Biol. & Med. 98:334, 1958.

21. Boone, I. U.; Rogers, B. S.; Maxfeld, J. R.; and Storer, J. B.: Nitrogen Mustard and Triethylene Melamine Content in Normal and Tumor Tissues after Intra-Arterial and Intravenous Injection in Rats, Cancer Res. 17:1120, 1957.
22. Kessner, D. M.; Borowsky, B. A.; and Recant, L.: Effect of 6-dimethyl-aminopurine-3-amino-d-ribose on Adenosine Triphosphate Formation in Yeast, Proc. Soc. Exper. Biol. & Med. 98:766, 1958.
23. Fisher, E. R. and Gruhn, J.: Aminonucleoside Nephrosis in Rats, A.M.A. Arch. Path. 65:545, 1958.
24. Agosin, M. and Von Brand, J.: The Influence of Puromycin on the Carbohydrate Metabolism of Trypanosoma Equiperdum, Antibiotics & Chemother. 4:624, 1954.
25. Alexander, C. S. and Hunt, V. R.: Unpublished observations.
26. Vernier, R. L.; Papermaster, B. W.; and Good, R. A.: Amino-nucleoside Nephrosis. I. Electron Microscopy of the Renal Lesion in Rats, J. Exper. Med. 109:115, 1959.
27. Hewitt, R. I.; Gumble, A. R.; Wallace, W. S.; and Williams, J. H.: Experimental Chemotherapy of Trypanosomiasis; IV. Reversal by Purines of the In Vivo Activity of Puromycin, and an Amino-Nucleoside Analog, Against Trypanosoma Equiperdum, Antibiot. & Chemother. 4:1222, 1954.
28. Hewitt, R. I.; Gumble, A. R.; Wallace, W. S.; and Williams, J. H.: Experimental Chemotherapy of Trypanosomiasis; V. Effects of Puromycin Analogues Against Trypanosoma Equiperdum in Mice, Antibiot. & Chemother. 5:139, 1955.
29. Lowry, B. A.; Davoll, J.; and Brown, G. B.: The Utilization of Purine Nucleosides for Nucleic Acid Synthesis in the Rat, J. Biol. Chem. 197:591, 1952.
30. Brown G. B. and Roll, P. M.: *The Nucleic Acids*, Chargoff, E., and Davidson, J. N. (Ed.) New York, Academic Press, 1955, Vol. 2, p. 341.
31. Hutchings, B. L.: *The Chemistry and Biology of Purines*, Wolstenholme, C. E. W., and O'Conner, C. M. (Ed), Boston, Little Brown & Co., 1957, p. 177.
32. Hartman, M. E.; Hartman, J. D.; and Baldridge, R. C.: Inhibition of Amino-nucleoside Nephrosis by Adenine, Proc. Soc. Exp. Biol. & Med. 100:152, 1959.
33. Natelson, S.; Scott, M. L.; and Beffa, C. A.: Rapid Method for the Estimation of Urea in Biologic Fluids, Am. J. Clin. Path. 21: 275, 1951.
34. Kingsley, G. R.: Direct Biuret Method for Determination of Serum Proteins as Applied to Photoelectric and Visual Colorimetry, J. Lab. & Clin. Med. 27:840, 1942.
35. Turner, T. J. and Eales, L.: Quantitative Determination of Cho-

lesterol in Serum with P-toluene Sulphonic Acid, Scandinav. J. Clin. & Lab. Invest. 9:210, 1957.

36. Van Handel, E.; and Zilversmit, D. B.: Micromethod for the Direct Determination of Serum Triglycerides, J. Lab. & Clin. Med. 50:152, 1957.
37. Bendick, A.; Russell, P. J., Jr.; and Brown, G. B.: On the Heterogeneity of the Desoxyribonucleic Acids, J. Biol. Chem. 203:305, 1953.
38. Philips, F. S.; Thiersch, J. B.; and Bendich, A.: Adenine Intoxication in Relation to In Vivo Formation and Deposition of 2, 8-Dioxyadenine in Renal Tubules, J. Pharmacol. & Exper. Therap. 104:20, 1952.
39. Stone, R. E.; and Spies, T. D.: Adenine, Its Failure to Stimulate Hematopoiesis or to Produce Pellagra in a Case of Pernicious anemia, Am. J. M. Sc. 215:411, 1948.

Staff Meeting Report

Effects of Irradiation on the Phosphatides from Normal and Tumor-Bearing Mice*†

Theresa C. Lee, Ph.D.,‡ Robert J. Salmon, M.S., F.R.I.C.§
Donn G. Mosser, M.D.,¶ and Merle K. Loken, Ph.D.**

An excellent review has been published by Haven and Bloor[1] setting forth evidence that phospholipid metabolism is intimately associated with tumor growth. These authors found that in rats, phospholipids accumulate in the tumors to a greater extent than in other tissues of the body. The tumor-bearing animals showed pronounced lipemia when compared with the controls. Furthermore, this lipid material was peculiarly resistant to alkaline hydrolysis.

Haven and Levy[3] observed that one quarter of the phospholipid of the peripheral, rapidly growing part of the rat carcinosarcoma 256 consisted of sphingomyelin. This was a much greater proportion than that found in the central part of the tumor, or in any other part of the animal, with the exception of the brain. The ratio of the phosphorus to the phospholipid isolated was higher than it would be in the ester form. In a more recent publication, Bloor and Haven[4] reported that the phospholipid and cholesterol content of the intestine of rats bearing large tumors increased, while that of neutral fat decreased.

THERESA C. LEE

Schulman and associates[5] found a 45 per cent increase in the turnover rate of phospholipid and a 124 per cent increase in phosphoprotein phosphorus in human gastric carcinoma as compared with normal gastric mucosa. The report of Kosaki and associates[6] of a

*This paper was presented at the Staff Meeting of the University of Minnesota Hospitals on May 20, 1960.
†Supported in part by grants from the United States Public Health Service, Minnesota Division of the American Cancer Society and the Graduate School, University of Minnesota
‡Associate Scientist, Division of Radiation Therapy
§Research Associate, Division of Radiation Therapy; Fellow, Royal Institute of Chemistry, London.
¶Associate Professor and Director, Division of Radiation Therapy
**Assistant Professor, Division of Radiation Therapy

peculiar phospholipid in human cancer tissue and also in the blood of patients with cancer has aroused great interest in these compounds.

Whether or not irradiation has any effect on the metabolism of phospholipids has been much debated. Rosenthal[7] noted that after total body irradiation, pronounced lipemia developed. Entenman and his associates[8–10] showed that roentgen irradiation increased the plasma phospholipid of a number of different animal species. The rate of increase was different for lack of the different species and could be related to radioresistance of the species. Entenman *et al.* found no increase in the concentration of phospholipids in the liver after irradiation, but they did observe an increased uptake of P^{32} into liver phospholipid because of an increase in liver substance.

Thompson and his associates[11] were unable to find any effect of irradiation on the uptake of P^{32} into the phospholipids of the thymus gland of rats. Cornatzer and his co-workers[12] reported that whole body irradiation had little effect on the uptake of P^{32} into the phospholipids of tissue slices of liver and kidney; they did observe a small reduction in phospholipid synthesis in the spleen.

Morehouse and Searcy,[13,14] studying the uptake of C^{14} labeled acetate into the lipids of irradiated rats, found that its incorporation in the neutral fat of the liver increased twofold in the nonfasted animals and sixfold in the fasted animals. The amount of phospholipid synthesized in the livers of the irradiated animals was found to have doubled. Furthermore, the rate of incorporation of the isotope into the neutral fat of the intestine was observed to have remained unchanged by irradiation, whereas the synthesis of phospholipid in the intestinal wall was reduced 50 per cent by irradiation.

The great diversity of results from whole body irradiation upon the lipid metabolism may be due in part to the difference in techniques used for isolating the constituents from the tissues or to the difference in doses of irradiation. In fact, the various organs of the same animal appear to respond quite differently to the same dose of irradiation;[14] thus, different tissues have been shown to exhibit different time sequences of enzyme inactivation and recovery following irradiation.[15]

Vermund and associates demonstrated a periodicity in the phospholipid metabolism of mouse liver,[16] the phase varying in different animals. Thus, variations in the timing of experiments of different workers could produce large differences in the results.

Most of the studies described above were concerned with changes in total lipids or total phospholipids. Only rarely were

attempts made to isolate a single group of lipids, such as the choline containing phospholipids. In recent years it has become clear that there exists a great variety of phospholipids, many of which have specific functions.[17] Therefore, we felt that an effort should be made to discover whether or not roentgen irradiation has a specific effect on some of these functionally specialized phospholipids which may actually constitute only a small fraction of the total phospholipid complex. This report describes a study of some of the effects of roentgen irradiation on the individual phospholipids of liver, spleen, and ascites tumor cells of mice.

Experimental Procedure

A dozen mice of the C3H strain were each given an intraperitoneal injection of 0.2 ml. saline suspension of about 200,000 Ehrlich ascites cells. Seven or eight days later five of the animals which showed an abdominal distention indicative of sufficient growth of the ascites tumor were subjected to a dose of 800 r total body irradiation. The mice were placed in individual compartments of a lucite box and were exposed to roentgen rays from a machine operated at 220 KVP, 15 MA, 0.25 mm. Cu., and 1 mm. Al. filter (HVL 0.95 mm. Cu.). An equal number of tumor-bearing animals were sham irradiated. Ten animals without tumors were similarly treated (five were irradiated and five were sham treated). Immediately after irradiation, all experimental animals were given a subcutaneous injection with a saline solution of radioactive phosphate (1 μc per gram body weight). At either two or four hours after the injection of the P^{32}, the ascitic fluid was withdrawn with a needle and syringe from the tumor-bearing animals and was spun down in a refrigerated centrifuge at 1,000 r.p.m. for thirty minutes. The cells were washed three times with deionized and doubled distilled water. Immediately following withdrawal of the ascitic fluid, the animals were killed, and the liver and spleen removed and weighed.

After the tissues and cells had been homogenized with methanol in a glass homogenizer, the homogenate was diluted with two volumes of chloroform. Homogenates from each of the four groups were pooled, each pooling group consisting of five animals. The mixtures were boiled under reflux for 15 minutes. After brief cooling, the warm extracts were filtered and then quickly evaporated to dryness in an evacuated desiccator. The residue was dissolved in 3 ml. of chloroform and an aliquot was taken for the determination of total phosphorus. The rest of the phosphatide solution was used for separation of the constituents by silicic acid column chromotography as

described by Marinetti.[18] Ten grams of silicic acid was suspended in a mixture of equal volumes of chloroform and methanol and slurried into a glass column 1.2 cm. in diameter. When the acid had settled, the column was washed with 25 ml. of chloroform. Elution was carried out with chloroform, chloroform-methanol mixtures (4:1) (2:1) (1:1) and finally with pure methanol. Three-ml. fractions were collected at a flow rate of 1 ml. in eight minutes. The radioactivity of each fraction was determined by drying 0.2 ml. in a small aluminum cup which was fitted over the end window of a Geiger tube. After a correction was made for decay, the activities were plotted against the volume of eluate (Figures 1-6).

Fractions were then pooled according to the peaks, and aliquots were taken for the accurate determination of phosphorus and radioactivity. The sample was digested with sulphuric and nitric acid and after the volume was made up, an aliquot was counted and the phosphorus determined by the conventional Fiske and Subbarow method. From these data the specific activity and the total phosphorus for each peak were determined.

Partition chromatography was carried out on the remainder of the material by the method of Marinetti.[18] The dried residue from the pooled fraction was dissolved in a very small volume of isoamyl alcohol-benzene mixture (1:1 v/v) and spotted on silicic acid impregnated paper. The chromatogram was developed by ascending technique in di-isobutyl ketone-acetic acid-water (40:30:7).

For identification of the spots, the following reagents were used: ninhydrin, rhodamine 6G, phosphomolybdic acid, hexanitrodipicrylamine, Fleury's reagent.[19] Radioautograms were also made.

Results

Figures 1-6 show the curves obtained by plotting the radioactivities of the fractions against the volumes of eluate from the silicic acid column. These have been grouped in pairs for ease in comparison. Figure 1 compares the activities of the phospholipids from normal mouse liver (upper curve) with those from the liver of a mouse with ascites tumor. For purposes of description, the activity curve is considered in seven sections. Section one consists of a very small peak eluted by chloroform and has been found in all tissues so far examined; the material in this peak is ninhydrin negative and does not contain choline.

Section two follows immediately after methanol is added to the eluant and consists of a very prominent peak containing two ninhydrin positive substances, which are probably phosphatidylethanolamine and phosphatidylserine. The third section

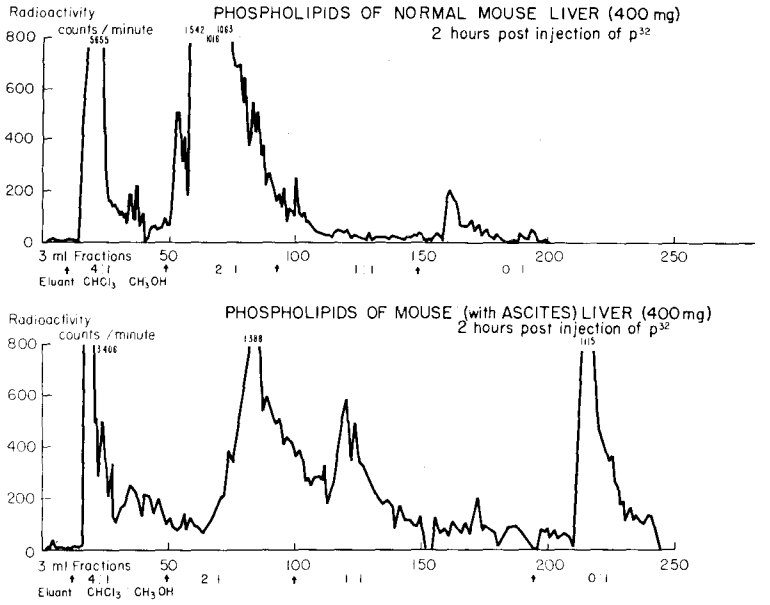


Fig. 1

consists of a series of small peaks eluted by chloroform-methanol mixture of 4 volume to 1 volume; these compounds contain inositol and do not give a positive phosphomolybdic acid test for choline. Sections four and five contain the most prominent peak of all. The first part of this peak, in which the activity rises very sharply, contains lecithin principally, but it also has another phospholipid with a lower Rf value than that of lecithin. The descending part of the peak (section 5) contains lecithin in addition to a phospholipid with an Rf value corresponding to that of sphingomyelin. Section six is a relatively small peak with an Rf value corresponding to sphingomyelin. Section seven contains a peak which is insignificant in liver from a nontumor-bearing mouse, but is very large in the livers of the animals with ascites. This peak gives positive tests for sphingomyelin but exhibits a lower Rf value than does that compound.

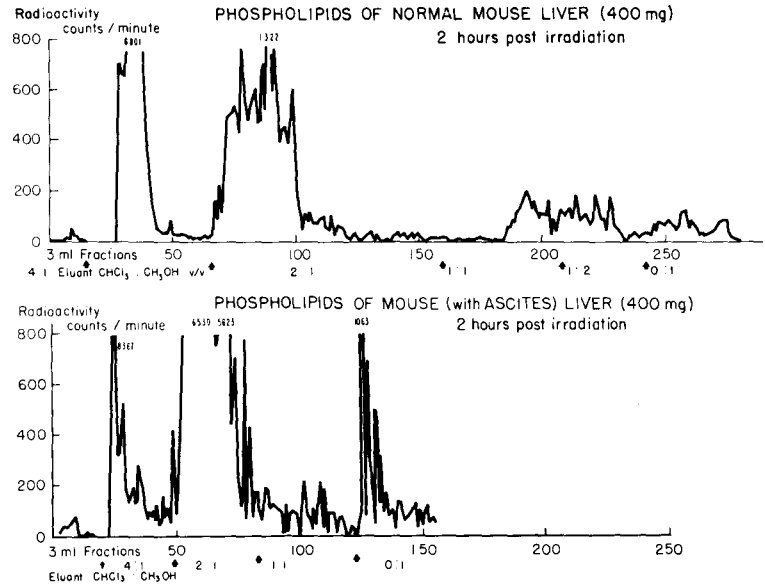

Radioactivity
counts / minute
PHOSPHOLIPIDS OF NORMAL MOUSE LIVER (400 mg)
2 hours post irradiation
800
600
400
200
0
6801
1322
3 ml Fractions
50
100
150
200
250
4 : 1 Eluant CHCl3 : CH3OH v/v
2 : 1
1 : 1
1 : 2
0 : 1
Radioactivity
counts / minute
PHOSPHOLIPIDS OF MOUSE (with ASCITES) LIVER (400 mg)
2 hours post irradiation
800
600
400
200
0
6539 5823
4361
1063
3 ml Fractions
50
100
150
200
250
4 : 1
2 : 1
1 : 1
0 : 1
Eluant CHCl3 : CH3OH

Fig. 2

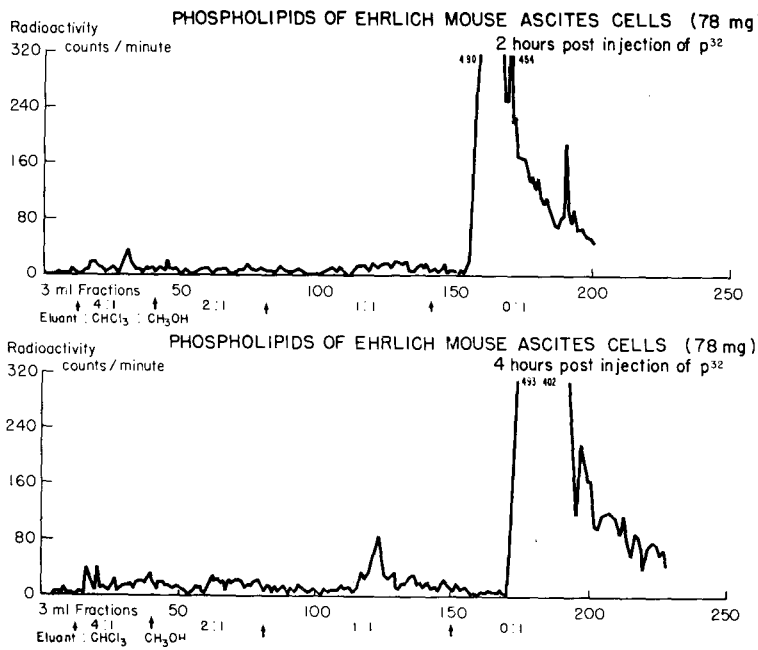

Radioactivity
counts / minute
PHOSPHOLIPIDS OF EHRLICH MOUSE ASCITES CELLS (78 mg)
2 hours post injection of p32
320
240
160
80
0
490
454
3 ml Fractions
50
100
150
200
250
4 : 1
2 : 1
1 : 1
0 : 1
Eluant : CHCl3 : CH3OH
Radioactivity
counts / minute
PHOSPHOLIPIDS OF EHRLICH MOUSE ASCITES CELLS (78 mg)
4 hours post injection of p32
320
240
160
80
0
493 402
3 ml Fractions
50
100
150
200
250
4 : 1
2 : 1
1 : 1
0 : 1
Eluant : CHCl3 : CH3OH

Fig. 3

Figure 2 compares the activity curves of the phospholipids of liver in a normal mouse (upper) and in a mouse with ascites (lower), both animals having been irradiated two hours previously. The effect of irradiation can be seen by comparing the upper curve of Figure 1 with the upper curve of Figure 2 and similarly the lower curves of these two figures. Figure 3 compares the activity curves of the phospholipids of ascites cells two and four hours after the injection of the P^{32} into the host. Note that peak seven is by far the largest of all the phospholipids of the tumor cells and that its activity is apparently still increasing at the four-hour period. Figure 4 shows the comparison of the activity curves of nonirradiated and irradiated ascites cells four hours after irradiation.

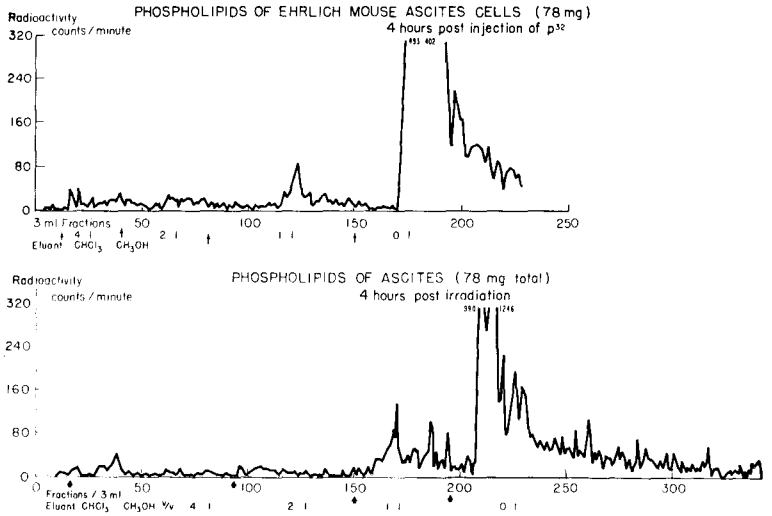


Fig. 4

Figure 5 compares the activities of the phospholipids of normal mouse spleen with those of normal mouse spleen after irradiation. Figure 6 compares the activities of the phospholipids of the spleen of mice with ascites tumors, both irradiated and nonirradiated. The column yielding the data for upper curve was unfortunately cut off before elution was complete and so does not show peak seven, which is extremely prominent on the lower curve. Peak three is relatively very prominent in the nonirradiated spleen of the tumor-bearing mice. Irradiation appears to change the organization of the spleen so that the regular elution pattern of phospholipids is hardly recognizable.

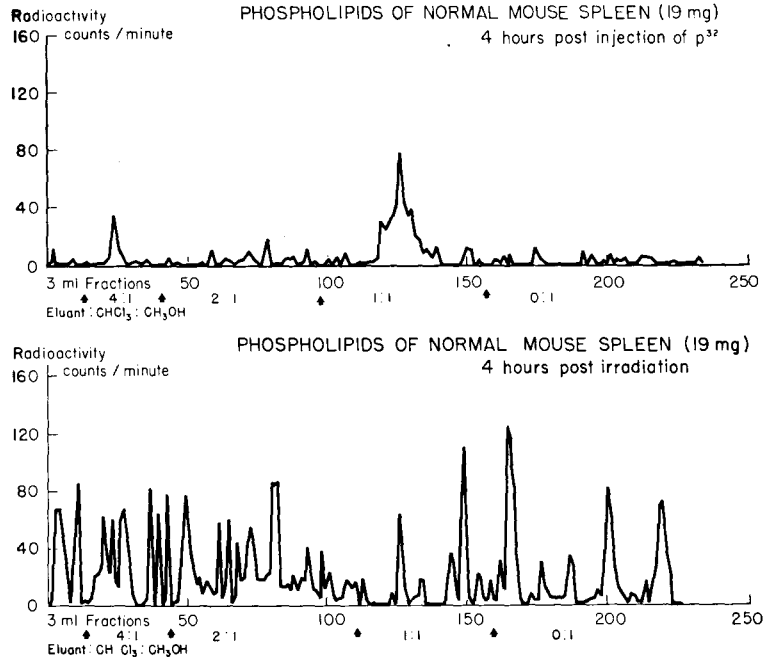

Radioactivity
counts / minute
PHOSPHOLIPIDS OF NORMAL MOUSE SPLEEN (19 mg)
4 hours post injection of p32
160
120
80
40
0
3 ml Fractions
50
100
150
200
250
4:1
2:1
1:1
0:1
Eluant : CHCl3 : CH3OH
Radioactivity
counts / minute
PHOSPHOLIPIDS OF NORMAL MOUSE SPLEEN (19 mg)
4 hours post irradiation
160
120
80
40
0
3 ml Fractions
50
100
150
200
250
4:1
2:1
1:1
0:1
Eluant : CH Cl3 : CH3OH

Fig. 5

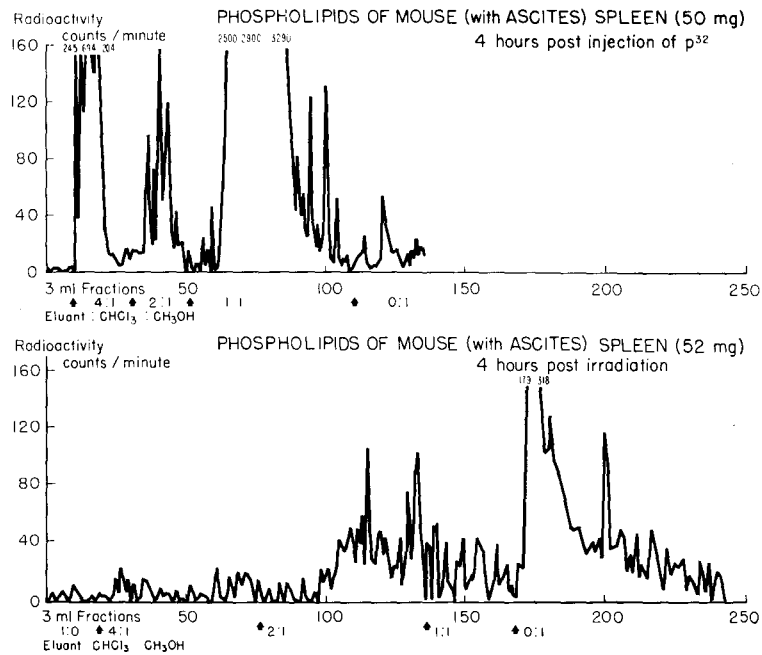

Radioactivity
counts / minute
PHOSPHOLIPIDS OF MOUSE (with ASCITES) SPLEEN (50 mg)
4 hours post injection of p32
160
120
80
40
0
3 ml Fractions
50
100
150
200
250
4:1
2:1
1:1
0:1
Eluant : CHCl3 : CH3OH
Radioactivity
counts / minute
PHOSPHOLIPIDS OF MOUSE (with ASCITES) SPLEEN (52 mg)
4 hours post irradiation
160
120
80
40
0
3 ml Fractions
50
100
150
200
250
1:0
4:1
2:1
1:1
0:1
Eluant CHCl3 CH3OH

Fig. 6

Table 1 indicates that when ascites cell tumors are present, the radioactivity of the phospholipids of the liver increases. Irradiation does not appear to change the specific activity of peaks one, two, and four. The specific activity of peak three appears to be reduced for hours after irradiation, and the specific activity of peak six is increased two hours after irradiation but is reduced at the four-hour period. The amount of phosphorus of peak seven is very small in normal liver; its turnover, however appears to be high and to be increased initially but later to be diminished by irradiation.

TABLE 1

SPECIFIC ACTIVITIES OF PHOSPHOLIPID PEAKS
(counts/minute/μg P)

Peak	Nonirradiated		Irradiated	
	2 hour	4 hour	2 hour	4 hour
	Livers of Normal Mice			
1	107	32.8	66.3	30.4
2	99.0	138.3	95.5	149.0
3	63.7	95.1	63.4	77.7
4	64.2	147.0	69.9	166.4
5	111.8	172.0	92.4	43.9
6	28.3	60.5	230.6	142.8
7	106.2	140.2	283.3	214.3
	Livers of Ascites-bearing Mice			
1	111.0	92.0	15.3	16.9
2	147.4	198.3	124.3	144.0
3	125.2	256.8	103.6	144.8
4	198.0	238.6	157.2	193.3
5	136.7	221.0	55.6	230.9
6	128.3	156.0	149.0	99.4
7	133.5	230.0	349.0	178.7

DISCUSSION

The occurrence in the ascites cells of the substance which is eluted from the silicic acid column near the point for the expected elution of sphingomyelin, and its increase in the liver of ascites-bearing animals seem especially noteworthy. This might be supposed to be the compound that Haven found in

particularly high amounts in rat sarcoma 256,[3] but it does not have the correct Rf value for sphingomyelin. Furthermore, the results to date appear to indicate that its specific activity is increased immediately after irradiation but is reduced four hours later. This fraction has not been obtained in pure form at present. When subjected to chromatography on Whatman 1 paper using a mixture of butanol-pyridine-water (1:1:1) as developing fluid, the main fraction had an Rf of 0.6 and was ninhydrin negative. Two ninhydrin positive compounds, however, were present in this fraction with respective Rf values of 0.23 and 0.29. Radioautograms showed that the majority of the radioactivity was associated with the fast moving spot, but two other active substances were closely allied to the ninhydrin spots. The chromatographic behavior of these latter suggest similarity to the reported "malignolipin" of Kosaki and his associates.[6]

Another interesting finding is that the fraction is associated with substances involved in the well-known phenomenon of the yellowing of oils. In extracts containing the lipids from normal animal tissue, a yellow substance develops as the extracts are permitted to stand in air. This material is eluted from the the column by the chloroform. In the extracts from tumor and tissues of animals bearing tumors an even more intense discoloration occurs. While some of this fraction is eluted by chloroform, more is eluted by methanol. It is tempting to speculate whether or not fat soluble quinones such as tocopherylquinone, or coenzyme Q10 might be present in this fraction.

Calculation of the total phosphorus from the columns indicated incomplete recovery of the phosphorus. We then discovered that by continuing the elution with a mixture of methanol-chloroform and water (4:1:1) another large peak of activity was obtained. In the past few years, several investigators have found[20,21,22] a number of amino acids and peptids closely associated with phospholipids. Gaby and associates[23] suggested that the phospholipid-amino acid fraction isolated by them may serve to transport amino acids across cell membranes.

A number of the peaks isolated in our laboratories show several ninhydrin positive spots. The investigation of the nature of this material is being continued.

Further improvement in technique of resolution from the silicic acid column and necessary refinements in the analytical procedures are now being attempted.

References

1. Haven, F. L. and Bloor, W. R.: Lipids in Cancer, Adv. Cancer Res. 4:237, 1957.
2. Haven, F. L.; Bloor, W. R.; and Dawson, J. B.: Concentration of Lipid Phosphorus in Tumor and Carcass of Rats Growing Walker Carcinoma 256, Proc. Soc. Exp. Biol. & Med. 80:697, 1952.
3. Haven, F. L. and Levy, S. R.: Occurrence and Rate of Turnover of Tumor Sphingomyelin, J. Biol. Chem. 141:417, 1941.
4. Bloor, W. R. and Haven, F. L.: The Weight and Lipid Content of the Intestines in Rats with Walker Carcinoma 256, Cancer Res. 15:173, 1955.
5. Schulman, J. Jr.; Falkenheim, M.; and Gray, S. J.: The Phosphorus Turnover of Carcinoma of the Human Stomach Measured with Radioactive Phosphorus, J. Clin. Invest. 28:66, 1949.
6. Kosaki, T.; Ikoda, T.; Kotani, Y.; Nakagawa, S.; and Saka, T.: A New Phospholipid, Malignolipin, in Human Malignant Tumors, Science 127:1176, 1958.
7. Rosenthal, R. L.: Opalescence of Serum After Total Body X-irradiation as a Prognostic Sign of Death, Science 110:43, 1949.
8. Neve, R. A. and Entenman, C.: Changes in Plasma Phospholipids following Whole Body X-irradiation, Fed. Proc. 10:367, 1951.
9. Entenman, C. and Weinman, E. O.: Effect of X-irradiation on the Incorporation of Inorganic P-32 into Phospholipids by Rat Liver Slices, Fed. Proc. 11:44, 1952.
10. Entenman, C.; Neve, R. A.; and Olmstead, C. A.: Species Differences in Plasma Phospholipid Levels as Influenced by Whole Body X-irradiation, Fed. Proc. 12:40, 1953.
11. Thomson, J. F.; Tourtellotte, W. W.; and Carttar, M. S.: Some Observations on the Effect of Gamma Radiation on the Biochemistry of the Rat Thymus, Proc. Soc. Exp. Biol. & Med. 80: 268, 1952.
12. Cornatzer, W. E.; Davison, J. P.; Engelstad, O. D.; and Simonson, C.: Effect of Whole Body X-irradiation on Lipids in the Liver, Kidney and Spleen of Fasted Rats, Rad. Res. 1:546, 1954.
13. Morehouse, M. G. and Searcy, R. L.: C-14 Acetate Incorporation into Liver Lipids and Glycogen of Irradiated Rats, Science 122: 158, 1955.
14. Morehouse, M. G. and Searcy, R. L.: In Vivo and In Vitro Lipogenesis in the Irradiated Rat, Fed. Proc. 15:316, 1956.
15. Nygaard, O. F. and Potter, R. L.: Effect of X-radiation on Deoxyribonucleic Acid Metabolism in Various Tissues of the Rat, Rad. Res. 12:120, 1960.
16. Vermund, H.; Halberg, F.; Barnum, C. P.; Nash, C. W.; and Bittner, J. J.: Physiologic 24-hour Periodicity and Hepatic Phospholipid Metabolism in Relation to the Mouse Adrenal Cortex, Am. J. Physiol. 186:414, 1956.

17. Rapport, M. M.; Alonzo, N. F.; Graf, L.; and Skipski, V. P.: Immunological Studies of Organ and Tumor Lipids, IV. Chromatographic Behavior of the Lipid Hapten of Rat Lymphosarcoma: Dependence of in vitro Measurements on Lipid Interactions, Cancer 11:1125, 1958.

18. Marinetti, G. V.; Eroland, J.; and Kochen, J.: Quantitative Chromatography of Phosphatides, Fed. Proc. 16:837, 1957.

19. Block, R. J.; Durrum, E. L.; and Zweig, G.: *A Manual of Paper Chromatography and Paper Electrophoresis,* 2nd ed., New York, Academic Press, Inc., 1958, p. 203.

20. Westley, J.; Wren, J. J.; and Mitchell, H. K.: Phospholipids containing Amino Acids Other than Serine, J. Biol. Chem. 229:131, 1957.

21. Hipp, N. J.; Groves, M. L.; and McMeekin, T. L.: Phosphopeptides obtained by Partial Acid Hydrolysis of α-Casein, Abstracts, Division of Biological Chemistry, American Chemical Society, 128th Meeting of the American Chemical Society, Minneapolis, Minnesota, 1955, p. 22c.

22. Becker, G.; Bode, F.; and Schrade, W.: Über Lipopeptide des Blutes und Gewebes, Klin. Wchnschr. 31:593, 1953.

23. Gaby, W. L.; Hadley, C.; and Kaminski, Z. C.: A Study of Lipids of Penicillium Chrysogenum, J. Biol. Chem. 227:853, 1957.

Staff Meeting Report

Subacute Bacterial Endocarditis: A Review of Cases Seen at the University of Minnesota Hospitals, 1939-1959*†

George A. Pankey, M.D.‡

Subacute bacterial endocarditis (SBE) is considered an infection of the endocardium, usually caused by microorganisms of low virulence, resulting in the clinical signs of chronic infection.[1] The term "subacute" is somewhat inappropriate, considering the chronicity of the disease in most of the patients. With present antibiotic therapy, more patients with endocarditis caused by bacteria of greater virulence are kept alive and reach the more chronic stage. (In this paper the pre-penicillin years of 1939-1942 are included for mortality comparisons.)

GEORGE A. PANKEY

In compiling the present survey, we reviewed the histories of 167 patients with SBE. Eleven of these patients each had one recurrence, and four each had two recurrences. For analysis of clinical and laboratory data, each recurrence is considered as a separate patient, bringing the total number to 186. Fifty-four patients with acute bacterial endocarditis were seen during the same 21 years (Table 1).

Table 2 shows the distribution of the patients by age and sex. Most reported series list the highest incidence in the third and fourth decades with a much smaller incidence in the second decade and a rare occurrence in the first decade. In this series, however, 20 patients with congenital heart disease are included in the first two decades, representing 37 per cent of the total for these periods. This, undoubtedly, is a reflection of the large number of patients with congenital heart disease referred to the University of Minnesota Hospitals.

Table 3 shows the type of underlying organic heart disease.

---

*This report was given at the Staff Meeting of the University of Minnesota Hospitals on March 25, 1960.

†This work was supported by a National Heart Institute graduate training grant.

‡Medical Fellow, Department of Medicine.

TABLE 1

DISTRIBUTION OF CASES OF BACTERIAL ENDOCARDITIS BY SEX

	Males	Females	Total
Subacute bacterial endocarditis	85	82	167
SBE with one recurrence	8	3	11
SBE with two recurrences (patients)	1	3	4
(cum.)	2	6	8
Total SBE	95	91	186
Acute bacterial endocarditis	34	20	54
TOTAL	129	111	240

TABLE 2

DISTRIBUTION OF PATIENTS WITH SUBACUTE BACTERIAL ENDOCARDITIS BY AGE AND SEX

Age	Males	Females	Total	Per cent
0 - 10	10	9	19	11.4
11 - 20	14	21	35	20.9
21 - 30	11	14	25	14.9
31 - 40	9	19	28	16.8
41 - 50	11	7	18	10.8
51 - 60	13	6	19	11.4
61 - 70	11	3	14	8.4
71 - 80	6	3	9	5.4
TOTAL	85	82	167	100

Most reported series have indicated that organic heart disease almost always precedes the development of SBE.[1] In this series, 37 patients gave no histories of underlying organic heart disease, and autopsies, performed on 19 of these, revealed valvular lesions. Rheumatic heart disease was found to be equally common in males and females. At one time SBE, was reported as developing in 25 per cent of patients with rheumatic heart disease and in an even greater per centage of those with congenital heart disease.[1] The incidence has decreased with the use of prophylactic antibiotics for dental extractions, operations, and other procedures requiring manipulation of the mucous membranes.

Primary factors that could have initiated the bacteremia which results in the development of SBE are often neglected in

TABLE 3

UNDERLYING HEART DISEASE IN SUBACUTE BACTERIAL ENDOCARDITIS

	Males	Females	Total	Per cent
Rheumatic heart disease	47	47	94	56.3
Congenital heart disease	16	19	35	20.9
None mentioned	22	15	37	22.2
Atherosclerosis of aortic valve	0	1	1	0.6
TOTAL	85	82	167	100

recording the history of these patients. In this series, a possible predisposing factor was mentioned for two-thirds of the patients (Table 4). Important points to note from Table 4 are: the high incidence of history of coryza, flu, or sore throat; the well-recognized danger of dental manipulations; and the incidence among elderly men who have undergone diagnostic and operative genito-urinary procedures. Subacute bacterial endocarditis occurred following normal spontaneous delivery in three obstetric patients with organic heart disease and following abortion in two other obstetric patients. In three patients SBE developed after cardiac catheterization. Fungal endocarditis is likely to develop in heroin addicts who give themselves injections intravenously; no blood

TABLE 4

INITIATING FACTORS IN SUBACUTE BACTERIAL ENDOCARDITIS

	Males	Females	Total	Per cent
Upper respiratory infections	30	28	58	46.8
Dental manipulations	18	15	33	26.6
Operations (5 TUR's included)	8	5	13	10.5
Delivery and/or abortion	0	5	5	4.0
Urinary catheterization	4	0	4	3.2
Cardiac catheterization	0	3	3	2.4
Traumatic injury	1	1	2	1.6
Genito-urinary infection	0	2	2	1.6
Cystoscopy	1	0	1	0.8
Heroin addict	1	0	1	0.8
Burn	0	1	1	0.8
Insect bite	1	0	1	0.8
TOTAL	64	60	124	100

TABLE 5

SYMPTOMS IN 186 PATIENTS WITH SUBACUTE BACTERIAL ENDOCARDITIS

	Males	Females	Total	Per cent
Fever	70	71	141	75.8
Weight loss	56	52	108	58.1
Fatigue	51	54	105	56.5
Anorexia	51	43	94	50.5
Weakness	44	41	85	45.7
Chills	35	47	82	44.1
Sweats	41	36	77	41.4
Joint pains	30	41	71	38.2
Petechiae	23	28	51	27.4
Central nervous system symptoms	22	27	49	26.3
Osler's nodes	13	30	43	23.1
Anemia	6	24	30	16.1

cultures were obtained from the one heroin addict in this series, but autopsy disclosed SBE of the mitral valve.

In the majority of patients, symptoms had persisted for several months before admission (average, four months). Weakness, fatigue, and fever were the most prominent presenting complaints. Six patients were admitted following cerebrovascular accidents. Histories of heart disease prior to development of SBE were given by 70 per cent of the patients.

Table 5 shows the symptoms of the 186 patients. Fever was the most common symptom and was associated with chills in 58 per cent. Frequent periods of remission often accounted for the delay in seeking medical attention.

Joint pains occurred in 71 patients; this finding led to some confusion with the diagnosis of acute rheumatic fever, but chills are extremely rare in the latter condition. Symptoms suggestive of embolic phenomena occurred fairly frequently.

Table 6 shows the physical findings in the 186 patients. Organic heart murmurs were detected in all patients except the heroin addict. Fever, mainly intermittent and remittent, occurred in 177 patients; it was absent or low grade in eight patients and was not recorded in one. Petechiae were most commonly seen in the conjunctivae; the second most common site was the skin, especially on the extremities. Splinter, fundal, and mucous mem-

TABLE 6

PHYSICAL FINDINGS IN 186 PATIENTS WITH SUBACUTE BACTERIAL ENDOCARDITIS

	Males	Females	Total	Per cent
Murmur(s)	94	91	185	99.5
Fever	91	86	177	95.2
Cardiomegaly	74	65	139	74.7
Petechiae	69	62	131	70.4
Splenomegaly	48	50	98	52.7
Tachycardia on admission	39	51	90	48.4
Pallor	38	50	88	47.3
Hepatomegaly	49	35	84	45.2
Congestive heart failure	27	28	55	29.6
Poor oral hygiene	34	21	55	29.6
Hepatosplenomegaly	28	26	54	29.0
Positive neurological findings	23	27	50	26.9
Clubbing	27	18	45	24.2
Changing murmurs	23	8	31	16.7
Suspected emboli (to spleen, kidneys, etc.)	6	18	24	12.9
Osler's nodes	6	15	21	11.3
Trombophlebitis	3	8	11	5.9
Janeway lesions	5	5	10	5.4

brane petechiae were noted in one-fourth of the patients. A cuff test was positive in 64 per cent of the 58 patients tested.

Forty-five patients had clubbing, excluding those with cyanotic congenital heart disease. It is important to note that changing murmurs, Osler's nodes, and Janeway lesions did not occur frequently. The physical findings were all fairly evenly distributed between males and females.

Since blood cultures are of prime consideration in the diagnosis of SBE, it is important to remember that: 1) If the culture is going to be positive, it will probabdly be so in the first five attempts, since in SBE bacteremia is usually steady rather than intermittent;[2] 2) approximately 15 per cent to 20 per cent of patients will have sterile blood cultures, regardless of the number taken; 3) the blood should be cultured both aerobically and anaerobically for three weeks before being pronounced sterile; 4) fungal cultures should be made if bacteria are not readily

cultured; and 5) in a small percentage of patients, bone marrow cultures are positive, while the blood remains sterile.[1]

All microorganisms cultured should be tested for sensitivity to appropriate antibiotics. Of the total number of patients with SBE, 31 had sterile blood cultures. Four of these latter had had positive blood cultures reported by their referring physician, and two had organisms cultured from the involved valve at autopsy; in all six cultures the organism was *Streptococcus viridans*. Nine more had the diagnosis confirmed at autopsy, three others had positive cerebrospinal fluid cultures, and one other had a positive urine culture. The physicians seeing the 12 remaining patients all agreed with the diagnosis of SBE; moreover, these patients exhibited fever, murmurs, anemia, and increased erythrocytic sedimentation velocities. Excluding four patients who had no blood culture, 83 per cent of the cultures were positive—a finding that agrees with those of other reported series.[1] Subacute bacterial endocarditis was suspected as being the diagnosis based on clinical and laboratory examination in more than 90 per cent of the patients finally diagnosed as such. This is reflected in the small number of cases in which no blood cultures were drawn.

Table 7 shows the results of the blood cultures. *Streptococcus viridans* was the most frequently cultured microorganism, and if the six probable ones mentioned previously are included, the percentage of cultures containing this organism rises to 60 per

TABLE 7

BLOOD CULTURE RESULTS IN 186 PATIENTS WITH SUBACUTE BACTERIAL ENDOCARDITIS

Organism	Total	Per Cent
Streptococcus viridans	95	51.1
Streptococcus viridans plus other(s)	8	4.3
Staphylococcus albus	23	12.3
Streptococcus fecalis	10	5.4
Streptococcus fecalis plus one other	2	1.1
Pseudomonas	3	1.6
Staphylococcus aureus	3	1.6
Brucella abortus	2	1.1
Micrococci	2	1.1
Pneumococcus	1	0.5
Alkaligenes	1	0.5
Gamma streptococcus	1	0.5
Sterile	31	16.7
No cultures	4	2.2
TOTAL	186	100

cent. Three different species of bacteria were cultured from the blood of two patients: *Streptococcus viridans, Pseudomonas,* and *Alkaligenes* in one; and *Streptococcus viridans,* beta hemolytic streptococci, and coagulase-negative staphylococci in the other. (Coagulase-negative staphylococci are classified as *Staphylococcus albus* in Table 7.) Seven of the patients with coagulase-negative staphylococci had only one positive blood culture, but four of these were proven at autopsy to have SBE. Sensitivities to penicillin were determined on 33 cultures of *Streptococcus viridans,* and 73 per cent were found to be sensitive to less than one unit per ml. This sensitivity has not decreased in recent years as did that of coagulase-positive staphylococci. Twelve grew out *Streptococcus fecalis,* which was more resistant to penicillin. Coagulase-positive staphylococci, classified as *Staphylococcus aureus* in Table 7, were cultured from three patients. *Candida albicans* was cultured from one patient in association with *Streptococcus fecalis.* This was the only species of fungus cultured. The percentage of sterile blood cultures corresponds closely to that reported in other series.[1]

Thirty-five per cent of the patients had completely normal urinalyses. Pyuria was the most frequently found urinary abnormality. The average of the lowest hemoglobin values was 10 gm/100 ml. with 118 patients having hemoglobin values below 11 gm/100 ml. Reticulocyte counts were elevated in 19 of 27 patients. Platelet counts were below 100,000 in 7 of 24 patients. Histiocytes were looked for in 44 patients and found in 32 per cent of these. Forty per cent of all patients in this series had their highest white blood cell counts below 10,000, indicating that leukocytosis is not a very reliable sign of infection in SBE. The differential leukocyte counts were normal in most patients, but five or more monocytes were observed in 37 per cent of the patients, and four or more eosinophils occurred in 29 per cent, indicating the chronicity of the infection. Ninety-four per cent of the patients had elevated erythrocytic sedimentation velocities. The 6 per cent with normal sedimentation velocities included some previously treated patients and some with polycythemia secondary to cyanotic congenital heart disease. Blood urea nitrogen levels were determined in 108 patients and found to be elevated in 44 per cent. Some have suggested that elevated blood urea nitrogen accounts for sterile blood cultures. In this series, however, 40 out of 47 patients with elevated blood urea nitrogen levels had positive blood cultures, while 17 with normal levels had sterile blood cultures. The albumin-globulin ratio of serum proteins was reversed in the majority of patients on whom this determination was made.

TABLE 8

ELECTROCARDIOGRAPHIC FINDINGS IN 147 PATIENTS WITH SUBACUTE BACTERIAL ENDOCARDITIS

ECG	SBE*	
	Total	Per cent
Normal	65	44.2
Abnormal with normal sinus rhythm	51	34.7
Atrial fibrillation on admission	6	4.1
Developed atrial fibrillation	8	5.4
First degree atrioventricular block	17	11.6

*SBE = Subacute bacterial endocarditis

Table 8 shows the electrocardiographic findings. Note that 96 per cent of the patients who had, electrocardiograms exhibited a sinus rhythm on admission. It is also obvious that SBE can occur in the presence of atrial fibrillation. The incidence of first degree atrioventricular block is similar to that recorded in othes series.[1]

Table 9 shows the seven most frequent abnormal physical and laboratory findings; interestingly, splenomegaly is not included in this group.

The outcome of the disease in these 167 patients is shown in Table 10. A form letter was sent to all the patients discharged from the hospital in order to determine the state of their health. If no answer was obtained from the patient, a similar form letter

TABLE 9

OCCURRENCE OF ABNORMAL PHYSICAL AND LABORATORY FINDINGS IN PATIENTS WITH SUBACUTE BACTERIAL ENDOCARDITIS

	Per Cent	Comment
Murmurs	99.5	
Fever	95.2	
Increased erythrocytic sedimentation velocity	94.3	27 not done
Positive blood culture	83.2	4 not cultured
Cardiomegaly	74.7	
Petechiae	70.4	
Anemia	64	4 not done

TABLE 10

FATE OF 167 PATIENTS WITH SUBACUTE BACTERIAL ENDOCARDITIS

	Total	Per Cent
Died in hospital of subacute bacterial endocarditis	68	40.7
Died in hospital of other causes	4	2.4
Died within six months after discharge	17	10.2
Died six to twelve months after discharge	2	1.2
Died one to two years after discharge	3	1.8
Lived longer than two years (present status unknown)	17	10.2
Living now	44	26.3
Completely unknown	12	7.2
TOTAL	167	100

was sent to the referring phsician. By this method, information was obtained for 83 of 95 patients discharged from the hospital. Nine of the 16 patients seen in 1958 and 1959 are living now, thus somewhat distorting the size of that group. Three patients lived for more than two years after their first attack of SBE and died in the hospital of a recurrence: they are included in the "Died in the Hospital" group. Fifty-one per cent of the patients died from the infection itself in the hospital or within six months of discharge. Eleven of these died within three months of discharge, and the remaining six exhibited signs of infection until death. Excluding the three patients who were seen in 1959 and are alive now, and the 12 whose fate is unknown, 63 were considered cured of SBE. In addition, in four patients the SBE was regarded to have healed when seen at autopsy. Therefore, 67 patients—i.e., 44 per cent of the 152 patients with known outcomes—are considered cured of SBE. When these figures are broken down by age, we see that approximately two-thirds of the patients over 20 years old died in the hospital or within six months of discharge.

Table 11 shows the mortality from SBE during the pre-penicillin years, 1939 through 1942, and for two groups during the penicillin era. Of the 45 patients in the years 1939 through 1942, two are living now; two are unknown, and one lived for more than two years. Both of those living now had had rheumatic heart disease, and *Streptococcus viridans* was isolated as

TABLE 11

MORTALITY IN SUBACUTE BACTERIAL ENDOCARDITIS FROM 1939 THROUGH 1959

Years	Total Cases	Deaths in Hospital or Within Six Months after Discharge	Per Cent
1939 - 1942	45	38	84.4
1943 - 1954	82	36	43.9
1955 - 1959	40	15	37.5
TOTAL	167	89	53.3

the causative microorganism in both cases. One received no therapy for SBE, and the other received sulfonamide therapy for 58 days. Many reported series show a lower mortality than that found in this hospital,[1] but, definitions of what constitutes a cure of the infection have varied considerably throughout the country. The fall in mortality with penicillin therapy is readily apparent from Table 11.

Table 12 shows the valve or valves involved in patients with underlying rheumatic heart disease. The mitral valve was generally most frequently involved, but in male patients aortic lesions were more common. Mitral vegetations were usually associated with primary mitral insufficiency. In four patients, however, severe mitral stenosis was noted at autopsy to be associated with SBE.

Table 13 shows the location of valvular lesions in patients with no underlying organic heart disease—a diagnosis made at autopsy in 19 of the 37 patients. Again, mitral lesions predominate. The tricuspid valve was not mentioned as being involved in any patient in the entire series.

TABLE 12

LOCATION OF VALVULAR LESIONS IN PATIENTS WITH SUBACUTE BACTERIAL ENDOCARDITIS WITH RHEUMATIC HEART DISEASE

Valves Involved	Autopsy Diagnosis	Clinical Diagnosis	Total	Per Cent
Mitral	12	30	42	44.7
Aortic	10	17	27	28.7
Mitral and Aortic	11	14	25	26.6
TOTAL	33	61	94	100

TABLE 13

LOCATION OF VALVULAR LESIONS IN PATIENTS WITH SUBACUTE BACTERIAL ENDOCARDITIS WITH NO UNDERLYING HEART DISEASE

Valves Involved	Autopsy Diagnosis	Clinical Diagnosis	Total	Per Cent
Mitral	10	16	26	70.3
Aortic	4	2	6	16.2
Mitral and Aortic	5	0	5	13.5
TOTAL	19	18	37	100

In the patients with congenital heart disease, the most frequently observed defects were: isolated interventricular septal defect, tetralogy of Fallot, and patent ductus arteriosus. An interatrial septal defect was present in six cases but was the site of SBE in only two. Several of the patients have had successful cardiac surgery following an attack of SBE.

Autopsises were performed in this hospital on 59 patients with SBE; findings on one other patient, on whom an autopsy was performed at another hospital, are included in Tables 14 and 15.

Table 14 shows autopsy findings exclusive of those relating to the central nervous system in the 60 patients. Fifty-six patients had active endocardial lesions and four had healed lesions. Congestion was a frequent finding and is the chief source of trouble in patients who are cured of the infection. The suspicion of emboli to the spleen and kidney was frequently confirmed. Pneumonia was common, and the focal embolic glomerulonephritis of SBE occurred in 10 patients. Splenitis, or the so-called septic spleen, was also frequently noted. Coronary embolism occurred in three patients. Seven of the perforated valves were aortic, six of these perforations occurring in males. The pericarditis found was usually fibrinous or fibrous. Mycotic aneurysms—five of them cerebral—occurred in a total of 11 patients. Two patients had amyloidosis. The low incidence of abscess formation reflects the decreased invasiveness of the microorganism usually associated with SBE.

Table 15 shows the autopsy findings relative to the central nervous system. Suppurative encephalitis secondary to septic embolization was frequent. Subarachnoid hemorrhage or intracerebral hemorrhage or both were noted in 15 per cent. In several cases this was associated with heparin therapy; this type of treatment was formerly advocated for SBE, but it is now

TABLE 14

NON-NERVOUS SYSTEM AUTOPSY FINDINGS IN 60 PATIENTS WITH SUBACUTE BACTERIAL ENDOCARDITIS

	Total	Per Cent
Active endocardial lesion(s)	56	93.3
Congestion	41	68.3
Splenic infarct	33	55.0
Renal infarct	23	38.3
Pneumonia	22	36.7
Glomerulonephritis	17	28.3
Embolic	10	—
Acute	4	—
Subacute	2	—
Chronic	1	—
Splenitis	13	21.7
Other embolic phenomena	11	18.3
Pulmonary	5	—
Coronary	3	—
Femoral	1	—
Liver	1	—
Superior mesenteric	1	—
Perforated valves	9	15.0
Pericarditis	9	15.0
Mycotic aneurysm	6	10.0
Myocarditis	5	8.3
Pericardial effusion	5	8.3
Amyloid	2	3.3
Splenic abscess	2	3.3
Renal abscess	2	3.3
Myocardial abscess	1	1.7

known to aggravate the hemorrhage associated with embolic phenomena and mycotic aneurysmal leaks. The brain was normal in five patients, and in four others only cerebral sclerosis was found.

Although no detailed analysis of the treatment of the patients in this series can be given, a few points are worth noting: Antibiotic treatment was extremely variable as to amount and duration. After 1942, penicillin and streptomycin were the most commonly used antibiotics. Ignorance of what constituted adequate therapy for the individual patient seemed to be the

TABLE 15

CENTRAL NERVOUS SYSTEM AUTOPSY FINDINGS IN 60 PATIENTS WITH SUBACUTE BACTERIAL ENDOCARDITIS

	Total	Per Cent
Suppurative encephalitis	15	25.0
Brain abscess	2	3.3
Subarachnoid hemorrhage and/or intracerebral hemorrhage	9	15.0
Mycotic aneurysms	5	8.3
Meningitis	1	1.7
Normal	5	8.3
No brain examination	19	31.7

most common error in management. Only one patient lived longer than a year during the period when no antibiotic treatment was given.

On the basis of this series and others reported in the literature, the following treatment schedules seem most adequate: For *Streptococcus viridans* sensitive to less than 0.2 units per ml. of penicillin, one million units of aqueous penicillin is given intramuscularly every six hours for four to six weeks. Aqueous penicillin is preferred because it gives high peak levels and probably entails fewer sensitivity reactions. For every million units of aqueous penicillin given intramuscularly per day in divided doses, the blood level rises by approximately one unit. The aim in the treatment of SBE is to obtain a blood level five to ten times the level to which the bacteria are sensitive.[3] Thus, if the organism is sensitive to 2 units per ml. of penicillin, 10 million to 20 million units of penicillin should be given per day. A continuous intravenous drip of penicillin should be employed if the total amount exceeds 8 to 12 million units, or less if the patient cannot tolerate intramuscular injection. If the *Streptococcus viridans* is resistant to more than 1 unit per ml. of penicillin, 2 gm. of streptomycin a day should be given for three to four weeks, and then 1 gm. a day for two to three weeks. One-half gm. of Benemid® may be given every six hours, since by blocking the urinary excretion of penicillin, it raises the blood levels even higher.

For staphylococci sensitive to 1 unit or less per ml. of penicillin, 1 million units may be given intramuscularly every two to three hours for six weeks, but, an intravenous drip is usually necessary. If the staphylococci are resistant to more than 1 unit per ml. of penicillin, 2 to 3 gm. per day of Vancomycin® should

be given for two weeks and the penicillin dose doubled or tripled. Benemid should also be used, as extremely high blood levels of penicillin may overcome moderate resistance of the staphylococci.

In *Streptococcus fecalis* SBE treatment should begin with 20 million units of penicillin per day intravenously, with Benemid, and 2 gm. of streptomycin a day. This treatment should be continued for six to eight weeks. Up to 100 million units of penicillin per day has been given for this type of SBE. Vancomycin, Novobiocin® or Bacitracin, or all three may be necessary. If the blood cultures are negative, treatment is the same as for *Streptococcus viridans* sensitive to 0.2 units per ml. of penicillin; but if no response occurs during the first week, the treatment schedule described above for *Streptococcus fecalis* is instituted. These patients are usually the most difficult to cure. Heparin therapy is contraindicated, and if thrombophlebitis develops, ligation of the veins is the treatment of choice.

If the patient is allergic to penicillin, a bacteriostatic and a bactericidal antibiotic are aften combined, such as erythromycin and streptomycin. A bactericidal antibiotic should always be included, in spite of results of sensitivities.

In some patients with penicillin allergy associated with a micro-organism sensitive to penicillin but resistant to other antibiotics, adrenal cortical steroids may be given with penicillin to prevent reactions. Sensitivity studies should be performed on all species of bacteria cultured.

The recommended antibiotic prophylaxis for patients with rheumatic or congenital heart disease is as follows: For dental or other oral surgery, 600,000 units of penicillin is given intramuscularly daily two days before, one hour before, and daily two days after the procedure. If the patient is allergic to penicillin, erythromycin 250 mg. four times a day may be given for the five days. For childbirth, catheterization, genito-urinary surgery, and other manipulative procedures, 1 gm. per day of streptomycin is added to the penicillin administered during the 5-day period. A five-day course of tetracycline or Chloromycetin® may be used instead of penicillin but still in association with streptomycin.[4]

In conclusion, this review of 167 patients with subacute bacterial endocarditis (19 of whom had recurrences) for the most part confirms the findings reported in other series. Differences may be explained in part by the fairly unique patient population of the University of Minnesota Hospitals. It was concluded that the importance of prophylactic antibiotics must be stressed

for all patients with known organic heart disease who undergo procedures that may give rise to bacteremia.

Acknowledgment: This work was done under the supervision and guidance of Dr. Wesley W. Spink.

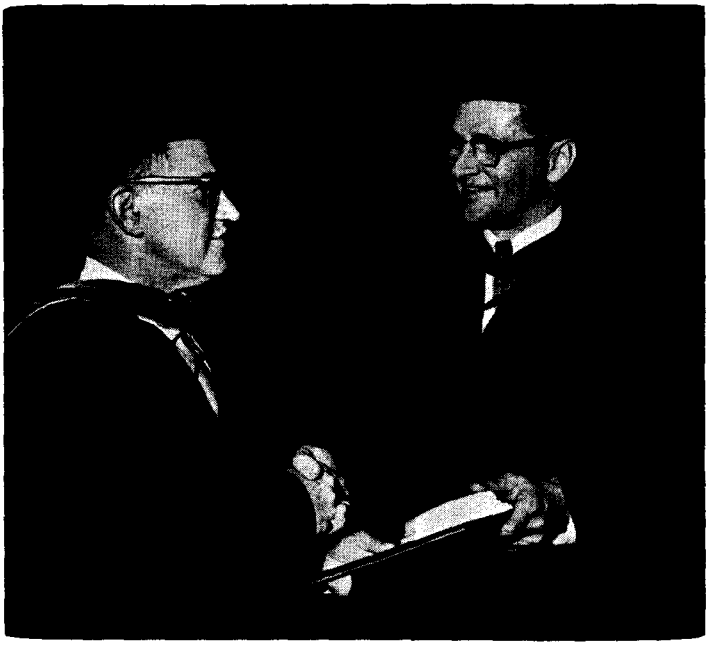
References

1. Kerr, Andrew, Jr.: *Subacute Bacterial Endocarditis,* Springfield, Illinois, Charles C Thomas, 1955.
2. Belli, James, and Waisbren, Burton A.: The Number of Blood Cultures Necessary to Diagnose Most Cases of Bacterial Endocarditis, Am. J. M. Sc. 232:284, 1956.
3. Tamulty, P. A.: The Management of Bacterial Endocarditis, A.M.A. Arch. Int. Med. 105:126, 1960.
4. Finland, M.: Chemoprophylaxis of Infectious Diseases, Disease-a-Month December, 1959.

Medical School News

122 SENIORS RECEIVE MD DEGREES

One hundred twenty two members of the Class of 1960, University of Minnesota Medical School, received medical degrees at graduation exercises June 11, 1960. Dr. Robert B. Howard, Dean of Medical Sciences, conferred the diplomas.



Dr. Leo G. Rigler (left) receives Outstanding Achievement Award from Dr. Robert B. Howard, Dean of Medical Sciences.

A day earlier, colorful Recognition Day Exercises were held at the Medical School for the graduating seniors. Dr. Leo G. Rigler (Med. '20), Executive Director, Cedars of Lebanon Hospital, Los Angeles, California, received the University's Outstanding Achievement Award, and was main speaker. His topic was "the Medical Citizen."

Kenneth P. Manick, President of the Senior Class and Philip J. Worrell were both awarded medals for outstanding scholarship by Dr. E. C. Bayley (Med. '25) of Lake City, President of the Southern Minnesota Medical Association. Lawrence W. DeSanto received the Mediclinics award for scholastic and professional achievement from Dr. Arthur C. Kerkhof (Med. '27),

Chairman of that group. C. Carlyle Clawson was given the Borden Award for medical research achievement as a student.

Dr. Moses Barron (Med. '11) administered the *Declaration of Geneva* to the graduates before a capacity audience of 550 wives, children, parents and friends at Mayo Auditorium.

An analysis of the internship plans show Minnesota and California were popular choices among the graduates. Fifty-two physicians chose to remain at Minnesota hospitals, and 34 accepted appointments in California. Distance honors went to two members who will intern in Honolulu, Hawaii.

Listed below are names of graduates and their internship appointments:

ACUFF, EUGENE L.
Iowa Methodist Hospital
Des Moines, Iowa

ALBRECHT, PAUL G.
Bethesda Hospital
St. Paul, Minn.

ALBRIGHT, JOHN G.
Bethesda Hospital
St. Paul, Minnesota

ALLEN, JAMES R.
St. Mary's Hospital
Minneapolis, Minnesota

ANDERSON, LOREN A.
University Hospital
Jackson, Mississippi

ANDERSON, RICHARD O.
Strong Memorial Hospital
Rochester, New York

ASSIMACOPOULOS, COSTAS
Univ. of Minnesota Hospitals
Minneapolis, Minnesota

BAAB, GARY H.
San Joaquin General Hospital
Stockton, California

BAKER, C. ROBERT
San Diego County General Hosp.
San Diego, California

BEAN, DAVID W.
St. Luke's Hospital
Duluth, Minnesota

BECKMAN, DONOVAN L.
San Joaquin General Hospital
Stockton, California

BENJAMIN, ROGER J.
Ancker Hospital
St. Paul, Minnesota

BERMAN, JULIAN L.
Mount Zion Hospital
San Francisco, California

BOHLAND, ERNEST T.
St. Mary's Hospital
Minneapolis, Minnesota

BOLINE JON E.
Tripler Army Hospital
Honolulu, Hawaii

BRACE, RAY I.
U. S. Public Health Serv. Hosp.
Staten Island 4, New York

BROMAN, HAROLD R., JR.
Bethesda Hospital
St. Paul, Minnesota

BUGBY, ROBERT D.
Minneapolis General Hospital
Minneapolis, Minnesota

CASEY, THOMAS G.
Southern Pacific General Hosp.
San Francisco, California

CHRISTIAN, WILLIAM L.
St. Mary's Hospital
Minneapolis, Minnesota

CLAWSON, C. CARLYLE
Broadlawns County Hospital
Des Moines, Iowa

DAMBERG, SHELDON W.
St. Luke's Hospital
Duluth, Minnesota

DAVIDSON, JOSEPH
U. S. Public Health Serv. Hosp.
San Francisco, California

DESANTO. LAWRENCE W.
St. Mary's Hospital
Duluth, Minnesota

DEWERD, DANIEL L.
Southern Pacific General Hosp.
San Francisco, California

DIAMOND, ROBERT A.
Harbor General Hospital
Torrance, California

DOYLE, OWEN P.
Southern Pacific General Hosp.
San Francisco, California

ECKERT, JOSEPH F.
Brooke General Hospital
San Antonio, Texas

EICHELBERGER, DALE L.
Harbor General Hospital
Torrance, California

EILERS, WILLIAM B.
Ancker Hospital
St. Paul, Minnesota

ELIASON, ORLAND D.
Brackenridge Hospital
Austin, Texas

ERICKSON, JAMES L.
Queen's Hospital
Honolulu, Hawaii

FETT, JAMES D.
Kansas City General Hosp.
Kansas City, Missouri

GAMBILL, HAROLD D.
Highland Alameda County Hosp.
Oakland, California

GARBER, JAMES J.
Harbor General Hospital
Torrance, California

GEARY, WENDELL G.
U. S. Public Health Serv. Hosp.
Seattle, Washington

GENDEIN, ALVAN R.
Mount Sinai Hospital
Minneapolis, Minnesota

GOLDFARB, MACE G.
Minneapolis General Hospital
Minneapolis, Minnesota

GREENE, GORDON O.
Springfield City Hospital
Springfield, Ohio

GROVER, JOHN A.
Orange County General Hospital
Orange, California

HAALAND, ELIZABETH M.
Denver General Hospital
Denver, Colorado

HELSETH, HOVALD K., JR.
University Hospital
Jackson, Mississippi

HENRY, JOHN C.
Charles T. Miller Hospital
St. Paul, Minnesota

HENRY, RICHARD T.
Springfield City Hospital
Springfield, Ohio

HERMAN, NORMAN P.
Mount Sinai Hospital
New York, New York

HERRED, CLEMENT N.
Charity Hospital
New Orleans, Louisiana

HILLER, BRUCE H.
St. Mary's Hospital
Minneapolis, Minnesota

HODGSON, CORBIN J.
St. Mary's Hospital
Duluth, Minnesota

HOPKINS, DAVID S.
Pierce County Hospital
Tacoma, Washington

HUNT, VINCENT R.
Bethesda Hospital
St. Paul, Minnesota

IVERSLIE, PHILIP C.
Minneapolis General Hospital
Minneapolis, Minnesota

JENIKE, CLARENCE A.
St. Mary's Hospital
Minneapolis, Minnesota

JOHNSON, ALAN R.
Ancker Hospital
St. Paul, Minnesota

JOHNSON, ARTHUR G.
St. Joseph's Hospital
St. Paul, Minnesota

JOHNSON, ROBERT L.
Denver General Hospital
Denver, Colorado

JOHNSON, THOMAS P.
Santa Barbara Cottage Hosp.
Santa Barbara, California

JONAS, EUGENE R.
Minneapolis General Hospital
Minneapolis, Minnesota

KALINA, ROBERT E.
U. of Oregon Medical School
Portland, Oregon

KAPLAN, ARNOLD P.
Mount Sinai Hospital
Minneapolis, Minnesota

KEENAN, CHARLES E., JR.
Queen of Angels Hospital
Los Angeles, California

KELLY, JOHN C.
Ancker Hospital
St. Paul, Minnesota

KENEFICK, THOMAS P.
Philadelphia General Hosp.
Philadelphia, Pennsylvania

KINNEY, WILLIAM N.
Ancker Hospital
St. Paul, Minnesota

KNAPP, JAMES F.
Highland Alameda County Hosp.
Oakland, California

KNIP, ROBERT J.
Minneapolis General Hospital
Minneapolis, Minnesota

KOCH, MICHAEL F.
San Joaquin General Hospital
Stockton, California

LALONDE, JOHN B.
Philadelphia General Hospital
Philadelphia, Pennsylvania

LANSKY, SHIRLEY B.
Univ. of Minnesota Hospitals
Minneapolis, Minnesota

LARKIN, JOHN E.
Detroit Receiving Hospital
Detroit, Michigan

LELWICA, THADDEUS J.
Sacramento County Hospital
Sacramento, California

LEVITT, MICHAEL D.
Univ. of Minnesota Hospitals
Minneapolis, Minnesota

LOMMEN, MORRIS L.
Ancker Hospital
St. Paul, Minnesota

LUFKIN, MURRAY W.
U. S. Naval Hospital
Oakland, California

MACARTHUR, JOHN D.
Peter Bent Brigham Hospital
Boston, Massachusetts

MACGIBBON, JAMES D.
Minneapolis General Hospital
Minneapolis, Minnesota

MANICK, KENNETH P.
Minneapolis General Hospital
Minneapolis, Minnesota

MARQUARDT, JAMES E.
Los Angeles County Hospital
Los Angeles, California

MCKELVEY, JOHN M.
Strong Memorial Hospital
Rochester, New York

MCLEOD, JOHN A.
Ancker Hospital
St. Paul, Minnesota

MECKLENBURG, FRED E.
U. S. Public Health Serv. Hosp.
Seattle, Washington

MERCIL, CHARLES B.
St. Luke's Hospital
Duluth, Minnesota

MORSE, ROBERT M.
San Joaquin General Hospital
Stockton, California

MORSE, ROGER D.
Pierce County Hospital
Tacoma, Washington

MOSS, RONALD A.
Minneapolis General Hospital
Minneapolis, Minn.

NELSON, RONALD J.
Ancker Hospital
St. Paul, Minnesota

NYDAHL, BRUCE C.
Highland Alameda County Hosp.
Oakland, California

OCHSNER, JOHN A.
Milwaukee County Hospital
Milwaukee, Wisconsin

OLSON, BARBARA F.
Ancker Hospital
St. Paul, Minnesota

OLSON, RICHARD E.
San Diego County General Hosp.
San Diego, California

OSEID, BETTY J.
Univ. of Minnesota Hospitals
Minneapolis, Minnesota

OSEKOWSKY, HENRY J.
Minneapolis General Hospital
Minneapolis, Minnesota

PETERSON, CHARLES R.
U. of Oregon Medical School
Portland, Oregon

PETERSON, WILLARD C.
Univ. of Minnesota Hospitals
Minneapolis, Minnesota

POLLARA, BERNARD
U. S. Public Health Serv. Hosp.
Seattle, Washington

PRINCELL, ROGER H.
Springfield City Hospital
Springfield, Ohio

REISDORF, GEORGE E.
Minneapolis General Hospital
Minneapolis, Minnesota

ROBERTSON, DENNIS M.
San Bernardino County Hospital
San Bernardino, California

ROWE, RICHARD G.
St. Luke's Hospital
Duluth, Minnesota

RUSTAD, ELLIOTT L.
Santa Clara County Hospital
San Jose, California

SCHOTTLER, JERRY L.
San Bernardino County Hospital
San Bernardino, California

SCHUNK, PETER A.
San Bernardino County Hospital
San Bernardino, California

SEGAL, SHELDON J.
Ancker Hospital
St. Paul, Minnesota

SEVERSEIKE, ODEAN M.
Minneapolis General Hospital
Minneapolis, Minnesota

SIEWERT, DAVID E.
St. Luke's Hospital
Duluth, Minnesota

SILVERSTEIN, PAUL M.
Minneapolis General Hospital
Minneapolis, Minnesota

SIMS, WARREN F., JR.
Mary Fletcher Hospital
Burlington, Vermont

SOHLBERG, OLOF S.
San Bernardino County Hosp.
San Bernardino, California

STREU, RICHARD E.
Pierce County Hospital
Tacoma, Washington

SWENSON, JAMES D.
St. Luke's Hospital
Duluth, Minnesota

TELANDER, GERALD T.
San Joaquin General Hosp.
Stockton, California

THOMPSON, GERSHOM J.
Highland Alameda County Hosp.
Oakland, California

VILLELLA, RONALD L.
Ronald L. Madigan Army Hosp.
Tacoma, Washington

VONTVER, LOUIS A.
Harbor General Hospital
Torrance, California

WEINMAN, DARRELL T.
Santa Clara County Hospital
San Jose, California

WEISBERG, MARTIN G.
Minneapolis General Hospital
Minneapolis, Minnesota

WEMPNER, JON D.
Pierce County Hospital
Tacoma, Washington

WHITESELL, LLOYD A., JR.
Charity Hospital
New Orleans, Louisiana

WIGG, NORMAN P.
Methodist Hospital
Minneapolis, Minnesota

WILKOWSKE, CONRAD J.
Minneapolis General Hospital
Minneapolis, Minnesota

WONG, EDWARD T.
U. of California Hospitals
San Francisco, California

WORRELL, PHILIP J.
Minneapolis General Hospital
Minneapolis, Minnesota

WRIGHT, THOMAS J.
Santa Clara County Hospital
San Jose, California

COMPREHENSIVE CLINIC PROGRAM BEGINS

Junior-Senior medical students were given greater responsibility for the care of outpatients at University Hospitals under a new Comprehensive Clinic program inaugurated June 20, 1960 at the University of Minnesota Medical School. Dr. Richard M. Magraw, Assistant Dean and Associate Professor of Psychiatry and Internal Medicine, is Director of the program, which is the heart of a major curriculum change.

Fifty four students just entering the Junior-Senior biennium are now planning and integrating the total care of outpatients who are assigned to them from the Adult Medical Admission Clinic, Pediatrics Admissions Clinic, and North Clinic. To fit the program into the student's busy schedule, the Outpatient Department at the University Hospitals has been placed on an appointment schedule.

Under careful supervision, the medical student is now responsible for integrating all aspects of the outpatient's care, and for the care provided directly by himself. Each student will be assigned to the Comprehensive Clinic program for a six months period, and will care for from 40 to 60 patients during that period. He will retain responsibility for the patient until discharge or hospitalization. The student will present the patient at other specialty clinics when other consultation seems necessary.

The objectives of the new Comprehensive Clinic program, according to Dr. Magraw, are:

1. To make Medical School teaching and experience more realistic and scientific by re-emphasizing the patient as the unit of practice and teaching

2. To permit continuity in the student's work with patients

3. To increase the student's motivation for learning by making him a responsible participant in the patient's care rather than a participant in a teaching exercise

4. To provide an opportunity to observe and evaluate the student's performance in the role of a doctor

Dr. James B. Carey, Jr., former Curriculum Coordinator, helped draw up the new program. Dr. Warren Warwick is Assistant Director of the new program and Director of the Pediatric Clinic.

Departmental News

ANATOMY

Dr. R. Dorothy Sundberg, Associate Professor, was elected an advisory editor of the journal BLOOD. Her five-year appointment began Jan. 1, 1960.

CLINICAL PSYCHOLOGY

Dr. William Schofield, Professor, attended a meeting of American Association of Medical Colleges May 4-5 in New York City. He reports the Psychological Corporation of New York has been appointed by AAMC to administer and develop its 1960 Medical College Aptitude Test program. Dr. Schofield has been a member of AAMC's Committee on Research and Education since 1956.

PSYCHIATRY & NEUROLOGY

Dr. Donald W. Hastings, Professor and Head of the Department, and Dr. Burtrum C. Schiele, Professor of Psychiatry, were co-directors of the continuation course for General Physicians on the use of new psychotherapeutic drugs held May 16-18 at the University of Minnesota.

UNIVERSITY HOSPITALS

A $6,000 contribution to provide heart surgery equipment was presented to the University of Minnesota Hospitals May 18, 1960 by the Auxiliary of the Variety Club of the Northwest. The funds were raised during the 1959-60 presidency of the late Mrs. Morris Chalfen. Gerard Frawley, Administrator of the Variety Club Heart Hospital, accepted the check for the University at a special luncheon, and Dr. Robert A. Good, American Legion Memorial Heart Research Professor of Pediatrics, spoke on research at the Heart Hospital.

PHYSIOLOGICAL CHEMISTRY

Dr. David Glick Professor, was Chairman of a section of the National Academy of Science symposium on experimentation below the microgram range held May 15-18 at Washington, D.C. He also presented a paper on "X-Ray Absorption of Biological Material for Submicrogram Analysis."

MEDICINE

Dr. Cecil J. Watson, Professor and Head, was elected President of the Association of American Physicians on May 4, 1960. During the period March 6-11 he served as Visiting Physician at Massachusetts General Hospital, Boston, delivering the annual Chester Jones Lecture at that institution. He lectured at the St. Louis University Medical School April 1, and presented a paper on porphyrins before the National Academy of Science on April 25, 1960 in Washington, D. C.

Dr. Dennis J. Kane, Instructor, spoke on "Diet in Cardiac Management" at the Upper Midwest Hospital Conference in Minneapolis May 11-13 in Minneapolis. James A. Hamilton, Director of the Course in Hospital Administration; Dr. Robert L. Vernier, Assistant Professor of Pediatrics; and Dr. Robert K. Ausman, Medical Fellow in Surgery, also spoke.

MINNESOTAN TO HEAD A.M.A.

A 1921 graduate of the University of Minnesota Medical School, Dr. Leonard W. Larson of Bismarck, N.D., has been named President-elect of the American Medical Association.

Dr. Larson was given organized medicine's highest honor at the A.M.A.'s 109th annual meeting June 16, 1960 in Miami Beach, Fla. He will succeed to the presidency in June, 1961, and has served as Chairman of the A.M.A.'s Board of Trustees.

A 62-year-old native of Clarkfield, Minn., Dr. Larson a partner in the Quain and Ramstad clinic in Bismarck, which has a 35-man medical staff. His own specialty practice is pathology.

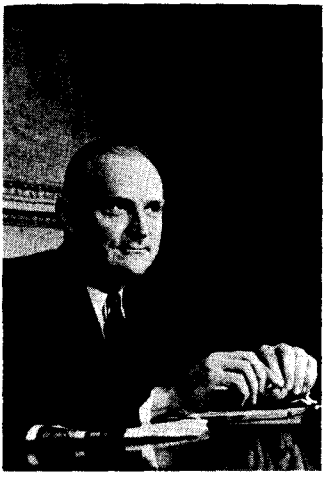
The last Minnesotan to head the A.M.A. was Dr. E. Starr Judd, Mayo Clinic, in 1931.

Medical Foundation News

PRESIDENT WILSON TO GIVE FOUNDATION DAY ADDRESS

President-elect O. Meredith Wilson of the University of Minnesota has accepted an invitation to speak at Minnesota Medical Foundation Day ceremonies on September 26, 1960 at the Medical School.

The title of his address has not been announced, but his remarks will constitute one of his first introductory statements to the University of Minnesota family. Now Chancellor of the University of Oregon, Dr. Wilson becomes President of the University of Minnesota on July 1, 1960, succeeding Dr. James L. Morrill, who has retired.



O. Meredith Wilson

The Minnesota Medical Foundation traditionally distributes its scholarship awards to medical students on Foundation Day, which also serves as an orientation session for the students and marks the opening day of the Fall school term.

The awards ceremony will be held at 11:15 a.m. in Mayo Auditorium, with President Wilson's address to follow. Preparations are being made to accommodate a capacity audience of medical school faculty, staff, and students, as well as members of the Minnesota Medical Foundation, who will receive special invitations.

The Foundation's annual business meeting and luncheon will follow at 12:15 p.m. in the Junior Ballroom, Coffman Memorial Union. Parents of scholarship recipients will be special guests.

Student News

MEDICAL SCHOOL GOLF TOURNAMENT

Duncan MacGibbon, Senior medical student, and Dr. A. A. Urrutia, Surgery Intern, were winners of the Medical School Golf Tournament played May 7 at the University of Minnesota Golf Course. MacGibbon's 82, and Dr. Urrutia's 86 were low gross scores achieved among the 55 student and faculty golfers who participated.

The tournament was sponsored by the Minnesota Chapter, Student American Medical Association, with awards provided by the Minnesota Mutual Life Insurance company.

MEDICAL SCHOOL ENROLLMENT

Spring quarter enrollment in the Medical School was 473, six more students than were enrolled in the comparable period one year ago.

JUNIOR CLASS OFFICERS

Members of the junior class, University of Minnesota Medical School, have chosen Keith Burnes, St. Paul, Minn., as President. Class Representatives during the group's junior year (1960-61) will be David Culligan, St. Paul, and John Sutherland, North St. Paul.

All will be delegates to the Medical Student council.

ALUMNI DEATHS

Dr. Elmer J. Lillehei (Med. '24) died May 14, 1960. He was 65 years old and had practiced in Robbinsdale, Minnesota since 1926. He was a member of the Masonic Lodge, the American Medical Association, and the Minnesota State Medical Association, Dr. Lillehei was an uncle of Dr. C. Walton Lillehei (Med. '41), Dr. James Lillehei (Med. '47), and Dr. Richard Lillehei (Med. '51).

Dr. Jacob Biedermann (Med. '04) died March 1, 1960. He had retired from practice in 1958 following 43 years as a physician in Thief River Falls, Minn. He was 84 years old, and spent his early years of medical practice at Argyle, Minn.

Dr. Maurice D. Cooper (Med. '07) died March 25, 1960 at the age of 78 years. He had practiced in Winnebago, Minn. since 1908. In recent years he was associated in the practice of medicine and surgery with Dr. John L. Mills (Med. '20). Dr. Cooper was a lifetime civic leader in his community, and had been tendered a testimonial dinner in his honor in 1946.

Dr. Claude L. Haney (Med. '06) died June 15, 1960 in Duluth, where he had lived and practiced medicine for 55 years. He was on the staffs of St. Luke's, St. Mary's, and Miller hospitals, all in Duluth. He was active in state and local medical societies, and served as a captain in the Army Medical Corps in World War I. Among survivors is his daughter, Mrs. Ancel Keys (Margaret), of St. Paul.

Memorial Gifts

Recent contributions to the Minnesota Medical Foundation have been received in memory of:

Mrs. Harriett Atkinson
St. Paul, Minn.

Memorial gifts are a practical means of honoring the memory of a friend or loved one while providing needed assistance for the University of Minnesota Medical School. Dignified acknowledgments are made by the Foundation to both the donor and to the family of the deceased.

Alumni Notes

◆ 1909

Henry W. Meyerding, President of the International College of Surgeons since 1958, opened the College's 12th biennial international congress in Rome, Italy, May 15-18, 1960. He is a native of St. Paul and Emeritus Professor of Orthopedic Surgery in the Mayo Foundation, Graduate School, University of Minnesota, at Rochester, Minn. He retired in 1949 after 39 years on the Mayo Clinic staff.

◆ 1921

Chester L. Oppegaard, Crookston, Minn., was elected President of the Minnesota State Medical Association at its 107th annual meeting May 23-25 in Rochester, Minn. Dr. Oppegaard will take office Jan. 1, 1961, succeeding **Dr. Clarence Jacobson (Med. '25),** of Chisholm, Minn.

◆ 1922

Gordon R. Kamman, St. Paul, has been appointed head of the Health Council of the Greater St. Paul Community Chest and Council. He is a Clinical Associate Professor of Psychiatry at the University of Minnesota Medical School, and chairman of the neuro-psychiatric department at Miller Hospital, St. Paul.

Malcolm G. Gillespie of Duluth, and **Dr. Louis Lick (Med. '47)** of St. Paul were recently elected to the Board of Directors of the Minnesota Surgical Society. **Dr. Lyle J. Hay (Med. '37)** of Minneapolis is also on the Board.

◆ 1928

Kenneth R. Nelson has been appointed Chief Medical Officer of the United States Coast Guard. He assumed the post July 1, 1960, and has been commissioned in the U. S. Public Health Service for the past 31 years. Dr. Nelson is a native of Minneapolis, and has spent about one-half of his professional career in hospital administration. He has recently served as Chief of the USPHS Division of Hospitals.

◆ 1929

Robert N. Barr, executive officer of the Minnesota Department of Health, is serving as Secretary of the Minnesota Water Pollution Control Commission.

◆ **1935**

Ralph V. Platou, Professor and Head of the Department of Pediatrics at Tulane University Medical School, New Orleans, La., visited the Duluth Clinic and addressed the St. Louis County Medical Society during May. His topic was "From the Mouth of Babes," a discussion of stigmata and diagnostic aids from oral examination of infants. He is the brother of the late Dr. Erling S. Platou, (Med. '20) a founder of the Minnesota Medical Foundation.

◆ **1936**

Dean S. Fleming of the Minnesota Department of Health, **Dr. John G. Mayne (Med. '46)** of Rochester, and **Dr. J. Arthur Myers (Med. '20)** of Minneapolis represented the Minnesota Tuberculosis and Health Association at the 65th annual meeting of the National Tuberculosis Association May 15-19 in Los Angeles, Calif.

◆ **1937**

Raymond L. Eck, practicing in Lewistown, Montana, was elected president of the Montana State Board of Medical Examiners for 1960.

Hendrik J. Svien, neurosurgeon at the Mayo Clinic, Rochester, was among guest speakers at the Tenth Annual Midwest Podiatry-Chiropody Conference last Spring in Chicago, Ill. He spoke on neurosurgical conditions which manifest themselves in the feet.

◆ **1943**

Lester W. Carlander, Jr., is serving as Chief of Staff at Fairview Hospital, Minneapolis. Chief-elect is **Dr. Julian V. Petit (Med. '40);** Secretary is **Kenneth B. Romness (Med. '50);** and Treasurer is **George W. Haugen (Med. '44).**

◆ **1944**

John G. Rukavina, St. Paul, Assistant Clinical Professor in the Division of Dermatology, University of Minnesota Medical School, was a member of the guest faculty at a postgraduate course for internists at the University of Michigan Medical School April 25-29, 1960. He lectured on "Chelating Agents in Scleroderma," "The Amyloidoses," and "The Use of Unsaturated Fats in the Treatment of Essential Zanthomas."

Edgar C. Burseth of Mora, Minn. was elected President of the Kanabec County Association for Mental Health.

Clark W. Truesdale, Glencoe, Minn. physician, has been appointed to the Governor's Citizens Committee on Aging, and will serve with a subcommittee dealing with nursing home classification.

Robert Goltz, Minneapolis physician, was elected new President of the Minnesota Academy of Occupational Medicine. **Dr. Earl Opstad (Med. '46)** was named vice president, and **Dr. Lyman B. Clay (Med. '36)** was elected secretary. Both are from Minneapolis.

◆ **1950**

Arthur B. Quiggle was a faculty member May 23-25 at the Institute of Care of the Aged in Geriatric Homes conducted at the University of Minnesota Center for Continuation Study. Dr. Quiggle spoke on "Activities of Daily Living for Residents of Geriatric Homes." He is a Clinical Instructor in Physical Medicine at the University of Minnesota Medical School, and on the staff at St. Barnabas Hospital, Minneapolis.

◆ **1954**

Isaac M. Prlina, staff physician at the East Range Clinic in Virginia, Minn., was recently certified by the American Board of Urology.

Dr. Arthur N. Larson has joined the staff of the Mesaba Clinic, Hibbing, Minn. He interned at the University of Minnesota Hospitals.

◆ **1957**

Martin W. Orbuch has been discharged from the U. S. Navy after completing his military service at the Naval Hospital, Portsmouth, Va. He was a Lieutenant in the Medical Corps.

◆ **1958**

Lt. Ralph J. Langsjoen, U.S. Navy Medical Corps, has been transferred to duty at the Naval Air Station, Miramar, Calif. He was formerly aboard the USS Renville.

◆ **1959**

Lt. Lawrence R. Ringhofer, Jr., U.S. Navy Medical Corps, has been transferred to duty at the Navy Recruiting Station, Indianapolis, Ind. He was formerly a physician at the Naval Hospital, Portsmouth, Va.

Coming Events

University of Minnesota Medical School

(Tentative Schedule)

COURSES IN CONTINUATION MEDICAL EDUCATION
DURING 1960-61

Oct. 3-5, 1960	Obstetrics for General Physicians
Oct. 20-22, 1960	Dermatology for General Physicians
Oct. 31-Nov. 4, 1960	Radiology for Specialists
Nov. 7-9, 1960	Physical Medicine for Specialists
Nov. 16-18, 1960	Ophthalmology for General Physicians (Refraction)
Dec. 1-3, 1960	Orthopedics for Specialists and General Physicians
Jan. 3-7, 1961	Introduction to Electrocardiography for General Physicians
Jan. 26-28, 1961	Otolaryngology for Specialists
Feb. 6-8, 1961	Anesthesiology for Specialists
Feb. 9-11, 1961	Surgery for Surgeons
Feb. 13-17, 1961	Neurology for General Physicians
Feb. 27-Mar. 1, 1961	Pediatrics for General Physicians
Mar. 13-15, 1961	Allergy for General Physicians
Mar. 17-18, 1961	Trauma for General Physicians
Mar. 27-29, 1961	Urology for Specialists
April 20-22, 1961	Otolaryngology for General Physicians
May 1-3, 1961	Ophthalmology for Specialists
May 8-10, 1961	Gynecology for General Physicians
May 15-19, 1961	Proctology for General Physicians
May 25-27, 1961	Internal Medicine for Internists
June 1-3, 1961	Office Psychotherapy for General Physicians
1960-61—All year	Cancer Detection for General Physicians

Courses are held at the Center for Continuation Study or at the Mayo Memorial Auditorium on the campus of the University of Minnesota. Usual tuition fees are $10 for a one-day course, $40 for a three-day course, and $65 for a one-week course. These are subject to change under certain circumstances.

Register early. For further information write to:

DIRECTOR
DEPT. OF CONTINUATION MEDICAL EDUCATION
1342 Mayo Memorial — University of Minnesota
Minneapolis 14, Minnesota

A Word About Memorial Gifts

The Minnesota Medical Foundation welcomes your memorial gifts when an appropriate occasion arises. Memorial gifts serve the living and pay thoughtful tribute to the memory of a friend, associate, or relative. The Foundation will acknowledge gifts with suitable cards mailed promptly to both the donor and the family of the deceased. The gift will help finance the Foundation's program of support for the Medical School of the University of Minnesota.

Many people have adopted the appropriate custom of sending memorial gifts to worthy organizations in time of bereavement or other occasion. Such funds have lent significant strength to the fight against the major diseases known to Americans.

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