VOLUME XXXI, NUMBER 2 : NOVEMBER 1, 1959

MEDICAL BULLETIN

IN THIS ISSUE

Aminopeptidase Studies

Insulin Assay

University of Minnesota Medical Bulletin

UNIVERSITY OF MINNESOTA

Medical Bulletin

Official Publication of
UNIVERSITY OF MINNESOTA HOSPITALS
MINNESOTA MEDICAL FOUNDATION
MINNESOTA MEDICAL ALUMNI ASSOCIATION

VOLUME XXXI *November 1, 1959* NUMBER 2

CONTENTS

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

Staff Meeting Report

Morphologic Histochemical Studies of Aminopeptidase in Surgical and Autopsy Specimens*

Mitchell Rosenholtz, M.D.† and Lee W. Wattenberg, M.D.‡

The last two decades have seen a remarkable increase in the number of techniques for the histochemical demonstration of enzymes in tissue sections. Thus, Pearse[1] observed that whereas in 1939 less than five specific enzymes could be demonstrated in tissue sections by histochemical techniques, 45 could be so studied in 1958. He speculated that use of these techniques in tissues whose morphologic integrity remained intact could greatly extend the limits of cellular pathology of a more classical sort. He quoted Young, who expressed the belief that such specialized methods would allow investigators to "describe the organization of cellular events," and Cameron and Muzaffar, who stressed detection of "early functional changes." Gössner[2] too discussed the value of enzyme histochemistry for relating structure and function.

The application of the techniques of enzyme histochemistry to autopsy specimens might be expected to broaden greatly the value of the postmortem examination. Thus the pathologist might clarify not only the morphologic aspects but also some of the physiologic aspects of the patient's disease. The interest in this laboratory in such application led to review of the literature on the effects of certain forms of tissue injury and tissue death on enzyme activity. Bayerle *et al.*,[3] in 1936, carried out a study of arginase activity in necrotic parts of certain animal tumors, in experimentally produced renal infarcts, and even in some human infarcts found at autopsy. Most of the studies of the enzymic aspects of autolysis, however, as seen in autopsy specimens and in tissues subjected to controlled periods of autolysis, have been carried out within the last five years.[4–11] Most of the observations of enzyme activity alterations as a function of

*The report was given at the Staff Meeting of the University of Minnesota Hospitals on October 9, 1959.

†This study was carried out during Dr. Rosenholtz's tenure as a U. S. Public Health Service Trainee in Cancer Research.

‡Associate Professor, Department of Pathology

anoxia, tissue necrosis, or vascular occlusion in a given tissue have likewise been recent.[3, 11–19]

A technique commonly employed to produce tissue death has been described by Jennings and co-workers.[13] They ligated a coronary artery branch in dogs and studied by quantitative techniques the enzyme activity of myocardial homogenates as a function of time after ligation. They found that the activity of transaminase, lactic dehydrogenase, and succinic dehydrogenase fell slowly in the first to the fourth hours, then fell more quickly, finally leveling off at 30–50 per cent of normal at about 15 hours after ligation. Kent and Diseker[14] used morphologic enzyme histochemistry to study infarcted dog myocardium; they observed that succinic dehydrogenase definitely decreased at 15 hours and was totally absent at 24, whereas alkaline phosphatase showed no change in 48 hours. Gavan and Kaufman,[7] using morphologic histochemical techniques, found in studies of vascular occlusion of one kidney in the rat that the activity of succinic dehydrogenase at 0 hours was 4+; at 12 hours, 2+; and at 36 hours, 0. Cytochrome oxidase activity fell more slowly, being 4+ at 0 hours, 3+ at 18 hours, 1+ at 36 hours, and absent at 48 hours. Rudolph and Scholl[17] described similar studies of succinic dehydrogenase, alkaline phosphatase, and adenosine triphosphatase.

A second type of study has used autolyzed tissue as the experimental object. The studies to be reported employed morphologic histochemical techniques to demonstrate a specific enzyme; but previous studies of autolyzing tissue homogenates have also been of interest. For example, Bayerle and associates[3] found an increase in arginase activity during early autolysis which they correlated with histological dissolution of cell nuclei; they concluded that enzyme liberation occurred during autolysis. Pieces of liver, devascularized but allowed to remain in the peritoneal cavity, were studied by Berenbom *et al.*[4] Six hours after liver devascularization, alkaline and acid phosphatase, esterase, and L-leucyl-glycine peptidase showed at least 68 per cent of the activity they had had at zero time. Furthermore, at 48 hours significant activity could still be shown. Succinic dehydrogenase and cytochrome oxidase activity, however, fell off rapidly; they were 36 per cent and 61 per cent of their control values respectively at six hours and were completely absent at 24 hours. Berenbom and associates[5] obtained similar results employing mouse livers incubated at 37° C. outside the host.

Smith and co-workers[10] have shown that aqueous homogenates of rabbit cerebellum which were obtained at autopsy and kept at 24° C. for specific periods thereafter, then frozen and

later analyzed demonstrated no more than ± 14 per cent change during 24 hours in activity of the following enzymes: acid and alkaline phosphatase, lactic dehydrogenase, malic dehydrogenase, glutamic dehydrogenase, and hexokinase. Only phosphofructokinase showed a pronounced fall, and that occurred within two hours. In the same paper,[10] the authors described quantitative histochemical studies of the three layers of the rabbit cerebellum in animals kept at room temperature for as long as six hours after death. Again, except for phosphofructokinase, all enzymes showed a change in six hours of only ± 7.6 per cent from values immediately after death. Smith *et al.* quoted others who suggest that a period not exceeding 24 hours after death would be satisfactory for quantitative studies under certain circumstances. In a more recent investigation, this group found that after six hours of autolysis at room temperature, 14 of 15 enzymes were stable as compared to those described above.[9] Neither high fever of two hours' duration prior to death nor uremia of two days' duration significantly altered their results.

In addition to quantitative studies of enzyme activity employing tissue homogenates, morphological histochemical techniques have also been used in the study of autolysis. Gössner,[8] for example, kept a kidney removed from a mouse at 37° C. and studied sections histochemically and histologically at different times. He found at 12 hours, when karyorrhexis was maximal and karyolysis was beginning, that esterase, acid phosphatase, and alkaline phosphatase were beginning to show decreased activity. On the other hand, the rate of enzyme disappearance was slowed when autolysis occurred at lower temperatures. Persistence of enzyme activity (for as long as 48 hours after death) was demonstrated in mouse tissues removed from animals kept at 15° C. after death. With these extended periods of autolysis, however, some diffusion of enzyme activity could be demonstrated by morphologic histochemical techniques.

Gössner expressed the belief that these hydrolytic enzymes depended for their activity on connection within the cell to some carrier structure *(Trägerstruckture)* ultimately broken up by the autolytic process itself. A similar relation between cellular integrity and enzyme activity has been noted by others.[3,4,10] Kent,[20] in a histochemical study of the autolysis of dog myocardium, liver, and kidney at 37° C., noted that succinic dehydrogenase fell sharply in four to 16 hours; alkaline phosphatase fell only slightly beginning at 15 to 48 hours, but it gave a less sharp localization after several days. Kent also found that low temperatures slowed the disappearance of enzyme activity; for example, he observed that succinic dehydrogenase in tissues kept

at 4° C. showed little loss of activity for as long as 144 hours.

Only in the last decade have techniques been perfected to study the early changes of autolysis. Two groups of investigators[4,5,7] have independently reported relatively rapid falls in succinic deyhdrogenase and cytochrome oxidase activity. A group at University College in London, using the techniques of *in vitro* autolysis and isolated perfusion of the rat liver, has concluded that the basic defect in autolysis is deterioration of the system that carries out oxidative phosphorylation with resulting injury to the mitochondria.[6,21] A similar suggestion has been made by Berenbom *et al.*[4] Other postulated defects include: a loss of cofactors,[21] an increase in inhibitor concentrations,[12] and a decline in concentrations of those enzymes which are energy requiring rather than energy producing.[21]

The results of experiments described above have suggested to several groups of investigators that both orthodox biochemical and histochemical enzyme techniques are applicable to the study of postmortem specimens in the human.[8,10,11,14] The data of Smith *et al.*[10] showed the possible value of using autopsy material for enzyme studies with proper control studies of enzyme activity as a function of the duration of autolysis. The obvious importance of temperature both of the cadaver and of the tissue prior to fixation has been suggested by others.[8,20] Other factors shown to affect enzyme activity in autopsy specimens include: organs studied,[8] species and individual differences,[22,23] and bacterial contamination.[20]

Wachstein and Meisel[11] have reported one of the few published studies using autopsy material. In investigating myocardial sections from cases thought clinically to be acute myocardial infarction, these authors stated that the enzyme studies might be more effective than routine histological techniques in characterizing the morphologic changes. Others have also made such suggestions.[14] Wachstein and Meisel pointed up, however, the obvious difficulty in dealing with clinical material of establishing the time of onset of a given clinical situation—in their case, coronary occlusion.

Materials and Method

The morphologic histochemical studies to be described here have employed a technique for demonstrating aminopeptidase in autopsy specimens. These studies were initiated in this laboratory more than two years ago—shortly after two different techniques had been described for localizing this enzyme by morphologic histochemical means.[22,23] Both of these methods depended on the use of an artificial substrate which, when hy-

drolyzed at the sites of enzyme activity, produced β-naphthylamide. The latter could be demonstrated visually by diazotization, which produced a bright red azo dye.

The potential usefulness of this procedure as an investigative tool for the pathologist was suggested by two studies: The first was a paper in 1956 in which Burstone[24] observed an abnormally high activity of the enzyme in the stroma around certain neoplasms. Burstone, as well as Braun-Falco,[25] suggested a possible relationship between the stromal activity and the invasiveness of the tumor. The second was a report by Wattenberg[26] of a demonstrable enzymatic interrelationship between the small bowel mucosa, intestinal metaplasia of the gastric mucosa, and gastric carcinoma—an interrelationship of interest in light of the suggestion of others[27–31] that intestinal metaplasia might be a precursor of gastric cancer.

The work of various investigators had, therefore, suggested the applicability of morphologic enzyme histochemical studies to autopsy material. The investigations to be reported have been concerned primarily with the morphologic histochemical demonstration of aminopeptidase activity in a number of malignant neoplasms, in sections of normal liver, and in certain hepatic lesions. They have been concerned secondarily with the effects of autolysis on aminopeptidase activity.

Tissues Employed

The studies described in this paper were performed on specimens obtained after death. Tissues were removed from laboratory animals immediately after they were killed. Rabbits and guinea pigs were killed by an overdose of ether; all other animals were killed by sudden transection of the spinal cord. Most of the human specimens were obtained at autopsy, usually within

TABLE 1

ORGANS STUDIED

Human:	Liver	Kidney	Duodenum	Ovary
	Spleen	Gallbladder	Pancreas	Adrenal
	Lung	Breast	Prostate	Lymph Node
	Colon			
Monkey:	Liver	Gallbladder	Pancreas	Common Bile Duct
Mouse:	Liver	Kidney	Duodenum	
Guinea Pig:	Liver	Gallbladder		
Rat:	Liver	Kidney		
Rabbit:	Liver	Kidney		
Dog:	Liver			
Hamster:	Liver			

10 hours of death. Some, however, were obtained later, having been removed from the body and kept after autopsy at 4° C. in a moist environment. Tables 1, 2, and 3 outline the organs and the pathological entities studied:

TABLE 2

MALIGNANT SPECIMENS STUDIED

Organ	Primary Lesions	Metastatic Lesions In Liver	Metastatic Lesions In Other Organs
Stomach	6	2	2
Gallbaldder	5	4	2
Liver and Bile Ducts	4		
Breast	1	5	
Large Intestine	2	3	
Pancreas	5	4	1
Kidney	1	1	
Lymphoma	2 (spleen)	2	
Appendix			1
Oral Cavity		1	
Cervix		1	
Ovary	1		
Thyroid	1		
Prostate	2		
Small Bowel (carcinoid)		1	
Melanoma		1	1

TABLE 3

NONMALIGNANT SPECIMENS STUDIED

Hepatic cirrhosis	3
Liver with biliary obstruction	4
Liver with shock	4
Chronic passive congestion of the liver	3
Hepatic granuloma	1
Arterionephrosclerosis	1
Shock kidney	3
Colonic polyp	1

Preparation of Sections and Histochemical Procedures

Blocks of tissue to be studied were frozen and stored at −76° C. until used. The detailed method of handling tissues in this laboratory—including freezing, sectioning, washing, staining for aminopeptidase with the method of Burstone and Folk,[22] and mounting—has previously been described.[26,32] Following the staining, some of the slides processed by the Burstone and Folk method were lightly counterstained with hematoxylin. In addition to sections used for the histochemical procedure, other sections were stained with hematoxylin-eosin.

An alternate method, described by Nachlas,[23] was used in this laboratory to make more permanent preparations. With this method some of the slides were counterstained with 0.1 per cent Bismarck Brown Y for two minutes. In this modification, Garnet GBC, which is used in the method of Burstone and Folk, was replaced by Diazo Blue B in an equal weight, and the tris buffer was replaced by 0.1 M acetate buffer at a *p*H of 6.4. The incubation times with the Garnet GBC method were: kidney, 5 minutes; liver, 20 minutes; stomach, 15 to 30 minutes; other organs, 20 to 30 minutes. In general, the times appropriate to the Diazo Blue B method were 50 per cent greater than those for the Garnet GBC studies. In order to decrease possible diffusion artefacts, incubation periods were kept at a minimum.

With the Garnet GBC procedure, the presence of aminopeptidase activity was indicated by the deposition of a red azo dye. The Diazo Blue B technique as employed with a copper chelation step produced a blue-purple color at sites of enzyme activity. Because the GBC method has given more intense reactivity and sharper localization most of the pictures to be shown illustrate the results of that technique. Such preparations were not as satisfactory for long term storage, however, for their sites of activity were transformed from very fine red granules to coarse yellow crystals in a period ranging from one hour to several days, whereas preparations made using Diazo Blue B lost their activity rather slowly over several months.

Results

Effect of Autolysis and Prolonged Storage on Aminopeptidase Activity

A number of tissues, both human and animal, were allowed to autolyze in a moist environment. Representative samples were then taken for morphologic histochemical studies, and the activities of similar areas from the same tissues were compared by visual microscopic examination. Table 4 outlines the results of

a group of such studies. In all the studies, it will be seen that activity persisted for at least nine hours after death; by that time most autopsies could be completed and tissues obtained. In some studies activity decreased little or not at all during the periods of observation. In none of these studies did enzyme activity appear during autolysis at sites originally inactive, nor was any increase in activity observed in structures which had originally shown positive reactions.

TABLE 4

EFFECT OF AUTOLYSIS AT 22° C. ON AMINOPEPTIDASE ACTIVITY*

Organs	Species	Aminopeptidase Activity* Hours of Autolysis 0	3	6	9	12	15	18
Kidney	Human		3–4+	3+	2–3+	3+		3+
Liver	Human		1–2+	1+	1+	(tr. to 1+)		
Pancreas	Human		1–2+	2+	2+	1–2+		
Kidney	Rabbit	3+	3+	3+	3+			
Liver	Monkey		3+	2+	2+			

*Aminopeptidase activity as estimated from visual microscopic examination of tissue sections from trace (tr.), weak to 4+, very intense

A number of human autopsy specimens studied had autolyzed from 20 to 70 hours at 4° C. before being frozen. Nevertheless, some of the most intensely positive tissues have come from this group. The effect of long term storage at −76° C. has also been studied. Very satisfactory enzyme activity has been demonstrated after storage periods as long as 13 months.

Aminopeptidase Activity in Primary and Metastatic Malignant Tumors

Carcinoma of the gallbladder: Five primary and six metastatic lesions from a total of six patients were studied. All but one tumor site showed enzyme activity. The one negative specimen was a liver metastasis, and interestingly, the primary lesion from which this metastasis originated revealed patchy activity, the activity of the positive areas being quite intense. The pancreatic metastasis, unlike the hepatic metastasis, showed the most intense reaction yet seen in our investigations. The intensity of color of several of these preparations has been so great that small clumps of isolated tumor cells which were positive for aminopeptidase could easily be identified.

Liver and bile ducts: Four primary lesions arising in hepatic parenchyma were examined. Histologically each consisted predominantly of a moderately well differentiated adenocarcinoma. Three showed moderate activity, and in one tumor, the very intense activity in the well differentiated areas shaded off into little or no activity in areas which histologically showed less differentiated tumor components. In these studies of malignant tumor generally, no correlation has been found between degree of enzyme activity and degree of differentiation. The finding described in this cholangiocarcinoma was therefore exceptional.

Carcinoma of the stomach: Six primary and four metastatic lesions from six patients were examined. All were positive except for one primary lesion and the liver metastasis arising from it. In primary and metastatic gastric lesions, the variation of staining from one area of a tumor to another was more apparent than in tumors of the gallbladder or bile ducts. In some gastric lesions a large portion of a given tumor showed no activity. In the gastric tumors studied, primary lesions which were positive have not been the origin of metastatic lesions without activity, and no positive metastases have arisen from primary lesions that were free of aminopeptidase activity.

Carcinoma of the breast: Six specimens from six patients were studied—one primary lesion and five metastatic lesions, all of which were metastatic to the liver. The one primary lesion showed areas of moderate activity interspersed with varying amounts of tumor having no activity. Of five liver metastases, only one was positive; this was from the only male patient included in this small series, and the primary lesion was not examined.

Carcinoma of the pancreas: Five primary lesions and five metastatic lesions from a total of five patients were studied. Of these, only one, a liver metastasis, demonstrated aminopeptidase activity, and that was minimal. This aminopeptidase positive specimen is of interest for two reasons: First, a representative sample of the primary lesion was examined and found to be free of enzyme activity. Second, from a technical standpoint this specimen showed the great stability of the enzyme, since activity was demonstrated in the liver nodule after it had been kept at −76° C. for almost 13 months.

Other malignant lesions: The only other positive specimens were a nodule of melanoma in the spleen and an area from an undifferentiated thyroid carcinoma. Neither of these showed an intense reaction. Furthermore, the activity described in the splenic lesion appeared to be in the connective tissue around the nodule rather than in the tumor itself. This is the only ma-

lignant neoplastic specimen described in this study in which the aminopeptidase activity of the stroma near the edge of the tumor has appeared significantly higher than that of the stroma more distant from the edge.

Hepatic and biliary studies: Fifteen specimens of normal human liver have been examined using the aminopeptidase reaction. The findings, similar to those described by others,[22] were: 1) a very intense reaction by the branches of the biliary duct system in the small portal triads; 2) moderate activity at the presumed sites of the small bile canaliculi between the cords of hepatic cells; 3) minimal to moderate activity in the hepatic parenchymal cells. In some sections the outlining of the bile canaliculi has been striking; in most, however, this has not been the case. Rather, there has been only a diffuse cytoplasmic staining of the hepatic cord cells without localization to the area between the cells. The intense reaction of the small biliary ducts in portal triads was in obvious contrast with the minimal reaction of the ducts in the larger portal triads. Unfortunately, human autopsy studies of the common duct and hepatic ducts have been unsuccessful because autolysis has proceeded to the point of dissolution of the mucosal elements. Others have noted that the human common duct was positive.[33] A gallbladder, surgically removed and free of autolysis, showed a moderately positive mucosa.

Animal livers have been studied in an attempt to find a liver with similar activity to that in the human. Of livers from dog, monkey, rat, mouse, guinea pig, hamster, and rabbit, only that of the monkey appeared comparable, but the gallbladder, hepatic duct, and common bile duct of the monkey were observed to be negative. Using the technique described, we found the other animal livers to be negative both in the bile ducts and hepatic parenchyma.

Three types of non-neoplastic hepatic lesions have been studied. In liver sections from three patients with Laennec's cirrhosis, the small bile ducts in the portal scars were strikingly outlined by their intense reactivity. In most of these studies, the small ducts were proliferating in these portal scars. On the other hand, small cords of hepatic parenchymal cells trapped in the areas of scar have shown, as might have been expected, only minimal to moderate activity.

Livers from patients with clinical hypotension presumably associated with decreased perfusion of the hepatic parenchyma have shown a decrease in enzyme activity centrally associated with the atrophy observed histologically. Decrease in enzyme activity independent of atrophy has not been demonstrated.

The third type of hepatic lesion studied has been extrahepatic biliary obstruction of varying duration. In these studies, too, the small bile ducts showed intense activity whether they were proliferating or not. On the other hand, the enzyme activity of hepatic parenchymal cells in the central part of the lobules was reduced compared to that occurring near the portal spaces. Because the excess bile pigment in these sections was also mainly central, studies were performed in which bile was added to the reaction mixtures to establish its influence on the reaction. In concentrations of 1.6 per cent by volume, bile appeared to suppress the reaction somewhat. However, the decrease in activity seen centrally in the livers subjected to extrahepatic biliary obstruction was more definitely related to the central atrophy seen in hematoxylin-eosin preparations than to the amount of excess bile in the area—a finding comparable to that described above in livers from patients with terminal hypotension.

Discussion

Studies of enzyme activities in autopsy specimens, potentially a source of much new and useful information for the pathologist, have only begun to appear in the last five years. Smith and associates[10] have stressed the importance of proper control studies if such techniques are to be used. They emphasized the need to validate the applicability of each enzyme studied in postmortem work with special reference to the fall of activity as a function of time after death. In that respect, the studies of autopsy material described in this paper have been very encouraging. We have observed intense aminopeptidase activity as long as 80 hours after death. On the basis of visual interpretation of color intensity, there appeared to be only a minimal diminution in activity of this enzyme during periods of time that might elapse between death and autopsy. Some of the studies were performed on specimens allowed to autolyze at 4° C. for many hours after the autopsy was completed. The localization of enzyme activity in such tissues was naturally less satisfactory than in tissues which had autolyzed for a shorter period of time, but hematoxylin-eosin preparations of the same tissues showed comparable alterations in cellular detail. Furthermore, none of the animal or human tissues subjected to controlled periods of autolysis have demonstrated activity at sites that were free of activity in the least autolyzed blocks examined.

In discussing studies of aminopeptidase activity in neoplasia, we have chosen to include the following considerations: 1) the presence of enzyme activity in a number of malignant tumors,

both primary and metastatic; 2) the possible use of morphologic enzyme studies of aminopeptidase as a diagnostic tool; 3) inconsistencies in activity between primary and metastatic lesions; 4) the use of the aminopeptidase technique in studies of the basic character of a specific malignant tumor, as described in a previous report from this laboratory;[32] and 5) enzyme activity in the stroma around the malignant tumors studied.

1) Aminopeptidase activity has been demonstrated in a variety of malignant tumors, both primary and metastatic. All but two specimens from the group of gallbladder and biliary duct carcinomas have had some activity. This activity has frequently been very intense, but most specimens had some areas that were free of activity. Gastric and breast carcinomas have been less consistently positive. Of 10 specimens from a series of pancreatic carcinomas, only one liver metastasis has been positive. None of the five colon carcinomas studied has shown any activity.

2) The use of morphologic histochemical studies of aminopeptidase as a diagnostic tool in surgical pathological studies was suggested by Willighagen and Planteydt.[33] They recommended this application of the technique in examining lymph node biopsies and regional lymph nodes and lines of resection in cancer surgery. The results described here confirmed the usefulness of this method in outlining small clumps of tumor cells at such sites; positive reactions, however, have been seen not only in biliary duct and gastric carcinoma, as noted by Willighagen and Planteydt, but also in a high percentage of gallbladder carcinomas and occasionally in pancreatic carcinoma.

In applying this method as a diagnostic tool, the investigator should expect a number of false negatives, for it is well known that malignant cells often lose specialized enzymes in the process of becoming neoplastic.[34] On the other hand, this study and another from this laboratory have shown that histologically poorly differentiated tumor may have intense aminopeptidase activity.[31]

Studying serum and urinary levels of "leucine aminopeptidase" has also been suggested as a diagnostic technique in cases of malignant neoplasm.[35–37] Elevated levels have been ascribed by different groups to a variety of causes, including: carcinoma of the colon,[36] carcinoma of the pancreas,[37] and hepatocellular disease such as hepatitis.[35] Rutenberg and co-workers[37] have suggested that the high serum levels in cases of carcinoma of the pancreas were related to either extrahepatic or intrahepatic biliary obstruction rather than to necrosis of tumor cells or normal acinar cells. The observations described in this paper

have supported their hypothesis to the extent that pancreatic carcinomas have rarely been shown to have aminopeptidase activity.

3) As suggested, inconsistencies in enzyme activity between primary lesions and their metastases have been observed in these studies. Both a positive primary tumor (gallbladder) with a negative metastasis, and the reverse (pancreas) have been seen. But the area studied from a liver metastasis, for example, may represent considerably less than 1 per cent of the total volume of metastatic tumor in that organ; therefore, one cannot state with assurance that all the hepatic metastases were free of enzyme. If there has been no sampling error, however, the contrast in enzyme activity between a primary tumor and its metastasis may have some yet unexplained significance with reference to the usual sites of metastases or to the mode of growth and expansion of metastatic tumor nodules.

4) Another application of morphologic histochemical studies of aminopeptidase activity used to characterize the basic behavior of neoplasia has previously been reported from this laboratory.[32] In that investigation Wattenberg demonstrated that gastric mucosa was essentially free of activity, while normal small bowel, areas of intestinal metaplasia of the gastric mucosa, and some gastric carcinomas were positive for aminopeptidase. He speculated that other important metabolic similarities between normal small bowel mucosa and gastric tumor cells might ultimately be established, with important implications for the biochemist and the chemotherapist.

5) A final implication of the work described here on neoplastic growth relates to the work of Burstone[24] and Braun-Falco.[25] Both have observed that the stroma around a malignant neoplasm was more active than the stroma distant from the tumor. Of the tumors described in this paper, only one, a malignant melanoma which had metastasized to the spleen, has shown intense stromal activity. The technique used here, however, was not identical to that of Burstone. In previous studies carried out in this laboratory, a high stromal activity has been found in a number of basal cell carcinomas, in several rectal adenocarcinomas, and in several squamous cell carcinomas.[38] It would appear that while high aminopeptidase activity occurs in the stroma of malignant tumors, it does so in only a small proportion of these lesions.

Studies of non-neoplastic pathologic material were concentrated mainly on the liver. The normal pattern in the liver also has been of interest. The findings of others[22] that the small bile ducts in the portal triads were very active, that the areas of the

small bile canaliculi were moderately active, and that the hepatic parenchymal cells were minimally active have been confirmed. Studies of cirrhotic livers or those subjected to extrahepatic obstruction have demonstrated the striking ability of this technique to outline small bile ducts, proliferating or not. The difference in activity between the ducts and the hepatic cells has been used in this laboratory to distinguish proliferating bile ducts from small narrow cords of hepatic cells trapped in the areas of fibrosis seen in Laennec's cirrhosis. Such a technique might be of great value to the surgical pathologist or the experimental pathologist. Unfortunately, however, among the laboratory animals studied by this technique, only the monkey showed reasonably high activity in the small bile ducts. Further experiments are in progress in an attempt to find a more suitable animal.

Summary

The literature on the effects of infarction and autolysis on enzyme activity has been reviewed with particular reference to the applicability of morphologic enzyme histochemistry to autopsy material. Many valid investigations can be made using postmortem specimens, but appropriate control studies must be carried out.

The technique used in this laboratory to demonstrate aminopeptidase in tissues has been outlined, and a number of studies on the autolysis of human and animal tissues have been described. Aminopeptidase activity in tissues obtained at autopsy and tissues obtained up to 70 hours after autopsy and kept at 4° C. has been sufficiently high to make morphologic enzyme histochemical studies of value.

Aminopeptidase activity has been found in a variety of malignant neoplasms, primary and metastatic. Of the malignant tumors studied, carcinomas of the gallbladder and biliary ducts have shown the highest percentage of tumors with positive activity. Gastric and breast carcinomas have been less consistently active, while only one of ten specimens from the pancreas was positive. Colon carcinomas have been negative.

These studies have been discussed with reference to the demonstration of aminopeptidase as a diagnostic tool, the discrepancy in activity between primary and metastatic lesions, a previous study from this laboratory of the cellular origin of gastric carcinoma, and the reports of others on serum and urinary levels of aminopeptidase. Increased activity in the stroma around rapidly growing tumors noted by others has been observed in only one of the malignant neoplasms described in this study.

In addition to its usefulness in the evaluation of neoplasia,

the histochemical demonstration of aminopeptidase has been of value in studies of the liver, both normal and pathologic. The aminopeptidase activity of the small biliary ducts has been shown to be intense compared to that of the hepatic cord cells, which was only moderate. This difference in activity has been used to distinguish proliferating bile ducts from the small cords of hepatic parenchymal cells seen in the scars of cirrhotic livers. The possible usefulness of this method for the experimental pathologist studying a variety of liver diseases has been noted.

Acknowledgments

The technical assistance of Mrs. Delores Thompson and Mrs. Mary Erickson has been greatly appreciated. Drs. Frederick Hoffbauer and James R. Dawson and Mr. Alvin Shemesh have also been very helpful.

References

1. Pearse, A. G. E.: Extension of the Limits of Cellular Pathology: The Role of Enzyme Histochemistry, J. Clin. Path. 11:520, 1958.
2. Gössner, W.: Methoden der Enzymhistochemie und Ihre Bedeutung für die morphologische Forschung, Deutsche med. Wchnschr. 83:1752, 1958.
3. Bayerle, H.; Borger, G.; and Mayr, T.: Untersuchungen zur Pathologische Physiologie des Infarkts, Ztschr. Physiol. Chem. 243: 227, 1936.
4. Berenbom, M.; Chang, P. I.; and Stowell, R. E.: Changes in Mouse Liver Undergoing Necrosis in Vivo, Lab. Invest. 4:315, 1955.
5. Berenbom, M.; Chang, P. I.; Betz, H. E.; and Stowell, R. E.: Chemical and Enzymatic Changes Associated with Mouse Liver Necrosis in Vitro, Cancer Res. 15:1, 1955.
6. Dawkins, M. J. R.; Judah, J. D.; and Rees, K. R.: Factors Influencing the Survival of Liver Cells during Autolysis, J. Path. & Bact. 77:257, 1959.
7. Gavan, T. L. and Kaufman, N.: Experimental Renal Infarction, A.M.A. Arch. Path. 60:580, 1955.
8. Gössner, W.: Untersuchungen über das Verhalten der Phosphatasen und Esterasen während der Autolyse, Arch. path. Anat. 327:304, 1955.
9. Robins, E.; Smith, D. E.; Daesch, G. E.; and Payne, K. E.: The Validation of the Quantitative Histochemical Method for Use on Postmortem Material: II. The Effects of Fever and Uraemia, J. Neurochem. 3:19, 1958.
10. Smith, D. E.; Robins, E.; Eydt, K. M.; and Daesch, G. E.: The Validation of the Quantitative Histochemical Method for Use on Postmortem Material, Lab. Invest. 6:447, 1957.

11. Wachstein, M. and Meisel, E.: Succinic Dehydrogenase Activity in Myocardial Infarction and in Induced Myocardial Necrosis, Am. J. Path. 31:353, 1955.

12. Goebel, A. and Puchtler, H.: Ueber das Verhalten der Histochemische Nachweisbaren Alkalischen Phosphatase und 5 Nucleotidase im Sauerstoffmangel und im Niereninfarkt, Arch. path. Anat. 326:119, 1954.

13. Jennings, R. B.; Kaltenbach, J. P.; and Smetters, G. W.: Enzymatic Changes in Acute Myocardial Ischemic Injury, A.M.A. Arch. Path. 64:10, 1957.

14. Kent, S. P. and Diseker, M.: Early Myocardial Ischemia, Lab. Invest. 4:398, 1955.

15. Merrill, J. M.; Lemley-Stone, J.; and Meneely, G. R.: Effect of Acute Anoxia on Glutamic Oxalacetic Transaminase Content of the Myocardium of the Rat, Am. J. Physiol. 190:522, 1957.

16. Peters, E. R.: Histochemical Localization of Certain Oxidative Enzymes during the Production of Infarcts in the Rat Kidney, Am. J. Path. 31:590, 1955.

17. Rudolph, G. and Scholl, O.: Histochemische Untersuchungen zum Fermenthaushalt des experimentallen Niereninfarktes, Beitr. path. Anat. 119:13, 1958.

18. Wachstein, M. and Meisel, E.: Influences of Experimental Renal Damage on Histochemically Demonstrable Succinic Dehydrogenase Activity in the Rat, Am. J. Path. 30:147, 1954.

19. Zimmermann, H.: Experimentelle histologische, histochemische, und funktionelle Untersuchungen zur Frage der Nierenschädigung nach temporärer Ischaemie, Beitr. path. Anat. 117:65, 1957.

20. Kent, S. P.: Effect of Postmortem Autolysis on Certain Histochemical Reactions, A.M.A. Arch. Path. 64:17, 1957.

21. Gallagher, C. H.; Judah, J. D.; and Rees, K. R.: Enzyme Changes during Liver Autolysis, J. Path. & Bact. 72:247, 1956.

22. Burstone, M. S. and Folk, J. E.: Histochemical Demonstration of Aminopeptidase, J. Histochem. 4:217, 1956.

23. Nachlas, M. M.; Crawford, D. T.; and Seligman, A.: The Histochemical Demonstration of Leucine Aminopeptidase, J. Histochem. 5:264, 1957.

24. Burstone, M. S.: Histochemical Demonstration of Proteolytic Activity in Human Neoplasms, U. S. Nat. Cancer Inst. J. 16:1149, 1956.

25. Braun-Falco, O.: Ueber die Histotopographie der Aminopeptidase bei Hauttumoren, Klin. Wchnschr. 35:50, 1957.

26. Wattenberg, L. W.: The Microscopic Histochemical Demonstration of Steroid-3 β ol Dehydrogenase in Tissue Sections, J. Histochem. 6:225, 1958.

27. Konjetzny, G. E.: Ueber die Beziehungen der chronischen Gastritis mit ihren Folgeerscheinungen und des chronischen Magenulcus zur Entwicklung des Mangenkrebses, Beitr. klin. Chir. 85: 455, 1913.
28. Morson, B. C.: Carcinoma Arising from Areas of Intestinal Metaplasia in the Gastric Mucosa, Brit. J. Cancer 9:377, 1955.
29. Morson, B. C.: Gastric Polyps Composed of Intestinal Epithelium, Brit. J. Cancer 9:550, 1955.
30. Warren, S. and Meissner, W. A.: Chronic Gastritis and Carcinoma of the Stomach, Gastroenterology 3:251, 1944.
31. Hurst, A. F.: Precursors of Carcinoma of the Stomach, Lancet 2:1023, 1929.
32. Wattenberg, L. W.: Histochemical Study of Aminopeptidase in Metaplasia and Carcinoma of the Stomach, A.M.A. Arch. Path. 67:281, 1959.
33. Willighagen, R. G. J. and Planteydt, H. T.: Aminopeptidase Activity in Cancer Cells, Nature 183:263, 1959.
34. Greenstein, J. P.: *Biochemistry of Cancer*, Second Ed., New York, Academic Press, 1954.
35. Fleisher, G. A.; Butt, H. R.; and Huizenga, K. A.: Serum Peptidase in Hepatic Disease, Ann. New York Acad. Sc. 75:363, 1958.
36. Goldbarg, J. A. and Rutenberg, A. M.: The Colorimetric Determination of Leucine Aminopeptidase in Urine and Serum of Normal Subjects and Patients with Cancer and Other Diseases, Cancer 11:283, 1958.
37. Rutenberg, A. M.; Goldbarg, J. A.; and Pineda, E. P.: Leucine Aminopeptidase Activity—Observations in Patients with Cancer of the Pancreas and Other Diseases, New England J. Med. 269: 469, 1958.
38. Wattenberg, L. W.: Personal Communication.

Staff Meeting Report

Insulin Secretion and the Problem of Insulin Assay*

Frederick C. Goetz, M.D.,† Eugene A. Johnson, Ph.D.‡ and Richard Egdahl, M.D., Ph.D.§
With the assistance of John William Maney,¶ Virginia Betlach, B.A.†† and Charlene Schmidt‡‡

Real progress in the understanding of the function of an endocrine organ or tissue requires a satisfactory method of measuring in the blood the hormone produced by the tissue. This is notably true of the secretions of the adrenal cortex and of the thyroid gland. Another hormone, insulin, which is produced by the beta cells of the pancreatic islets, was first isolated in crystalline form in 1926, and since then its chemical composition has been completely worked out. Nevertheless, we still have no reliable and relatively simple method for measuring insulin in blood and body fluids.

The reasons for seeking such a method are compelling. Very little is known of the mechanisms regulating formation and release of insulin into the circulation beyond the fact that elevation of the blood glucose concentration is followed by increased evidence of insulin activity in blood leaving the pancreas. The nature and specificity of this stimulus, and its quantitative aspects, are largely unexplored. The problem takes on special significance when one considers the possibility that substances other than glucose might also enhance insulin production or release. Much remains to be learned about human diabetes. The probability that some diabetic patients are totally without insulin while others have definite or possibly normal amounts of available insulin has been strongly supported by Wrenshall's assays of the insulin content of the pancreas after death. The rela-

* This report was given at the Staff Meeting of the University of Minnesota Hospitals on October 16, 1959.
† Assistant Professor, Department of Medicine
‡ Associate Professor, School of Public Health
§ Formerly Instructor, Department of Surgery; at present, Department of Surgery Medical College of Virginia, Richmond, Va.
¶ Principal Laboratory Animal Attendant
†† Junior Scientist, Department of Medicine
‡‡ Senior Laboratory Technologist, Department of Medicine

tionship between circulating insulin in the blood and the onset, course, and complications of diabetes is of intense interest. Insulin levels in the blood are of great importance to the clinician and also to the physiologist in treating hypoglycemia, acromegaly, and hyperadrenalism, and in managing fever and stress.

The difficulties in achieving a satisfactory assay for insulin lie in the nature of insulin itself: Insulin is a small protein or large polypeptide consisting of amino acids arranged in two connecting chains with a total molecular weight of about 6000. Although the molecule exhibits a rather large proportion of the sulfur-containing amino acid cystine, so far no chemically unique feature has been discovered to distinguish insulin from other proteins. The insulin unit of pharmacology is defined by biologic assay according to its ability to depress blood sugar in rabbits. Pure insulin has biologic activity of the order of 25 units per milligram of protein. Using the figure 25 milliunits of insulin per ml. of plasma (probably a very excessive estimate except perhaps for pancreatic venous blood), one meets the difficult task of measuring 2.5 units, or 0.1 mg., of protein in 100 ml. of plasma containing 6000–7000 mg. of a complex protein mixture — this is the crux of the problem.

Current Approaches

Two *immunologic* approaches have shown some promise in recent years. Arquilla and Stavitsky[1] prepared an antiserum to beef or pork insulin in rabbits, then used red blood cells coated with insulin as a testing system. Berson and Yalow[2] have used insulin labeled with radioactive iodine in combination with sera containing large amounts of insulin antibodies. At present neither method quite reaches the level of sensitivity needed for determining human peripheral blood levels of insulin.

The *biologic* approaches to the problem are summarized in Table 1. The earlier methods of Bornstein[3] and of Evelyn Anderson used living rats or mice specially sensitized by hypophysectomy, adrenalectomy, and alloxan administration. Bornstein's report of the first measurements of insulin activity in the peripheral blood of normal and diabetic humans enormously stimulated interest in the problem; however, his method has been difficult for others to reproduce. More recently, interest has centered on the response to insulin *in vitro* of tissues removed from normal rats—diaphragm muscle[4,5,6] and epidymal fat[7]; disappearance of glucose from the medium, or appearance of radioactive carbon dioxide have been observed. These methods in general have the important advantage of high sensitivity;

on the other hand, they require relatively great skill. Further, the responses observed are somewhat removed from that by which insulin activity has customarily been defined (hypoglycemia), and they are not as specific as they might be. Some inconsistencies among results of various workers using the same general method are hard to explain; differences in calculation methods may account for some of the major discrepancies (cf. Randle vs. Vallance-Owen). The rat diaphragm assay, however, has shown an increase in insulin activity with increasing dilution of the plasma sample, and this peculiar phenomenon has not been entirely explained.

TABLE 1

SOME METHODS FOR ASSAY OF PLASMA INSULIN

Method	Applicable Range (Milliunits)	Normal Values (Milliunits per ml.)	95% Confidence Limits	Index of Precision $\lambda = \frac{s}{b}$
Immunologic (Red cells coated with insulin) Arquila, 1956	2.5 –250	–	–	–
A.D.H.A. Rat Bornstein, 1951	0.05– 0.5	0.1 – 0.4	–	–
Rat Diaphragm Vallance-Owen, 1954	0.02– 2	0.04– 0.8	–	–
Rat Diaphragm Randle, 1954	0.1 – 32	9 –22	2/5–2.5	0.36
Rat Diaphragm Groen, 1952-'59	0.03– 10	0.1 – 4.6	1/3–3	0.55
Rat Fat Pad Martin, 1958	0.01– 0.5	0.05– 0.35	–	0.31
Mouse Hypoglycemia Present Authors	1.2 – 20	Less than 1.2	1/3–3	0.78

Present Method

There is considerable justification, therefore, for pursuing the development of assay methods depending on hypoglycemia. Our first work used an adaptation of Anderson's method, with alloxan-diabetic hypophysectomized (ADH) mice as the test animals.[8] The preparation of the animals was difficult, and the method was difficult to standardize, but it could detect as little as 2 milliunits of insulin. This method allowed us to show that

in fractions of pooled human plasma, insulin activity probably was mainly associated with beta globulin.

The present method also uses living mice, but it is simplified in that the animals are not previously subjected to operation nor treated with alloxan. Instead, their preparation on a high-carbohydrate diet and incubation during the assay at 37° C. appear to sensitize them to insulin, so that the minimum amount detected is equal to that of the older method with ADH mice.*

The assay as presently developed is carried out in the following manner: female mice, 10 to 15 grams in weight, are secured from the Jackson Laboratories, Bar Harbor; a single inbred strain is used (DBA_1). They are fed on bread and lettuce and kept in a room alternately light and dark in 12-hour periods.† After seven days under these conditions, the mice are considered ready for experimental use. Twenty-four mice are fasted overnight, beginning at 4:00 p.m. Starting at 10:00 the next morning, the mice are given intraperitoneal injections of 0.5 ml. of the solution to be tested per 15 gm. of mouse. Six mice are injected with undiluted plasma; six with plasma diluted 1:4; six with glucagon-free insulin, 5 milliunits; and six with insulin, 1.25 milliunits. The animals are placed in an incubator for one hour at 37° C., and then 0.1 ml. blood is taken from the tail and analyzed for glucose by the method of Somogyi and Nelson. The potency of the unknown plasma sample is estimated by a formula which assumes a common slope in the response curves of unknown and standard; this assumption appears justified by our experience so far.

Under these conditions, mice injected with 0.6 milliunits or less of insulin show blood sugars of about 50 mg. per 100 ml.; mice injected with 10 milliunits or more show blood sugars of about 10 mg. per 100 ml. and often die in hypoglycemia. The useful range of the assay lies between these limits. The "95% confidence limits" are from about one-third to three times the estimated potency of the sample. These limits, although unacceptably wide for many biological assays, are about the same as those described for other plasma insulin assay methods. Further work is in progress to increase the precision of the assay; at present it appears sufficiently precise to give useful information in the circumstances in which we have applied it.

---

*The experience and advice of Dr. Gerald Wrenshall of Toronto was invaluable in choosing conditions for the assay; Wrenshall has worked with a mouse-convulsion method designed for larger amounts (40 to 100 milliunits) of insulin.

†At the suggestion of Dr. Franz Halberg

Application of the Method

1) Pancreatic venous blood in the normal dog

Effect of glucose administration: Cross-circulation experiments established many years ago that elevation of blood glucose concentration leads to hypoglycemic activity in pancreatic venous blood, but quantitative information on this finding is almost totally lacking. For our studies Dr. Egdahl has devised a method of inserting a cannula into the splenic vein of the dog which allows collection of pure pancreatic blood intermittently over a period of two to four hours; he has described the general method in detail elsewhere.[9] Blood is collected only from the tail of the pancreas and does not include the total venous drainage of the pancreas. But since the tail is the region richest in islets, hence by rough estimate about half the islets are represented. After each experiment is completed and after each dog is killed, India ink is injected up the cannula in order to demarcate the area drained by the cannula; only experiments in which anatomical relationships were revealed as satisfactory by this test are described.

A typical experiment is summarized in Table 2. Noteworthy is the sharp increase in venous flow through the cannula which occurred five minutes after administration of intravenous glucose; this was accompanied by an obvious arterialization of the blood in appearance and a sharp drop in hematocrit level. These changes disappeared in an hour. The pronounced increase in hypoglycemic activity (HGA) resulted from both increased flow and increased plasma concentration.

TABLE 2

PANCREATIC VENOUS BLOOD ANALYSES

Dog No. 25—Female—18.4 kg. Nembutal® anesthesia, 30 mg/kg. Fasted 16 hours. Cannula placed, 11:00 A.M.

	11:20 A.M.	11:45 A.M.	5 Min.	30 Min.	60 Min.
Femoral Artery Blood Glucose (mg.%):	98	Glucose, 3 gm/kg IV = Zero Time	876	624	456
Pancreatic Venous Blood:					
Glucose (mg.%)	72		858	634	394
Flow (cc/min)	3		17	8	3
Hematocrit, %	44		24	32	41
Plasma HGA, mU/ml	2		3	3	35
Plasma HGA, mU/min.	3		39	15	63

From the results of eight similar experiments, summarized in Table 3, it may be noted that: 1) the preliminary sample is usually, but not always, inactive (less than 1.2 milliunits per ml.), and 2) the rate of insulin secretion after glucose is sometimes very high. On the other hand, in two of the experiments no response to glucose was seen. It is of interest to compare these results with those of Anderson,[10] who found 0.25–0.63 milliunits insulin per ml. in similar experiments. Our results would indicate a total insulin output in the range of 2 to 5 units in the first hour after massive glucose administration. This figure seems compatible with the 10 to 20 units a day required to maintain a depancreatized dog, since it can be assumed that the secretion rate is at a basal level of 0.2 or 0.3 units an hour during a good part of the day.

TABLE 3

HYPOGLYCEMIC ACTIVITY IN PANCREATIC VENOUS PLASMA* BEFORE AND AFTER INTRAVENOUS GLUCOSE, 2 OR 3 GM/KG

Dog Number	Preliminary Sample	Time in Minutes After Glucose Injection					
		5	15	30	60	90	120
1				Inactive			
2 (Head)				Some Activity (>4)	Active (±20)	Some Activity (>4)	Some Activity (>4)
3	0			Some	14	Some	
4	2			101	8		3
25	3	39		15	63		
26 (Arterial injection)	5		23	2	11		
29	0	0	0	0	0		
32	0	0	0	6	11		

*Expressed as milliunits insulin per minute

Effect of intravenous tolbutamide administration: One of the most intriguing aspects of the drug tolbutamide (Orinase®), now widely used in treating diabetic patients of the stable variety, is its mode of action in lowering the blood sugar. Some earlier studies[11] showed a widening of the difference between capillary and venous blood sugar levels when tolbutamide was given in a single dose to normal persons—an effect very similar to that produced by intravenous injection of insulin. This and other work strongly implied that insulin release from the pancreas might play a part in the acute response to tolbutamide.

Experiments designed to examine this possibility directly are summarized in Table 4. The technique in all respects was like that in the glucose experiments already described. In most of the experiments, it can be seen that pancreatic hypoglycemic activity rises somewhat after tolbutamide, and in about half of the experiments, it can be seen that pancreatic hypoglycemic to account for the acute hypoglycemia produced by the agent. The reasons for the negative experiments are not clear, but it will be remembered that in some of the glucose experiments no insulin output was observed.

TABLE 4

HYPOGLYCEMIC ACTIVITY IN PANCREATIC VENOUS PLASMA* BEFORE AND AFTER INTRAVENOUS TOLBUTAMIDE, 50 MG/KG

Dog Number	Preliminary Sample	Time in Minutes After Tolbutamide: 5	15	30	60	90	120	180	240	2-Fold Rise?	5-Fold Rise?
10	0			6	10	16	1			Yes	Yes
11	1		5		4	6	0			Yes	Yes
14	0			0	6	3	2			Yes	Yes
16	2		11	4						Yes	Yes
17	6		9		2					No	No
18	6	16		4	5					Yes	No
18†	0	15		2	0					Yes	Yes
20	2	13		12						Yes	Yes
20†	7		3	7	1					No	No
40	3			2		1	0	0		No	No
41	6			9		11	5		13	Yes	No

*Expressed as milliunits insulin per minute
†2 days later

It remained to be shown that the hypoglycemic activity found was not due to tolbutamide itself, present in the plasma sample. This was demonstrated by: 1) treating a number of plasma samples with cysteine, which terminated the hypoglycemic activity and is known to destroy insulin but not tolbutamide; and 2) measuring the tolbutamide content of the plasma samples and showing that tolbutamide in this concentration was not effective in the assay procedure. In an alloxan-diabetic dog, no activity was found after tolbutamide had been administered. Furthermore, the negative tolbutamide experiments in normal dogs indicated that the plasma content of tolbutamide was not sufficient by itself to affect the mouse assay.

2) Clinical studies

The method has not been widely applied as yet to human plasma samples, but some examples of its use may be of interest:

Normal human peripheral venous plasma has not shown activity in the assay up to this time, whether taken from fasting subjects or from subjects who had ingested glucose. This finding indicates that insulin content was less than 1.2 milliunits per ml., which agrees with those of most other investigators.

In two *acromegalic* subjects, no activity was found in venous samples after glucose ingestion. This contrasts with the results of Randle,[5] who noted levels of 40 to 527 milliunits per ml. of plasma in acromegalic patients. Such levels would be readily detectible by the present method; they would, in fact, produce convulsions and death in the sensitive strain of mice employed.

In contrast to these negative results, low but definite amounts of hypoglycemic activity (about 2 milliunits per ml.) were observed in the plasma of a patient with severe attacks of hypoglycemia, though only after glucose administration. This patient was subsequently found to have an islet cell adenoma, containing a large amount of insulin by assay. She showed the interesting phenomenon of temporary mild diabetes in the first few days after surgery. It is of considerable interest, in relation to this, to note that a sample of plasma obtained from veins draining the tail of her pancreas shortly after removal of the tumor showed hypoglycemic activity as determined by the mouse assay. This suggests that the temporary diabetes seen in this patient may *not* have been due to suppression of function of the normal islets, as has usually been suggested.

Of great interest also was the opportunity to examine plasma from an unusual case of diabetes in a young woman under study by Dr. James Field at the Clinical Center of the National Institutes of Health.[12] This remarkable patient displayed extreme insulin resistance, with blood glucose levels above 2000 mg. per 100 ml. despite administration of many thousands of units of insulin per day; yet after withdrawal of insulin treatment she showed no evidence of ketosis and seemed to improve. More than three months after insulin withdrawal, Dr. Field found insulin activity in her plasma with the rat diaphragm assay. In the same sample, hypoglycemic activity equal to about 5 milliunits of insulin per ml. was detected. Dr. Field has suggested that this may be an example of tissue insensitivity to a hormone, analogous to Albright's pseudohypoparathyroidism—a previously unrecognized form of diabetes.

Summary

The present method, then, may already have some applications in special clinical problems. Its disadvantages, in relation to other methods, include lack of extreme sensitivity; though

not very precise, it appears not much worse than other methods in this regard; moreover, it is time-consuming and progress is slow. As for its advantages, it is close in principle to the original and generally accepted definition of insulin activity; calculation of relative potency is straightforward, with no puzzling discrepancies appearing on dilution of the sample; and no difficult techniques are required. Like all biological assays for insulin, however, this method represents a summation of positive and negative biological effects rather than a measure of the absolute amount of hormone. Further work will be directed at increasing the precision of the assay and developing concentration methods which can be applied to human peripheral venous plasma.

Application of the method to pancreatic venous blood in the Nembutal®-anesthetized dog revealed resting values usually below 2 milliunits per minute, but sometimes as high as 6 milliunits. After massive glucose administration, peak values between 10 and 60 milliunits per minute were seen in the first hour. After tolbutamide administration, peak values of 5 to 16 milliunits per minute were observed, supporting the hypothesis that insulin release from the pancreas plays a major role in the acute effects of this drug.

These studies were supported mainly by Grant A-1556 from the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service, and also by grants from the Graduate School of the University of Minnesota, the Upjohn Company, and Eli Lilly and Company.

References

1. Arquilla, E.R. and Stavitsky, A. B.: The Production and Identification of Antibodies to Insulin and Their Use in Assaying Insulin, J. Clin. Invest. 35:458-466, 1956.
2. Berson, S. A.; Yalow, R. S.; Bauman, A.; Rothschild, M.; and Newerly, K.: Insulin (I^{131}) Metabolism in Normal Human Subjects: Demonstration of Insulin Binding Globulin in the Circulation of Insulin Treated Subjects, J. Clin. Invest. 35:170-190, 1956.
3. Bornstein, J. and Lawrence, R. D.: Plasma Insulin in Human Diabetes Mellitus, Brit. Med. J. 2:1541-1544, 1951.
4. Vallance-Owen, J. and Hurlock, B.: Estimation of Plasma-Insulin by the Rat Diaphragm Method, Lancet 1:68-70, 1954.
5. Randle, P. J.: Assay of Plasma Insulin Activity by the Rat-Diaphragm Method, Brit. Med. J. 1:1237, 1954.
6. Willebrands, A. F.; v. d. Geld, H.; and Groen, J.: Determination of Serum Insulin Using the Isolated Rat Diaphragm, Diabetes 7:119-124, 1958.

7. Martin, D. B.; Renold, A. E.; and Dagenais, Y. M.: Lancet 2: 76-77, 1958.
8. Goetz, F. C.; Beigelman, P. M.; and Thorn, G. W.: A Method of Insulin Bio-Assay and Its Application to Human Plasma Fractions, Proc. Soc. Exp. Biol. & Med. 86:484-486, 1954.
9. Egdahl, R. H.: Mechanism of Blood Enzyme Changes Following the Production of Experimental Pancreatitis, Ann. Surg. 148:389-400, 1958.
10. Anderson, E.; Wherry, F.; and Bates, R. W.: Factors Influencing Secretion of Insulin, Fed. Proc. 18:4, 1959.
11. Goetz, F. C.; Gilbertsen, A. S.; and Josephson, V.: Acute Effects of Orinase on Peripheral Glucose Utilization, Metabolism 5:788-800, 1956.
12. Field, James: Personal communication.
13. Wrenshall, Gerald A.; Bagach, A.; and Ritchie, R. C.: Extractable Insulin of Pancreas, Diabetes 1:87-107, 1952.

Some Thoughts on the Nature and Meaning of Scholarship*

Theodore C. Blegen†

There are many definitions of scholarship. Most of us, when we hear the word, think of high excellence in learning, of originality, and creative achievement, but in its simplest meaning a "scholar" is just somebody who attends a school. He may be anywhere in the scale of grades; he may be good or bad; but by the dictionary definition he is a scholar even if his IQ is below the freezing point.

Such an IQ will not carry him through Medicine or into the Graduate School, but we need to remember that in our system of universal education, everybody is challenged to come up to his potentiality—to measure up to what God or his genes make it possible for him to do or be. I mention this at the outset because one of the problems—and tragedies—of education is the gap, for many individuals, between what they could do and what they actually accomplish—between their potentiality and achievement. Failure to use one's talents to the uttermost means the sad refrain of "too little, too late." Talent is highly important, but education is more than talent. It means interest, determination, hard work. It means using our little gray cells to their full potentiality. In short, it means doing the best one can with what one has. This, I suppose, is what Thomas Edison had in mind when he said that genius is one per cent inspiration and ninety-nine per cent perspiration. Some of us might make a little change in the percentages, but we recognize wisdom and experience in the inventor's words.

In a better and more accepted sense, scholarship means quality and excellence applied to studies, training, ideas, and problems. It is difficult to come up with a puncture-proof definition, and I doubt that we need one. I think it worthwhile, however, to remind ourselves of some of the basic elements or characteristics of scholarship.

Elements of Scholarship

One is curiosity. A poet has said that "America is West and the winds blowing." Scholarship is questions and answers blowing. It is the process of hunting for answers. It is Sherlock Holmes and the pursuit of clues. It is inquisitiveness with in-

*This address was delivered as the Minnesota Medical Foundation Lecture on September 28, 1959.

†Dean, University of Minnesota Graduate School

formed purpose. Curiosity kills cats, breeds gossip, and pulls skeletons out of closets; but it is a trail to knowledge and truth if disciplined by training, observation, and purpose. Kipling was a poet and story-writer, but he used the servants of scholarship. Do you remember his lines?

> I keep six honest serving men
> (They taught me all I knew).
> Their names are *What* and *Why* and *When*
> And *How* and *Where* and *Who*.

Such servants, especially What and Why and How, must have helped an English scholar who one day noticed that his bacterial culture had been invaded by a mold that killed off the germs; and the same assistants stood at the side of a French chemist who, among other things, attacked the problems of anthrax and hydrophobia. Sir Alexander Fleming and the great Pasteur were curious. They asked questions and hunted for answers. And there is not a medical scientist in the University of Minnesota who has not made use of What and Why and When, and How and Where and Who, in the many researches that have brought great distinction to this school of medicine.

A second characteristic worth noting is patience, coupled with the will to work and with the use to the limit of the intellectual potentiality that I have mentioned. There are blind alleys in scholarship and in research in all its varied fields. Hopeful leads often come to negative ends. Frequently goals seem to retreat the more one studies and knows. The scholar, pursuing one course, may change it because of unforeseen openings. He knows frustration, but he does not give up, and sometimes he has a taste of triumph. We hail tested and proved results, and now and then a scholar wins acclaim and high awards. I recall the story of one of my colleagues at the Mayo Foundation of the Graduate School who worked twenty years on a research problem. I once heard him say that he worked for the first ten years without coming to any turning point. Then there was a turn, but Dr. Kendall worked ten more years before he came up with cortisone. With a colleague he won the Nobel Prize, but I suspect that many people who applauded him for the prize knew little about his two long decades of patient effort. Patience, by the way, can mean a good many things, all interrelated. The dictionary suggests a flock of synonyms, including endurance, fortitude, perseverance, long-suffering, and self-control. Underlying these basic traits, I think, is the scholar's unwillingness to settle for anything that is meretricious or, as it is put in vigorous slang, "phony." And this unwillingness goes

along with another trait that I shall only mention. True scholarship is not arrogant. It does not boast. It does not covet headlines. It recognizes the limitations of man's knowledge; it is honest; it shuns claims that cannot be sustained. It detests and rejects the "phony."

"Ideas in Motion"

But let me now turn to the third characteristic that I want to emphasize in this quick analysis. If scholarship is questions, it also is ideas in motion. It is imagination backed by knowledge and fired by intellectual resourcefulness. Ideas are vital to the emergence of principles and to the thousand-fold applications given to principles. And ideas in scholarship and research offer a happy field for comment in medical circles, because medical science beautifully exhibits and illustrates their basic importance. Research—one of the arms of scholarship—starts from and reaches out from principles. There is universal admiration and respect for the achievements of medical science. To laymen they seem like magic, but they are not magic. They are ideas plus curiosity, patience, training, experiment, and sweat—in a word, they are ideas in motion. Chiefly, I suppose, the achievements are basic ideas or principles put to use in uncounted ways. The National Science Foundation has published a report pointing out that modern medicine is largely built upon only five basic ideas or principles. They are not machines or techniques or clever phrases. They are ideas worked out in the minds of thinkers, of men who combined imagination with observation, who labored patiently at benches and in the smells of laboratories. The five ideas or principles were just these: the germ theory, nutrition, hormones, genetics, and cells. Five—but how many thousands of forms and turns have not their applications taken? How much and far-flung the research that has proceeded, to the benefit of mankind, from these five principles! I take my illustration from medicine, but the National Science Foundation tells us also that an army of engineers is waiting for scholars to come up with some new principles, some idea, some fundamental starting point for new departures. It is reassuring to have that trained army, but the supplies behind it are basic knowledge and its advance starts from formulated and tested principles.

More to the Story

Curiosity, patience, and ideas are basic to scholarship, but they are not the full story. They are not enough, as the nurse Edith Cavell once said of patriotism. We all realize that many other factors play into the total picture. There is such a thing as intelligence of high potentiality, and in college and graduate

school your faculties are constantly on the look-out for it, ready to give it encouragement and support. There is the long and testing road of training, calling for grit and endurance. There are the rich resources of great institutions of learning with their libraries, staffs, equipment, laboratories, and other facilities, all of which are designed to aid scholars and scholars-in-training. There is the air of freedom, of scholarship and research unhampered. Science unfree is no science at all. In the atmosphere of freedom there is the indispensable discipline of criticism—criticism of ourselves, of others, and by others. There is an inescapable sense of team-work, of being a part of a whole and contributing to a total that relates one to many minds and places. In science this means the world, with no provincial boundaries. And there is the heritage of the past—a past that comes up to this fleeting line of present, the past with its accumulated store of knowledge and of ideas and institutions, built through the many generations of man. We cannot encompass it all. It is too big for that, but we must have understanding of it, generally and also in our special studies and professions.

I am not trying to make a catalog of everything that goes into scholarship and research, and your eyes may see some barns on the landscape that mine have missed or that I have chosen not to describe here. But I am confident that I have stressed some points that are fundamental to this exciting enterprise that we call scholarship—an enterprise crucial to us in America and to the world as we face the future.

A Double Resource

I now want to look at two related matters that are relevant to my theme. Scholarship is a double resource—a resource for us in our individual lives and for the society of which we are a contributing part. In this connection I should like to make the point that scholarship reaches beyond our professions and specialized work. If measurable excellence in learning is the heart of scholarship, it is of concern to everybody. From home to business and profession, from community affairs to reading and recreation, everywhere, we as people and citizens must draw upon knowledge and understanding, on memory and reasoning—if we are to play our roles in life well and richly. We all try to understand and interpret ourselves in relation to the life about us. We have to see ourselves in the context of past and present. We must all meet and try to solve problems that call for study and action. Whatever our degree of excellence in learning, it comes into play every hour and day, and we must use it and depend upon it. The insistence on universal education in this

country is no passing fad. It springs from a widespread and sound conviction that our civilization must have the very highest degree of knowledge and intelligence that we can develop—if we are to live satisfying lives, if we are to make good the dreams on which our society was founded, and, I may add, if we are to meet with wisdom the responsibilities of leadership in the troubled world of today.

Recruitment for Research

That is one matter. The other has to do with research, and now I address myself primarily to my colleagues, who are famed for their contributions as research scholars. How shall we recruit high research talent for the tomorrows that stretch into future years? Where shall we find that talent? How identify it? Most of us believe that our future depends on research, and many people are worried about the supply of talent. I think of our teachers in schools and colleges as focal points for searching out and encouraging highly promising young people to take to the hard but fascinating road of scholarship. Many suggestions have been made of effective ways of increasing the supply of talent. One very simple one that I favor is that of helping young students to get a taste of what research means by doing research, however little and modest the experience. I am not now speaking of graduate students who of course are trained in research, but of students at earlier levels. Some time ago I read a book entitled *The Making of a Scientist* in which the author, Anne Roe, said that it "is the discovery that a boy can himself do research that is more important than any other factor in his final decision to become a scientist." I know from experience what an early taste of research can mean. When I was young, already interested in History—which became my field—I got the opportunity of sorting out and working over a collection of important manuscripts that no historian had seen or used, and this opportunity led to my first published historical article. For me it was a stirring and heady taste of research, and I must confess that the taste—and inspiration—has lingered with me through all the years since I first thumbed over and studied those unique records.

On this personal note of research taste, I close, with my good wishes to you who are students and to my colleagues in the medical sciences, and with my congratulations to the Minnesota Medical Foundation on its splendid, constructive support of medical education, scholarship, and research.

SCHOLARSHIP WINNERS — Happy with their scholarship aid from the Minnesota Medical Foundation are 26 University of Minnesota medical students. Their awards, based on financial need and scholarship, were presented Sept. 28 on Minnesota Medical Foundation Day. All were for $500, except one for $750. Dr. Herman E. Drill, (Med. '28), president of the Foundation, conferred the awards. Posed as a group are L-R. 1st Row: Dr. Herman E. Drill; Rolf Larson; Albert Roth; Lowell Van de Riet; George Gerstenkorn; Solveig Stepperud. 2nd Row: W. Cunningham; Nancy Jo Engeset; John Cich; John Leary; Schrae LaPlant; John Sutherland. 3rd Row: Bernie Hanson; David Hopkins; Kenneth Manick; Paul Mertens; Herman Langner; Lawrence Schut; Michael Koch. 4th Row: Henry Knudsen; Robert E. Olson; James Guthrie; Bruce Jensen; Dale Kaye; Herbert Hobday; Darryl Washa and Jon Parsons.

Departmental News

RADIATION THERAPY

Dr. Harold O. Peterson, Head of Radiology, and Dr. Donn G. Mosser, Director of Radiation Therapy, arranged the courses of instruction given at the 1959 annual meeting of the American Roentgen Ray Society in Cincinnati, O., in September. Dr. Mosser presented a course on the "Methods of Cancer Therapy in European Radiotherapy Centers." He also addressed the Wisconsin Radiological Society's annual meeting in Milwaukee Sept. 12 on the topic "The Place of $Cobalt^{60}$ Teletherapy in Radiotherapeutic Practice."

Dr. John F. Dillon was appointed Assistant Professor of Radiology and Radiation Therapy. He received his doctor of medicine degree from George Washington University School of Medicine in 1947 and comes to Minnesota from his former post at the Medical College of Georgia, where he was an associate professor of radiology and chairman of the isotope committee.

Participating in the Southeast Missouri Cancer Conference Oct. 4 in Cape Girardeau, Mo., were Drs. Stuart Arhelger, Clinical Assistant Professor of Surgery; Alvin Schultz, Clinical Assistant Professor of Medicine, and Donn Mosser.

SURGERY

"Open Heart Surgery," a film portraying open heart surgery on a six year old patient at the University of Minnesota Hospitals and produced by CBS-TV, was named winner of a 1959 Blakeslee Award sponsored by the American Heart Association. Dr. C. Walton Lillehei and his associates performed the surgery. The film was telecast nationally March 9, 1958, as part of the "Conquest" television series.

The American Heart Association applauded the film as "distinguished in its technical skill, a dramatic insight into progress in open heart surgery, and a leading achievement of network television in presenting a major advance in cardiovascular research."

The Blakeslee Awards are given in memory of the late Howard W. Blakeslee, noted science reporter, and are for outstanding reporting in the field of heart and circulatory diseases.

CONTINUATION MEDICAL EDUCATION

Dr. W. Albert Sullivan, Director, Department of Continuation Medical Education, was elected to a three-year term on the Board of Directors of the American Cancer Society, Minnesota Division.

PHYSICAL MEDICINE AND REHABILITATION

Dr. Frederic J. Kottke, Professor and Head, was elected President of the American Congress of Physical Medicine and Rehabilitation at its annual meeting in Minneapolis during September.

Dr. Glenn Gullickson, Assistant Director of the Rehabilitation Center, was elected assistant to the executive director of the ACPMR at the same meeting.

Dr. Kottke received a citation of meritorious service Oct. 7 from President Eisenhower's Committee on Employment of the Physically Handicapped. Given for "exceptional contributions in advancing employment of the handicapped," the honor was bestowed at festivities in Minneapolis noting "National Employ the Physically Handicapped Week."

Dr. Harold M. Sterling, Instructor, left the University Hospitals Sept. 30 to become Medical Director of the Joseph P. Kennedy, Jr., Memorial Hospital, Brighton, Mass.

Dr. William G. Kubicek, Professor and Clinical Physiologist, recently returned from an extended tour of South America visiting rehabilitation centers and attending the International Congress of Physiology in Buenos Aires, Argentina.

Hazelle M. Erickson, RPT, Rehabilitation Coordinator, returned from a European tour where she attended the World Confederation of Physical Therapists meeting in Paris.

Dr. Kottke lectured on "Total Rehabilitation" to the October meeting of the St. Louis County Medical Society in Duluth.

New members of the Department are Mrs. Ann Armstrong, speech pathologist; Mrs. Marcia Larson, occupational therapist; Allan C. Yater, vocational counselor; Lawrence Young, research fellow; Darlene Theisen, research fellow; Helen Skowlund, physical therapy instructor; and Dr. Harper Willis, medical fellow.

Staff members married recently were Nancy Munter, RPT, to David Folkestad on July 18; Joan Lundquist, RPT, to Dr. Po Ya on Aug. 1; Ruth Hultkrans, OTR, to Albert W. Hastings on Aug. 29; Gayle Mattson, OTR, to James Kincannon on Sept. 5; and Marilyn Kalitowski to Lawrence Daly on Sept. 26.

A daughter, Elizabeth Ann, was born to Mr. and Mrs. John D. Allison Aug. 31. Mr. Allison is an Instructor in physical therapy.

A girl, Ellen Elizabeth, was born Sept. 10 to Mr. and Mrs. Peter F. Briggs. Mr. Briggs is an assistant professor.

ANESTHESIOLOGY

Drs. Joseph J. Buckley, James H. Matthews and Arthur J. Oswald attended the annual meeting of the American Society

of Anesthesiologists Oct. 3–9 in Miami, Fla. Dr. Matthews was a member of the program committee, and Drs. Buckley and Oswald presented papers titled "An Assessment of Respiratory Efficiency in the Postoperative Patient," and "The Role of the Anesthesiologist in the Care of Polio and Tetanus," respectively.

Dr. Buckley and Dr. F. H. Van Bergen, Head of the Department, attended the annual meeting of the Academy of Anesthesiology in Rochester, Minn.

NEUROLOGY

Dr. Erland Nelson has taken a year's leave of absence to do research in neuropathology and electron microscopy at the Max Planck Institut für Hirnforschung in Munich, Germany.

Dr. Lucien Rubinstein of the Bernard Baron Institute of Pathology, London Hospital, London, England, has joined the Division and assumed Dr. Nelson's lecturing duties.

Drs. Frank Morrell and Fernando Torres presented papers before the 21st International Congress of Physiological Sciences Aug. 9–15 in Buenos Aires, Argentina. Dr. Morrell's paper was titled "The Mirror Focus As a Model of Neural Learning." Dr. Torres' was "Activity of Isolated Cerebral Cortex.

After the congress, Dr. Torres gave a series of lectures to the "Ateneo Neurologico de Buenos Aires" by invitation of that institution, lectured to the Colombian Institute for the Nervous System at Bogota, Colombia, and received full membership in the Medical Academy of Colombia.

Dr. Morrell presented a paper titled "Lasting Changes in Synaptic Organization Produced by Continuous Neuronal Bombardment" to an International Symposium on Brain Mechanisms and Learning in Montevideo, Uruguay Aug. 3. He has also been appointed to the Editorial Board of the publication "Neurology."

Dr. A. B. Baker, Professor and Director of the Division of Neurology, has been elected to the Norwegian Academy of Science.

Dr. Maynard Cohen was appointed a member of the Postgraduate Training Committee of the National Institute of Neurological Diseases and Blindness.

MEDICINE

Dr. Frederick C. Goetz, assistant professor of Medicine, was named chairman of Diabetes Detection week, Nov. 15–21. The Twin Cities Diabetes Association annually sets aside a period for an educational effort aimed at finding undiagnosed cases of diabetes in the area, and to alert unsuspecting diabetics to the need for medical care, according to Dr. Goetz.

Faculty News

DR. McQUARRIE HONORED

Dr. Irvine McQuarrie, Emeritus Professor of Pediatrics at the University of Minnesota Medical School, was awarded an honorary Doctor of Science degree by Northwestern University's Medical School Sept. 29 during the School's Centennial Celebration.

For many years he was Chairman of the Department of Pediatrics at Minnesota. Dr. McQuarrie retired in 1955 and is now Consultant in Research at the East Bay Children's Hospital, Oakland, Calif.

MEDICAL FOUNDATION GIVES INITIAL RESEARCH AWARD

Awarding of the first medical research grant in the history of the Minnesota Medical Foundation was completed Oct. 30.

The grant, for $13,716, went to Dr. Frederick C. Goetz of the Department of Medicine, University of Minnesota Medical School, to finance a two year research project aimed at developing substitutes for insulin in the treatment of diabetes. A team of physicians and technicians will participate.

The funds were willed to the Medical Foundation by the late Eva Rhodes Freeman of Minneapolis with a request that an attempt be made to find a less painful method of treating the diabetic. The donor's mother was a diabetic and the funds were presented to the Foundation as a memorial.

Dr. Goetz' study will emphasize an investigation of factors regulating secretion of insulin by the islet cells of the pancreas with special emphasis on the effects of tolbutamide and other synthetic substitutes for insulin; and a qualitative and quantitative estimation of individual fatty acids in the serum of diabetic patients by liquid-gas chromotography, with special reference to changes produced by oral hypoglycemic agents.

Dr. Goetz said that the project will get underway immediately.

UNIVERSITY GETS USPHS TRAINING GRANTS

The U. S. Public Health Service has awarded $100,560 to the University of Minnesota to support 1960 training programs for research scientists.

Staff members sharing in the awards are Dr. John A. Anderson, Professor and Head, Department of Pediatrics, Medical

School, $58,016 for a training program in Research Pediatrics; Dr. Ralph L. Kitchell, Professor and Head, Department of Veterinary Anatomy, $27,732 for a training program in Anatomy; and Dr. Sheldon C. Reed, Professor and Director of Zoology, $14,821 for a training program in Human Genetics.

USPHS announced 98 grants totalling nearly $3.5 million for such training programs in eleven basic medical and health related sciences. Fifty-three institutions and universities shared in the awards, given for new and ongoing programs.

AMERICAN LEGION HONORED BY REGENTS

The Regents' Award of the University of Minnesota was bestowed on the Minnesota American Legion and its Auxiliary during the Legion's 41st annual convention in Minneapolis.

The Legionnaires were cited for their establishment and support of the American Legion Memorial Heart Research Professorship. The post has been held since 1954 by Dr. Robert A. Good, Professor of Pediatrics.

Established in 1950 to study the causes, prevention and treatment of rheumatic fever and heart diseases, the professorship is supported by a $500,000 endowment created by Minnesota American Legion members and Auxiliaries.

Dr. Good conducts heart research in specially equipped laboratories on the fourth floor of the Variety Club Heart Hospital in the University's medical center.

MEDICAL STUDENTS WIN SCHOLARSHIPS

Four freshmen medical students at the University of Minnesota won $2,000 scholarships from the National Foundation to aid their medical studies during the next four years.

James H. House, Wood Lake, Minn., John M. Vener, Ward Springs, Minn., Dennis Jacobsen, Sioux Falls, S. D., and Orville Swenson, Mabel, Minn. were among nine University students who qualified for March of Dimes scholarship aid. Other winners were in the fields of Physical Therapy and Occupational Therapy.

Messrs. House and Jacobsen attended South Dakota State college and Messrs. Vener and Swenson are graduates of College of St. Thomas and St. Olaf college, respectively.

Group Health Mutual, Inc., St. Paul insurance firm, awarded scholarships this year to Edward Ellis, Minnesota City,

Minn., Clifford Pesonen, Minneapolis, Rodney Biltonen, Virginia, Minn., and James Runquist, Austin, Minn. Their scholarships ranged up to $2,000 each.

MEDICAL STUDENT DROWNS

Lloyd Austin Yates, 27, senior medical student and a graduate student in the Department of Anatomy, was drowned Sept. 2 in a swimming accident at Woods Hole, Mass.

He had been a teaching and research assistant in the Department of Anatomy for the past five years and he was spending the summer working at the Marine Biological Laboratory on a diabetes research project, with Dr. Arnold Lazarow. His home was in Seattle, Wash.

Mr. Yates is survived by his wife, Barbara, and two children. A memorial fund in his honor has been established with the Minnesota Medical Foundation, and a memorial service was held Sept. 18 at the Center for Continuation Study.

Memorials

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

Mrs. Dorothy Heegaard, Minneapolis

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

◆ **1920**

J. Arthur Myers, Professor Emeritus, School of Public Health, received the Outstanding Achievement Award of the Board of Regents Sept. 28 in ceremonies inaugurating the 1959–60 school year.

◆ **1925**

Clarence Jacobson, Chisholm, was named president-elect of the Minnesota State Medical Association at its 1959 annual meeting.

◆ **1927**

Arthur C. Kerkhof was elected president of the Minnesota Heart Association in July.

◆ **1928**

Irwin L. V. Norman was appointed medical director of the Chase Manhattan Bank in New York City. He retired from the Navy April 30, 1959 with rank of Rear Admiral, Medical Corps. He is a native of Willmar.

◆ **1931**

Corrin H. Hodgson, consultant in internal medicine at Mayo Clinic, was promoted to associate professor of medicine in the Mayo Foundation.

◆ **1933**

Horace DeLien is completing his first year as chief of the Division of International Health, U.S. Public Health Service, Washington, D.C. Prior to his appointment he spent seven years as chief of the U.S.P.H.S. Public Health Division, United States Operations Mission, Philippine Islands.

◆ **1936**

Leonard A. Titrud, who is a Colonel in the army reserve, completed two weeks of active duty training at Fort Riley, Kansas in July. He is a neurosurgeon in private practice in Minneapolis.

Richard L. Varco was elected 1st vice president of the Minnesota Heart Association in July.

◆ **1940**

Laurence M. Hursh, chief of the medical research branch at the Army Surgeon General's office, Washington, D.C., was re-

cently promoted to full colonel. He supervises an Army in-service research program of $2 million and a contractural research program of $2 million.

♦ **1941**

Robert W. Hollenhorst, consultant in ophthalmology at Mayo Clinic, has been advanced to an associate professor of ophthalmology in the Mayo Foundation.

♦ **1942**

Virgil J. P. Lundquist was elected president of Minnesota Medical Alumni Association. The Association has signed up 2,000 members in its initial reorganizational effort.

♦ **1943**

Lester N. Dale, practicing at Red Lake Falls, Minn., received an honorary life membership in the Minnesota Podiatry Association Sept. 12 "for guiding podiatry toward better interprofessional and public relations."

♦ **1944**

David D. Daly, Mayo Clinic, was promoted to associate professor of Neurology in the Mayo Foundation, Rochester.

Scott N. Swisher, Jr., Associate Professor of Medicine in the University of Rochester School of Medicine, New York, lectured Aug. 24 at the Mayo Clinic on "Mechanism of Iso-Immune and Auto-Immune Hemolytic Processes."

♦ **1949**

Capt. Floyd K. Garetz is now on active duty with the U.S. Army as a newly commissioned medical service officer.

Louis G. Stuhler was awarded the degree of Master of Science in medicine from the University of Minnesota July 16, 1959. He has completed a Fellowship in Medicine at the Mayo Foundation and is now practicing in Honolulu, Hawaii.

♦ **1950**

John A. Culligan received a Master of Science Degree in Surgery on June 13, 1959 from the University of Minnesota. He is now practicing in Minneapolis.

Michael P. Sperl, Jr., received a degree of Master of Science in Neurosurgery from the University of Minnesota June 13, 1959, and has gone into practice in St. Paul.

◆ **1952**

L. V. Kragh, fellow in plastic surgery at Mayo Foundation, received the degree of Master of Science in Plastic Surgery from the University of Minnesota on Aug. 20, 1959.

◆ **1954**

Thomas M. Parker was awarded a Master of Science degree in Pathology from the University of Minnesota in June, and will be located in Charleston, S.C.

◆ **1955**

Joseph S. Massee was appointed a fellow in Obstetrics and Gynecology at Mayo Foundation in July.

◆ **1956**

Robert A. Murray, Jr., is now serving a fellowship in Pathology at the Mayo Foundation.

Walter C. Stolov, second year resident in Physical Medicine and Rehabilitation at the University, was named winner of the Sixth Essay Award of the American Congress of Physical Medicine and Rehabilitation. His paper was titled "Rehabilitation of the Bladder in Injuries of the Spinal Cord."

◆ **1957**

John W. Pollard was appointed a fellow in Medicine at the Mayo Foundation in July.

◆ **1958**

Richard W. Fardal, Army 1st Lieutenant, is now on duty at the Tripler U. S. Army hospital in Honolulu, Hawaii. He entered the Army in June 1959.

Capt. Leslie W. Jacobson has received special military orientation training as a newly commissioned medical officer at Ft. Sam Houston, Texas.

Lowell H. Kleven, U.S. Navy medical corps, has been transferred to sea duty with the Fleet Marine Force of the Third Marine Division as he completes his military service.

Charles V. Allen and Thomas J. Lehar were appointed fellows in Medicine at the Mayo Foundation in July.

Lt. Charles D. Lufkin was transferred to sea duty aboard the navy seaplane tender USS Floyds Bay following completion of his internship at the naval hospital in Oakland, Calif.

Troy G. Rollins has taken up duties as dermatologist at the Woodland Clinic, Woodland, California.

Robert R. Rynearson was appointed a fellow in Psychiatry at the Mayo Foundation in July.

♦ **1959**

First Lt. Dean T. Schamber, Atherton, Calif., is interning at the Tripler Army Hospital in Honolulu. His wife, Sharon, is with him in Hawaii.

EDITOR'S NOTE: The MEDICAL BULLETIN invites your contributions to the Alumni Notes column. Send your news to The Editor, UNIVERSITY OF MINNESOTA MEDICAL BULLETIN, 1342 Mayo Memorial, University Campus, Minneapolis 14, Minnesota. Personal news welcome.

Join the
Minnesota
Medical Foundation
Today!