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Staff Meeting Report

The Hormonal Effects of the Islets of Langerhans*

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The pancreatic islet tissue was first described by Paul Langerhans in 1869[1] and was later studied in great detail by Laguesse.[2] The existence of different cell types within the islets of Langerhans, although noted earlier, was carefully investigated by Lane.[3] Working in Professor R. R. Bensley's laboratory, Lane studied the solubility of the granules within the islet cells of the guinea pig. By employing appropriate fixing methods he was able to stain selectively either the alpha or the beta cell granules; he found that these could be clearly differentiated from each other and from the zymogen and prozymogen granules of the pancreatic acinar cells. Subsequently, Bensley[4] was able to stain simultaneously both the alpha and beta cells using his aniline acid fuchsin, methyl green stain. The existence of a third cell type in the islet tissue of the human pancreas stained with the Mallory (Heidenhain) azan method was reported by Bloom,[5] but it has not been observed in all the species studied. The aldehyde fuchsin method introduced by Gomori[6] in 1950, modified slightly by various investigators, has proved to be one of the most reliable procedures for demonstrating the beta cell granules; the beta cell granules are stained a deep purple, while the alpha cell granules are unstained.

Various silver staining methods have also been used to stain the pancreatic islet tissue. Mankowski[7] injected silver nitrate and observed the appearance of black specks within the islets; Van Campenhout,[8] impregnating blocks of pancreatic tissue with silver nitrate, found that the granules of the pancreatic alpha cells as well as those of the intestinal enterochromaffin cells were stained with silver. Although Ferner[9] and others using silver impregnation methods noted that the alpha cell granules were selectively stained with silver, Gomori[10] and Masson[11] were unable to stain the alpha cells using other silver staining methods.

Bell[12] and the Toronto group[13] have reported a good correlation between the number of beta cell granules as estimated cytologically

*This report was given at the Staff Meeting of the University of Minnesota Hospitals on April 25, 1958.

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using aldehyde fuchsin stained material, and the actual insulin content as determined by bioassay. There is good evidence, therefore, that in the normal pancreas the beta cell granule, as stained by the aldehyde fuchsin method, represents stored insulin or a precursor of insulin.

In considering the interrelationship of diabetes, insulin synthesis, and the beta cell, it is important to realize that there are a number of separable steps: The insulin molecule must first be synthesized from its constituent amino acids, after which it can either be secreted directly into the bloodstream or stored as a secretion granule in the beta cell. In the normal person the stored insulin must be released from the beta cell when it is needed and it must be able to reach the peripheral tissue without being destroyed. Finally, the peripheral tissues must be able to respond to insulin. Diabetes could result from any of the following: an absence of beta cells, a block in the insulin synthesizing mechanism, an inability to release stored insulin, an increased rate of destruction of insulin, or a lack of responsiveness of the target organs to insulin.

Beta Cells

Synthesis of Insulin

Some of the steps involved in the synthesis of insulin are illustrated in Figure 1. The number of the component amino acid residues found in the insulin molecule is indicated by the numbers in parentheses; those amino acids that are marked with an asterisk are essential and must be supplied in the diet. The synthesis of insulin depends among other things upon an adequate supply of these essential amino acids being available to the beta cell. The sulfur-containing amino acids may be particularly important for insulin synthesis since they constitute 12 per cent of the amino acid residues in the insulin molecule.[14] These must be supplied in the diet either as cystine (or cysteine) or as methionine, which can be converted to cystine within the body. A diet low in the sulfur-containing amino acids has been shown to decrease the insulin content of the pancreas.[15]

The component amino acids are joined in a specific sequence by acid amide linkages to form two component polypeptide chains, the A and B chains, which contain 21 and 30 amino acids, respectively.[16] The arrangement of the amino acids within the polypeptide chain is not linear: the backbone of the chain is believed to be folded into a compact spiral structure in which the carboxyl group of a given amino acid in the chain is adjacent to the amide group of an amino

SYNTHESIS OF INSULIN

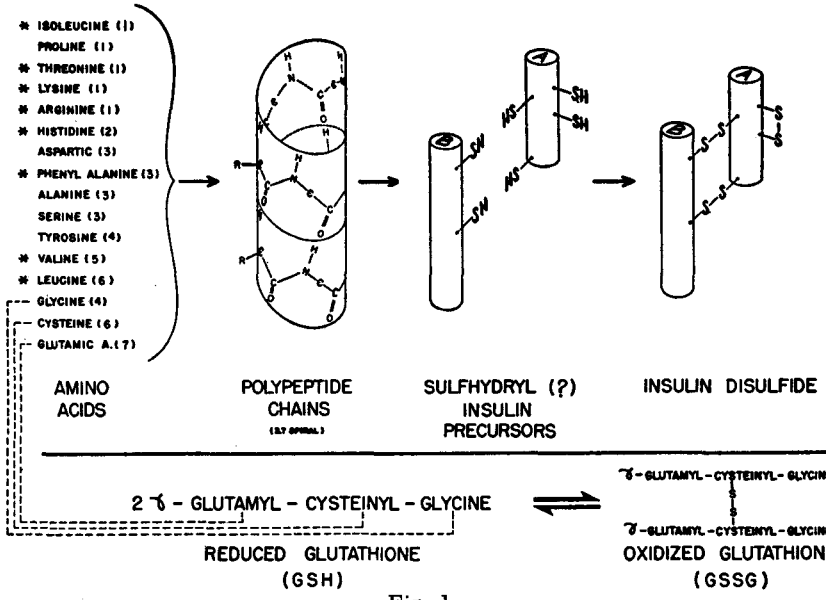

Fig. 1

acid four units along the chain (Fig. 1). Thus hydrogen bonding provides the forces needed to hold the spiral together. The two component A and B chains, however, are held together by two interchain disulfide bridges; in addition there is a third intrachain disulfide bridge within the A chain.[16] In Figure 2 the amino acids are shown entering the beta cell, where they are incorporated into the insulin molecule. The primary synthesized insulin molecule, which has a molecular weight of 6,000, is illustrated in the figure as two cross-linked spiral structures. Practically nothing is known about the mechanism or the enzymes by which the beta cells synthesize insulin. Since the mitochondria are active metabolic sites within the cell, they may well play a role in insulin synthesis by supplying the energy needed for amino acid transformation and peptide bond synthesis. The insulin synthesis may actually take place in another portion of the cytoplasm, for in the liver cell, at least, protein synthesis can occur in other isolated cytoplasmic fractions of the cell, e. g., the microsome fraction.

It has been postulated on purely hypothetical grounds[17] that the component peptide chains of insulin might be synthesized separately, possibly as the sulfhydryl peptide chains, and that the two sulfhydryl

peptide chains might be joined together enzymatically to form the insulin molecule by oxidizing the sulfhydryl groups to disulfide bonds (Figure 1). This would be analogous to the formation of oxidized glutathione,[18] in which two sulfhydryl containing tripeptide molecules are joined together enzymatically in similar fashion by oxidizing their sulfhydryl groups with the formation of an interpeptide disulfide bridge.

Investigators in this department began to use the toadfish as an experimental animal because in this species the insulin producing cells are separated from the exocrine pancreas and the islet tissue is

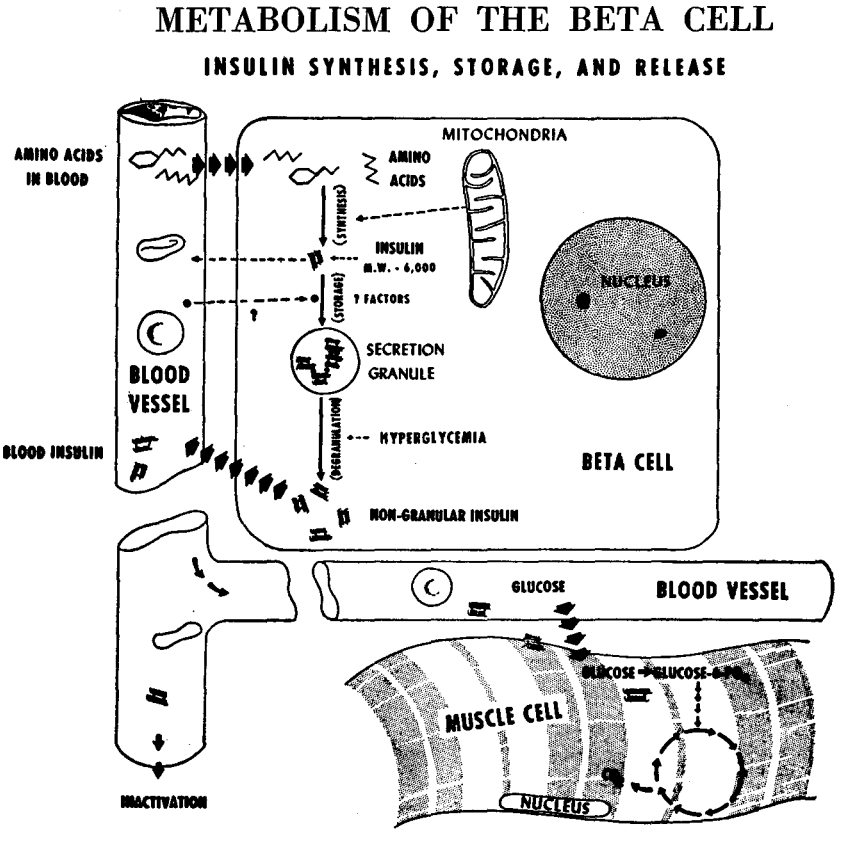

Fig. 2

concentrated into a segregated body known as the principal islet. In mammals, by contrast, the islet cells are scattered throughout the pancreas and are segregated into a million or more individual structures which total only one per cent of the weight of the pancreas. Austin Yates[19] has been seeking evidence of a sulfhydryl peptide insulin precursor in islet tissue. He has used insulin labeled with radioactive iodine (I^{131}) as a marker to follow the distribution of insulin constituents and has separated certain peptide components from fish islet tissue by paper electrophoresis. Nothing is known about the enzymes that synthesize the specific peptide chains of insulin or the factors that join or fold the peptide chains into the highly specific insulin molecule. An interference in insulin synthesis could conceivably occur at any of the many stages in the process.

Storage of Insulin

Although the newly synthesized insulin may be directly excreted into the blood stream, it may also be stored within the beta cell for later use as a secretion granule. The insulin that has been isolated from the pancreas by chemical means has a molecular weight[20] of about 6,000, and it is soluble at neutral pH.[21] By contrast granules containing insulin and presumed to be secretion granules can be centrifuged out of fish islet tissue homogenates.[22,23] The insulin or insulin precursor that is stored as, or in, the beta cell granule therefore appears to be insoluble at the pH of the cell.

The component insulin molecules (M.W. = 6,000) within the secretion granule are held together by an unknown factor, which is represented in Figure 2 by the black dots. Okamato, using a histochemical method, has shown that the islet tissue contains large amounts of zinc.[24] Microchemical determinations of the zinc content of islet tissue by Maske *et al.*[23] and by Davidson[25] have demonstrated that the islet tissue of the fish contains two to three times more zinc than does the liver or kidney. Most of this zinc in the islet tissue can be removed by centrifugation and can be recovered from the granule fraction. The insulin that is isolated from the pancreas by standard biochemical procedures likewise contains some zinc.[26] Indeed, it has been suggested that the insulin monomers of molecular weight equal to 6,000 may be cross-linked by zinc[27] and that the histidine residues in the B chains of insulin are joined together by metal binding with zinc.[28] It has been reported that the addition of zinc to insulin solutions at neutral pH and in the absence of phosphate ion, markedly decreases the solubility of insulin.[29] Although zinc may play a role

in the aggregation of insulin, little is known about the precise nature of the ultra structure of the secretion granule or the way in which insulin is stored within the granule. Although there may be a relationship between zinc, insulin, and the beta cell granule, it may be nonspecific, for alpha cells may likewise contain large amounts of zinc.[30]

It is important to have an accurate estimate of the volume of beta cells within the pancreas as well as to determine granule content within the beta cell. For this purpose an appropriate instrument has been designed,[31] consisting of a motor driven integrating stage and a bank of electronically controlled counters. This apparatus is used for scanning tissue sections, the volume of each tissue component being read directly from the instrument dial. Using this instrument, Carpenter and the author have determined the volume of islet tissue and have measured the separate volumes of the alpha and beta cells within the rat pancreas under various experimental conditions.[32] Studies of the volume of the alpha and beta cells under various experimental conditions are being continued in this laboratory. Application of this integrating scanning procedure to the study of electron micrographs will make it possible to measure the volume of the granules within the beta cell. Clawson *et al.*[33] have successfully used this method to determine the volume of the mitochondria components within the cell.

Release of Insulin

The beta cell releases its stored insulin into the pancreatic vein under the stimulus of hyperglycemia. When the glucose level of the blood perfusing the isolated pancreas is increased to hyperglycemic levels, the pancreas responds by releasing more insulin into the pancreatic vein.[34] Likewise, the transitory elevation of the blood sugar that occurs after the injection of a single intraperitoneal dose of glucose, is followed by a transitory partial degranulation of the beta cells;[35] the number of beta granules returns to normal as the blood sugar is restored. The release of the stored insulin from the beta cell must be associated with an active disaggregation of the beta granules and with a solubilization of the stored insulin. This solubilization may thus provide the basis for a rapid release of insulin into the bloodstream. Although the stimulus of hyperglycemia clearly can bring about the release of insulin, the mechanism of this release is not known.

In order to provide a better understanding of the means by which hyperglycemia and hormonial agents affect the beta cell, C. T. Friz of this department has been making a systematic study of the meta-

bolic activity of islet tissue, using the following procedure: A single toadfish islet is removed and cut into eight slices; these are placed in the cartesian diver microrespirometer, and the oxygen uptake is measured under various experimental conditions. It has been noted that the addition of phosphate ion stimulates the metabolic activity of islet tissue.[36] The effects of adding various substrates and certain inhibitors of specific metabolic steps have also been studied.[37] The evidence obtained to date indicates that glucose is metabolized in islet tissue by means of the glycolytic and tricarboxylic acid cycle. When glucose is added to islet slices it may be converted to glycogen; a progressive increase in the glycogen content of islet tissue occurs when increasing concentrations of glucose are added to the incubating media.[38] Once the basic metabolic pathways in islet tissue have been established, it may be possible to determine the mechanism by which glucose brings about a release of insulin from the beta cell and also to study the way in which hormonal substances such as growth hormone or cortisone affect the islet tissue's metabolic and functional activities.

One must remember that even though the pancreas may be able to synthesize and store insulin normally, a defect in the insulin release mechanism could produce diabetes. Thus, if the pancreas of a given diabetic subject were unable to respond to the stimulus of hyperglycemia by releasing the insulin stored within the beta cell, then the insulin content of this subject's pancreas might well be "normal." In this case there would be no insulin in the blood. On the other hand, with only a partial impairment in the insulin release mechanism, the pancreas might be able to liberate insulin in response to a greater than usual hyperglycemic stimulus. In this instance if the blood sample were drawn from a diabetic subject at a time when his sugar level was markedly elevated, the blood insulin level might approach the "normal range" – notwithstanding the decreased capacity of the pancreas to release insulin into the blood stream (at a normal blood sugar level) in response to a physiologic hyperglycemic stimulus. The absolute insulin contents of the blood or pancreas, then, may not indicate the ability of the pancreas to secrete insulin in response to the usual physiologic stimulus. Thus if we are to differentiate adequately among the possible types of human diabetes, it will be important to be able to measure the capacity of the pancreas to secrete insulin into the blood stream with reference to the absolute blood sugar level at the time the blood sample was taken, and in response to controlled levels of induced hyperglycemia.

Alpha Cells

Earlier investigators had suggested that the alpha cells of the pancreas had functional significance.[4] In 1923 Murlin *et al.*[39] reported the present of an insulin contaminant, which he named glucagon. Upon intravenous administration, glucagon-containing insulin preparations produced an initial transitory elevation in the blood sugar preceding the hypoglycemia that characteristically follows insulin administration. Glucagon, later isolated from the pancreas and purified,[40] increases the blood sugar presumably because it activates the liver phosphorylase system and thereby increases glycogen breakdown in the liver.[40] It has been suggested that islet tissue was the source of glucagon because following duct ligation it was possible to extract increased amounts of this substance from the pancreatic remnant in which only the islet tissue and the ducts remained. Similarly, the glucagon content in the tail of the pancreas was ten times greater than that of the head. Thus, the amount of glucagon extractable from the pancreas parallels the islet tissue content.[41]

In attempts to pinpoint the site of glucagon synthesis within the islet tissue, several studies have implicated the alpha cells as the most probable source. It has been noted that the amount of glucagon which can be extracted from the pancreas is not decreased following destruction of the beta cells by alloxan;[41] similarly, glucagon can be extracted from the duct-ligated, alloxan-injected pancreas.[42] Thus, the destruction of both the acinar and the beta cells does not decrease the glucagon content of the pancreas. The concept that the alpha cells are the site of glucagon synthesis is complicated by the finding that a chemical constituent with physiologic properties similar to those of glucagon has also been isolated from the gastrointestinal tract of some species.[41] The finding that the injection of cobalt[43] or Synthalin®[44] produces a selective injury to the alpha cells in the islet tissue suggested that these compounds would be useful in elucidating the cellular source of glucagon synthesis. It has been shown, however, that while these compounds produce hydropic degeneration and degranulation of alpha cells, they do not cause them to disappear completely.[45]

The studies on the glucagon content of the pancreas following cobalt and Synthalin treatment, as well as the study of the metabolic alteration produced in both normal and diabetic animals following the administration of these drugs, are conflicting and confusing. Although it is probable that the pancreatic alpha cells are the site of glucagon synthesis, there are unfortunately no available methods for the posi-

tive cytochemical identification of glucagon within the alpha cell. One may postulate that the glucagon which is synthesized may presumably be stored as a secretion granule within the cell and may be disaggregated and released into the blood stream as needed. Until an adequate cytochemical method is available, however, it seems probable that any correlated study of the cytological and functional states would be complicated and difficult to interpret; for even were the alpha cells depleted of stored glucagon, the cytoplasm of these cells might not show any change if the cells were stained by a nonspecific staining procedure.

Delta Cells

The delta cells, which were described in the islet tissue of some species more than 25 years ago, have been largely ignored. Although these cells have not been seriously considered as a source of glucagon, this possibility can not be excluded, because most of the evidence cited in support of the alpha cell origin of glucagon is indirect, i. e., it is based primarily on the exclusion of both the acinar and beta cells as a possible source of glucagon. Histologists, primarily because of their training, usually have an unquestioning faith in the cytologically differentiated structures they see under the microscope. The failure to demonstrate physiologic or biochemical significance for these structures usually does not decrease the histologist's belief in their importance. Experience has shown that it is not possible to demonstrate the effect of a hormone which acts on an enzyme or an intermediary metabolic step that has not yet been discovered, and that many decades may elapse between the time a cell type is discovered and the time its effect is explained. The pancreatic delta cells may some day be shown to have an important endocrine function. Therefore, in thinking about the role of the pancreatic islet tissue, we must include the possibility that one or more additional hormonal factors may play a physiologic and metabolic role.

References

1. Langerhans, P.: Beitrage zur Mikroskopischen Anatomie der Bauchspieldruse, Inaug, Diss. Berlin, 1869.
2. Laguesse, E.: Recherches sur l'histogenie du pancreas chez le mouton, J. de l'Anat. et Physiol, Paris, 21:475, 1895.
3. Lane, M. A.: The Cytological Characters of the Areas of Langerhans, Am. J. Anat. 7:409, 1907.
4. Bensley, R. R.: Structures and Relationships of the Islets of Langerhans. Harvey Lectures, Series X, pp. 251, 1914-15.

5. Bloom, W.: New Type of Granular Cell in Islets of Langerhans of Man, Anat. Rec. 49:363, 1931.
6. Gomori, G.: Aldehyde-Fuchsin: New Stain for Elastic Tissue, Am. J. Clin. Path. 20:665, 1950.
7. Mankowski, A.: Ueber die Mikroscopischen Veränderungen des Pankreas nach Unterdimnung (einzelner) Teile. Arch. F. mikr. Anat. u. Entwicklungsgesch. 59:286, 1902.
8. Van Campenhout, E.: Argentaffin Cells of Pancreas, Proc. Soc. Exper. Biol. & Med. 30:617, 1933.
9. Ferner, H.: Beiträge zur Histobiologie der Langerhans'schen Inseln des Menschen mit besonderer Berücksichtigung der Silberzellen und ihrer Beziehung zum Pankreasdiabetes, Virchows Archiv. für Path. Anat. 309:87, 1942.
10. Gomori, G.: Studies on Cells of Pancreatic Islets, Anat. Rec. 74:439, 1939.
11. Masson, P.: Carcinoids (Argentaffin-Cell Tumors) and Nerve Hyperplasias of Appendicular Mucosa, Am. J. Path. 4:181, 1928.
12. Bell, E. T.: The Incidence and Significance of Degranulation of the Beta Cells in the Islets of Langerhans in Diabetes Mellitus, Diabetes 2:125, 1953.
13. Wrenshall, G. A.; Hartroft, W. S.; and Best, C. H.: Insulin Extractable from the Pancreas and Islet Cell Histology, Diabetes 3:444, 1954.
14. Du Vigneaud, V.: The Sulfur of Insulin, J. Biol. Chem. 75:393, 1927.
15. Griffiths, M.: The Mechanism of the Diabetogenic Action of Uric Acid, J. Biol. Chem. 184:289, 1950.
16. Sanger, F.: Some Chemical Investigations on the Structure of Insulin, Cold Spring Harbor Symp. Quart. Biol. 14:153, 1949.
17. Lazarow, A.: Alloxan Diabetes and the Mechanism of Beta Cell Damage by Chemical Agents, In *Experimental Diabetes and Its Relation to Clinical Disease,* Oxford, Blackwell Scientific Publications, 1954, p. 49.
18. Hopkins, F. G. and Dixon, M.: On Glutathione. II. A Thermostable Oxidation-Reduction System, J. Biol. Chem. 54:527, 1922.
19. Yates, Austin: Unpublished observations.
20. Sanger, F.: Structure of Insulin, Bull. Soc. Chem. Biol. 37:23, 1955.
21. Jensen, H. F.: *Insulin: Its Chemistry and Physiology,* London, Oxford University Press, 1938.
22. Lazarow, A.: Unpublished observations.
23. Maske, H.; Munk, K.; Homan, J. D. H.; Houman, J.; and Matthijsen, R.: Ueber die Verteilung von Insulin und Zink in verschiedenen Zellbestandteilen der Rieseninseln bei Pleuronectiden, Zeit. Naturforsch. 116:407, 1956.
24. Okamato, K.: Biologische Untersuchungen der Metalle. VI. Histochemischer Nachweis einiger Metalle in den Geweben, besonders in den Nieren und deren Veränderungen, Trans. Soc. Path. Jap. 32:99, 1942.
25. Davidson, Joseph: Zinc Content of Toadfish (Opsanutau) Islet Tissue, Anat. Rec. 130:403, 1958.
26. Scott, D. A.: Crystalline Insulin, Biochem. J. 28:1592, 1934.
27. Tanford, C. and Epstein, J.: The Physical Chemistry of Insulin. II. Hydrogen Ion Titration Curve of Crystalline Zinc Insulin; The Nature of Its Combination with Zinc, J. Am. Chem. Soc. 76:2170, 1954.

28. Lindley, H.; and Rollett, J. S.: Investigation of Insulin Structure by Model-Building Techniques, Biochem. et Biophys. Acta 18:183, 1955.
29. Hallas-Moller, K.; Petersen, K.; and Schlichtkrull, J.: Crystalline and Amorphous Insulin Zinc Compounds with Prolonged Action, Science 116:394, 1952.
30. Wolff, I. H. and Ringer, D.: Histochemical Study of [Pancreatic] Island Zinc, Naturwissenschaften 41:260, 1954.
31. Lazarow, A. and Carpenter, A-M. Volume Quantitation of Tissue Components Using a Motor-Driven, Electronically-Controlled Integrating Stage, J. Histo. & Cyto. Chem. In press.
32. Carpenter, A-M. and Lazarow, A.: Volume Dtermination of Pancreatic Components [Islet: Acinar and Alpha:Beta Cell Ratios] Using a Motor-Driven Integrating Stage, J. Histo. & Cyto. Chem. In press.
33. Clawson, Carlyle; Carpenter, A-M.; Vernier, R.; Hartmann, F.; and Lazarow, A.: Volume Ratio of Mitochondria, Nucleus, and Cytoplasm of Rat Liver. Grid Scan of Electron Micrograghs Compared with Integrating Stage Scan of Histologic Slides, J. Histo. & Cyto. Chem., In press.
34. Anderson, Evelyn and Long, J. A.: Effects of Hyperglycemia on Insulin Secretion as Determined with the Isolated Rat Pancreas in a Perfusion Apparatus, Endocrinology 40:92, 1947.
35. Gomori, G.; Friedman, N. B.; and Caldwell, D. W.: Beta Cell Changes in Guinea Pig Pancreas in Relation to Blood Sugar Level, Proc. Soc. Exper. Biol. & Med. 41:567, 1939.
36. Lazarow, A.; Cooperstein, S. J.; Bloomfield, D. K.; and Friz, C. T.: Studies on the Isolated Islet Tissue of Fish. II. The Effect of Electrolytes and Other Factors on the Oxygen Uptake of Pancreatic Islet Slices Using the Cartesian Diver Microrespirometer, Biol. Bull. 113:414, 1957.
37. Friz, C. T.: Unpublished observations.
38. Cascarano, Joseph: Unpublished observations.
39. Murlin, J. R.; Clough, H. D.; Gibbs, C. B. F.; and Stokes, A. M.: Aqueous Extracts of Pancreas. I. Influence on the Carbohydrate Metabolism of Depancreatized Animals, J. Biol. Chem. 56:253, 1923.
40. Sutherland, E. W.: The Effect of the Hyperglycemic Factor of the Pancreas and of Epinephrine on Glycogenolysis. VI. Mechanisms of Hormone Action, in *Recent Progress in Hormone Research*, New York, Academic Press, Inc., 5:441, 1950.
41. Sutherland, E. W. and DeDuve, C.: Origin and Distribution of the Hyperglycemic-Glycolytic Factor of the Pancreas, J. Biol. Chem. 175: 663, 1948.
42. Cavallero, C.: Études sur le facteur hyperglycémiant du pancreas [glucagon], Rev. Canad. de Biol. 12:509, 1953.
43. Van Campenhout, E. and Cornelius, G.: Experimental Destruction of Alpha Cells of the Endocrine Islets of the Pancreas in the Guinea Pig. Compt. rend. Soc. Biol. 145:933, 1951.
44. Davis, J. C.: Hydropic Degeneration of the Alpha Cells of the Pancreatic Islets Produced by Synthalin, Am. J. Path. Bact. 64:575, 1952.
45. DeDuve, M. and Stahl, J.: Glucagon, the Hyperglycemic Glycogenolytic Factor of the Pancreas, Lancet 2:99, 1953.

Staff Meeting Report

Intraocular Foreign Bodies: Prevention, Diagnosis, and Treatment*

Richard A. Ness, M.D.†

Introduction

Although the subject of this paper lies primarily in the domain of the ophthalmologist, it should be of interest to every physician and particularly to the general practitioner, who is often the first to see the patient with ocular complaints or ocular injuries. It is his recognition of the seriousness of symptoms that sometimes seem trivial and his prompt referral to an ophthalmologist that may save an eye.

Prevention

The initial treatment of intraocular foreign bodies begins with prevention of the accident. Industry has long recognized the seriousness of eye injuries with the attendant loss of personnel and the expense of compensation. Thus wearing safety glasses has been made compulsory for employees in hazardous occupations. The "Wise Owl Club" has been established in industry to promote eye safety by educational propaganda. When an employee is involved in an accident and his eyes are saved from injury because he has been wearing safety glasses, he is initiated into the club with appropriate publicity. The effect on the other employees is obvious; but as in any other random sample, there are those who through stubbornness or carelessness will not wear or forget to wear their safety glasses. From this group come the bulk of the industrial accidents to the eye.

Other groups of accident prone individuals are the do-it-yourself hobbyists and the self-employed, especially small garage operators and farmers. As will be seen in our analysis of cases, ninety per cent of the intraocular foreign bodies are ferrous and are caused by pounding steel on steel. There was the case of a young man hammering on the drive shaft of his automobile; another case was that of a farmer hitting the plow with a sledge hammer at the end of every row to

*This report was given at the Staff Meeting of the University of Minnesota Hospitals on May 2, 1958.
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knock the dirt loose. It is these self-employed and the hobbyists to whom safety glasses are not readily available, and it is they who must be educated.

Information for the physician's office on the prevention of blindness may be obtained from the Minnesota Society for the Prevention of Blindness, Palace Building, Minneapolis, Minnesota, and from the Minnesota Services for the Blind, 476 St. Peter Street, St. Paul, Minnesota.

Safety devices consist of the hardened or tempered lens, the laminated lens with a plastic binder between two surfaces of glass, and various types of safety goggles. Information on these is obtainable through Bausch and Lomb, American Optical Company, or through local opticians.

Emergency Treatment for Intraocular Foreign Bodies

The treatment begins with early recognition of symptoms by the patient and his doctor. The initial symptoms may seem so trivial as to be shrugged off by the patient, but this should not mislead the doctor into an erroneous diagnosis. Often the patient is told that he has conjunctivitis, is given a tube of ointment, and is asked to come back in a few days. The site of entry may not be obvious. With a history of hammering steel on steel and symptoms of ocular injury, it is the duty of every physician to order x-rays. As in any examination of the eye, a record of the visual acuity should be made if possible before any manipulation, because of its prognostic value and because of medicolegal implications.

A lateral x-ray of the skull should be made with the affected eye closest to the film, and also a postero-anterior film with the face tilted 15–20 degrees upward from the horizontal plane, so that the petrous pyramids are not projected onto the orbits (Caldwell position). If a foreign body is found, several films may be taken with the eye looking first up then down to determine whether or not there is a displacement of the foreign body. If the foreign body is in the globe or in the epibulbar structures, it should be displaced with movements of the eye, unless it is in the center of the globe. With the x-rays as a guide, it is sometimes possible to see the foreign body using plus lenses in the ophthalmoscope if the foreign body is in the vitreous.

Assuming a positive diagnosis, the general physician should refer the patient to the ophthalmologist for whatever salvage is possible. The general physician should start antitetanus therapy, either antitoxin or a booster shot of toxoid, and antibiotics should be administered

after proper inquiries about possible sensitivity. Although medical opinion differs, the general approach is to administer large amounts of penicillin against possible gram-positive soil organism contamination and the gram-positive conjunctival contaminants, and in addition to use a broad spectrum antibiotic such as chloramphenacol or tetracycline. Usually by the time cultures of infecting organisms are available, panophthalmitis has developed and the eye is lost; therefore early and large doses of antibiotics are indicated.

Cleansing and débridement of the ocular wound should not be attempted before the patient reaches the ophthalmologist. Prolapsed iris has been known to be mistaken for a blood clot, and the result from attempted removal was disastrous. If the eye appears severely injured or a bulbar laceration is obvious, both eyes should be occluded with eye pads or gauze and elastoplast dressings, a good treatment in any event. No medication should be put on the eye, particularly if a laceration is present. Ointment not only obscures the ophthalmoscopic picture but also may be aspirated into the lacerated globe.

Treatment by the Ophthalmologist

Assuming the emergency care has been initiated, the ophthalmologist's job is to remove the foreign body if possible, replace or excise prolapsed intraocular contents, and restore the continuity of the cornea or sclera. If the eye is beyond repair or panophthalmitis is obvious, enucleation or evisceration is indicated.

Removal of the foreign body depends first on the nature of the intraocular material (vegetable or mineral, ferrous or non-ferrous) and second upon adequate localization. The majority of foreign bodies are steel and are radio-opaque and can be extracted with a magnet, but greater localization is required than that given by lateral skull and postero-anterior (Caldwell) films.

There are more specialized radiographic techniques for the localization of intracoular foreign bodies: In the Sweet method, generally used at the University Hospitals, the foreign body is located geometrically in relation to two metal reference beads (one of which is touching the lids and the other exactly 10 mm. in front of the cornea), and their positions related in two postero-anterior films taken with a known lateral displacement of the x-ray tube. In the Comberg technique, a contact lens containing four lead dots is placed on the eye and postero-anterior and lateral films are taken to relate the foreign body to the four dots. In another technique, a metallic ring is sutured to the eye at the limbus and again posterio-anterior and lateral films are

taken. When there are multiple foreign bodies in the globe and orbit, these methods of localization may not be possible.

Since the Berman Locator has become available, the task of locating and removing intraocular metallic foreign bodies has become infinitely easier. This apparatus consists of a portable tuning console operated by an assistant and a movable probe manipulated by the surgeon to localize the intraocular foreign body at the time of surgery. The probe creates a weak electromagnetic field that is altered by approaching a metallic foreign body. As the probe approaches a foreign body, there is a deviation on an indicator and an increase in the pitch of an audible tone from a loudspeaker. By this means a point is located perpendicular to the tangent at the surface of the globe closest to the metallic intraocular foreign body, the shortest route for removal.

If the foreign body is in the anterior chamber or is embedded in or immediately behind the iris, it can occasionally be drawn into the anterior chamber whence it can be removed. Sometimes a magnetic foreign body can be backed out through the wound of entry. If this fails, the foreign body may be removed via a limbal incision with a keratome. If the intraocular foreign body is embedded in the lens or has injured the lens, a cataract extraction may be indicated either at the initial surgery or at a later date.

When the intraocular magnetic foreign body is in the posterior part of the globe, it can sometimes be drawn to the pars plana portion of the ciliary body whence it may be extracted through a scleral incision, minimizing trauma to the retina. If the foreign body is located in the posterior pole or is embedded in hemorrhage and exudate so that it cannot be moved any great distance, it may be extracted through a scleral wound at its closest point to the surface. A barrage of diathermy punctures is placed about the proposed wound of exit. This serves both to coagulate the underlying choroidal vessels and to seal the resulting retinal tear when the foreign body is extracted. When the magnetic foreign body cannot be drawn to the sclera for extraction, a ferrous probe is inserted into the posterior cavity of the globe and magnetized in a desperate hope of attracting and extracting the foreign body.

When the intraocular foreign body is composed of vegetable or non-magnetic mineral matter, the case is much more hopeless. Rarely it is possible to extract these under direct vision. For such removal under direct vision the Thorpe Endoscope, a small cystoscope-like instrument, has been designed for introduction into the eye.

Complications

Intraocular foreign bodies in the posterior part of the globe and lying in the vitreous are surrounded by a medium as compatible for growth of bacteria as anything found in the bacteriological laboratory. The danger of infection and panophthalmitis is always present, and antibiotic therapy is therefore indicated before a culture of infecting organisms becomes possible. Generally speaking, an eye with a gross intraocular infection is an eye lost.

With perforating wounds of the anterior segment of the eye involving the ciliary body or the iris, if the injured eye is not promptly enucleated, a bilateral uveitis develops in as many as three to five per cent of the cases. This is called sympathetic ophthalmitis. Enucleation of the injured eye if performed within two weeks following the injury gives almost complete protection and if performed within a week protection is complete. The clinical picture of the disease differs little from that of other forms of granulomatous endophthalmitis. The inflammatory reaction develops simultaneously or almost simultaneously in both eyes and in the early stages proceeds apparently uninfluenced by conventional therapy. Heavy "mutton-fat" corneal deposits, the development of posterior synechiae, and a turbid aqueous are seen on slit-lamp and biomicroscopic examination. Histologically, there is a granulomatous inflammation of the uveal tissues, with characteristic nests or areas of almost homogeneous epithelioid cells alternating with zones of lymphocytic infiltration. The etiology of this rare disease is still unknown. Treatment, again, is enucleation of a severely injured, blind eye. If the eye is not enucleated, prophylactic treatment for sympathetic ophthalmitis should be started by the ophthalmologist as soon as the patient enters the hospital or has had surgery. The classic treatment of sympathetic ophthalmitis is foreign protein fever therapy. With recent developments, the prophylaxis of sympathetic ophthalmitis has been supplemented by adrenocorticotropic hormone and the corticosteroids. The preventative treatment and follow-up examinations for sympathetic ophthalmitis should be undertaken only by the ophthalmologist.

The cataract or complete disruption of the lens may result from ocular trauma. The iris may prolapse through wounds of the cornea or of the limbal area, and the uveal tissue may prolapse through wounds posterior to the limbus. Vitreous may exude and be lost through such lacerations. As massive vitreous hemorrhages are organized a subsequent retinitis proliferans may develop. Retinal tears due to penetration of the foreign body or due to extraction of the foreign

body may result in a retinal detachment. Retinal detachment may also result from the pull from an organizing retinitis proliferans. A secondary glaucoma may result from massive hemorrhage or blockage of the chamber angle due to a disrupted lens.

It may not be possible to extract the foreign body, in which case it is retained in the globe. If it is relatively inert, such as glass, sand, or rock, and if the infection is overcome, no serious consequences may result. Limestone, however, causes a marked ocular reaction. If the retained material is ferrous, a condition known as siderosis results. The iron pigments have a predilection for seeking the epithelial structures of the eye. A blind, shrunken eye may result. Copper, found in many alloys such as brass and as a trace metal in aluminum, when retained inside an eye results in a condition called chalcosis, often accompanied by a degeneration similar to that caused by iron. Another complication produced by intraocular foreign bodies is double perforation, a condition in which the foreign body may have perforated through the globe and may be located in the retrobulbar structures.

Case Summaries

From June, 1952, through April, 1958, 31 patients were treated for intraocular foreign bodies at the University of Minnesota Hospitals. Twenty-eight of these foreign bodies were ferrous and had entered the eye as a result of hammering steel on steel; all 28 patients were male, ranging in age from eight to sixty-five years, with an average age of thirty-six years. Two cases (6.5 per cent) were due to lead shotgun pellets, one in a man thirty-five years old, the other in a woman twenty-nine years old (the only female in the entire series). The one remaining case was caused by a dynamite cap explosion affecting the eyes of a child six years of age, with multiple brass foreign bodies in both orbits, resulting in the loss of one eye and vitreous hemorrhage in the other. There were no intraocular foreign bodies due to BB guns or fireworks.

The left eye was injured in 11 cases (35.5 per cent), the right eye in 20 cases (64.5 per cent). In one case both eyes were injured by the accident, but only the left contained the foreign body.

The intraocular foreign body was removed via the anterior chamber route in five instances (16.1 per cent) and via the posterior route in 22 cases (70.9 per cent). The involved eye was removed at the time of initial surgery in three cases (9.7 per cent). The intraocular foreign body was not removed in one instance. It was necessary to

remove and replace the extraocular muscles in eight patients (25.8 per cent).

The follow-up information, which is not entirely complete, indicates that vision is 20/20 in six patients (19.4 per cent) and 20/50 or better in 11 (35.5 per cent). Cataracts occurred in 12 patients (38.7 per cent). Seven patients (22.6 per cent) lost the injured eye at the time of initial surgery or later. It was judged that 87.1 per cent of the accidents resulting in intraocular foreign bodies among patients in this series were preventable.

Summary

The vast majority (90 per cent) of intraocular foreign bodies resulted from hammering metal on metal. The practitioner should inquire about a history of hammering in cases of seemingly trivial eye injuries. In all questionable instances x-rays of the orbit should be taken. Antitetanus therapy and adequate antibiotics should be instituted early. Salvage of the eye depends on early removal of the intraocular foreign body by the ophthalmologist, using all the aids available to him.

SOURCE MATERIAL

1. Friedenwald, J. S.; Wilder, H. C.; Maumenee, A. E.; Sanders, T. E.; Keyes, J. E. L.; Hogan, M. J.; Owens, W. C.; and Owens, E. U.: *Ophthalmic Pathology: An Atlas and Textbook,* Philadelphia, W. B. Saunders Co., 1952.
2. Hughes, W. F. Jr.: *Office Management of Ocular Diseases,* Chicago, The Year Book Publishers, Inc., 1953.
3. Pamphlet: Berman Metal Locator, Berman Laboratories, 112 Rockaway Blvd., Ozone Park 20, New York.

Editorial

Importance of the Internship

American medical education is stereotyped in many respects. Although rumblings of change are occasionally heard, the usual sequence is at least three years of liberal arts preparation; four years in medical school; and then an apprenticeship as an intern for at least one year in a hospital. The internship is almost a unique feature of the training of American doctors. Achieving entrance into a medical school and being accepted as an intern in the hospital of his choice are two highlights in the life of any aspiring physician.

Selecting a hospital for an internship should not be done lightly. The internship is a period when the young physician learns more about the nature of disease and the management of patients than he does in any other comparable amount of time in his career. During his internship he crystallizes and correlates the knowledge obtained in medical school. He acquires habits and learns techniques that will be part of his daily routine in the practice of medicine for the rest of his life. During this training, therefore, it is essential that he have competent supervision. Every hospital with interns should be a "teaching hospital." The senior staff should take a personal interest in the development and progress of the interns.

The success of a medical school is measured largely by the accomplishments of its graduates. At this time of year it is interesting to note where the graduates in the class of 1958 of the University of Minnesota Medical School are going, and what type of internship they have selected. Perhaps the most significant feature is that over 90 per cent of this year's graduates have chosen rotating internships. Of the 112 graduates, 31, or 27 per cent, are headed for California. This migration of Minnesotans to California has now become an established trend, with active clusters of Minnesota doctors to be found on the West Coast. Is this a studied attempt to exchange the cold and ice of this north country for the sun (and gold) of California? Thirty-seven graduates will serve internships in the Twin Cities, and nine will be in hospitals in Duluth. Fourteen graduates will go into institutions connected with the Federal Government: eight into hospitals of the United States Public Health Service, and six into Army, Navy, or Air Force installations.

While the majority of graduates from Minnesota prefer a rotating

internship, past experience has shown that such a selection is quite often only a preparatory step toward further postgraduate training in a specialty, and a number of our graduates have gone on to full time academic positions after a rotating internship.

The internships selected by the graduates of Minnesota can be contrasted with those chosen by the graduates of Harvard Medical School, among whom a high proportion have tended to enter immediately into specialty training and a good number to pursue an academic career. *The Harvard Medical Bulletin* for May, 1958, lists the internships selected by the 135 graduates in the class of 1958. Forty-three, or only 31 per cent, will start a rotating internship, while 86, or 63.7 per cent, have selected straight internships; 50 of them have chosen medical appointments, and 36, surgical internships. About 30 per cent of the class will remain in Boston hospitals.

A major factor in determining an individual's choice of internship appears to be economic. Approximately 75 per cent of the graduating students at Minnesota are married, and many of them have children. Hospitals are often selected because they offer the highest income. It is to be hoped that those institutions offering economic security will also present a thorough and sound training. While medical educators should concern themselves with the medical curriculum of the undergraduate students in medical schools, the objectives and standards at the postgraduate level cannot be neglected.

Medical School Activities

Faculty News

The annual Benjamin Rachford Lectures were presented by DR. ROBERT A. GOOD, Professor, Department of Pediatrics, at the Cincinnati Children's Hospital, Cincinnati, Ohio, in February, 1958. His lectures were entitled "Disturbance in Gamma Globulin and Antibody Synthesis" and "Diffuse Renal Disease of Childhood."

DR. RAY ANDERSON, Assistant Professor, Department of Pediatrics, attended the Symposium on Genetics in Medical Research, April 7, 1958. The primary purpose of the symposium was to promote interest in medical or human genetics in the medical teaching centers of the country.

DR. JOHN A. ANDERSON, Professor and Head, Department of Pediatrics, presented the annual Samuel Clauson Lecture at the University of Rochester, New York, on February 11, 1958. His presentation was entitled "Ammonia Metabolism, Clinical Significance." He also presented a series of lectures to the Honolulu Pediatric Society and the staff at the Kauikeolani Children's Hospital, Honolulu, Hawaii, March 31 through April 4.

The International Council for Health and Travel has announced the recipients of the Purdue Frederick Medical Achievement Travel Awards for outstanding medical and scientific activities. Two of the award winners are from the United States, both of the University of Minnesota: DR. C. WALTON LILLEHEI, Professor, Department of Surgery, and DR. EDGAR V. ALLEN, Professor of Medicine, Graduate School, Mayo Foundation. The awards will enable the winners to attend medical meetings of their choice here and in Europe, thereby "furthering the international exchange of medical ideas and information on a physician-to-physician basis."

DR. FRED GOETZ, Assistant Professor, Department of Medicine, presented a paper, "Insulin Secretion by the Pancreas," at the American Physiological Society meeting in Philadelphia, April 16.

The Minnesota Heart Association's Board of Directors conferred its distinguished service award on DR. JOHN F. BRIGGS, Clinical Professor of Medicine, honoring him for his faithful and inspirational service in founding the Association in 1948, and for his leadership in the ensuing years. The Association also announced the appointment of DR. HELEN M. WALLACE, Professor of Maternal and Child Health,

School of Public Health, as chairman of a newly created committee on school health.

Dr. C. J. Watson, Professor and Head, Department of Medicine, has been invited to give the Alpha Omega Alpha Lecture at the University of Arkansas Medical School, Little Rock, on May 23.

Recently elected an active member of the New York Academy of Sciences is Dr. Josef Brozek, Professor, Division of Physiological Hygiene. He also became a corresponding member of the Anthropological Association, Brno, Czechoslovakia.

Dr. Eugene A. Johnson, Assistant Professor, School of Public Health, presented a paper, "Monitoring and Evaluating Treatment Effect in Epileptics By A Graphical Sequential Test," at the Indianapolis meeting of the American Association for the Advancement of Science, held in December, 1957. Several new appointments have been made to the School of Public Health: Dr. Richard M. McHugh, who served as Visiting Lecturer last year while on leave from Iowa State College, has accepted a permanent appointment as Associate Professor. Mr. Byron William Brown, Jr., has rejoined the school as Lecturer after spending last year as Assistant Professor of Biostatistics in the Department of Public Health and Preventive Medicine at Louisiana State University.

Student News

On May 6, the following members of the Senior Class were received into membership in Alpha Omega Alpha, honorary medical fraternity:

James M. Anderson
Raymond G. Armstrong
William D. Backar
Louis W. Banitt
David A. Berman
James G. Cardle
Paul L. Felion
Gerald A. Gretsch
Glen A. Hartquist
Eugene G. Hunder
Loren R. Leslie
John I. Levitt
Charles D. Lufkin
Eugene T. O'Brien
Ray P. Rasmussen, Jr.
Barbara A. Rosine
Carl E. Sandberg
Leonard D. Schloff
Floyd J. Swenson

In addition, the following members of the Junior Class were also initiated:

John D. Banovetz
Benjie L. Goldfarb
Ronald John Nelson

Faculty Publications

ANDERSON, JOHN A.; ZIEGLER, M. R.; and DOEDEN, D.: Banana Feeding and Urinary Excretion of 5-Hydroxylindoleacetic Acid, Science 127:236, 1958.

BROZEK, J.: Biology of Human Aging, Newsletter Gerontological Soc. 5:7, 1958.

BROZEK, J.: Multilingual Reporting of Scientific Data, Science 127: 764, 1958.

BROZEK, J.: Physiological Psychology, Ann. Rev. Psychol. 9:71, 1958.

CROWELL, R. L. and SYVERTON, J. T.: Effects of Coxsackie B-3 Virus Infection on Cathepsin and Transaminase Activities of Mammalian Cells in Continuous Culture, Fed. Proc. 17:508, 1958.

FERGUSON, R. B. and LICHSTEIN, H. C.: Comparison of Microorganisms for the Assay of Bound Biotin, J. Bact. 75:366, 1958.

GOOD, R. A.; ABERNATHY, R. S.; and STREM, E. L.: Gamma Globulin Treatment of Asthma: A Double-blind Controlled Study, Proc. Am. Acad. Allergy, 14th Ann. Meeting, 1958.

GOOD, R. A.; ARHELGER, R.; SMITH, F.; BRUNSON, J.; and VERNIER, R.: Influence of Endotoxin on Response of Rats to Nephrotoxic Serum, Fed. Proc. 17:1672, 1958.

GRANDE, F.; ANDERSON, J. T.; and KEYS, A.: Changes of Basal Metabolic Rate in Man in Semistarvation and Refeeding, J. Appl. Physiol. 12:230, 1958.

GRANDE, F.; TAYLOR, H. L.; ANDERSON, J. T.; BUSKIRK, E.; and KEYS, A.: Water Exchange in Men on a Restricted Water Intake and a Low Calorie Carbohydrate Diet Accompanied by Physical Work, J. Appl. Physiol. 12:202, 1958.

HARRIS, S. J.; BROZEK, J.; and SMITH, K. U.: The Effects of Caloric Restriction on the Travel and Manipulation Components of Human Motion, Internat. Z. angew. Physiol. einschl. Arbeitsphysiol. 17:34, 1958.

JENSEN, R. A.: Guest Editorial, Mental Hygiene, January 1958.

KEYS, A.; ANDERSON, J. T.; and GRANDE, F.: Essential Fatty Acids, Lipid Metabolism and Atherosclerosis, Letter to Lancet, 1:742, 1958.

KEYS, A.; KIMURA, N.; KUSUKAWA, A.; BRONTE-STEWART, B.; LARSEN, N.; and KEYS, M.: Lessons From Serum Cholesterol Studies in Japan, Hawaii, and Los Angeles, Ann. Int. Med. 48:83, 1958.

WEEKLY CONFERENCES OF GENERAL INTEREST

Physicians Welcome

Monday,	9:00 to 10:50 A.M.	OBSTETRICS AND GYNECOLOGY Old Nursery, Station 57 University Hospitals
	12:30 to 1:30 P.M.	PHYSIOLOGY- PHYSIOLOGICAL CHEMISTRY 214 Millard Hall
	4:00 to 6:00 P.M.	ANESTHESIOLOGY Classroom 100 Mayo Memorial
Tuesday,	12:30 to 1:20 P.M.	PATHOLOGY 104 Jackson Hall
Thursday,	11:30 A.M. to 12:30 P.M.	TUMOR Todd Amphitheater University Hospitals
Friday,	7:45 to 9:00 A.M.	PEDIATRICS McQuarrie Pediatric Library, 1450 Mayo Memorial
	8:00 to 10:00 A.M.	NEUROLOGY Station 50, University Hospitals
	9:00 to 10:00 A.M.	MEDICINE Todd Amphitheater, University Hospitals
	1:30 to 2:30 P.M.	DERMATOLOGY Eustis Amphitheater University Hospitals
Saturday,	7:45 to 9:00 A.M.	ORTHOPEDICS Powell Hall Amphitheater
	9:15 to 11:30 A.M.	SURGERY Todd Amphitheater, University Hospitals

For detailed information concerning all conferences, seminars, and ward rounds at University Hospitals, Ancker Hospital, Minneapolis General Hospitals, and the Minneapolis Veterans Administration Hospital, write to the Editor of the BULLETIN, 1342 Mayo Memorial, University of Minnesota, Minneapolis 14, Minnesota.