VOLUME XXVIII • NUMBER 16

UNIVERSITY OF MINNESOTA

Medical Bulletin

OFFICIAL PUBLICATION OF THE
UNIVERSITY OF MINNESOTA HOSPITALS
THE MINNESOTA MEDICAL FOUNDATION
AND THE MINNESOTA MEDICAL ALUMNI
ASSOCIATION

IN THIS ISSUE:

Effects of Acidosis
Scoliosis
Radiation Effects

University of Minnesota Medical Bulletin

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VOLUME XXVII | *June 15, 1957* | NUMBER 16

CONTENTS

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

Staff Meeting Reports

The Impact of Acidosis on the Human Fetus*

Robert C. Goodlin, M. D.[1] and Irwin H. Kaiser, M. D., Ph. D.[2]

Acid-base balance of the fetus has been a matter of study for almost 75 years. Cohnstein and Zuntz in 1884 reported increased blood carbon dioxide values in the fetal goat. Their work has been continued, particularly by Barcroft and his associates, until at the present time, fetal electrolyte and gas values are known for a number of species.

It is simpler to study intrauterine physiology in the common farm animals than in primates. The bicornuate uterus of these farm animals allows study of multiple pregnancy, a single fetus at a time. The third stage of labor is frequently prolonged, allowing ample time for fetal study. In the ewe for instance, the fetus may be delivered by cesarean section, and the placenta, which is contra-decidual in type, does not immediately separate. Fetal respiration can be prevented by using a nose bag filled with amniotic fluid. It is assumed that, under these circumstances, the newborn lamb continues to function as a fetus. The uterus simplex and the deciduate placenta, characteristic of most primates, ordinarily react to the removal of the fetus by immediate placental separation. It is possible, however, to perform intrauterine studies on at least one primate. The rhesus monkey usually has two placentae with interplacental vessels in an extra-amniotic position. It is then possible to obtain fetal blood samples by hysterotomy, leaving the pregnancy intact.

Because of species differences, information obtained on animal fetuses must be applied to the human fetus with reservations. For example, the structure and function of the placenta, the identity of the blood sugars, the chemical characteristics of hemoglobin, and the size and number of erythrocytes differ in the various mammals.

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* This is an abstract of a report given at the Staff Meeting of the University of Minnesota Hospitals on May 31, 1957. A copy of the complete report, including tables, graphs, and references, may be obtained by writing to the Editor, UNIVERSITY OF MINNESOTA MEDICAL BULLETIN, 1342 Mayo Memorial, Minneapolis 14.

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To date, no one has reported the study of human fetal blood samples obtained in the course of a continuing pregnancy. On a few occasions, we have drawn blood samples from pre-viable fetuses with prolapsed cords. These are unphysiological conditions and data thus obtained must be interpreted with caution.

Studies on the human fetus have, therefore, generally been limited to the time of delivery. This limitation in turn has introduced experimental variables due to changing intrauterine conditions in the process of delivery. In addition, many physiologic conditions change significantly during the course of the pregnancy. The data obtained at delivery should be cautiously interpreted in estimation of intrauterine conditions at any other time.

Probably the earliest report of acid-base balance in the human fetus at term was that of W. Blair Bell in 1928. He noted that the fetal blood pH was uniformly lower than the maternal blood pH. Numerous reports have since confirmed that the human fetus exists at a higher blood hydrogen ion concentration than its mother or the normal adult.

In instances of asphyxia neonatorum, the infant has been found to be in a severe degree of acidosis and hypoxia. Both the low pH and the decreased oxygen saturation have been held responsible for the high fetal loss found in this condition. As far as is known, the fetus has never been observed to be in a state of relative alkalosis in comparison with its mother.

It seemed reasonable to study acidosis in the fetus by producing acidosis in patients with apparently normal pregnancies and by studying infants born of diabetic mothers.

Method

The maternal acidosis was produced with ammonium chloride. Ammonium chloride has long been used in the laboratory as a means of producing metabolic acidosis and clinically it is frequently used as a mild diuretic agent. As long as renal function is adequate, fetal deaths have not been reported from its extensive use. Patients in the thirty-ninth week of gestation and not in labor were given 6 grams of enteric coated ammonium chloride a day until the time of delivery. The longest period of constant medication was 28 days. Blood was collected from the umbilical vessels in heparinized, oiled syringes as soon as possible after delivery, nearly always before the infant had

begun to breathe. Umbilical artery, umbilical vein, and maternal vein samples were obtained and placed in ice. At the time of cesarean section, the cord was delivered first and the blood sampls obtained before any effort was made to deliver the infant.

Results

Blood was collected without stasis from the antecubital veins of patients in the thirty-ninth week of gestation. These patients were not in labor and had not received ammonium chloride. As the blood flow is markedly increased in the upper extremities during pregnancy, blood from the antecubital vein of pregnant women contains more oxygen than that from non-pregnant women. This source of maternal blood was chosen because of the technical difficulties in obtaining maternal arterial blood at the time of delivery. Values obtained for blood pH, bicarbonate (HCO_3), and carbon dioxide partial pressure (pCO_2) were slightly below the normal for non-pregnant individuals. Maternal blood lactic acid levels are significantly elevated in the third trimester of pregnancy, and the respiratory minute volume is known to increase progressively as term approaches. These facts indicate that the normal pregnant woman at term has a mild compensated metabolic acidosis.

Blood chemical determinations were made at the time of delivery in 11 patients who had received no ammonium chloride. Despite the fact that all but one were in labor, with its associated frequent hyperventilation and acetonuria, the maternal values were not materially different from those of the thirty-ninth week of gestation.

The blood of the fetus has a lower pH than that of its mother, and the pH of blood of the umbilical artery is lower than that of the vein. The oxygen content of the maternal peripheral vein is approximately equal to that of the umbilical vein, but the oxygen saturation of the former is very much higher. The fetal pCO_2 and lactic acid are higher than those of normal adults, with the umbilical artery having the highest values. The fetal values of potassium and phosphate are higher than those of the mother. The maternal blood proteins are in greater concentration than those of the fetus.

Ammonium chloride shows its most profound effect on blood pH during the first 3 or 4 days of administration. After this period of time, the compensatory production of ammonia by the renal tubules becomes sufficient to return the blood pH to less abnormal values.

In our group of controls, the average maternal peripheral vein pH in 86 patients at term but not in labor was 7.38. In 41 patients at the time of delivery, the average maternal pH was 7.37, an insignificant change. In the newborn babies of these controls, the mean umbilical vein pH was 7.32 and that of the umbilical artery, 7.28. Maximum fall in pH values occurred by the third or fourth day of ammonium chloride medication, and they were relatively stable thereafter. In 11 patients in whom the fetal pH values were low, more complete chemical analyses were done. Values for pH, HCO_3, pCO_2, chloride lactic acid, and oxygen saturation were different from those of controls. From the known action of ammonium chloride, the changes in the values for pH, HCO_3, and chloride were expected. The elevated fetal lactic acid and pCO_2 and decreased oxygen saturation were unexpected.

Six of these infants showed a severe degree of acidosis. The pH was low, the pCO_2, potassium, calcium, phosphate, and lactic acid were elevated, and the oxygen saturation was markedly reduced as compared with the controls. The elevated calcium and phosphate have been noted in experimental and clinical respiratory acidosis. Elevated potassium has been noted in respiratory acidosis of short duration in which the kidney has not had sufficient time to excrete the excess serum potassium. By producing a metabolic acidosis in the mother, an increased acidosis of respiratory type has been produced in the fetus. Eastman, Noguchi, and Wilson and associates have reported low values for pH and oxygen saturation and high values for pCO_2 in asphyxia neonatorum. In the six severely acidotic infants in the present series, pCO_2 and pH were equal to any reported in asphyxia neonatorum, and oxygen values approached those reported. Except for the marked cyanosis present at birth, these infants all appeared essentially normal and did well in the hospital.

In 1954, Lowrey, Graham and Tsoa reported studies of blood electrolyte balance in 11 infants born of diabetic mothers. They found extremely variable chloride and total base levels, and in a large proportion of the infants pH was low. Three of the four infants with low pH values, however, were born to mothers who had received ammonium chloride. The authors postulated a direct correlation between the degree of fetal acidosis and the severity of the abnormal clinical picture present in these infants. It seemed worthwhile to repeat these studies in the light of our experience with ammonium chloride acidosis.

We studied 18 infants born to diabetic mothers. Sixteen of the

infants survived. One infant died of hyaline membrane disease, and the other of prematurity. The umbilical vein pH of the first was 7.18 and of the second 7.29. The lowest pH which was found in the entire series was that of a 13-pound infant delivered with mid-forceps. The umbilical artery pH was 6.75. After a stormy hospital course, the infant survived and at last reports was doing well.

When compared with the controls, the only significant chemical findings of the diabetic mothers and babies were the low maternal and fetal values for pH and chlorides. The glucose values for both infant and mother varied markedly, depending upon the circumstances of glucose and insulin administration prior to delivery. There was no correlation between chemical values and the clinical course of the infants in our study. Both the pH and chloride values observed in the diabetic patients are thought to represent the mild acidosis seen in pregnant diabetic women. Many of these patients were on Diamox® throughout the third trimester.

In all of our study groups, the fetal chloride level varied with the maternal value. Values for chloride in maternal, umbilical artery, and umbilical vein plasma in 28 deliveries suggest the occurrence of a chloride shift at the placenta.

Utilizing data from 89 deliveries, maternal peripheral vein pH was plotted against the umbilical vein pH. With the exception of the seven infants in whom umbilical vein pH was below 7.15 and all of whom were born to mothers receiving ammonium chloride, a straight line relationship was obtained. Fetal pH appears to be related to maternal pH.

In 19 cases, the mothers were given uncoated ammonium chloride during labor. Uncoated ammonium chloride, if tolerated, has a rapid onset of action. While the individual groups were small and the findings not statistically significant, the data suggest that the establishment of the usual maternal-fetal pH gradient requires about 4 hours in the presence of a changing maternal pH. The same time lag was noted before re-establishment of a steady state in 11 patients in whom the ammonium chloride had been discontinued and the maternal pH was therefore changing in the opposite direction. Approximately one-half of the infants born of diabetic mothers had increased maternal-fetal pH gradients. This had no correlation with the clinical course of these patients. There was little correlation between oxygen saturation and pH, between oxygen saturation and lactic acid levels, or between lactic

acid and pCO_2. Fetal pH and fetal lactic acid, however, were correlated quite well as were oxygen saturation and pCO_2.

Discussion

The fetal acidosis present in the normal newborn is characterized by slightly decreased serum HCO_3 and elevated pCO_2, and has been considered both metabolic and respiratory in origin. Because the pCO_2 is elevated and there are associated decreases in oxygen saturation and other laboratory findings characteristic of respiratoy acidosis, many observers think of the normal newborn as being in a state of respiratory acidosis at the time of delivery. The normal adult pH level is usually found in the normal newborn by 4 to 6 hours after birth. Many premature infants, however, are acidotic for a much longer period of time. Conditions associated with decreased respiratory function, as hyaline membrane disease and pneumonia, are also associated with prolonged acidosis. The laboratory findings of an infant with hyaline membrane disease are not unlike those of the normal fetus.

The etiology of the observed fetal acidosis is a matter of controversy. The increased levels of lactic and pyruvic acid noted in the normal newborn are the basis for considering the acidosis to be metabolic in origin. Others, noting the elevated pCO_2 and decreased partial pressure of oxygen relative to those of the normal adult, consider the placenta to be an inefficient respiratory organ as compared with the lung. The decreased pH may represent a respiratory acidosis due to inadequate carbon dioxide excretion.

The elevated fetal lactic acid has been known since Bell's work in 1928, and at present is considered to be fetal in origin. It seems highly probable that the fetus makes use of anaerobic pathways of metabolism which are lost soon after birth. In instances of asphyxia neonatorum, lactic acid is markedly increased. In newborn rats, exposed to a pure nitrogen atmosphere, blood lactic acid is five times normal. The survival time of such young rats is increased by subcutaneous injections of glucose. The ability of newborn rats to survive in a nitrogen atmosphere is decreased from 50 to 3 minutes if they are first injected with an inhibitor of sulfhydryl enzymes. Sulfhydryl enzymes are needed to convert glucose to pyruvic and lactic acids. These facts strongly suggest that anaerobic glycolysis is a mechanism which increases tissue survival under conditions of hypoxia.

The exact signficance of elevated lactic acid is difficult to evaluate, for with human fetal tissue *in vitro* no increase in the accumulation of lactic and pyruvic acid results from hypoxia. Evidence suggests that in instances of anaerobic glycolysis some intermediate beyond pyruvate, other than CO_2, is accumulated. The measured lactic acid production in hypoxia experiments would protect newborns only one-tenth as long as actual protection can be measured. The whole subject is further confused by the possibility that the lactic acid represents in the fetus a link between the metabolism of fats and glucose.

As was noted in data presented, the fetal blood lactic acid levels correlated well with fetal pH. There was little correlation between lactic acid and oxygen saturation or lactic acid and pCO_2. With the exception of one report, lactic acid has been found to be uniformly decreased in respiratory acidosis in the adult. Diabetic acidosis excepted, acidosis both *in vivo* and *in vitro* decreases glucose metabolism and lactic acid formation. The association of increased fetal lactic acid, acidosis, and hypoxia has been thought to represent metabolic acidosis secondary to increased anaerobic glycolysis, in turn secondary to hypoxia. Hypoxia has been considered the initiating event. Our data suggest that the increased lactic acid in the instances of severe acidosis associated with ammonium chloride acidosis probably represents the fetal reaction to the associated hypoxia, which is secondary to the ammonium chloride acidosis.

Oxygen saturation was found to be correlated with pCO_2. There was no apparent correlation between oxygen values and pH or lactic acid. Following the development of the infant oximeter, numerous investigators have reported oxygen saturation values in infants at the time of birth. Except in those with extremely low values, no correlation has been reported between fetal oxygen values at the time of delivery and the clinical course of the infant. This has become more apparent as the result of several studies attempting to correlate cerebral palsy and oxygen saturation at birth. Apparently, the oxygen saturation at birth reflects a transitory state which has no value in predicting long-term effects upon the infant.

Our data show that the maternal oxygen values do not change significantly with acidosis. The maternal supply of oxygen at the placental barrier is not decreased. While carrier mechanisms have been postulated to explain the transport of different substances across the placenta, none has been suggested for oxygen. It is then difficult

to postulate a decreased oxygen supply to the umbilical vein in instances of fetal hypercapnia. The decreased oxygen saturation associated with increasing pCO_2 probably represents the effect of pCO_2 and pH on the oxygen dissociation curve, causing the curve to shift to the right. The expected oxygen saturation with the values of pCO_2 and pH found in these data is not predictable on the basis of information currently available; nevertheless, the hypoxia noted is probably the effect of induced acidosis.

Increased hemoglobin in the human fetus has been assumed to be the result of erythropoietic response to the decreased fetal oxygen saturation. The elevated hemoglobin may also represent a response to the observed elevated pCO_2 as there is some evidence that elevated pCO_2 is associated with increased hemoglobin and bone marrow activity. In comparative mammalian physiology, one finds that fetuses with significant blood levels of fructose at term have hemoglobin values comparable to the maternal, while those with insignificant blood fructose levels have high hemoglobin values. The human fetus is in the latter group.

The transfer of carbon dioxide has been assumed to take place across the placenta as it does across the alveolar membrance of the lung, that is, as a gas. Our data show that the pCO_2 is always higher on the fetal side. The pCO_2 gradients, however, varied markedly under the various circumstances of the different clinical categories. The bicarbonate ion, unlike the pCO_2, maintained relatively constant gradients. It may be that carbon dioxide moves across the placenta, at least in part, in the form of the bicarbonate ion.

During the latter part of gestation, fetal respiratory movements in rabbits, sheep, and goats are largely controlled by the oxygen tension of the fetal blood, not by carbon dioxide values. Eastman could find no correlation between the carbon dioxide tension of infants' blood and their ability to initiate respirations. While the facts concerning the effect of carbon dioxide on neonatal respiration are few, they indicate that the respiratory center is unresponsive at the time of birth to elevated pCO_2. The fetal homeostatic mechanisms, with no functioning respiratory organ comparable to the lung in the adult, are perhaps unaffected by pCO_2 levels. The newborn's respiratory center becomes responsive to carbon dioxide 2 hours after birth.

With this interpretation of the significance of fetal pCO_2 in mind,

the ability of the fetus to function with its known low blood levels of carbonic anhydrase is more readily understood.

If the fetal pCO_2 value has little significance, the usual adult criteria for determining whether acidosis is metabolic or respiratory in origin are not valid in the fetus.

The acidosis observed in the normal fetus probably is a metabolic acidosis resulting from the high level of anaerobic glycolysis known to occur. The decreased oxygen saturation and increased pCO_2, which in the adult are associated with respiratory acidosis, in the fetus are both secondary to the decreased pH. With the advent of adequate respiratory function, the products of anaerobic glycolysis are oxidized and the newborn's pH reaches the normal adult level a few hours after birth.

Conclusion

The laboratory findings of acidosis, hypoxia, and hypercapnia in the human fetus at the time of delivery have little or no correlation with the clinical status of the newborn.

Notice

In the staff meeting report entitled "The Use of Radioactive Phosphorus in the Diagnosis of Intraocular Tumors," by Robert M. Lundblad and James F. Marvin, which appeared in the May 15, 1957, issue of this BULLETIN, Vol. XXVIII, pages 398-406, acknowledgment of the financial support of the Minnesota Division of the American Cancer Society was inadvertently omitted. The authors and the Editor wish to apologize for this omission.

Staff Meeting Report

The Management of Scoliosis*

Donald C. Meredith, M. D.[1] and John H. Moe, M. D.[2]

Scoliosis is lateral rotatory curvature of the spine. The alteration in the spine, however, is representative of the pathological changes in other tissues and is an index of the type and degree of deformity of all the tissues.

Mechanics Of Scoliosis

In order to understand why scoliosis persists and progresses, we must recall some basic engineering principles. Lateral motion of the normal spine is accompanied by vertebral rotation and rotation of attached appendages. As Schmidt has pointed out, the engineering principle pertaining to a bent rod, "Eccentric loading efficiency increases with deflection," may be applied to any curvature of the spine. In simple terms, it states that the more it is bent or curved, the easier it is to bend it further; therefore, many curves progress.

In scoliosis there occur through continuous altered function (Wolff's law) changes in the external conformation and internal architecture of the vertebra and attached appendages, which prevent the spine from returning to its normal anatomical position; therefore, many curves persist.

Curves seen on x-ray may be structural or functional, as defined by Schmidt. The term "structural curve" implies that there are intrinsic changes of acquired or congenital origin, such as abnormal rotation, wedging, tilting, or gross malformation of vertebra with or without contracted soft tissue.

A functional curve is similar to that in a normal spine on lateral flexion. No intrinsic changes are present and the curve corrects com-

* This is an abstract of a report given at the Staff Meeting of the University of Minnesota Hospitals on June 7, 1957. A copy of the complete report, including tables and references, may be obtained by writing to the Editor, UNIVERSITY OF MINNESOTA MEDICAL BULLETIN, 1342 Mayo Memorial, Minneapolis 14.

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pletely when the extrinsic deforming forces are removed. When the body is in balance, any angular deviation in one direction is equal to an angular deviation in the opposite direction. This is called the law of balance. Ferguson prefers to denote curvatures as primary and secondary. A primary curve, then, is a lateral structural curve resulting from a force acting contrary to the law of balance. Secondary curves, therefore, are compensatory curves. As Cobb has pointed out, when a secondary curve is of long standing and crosses the midline, it nearly always takes on structural changes. The longer the curve, the more deformity will be present; the shorter the curves and the greater the number of curves, the less the deformity.

Assuming there are no deforming elements below the pelvic crest, the simplest form of scoliosis consists of three curves: a primary curve with secondary curves above and below which are attempting to return the spine to the erect position. If the secondary curves are successful in the attempt the spine is compensated; if not, it is decompensated. A patient whose curve is compensated will have his shoulders, eyes, and pelvis level with each other and with the floor when he is standing. The patient with a decompensated curve will present a list or deviation of the trunk from the midline; a dropped shoulder or pelvic obliquity may be present. Cobb has stated that the patient always lists toward the convexity of the primary curve and that the high side of the pelvis is on the concavity of the primary curve.

ETIOLOGY

Idiopathic Scoliosis

Idiopathic scoliosis is a fairly characteristic clinical entity, but the characteristics differentiating it from the curves resulting from poliomyelitis and from other types are not always entirely distinct. Various authors have implicated mechanical, neurogenic, embrylogic, developmental, and endocrinologic factors, but none of these theories have been proven. Many idiopathic curves do not progress to a deforming state and do not require treatment.

Post-Poliomyelitis

Scoliosis is due primarily to muscle imbalance.

Congenital Anomalies

Probably the commonest anolamy seen is the hemi-vertebra, but there may be others such as unsegmented vertebra and fused ribs.

These curves must be closely watched for they are unpredictable and may progress rapidly.

Thoracogenic

Scoliosis following emphysema, thoracoplasty, osteomyelitis of ribs, and radiation therapy in children often produces severe deforming curves in the thoracic region.

Osteochondrodystrophies, Muscular Dystrophies, Cerebral Palsy, and Friedreich's Ataxia

Scoliosis in these conditions is rare.

Neurofibromatosis

Scoliosis is relatively rare. Curves of the idiopathic pattern are occasionally seen associated with *cafe au lait* skin markings. They do not necessarily differ from idiopathic scoliosis in progress and treatment; however, the presence of neurofibromata near the spine may cause developmental defects often similar in appearance to congenital anomalies. Such defects produce sharp angular curves which are usually progressive.

Idiopathic Scoliosis

A true understanding of any disease is based on the knowledge of its etiology and natural history; with little knowledge of the former, the latter is of great significance for rational management. In idiopathic scoliosis the majority of the characteristic features of the primary curve pattern are present at the onset of the deformity and rarely change. As the curve progresses, one or two vertebrae may be added to the curve, but the apex, location of the curve, and direction of rotation are unchanged. In general, the majority of idiopathic curves are right thoracic, they occur predominately in females, and they usually appear just prior to adolescence. According to Cobb, fewer that 5 per cent of patients require spinal fusion, and 80 per cent of them never show progression to a noticeable deformity. In our own experience, most patients with idiopathic curves come in for treatment of an obvious deformity, and the less severe, non-progressive curves are seen more rarely. According to Risser, idiopathic curves may progress until spinal growth ceases. Spinal growth ceases simultaneously with completion of excursion of the iliac apophysis across the iliac crest to the posterior superior iliac spine (Risser's sign).

Idiopathic scoliosis may be classified into five main patterns after Ponseti and Friedman. According to them the primary lumber curve is the most benign and least deforming of all idiopathic curves. Its apex is usually the first or second lumbar vertebra.

The thoraco-lumbar curve, with its apex at T11-12, clinically is intermediate between thoracic and lumbar curves in regard to its characteristics. These curves, as a whole, are not severely deforming, but the incidence of severe curves is significantly higher than in the lumbar group.

The thoracic curve, according to these authors, progresses more rapidly, becomes more severe, and produces greater clinical deformity than any other pattern. Its apex is at T 8-9.

Combined thoracic and lumbar curves (double primary) are symmetrical, the axis of rotation being toward the convexity of the respective curves. The prognosis is good. The body is usually well aligned, and the curves compensate each other.

The cervico-thoracic curve is rare. The apex is at the T 3 level.

In our series of 130 idiopathic curves, 42 per cent were right thoracic and 31 per cent were right thoraco-lumbar curves. A majority of these were severely deforming.

Paralytic Scoliosis

The curve patterns in paralytic scoliosis vary according to the degree and location of muscle paralysis and weakness. A high percentage of paralytic curves are right thoracic. There is no sex predominance. Scoliosis may develop 10 to 15 years following poliomyelitis. Colonna and Vom Saal analyzed 500 cases of polio and found scoliosis developed in 150 or 30 per cent of the patients. These authors stated that thoracic curves are due to paralysis or weakness of the scapular muscles. J. I. P. James stated that paralysis of limb muscles is unrelated to the development of scoliosis but that the intercostal muscles and lateral abdominal flexors produce scoliosis when weaker on the convex side of the curve. Both authors agreed that lumbar curves are due to paralysis or weakness of the spinal muscles. The prognosis, unlike that in idiopathic scoliosis, is related to the age of onset of the curvature and the degree of muscle imbalance rather than the site of the primary curve. Paralytic curves in general are longer, more progressive and deforming, and may progress after completion of spinal growth. If soft tissue contrac-

tures exist below the pelvic crest, they must be treated before correction and fusion is attempted.

Measurement of the Angle of the Curve

A standing 14 by 17 inch x-ray of the spine showing the complete curve to be measured is essential. An accurate and simple method of measuring the degree of curvature on x-ray has been described by Cobb. The top vertebra of the curve is the highest one the superior surface of which tilts to the side of the concavity of the curve to be measured. The bottom vertebra is the lowest one with the inferior surface tilting to the side of the concavity. The angle formed by erecting intersecting perpendiculars from the superior surface of the top and the inferior surface of the bottom vertebra of the curve is the "angle of the curve".

Correction of the Curve

Three forces are necessary in the correction of lateral curvature of the spine: distraction, lateral pressure, and derotation. These forces are incorporated in the Risser localizer cast and the Milwaukee brace. The Risser localizer cast, like the Milwaukee brace, holds the compensatory curves relatively straight while the major curve is corrected, eliminating the danger of creating increased structural changes in the compensatory curves such as has occurred in wedging cast correction. Although Risser has stated that the localizer cast will permit ambulation without loss of correction, this is not a common experience. All curves are increased by gravity and maximal correction cannot usually be maintained except in the supine position.

Treatment of Scoliosis

Conservative Management

Mild curves are often associated with poor posture and in these cases postural exercises will improve the patient's appearance. Exercise fails to reduce the amount of angulation of a curve, but compensation can be improved by exercises in many instances.

Certain curves can be improved by creating a pelvic obliquity by the wearing of a high shoe and a buttock pad. Such measures must be used with caution, as only mild curves which are mostly functional will be improved thereby.

Cast correction as a means of conservative management will be effective only if the primary curve can be fully corrected or recor-

rected. In such instances, casts applied and worn for a period of several months may sometimes result in definite and persistent improvement in the angle of the curve. If the child is young, cast applications may be repeated at intervals. If the child is approaching termination of spinal growth, such correction will often be maintained permanently. It must be emphasized that conservative treatment fails if the curve is severe, structural, and progressive.

Operative Treatment

Hibbs performed the first spinal fusion for scoliosis in 1914. Attempts to fuse corrected curves have in the past led to many failures, for the bone graft did not sustain the correction in a high percentage of cases. It is now known that most of these failures occurred because of unrecognized pseudarthroses.

The improvement in surgical technique of bone grafting and improved measures for the prevention of surgical shock in recent years have promoted an upsurge of enthusiasm for maintenance of correction by surgical bone graft.

Spinal fusion will stop the increase in the scoliotic deformity and will maintain a previously gained correction of the scoliotic deformity if external protection is afforded during consolidation.

Correction is possible and will be proportionate to the mobility in the area of the curvature. Every curve that is flexible must have a structural and functional element. The functional component of the curve is pliable and may be corrected by side bending. The structural curve is rigid and only partially corrected by forceable means.

The minimum fusion area, determined by x-ray before correction is obtained, is the area between the end vertebrae of the primary curve. The maximum fusion area is from parallel to parallel vertebrae as seen on x-ray after the curve has been corrected.

The fusion area in poliomyelitis is usually from parallel to parallel vertebrae. In some idiopathic curves fusion of the minimum area is adequate. In others it may be necessary to include the entire maximum fusion area.

Spinal fusion is indicated in scoliosis for correction of cosmetic deformity and in progressing curves. Pain is usually not a factor in the developing curve, but fusion may be done later for pain resulting from secondary degenerative arthritis, root compression, and pressure of the ribs on the iliac crests.

Spinal fusion may be safely undertaken in the young child if the proper area is selected, but most fusions are done between the ages of 11 and 15 years. The danger of lordosis developing in a young child following fusion has been overemphasized. It is not desirable to fuse mild, stable, and unprogressive curves, or a severe curve if structural enough to permit little, if any, correction.

A review of 265 cases of idiopathic and paralytic scoliosis from the senior author's private practice and from the scoliosis service at Gillette Hospital for Crippled Children, St. Paul, Minnesota, has recently been undertaken. Patients treated between 1947 and 1956 are included. Most have been followed from 2 to 8 years after surgery.

A total of 380 patients have been treated by correction and fusion since 1947. Of these, 115 are still under treatment, and the follow-up period is too short for adequate evaluation.

TABLE

ANALYSIS BY DIAGNOSIS OF PATIENTS TREATED FOR SCOLIOSIS SINCE 1947

Idiopathic	151
Paralytic	179
Congenital	48
Thoracogenic	1
Friedreich's Ataxia	1

Patients with paralytic curves who were found to have double primary curves have not been included in this survey other than for the purpose of determining the percentage and number of pseudarthroses. In instances of idiopathic scoliosis the single curve with the greatest angulation and structural change was used for comparative evaluation.

In classifying results of treatment the cosmetic appearance has been correlated with the correction of angulation shown on x-ray, since x-ray evidence of corrected angulation is only one criterion by which end results should be judged.

As Schmidt has pointed out, a fused column is easier to maintain in balance than a flexible column. The regaining of compensation and consequent improved appearance can sometimes be obtained with little or no x-ray evidence of curve correction. In evaluating the cosmetic appearance of the patients, repeated observations were

made, and the patient's opinion and that of the parents were considered. A good result is apparent to everyone; a fair result indicates improvement and reasonable satisfaction; a poor result indicates general dissatisfaction on the part of all people concerned.

Methods of Correction

The turnbuckle cast was used between 1948 and 1953. The Risser localizer cast has been used on most patients since 1933. The Milwaukee brace of Blount and Schmidt has also been used.

Idiopathic Scoliosis. For purposes of this study, no curve was termed idiopathic when there was a history of poliomyelitis even in the absence of demonstrable muscle imbalance. No curve with vertebral anomalies, whether congenital or associated with neurofibromatosis, was included in this group.

Of the 265 patients with scoliosis observed sufficiently long to permit evaluation of treatment, 130 had the idiopathic variety. This group included 116 (89.6 per cent) females and 14 (10.4 per cent) males. The average age at the time of fusion was 13.8 years. The youngest patient was 9 years old, the oldest 40. Eighty-one per cent of the fusions were done in patients between the ages of 11 and 17.

Evaluation of correction in idiopathic scoliosis. The percentage gain of correction in a given patient was obtained by subtracting the final degree of angulation from the maximum precorrection angulation shown in an upright x-ray. This difference was then divided by the precorrection angulation. A standing x-ray taken when the patient was last seen was used for the final evaluation.

The average gain of patients in our series was 40 per cent. Patients in whom solid fusions without pseudarthroses were obtained gained an average of 43 per cent. Those in whom pseudarthroses developed gained an average of 22 per cent, and when pseudarthroses persisted average gain was only 13 per cent.

When the average percentage gain was correlated with the cosmetic evaluation, it was found that 101 patients rated as good cosmetically showed an average gain of 45 per cent, 20 patients rated as fair gained 24 per cent, and eight rated as poor showed only 5 per cent gain.

In this series of idiopathic curves, results were regarded as good cosmetically in 78 per cent of the total number of patients. When solid fusions were present with no evidence of pseudarthrosis at any

time, 86 per cent of the results were rated good. When pseudarthroses were present in the graft, 53 per cent of results were rated good, and with persistent graft defects results were rated good cosmetically in only 25 per cent.

Factors which may cause loss of correction in the treatment of scoliosis are: too short a fusion, removal of external protection before the fusion mass has become consolidated and mature, and pseudarthroses. Pseudarthroses account for nearly all major losses of correction. In this study the percentage of gain in correction was markedly reduced by the development of pseudarthroses and particularly by the presence of persisting defects. The average loss of correction in all patients was 12 degrees. In the presence of persistent pseudarthroses the average loss was 39 degrees.

Incidence of pseudarthroses in idiopathic scoliosis. The cases of 130 patients with idiopathic scoliosis were analyzed. In this group 165 separate spinal fusions were performed. Operations performed for repair of pseudarthrosis numbered 22.

"Early type" fusion. The method of fusion performed between 1948 and 1952 is designated as the "early type" of fusion in this study. The surgical exposure in this group was confined, and careful soft tissue removal and decortication were inconsistent. Flaps of bone from the laminae and spinous processes were turned down in a crossing fashion. The joints and transverse processes were not exposed. Bone in the form of bank bone or augotenous bone was added. Pseudarthroses developed in 19 (55 per cent) of 34 patients so treated.

Cobb method of fusion. This type of fusion, as used during 1952 and 1953, consisted of meticulous subperiosteal removal of all soft tissue from the spines, laminae, and transverse processes. Multiple, interlocking small bone flaps were raised from the laminae and transverse processes of adjacent vertebra. Bone was added generously. Facet areas were exposed but the joints were not disturbed. Of 30 patients treated by means of the Cobb method of fusion, pseudarthroses developed in two (6 per cent).

Facet fusions. The technique used was essentially that of Cobb with the facets opened, denuded of cartilage, and filled with blocks of bone. Ten instances of pseudarthroses occurred in 66 patients so treated, an incidence of 15 per cent.

It would appear from these data that in idiopathic scoliosis the

addition of facet fusion to the Cobb technique does not favorably influence the incidence of pseudarthroses.

In 98 patients treated at Gillette Hospital, there were 31 instances of pseudarthrosis, while in 32 private patients, no pseudarthroses occurred.

Analysis of Paralytic Scoliosis

There were 136 patients with paralytic scoliosis, 81 (59 per cent) females and 55 (41 per cent) males. The average age at time of fusion was 13.4 years. The youngest patient submitted to surgery was 3 years, the oldest 20 years.

The average gain in patients with paralytic scoliosis was 44 per cent. In the patients whose fusions become solid without evidence of pseudarthroses at any time, average gain was 47 per cent. When pseudarthrosis occurred gain was 39 per cent, and 29 per cent in the presence of persistent pseudarthrosis.

Patients with results rated as good cosmetically showed an average gain of 49 per cent. Those with fair results gained 21%, while those with poor results showed an average gain of 4%.

In the total series of patients with paralytic curves, 78 per cent had results rated as good. In patients with evidence of graft defect at any time, 85 per cent of results were rated good. When pseudarthroses were present, 67 per cent of results were good, while of those in whom the pseudarthrosis persisted, good cosmetic results were obtained in 42 per cent.

The average loss of correction in the series was 12 degrees. With pseudarthroses persisting, there was an average loss of 31 degrees.

Incidence of pseudarthroses in paralytic scoliosis. Patients with paralytic curves numbered 136, and 212 separate fusion operations were performed in this group. The "early type" of fusion resulted in pseudarthroses in 17 of 26 cases (65 per cent); the Cobb type of fusion in 14 of 28 cases (50 per cent); and the facet type of fusion in 25 of 82 cases (30 per cent).

LOCATION OF PSEUDARTHROSES

The thoracic spine fuses solidly in a much higher percentage of patients than do the thoraco-lumbar and lumbar segments. The high incidence of curves limited to the thoracic spine in idiopathic scoliosis and the relative stability of these curves contribute to the

high occurrence of solid fusions in this group. Paralytic curves involve a greater portion of the lumbar spine, are more collapsing and flexible, and tend to be longer. The transitional area between the thoracic and lumbar vertebrae is the most vulnerable area in the spine. In this series, 135 separate pseudarthroses occurred. Multiple defects developed in many patients.

Above T6 there were six defects, above T10 there were 27. A total of 53 defects were found in the T11-12 and the T12-L1 interspaces.

Operative Mortality

No operative deaths occurred in this series. In the group of 265 patients analyzed there were 377 operations for spinal fusion and 69 operations for repair of pseudarthroses, a total of 446 operative procedures.

Summary

Idiopathic Scoliosis

One hundred and thirty cases of idiopathic scoliosis were analyzed with respect to the percentage of correction obtained and the incidence and effect of pseudarthrosis in the graft. Three methods of fusions were analyzed.

The average gain in correction was 40 per cent. In the absence of pseudarthroses, the average gain was 43 per cent. Patients with graft defects showed an average gain of 22 per cent, and those with persistent defects showed an average gain of 13 per cent.

Patients whose results rated as a good cosmetically showed an average gain of 45 per cent while those with poor cosmetic results gained only an average of 5 per cent.

Fusions performed in the early years of this series resulted in pseudarthroses in 55 per cent of the patients while the Cobb method of fusion, used later, resulted in pseudarthroses in only 6 per cent. Facet fusions with autogenous bone gave no further improvement in results.

Paralytic Scoliosis

One hundred and thirty-six cases of paralytic scoliosis were analyzed. Double curves were omitted in evaluating the percentage of gain in correction.

The average gain in correction was 44 per cent. In the absence

of pseudarthrosis the average gain was 47 per cent. Patients with pseudarthroses averaged 39 per cent gain, and those with persistent defects averaged 29 per cent gain.

Patients whose results were rated as good cosmetically showed an average gain of 49 per cent, while those with poor cosmetic results showed a 4 per cent gain in correction.

Early fusions were followed by pseudarthrosis in 65 per cent of cases while the Cobb type of fusion resulted in graft defects in 50 per cent. Facet fusions resulted pseudarthrosis in 30 per cent, but when autogenous bone was added, defects occurred in only 13 per cent.

Conclusions

Correction and fusion of the scoliotic spine is uniformly productive of results entirely satisfactory cosmetically and functionally provided that the proper fusion area is selected and the fusion is successful in providing a solid and mature graft. The presence of pseudarthroses is the prime reason for loss of correction. Most pseudarthroses can be discovered, repaired, and protected until solid without appreciable loss of correction. A meticulous type of fusion, with wide exposure, with all soft tissue removed, and with facets carefully fused is productive of the best overall results.

Staff Meeting Report

The Effect of Ionizing Radiation on Some Biological System with Special Reference to Their Inactivation of Pepsin*†

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Halvor Vermund, M.D.,[3] and James F. Marvin, Ph.D.[4]

A better understanding of mechanisms by which ionizing radiations affect biological systems is most certainly an objective of everyone associated with radiation therapy. Any significant achievements toward this goal necessitate an appreciation of certain aspects of physics, chemistry, and biology which together with radiotherapy constitute an ever growing discipline known as "radiation research." Only through the effective functioning of this discipline can the effects of ionizing radiations on biological systems be understood. Each advancement in this area has improved the clinical program of radiation therapy. It is likely that new discoveries will continue this trend. Furthermore, no field of human endeavor can stop asking and attempting to answer questions of this basic nature without incurring the risk of deterioration of aptitude, attitude, and morale within its ranks.

Believing then that the most important questions which continually confront the radiotherapist can be attacked most effectively through research, we are endeavoring to promote "radiation research". At the present time our major research efforts involve the investigation of:

a. Metabolism of tumor cells using radioactive phosphorus (P^{32})

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* This is an abstract of a report given at the Staff Meeting of the University of Minnesota Hospitals on June 14, 1957. A copy of the complete report, including references and additional tables and graphs, may be obtained by writing to the Editor, UNIVERSITY OF MINNESOTA MEDICAL BULLETIN, 1342 Mayo Memorial, Minneapolis 14.

† Financial support for this work has come from the Atomic Energy Commission and the Graduate School of the University of Minnesota. We are grateful for the technical assistance of Miss Ardis Brown and Mrs. Kathleen Terrill in some phases of these studies.

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to tag phosphorylated compounds, the most notable of which are precursors to the nucleic acids.

b. The effect of ionizing radiations and the administration of chemicals on metabolic pathways in cells of normal and tumor-bearing animals.

c. The effects of irradiation on the host-tumor relationship in mice.

d. Dosimetry problems using fixed and rotating fields from a Cobalt-60 (Co^{60}) teletherapy unit.

e. Improved radiation detection devices with associated electronic apparatus for automatic recording of radioactive emanations in both *in vivo* and *in vitro* systems.

f. The effect of ionizing radiations on enzymatic activity.

Information on several of these projects has been presented in past years. The present discussion concerns some of our recent investigations using the protease pepsin. A brief review of pertinent literature is in order first, however.

As early as 1896, only a few months after Roentgen's discovery, bold attempts were made to treat patients without any knowledge about the biological effects of the x-rays. The results indicated that the rays had profound effects, manifested at first by the reactions on the skin (erythema, dermatitis, and necrosis). Sjögren and Steinbeck have been credited with the first successful attempt, in 1899, to cure a cancer of the skin using radiation therapy. Since that time, many manifestations of the biological effects of ionizing radiations have been noted. Among these are lethality, lymphopenia, granulocytopenia, anemia, epilation, genetic mutations, respiratory changes, protein denaturation, enzyme inactivation, and a host of others.

These biological effects are initiated by the absorption of radiant energy quanta (photons) in the system under consideration. These photons may interact with the atoms of a biological system to remove orbital electrons (ionization by the photoelectric and Compton effects) or displace electrons from stable orbits to higher energy orbits (excitation). High energy photons may react with nuclei to produce a positron and electron (pair production). The ionization resulting from these interactions is considered to be the most important cause of the biological changes.

Because of the importance of the orbital electrons in the mainte-

nance of molecular structure, ionization may cause molecular decomposition and, consequently, changes in the physical and chemical properties of the system. The ejected electron will be able to produce further ionization depending upon its kinetic energy derived from the initial photon encounter. Thus, the absorption of one photon may result in a large number of secondary electrons being formed, many with high kinetic energy and all of which are considered significant in the ultimate biological effect.

The maintenance and growth of most biological structures are dependent upon a multitude of chemical reactions which must be kept in delicate balance. The absorption of radiant energy with subsequent ionization and formation of highly reactive radicals from water often leads to a change in this balance with ultimate modification and/or destruction of the system. Because of the inherent complexity of most biological systems, there is difficulty in assaying the immediate effects of photon absorption. To gain information on the mechanisms involved, it is oftentimes advantageous to utilize simple systems *in vitro* such as enzymes and colloids.

In most biological systems, the major constituent is water. Inasmuch as the ionization produced by radiation occurs at random in the system, it is expected that the majority of biological action following irradiation is the result of radicals or molecular fragments formed in the water. This reasoning led investigators in the early 1930's to postulate the existence of "activated water." Further studies showed the existence of hydrogen and oxygen gases formed in irradiated water. Hydrogen peroxide is also present in water exposed to high doses of roentgen rays under aerobic conditions. The formation of atomic hydrogen and OH radicals was probably first postulated in a paper by Risse. Further evidence that "activated water" could be identical with H atoms and OH radicals is credited to Weiss. The formation of the perhydroxyl (HO_2) radical in irradiated water has been discussed by Burton and others.

Certain of these radicals are expected to recombine depending upon their spatial distribution. The remainder are capable of initiating oxidative and reductive reactions depending upon the characteristics of the solute and its pH. The H radicals may participate in reduction processes while the HO_2 and the OH radicals with their great affinity for electrons are expected to cause oxidation of molecules in the system being irradiated. It has also been shown, however, that the

OH radicals may act as an agent of reduction and under certain conditions, H atoms can cause oxidation by dehydrogenation. Stenstrom and co-workers and Fricke and his associates, working with solutions of inorganic and organic compounds, and Dale, working with enzymes, obtained evidence that the action of ionizing radiations on water has a pronounced effect on the solute molecules.

The biological effects of ionizing rays brought about through the aqueous medium have been termed the "indirect effect" of radiation as opposed to the "direct effect" in which the rays act by ionization or excitation of the solute molecules. In the deamination of glycine, the yield is nearly the same for dry material as it is for a 20 per cent solution. It cannot be assumed, however, that glycine when dissolved does not change its properties and, therefore, its response to a direct hit. Certain biological systems, such as seedlings, spores, tumor cells, and cell cultures have shown a radiosensitivity which is dependent upon water content. Experiments by Patt and co-workers have shown that radiosensitivity of certain animals may or may not be altered by dehydration. The decrease in radiosensitivity with dehydration in some systems may be explained in terms of a shift from indirect to the direct effect. At the present time, however, one cannot obtain absolute values for each of the two modes of action. From the biochemical point of view, both the direct and the indirect effects may be of biological importance. The average water content in most of these systems is about 75 per cent or higher so that it is reasonable to assume that the indirect mode of action of radiation is of major importance.

According to the concept of indirect action, the localization of an effect within a biological system will depend upon the nature of the system as well as upon the distribution of the radicals in space. A dense ionization track, such as would be produced by an alpha particle, is believed to consist of a dense central core of H^+ and OH ($H_2O^+ \rightarrow OH + H^+$) surrounded by a sheath of H and OH^- ($H_2O^- \rightarrow OH^- + H$). In environments with relatively poor diffusion kinetics, the reaction will be confined to a very limited space surrounding the ion track. The H atoms readily combine to form hydrogen gas whereas any oxygen gas formed is likely a byproduct of the decomposition of hydrogen peroxide. Through diffusion of these radicals, H_2O may again be formed. Contrasted with this is the effect expected with low density ion paths in which the initial distribution is considered to be more random in character.

Hydrogen peroxide production is considered to be a secondary product of the reaction of two perhydroxyl radicals with the release of oxygen. With x-rays as the source of ionizing radiation, the yield of H_2O_2 is negligible in the absence of oxygen. The reaction of two OH radicals to produce H_2O_2 is probable only in cases of high ion density radiations in which these radicals are formed in close proximity. In the irradiation of water with x-rays under aerobic conditions, the yield of H_2O_2 has been estimated to be 54 micrograms/cc/10^6r. A measurement by Bonet-Maury is in good agreement with this. The pH appears to have a profound effect on peroxide production, declining sharply above pH 8 and rising abruptly below pH 3. In the majority of *in vivo* systems, the presence of catalase is expected to minimize any peroxide effect.

Dissolved oxygen has a profound effect on the ultimate destruction of the radicals formed in water by ionizing radiations although this effect is smaller for ionizing radiations producing heavily ionized tracks (i. e. alpha particles) than it is for x-rays. The presence of oxygen probably permits the formation of the highly oxidative perhydroxyl radical with H atoms. The removal of the H atoms in this manner permits the OH radicals to exist for a longer period and, therefore, engage in more extensive reactions. Thus, oxygen is expected to increase the radiosensitivity of biological systems, whereas anaerobic conditions or the presence of oxygen acceptors will likely produce the opposite effect.

A decrease in radiosensitivity by lowering the oxygen tension in the tissues of the exposed area was first discussed by Schwarz. Holthusen in 1921 elaborated on this phenomenon in his findings of increased radioresistance of *Ascaris* eggs irradiated anaerobically. In 1933, Crabtree and Cramer presented a detailed account of the influence of anoxia and related chemical factors on the radiosensitivity of tumor cells. The possible relation between this phenomenon and the reactions of activated water was suggested by Thoday and Read in 1947.

Oxygen deprivation during exposure to roentgen rays has been shown to decrease the following radiation effects: oxidation of quinhydrone, auxin, glutathione, and other thiols; deamination of amino acids; inactivation of bacteriophage suspended in buffer; destruction of tumor cells; and lethality to bacteria.

Lacassagne and Dowdy have shown that reduced oxygen tension

decreases the mortality among mice and rats exposed to roentgen rays. Also complete asphyxia for 20 minutes before irradiation provides marked protection to newborn mice. Investigations of the usefulness of oxygen as an adjunct to clinical radiation therapy have been reported.

On the other hand, it was shown by Dale that the inactivation of carboxypeptidase by x-rays is unaffected by oxygen concentration. Furthermore, the lack of oxygen affords no protective effect from exposure to alpha rays, which may be explained in terms of the spatial distribution of the radicals formed in water. Bacteria grown and irradiated anaerobically are 10 times more resistant than their aerobic counterparts, and the shape of the survival curve appears to be dependent on the environment for growth while the oxygen tension in the medium during irradiation determines the slope of this curve.

Chemical modification of the effects of ionizing radiations has received the attention of many investigators and is of more than academic interest when considered in terms of either protection of an exposed biological system or increased radiosensitivity of malignant relative to normal tissues. A compound producing the latter effect may be said to potentiate the effect of local irradiation or, synonymously, to improve the therapeutic ratio.

Since the advent of atomic energy, the investigation of protective agent has been accelerated for obvious reasons. The most potent protective agents known today contain thiol groups, although side chains with amino groups also appear to be significant in this regard. Glutathione, cysteine, and beta-mercaptoethylamine (cysteamine) have been used in our laboratories. Of these, cysteamine appears to be the most effective protective agent of both *in vivo* and *in vitro* systems exposed to roentgen rays.

Improvement of therapeutic ratio has received considerable attention at this hospital during the past 2 years. In connection with a project under the direction of Dr. Schwartz of the Department of Medicine, we have been concerned especially with a study of some relationships of porphyrins to tumors and to radiosensitivity. We have found that certain porphyrins, particularly a copper complex of hematoporphyrin, may provide this potentiation, although further investigation is required. Other investigators have attempted to potentiate the effects of ionizing radiations in *in vivo* systems by adrenal insufficiency, increased metabolic activity, vitamin deficiency, and cortisone administration. Among the compounds reported to potentiate radiation effects have

been colloidal lead orthophosphate, guanazole, 8-azoguanine, urethane, and malonic and maleic acids. Studies in our laboratories indicate that folic acid analogues may also be added to this list.

In most biological fluids irradiated with moderate doses, it may be shown by calculation that the number of molecules changed in solution is extremely small. One to two ionizations are formed on the average per cubic micron per roentgen of x-rays delivered. With an ionic yield M/N (molecules changed per ion pair formed) of unity, only an insignificant number of the total molecules present in a biological system will be changed with x-ray doses in the therapeutic range. If one considers the transfer of energy via the "activated water" to induce chemical changes, however, an appreciable percentage of a solute may undergo change with reasonable doses.

Ionizing events occurring at random within a volume are expected to have a greater effect for a given concentration of large solute molecules than for the same concentration of small molecules because of the greater percentage of the volume occupied by the solute. Also, with large molecules, it is to be expected that the probability of recombination of the active radicals will be smaller. Oftentimes the assay of changes in large molecules is difficult to make. Considerable alterations in structure are expected to occur, some of which may not be detectable. Changes which are considered most readily detectable with large molecules are those occurring in enzymes and, inasmuch as these molecules are intimately associated with the metabolic function of biological systems, an analysis of these changes is considered to be of value in interpreting at least a part of the radiation syndrome as exhibited *in vivo.*

To study the effects of ionizing radiations on enzymes, we have chosen the proteases, pepsin and trypsin, primarily because they are available in crystalline form and have been used by several investigators in related studies. The early work on radiation inactivation of pepsin by Hussey and Thompson and later investigations by Northrop have been interpreted by Stenstrom, Fricke and others as indicating an indirect effect of x-rays mediated via the aqueous phase. More recent studies with pepsin by Anderson were concerned with an interpretation of a delayed inactivation of pepsin following irradiation. This was also studied by McDonald, who in addition has investigated the effect of pH, temperature, and solvent on the x-ray sensitivity of trypsin.

Pepsin has a molecular weight of 35000 ± 3000, has an isoelectric

point less than pH 1, and is optimally active near pH 2, although this optimal pH may be shifted in either direction depending upon the substrate. Trypsin, on the other hand, has a molecular weight of 22000 2000, has an isoelectric point near pH 11, and hydrolyzes protein substrate with maximum velocity between pH 7 and 9.

Materials and Methods

The pepsin used in our studies has been USP crystalline enzyme. Trypsin was purchased under the trade name Tryptar® (lyophilized crystalline trypsin, Armour Laboratories). Egg albumin (Mallinckrodt Laboratories) dissolved in buffer of appropriate pH and rendered radioactive by the addition of microcurie amounts of radioactive iodinated serum albumin (RISA,® Abbott Laboratories) served as substrate. When albumin to which iodine has been bonded is used as a substrate, it has been shown that the radioactivity in the split products is proportional to the enzyme activity.

The enzyme was dissolved in various media depending upon the nature of the investigation. These enzyme solutions were then exposed to roentgen radiation, always maintaining adequate non-irradiated control solutions. Pyrex tubes containing enzyme were arranged in cart wheel fashion on a presd wood phantom in the field of a General Electric Maximar x-ray machine operated at 220 KVP, 15 MA and no filter (HVL = 0.35 mm. Cu). Using a FSD of 35 cm., an output of 455 r/min (with backscatter) was obtained.

After completion of the irradiation, a fixed amount of buffered substrate was added to each tube in the series and all tubes were incubated in a water bath at 37° C. for one hour. At least two analyses were made for each experimental point. For studies with pepsin, a phosphoric acid buffer was the solvent for the albumin substrate to maintain an incubation pH of 2.0. A phosphate buffer was used in a similar manner to maintain a pH of 7.7 for incubation of trypsin. After incubation, instantaneous inactivation of the enzyme and precipitation of both enzyme and substrate were achieved by the addition of concentrated sulfosalicylic acid. The radioactive iodine freed from the digested albumin molecules by the action of the enzyme remained in the supernatant fluid after centrifugation for five minutes at about 4000 times gravity. The gamma radiation emitted by the iodine in an aliquot of the supernatant was determined in a well scintillation counter as a measure of the enzyme activity.

Results

Experiments using both the RISA method as presented and the Anson hemoglobin method showed good agreement when pepsin activity was measured as a function of either pepsin concentration or time of incubation. In comparable experiments with trypsin, it was found that the Anson method and the spectrophotometric method of Kunitz are both more sensitive than is the technique using RISA. Because of similarity in results obtained when either of these enzymes was exposed to ionizing radiations under various experimental conditions, only those data obtained from some of the pepsin studies will be discussed in the following paragraphs.

The results of typical experiments on the inactivation of a solution of crystalline pepsin in deionized water or in water containing 0.05N acid by x-rays are shown in Figure 1. The inactivation of this

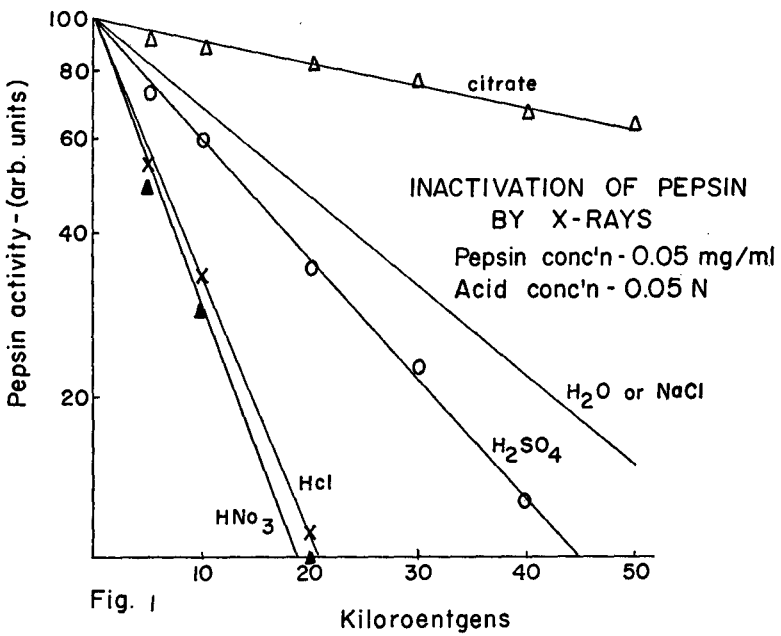

Fig. 1

enzyme is a first order reaction as evidenced by the straight line obtained when plotting the logarithm of the pepsin activity as a function of the radiation dose. At first glance, this result seems to imply a direct effect of the roentgen rays because in such a case the enzyme would

be inactivated in proportion to its concentration which represents a first order reaction. When this type of experiment was repeated using initial concentrations varying over a wide range, however, it was found that the ionic yield (M/N) was constant over a wide range of concentrations so that the inactivation was no longer proportional to the original concentration as would be expected from the direct or target theory.

This phenomenon is explainable in terms of the dilution theory which postulates that when the concentration of the solute is very low, many of the radicals formed by radiation will recombine before drifting to the sites of solute molecules so that the yield will depend upon concentration. Above a certain concentration, all of the radicals formed will react with solute molecules so that the ionic yield, M/N, will be constant over a wide range of concentrations. Furthermore, if one has in solution two solutes, which are assumed to be capable of reacting with radicals, it is expected that they will compete for these intermediate products formed in water by ionizing radiations. Because of this, there are fewer radicals available for reaction with either of the solutes than there would be if this solute were alone in solution. The radiation effect on either of them, singly, is reduced with the result that an apparent "protection" exists.

The exponential relationship existing between the chemical change of an organic substance and the dose of roentgen rays may be explained in terms of this protection effect as a special case of two solutes. Before the irradiation is started, only the active type of molecule exists. By the action of radiation, originally active molecules lose their activity and become a second kind of solute, inactive molecules, which are still capable of further reactions. These latter reactions are not measured since the usual criterion for evaluation of the radiation effect is the change in the original active molecules.

It appears that a significant sensitization to the effect of x-rays obtains when pepsin is in the acid solution. The mechanism of this effect is not known although it is conceivable that in acid solutions in which this enzyme is most active, it may be in an ionic state which is more vulnerable to radiation. Citric acid, on the other hand, affords considerable protection even though the pH's of the solutions are much the same. This protection is believed to be associated with the ability of organic molecules to compete for active radicals formed in water, or as has been postulated recently, certain compounds may provide chem-

ical linkages to sensitive sites on the solute molecules and thus account for their effectiveness as protective agents.

The effect of pH during irradiation on peptic activity was studied by varying amounts of 0.005N citric acid and 0.005N sodium hydroxide to give a pH range from 2.2 to 12.1. Changes in the radiosensitivity of pepsin due to the changes in the concentration of citrate and sodium ions necessary to effect the pH shift were found to be negligible at the concentrations used.

It was found that most of the pepsin is irreversibly denatured if the pH of the solution is permitted to go above pH 6.5, as has been reported by several investigators. In these reports, it has been stated that the denaturing of pepsin in the interval of pH above 6.0 is a first order reaction with the rate proportional to the concentration of the hydroxyl ions.

Dome-shaped curves, typical of studies of enzyme activity as a function of pH, were obtained when irradiated pepsin was incubated with substrate at pH varying from 1.0 to 7.0. Pepsin was irradiated either at pH 2.5 using 0.01N citric acid as the solvent or near pH 5.5 using de-ionized water. It was found there is a shift of the pH optimum for peptic hydrolysis toward lower pH with the enzyme irradiated in de-ionized water from that found for either non-irradiated pepsin or that irradiated in citrate.

Autolysis possibly accounts for the slight decrease in the activity of unirradiated pepsin which was noted as its concentration was increased (Table 1). On the other hand, the activity was definitely dependent on concentration when solutions of the enzyme were exposed to 30,000 and 50,000 roentgens. The smaller percentage inactivation of pepsin with increased concentration is attributed to a protective effect afforded by enzyme molecules previously inactivated.

TABLE 1

Dose of radiation	*Pepsin Concentration (mg./ml.)* 0.01	0.025	0.05	0.1	0.25	0.5
0 r	100	85 ± 5	80 ± 9	82 ± 3	82 ± 2	82 ± 3
30 Kr	0	2 ± 1	8 ± 4	27 ± 3	45 ± 5	60 ± 10
50 Kr	0	0	0	3	10 ± 3	24.5 ± 8

Pepsin activity (arbitrary units) as a function of its concentration during exposure. Solvent: H_3PO_4 buffer (0.1 M). The data tabulated are average values together with deviations found in three experiments.

The effect of 0.25 mg./ml. ($4x10^{-4}M$) "hematoporphyrin" (Mann Chemical Company*) on the radiosensitivity of pepsin is shown in Table 2. Under the conditions of this experiment, porphyrin produced

TABLE 2

X-ray Dosage (Kiloroentgens)	*Per cent Inactivation of:* *Pepsin in Buffer*	*Pepsin in Buffer and Hematoporphyrin*
0	0	10.5
2.5	0.2	11.
5	7.5	13.5
10	12	16.0
20	18.5	19.0
30	30	22.0
40	39.6	23.0
50	56	26
60	69	28.5

Per cent inactivation of pepsin by roentgen rays. Pepsin dissolved in 0.1 M buffer (pH 1.8) with and without Mann hematoporphyrin added. Pepsin concentration: 0.05 mg./ml. Hematoporphyrin concentration: 0.25 mg./ml.

an inactivation of pepsin equivalent to that produced by 10,000 roentgens. Above 20,000r, this concentration of porphyrin afforded definite protection of the enzyme. If the inactivation due to porphyrin is ignored, it may be concluded that the dose of radiation can be an important factor in determining whether the radiosensitivity of this system is increased or decreased. Evidence of a similar response in *in vivo* systems treated with porphyrin has been considered elsewhere.

In Figure 2, a comparison is made of the protective capacities of glucose and "hematoporphyrin" on an equimolar basis. The maximum protection afforded was very nearly the same for both compounds. With porphyrin, however, this maximum protection was achieved at less than one-tenth the molar concentration of that required with glucose. The appearance of a pronounced dome-shaped curve following exposure of pepsin to 50,000r in solution with various concentrations of porphyrin is worthy of note. The explanation appears to embody two properties of porphyrin, which at high concentrations is capable of inactivating the enzyme, whereas at moderate concentrations, this toxic effect becomes less pronounced while the protection afforded the

* Studies made by S. Schwartz, M.D., of the Department of Medicine, University of Minnesota Hospitals, and by chemists of General Mills Research Laboratory and of Merck and Company have shown that "hematoporphyrin" prepared in their laboratories as well as all other commercially available "hematoporphyrin" is composed of a mixture of several porphyrins.

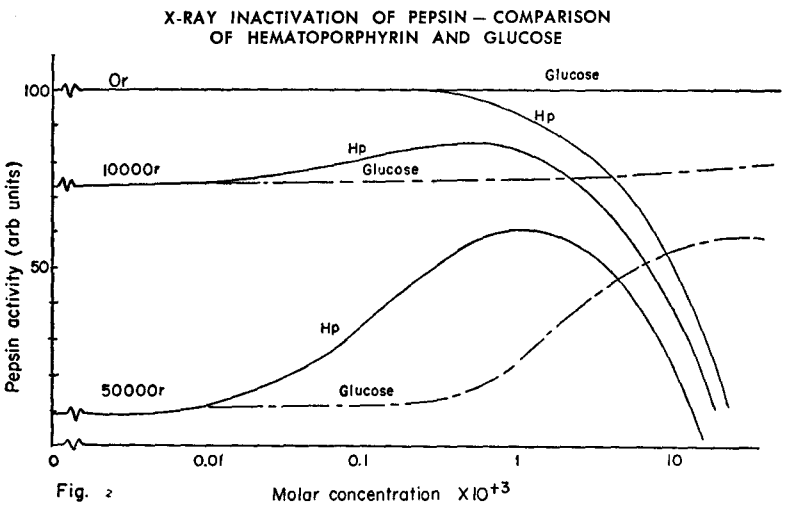

Fig. 2

pepsin by the porphyrin molecules becomes a more important factor. At very low concentrations, the toxicity as well as the protective quality of porphyrin completely disappear. Experiments in which mice were exposed to total body x-irradiation following intraperitoneal injections of various concentrations of "hematoporphyrin" yielded results which have been interpreted in a similar manner.

The sulfhydryl compounds, cysteine, and cysteamine (beta-mercaptoethylamine), have been found to protect pepsin in much the same manner as glutathione was found to protect ribonuclease which is also a non-sulfhydryl enzyme. In equimolar concentration, cysteamine affords the greatest protection of irradiated systems followed closely by cysteine. This protection parallels that found when these compounds were administered to mice before exposure to total body x-irradiation. Glucose affords some protection but not to the same extent as that afforded by the sulfhydryl compounds. Other studies have shown that albumin is also a protective agent for pepsin exposed to x-rays.

We have also investigated the inactivation produced by hydrogen peroxide and by OH radicals formed chemically by the reaction of hydrogen peroxide and ferrous sulfate and the effect of oxygen tension on the radiosensitivity of the enzymes. We have found in this regard that hydrogen peroxide in concentrations well above those which could conceivably be produced by 50,000 roentgens does not appreciably alter

the activity of pepsin. When ferrous sulfate is added to the mixture, however, there is a very marked inhibition of peptic activity. (Table 3). This is attributed to the formation of OH radicals according to the

TABLE 3

Pepsin in Solution with	*Time of Exposure (Minutes)* 1	15	30	60	90.
H_2O	10.0	10.0	10.0	10.0	10.0
H_2O + $FeSO_4$ (2 x 10^{-3}M)	9.8	9.8	9.8	9.2	9.0
H_2O + $FeSO_4$ (4 x 10^{-3}M)	9.6	9.5	10.2	9.2	9.0
H_2O_2	10.0	9.9	9.8	9.2	9.1
H_2O_2 + $FeSO_4$* (10^{-3}M)	8.1	7.8	7.3	7.7	7.4
H_2O_2 + $FeSO_4$* (2 x 10^{-3}M)	6.5	6.1	5.9	6.0	6.2
H_2O_2 + $FeSO_4$* (3 x 10^{-3}M)	3.1	3.5	3.3	3.0	2.8
H_2O_2 + $FeSO_4$* (4 x 10^{-3}M)	1.8	2.0	1.8	1.5	1.5

Inactivation of pepsin by OH radicals with controls to determine the effect of various chemicals alone (pepsin activity in arbitrary units). *OH radicals formed.

reaction ($FeSO_4 + H_2O \rightarrow OH^- + Fe^{+++} + SO_4^{=} + OH$). The OH radicals appear to be the most important single factor in producing inactivation of pepsin. This conclusion is based on studies in which pepsin was irradiated under anaerobic conditions. Under these conditions, we found that the inactivation was identical to that produced when the radiation was carried out in the presence of air.

Our most recent investigations have been concerned with a delayed inactivation of pepsin following exposure to various doses of radiation up to 100 Kr. Both conventional x-ray (220 KV) and Co^{60} gamma rays delivered from one of our teletherapy units or from a high intensity Co^{60} source available at the General Mills Research Laboratory have been used as sources of ionizing radiations.

Delayed inactivation of pepsin has been assayed using our RISA method and the Anson hemoglobin method and more recently by a spectrophotometric technique developed in our laboratories. The sensitivities of these methods are comparable, and it was gratifying to find that results obtained by these methods agreed very well. Our most consistent data have been obtained using the RISA method.

During these studies, it was noted that pepsin (0.05 mg./ml.) in 0.005 M buffer at pH 4.5 to 5.0 that had been partially inactivated by roentgen rays continued to lose activity after completion of the irradiation. This delayed inactivation was found to depend upon dose of irradiation, enzyme concentration, pH, nature of the solvent, time, quality of the ionizing radiation, and temperature. Similar temperature

dependence has been reported for trypsin, hemoglobin, thymus nucleic acid, and pepsin.

There was a slight inactivation of unirradiated pepsin stored at room temperature for 24 hours, with a greater decrease in activity as the temperature was raised. In order to study any delayed inactivation following irradiation, it was necessary to adjust for this spontaneous loss. This was done by including adequate controls, i.e., at least five independent measurements of the activity of unirradiated enzyme.

This spontaneous inactivation is also dependent upon enzyme concentration, pH, and nature of the solvent. Because dissolved pepsin is most stable near pH 4.5, this pH has been used in the majority of our studies. The enzyme concentration was governed by our assay methods. We found that spontaneous inactivation increased with enzyme concentration. It is probably a first order autolytic reaction as has been reported by Kunitz, Northrop, and others.

Table 4 shows the effect of storing pepsin at 25°, 37°, and 45° for

TABLE 4

Per cent of Unirradiated Pepsin Activity as Assayed by Two Methods (±S. E.)

Method	*Anson*	*RISA*	*Anson*	*RISA*	*Anson*	*RISA*	*Anson*	*RISA*
Delay time (hr)	0	0L	24 hr	24 hr	24 hr	24 hr	24 hr	24 hr
Delay temperature	–	–L	25°	25°	37°	37°	45°	45°
0 r	100	100	100	100	100	100	100	100
10 Kr	81±	85±	81±	76±	76.6±	79±	71.3±	77±
	1.6	1.8	4.1	3.7	6	0.7	1.0	2.0
20 Kr	76±	75±	71	67L	66	68	64±	58±
	1.0	1.0					2.0	4.0
30 Kr	64±	65±	58.6±	58±	61±	54±	52±	50±
	1.7	1.0	3.2	3.4	2.9	1.1	2.4	2.8
50 Kr	48±	47±	37±	40±	39±	35±	32±	33.6±
	2.2	1.3	2.8	4.8	1.1	2.3	3.3	3.4

Delayed inactivation of pepsin following exposure to roentgen rays.

24 hours after exposure to doses of radiation up to 50,000r. Standard errors are indicated. When these data, the results of 14 separate experiments using both RISA and the Anson hemoglobin methods, are plotted on semi-logarithmic paper, straight lines are obtained indicating

that both the initial and the continued inactivations are unimolecular reactions.

It is evident that the delayed inactivation is markedly temperature dependent with increasing inactivation with increased temperature. This temperature dependence suggests that delayed inactivation reflects some internal change in the pepsin molecule produced by x-rays which enhances its sensitivity to thermal denaturation as has been postulated for other molecules by Fricke, Barron, and McDonald.

Caldecott has shown evidence of a continued inactivation of barley seeds following exposure to radiation and that this continued inactivation is very dependent on the presence of oxygen. He has shown, further, that the effect is present for a period as long as 6 hours following exposure. We studied the effect of oxygen on this delayed inactivation. Results to date indicate that oxygen tension affects neither the immediate inactivation nor the delayed inactivation of pepsin exposed to ionizing radiations. We have found evidence that cysteine tends to reduce the extent of the delayed inactivation following irradiation. This part of our investigation has been complicated by the fact that solutions of cysteine tend to be very acid, and consequently, more concentrated buffering action is required to maintain a pH of near 4.5 so that the pepsin will not be spontaneously inactivated on standing due to autolysis. With regard to dependence on the radiation quality, we have found that the delayed inactivation is somewhat smaller following exposure to Co^{60} gamma rays than it is following exposure to x-rays from a conventional x-ray therapy unit.

Studies of changes in the ultraviolet spectra of pepsin following irradiation, as well as iontophoresis and chromatography, have been initiated. It is hoped that these investigations will shed some light on the mechanisms by which the actual inactivation is produced. Furthermore, it is hoped that these studies will also aid in an evaluation of the continued inactivation of pepsin following exposure to ionizing radiations.

Summary

Using RISA and hemoglobin substrate for assaying enzymatic activity, various factors affecting the radiosensitivity of pepsin have been investigated. Among these are enzyme concentration, solvent, pH, temperature, concentration of inorganic and organic ions, and oxygen tension. Studies have also been made of the mechanism responsible for the gradual loss of activity of these enzymes after termination of ir-

radiation. This delayed inactivation is dependent upon temperature of storage of the irradiated enzyme, enzyme concentration, and the dose of radiation, but not on oxygen tension. The effects of radiation quality and the addition of various chemicals before and after radiation have also been investigated.

Editorial

As the Year Ends

As the current academic year draws to a close, the BULLETIN rounds out its second year of publication in its present, printed format. We believe that it has been a successful year for the BULLETIN, for it seems to have met with the continued approval of its readers.

During the past year many staff meeting reports appeared in their entirety. Those that were abridged were abridged far less drastically than was the case during the preceding year. Readers are once again invited and urged to let us know whether they prefer the short abstracts, such as were published during the first year of the new BULLETIN, or the complete papers and longer abridgments such as were printed during the past year.

We wish to extend our thanks to the authors of the various staff meeting reports, which this year hit a new high in excellence; to the Associate Editors, each of whom accepted the complete responsibility for one issue of the BULLETIN during the year; and to the girls of our office staff, Miss Gloria Norell and Miss Louise Liggett, without whose skillful preparation of the material to be printed the BULLETIN could not be published. Special thanks are due, too, to Miss Elva Lavers and her co-workers of the Mimeograph Department who once again have done their usual fine job in providing us with the mimeographed copies of the staff meeting reports which are handed out to those attending the staff meetings.

To all of our students, members of the Faculty, members of the Foundation, and friends of the Medical School, we express the hope that the coming summer will be a pleasant one, and that it will provide an opportunity to rest, to relax, and perhaps to recharge the intellectual batteries.

Medical School Activities

Recognition Exercises

Recognition Exercises honoring the Senior Class in Medicine were held on Friday, June 14, at 4:00 p. m. in the Mayo Memorial Auditorium. Held on the day preceding the all-University Commencement, the Recognition Exercises provided the Faculty an opportunity to honor its graduating Seniors in a more personal way than is possible at the larger University-wide event.

Following the invocation by RABBI LEWIS MILGROM, DEAN H. S. DIEHL welcomed the 110 Seniors and members of their families, and JOHN R. SHEFVELAND, *President* of the Senior Class, responded on behalf of his classmates. The Borden Award for outstanding research carried out as an undergraduate student was presented to SOLOMON J. ZAK. JOHN S. GLETNE was recipient of the Southern Minnesota Medical Association Award, recognizing the outstanding senior student in the Medical School on the basis of greatest proficiency during his junior and senior years in the clinical fields of medicine and surgery. The award was presented by DR. C. F. STROEBEL. ALLEN M. ANDERSON, RICHARD D. CUNNINGHAM, CARL B. HEGGESTAD, ROBERT J. OLSON, and JEROME H. SACKS received Mosby Scholarship Book Awards.

The Declaration of Geneva was administered to the class by DR. LEO G. RIGLER. This was followed by presentation of the University's Outstanding Achievement Award to DR. LOUIS H. RODDIS, member of the Class of '13, by DR. CHARLES W. MAYO on behalf of the Board of Regents. Dr. Roddis was honored "as a retired captain and surgeon of the United States Navy, a prominent author of books and articles on nautical medical history, outstanding and diligent editor of scientific publications, and efficient administrator of military hospitals in peace and in war."

The principal address of the evening was given by DR. PAUL DUDLEY WHITE. Dr. White's address, entitled "The Patient Leads the Way," provided a most fitting climax to the impressive ceremony.

Alpha Omega Alpha

On May 23, the following members of the Senior Class were re-

ceived into membership in Alpha Omega Alpha, honorary medical fraternity:

ALLEN M. ANDERSON
WILLIAM E. BRADLEY
PAUL E. BRENK
LELAND R. CHRISTENSON
PETER E. FEHR
ROBERT R. FOLEY
DONALD S. HARDER
NORMAN M. HORNS
ROBERT J. OLSON
JAMES P. ROBINSON
JEROME H. SACKS
GEORGE J. SCHROEPFER, JR.
WAYNE W. THOMPSON
FRANK W. VAN DE WATER
JOSEPH O. YOUNG

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

GERALD A. GRETSCH
GLENN A. HARTQUIST
EUGENE T. O'BRIEN

The Senior Class

The following is a complete list of all of the members of the Senior Class. With each physician's name we have listed where he will serve his internship.

ALLEN M. ANDERSON
Philadelphia General Hospital
Philadelphia, Pennsylvania

DANIEL C. ANDERSON
U. S. Public Health Service
San Francisco, California

WILLIAM W. BAAK
Ancker Hospital
St. Paul, Minnesota

JEROME M. BACH
U. S. Public Health Service
Baltimore. Maryland

LUCY J. BALIAN
Philadelphia General Hospital
Philadelphia, Pennsylvania

GEORGE B. BAUDER
Asbury Methodist Hospital
Minneapolis, Minnesota

JOHN H. BEGGS
Gorgas Hospital
Ancon, Canal Zone

PAUL G. BELAU
U. S. Public Health Service
Staten Island, New York

AVI BEN-ORA
San Joaquin County Hospital
Stockton, California

LAVONNE B. BERGSTROM
Minneapolis General Hospital
Minneapolis, Minnesota

JOHN A. BERMAN
Highland-Alameda County Hospital
Oakland, California

GEORGE C. BINGHAM
Mount Sinai Hospital
Minneapolis, Minnesota

WILLIAM E. BRADLEY
University of Minnesota Hospitals
Minneapolis, Minnesota

PAUL E. BRENK
United States Army
Fort Benning, Georgia

BANCROFT M. BROOKS
Kaiser Foundation
San Francisco, California

CHARLES T. BROWN
Bellevue Hospital, Third Surgical Division
New, York, New York

FRANK T. BROWN
Parkland Memorial Hospital
Dallas, Texas

JOHN J. CASEY
Bethesda Hospital
St. Paul, Minnesota

GEORGE R. CHAPMAN
Minneapolis General Hospital
Minneapolis, Minnesota

LELAND R. CHRISTENSON
Bethesda Hospital
St. Paul, Minnesota

GEORGE M. CROW
Mercy Hospital
Toledo, Ohio

RICHARD D. CUNNINGHAM
Tripler Army Hospital
Honolulu, Hawaii

WALTER J. DAWSON
St. Luke's Hospital
Cleveland, Ohio

JAMES P. DUDLEY
Ancker Hospital
St. Paul, Minnesota

JOHN C. EDGERTON
Brooke General Army Hospital
San Antonio, Texas

S. PAUL EHRLICH
U. S. Public Health Service
Staten Island, New York

E. DUANE ENGSTROM
St. Luke's Hospital
Duluth, Minnesota

JOSEPH H. EUSTERMAN
Abington Memorial Hospital
Philadelphia, Pennsylvania

LELAND L. FAIRBANKS
U. S. Public Health Service
New Orleans, Louisiana

PETER E. FEHR
Minneapolis General Hospital
Minneapolis, Minnesota

ROBERT R. FOLEY
Santa Clara County Hospital
San Jose, California

JOSEPH GELLER
Minneapolis General Hospital
Minneapolis, Minnesota

JOHN S. GLETNE
Santa Clara County Hospital
San Jose, California

M. MELVIN GOLDFINE
U. S. Public Health Service
San Francisco, California

ALAN L. GOLDSTEIN
St. Charles Hospital
Toledo, Ohio

THOMAS H. GONIOR
University of Minnesota Hospitals
Minneapolis, Minnesota

GARY D. GOOD
Salt Lake County Hospital
Salt Lake City, Utah

ASA B. GRAHAM
Mercy Hospital
Toledo, Ohio

DAVID G. HANEY
Wayne County General Hospital
Eloise, Michigan

DONALD S. HARDER
Ancker Hospital
St. Paul, Minnesota

JEROME L. HARTY
St. Luke's Hospital
Duluth, Minnesota

CARL B. HEGGESTAD
University of Minnesota Hospital
Minneapolis, Minnesota

ROBERT R. HILKER
St. Mary's Hospital
Duluth, Minnesota

NORMAN M. HORNS
Minneapolis General Hospital
Minneapolis, Minnesota

VINCENT W. HOVERSTEN
Santa Clara County Hospital
San Jose, California

GEORGE E. JACKISH
Hurley Hospital
Flint, Michigan

DWIGHT E. JAEGER
Ancker Hospital
St. Paul, Minnesota

FREDERICK W. JENSEN
San Francisco Hospital
San Francisco, California

PAUL A. JENSEN
Letterman Army Hospital
San Francisco, California

DORIS I. JOHNSON
Orange County Hospital
Orange, California

WENDALL A. JOHNSON
St. Mary"s Hospital
Duluth, Minnesota

HAROLD W. KEAIRNES
U. S. Public Health Service
San Francisco, California

MICHAEL J. KOZAK
Mount Sinai Hospital
Minneapolis, Minnesota

GERALD K. KVISTBERG
Minneapolis General Hospital
Minneapolis, Minnesota

RONALD R. KYLLONEN
Minneapolis General Hospital
Minneapolis, Minnesota

DEAN W. LARSON
Cleveland City Hospital
Cleveland, Ohio

MOSES LICHTIG
Montefiore Hospital
New York, New York

WILLIAM L. LIFSON
Milwaukee County Hospital
Milwaukee, Wisconsin

DALE D. LINDHOLM
St. Luke's Hospital
Duluth, Minnesota

RICHARD R. LUND
Minneapolis General Hospital
Minneapolis, Minnesota

RICHARD P. LYNCH
St. Mary's Hospital
Duluth, Minnesota

FRED A. LYON
Mount Sinai Hospital
Minneapolis, Minnesota

MERLE S. MARK
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Philadelphia, Pennsylvania

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Sacramento, California

JOSEPH P. MLINAR
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Philadelphia, Pennsylvania

JEROME H. MODELL
St. Albans Naval Hospital
Long Island, New York

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Minneapolis General Hospital
Minneapolis, Minnesota

GENE C. MUCHOW
Ancker Hospital
St. Paul, Minnesota

JOHN E. MULVAHILL
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St. Paul, Minnesota

JOHN A. NILSEN
Ancker Hospital
St. Paul, Minnesota

SOM N. NAYYAR
University of Minnesota Hospitals
Minneapolis, Minnesota

ROBERT J. OLSON
U. S. Public Health Service
San Francisco, California

RICHARD H. OPHEIM
Southern Pacific Hospital
San Francisco, California

MARTIN W. ORBUCH
Minneapolis General Hospital
Minneapolis, Minnesota

AUSMA V. OSS
St. Michael's Hospital
Newark, New Jersey

CHARLES A. PETERSON
U. S. Public Health Service
Boston, Massachusetts

JOHN A. PETERSON
Bethesda Hospital
St. Paul, Minnesota

PAUL H. POBOR
Los Angeles County Hospital
Los Angeles, California

JOHN W. POLLARD
Valley Forge Hospital
Valley Forge, Pennsylvania

CURTIS C. REEMSNYDER
Bethesda Hospital
St. Paul, Minnesota

CHARLES A. ROACH
Miller Hospital
St. Paul, Minnesota

JAMES P. ROBINSON
Walter Reed Army Hospital
Washington, D. C.

ARTHUR J. RUSHAY
Ancker Hospital
St. Paul, Minnesota

JOHN RYDBERG
Receiving Hospital
Detroit, Michigan

JEROME H. SACKS
University of Minnesota Hospitals
Minneapolis, Minnesota

NORMAN I. SACKS
Harbor County Hospital
Torrance, California

KEITH D. SAYTHER
San Joaquin Hospital
French Camp, California

JEROME J. SCHEREK
Ancker Hospital
St. Paul, Minnesota

GEORGE J. SCHROEPFER, JR.
University of Minnesota Hospitals
Minneapolis, Minnesota

STANLEY W. SHAPIRO
Mount Sinai Hospital
Minneapolis, Minnesota

JOHN R. SHEFVELAND
Harbor General Hospital
Torrance, California

DONALD A. SHUMRICK
San Francisco County Hospital
San Francisco, California

HENRY N. SMITH
Ancker Hospital
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STANLEY C. SOLLIE
Highland-Alameda County Hospital
Oakland, California

ANTON F. SPRAITZ
Ancker Hospital
St. Paul, Minnesota

LENNART E. SUTHER
Highland-Alameda County Hospital
Oakland, California

EDWIN J. TANQUIST
Gorgas Hospital
Ancon, Canal Zone

WAYNE W. THOMPSON
Minneapolis General Hospital
Minneapolis, Minnesota

FLORENCE A. TOUSSAINT
Queen's Hospital
Honolulu, Hawaii

ROMIL VALGEMAE
Minneapolis General Hospital
Minneapolis, Minnesota

FRANK W. VAN DE WATER
Denver General Hospital
Denver, Colorado
PAUL S. VAN PUFFELEN
St. Luke's Hospital
Duluth, Minnesota
RALPH F. WELLS
Brooke General Army Hospital
San Antonio, Texas
ROBERT W. WILKEN
Ancker Hospital
St. Paul, Minnesota
JOSEPH O. YOUNG
Tripler Army Hospital
Honolulu, Hawaii
SOLOMON J. ZAK
University of Minnesota Hospitals
Minneapolis, Minnesota

Faculty News

DR. RICHARD A. DEWALL, *Research Assistant,* Department of Surgery, shares the American Association for Advancement of Science-Ida B. Gould Memorial Award with DR. C. WALTON LILLEHEI, *Professor,* Department of Surgery. The award which consists of $1,000 was given to the two doctors for their pioneering developments in heart surgery. Presentation was made at a recent meeting of the American Association for Advancement of Science.

DR. MILAND E. KNAPP, *Clinical Professor,* Department of Physical Medicine and Rehabilitation, has received the distinguished service key from the American Congress of Physical Medicine and Rehabilitation.

IN MEMORIAM

Our friend and colleague, DR. JOHN P. STREET, *Instructor,* Department of Medicine, died on April 28, 1957, at age 31. Dr. Street, a 1950 graduate of the University of Minnesota Medical School, served his internship at Ancker Hospital, St. Paul. After completion of his military service, he became a Medical Fellow at the Minneapolis Veterans Administration Hospital. He joined the Faculty as an Instructor in July, 1956, and was a member of the full-time staff of the Medical Service of the Veterans Administration Hospital up to the time of his death. Dr. Street was a capable clinician and teacher whose promising career was cut short by his untimely death.

Dr. Street is survived by his wife, Charlotte, and an infant daughter. Members of the Faculty join in extending deepest sympathy to his family.

Index

CUMULATIVE INDEX: 1952-1957

From October, 1951, until June, 1955, the name of this publication was "Bulletin of the University of Minnesota Hospitals and Minnesota Medical Foundation." In October, 1955, the name was changed to "UNIVERSITY OF MINNESOTA MEDICAL BULLETIN."

C

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
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 Hospital and the Minneapolis Veterans Administration Hospital, write to the Editor of the BULLETIN, 1342 Mayo Memorial, University of Minnesota, Minneapolis 14.