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PNEUMOCOCCAL HEMOLYSIN

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New Horizons in Psychiatry

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Psychiatry suffered its literal baptism of fire in World War II. It survived and flourished because for the first time in its history it was able to demonstrate on a mass scale a practical aspect of its nature which gave it a respectable identity in the medical family. The venerable Franklin Ebaugh, chairman of the department of psychiatry at the University of Colorado from 1924 to 1953, told a group of us who were interns in 1949 that he was pleased to see that young physicians were becoming interested in psychiatry simply because they desired to become specialists in emotional and mental aberrations, and not because they were seeking magical solutions to their own problems. He made this generalization from his long and varied experience to accent the point of the changing status of the profession.

The immediate post-war period concentrated on education with emphasis upon the post-graduate phase. There was a sudden explosion of the number of returning physicians going into psychiatry, and a side benefit of this aroused interest was an increase in the number of medical personnel available to care for hospitalized patients. The upgrading of mental hospitals and patient care received attention not seen since the days of Dorothea Dix.

The phenothiazines and related psychotropic drugs were introduced in the early 1950's. They went hand in glove with the changing concepts of care for the mentally ill—to reduce hospital stay and renew attention to neglected and hitherto almost hopeless areas. Use of the new drugs ignited greater interest in the chemistry of the central nervous system. The previously held faint hope of magical control of mental illness with almost antibiotic-like efficiency became almost a certain promise if only enough research time and money were made available. The flood of data about neurohormones, the limbic system, the hypothalamus, and metabolic disorders which came from laboratories continues today with only slight abatement. However, the promise of magical control of emotional and mental disorders via the new chemical means remains unfulfilled, however great were the benefits from them.

The 1960's might be called the decade of social awareness. A rising crescendo of voices demanded that the same attention be given to the quality of man's years as was being given to the quantity of them. It became painfully obvious that man's psyche could not be disected from his environment any more than his mind could be separated from his body. Many of the concepts of Adolf Meyer were rediscovered, rephrased, and amplified. The psychiatrist, the behavioral scientist, and the social scientist suddenly discovered that they were playing in the same ball game, and that they needed to establish compatible rules and regulations. Community and social psychiatry became embryonic modalities with indistinct boundaries and with even dimmer convictions about how they were to accomplish their goals. Progress has been made, but much more remains for the future.

During this post-war period of massive change and rapid advancement the entire medical profession became increasingly aware of the significance of human behavior and emotions in relation to organic disease. The acceptance of psychiatry as part of the medical team was hastened by advances such as organ transplant, intensive care units, and by information showing that in some cases emotional factors could, by changes in the autonomic nervous system and its end organs, determine the outcome of otherwise superb technical procedures. Departments of obstetrics-gynecology and pediatrics began to include the psychiatrist within their own domains as an integral part of the system to teach central aspects of behavior once ignored or given only token attention. A new breed of student began entering medical school with an increased social awareness and with demands to be taught more than the technicalities of medicine. Curriculum time for psychiatry more than quadrupled in most schools.

Just as psychiatry was gaining a hard-won acceptance and was beginning to enter the medical world as a full-scale citizen, a jarring note appeared. The American Psychiatric Association approved a plan to become effective 1 July, 1970 which would allow the psychiatrist to enter residency and complete certifiable

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training without an internship (Gregory, 1970). John Romano has aptly termed this a regressive move, and many of us agree (Romano, 1970). This plan could produce psychiatrists of doubtful maturity and questionable medical competence to understand the total patient. Perhaps no medical specialist so badly needs some clinical experience with the overall physiology and pathology of life as does the psychiatrist. Without the medical orientation and knowledge which can be built upon internship or a comparable experience, the psychiatrist may become indistinguishable from the clinical psychologist and Ph.D. social worker. Some of us do not hold that the changed and more clinical senior year of medical school is an adequate substitute for the internship.

Another very practical fact dictates against shortening the post-graduate educational period. The amount of data presently essential for the well rounded psychiatrist has multiplied astoundingly in the last two decades. There have been almost no compensating eliminations of material, and methods of transmitting the needed information and techniques have not improved measurably. Perhaps this decision to shorten training is a compliment to the increased sophistication and brilliancy of our medical students—and without question that is true of them—but it is hard to visualize this plan other than as a ploy to produce greater numbers at the sacrifice of quality.

What, then, of the future? All the advances of the post-war years must be considered as a continuum which will determine somewhat the way we go. There is first a decision to make about the general direction. Will psychiatry continue to be a medical specialty, or will it become another branch of psychology and head toward the elimination of medical requirements altogether? The present trend to reduce the medical training prior to residency, combined with the virtual elimination of neurology from many residency programs, is not a happy sign to those of us who identify as physicians. Some of us will resist this and actively attempt to reverse the trend.

The other and preferred orientation is medical. This route will produce the future psychiatrist whose basic orientation is, to borrow a word from James Weiss, operational (Weiss, 1970). This term is used to refer to "a marriage of experimentally derived principles and naturalistic clinical observations." To further quote Dr. Weiss:

> The operational psychiatrist is concerned not only with the biology and psychology of the "whole patient" but also with the social environment which is invariably involved in the predisposition, precipitation, and perpetuation of illness. He needs to understand and to use effectively the language of the culture (as well as that culture's and its people's attitudes, mores, biases, and concerns), to communicate with patients. He needs some appreciation of cultural differences and cross-cultural variations, without losing sight of the individual patient in that broad social matrix. He has an interest in limiting the two major defects from which psychiatry as a total discipline, despite its remarkable advances in the past half century, has suffered: a lack of sufficient researched knowledge and a lack of adequate proportion of psychiatrists who are both professionally and characterologically competent.

Operational obviously indicates flexible. A profession or specialty must reach a certain stage of maturity before the luxury of flexibility can be afforded. It appears that a degree of rigidity and fanaticism may be an essential, albeit unfortunate, defense for the insecure and the actually limited. Hopefully, psychiatry in the future will no longer require this defense, although it admittedly has used it in the past.

The operational psychiatrist in the future will have a subdivision dependent upon individual interests and talents. Some will work primarily with communities, and others with the basic building block of the community, the family. The recent growth of interest in forensic psychiatry will necessitate a sub-specialty in this field. Adolescent psychiatry already is becoming an entity. Psychiatric research will continue, but most of it will not be done by clinical psychiatrists. Hopefully, though the clinical psychiatrists will be an important cog in research machinery, they will act primarily as consultants to those more specifically trained for scientific research.

One thing will not change if the future psychiatrist is to be effective. The base line of knowledge and skill will still be drawn from a complete understanding of the structure, development, function and malfunction of the total individual. He may call himself a behavioralist, a psychoanalyst, an opeationalist, or a social or community psychiatrist, but he must operate from a thorough knowledge of the most basic unit of all society, the individual. All other information and technical skills will be layered upon this foundation and it must remain the core of the educational program.

It follows from this line of reasoning that the future psychiatrist may be primarily a consultant to other disciplines. One-to-one psychotherapy with individual patients will remain an important facet of his work. In fact, this type of contact is essential if the psychiatrist's skills as an expert in human behavior are to be maintained, but there will be many other professionals equally adept at this. Already there are those who feel that the lay analyst, the clinical psychologist, and the well-trained psychiatric social worker are as adept at individual psychotherapy as are many psychiatrists with less special or more general interests.

The future psychiatrist's identity will depend upon

his ability to correlate the psychological, pharmacological and sociological treatment modalities within the medical community. Each of these areas will have its own expert, but the psychiatrist will translate this expertise into a clinically usable form.

The work of the future psychiatrist, and all others in the field of mental health, will be dictated by a trend that is now quite well established. The major problem of the present, and it seems fairly certain that it will continue so, is with the broad category of personality disorders or behavioral disorders. The classical neuroses that filled the clinics a generation or two ago are declining in number and changing in symptomatology. The disorders of behavior including alcoholism, drug abuse, delinquency and violence are growing with alarming frequency. The explosive events of the past decade have caught us literally with our couches down! Our therapeutic modalities for these entities have been relatively ineffective, and even if this were not so, the problems of numbers and finances have made delivery of treatment in sufficient quantity virtually impossible. Disorders of personality and behavior constitute psychiatry's number one problem of the future.

It would take either colossal narcissism or unbelievable ignorance to make a concrete prediction about the future. And yet, those of us in medical schools must try to do this to some extent, and attempt to educate for the future with present knowledge based on past experience. The danger lies in making a prediction and then trying to manipulate events so as to make the prophesy self-fulfilling. Only two things appear absolutely certain if psychiatry is to maintain its identity as a medical specialty: 1. The future psychiatrist must have a thorough grounding in the physiological, psychological and sociological aspects of the human being. 2. He must be so flexible as to give any one of these three facets precedence when a given situation indicates the need.

Psychiatrists have long held that there should be more psychiatry in general medicine, and the non-psychiatric physicians have said that there should be much more medicine in psychiatry. Both groups have been perfectly correct and are being told by the consuming population that there must be more sociology in each of them. The future psychiatrist must and will work with his colleagues in medicine, not to achieve a utopia, but at least to approach this desirable situation in some degree.

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Pneumococcal Hemolysin*

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Diplococcus pneumoniae elaborates a heat labile substance capable of lysing rabbit erythrocytes *in vitro* and *in vivo* (Shumway and Pollock, 1965). It has been suggested (Shumway and Pollock, 1965) that this substance may be responsible for the hemolytic anemia which occurs in rabbits with pneumococcal septicemia (Shumway, 1958) but proof for this hypothesis is still lacking.

The purposes of this report are to describe a method of preparing a potent crude pneumococcal hemolysin, to define some of its characteristics, and to describe the effects of its administration to rabbits.

Methods

Preparation of Crude Hemolysin

The microorganism utilized in these studies was a strain of Diplococcus pneumoniae, type 1, kindly supplied by Herbert Morgan. As the microorganism grows in glucose-enriched brain-heart infusion broth, it elaborates a hemolysin which can be precipitated from the supernatant broth by ammonium sulfate. Because early investigations suggested that the hemolysin was an endotoxin (Cole, 1914), attempts were made to extract the hemolytic substance from bacterial cells rather than from the supernatant broth. Preliminary studies disclosed that hemolytically active extracts could be obtained when the bacteria were disrupted enzymatically with lysozyme, osmotically with distilled water, or mechanically with glass beads or high frequency sound. Sonic disintegration proved to be the simplest and most efficient method of cell disruption.

A potent crude hemolysin was prepared in the following manner: Three liters of brain-heart infusion broth (Difco) containing glucose in a final concentration of 500 mg/100 ml was inoculated with 300 ml of a 6 to 8 hr broth culture of Diplococcus pneumoniae, type 1. The broth was maintained at 37 C in an atmosphere of nitrogen and was mixed continuously with a magnetic stirrer. Aliquots were withdrawn at 30 to 60 min intervals to measure turbidity at 650 mμ, pH and glucose concentration. The pH, which decreased rapidly during the logarithmic phase of bacterial growth, was maintained between 7.0 and 7.5 by the gradual addition of sterile 5N sodium carbonate. Glucose concentration was maintained between 400 and 600 mg/100 ml by the addition of sterile 50% glucose solution. When the optical density of the culture reached its maximum (6–8 hr) the broth was placed at 4 C under nitrogen for 18 hr. It was then centrifuged at 18,000 G in a continuous flow system; this centrifugation and all subsequent manipulations were carried out at 5 C.

The sedimented bacteria were suspended in approximately two volumes of cold distilled water and subjected to high frequency sound (Branson Model S-75 Sonifier at full power) for a total of 10 min. The sonication was carried out at 1 min intervals so that the temperature of the bacterial suspension did not rise above 10 C. The material was then centrifuged at 18,000 G for 30 min, and the supernate, which contained the hemolysin, was removed and frozen at −20 C until further steps could be carried out.

Preliminary studies using step-wise fractionation disclosed that the hemolysin was precipitated from the bacterial liquor between 20 and 60% saturation with ammonium sulfate. All subsequent preparations of the hemolysin were made with the precipitate formed between these two concentrations of this salt. The precipitate was dissolved in distilled water and dialyzed against cold distilled water until the dialysis bath no longer reacted with Nessler's reagent. The dialyzed material was then divided into aliquots, freeze-dried, vacuum sealed and stored at −20 C until further use.

The hemolytic activity of this extract varied from batch to batch; ordinarily it required 0.006 to .01 ml of the material (equivalent to 87-145 μg of protein) to completely lyse 1 ml of human or rabbit erythrocytes. In its freeze-dried state the hemolysin maintained

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its potency for at least 5 years. When it was stored in solution at room temperature for 8 days, there was a four-fold reduction in its hemolytic activity; when stored in solution at −20 C for 12 months, there was a 16-fold reduction in its activity. In the latter instance, part of its activity could be restored by the addition of 0.4 M cysteine.

Hemolysin Assay

Maximal lysis of either human or rabbit erythrocytes by the hemolysin occurred within 60 min at 37 C. Two methods of quantitation were used for the studies to be reported here. The first involved the addition of 0.5 ml of a known dilution of the hemolysin to 1 ml of a 50% suspension of thrice washed rabbit or human erythrocytes in isotonic sodium chloride solution; this mixture was incubated for 60 min at 37 C, centrifuged at 3000 G for 5 min, and the amount of hemoglobin released into the supernatant solution was measured spectrophotometrically. Results were expressed as percentage of red cells lysed. This method was satisfactory for much of the early work where gross differences of hemolysis were evident, but when differences were more subtle, a more sensitive method of assay was needed.

For this latter purpose, serial dilutions of the hemolysin were made in 0.85% sodium chloride solution. One ml of a 3% suspension of thrice washed human or rabbit erythrocytes in isotonic saline was added to an equal volume of each dilution of hemolysin. The tubes containing these mixtures were mixed thoroughly and incubated for 60 min at 37 C. After incubation, the contents of the tubes were mixed again, centrifuged at 2000 G for 2 min, and the degree of hemolysis graded on a scale of from 0 to 4^+ (complete hemolysis). Results using this method were reproducible within one tube dilution.

Hemolysin Inhibition Titer

Two-fold serial dilutions of the serum under investigation were made using 0.85% sodium chloride as diluent. Five-tenths milliliter of each serum dilution was mixed with 0.5 ml of diluted hemolysin; the concentration of hemolysin used was one tube dilution less than that dilution which produced complete lysis of the standard red cell suspension. After the mixtures of serum and hemolysin had been incubated at 37 C for 15 min, 1 ml of a 3% suspension of washed rabbit erythrocytes was added to each tube. The contents of the tubes were thoroughly mixed, incubated at 37 C for 60 min, centrifuged at 3000 G for 2 min, and examined for hemolysis. The highest dilution of serum which completely inhibited hemolysis under these conditions was termed the "hemolysin inhibition titer." Results were reproducible from day to day within two tube dilutions, but whenever two or more sera were compared they were analyzed simultaneously.

TABLE I

Effect of Trypsin on Pneumococcal Hemolysin

Hemolysin Dilution	Trypsin-Percent	Percent Hemolysis
1 : 200	0	94
1 : 200	.0009	85
1 : 200	.009	2
0	.0009	1
0	.009	1

Crystalline trypsin was dissolved in M/15 phosphate buffer pH 7.3 and mixed with the crude bacterial extract (hemolysin) so that final concentrations were as above. Control tubes contained isotonic saline solution. After 30 min at 37 C, 0.5 ml of each mixture was added to 1 ml of 50% suspension of washed rabbit erythrocytes, tubes mixed and incubated at 37 C for 60 min and centrifuged. Percentage of cells lysed was measured spectrophotometrically.

TABLE II

Effect of Cholesterol on Pneumococcal Hemolysin

Hemolysin Dilution	Cholesterol mg/100 ml	Percent hemolysis
1 : 200	0	93
1 : 200	90	< 1
1 : 200	9	< 1
0	90	< 1
0	9	< 1

Cholesterol suspended in isotonic sodium chloride solution was mixed with bacterial extract (hemolysin) to give final concentrations noted above. Control tubes contained isotonic saline solution. After 30 min at 37 C, 0.5 ml of each mixture was added to 1 ml of a 50% suspension of washed rabbit erythrocytes in isotonic sodium chloride. Tubes were mixed thoroughly, incubated 60 min at 37 C and centrifuged. Percentage of cells lysed was measured spectrophotometrically.

As an additional control, at the time of each assay a "standard serum" of known inhibitory effect was also analyzed; these results were always within one tube dilution.

The animals used for *in vivo* studies were mature male albino rabbits. Blood was obtained from marginal ear veins and measurements of packed red cell volume and erythrocyte osmotic fragility were made by standard methods (Shumway and Pollock, 1965). The degree of hemoglobinemia was estimated qualitatively by inspection of supernatant plasma.

Results

The hemolysin present in this bacterial extract exhibited characteristics similar to those described by others working with "pneumococcal hemolysin," "pneumotoxin," or "pneumococcus hemotoxin" (Cohen, Halbert and Perkins, 1942; Cole, 1914; Cowan, 1934; Fleming and Neill, 1927; Halbert, Cohen and Perkins, 1946; Neill, 1926; Weiss, 1918). Hemolytic activity was abolished by heating to 56 C for 10 min and by exposure of the hemolysin to trypsin (Table I) or cholesterol (Table II).

Sodium cyanide 0.02m and disodium-EDTA 0.003m exhibited no inhibitory effects. Normal human sera and normal rabbits' sera exhibited slight inhibitory effect, the former more than the latter.

The effect of temperature upon the interaction of hemolysin and erythrocytes was noteworthy. When hemolysin and either human or rabbit red blood cells were incubated together for 60 min at temperatures between 4 C and 37 C, the degree of hemolysis was greater at the higher temperatures (Fig 1), At 4 C hemolysis was virtually absent. When erythrocytes which had been exposed to hemolysin for 60 min at 4 C were washed three times with 10 volumes of cold isotonic saline solution, resuspended to their original volume and incubated at 37 C for 60 min, lysis occurred. The supernatant material from the original mixture of hemolysin and erythrocytes failed to lyse fresh erythrocytes when exposed to them at 37 C for 60 min.

Earlier studies had shown that the intravenous administration of supernatant broth from a culture of Diplococcus pneumoniae to rabbits resulted in intravascular hemolysis (Shumway and Pollock, 1965). As will be demonstrated, this same phenomenon was observed after injection of crude bacterial extract. Because the characteristics of the hemolysin suggested that it was a protein or contained protein as an integral part of its structure, it was likely to be antigenic. The results of the following experiments are compatible with this hypothesis.

Normal mature male albino rabbits were selected at random. Blood samples were obtained for baseline measurements of red blood cell morphology, packed red cell volume and erythrocyte osmotic fragility; an

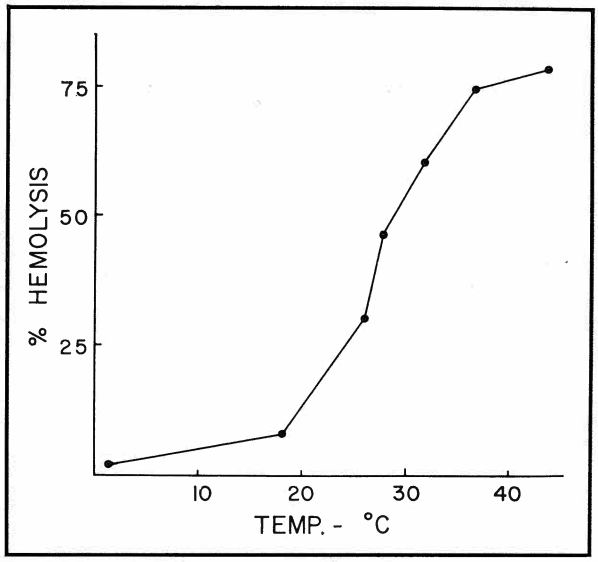

Fig 1—Effect of temperature upon the lysis of human erythrocytes by pneumococcal hemolysin.

aliquot of serum was frozen for subsequent determination of the "hemolysin inhibition titer." A standard amount of the crude bacterial hemolysin (0.2 ml/kg) diluted in isotonic sodium chloride solution was given intravenously to each rabbit, and 2 hr later a second blood sample was obtained to reassess the above mentioned parameters.

The results are summarized in Table III. Twelve of 22 rabbits exhibited unequivocal evidence of intravascular hemolysis as manifested by a 6% or greater decrease in packed red cell volume, an increase of erythrocyte osmotic fragility and hemoglobinemia. The animals' erythrocytes were found to be spherocytic or to have a crenated or spiculated appearance (Fig 2). Abnormalities of at least one or two of these parameters were evident in nine other animals. The one rabbit which failed to exhibit any evidence of hemolysis was subsequently found to have a high "hemolysin inhibition titer" (1/5120) without any known prior exposure to the hemolysin.

Nine of the rabbits which had been challenged by the intravenous injection of the hemolysin were given "booster doses" of the material (one-fourth original amount) intramuscularly, 9, 12, and 14 days after the original challenge. On the 21st day the sera of seven of the nine animals had an increased ability to inhibit the hemolysin *in vitro; the* "hemolysin inhibition titers" rose from 1/80 or less to values from 1/640 to 1/5120. When these immunized rabbits were challenged a second time by the intravenous injection of the standard amount of hemolysin, none exhibited any evidence of intravascular hemolysis (Fig 3; Table III).

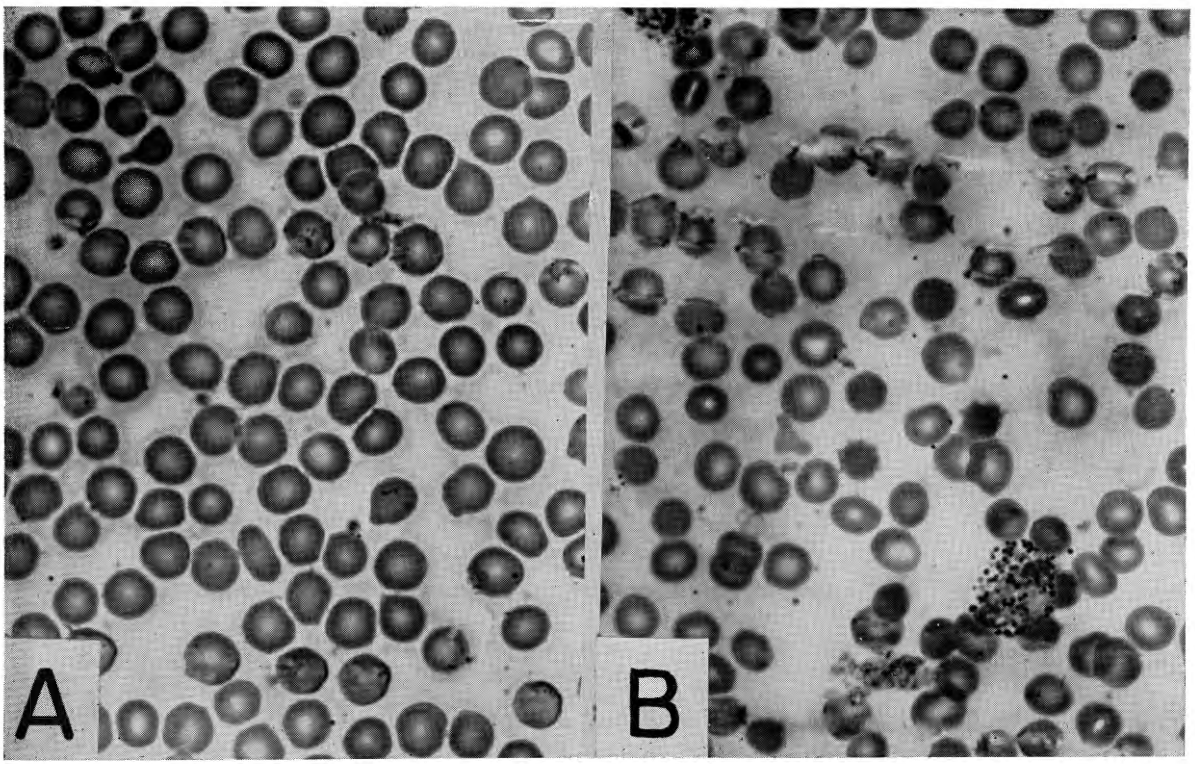

Fig 2—Photomicrographs of rabbit's blood smear (Wright Stain). (A) Normal rabbit. (B) Same animal 2 hr after intravenous administration of pneumococcal hemolysin. Note presence of spherocytes and "spiculated" erythrocytes.

TABLE III

Effects of Administration of Pneumococcal Hemolysin to Normal and Immunized Rabbits

	FIRST INJECTION OF PNEUMOCOCCAL HEMOLYSIN				SECOND INJECTION OF PNEUMOCOCCAL HEMOLYSIN			
Rabbit No.	Hemolysin Inhibition Titer	Change in* PCV %	Increase of** Erythrocyte Osmotic Fragility	Hemoglobinemia	Hemolysin Inhibition Titer	Change in PCV %	Increase of Erythrocyte Osmotic Fragility	Hemoglobinemia
127	1/10	−13	+	+				
128	1/10	−17	+	+				
130	1/20	−5	0	+				
133	<1/10	−1	0	+				
141	1/80	−3	+	+				
142	1/160	−3	0	+				
147	1/5120	−4	0	0				
151	<1/10	−23	+	+				
152	<1/10	−25	+	+				
183	1/40	−2	0	+				
184	<1/10	0	+	+				
185	1/10	−11	+	+				
186	<1/10	−14	+	+				
129	1/10	−4	+	+	1/640	0	0	0
131	1/20	−10	+	+	1/1280	+2	0	0
132	<1/10	−18	+	+	1/1280	−3	0	0
134	<1/10	−13	+	+	1/1280	−3	0	0
144	1/80	−11	+	+	1/80	+6	0	0
145	<1/10	−5	0	+	>1/5120	−1	0	0
146	<1/10	−3	0	+	1/2560	−3	0	0
153	1/80	−7	+	+	1/80	−5	0	0
157	1/40	−7	+	+	1/640	+2	0	0

* Packed red cell volume (hematocrit)

*+ Denotes an increase beyond two standard deviations of normal mean

A surprising phenomenon was noted in five of six rabbits that were studied in more detail. On the first and second day after the first intravenous injection of the bacterial product, the animals' "hemolysin inhibition titer" rose transiently, but by the fifth day the results returned to the previous low values. Coincident with the transient rise in "inhibition titer" these sera appeared opalescent as if hyperlipemic.

Discussion

Libman (1905) is credited with first describing the lysis of erythrocytes by Diplococcus pneumoniae. Nine years later Cole (1914) prepared an extract of pneumococcal cell bodies which lysed human, rabbit, sheep, and guinae pig erythrocytes. He noted that the hemolysin was inactivated by trypsin, cholesterol, heat (55 C for 30 min), and by the sera of animals which had been immunized with the material.

From 1914 to 1946 many investigators (Cohen, Halbert and Perkins, 1942; Cole, 1914; Cowan, 1934; Fleming and Neill, 1927; Halbert, Cohen and Perkins, 1946; Neill, 1926; Weiss, 1918) working with crude bacterial cell extracts studied the properties of the hemolysin and arrived at the following conclusions: The substance responsible for the hemolytic property of the pneumococcus is present within the bacterial cells, and under some conditions it is elaborated into the culture medium. The hemolysin is heat labile (56 C for 3 min) and is inactivated by trypsin, cholesterol, red blood cell stroma, and oxidizing agents. Oxidation by air and mild chemical oxidants is partially reversed by reducing agents. Halbert, Cohen and Perkins (1946) produced evidence which suggested that the hemolysin also exhibited dermotoxic and lethal toxicity. Recently, Kreger and Bernheimer (1969) found that the hemolysin behaved as an acidic protein with an isoelectric pH of 4.9 and a molecular weight of approximately 63,000. In some respects it is thought to resemble streptolysin 0; Todd

(1934) demonstrated that the sera from animals immunized with streptolysin 0 partially inhibited pneumococcal hemolysin. However, Tunevall (1953) noted that in human infections there was no significant neutralization of "pneumolysin" by antistreptolysin immune sera or of streptolysin 0 by "antipneumolysin" sera.

A great deal of effort was expended to determine the role, if any, which the hemolysin played in the pathogenesis of pneumococcal infections. All investigators who administered the material to experimental animals commented upon the *absence* of demonstrable hemolysis *in vivo*. Although it has not been shown that this substance plays a significant part in the disease process, this possibility has not been excluded.

With the advent of chemotherapeutic agents and antibiotics, interest in Diplococcus pneumoniae waned. In 1958 it was noted that pneumococcal septicemia in rabbits was associated with a spherocytic hemolytic anemia. (Shumway, 1958) Later it was found that the intravenous administration to rabbits of supernatant broth from cultures of Diplococcus pneumoniae, type 1, resulted in spherocytosis, increased erythrocyte osmotic fragility and intravascular hemolysis (Shumway and Pollock, 1965). This observation suggested that pneumococcal septicemia of rabbits might offer a model in which to test the hypothesis that this bacterial hemolysin plays a role in the pathogenesis of pneumococcal infections. The studies reported here represent a step toward this goal.

The results of these studies demonstrate that a crude extract of Diplococcus pneumoniae, type 1 cells, damages and lyses human and rabbit erythrocytes *in vitro* and rabbit erythrocytes *in vivo*. The characteristics of our material suggest that it contains the same products which other workers have described as "pneumolysin," "pneumococcal hemolysin," "pneumococcal hemotoxin" and "pneumotoxin." The observation that the sera of rabbits which have received multiple injections of the material are able to neutralize the hemolysin *in vitro*, suggests that the material is antigenic. This suggestion is strengthened by the demonstration that an immunized rabbit does not experience hemolysis when challenged by an intravenous injection of the active hemolysin.

Although the term "hemolysin" has been used to describe this bacterial product, the term should not be taken literally. Halbert, Cohen and Perkins (1946) presented evidence which suggested that the material was lethal to mice and dermotoxic to guinea pigs. Robert Post working with our crude material has demonstrated that it damages and destroys human AV_3 cells in tissue culture, and that this cytotoxic effect is abolished by heating the bacterial extract for

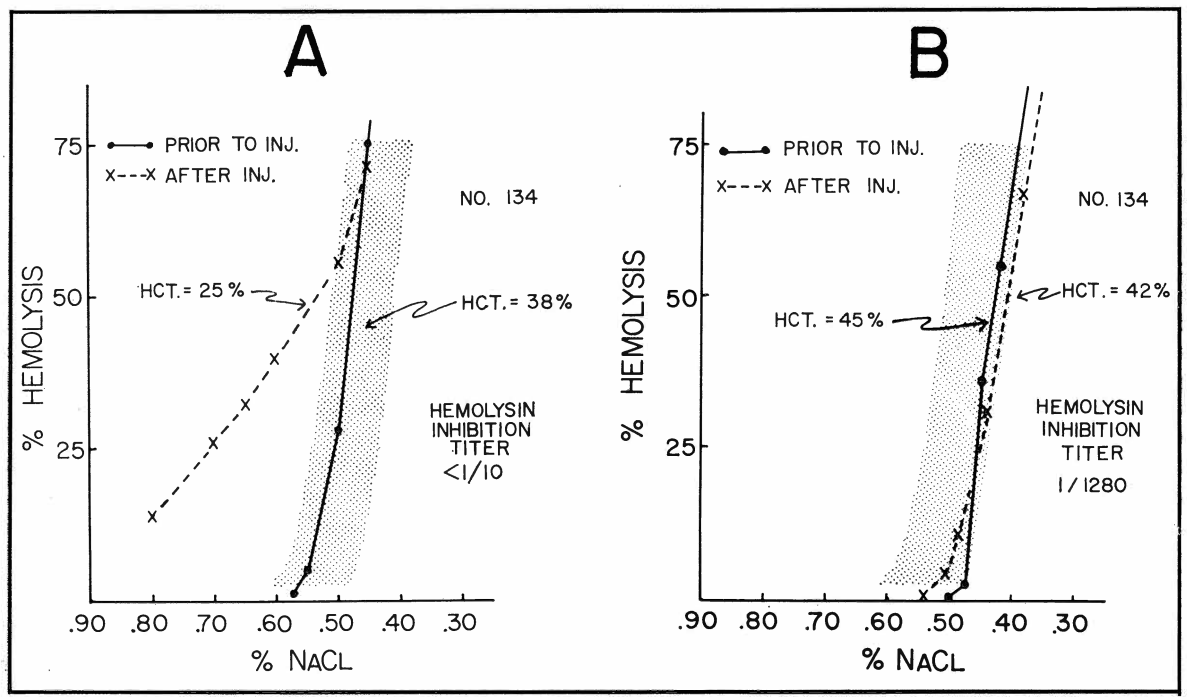

Fig 3—Effect of administration of pneumococcal hemolysin to a normal rabbit (A); and to same immunized animal (B) 21 days later. Shaded area represents the normal range (± 2 SD) of erythrocyte osmotic fragility. Measurements made 2 hr after injection of the hemolysin.

10 min at 56 C or by prior mixing of the extract with serum of an immunized rabbit. Thus, the "hemolysin" may have an affinity for and effect upon many cells including the erythrocyte.

The major weakness of this study and the work of others is the heterogeneity of the bacterial product. It is truly a crude extract; analytical disc gel electrophoresis of the material discloses the presence of many bands of protein. Before the question of its significance in the pathogenesis of pneumococcal infections can be answered and before its mechanism of action can be determined, a purified preparation must be obtained.

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Autoerythrocyte Sensitization or Psychogenic Purpura?

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The following report describes the occurrence, in four women, of an abnormal response to bruising, characterized by local pain, swelling and extension of bleeding into adjacent areas, often to a serious extent. The histories and laboratory investigations suggest that, in these patients, there has occurred a sensitization against one of their own body tissues, namely red blood cells.

The above quotation is taken from the original clinical report in which Gardner and Diamond (1955) described the syndrome of autoerythrocyte sensitization as an unusual purpuric syndrome in which crops of apparently spontaneous painful inflammatory ecchymoses occurred in four women who had sustained physical injury shortly before the onset of symptoms. They postulated that, as a result, their patients had become sensitized to their own erythrocytes and, subsequently, extravasation of blood during the unrecognized trivial injuries of everyday life induced the purpuric lesions. As predicted by this hypothesis, Gardner and Diamond could reproduce the typical bruises of autoerythrocyte sensitization by the intracutaneous injection of the patient's own blood. The offending agent appeared to be erythrocytic stroma; neither plasma, white cells nor hemoglobin elicited a response. One patient was found to be sensitive to the phosphatidyl serine of the red cell membrane (Groch, 1966). Since that time approximately 50 patients have been reported, most of whom were reviewed by Ratnoff and Agle (1968); Hersle and Mobacken (1969).

Gardner and Diamond (1955) postulated that fixed tissue antibody reacts with the red cell stroma to produce edema, increased capillary permeability, and extravasation of red cells into tissues.

As further patients with this syndrome were reported it became clear that in some otherwise typical cases the cutaneous response to blood might be consistently negative. Further doubt that the syndrome may have anything to do with autoimmunity is raised by a thorough study by Ratnoff and Agle (1968) of 27 women with the characteristic bruising. In nine patients the lesions could not be reproduced by the intracutaneous injection of autologous blood. All the patients had severe emotional disorders and the repetitious nature of their psychiatric problems suggested that emotional factors might be critical in pathogenesis. Although symptoms first appeared after physical injury or surgery in 19 of the 27 patients, a closer correlation could be obtained between severe emotional distress and the onset or exacerbation of purpura. A similar association was noted by McDuffie and McGuire (1965). In the experience of these workers and of others, symptoms of purpura usually appeared during the third and fourth decades of life. All known cases of this disorder have been in women.

Many attempts have been made to demonstrate the presence of antibodies directed against red blood cells in patients with autoerythrocyte sensitization. Gardner and Diamond (1955) found that their patients' red cells would not react with antihuman globulin antiserum, and studies for plasma hemolysins and agglutinins against erythrocytes were negative. These findings have been repeatedly confirmed. It has not been possible to demonstrate skin-sensitizing antibodies to blood or its constituents in serum by the Prausnitz-Kustner technique (Gottlieb, 1957; Kremer, 1967). Ratnoff and Agle (1968) were unable to detect antibodies in their patients' serums by hemagglutination of tanned red cells coated with stroma. These various negative studies make it unlikely that the patients have an immunologic sensitivity to blood,

stroma, or hemoglobin. They do not preclude the possibility that in these individuals the threshold of reactivity of the blood vessels to noxious stimuli has been lowered by nonimmunologic means. Agle and Ratnoff (1962) reviewed the evidence that the reactivity of the skin to histamine or thermal injury can be influenced by suggestion. The observation that the appearance of new ecchymoses in patients with autoerythrocyte sensitization is affected by emotional stresses may mean that the reactivity of blood vessels to stimuli is influenced by the central nervous system. This view is fortified by studies of the effects of hypnotic suggestion (Agle and Ratnoff, 1967). The possibility that psychological factors were directly concerned in the purpura was suggested by studies in some of the 27 patients in whom injection of either blood or normal saline into the skin of the anterior thigh each induced typical purpuric lesions. However, if injections were made on the posterior thigh and the sites covered so patients were unable to see them, either no lesions were produced or purpura occured at the saline injected sites only. The strong influence of the psyche was further documented when hypnotic suggestion was found to suppress reactivity to blood in a patient previously reactive, and vice versa (Caron, 1969).

The evolution of the cutaneous bruises is remarkable in that an inflammatory component differentiated the ecchymoses from those associated with injury or hemostatic defects. Usually the patient's attention was drawn to a fresh lesion by a subjective sensation described as stabbing, burning, tingling, cramping, throbbing, popping, or painful. The subjective prodromata constitute a central and diagnostic feature of autotrythrocyte sensitization. Either immediately, or within an hour or two, the patients became aware of erythema or puffiness at the site of the subjective sensation. Ecchymoses only became prominent several hours or a day after the subjective sensations, swelling, and erythema had been noticed. In some patients:

> An ecchymosis began as a ring around the central erythematous area, giving the appearance of a target, while in others, it was more often superimposed upon the erythematous region. In either case, the blue area usually spread rapidly for a distance of several centimeters around the zone originally affected. After a day or two, the swelling and erythema subsided, and the ecchymosis began to involute, disappearing in a week or two. Severe ecchymoses often had a mottled appearance, in which the areas around the hair follicles were more intensely blue than the regions between, a phenomenon seen in normal individuals who have been bruised. Although typical bruises were tender a day or two, pain often subsided more quickly, sometimes as soon as the patient was aware that the spots had turned blue. The bruises varied in diameter from a centimeter or two to 15 cm or more, encompassing, for example, most of the forearm of calf, although such large lesions were seen in only a few cases (Ratnoff and Agle, 1968).

Pain and tenderness were common findings in all patients (Ratnoff and Agle, 1968). Bruising occurred most often in the skin of the lower extremities, particularly the anterior and lateral thighs and legs. The frequency with which the patients or their families related flare-ups of cutaneous purpura to emotional tensions was notable; the relationship was clearly demonstrable in 19 of the 27 patients studied (Table). Biopsy of the early lesions when the affected area appeared erythematous, swollen, and warm revealed edema in the upper dermis; the capillaries of the dermis or subcutaneous tissues were surrounded by an infiltrate of mononuclear cells, most of which were small lymphocytes. Later, extravasated blood cells were evident. It should be mentioned at this point that all patients in Ratnoff and Agle's study (1968) underwent thorough examinations of their hemostatic mechanisms (including clotting times, prothrombin time, partial thromboplastin time, platelet count, bleeding time and clot retraction, as well as the agglutinating effect of ADP upon the patients' platelets) with uniformly negative results. Likewise, lupus erythematosus cell tests, antinuclear antibody, and serum electrophoresis were always negative. None of the patients had evidence of hemolytic anemia.

As mentioned previously, the syndrome is confined to adult women who usually have multiple systemic complaints. Prominent among these are severe headache; transient paresthesias; transient paresis; repeated syncope; diplopia; abdominal pain or distress; nausea; vomiting or diarrhea; chest pain; dyspnea; dysuria; frequency of urination; hematuria; menorrhagia; epistaxis; gastrointestinal hemorrhage; joint, muscle or back aches; and remarkable fluctuations of body weight. No organic cause was found for most of the patients' symptoms.

Extensive psychologic evaluation of all 27 patients was carried out with a remarkable uniformity of findings. Five components in the psychologic make-up of patients with this syndrome were almost always present: hysterical and masochistic character traits, problems in dealing with their own hostilities, and overt symptoms of depression and anxiety. Sixteen of the 27 patients had had psychiatric evaluation or care at some time during the years before the onset of purpura. The masochistic nature of the patients' personalities invariably suggested the possibility that their bruising was self induced. And, in fact, several patients in many different studies have been noted to induce

TABLE

Clinical Features of Autoerythrocyte Sensitization in 27 Patients

(Based on data from Ratnoff and Agle, *Medicine* 47 : 475, 1968)

Feature	Number of Patients (Total = 27)
Female	27
Age at Onset of Purpura	
31–40 years	10
21–30 years	8
14–20 years	4
41–50 years	4
51 years	1
Precipitating Factors in Production of Skin Lesions	
Injury	11
Surgical procedure	9
Emotional stress	23
Skin Lesions (in order of appearance)	
Preceded by subjective sensation (stabbing, burning, tingling, cramping, etc.)	27
Erythema and/or Puffiness	27
Tenderness and/or Pain	27
Ecchymosis	27
Distribution of Skin Lesions	
Legs	27
Arms and Wrists	20
Abdomen	10
Breasts	7
Face	8
Back	3
Skin Test Results (using intracutaneous injection autologous blood)	
Positive (ie, reproduction of characteristic skin lesion)	18
Negative (no response)	9
Emotional Characteristics of Patients with AES	
Depression	20
Suicidal attempt	5
Overt sexual problems	18
Hostility	15
Masochism or martyrism	15
Anxiety	14
"Hysteria"	14
Emotional lability	11

new lesions factitiously. Nevertheless, not all of the lesions are factitial.

DNA autosensitivity somewhat resembles autoerythrocyte sensitization but may be a different disorder (Chandler and Nalbandian, 1966). Spiera and Schwartz (1970) present a patient with the typical syndrome of autoerythrocyte sensitization including recurrent painful ecchymoses and a severe psychiatric disorder. Her lesions could be reproduced by subcutaneous injection of both autologous red cells and heterologous DNA. Other authors (Levin and Pinkus, 1961) feel that these two entities should be differentiated, since patients with DNA autosensitivity improve when given chloroquine; but no treatment including splenectomy, antimalarials, steroids, antihistamines, ethinyl-estradiol, anticoagulants, antibiotics, 6-MP, or vitamin C has been successful in patients with autoerythrocyte sensitization syndrome (Khan and Cash, 1970). The possible relationship between DNA sensitivity and autoerythrocyte sensitization remains unsettled (Ratnoff and Agle, 1968).

Therapy in this disorder, as outlined by McDuffie and McGuire (1965), should be limited to provision of emotional support, encouragement, and the lessening of environmental stresses. This, then, is a recommendation for "supportive psychotherapy."

> Long-term somatic treatment and investigations of physical complaints in which psychic factors are prominent rapidly fix the problem at a physical level and make more and more inaccessible the emotional antecedent. Accordingly, we recommend early diagnosis, a recognition and consideration of emotional antecedents that may be present, and resistance against the use of somatic therapy (Ratnoff and Agle, 1968).

The prognosis of autoerythrocyte sensitization is difficult to determine because of the fluctuating nature of the symptoms. Of the 27 patients studied, 10 had ecchymoses for 10 years, and one as long as 20 years. The severity of bruising varied from time to time. A decline in the severity of bruising or remission has occurred in at least eight cases (Ratnoff and Agle, 1968). Although the purpura often appeared to improve with the years, this change was not accompanied by an amelioration of the patients' multiple somatic complaints, which in most cases continued without let.

The studies of Ratnoff and Agle (1968), the most extensive studies done on this disorder, are consistent with the hypothesis that in patients with autoerythrocyte sensitization the purpuric lesions are related to emotional stresses. The mechanisms through which these stresses are translated to cutaneous bruising are unknown. A factitious origin for the patients' ecchymoses cannot be rigidly excluded, but this possibility seems inadequate to explain the symptom complex

observed. These authors postulate that the purpuric state observed may be an hysterical conversion reaction mediated through the autonomic nervous system and, therefore, that the term psychogenic purpura may be more appropriate than autoerythrocyte sensitization.

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Neoplasms of the Internal Auditory Canal*

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Introduction

Histopathological studies of tumors of the temporal bone are scarce. The authors examined a relatively large number of sectioned human temporal bones in search of small asymptomatic acoustic neurilemmomata and have reported the findings (Leonard and Talbot, 1970). During that search, several other neoplasms were encountered in the internal auditory canal. This report presents a clinical and pathological review of these lesions.

Method

Serially sectioned temporal bones from 490 autopsied patients were studied. Eight-hundred eighty-three temporal bones were considered suitable for inclusion in the study, specifically to assess the internal auditory canal for the presence of any type of neoplasm. The difference of almost 100 bones (883 vs 980) is accounted for in one of the following ways: One of the pair of bones was missing (30 bones); tissues were absent from the internal auditory canal on one side of a pair (49 bones); and the tissues were absent from the internal auditory canal on both sides of nine autopsy specimens (18 bones). The autopsy specimens were collected from The Johns Hopkins Hospital and the Baltimore City Hospital during the years 1929 to 1941— the majority of the bones gathered between 1929 and 1934. Approximately three-fourths of the specimens came from The Johns Hopkins Hospital. The specimens came from an unselected hospital population, but many of the patients were from the neurosurgical service. All of the bones were processed in The Johns Hopkins Otological Research Laboratory by methods standardized by the late Stacy Guild. Most of the bones were sectioned in a vertical direction. All patients had audiograms (pure tone, air conduction, and bone conduction) performed while alive. All bones collected and processed during the years named were studied.

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The study consisted of microscopic examination of every mounted section of the temporal bone in which any part of the internal auditory canal was included.

Findings

Tumors encountered included four small asymptomatic neurilemmomas, three known neurilemmomas that were operated upon and died postoperatively, two meningiomas, one medulloblastoma, one eosinophilic granuloma, and one astrocytoma.

Asymptomatic Acoustic Neurilemmoma

Four of these small lesions were found. These are described in detail in Leonard and Talbot (1970). Related findings are included in the other report.

Postoperative Acoustic Neurilemmoma

Case One (Pathology Number 12411). This 42-year-old male had noted the gradual onset of hearing loss in the left ear four years prior to treatment. This had progressed, along with tinnitus, until the loss became complete. About one year prior to treatment he noted frontal headaches, blurred vision, vertigo, and difficulty in walking. In the few months before coming to the hospital he noted blindness in the left eye, numbness in the left half of the face, partial loss of taste and smell, and falling to the left.

Physical examination revealed weakness in the left leg, positive Romberg to the left, total blindness in the left eye, loss of taste and smell (left), no caloric response on the left, and a 50 dB flat sensorineural hearing loss on the left. Diagnosis was a left cerebellopontine angle tumor, and partial removal was accomplished via an occipital craniotomy. The patient expired a few hours postoperatively.

The tumor was an acoustic neurilemmoma. The temporal bones (Fig 1, A and B) showed that the tumor almost filled the internal auditory canal and had spread all the way to the spiral ligament. There was little bone destruction.

Case Two (Pathology Number 12657). This 40-year-old female was admitted to the hospital because of headaches and left sided deafness. She had noted left tinnitus about three years previously, onset of hearing loss about a year later, and total deafness on

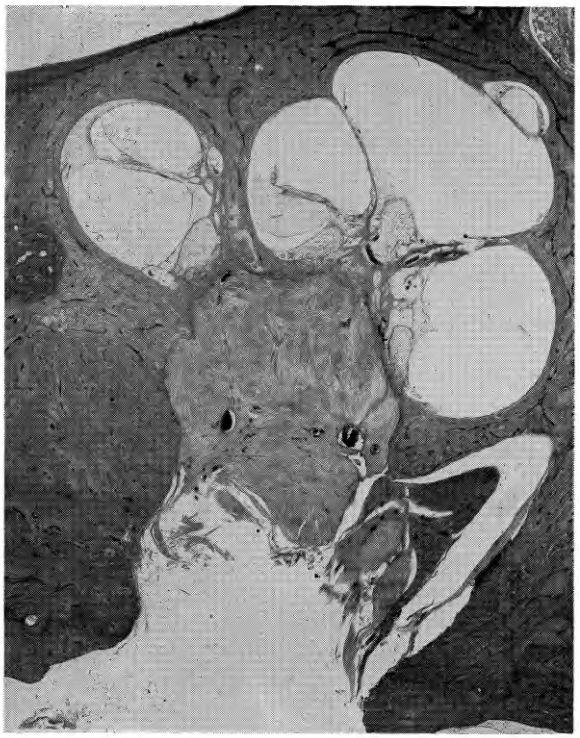

Fig 1 (A)—Path. No. 12411. Neurilemmoma completely filling the internal auditory canal (IAC). Facial nerve is beginning to exit from the canal at lower right. ×11. H & E stain.

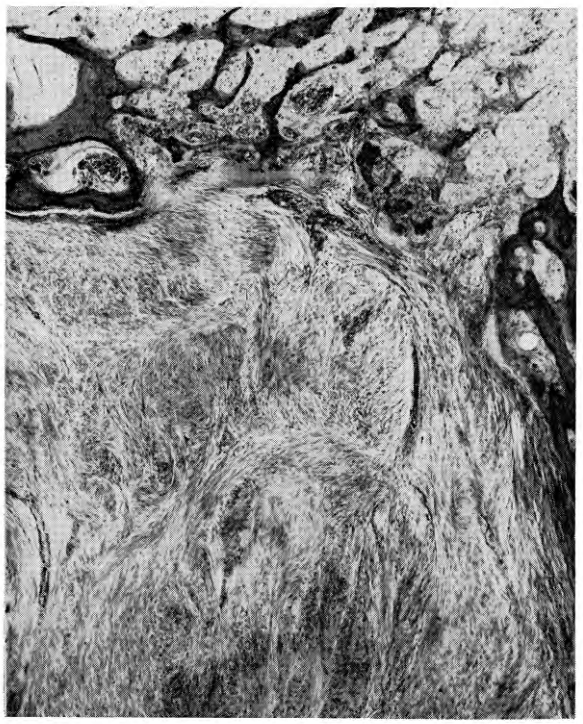

Fig 1 (B)—Path. No. 12411. Slide 1(A), at spiral ligament, shown at magnification of 165×.

the left one year prior to admission. Six months previous to admission she developed severe occipital headaches, momentary bouts of blindness, left facial numbness, and staggering to the left on walking. She had one month of occasional vomiting, and one week of difficulty swallowing.

Physical examination showed a positive Romberg to the left, ataxia, diplopia, left facial anesthesia, no spontaneous nystagmus, a greatly decreased left caloric exam, and a sloping sensorineural hearing loss on the left with approximately 60 dB hearing loss. Masking was not used; we believe this represents a nonresponsive cochlea with a shadow curve from the opposite side.

Diagnosis was a left cerebellopontine angle tumor. Partial removal was accomplished via an occipital craniotomy and the patient expired of surgical complications. The tumor was an acoustic neurilemmoma. The temporal bones revealed the tumor mass occupying about one-half of the internal auditory canal. The tumor had not caused much bony destruction and the facial nerve was intact.

Case Three (Pathology Number 12669). This 60-year-old male was admitted because of recurrent right facial pain. About two years prior to admission, he began having episodes of pain that extended from the right supraorbital area to the occiput. Pain became almost constant; six months prior to admission he was operated on for "tic douloureux" and was relieved for about two months. Twenty years earlier, he had had a right myringotomy for the removal of coal dust. A few months after this procedure, he lost all hearing on the right side.

Physical examination revealed decreased smell, slight diminution of sensation in the right trigeminal nerve, diminished right corneal reflex, no hearing on the right, and normal rotational vestibular tests. Diagnosis was a right cerebellopontine angle tumor, and at operation a large, orange sized tumor was removed. The tumor, a neurilemmoma, indented the brain stem. Removal was difficult and the patient expired a few hours later. The right temporal bone revealed residual tumor along the periphery of the internal auditory canal, extending to Scarpa's ganglion. The facial nerve was intact.

Meningioma

Case One (Pathology Number 16014). This 76-year-old housewife died a few hours after admission; cause of death was acute gastroenteritis. Past medical history revealed that the patient had had seven miscarriages, and seven of her children had died in early infancy due to various causes. At age 66, she was found to have central nervous system lues. History further revealed that she had had, for many years, unsteadiness while walking and particular difficulty while walking in the dark. In the four years prior to

her death, she developed tinnitus and a progressive hearing loss in the left ear to the point that she could hear only loud shouts. In the three months prior to her death, there had been occasional drainage from the left ear.

Physical examination revealed a central perforation in the anterior inferior quadrant of the left tympanic membrane, and there was active suppuration in the middle ear space. Audiogram consisted of only a pure tone, air conduction study. There was a flat 20 dB loss on the right, and a sloping 65 dB loss on the left, when measured with adequate masking. Neurological examination was sketchy, but revealed that the gait was staggering, and the Romberg test was markedly positive. Because of the acute illness at the time of admission consisting of profuse diarrhea, high fever, and dehydration, no other physical findings could be elicited.

At postmortem examination a complete intracranial autopsy was performed, and temporal bones were obtained. The left temporal bone showed evidence of chronic tympanomastoiditis, and there was a large, central perforation in the tympanic membrane. Examination of the left internal auditory canal revealed a small meningioma (4 × 4 mm in diameter) within and confined to the internal canal (Fig 2, A and B). The histological pattern of the meningioma was of the endotheliomatous subgroup, and it contained numerous vascular channels and psammoma bodies. We believe this tumor had its origin from an arachnoid villus within the left internal auditory canal. The tumor did not compress the VIIth or VIIIth nerves, nor did it invade them. This case is discussed in somewhat greater detail in a report by Nager (1964).

Case Two (Pathology Number 12258). This 44-year-old female had developed dull pain in the right cheek two years previously. The pain progressed, and a few months later there was gradual loss of hearing on the right, low pitched tinnitus, and vertigo. About one year prior to admission, the pain and tinnitus increased sharply. Six months later, vision became poor bilaterally. There was no nausea or vomiting.

Physical examination revealed evidence of greatly increased intracranial pressure with choked retinal discs. A ventricular tap confirmed this increased intracranial pressure. Hearing tests revealed a flat sensorineural loss of approximately 45 dB in the right ear (with masking), confirmed by tuning forks. No vestibular tests were performed. At craniotomy, a right cerebellopontine angle tumor, friable, hemorrhagic, and without a capsule, was removed. The VIIth and VIIIth nerves were injured during the operation, and the patient died on the first postoperative day.

The tumor was a large meningioma that had arisen from the surface of the cerebellum. Right temporal bone sections showed that the tumor had grown into the right auditory canal, completely filling this space.

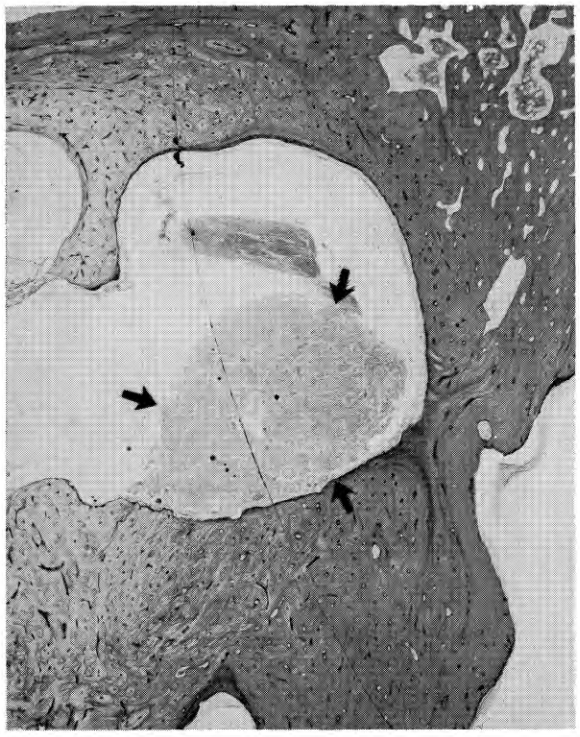

Fig 2 (A)—Path. No. 16014. Small meningioma (arrows) confined to the IAC. Slight bony erosion along the canal floor. ×11. H & E stain.

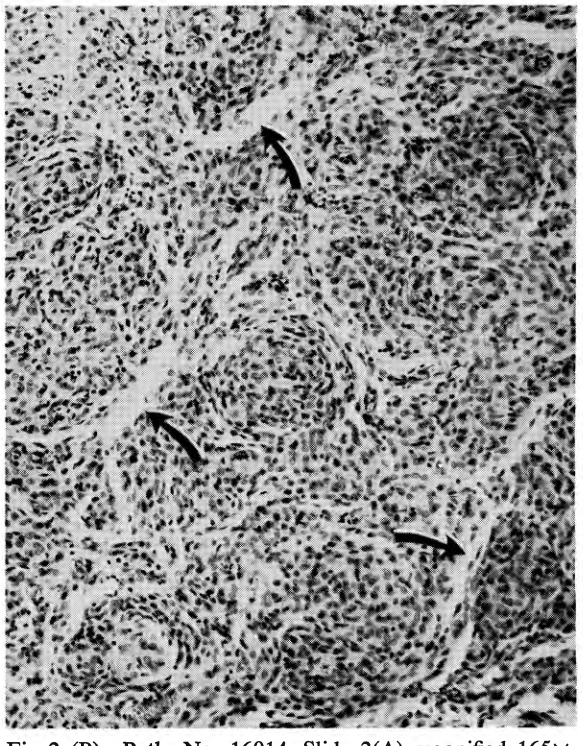

Fig 2 (B)—Path. No. 16014. Slide 2(A) magnified 165×. Arrows point to vascular channels within the tumor. ×165.

Medulloblastoma

Case (Pathology Number 11620). This 13-year-old female had abruptly stopped growing approximately two and a half years previous to admission. One year later, she began vomiting almost every morning and started having bilaterial frontal headaches. One year prior to admission, she began to lose visual acuity, worse on the right. Ten months prior to admission, she began fainting every two to three weeks. Six months prior to admission, she started staggering to the left and noted bouts of vertigo. Later came attacks of hiccoughs lasting two to three hours.

Physical examination revealed bilateral dilated pupils that reacted poorly to light, restricted visual fields and a right hemianopsia, a horizontal nystagmus to the left, and the fundi showed the discs to be pale and cupped. There were distended retinal veins. Neurological examination revealed a diminished right corneal reflex, Romberg positive to the right and rear, staggering to the right, and poor finger-to-nose reaction on the right. Hearing tests showed a bilateral flat sensorineural hearing loss of approximtely 30 dB on the right and approximately 40 dB on the left. The Weber did not lateralize, and the hearing level was confirmed with forks. Except for the spontaneous nystagmus, no evaluation was made of the vestibular apparatus. An exploratory craniotomy was performed and a large tumor of the left cerebellar lobe was encountered; no resection was attempted. The patient never regained consciousness and died the first postoperative day.

The tumor proved to be a cerebellar glioma which pathologically was termed a medulloblastoma. The temporal bones (Fig 3, A and B) showed the tumor had infiltrated both internal auditory canals and appeared to invade both the VIIth and VIIIth nerves on both sides.

Eosinophilic Granuloma

Case (Pathology Number 11083). This 33-year-old male had a lifelong history of chronic mastoiditis and discharge on the right side. He was admitted to the hospital because of an acute exacerbation and toxicity. On admission, the patient had a temperature of 100.4 F, tender neck nodes on the right, and tenderness over the right forehead and right maxillary antrum. Examination of the right ear canal showed mucopus present in the external canal, and there was a posterosuperior perforation in the tympanic membrane. Visualization of the middle ear through the perforation revealed granulation tissue. Hearing tests on admission revealed a 60 dB conductive hearing loss on the right, but when adequate masking was applied to the left ear, the right ear was found to be nonresponsive. This was confirmed with tuning forks. The admitting diagnoses were acute exacerbation of right chronic otitis media with cholesteatoma, acute meningitis, and the possibility of a brain abscess. The day after admission, a right complete mastoidectomy was performed and cholesteatoma was confirmed. Surgery did not include opening the sigmoid sinus, the inner ear, or going into the internal auditory canal. The patient did well and was discharged the sixth postoperative day. Ten days later, he was readmitted in a comatose state. He had been complaining of pain over the right ear in the interim and expired a few hours after admission.

At autopsy, the middle ear space was seen to contain what was pathologically termed an eosinophilic granuloma, which had extended into the inner ear and had completely filled the internal auditory canal (Fig 4, A and B). There was diffuse pachymeningitis.

Astrocytoma

Case (Pathology Number 12570). This 11-year-old male had begun having nausea and vomiting approximately one month prior to admission. Over the succeeding days, he began having some staggering to the left on walking, drowsiness, generalized headache which would occur every three to four days, and bouts of nuchal rigidity. Approximately two weeks prior to admission, he developed diplopia which lasted for three days. One week prior to admission, a lumbar puncture was performed and there was increased spinal fluid pressure. The family history revealed that a maternal aunt had had a cerebellopontine angle tumor.

Physical examination revealed vital signs to be normal. Neurological examination showed smell to be decreased. Left pupil was slightly larger than the right and both reacted poorly to light. There was no spontaneous nystagmus, but both fundi revealed disc edema and fundal hemorrhages. The left finger-to-nose test was clumsy, as was the left heel-to-knee. On Romberg testing, he swayed to the left. The audiogram revealed hearing to be normal bilaterally. No vestibular tests were performed. At craniotomy, a pineal tumor was removed and the operation was described as difficult. The patient expired a few hours postoperatively. On pathological examination, the tumor was found to be a glioma and was classified as an astrocytoma. The right temporal bone (Fig 5, A and B) revealed that the tumor had grown into the right internal auditory canal, filling about one-third of the canal. The tumor had not produced any local destruction.

Discussion

A complete microscopic study of the internal auditory canals in 883 temporal bones revealed four small asymptomatic acoustic neurilemmomas, three neurilemmomas which had been operated on, two meningi-

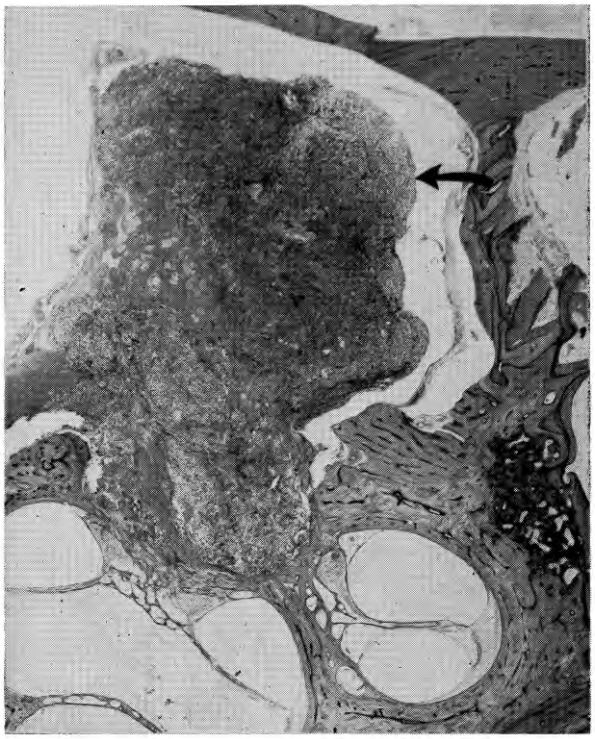

Fig 3 (A)—Path. No. 11620, Right. Cerebellar medulloblastoma (arrow) that expanded into the IAC and invaded the VIIth and VIIIth nerves. ×11, H & E stain.

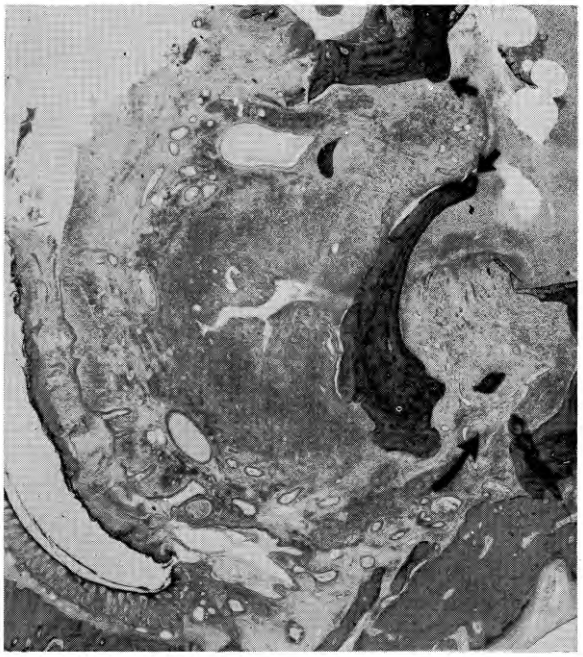

Fig 4 (A)—Path. No. 11083. Right middle ear with eosinophilic granuloma invading inner ear. Tympanic membrane and external ear canal are at extreme left. Tumor passes through the oval window (small arrows) and the round window (large curved arrow). The promontory is the bone between the 2 windows. X11. H & E stain.

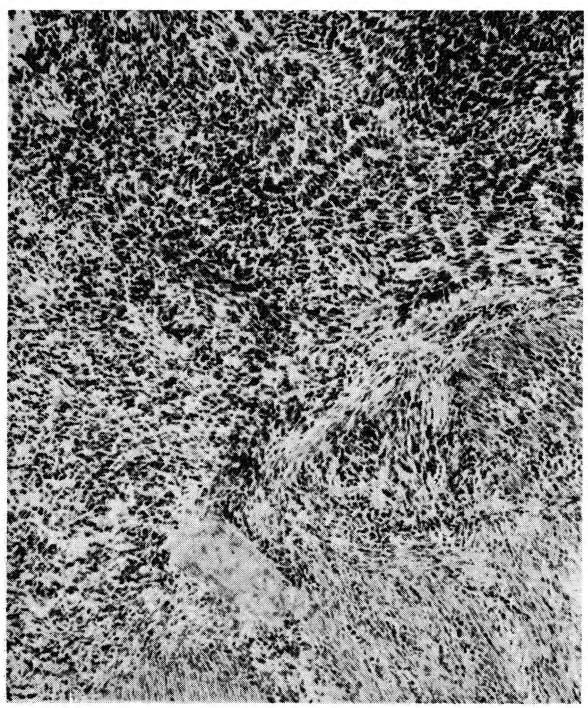

Fig 3 (B)—Path. No. 11620, Right. Same temporal bone section as 3(A). The tumor is identical to that seen in the opposite ear. ×125, H & E stain.

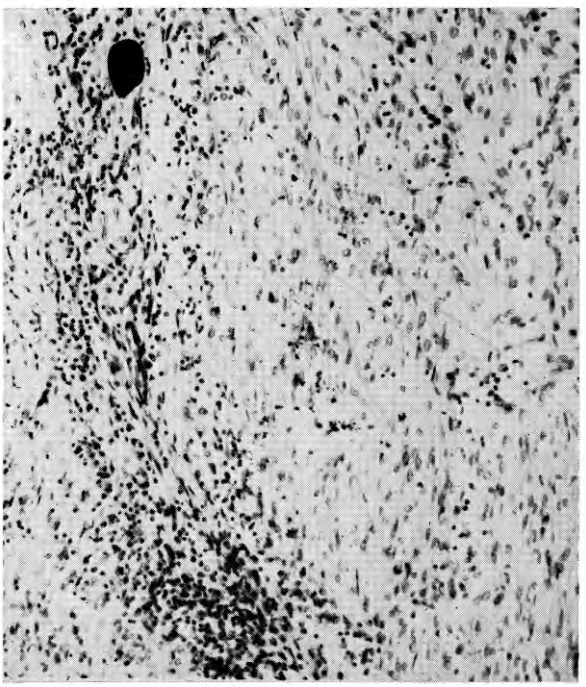

Fig 4 (B)—Path. No. 11083. Same temporal bone section as 4(A), with histologic pattern at 165× magnification. The tumor appeared to spread to the IAC by way of the neural channels in the spiral ligament.

omas, one medulloblastoma, one eosinophilic granuloma, and one astrocytoma.

As mentioned, the four small acoustic neurilemmomas were considered of sufficient importance to have been reported previously (Leonard and Talbot, 1970), in detail with clinical and pathological findings. Related data from other specimens, including cellular proliferations thought to be possible precursors to neoplasms, were also discussed.

The three postoperative acoustic neurilemmomas are presented here only for complete coverage of the neoplasms found. It is interesting that this study covered a period during which the neurosurgical service at The Johns Hopkins Hospital, headed by Walter Dandy, was especially active. Because acoustic neurilemmomas and other cerebellopontine angle tumors were of special interest to Dr. Dandy, it is a credit to his and his colleagues' skill that more of these patients did not come to autopsy. Obviously, a large number of these patients did die, either immediately postoperatively or at some later time, and a number of the temporal bones ultimately were received in the Otological Research Laboratory. However, in the years covered, the three cases presented were the only ones in which tumor was found in the sectioned temporal bones. The clinical and pathological picture of these three cases is classic.

One autopsy specimen was from a patient who had multiple neurofibromatosis, or von Recklinghausen's disease. He was a 13-year-old male (Pathology Number JHH 15681) who developed an intracranial tumor which proved on exploratory craniotomy to be a neurilemmoma of the third ventricle. The patient died postoperatively, and a complete autopsy was performed. No other central nervous system neuromata were found. Specifically, the entire VIIIth nerves were examined and there was no evidence of acoustic neurilemmoma. The relationship of multiple neurofibromatosis and acoustic neurilemmoma is well established. Hitselberger and Hughes (1964) reported 12 such cases with bilateral acoustic tumors in this syndrome.

Meningiomas were found within the internal auditory canal in two patients; in the first patient the small tumor was discovered accidentally. In the second patient there was a large meningioma in the right cerebellopontine angle. Preoperative diagnosis had been a benign neoplasm, probably a meningioma. It is fairly well accepted that meningiomas originate from the clusters of cells normally present at the tips of the arachnoid villi (Bailey and Bucy, 1931). Arachnoid villi are frequently present in the internal auditory canal, as they are in three other sites within the temporal bone—within the jugular foramen, in the region of the geniculate ganglion, and in the sulcus of the greater and lesser superficial petrosal nerves in the roof of the eustachian tube.

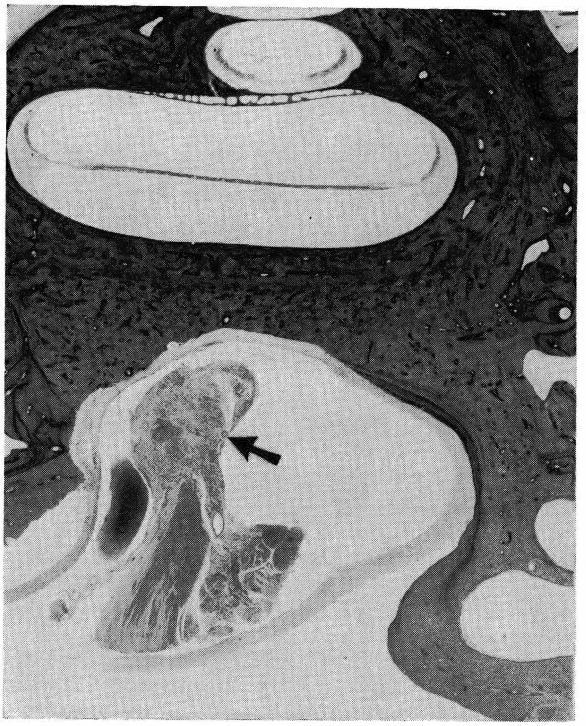

Fig 5 (A)—Path. No. 12570. Right IAC with neoplasm (astrocytoma) at end of the arrow. ×11. H & E stain. Special stains not used in this case (1930).

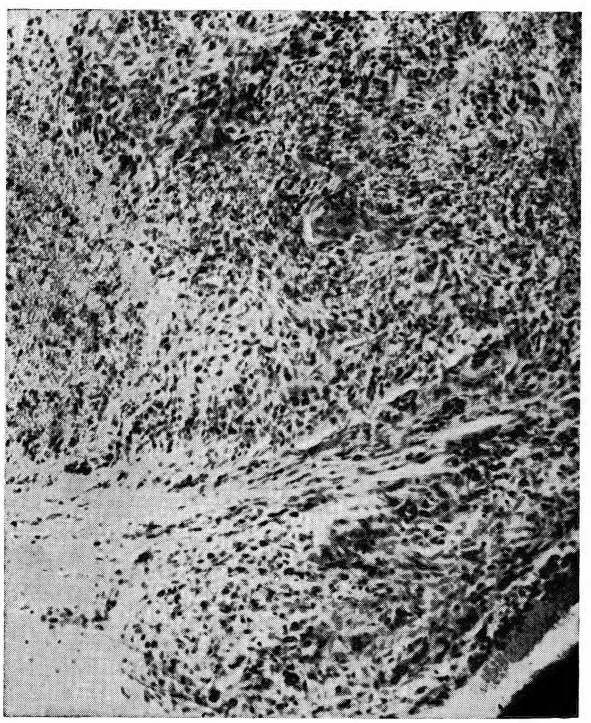

Fig 5 (B)—Path. No. 12570. Same temporal bone section as 5(A), magnified at ×165.

The medulloblastoma that was reviewed represents one of the highly malignant gliomas, this one having arisen in the cerebellum and spread to adjacent areas including the internal auditory canal.

The eosinophilic granuloma that was noted is, of course, not a true neoplasm, but is a particular type of chronic inflammatory response. It is a "new growth," and can cause local destruction. This case illustrates this point as there was considerable destruction within the temporal bone, including the inner ear and internal auditory canal. There can be little doubt that this lesion led directly to the death of this patient.

The final case reviewed was that of an astrocytoma. The entire clinical history of the patient included only a month. At operation, the neoplasm was listed as a pineal tumor, and pathologically it was described as an astrocytoma.

The authors are not implying that the cases found in the review of the temporal bone series represents the incidence of these lesions in the general population. These autopsied patients were drawn from an unselected hospital population, but because of the close liaison between the otolaryngological and neurosurgical services, a high percentage of the patients came from the neurological surgery service.

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Leonard JR, Talbot ML: Asymptomatic acoustic neurilemoma. Arch Otolaryng 91: 117, 1970

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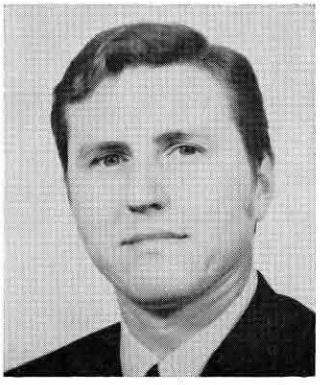
Contributors



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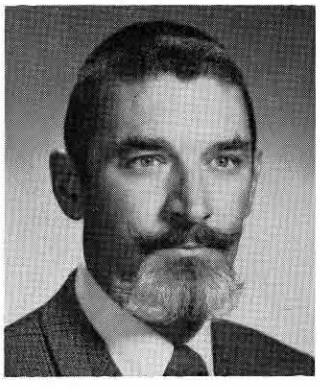

James L. Mathis *(New Horizons in Psychiatry)* is professor and chairman of the department of psychiatry at the Medical College of Virginia. After attending undergraduate school at The Citadel in South Carolina and the University of Missouri, he received his M.D. from St. Louis University School of Medicine. Dr. Mathis began his medical career in general practice, but in 1960 entered a psychiatric residency at the University of Oklahoma Medical Center where he went on to hold the positions of instructor and assistant professor of psychiatry. Before coming to MCV, Dr. Mathis was associate professor of psychiatry at Rutgers.

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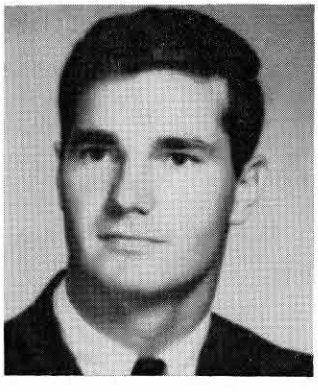

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Diagnosis: spasm reactor

Decision: Donnatal®

	each tablet, capsule or 5 cc. teaspoonful of elixir (23% alcohol)	each Donnatal No. 2	each Extentab
hyoscyamine sulfate	0.1037 mg.	0.1037 mg.	0.3111 mg.
atropine sulfate	0.0194 mg.	0.0194 mg.	0.0582 mg.
hyoscine hydrobromide	0.0065 mg.	0.0065 mg.	0.0195 mg.
phenobarbital	(¼ gr.) 16.2 mg.	(½ gr.) 32.4 mg.	(¾ gr.) 48.6 mg.
(warning: may be habit forming)			

Brief summary. Side effects: Blurring of vision, dry mouth, difficult urination, and flushing or dryness of the skin may occur on higher dosage levels, rarely on usual dosage. Administer with caution to patients with incipient glaucoma or urinary bladder neck obstruction as in prostatic hypertrophy. Contraindicated in patients with acute glaucoma, advanced renal or hepatic disease or a hypersensitivity to any of the ingredients.

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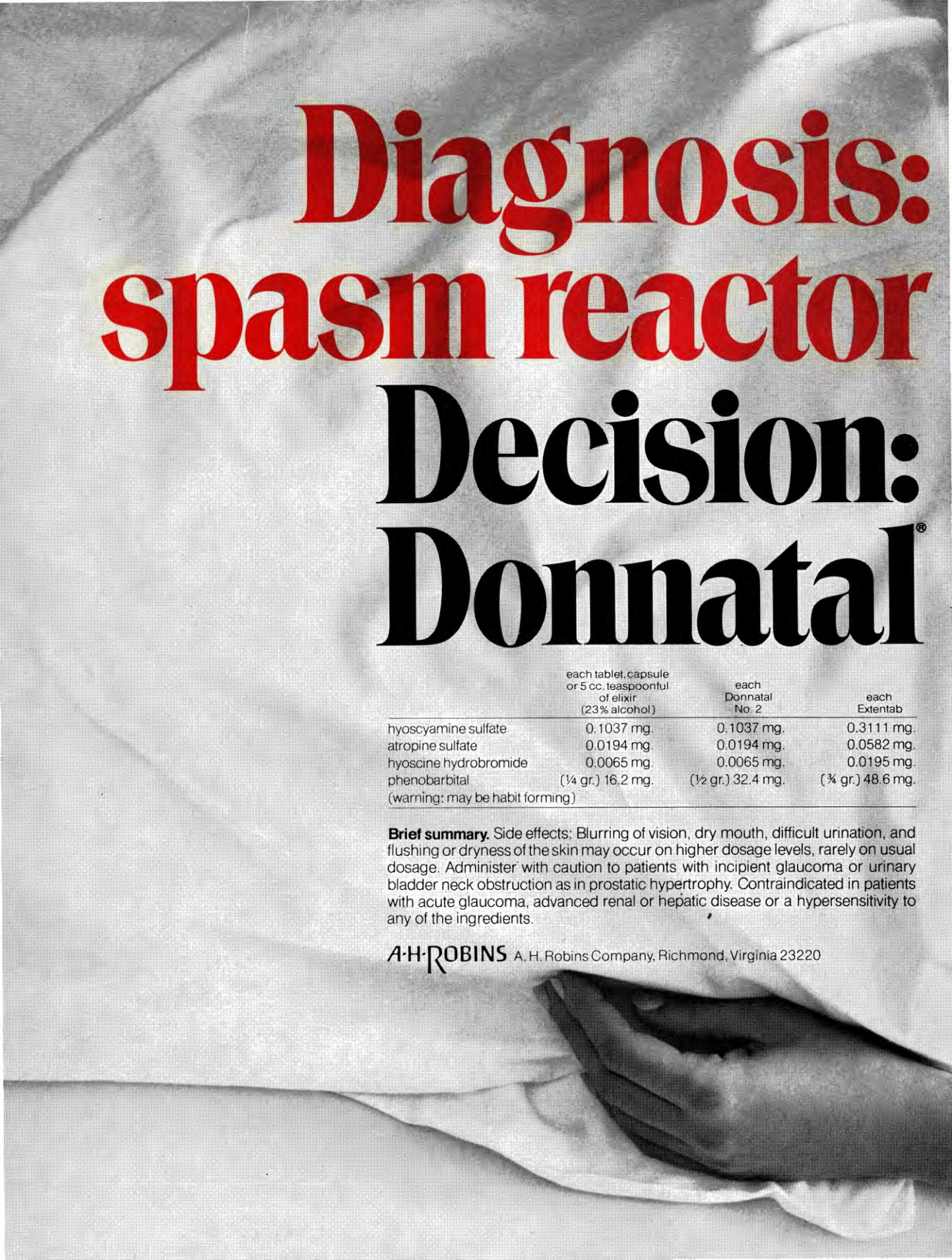

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