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STAFF MEETING REPORTS

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Staff Meeting Report

Stereotactic Surgery in Parkinson's Disease*†

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Stereo-encephalotomy, or stereotactic surgery, is the term given to the surgical technique in which circumscribed, destructive lesions are placed in subcortical ganglia or pathways with negligible injury to adjacent structures. This is accomplished through the use of electrodes whose placement in the depths of the brain are precisely determined by mechanical guides. The use of mechanical devices to produce discrete destructive lesions in the brains of experimental animals has been common in neurophysiological laboratories for almost 80 years; but only recently (since 1947) has this technique been applied to human patients. Many such devices have been constructed, most of them variations of the Horsley-Clarke stereotactic apparatus first described in 1908.[1]

The stereotactic method, as applied to animal experimental work, depends for its accuracy upon a fixed size and shape of skull. It cannot be used in animals exhibiting appreciable variation in skull architecture because the method relies upon a constant correlation between external skull landmarks and internal brain structures or targets. Animals such as cats and monkeys do have skulls of reasonably constant size and shape, and consequently they are widely used for neurophysiological investigation. The architecture of the dog's skull, on the other hand, is notoriously variable, and therefore, its usefulness for these purposes is sharply limited. The human skull is also subject to a wide variability in size and shape, and indeed, even the internal structure of the brain itself exhibits a distressing variability. This has been the chief reason for the 80-year delay in adapting the stereotactic method to subcortical surgery in the human.

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On the theory that stereotaxis could practically be applied to the human patient if *internal* brain landmarks or reference points were used, Spiegel and Wycis[2] developed such a method and reported its successful use in 1947. (Originally, they used the pineal gland as a reference point; the unreliability of that structure has been amply demonstrated, however, and now more dependable reference points are being used.) Stereotaxis was first applied to human patients by Spiegel and Wycis[3] in the field of psychosurgery. Lesions were placed in the dorsomedial nucleus of the thalamus, thereby achieving the effect of lobotomy with minimal intellectual deterioration. Encouraged by these results, they then placed lesions in the mesencephalic spinothalamic tract for the relief of pain. Since their original report, they have demonstrated that the stereotactic method can also be fruitfully applied to such diversified disorders as diseases of abnormal movements and subcortical epilepsy.[4,5] A number of stereotactic instruments have been devised and described by others, and fairly large series of cases in each of the above categories have been reported, so that human stereotaxis, although still in its infancy, can now be considered to have progressed beyond the theoretical stage.

The recent enthusiasm for surgery of the basal ganglia in diseases of abnormal movements led members of the Neurosurgical Division at the University Hospitals to become interested in the stereotactic method. To date, 27 operations have been carried out here on 22 patients with problems including Parkinson's disease, athetosis, dystonia, subcortical seizures, and psychosis. Obviously, the comparatively small number of cases and relatively short period of follow-up—15 months at most—precludes the development of statistically valid conclusions. We believe, however, that much can be learned from our experience, particularly in the patients with Parkinson's disease.

Parkinson's disease, first described by James Parkinson in 1817, is actually a complex of symptoms or a syndrome. Clinically, this constellation includes alternating tremor, cog-wheel rigidity, masklike facies, dysarthria, kyphosis, gait disturbances, poverty of movement, and disturbances of autonomic function. Tremor and rigidity, however, are the two cardinal features, and they account for most of the other symptoms. The pathologic changes associated with Parkinsonism are widespread, involving the central structures from the basal ganglia and thalamus rostrally through the reticular formation to the pons and medulla caudally. Even the cerebral cortex may be involved. There is no one specific site in which a lesion can be placed, or in which one has been observed, that will account for the

clinical picture. This clinical complexity should indicate that in all likelihood no single anatomical and physiological basis for all the stigmata of Parkinsonism will be found. Even the etiology has remained controversial.[6] Inflammatory and toxic encephalitis, cerebral arteriosclerosis, post-traumatic encephalopathy, and a number of other causes have been implicated. It seems clear, however, that in most of the estimated 400,000 cases of Parkinson's disease in this country, the disorder is more or less clearly related to the 1917-18 influenza pandemic.

In spite of these complexities, an understanding of the pathophysiology is now beginning to emerge. Within the past few years, several laboratories have demonstrated that in the experimental animal an alternating tremor can be produced either by stimulation or by discrete destructive lesions within the bulbar reticular formation.[7,8] Moreover, stimulation or coagulation within certain basal ganglia, particularly the globus pallidus, effectively alters the tremor thus produced.

Attempts at surgical alleviation of abnormal movements began at the turn of this century when Sir Victor Horsley[9] reported the alteration of abnormal movements in an adolescent boy produced by resecting a portion of the contralateral motor cortex. Since then, many additional cortical ablations have been reported.[10,11,12] It is the opinion of Bucy[13] and others that such cortical extirpations are inevitably followed by a spastic hemiparesis and hence entail exchanging one serious neurologic disorder for another.

Many other approaches to the problem have been tried, including section of a portion of the internal capsule,[14] partial section of the cerebral peduncle,[15] hemisection of the cord,[16] postero-lateral cordotomy,[17] sympathectomy,[18] and even prefrontal lobotomy. Each of these procedures has had specific drawbacks, some sufficient to discredit their use. Then in 1954, Cooper[19] reported gratifying results in patients with Parkinson's disease, first from ligation of the anterior choroidal artery and later from injection of destructive solutions into the basal ganglia. His enthusiasm was contagious, and subcortical surgery emerged as the most promising approach to the surgical alleviation of tremor and rigidty. Although an imposing number of cases of basal ganglia surgery have been reported by various authors during the past five years, many important questions are still unanswered. It is for this reason that the present project has been undertaken.

Inasmuch as Parkinsonism afflicts the young as well as the old and

exhibits a wide variety of clinical features in varying degree, any attempt to define the classic case is valueless. Consequently, the authors felt that an investigative series should be unselected and should include the many naturally occurring variations. In the present series, the chief indication for surgery was the presence of incapacitating tremor or rigidity, either unilateral or bilateral. Contraindicatons were limited to: (1) general medical contraindications, (2) mental deterioration, and (3) physiologic old age. We hope that as a result of this study it will be possible to define more accurately the indications and contraindications for this type of surgery.

Preoperative and postoperative evaluations are being conducted on the Medical Neurology Service under the direction of Dr. A. Iannone. These include detailed neurological examinations, electroencephalographic studies, and motion picture analyses. In addition, detailed objective measurements of tremor, rigidity, and motor function are carried out at predetermined intervals by Dr. David Webster at the Minneapolis Veterans Administration Hospital. The rather complex techniques involved in these studies will be described in greater detail in future reports. Although some weight is given to the estimates of the patient and his family concerning degree of disability, degree of improvement, etc., the detailed objective data supplied by these studies have been invaluable in the overall evaluation of each case.

In this clinic, the Spiegel-Wycis method of stereotaxis[20] has been adapted, and can be described briefly as follows: The procedure is carried out in two stages, the first being performed under general anesthesia, and the second under local anesthesia. The first stage is devoted to making the accurate x-ray studies necessary for localization of the intracerebral target. The second stage is devoted to studying the target area by means of stimulation and observation of the clinical response, patterns of evoked potentials, and under the usual circumstances, destruction of the target by means of electrocoagulation.

Through pneumoencephalography, two intracerebral reference points are routinely identified—the anterior and posterior commissures of the third ventricle. These are convenient reference points because: (1) they are readily demonstrable in a conventional pneumoencephalogram, and (2) they are near the usual target points within the basal ganglia. This latter fact is especially important, because the value of any structure as a reference point varies inversely with that structure's distance from the target. Error is considerably minimized

through the use of two reference points rather than one.* With the information thus derived, the area representing any desired subcortical structure can be plotted topologically through the use of a carefully devised brain atlas. Of course, given the demonstrated variability in brain structures,[21] one cannot predict a configuration of a particular structure that will hold for all patients. That is to say, should one produce a lesion large enough to destroy the entire globus pallidus, it is inevitable that in some patients a portion of that lesion will actually fall outside the globus pallidus and injure adjoining structures. It is possible, however, to plot an area which will represent a *portion* of the desired structure in *every* instance without encroaching on adjoining structures. In this clinic we concern ourselves primarily with this constant target area; should this prove insufficient for good clinical results, we then plan to probe the marginal zone with stimulation at a later date.

The actual technique is, in itself, not tedious. An electrode carrier is fastened to the patient's skull by means of implanted stainless steel screws. The carrier is graduated in all planes in millimeters and in degrees to give maximum versatility. The screws are countersunk in the skull and remain in place permanently; this permits reapplication of the instrument at any time in exactly the same position, utilizing the original coordinates and without the necessity of repeated air injections. A fine electrode, 1.2 mm. in outside diameter, is used to obtain electrograms, or for stimulation or coagulation; it is even adaptable for the injection of fluid substances. For purposes of coagulation, we have routinely used direct, anodal current. In the laboratory, we have found that 10 milliamperes of current applied for one minute will produce an ovoid shaped area of coagulation that measures approximately 4 by 2 millimeters. A planned pattern of such lesions is used to produce larger areas of destruction with some degree of axial orientation.

Table 1 summarizes the data on the 14 patients with Parkinson's disease who have been studied and subjected to pallidotomy up to this time. Several points are worth noting: The ages range from 41 years to 70 years, with the majority of patients being in the sixth decade of life. In only three patients was the disease essentially unilateral in distribution, while in 11 it was distinctly bilateral. Duration of the disease varied widely, from a minimum of three years to a maximum of 38 years. The last column represents an esti-

*Dr. H. O. Peterson has been of invaluable assistance in directing and contributing to this aspect of our investigation.

mated clinical consensus of the therapeutic results in regard to tremor and rigidity. Four patients are classed as excellent, representing 95 per cent or better reduction of both tremor and rigidity; five patients are classed as good, representing 66 per cent to 95 per cent reduction of symptoms; three patients are classed as fair, representing 33 per cent to 66 per cent reduction of symptoms; two are classed as poor—one because of a permanent hemiparesis, the other because of death due to a pulmonary embolus two and one-half weeks after operation. The patient's age and the duration of disease, while undoubtedly influencing the results of surgery, do not necessarily preclude satisfactory results; the results in the oldest patient in the series, age 70, is classed as excellent, and three patients with symptoms of 20 years or more duration are classed as either good or excellent.

TABLE 1

RESULTS OF PALLIDOTOMY IN PATIENTS WITH PARKINSON'S DISEASE

Patient	Age	Laterality	Duration of Symptoms	Clinical Result
	41	Unilateral	6 yrs.	Excellent
	47	Unilateral	20 yrs.	Excellent
	55	Bilateral	5 yrs.	Good
	57	Bilateral	38 yrs.	Fair
	49	Bilateral	20 yrs.	Good
	58	Bilateral	6 yrs.	Good
	51	Bilateral	10 yrs.	Good
	47	Bilateral	20 yrs.	Good
	61	Bilateral	25 yrs.	Fair
	70	Unilateral	5 yrs.	Excellent
	49	Bilateral	20 yrs.	Poor
	41	Bilateral	8 yrs.	Excellent
	66	Bilateral	3 yrs.	Poor
	55	Bilateral	31 yrs.	Fair

Table 2 summarizes the complications which have followed pallidotomy: 1. In H.F., age 55, the only patient in this series who had a bilateral pallidotomy, the first operation was uneventful. However, following a pallidotomy on the second side one month later, he experienced a curious alteration in mental abilities. While maintaining an I.Q. of 117, he exhibited judgement and emotional content at

the five-year old level. This regression persisted for three months, after which the patient returned rapidly to normal. 2. P.F., age 55, had advanced disease of 38 years' duration. In addition, he exhibited a hemiparesis as a sequel of encephalitis prior to the onset of the Parkinson's disease. Following unilateral pallidotomy, he exhibited a persisting pseudobulbar palsy. 3. L.J., age 49, suffered an apparently permanent hemiparesis of moderately severe degree. The other listed complications are self-explanatory.

TABLE 2

COMPLICATIONS FOLLOWING PALLIDOTOMY IN PATIENTS WITH PARKINSON'S DISEASE

Patient	Age	Duration of Disease	Complication
	55	5	Mental change, temporary
	57	38	Pseudobulbar palsy
	49	20	Permanent hemiparesis
	66	3	Death from pulmonary embolus 16 days after operation
	55	31	Homonymous hemianopsia

Table 3 concerns that single factor in which the patient and his family are most interested—rehabilitation. Considered realistically, this means different things to the patient who is confined to a wheelchair and incapable of self-care than it does to the patient who is working every day in spite of a handicap. The ability to feed himself is a major gain for the one, while the other takes this for granted. Considered in this light, we have simply tabulated the patients in terms of "real gain" over and above their capabilities prior to surgery. As is readily apparent from the figures, significant gains in rehabilitation were made by only half the patients. Particularly impressive is the fact that good or excellent reduction of tremor and rigidity is not synonymous with rehabilitation. Motivation, which is of paramount importance in this regard, remains exceedingly difficult to evaluate, both before and after surgery. Related to this observation is one important question which we hope to answer eventually, namely: Should this type of therapy be restricted to those most likely to utilize it for significant gain in rehabilitation, or is the comfort of the patient in itself a legitimate indication? The tendency in most clinics at the present time is to confine efforts to the former group.

TABLE 3

REHABILITATION OF PATIENTS WITH PARKINSON'S DISEASE

	Excellent	Good	Fair	Poor	Total
Significant gains	3	4			7
Minimal gains but more comfortable	1	1	2		4
Worse			1	1	2

DISCUSSION

Four distinct advantages make stereotactic surgery a desirable investigative tool:

(1) It is exceedingly accurate. Thus, destructive lesions of known size can be placed in a predetermined locus with minimal damage to surrounding structures. Some have argued that such accuracy is unnecessary in most of the subcortical surgery presently being done. We believe, however, that this accuracy is important both for the safety of the patient and for the interpretation of the altered physiology. This is especially true of thalamic lesions, in which the groups of nuclei have very diverse connections.

(2) Before any destructive lesion is made using the stereotactic method, electrostimulation is generally applied, thus giving a physiological demonstration of the position of the electrode.

(3) The surgeon can control the size of the destructive lesion made. As stated previously, one coagulation produces an ovoid area of destruction of approximately 4 by 2 mm. Six to 22 lesions have been produced in various patients, using as the endpoint the desired clinical effect rather than an arbitrarily chosen number.

(4) Reapplication of the stereoencephalotome in a precisely identical position is simple. Therefore, one can produce further lesions at intervals if necessary. Reapplication at intervals up to six months has been performed without repeated pneumoencephalography and without any guesswork.

Some authors have expressed the opinion that there is somatotopic localization within the globus pallidus with respect to tremor and rigidity. Inasmuch as the stereotactic methods lends itself to investigating such possibilities, we attempted to verify this hypothesis by varying the axis and direction of the lesions as they were progressively enlarged. At present, we believe that somatotopic localization has not been established and that reduction of tremor or

rigidity depends more upon the size of the lesion than upon its location within the nuclear mass under consideration.

We have repeatedly listed tremor and rigidity jointly as the two symptoms of paramount interest, and the impression may thus have been given that they respond similarly to surgical destruction of the globus pallidus. This is not the case. Rigidity has effectively been reduced in every instance with relatively small lesions, and there has been little tendency for recurrence. But tremor, on the other hand, has not been significantly reduced in every instance, has required large lesions for effective alteration, and exhibits a distressing tendency to recur in the weeks and months following surgery.

A few words concerning our experiences with stimulation of the various subcortical masses seem in order. In general, the results have been somewhat inconstant, and further work must be done before stimulation can be used as a reliable guide for the position of the electrode. Table 4 represents merely a preliminary step in that direction.

TABLE 4

RESULTS OF ELECTROSTIMULATION

Disease	Structure	Response
Parkinsonism	Medial globus pallidus	Tremor exaggerated
	Lateral globus pallidus	None
	Internal capsule	Flexion of contralateral extremities
Athetosis	Medial globus pallidus	Movements mildly exaggerated
	Anterior V.L. nucleus	Movements markedly exaggerated
	Posterior V.L. nucleus	Dyesthesias in contralateral extremities
Centrencephalic seizures	N. centrum medianum	Arrest reaction
	N. ventralis anterior	None
Psychosis	Dorsomedial nucleus	Evoked potentials in frontal scalp leads
Hemiballismus	Medial globus pallidus	Movements mildly exaggerated

The interpretation of the phenomena resulting from stimulation varies, of course, with the pathophysiologic aberrations present in each individual case. As is readily apparent from the data in Table 4, electrostimulation is important in the study of a variety of disease states in addition to Parkinsonism.

In summary, a method for carrying out subcortical surgery has been described, together with its application to the problem of Parkinson's disease. Experience with 14 patients with Parkinson's disease has been discussed, and a preliminary report on both the clinical and investigative aspects has been presented. The authors feel that much more data must be accumulated in order to permit correct evaluation of the place of surgery in diseases of abnormal movements.

References

1. Horsley, V. and Clarke, R. H.: The Structure and Functions of the Cerebellum Examined by a New Method, Brain 31:45, 1908.
2. Spiegel, E. A.; Wycis, H. T.; Marks, M.; and Lee, A. J.: Stereotaxic Apparatus for Operations on the Human Brain, Science 106:349, 1947.
3. Spiegel, E. A. and Wycis, H. T.: Thalamotomy and Mesencephalotomy; Neurosurgical Aspects, New York J. Med. 49:2275, 1949.
4. Spiegel, E. A. and Wycis, H. T.: Ansotomy in Paralysis Agitans, Arch. Neurol. & Psychiat. 71:598, 1954.
5. Spiegel, E. A. and Wycis, H. T.: Thalamotomy and Pallidotomy for Treatment of Choreic Movements, Acta Neurochir. 2:417, 1952.
6. Kurland, L. T. and Mulder, D. W.: Epidemiology, Incidence, Geographic Distribution, and Genetic Considerations of Parkinsonism. Presented at 6th Annual Meeting of Houston Neurological Society, Houston, Texas, March 13, 1958.
7. Jenkner, F. L. and Ward, A. A., Jr.: Bulbar Reticular Formation and Tremor, Arch. Neurol. & Psychiat. 70:489, 1953.
8. Ward, A. A., Jr.; McCulloch, W. S.; and Magoun, H. W.: Production of Alternating Tremor at Rest in Monkeys, J. Neurophysiol. 11:317, 1948.
9. Horsley, V.: The Function of the So-Called Motor Area of the Brain, Brit. Med. J. 2:125, 1909.
10. Bucy, P. C.: Cortical Extirpation in the Treatment of Involuntary Movement, Res. Pub., Assoc. Nerv. & Ment. Dis. 21:551, 1940.
11. Klemme, R.: Surgical Treatment of Dystonia. Res. Publ., Assoc. Nerv. and Ment. Dis. 21:596, 1940.
12. Meyers, R.: Surgical Procedure for Postencephalitic Tumor, with Notes on the Physiology of Premotor Fibers, Arch. Neurol. & Psychiat. 44: 455, 1940.
13. Bucy, P. C.: Relationship of the Pyramidal Tract and Abnormal Involuntary Movements, 1st Internat. Cong. Neurol., Brussels, 1957.
14. Browder, J.: Section of the Fibers of the Anterior Limb of the Internal Capsule in Parkinsonism, Am. J. Surg. 75:264, 1948.
15. Walker, A. E.: Cerebral Pedunculotomy for the Relief of Involuntary Movements, II. Parkinsonian Tremor, J. Nerv. & Ment. Dis. 116:766, 1952.

16. Oliver, L. C.: *Parkinson's Disease and Its Surgical Treatment*, London, Lewis, 1953, p. 87.
17. Putnam, T. J.: Relief From Unilateral Paralysis Agitans by Section of the Lateral Pyramidal Tract, Arch. Neurol. & Psychiat. 40:1049, 1938.
18. Gardner, W. J.: Surgical Aspects of Parkinson's Syndrome, Postgrad. Med. 5:107, 1944.
19. Cooper, I. S.: Surgical Occlusion of the Anterior Choroidal Artery in Parkinsonism, Surg., Gynec., & Obstet. 99:207, 1954.
20. Spiegel, E. A. and Wycis, H. T.: *Stereoencephalotomy (Thalamotomy and Related Procedures)*, Part I: Methods and Stereotaxic Atlas of the Human Brain, New York, Grune & Stratton, 1952.
21. Blundell, J. E.: Variability of Size and Position of the Basal Ganglia in Man, presented at 6th Annual Meeting of Houston Neurological Society, Houston, Texas, March 13, 1958.

Staff Meeting Report

Chelation: Experimental, Biochemical, and Clinical Studies in Dermatology*

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The role of oligometals in human health and disease is far from being precisely delineated or understood. Recent studies, both at the clinical and experimental level, have tended to elevate the trace-metals from the realm of the chemistry laboratory to that of speculative clinical application. Schroeder,[1] in a stimulating review, has raised the possibility that some of the diseases of man may represent in essence a disturbance of trace-metal function, deficiency, or accumulation; these diseases would include atherosclerosis, connective tissue diseases, some drug reactions, some neurological disorders, and vitamin B-complex deficiencies. Meyer and Rapport[2] have concluded as a result of in-vitro studies of connective tissues that the sulfated mucopolysaccharides of the ground substance seem to serve as cation exchange resins in a complex physiological system. Neuman and associates[3] found an excellent correlation between cation binding capacity and sulfate content. Investigating the electrochemical potential across various connective tissues, Engel and associates[4] were able to show that dense connective tissue (cartilage) contained ionic and bound calcium far exceeding that of blood. The calcium content, especially the bound calcium, of the skin of rabbits and monkeys was also noted to exceed that of blood. Interestingly, at the basic experimental level, Meyer[5] has noted that the protein of the chondroitin sulfuric acid-protein complex is distinct from collagen and is easily digested by trypsin with a relatively high yield of tryptophane and tyrosine. This emphasizes the need for a reorientation in our understanding of the dynamic connective tissue compartments in terms of amino acid relationships.

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*This report was given at the Staff Meeting of the University of Minnesota Hospitals on February 20, 1959.

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Schroeder[1] has applied these basic findings more practically by enumerating the situations in which therapeutic effects have presumably been brought about through the metal binding properties of such drugs as BAL, antithyroid drugs, antibiotics, analgesics, antipyretics, etc. An extension of this concept has led one group of investigators[6] to note that metal binding properties characterize many of the drugs previously employed in treating scleroderma—para-amino benzoic acid, citric acid, dihydrotachysterol, promin, ACTH and cortisone, penicillin, cinchoninic acid, and prednisone. The possibilities of the metal-binding denominator were further heightened by the recent clinical experiences of Klein and Harris,[7] who successfully treated a woman suffering from severe calcific scleroderma with intravenously administered disodium ethylene-diamine-tetra-acetic acid (also known as Edathamil,® and hereafter referred to as disodium EDTA).

Subsequently, Rukavina and associates[6] and Price and associates[8] reported their experiences in treating three cases of noncalcific, acrosclerotic scleroderma with disodium EDTA. These papers reviewed the broad pattern of data on metal binding; they also emphasized the clinical changes occurring in the subjects, along with the characteristic tryptophan abnormalities and their modification or correction by disodium EDTA, pyridoxine, and nicotinamide. In a subsequent report, Curtis[9] claimed striking clinical results in treating 40 cases of morphea and scleroderma with disodium EDTA.

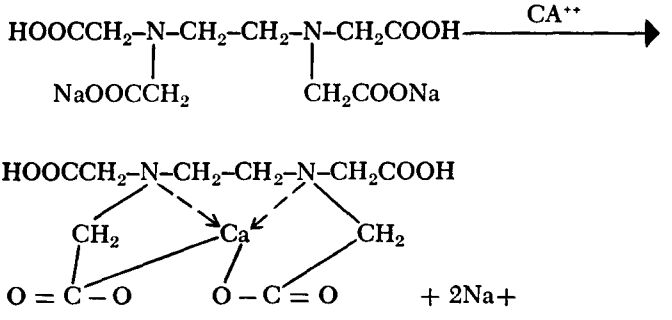
This paper will review our experience at University Hospitals with the use of disodium EDTA in scleroderma and sarcoidosis. We will describe the clinical and biochemical changes in four patients with scleroderma and the clinical and histopathologic changes in three patients with sarcoidosis.* We will also briefly discuss the chemistry and pharmacology of the metal-binding agents.

Chemistry and Pharmacology of Metal-Binding Compounds

"Chelation" in essence refers to a means of binding metals in the body, the word being derived from the Greek "chele" meaning claw. An example is the action of disodium EDTA, which forms a ring structure or chelate with a metal. The heterocyclic ring thus formed

*This experimental study of sarcoidosis was based on the recent report of Henneman and associates[10] of the effects of cortisone and sodium phytate, a calcium immobilizing agent, on a patient with sarcoidosis.

binds the metal ion, thereby preventing it from acting as a free ion. Calcium combines with disodium EDTA in this manner:[11]



Here the co-valent and coordinate bonds hold the metal to form a multimembered ring. Another means of binding metals in the body is known as a "complex," which exists in a non-ring form; penicillamine, for example, forms a simple complex between a sulfhydryl (–SH) group and copper.

The chemistry and pharmacology of metal-binding compounds are complicated and represent a frontier of ill-defined borders. The reader is referred to the standard studies by Martell and Calvin,[12] Calvin,[13] Williams,[14,15] and Chenoweth.[16]

A number of factors influence the formation and stability of a chelate or complex in the body. Two of the most important are the pH of body fluids and the stability constants of the metal compound formed. Ancillary factors include the relative concentrations of the available metal ions, the competition of body proteins for metal ions, and the concentration of the metal-binding agent in the tissues. Irving and Williams[17] have shown that the relative affinity of bivalent cations of the first transition series of the periodic table is as follows: Mn<Co<Ni<Cu<Zn, irrespective of the coordination or number of the ligands involved. Mellon and Maley,[18,19] using salicaldehyde complexes, arrived at a series in the following order of decreasing chelate stability: Pb++, Cu++, Ni++, Co++, Zn++, Ca++, Fe++, Mn++, Mg++.

Some evidence suggests that a chelating compound may affect cells by several mechanisms: (1) the sequestration of unwanted metals, (2) reaction with fixed intracellular metals, (3) catalysis, and (4) the improvement of absorption. Examples of such actions are the removal of toxic metals with BAL by producing a less toxic chelate,[20]

and the excretion of nickel-chelate complexes in nickel eczema following high citrus fruit ingestion.[21] Chelates of tissue ligands with extraneous metals interfering with normal function are exemplified by mercury and arsenic. The detoxification of the poison ivy (Rhus toxicodendron) toxin by chelation of zirconium exemplifies inhibition of a toxic ligand by chelation.[22,23]

Toxicity of EDTA

EDTA and its salts have a low degree of toxicity. Thus Foreman and associates[24] noted that most of the C^{14}-labeled calcium disodium EDTA administered intravenously to rats passed through the body unchanged, and that 95 to 98 per cent of the substance was excreted in the urine within six hours. Similar studies in human beings[25] indicated that excretion by this route occurred in 24 hours, involving both the glomerular and tubular apparatus. The drug did not invade erythrocytes and passed slowly into the spinal fluid. Poor cutaneous and gastrointestinal absorption was noted. Following administration of disodium EDTA to normal patients and to patients with osteoporosis, multiple myeloma, and osteoplastic metastases, Spencer and associates[26] studied blood calcium levels and urinary excretion of the drug. The blood calcium levels, they found, were maintained at the expense of the skeletal system, and the rate and extent of demineralization appeared to depend on the speed of infusion and the quantity of the chelating agent, rather than on the state of the skeletal structures.

Serious consequences following the use of EDTA have been most infrequent. Dudley and associates[27] administered disodium EDTA to two patients, a woman dying with osteolytic metastases and a child critically ill due to hypervitaminosis D. The findings, at autopsy in both instances, of severe renal tubular damage, engorgement of reticulo-endothelial cells with coarse, eosinophilic granules, and hemorrhagic manifestations were felt to be related to the drug. Clarke and associates[28] have expressed the belief that the sudden death of one of their patients under treatment with disodium EDTA for angina pectoris was due to calcium embolization. They also observed oral bullous lesions and dermatitis in five patients. Perry and Schroeder[29] noted in two patients a diffuse papulosquamous eruption with ulcerating lesions of the mucous membrane suggestive of avitaminoisis B; prompt recovery occurred following the interdiction of the drug and the administration of riboflavin, nicotinamide, and pyridoxine.

clinical results of these agents became apparent during the second week of therapy, when loosening of the indurated, bound-down skin of the face and extremities was noted. Reduction of pain on motion of the joints and healing of the ulcerations of the fingers were also observed. Subjectively the patients all felt an increase in the mobility of the skin and in joint motion. The dysphagia present in two cases did not appear to be affected. Before and after studies of range of motion and of circulation by oscillometry, histamine wheal reaction, and skin temperature studies showed no objective changes except in one patient in whom significant improvement was noted. Biopsy specimens secured before and after treatment revealed a deepening of the rete pegs, lessened sclerosis, and in one patient an apparent increase in fat.

Furthermore in these patients with acrosclerotic scleroderma, disodium EDTA produced profound alterations in tryptophan metabolism. This action appeared to be closely interrelated with that of magnesium or of other divalent metals or both. The complex metabolic pathway by means of which tryptophan is converted to nicotinic acid is affected by many agents. The first step in this pathway is the formation of formylkynurenine by the cleavage of the pyrrole ring of the indole nucleus.[8] Formylkynurenine is then rapidly converted to kynurenine. The further metabolism of kynurenine depends upon nicotinamide and vitamin B_6 as pyridoxal phosphate. Evidence suggests that the active form of pyridoxal phosphate consists of a complex with a metal cation. Tryptophan metabolism could therefore be altered by changing the levels of tryptophan or the amounts of niacin or pyridoxine, or by changing tissue levels of metal cations. Decreased activity could be expected of enzymes dependent upon pyridoxal phosphate if this substance were lacking, or if it existed in combination with a deficiency or imbalance of metal cations.

All four subjects were studied with reference to tryptophan metabolism before and after the exhibition of disodium EDTA and pyridoxine. Prior to the study they were placed on nut-free, liver-free diets. Twenty-four hour urine specimens were analyzed before and after tryptophan challenge with 2 gm. of L-tryptophan. Routine quantitative determinations were performed for the following substances: urinary N-methyl-2-pyridone-5-carboxamide, kynurenic acid, xanthurenic acid, aromatic amine fraction A, anthranilic acid glucuronide, o-aminophippuric acid, anthranilic acid, kynurenine, Na-acetylkynurenine, and 3-hydroxykynurenine. Paper chromatography

Disodium EDTA and Acrosclerotic Scleroderma

The following four patients with acrosclerotic scleroderma were treated at University Hospitals with disodium EDTA:

Case 1:

U. H. No .. This 34-year-old woman had had a two-year history of multiple joint arthralgia and purple-white color changes of the fingers on exposure to cold. Swelling and tightening of the skin of the fingers and face had followed, with limitation of motion in all extremities.

Physical examination revealed taut, waxen skin over the face, arms and hands, decreased acral pulsations, and pain in the joints on hyperflexion.

Laboratory data revealed a reversal of the albumin/globulin ratio, a cephalin-cholesterol flocculation of 2+, and a C-reactive protein precipitation reaction of 3+. Other laboratory data were within normal limits.

Case 2:

U. H. No. This 33-year-old woman had first noted swelling and stiffness of the hands seven years before admission. This was followed by cold-induced, blue-white color changes and ulcerations of the fingers, and dysphagia.

Physical examination revealed bound-down skin over the hands and arms, ulcerations of the fingers, and scattered areas of hyperpigmentation on the trunk.

Laboratory data were within normal limits with the exception of a cephalin-cholesterol flocculation of 3+. Esophageal x-rays demonstrated a stricture.

Case 3:

U. H. No. This 62-year-old woman had for 18 years experienced progressive swelling and pain in the arms, followed by tightening of the skin, cold-induced cyanosis of the fingers, arthralgia, dysphagia, and fingertip ulcerations.

Physical examination revealed bound-down skin of the face, arms, hands and fingers. Calcific plaques were present in the buttocks, and motion of the jaw, arms, and hands was markedly limited.

Laboratory data revealed reversal of the albumin/globulin ration, and a C-reactive protein precipitation reaction of 2+. Other studies were within normal limits.

Case 4:

U. H. No. This 37-year-old man had had a two-year history of swelling and hardening of the skin of the face, hands, and legs with increasing stiffness of these areas. Cyanotic color changes occurred in the hands during the summer and winter, followed by ulcerations. Dysphagia was also present.

Physical examination revealed hide-binding of the face, neck, abdomen, buttocks, and extremities, with limitation of motion. No ulcerations were noted.

Laboratory data were essentially normal.

Each of these subjects was given 3 gm. of disodium EDTA in 500 cc. of 5 per cent glucose in distilled water daily over a 15-day period. In addition, each was given 100 mg. of pyridoxine daily. The

was performed on all the aromatic amine fractions to provide a qualitative check on the quantitative determinations. All subjects excreted abnormal amounts of kynurenine, kynurenic acid, acetylkynurenine, and hydroxykynurenine following tryptophan "loading" prior to treatment. The tryptophan metabolism of two of the four subjects studied changed toward normal after 45 gm. of disodium EDTA and 1500 mg. of pyridoxine. These subjects with scleroderma almost certainly have abnormal tryptophan metabolism, and their patterns resembled the before and after tryptophan patterns of the cases described by Price and associates.[8]

One explanation of the biochemical data on tryptophan metabolism of both of our patients and of Price's patients is that in scleroderma (acrosclerosis) an abnormal urinary excretion of kynurenine and its metabolites occurs after oral administration of tryptophan. The administration of disodium EDTA and vitamin B_6 appears to decrease calcium and zinc (and possibly other cations), making it possible for the metal ion or ions normally functioning with pyridoxal phosphate to be utilized more efficiently. The data in the literature suggest that the metal which is unblocked is magnesium, but other metals may be involved.

Disodium EDTA and Sarcoidosis

Henneman and associates[10] have provided the rationale for the effects of disoudium EDTA on sarcoidosis. They demonstrated by means of complete calcium balance studies on three patients that hypercalcuria may be due to hypervitaminosis D in which this agent may: (1) exert a parathoromonelike action, (2) increase calcium absorption from the intestine, and (3) increase absorption of magnesium, sodium potassium, nitrogen, and iron. (Their work with sodium phytate has been mentioned previously.)

The following three patients with sarcoidosis were subjected to chelation with idsodium EDTA:

Case 1:

U. H. No. This 34-year-old white woman had had painless, reddish-brown, elevated lesions on the extremities 10 months before admission and mild dyspnea two months before admission. "Healed tuberculosis" was described five years earlier.

Physical examination revealed only the above lesions.

Laboratory data demonstrated a 1+ cholesterol flocculation and were otherwise within normal limits. An electrocardiogram revealed a primary atrioventricular block and bigeminy. A biopsy specimen of a skin lesion was interpreted as compatible with sarcoidosis.

Case 2:

U. H. No. This 55-year-old woman noted progressive involvement of the left leg, left arm, and face for three years, with annular, red-brown infiltrated plaques. She had also experienced fatigue and mild dyspnea.

Except for the skin lesions, physical examination yielded no remarkable findings.

Pertinent laboratory data demonstrated a sedimentation rate of 38 mm/ hour and reversal of the albumin/globulin ratio. Confluent densities were noted on the chest x-ray in both upper lobes.

Case 3:

U. H. No . This 55-year old woman had noted slowly progressive discolored lesions of the back, nose, and left arm for ten years prior to admission. A persistent cough and pain at the angles of the jaw occurred concomitantly. Persistent tearing of the eyes occurred later.

Physical examination demonstrated the reddish-brown plaques on the nose, left arm, and back. The lacrimation was felt to be due to rhinitis.

Pertinent laboratory and x-ray data revealed only a 1+ cholesterol flocculation and slight hilar prominence with a bilateral, nodular infiltrate in the upper lobes on chest x-ray. Histopathologic study of a skin lesion revealed findings consistent with sarcoidosis.

These patients each received 3 gm. disodium EDTA by infusion plus 75 mg. pyridoxine daily for 14 days. Improvement in the skin lesions was noted in all patients during the second week of treatment. Following treatment the lesions regressed slightly in one patient, flattened with the appearance of islands of normal skin in a second, and cleared leaving only areas of hyperpigmentation in a third patient. No objective changes occurred in the chest lesions, although one patient said his dyspnea had lessened. In two patients repeated histopathologic studies after treatment indicated less epithelioid and giant cell reaction. A second electrocardiogram, after chelation, showed no sign of the bigeminy previously observed in one patient, and a third tracing, made eight months later, was interpreted as normal.

Complications Occurring with Disodium EDTA

Like all potent chemical agents, the intravenous metal binding compounds may produce complications: Local burning or thrombophlebitis at the site of infusion has been noted. Hypocalcemia may supervene, but it is rare and is probably related to the rate of administration of the solution. When the compound is administered over a four-hour period this complication has not been observed. The renal tubular damage that occasionally occurs may be precluded by moderate dosage (3 gm. maximum daily dose) and by the judicious

use of rest periods. The drug should not be administered to patients with manifest renal disease. Toward the end of infusions of disodium EDTA, histaminelike reactions such as sneezing or nasal stuffiness may occur. Transitory elevations of blood sugar levels have been noted in cases of prolonged therapy. Transitory leukopenia may likewise occur, and liver function studies may reveal temporary abnormal cephalin flocculation levels. Vitamin B_6 deficiency may be seen with prolonged administration of the drug; the concomitant administration of pyridoxine phosphate appears to obviate this manifestation.

Seven and Johnson[30] have described an excessive chelation syndrome which may be produced by the administration of doses exceeding 3 gm. in 24 hours. This syndrome is characterized by an acute, febrile state occurring four to eight hours after the infusion, with myalgia, headache, nasal congestion, urinary urgency, chills, and leukopenia, all subsiding within 18 to 24 hours. These investigators believe that these events may be due to over-chelation of a specific metal, although the possibility of toxicity from a specific metal chelate cannot be excluded.

In summary, then, we have attempted to review briefly the chemistry and pharmacology of metal binding agents. We have likewise reported our clinical experiences with a potent chelating compound, disodium EDTA, in four cases of acrosclerotic scleroderma and three cases of sarcoidosis. Since we believe that acrosclerotic scleroderma is characterized by abnormal tryptophan metabolism, we have studied the effect of disodium EDTA upon this disorder, with the results described above.

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References

1. Schroeder, H. A.: Trace Metals and Chronic Diseases, Adv. Int. Med. 8:259, 1956.
2. Meyer, K. and Rapport, M.: The Mucopolysaccharides of the Ground Substance of the Connective Tissue, Science 113:596, 1951.
3. Neuman, W. F.; Boyd, S. E.; and Feldman, I.: The Ion Binding Properties of Cartilage. Fourth Conference on Metabolic Interrelations with Special Reference to Calcium. Ed. by E. C. Reifenstein, Jr. New York, Josiah Macy, Jr. Foundation, 1952, p. 100.

4. Engel, B.; Joseph, N.; and Catchpole, H. R.: Equilibrium of Calcium and other Ions in Connective Tissue, Fifth Conference on Metabolic Interrelation with Special Reference to Calcium, Ed. by E. C. Reifenstein, Jr. New York, Josiah Macy, Jr. Foundation, 1953, p. 105.
5. Meyer, K.: Connective Tissues, Transactions of the First Connective Tissue Conference, New York, Josiah Macy, Jr. Foundation, 1950.
6. Rukavina, J. G.; Mendelson, Charles; Price, J. M.; Brown, R. R.; and Johnson, S. A. M.: Scleroderma (Acrosclerosis); I. Treatment of Three Cases of the Non-Calcific Variety of Chelation (EDTA), J. Invest. Dermatol. 29:273, 1957.
7. Klein, R. and Harris, S. B.: Treatment of Scleroderma, Sclerodactylia and Calcinosis by Chelation (EDTA), Am. J. Med. 19:798, 1955.
8. Price, J. M.; Brown, R. R.; Rukavina, John G.; Mendelson, Charles; and Johnson, S. A. M.: Scleroderma (Acrosclerosis); II. Tryptophan Metabolism Before and During Treatment by Chelation (EDTA), J. Invest. Dermatol. 29:289, 1957.
9. Curtis, A. C. and Jansen, T. G.: The Prognosis of Localized Scleroderma, Arch. Dermatol. & Syphilol. 78:749, 1958.
10. Henneman, P. H.; Dempsey, E. F.; Carroll, E. L. and Albright, F.: The Cause of Hypercalcuria in Sarcoid and Its Treatment with Cortisone and Sodium Phytate, J. Clin. Invest. 35:1229, 1956.
11. Muller, S. A.: Scleroderma: The Relationship of Calcinosis and the Effects of a New Chelating Agent, EDTA, Master's Thesis, University of Minnesota. March, 1958.
12. Martell, A. E. and Calvin, M.: *The Chemistry of Metal Chelate Compounds,* New York, Prentice Hall, 1952.
13. Calvin, M.: Chelation and Catalysis, in *A Symposium on the Mechanism of Enzyme Actions,* Ed. by W. D. McElroy and B. Glass. Baltimore, Johns Hopkins Press, 1954, p. 221.
14. Williams, R. J. P.: Metal Ions in Biological Systems, Biol. Rev. 28:381, 1953.
15. William, R. J. P.: Models for Metallo-Enzymes. Nature 177:304, 1956.
16. Chenoweth, M.: Chelation as a Mechanism of Pharmacological Action, Pharmacol. Rev. 8:57, 1956.
17. Irving, H. M. and Williams, R. J. P.: The Stability of Transition Metal Complexes, J. Chem. Soc. 1952:370, 1953.
18. Mellon, D. P. and Maley, L.: Stability Constants of Internal Complexes, Nature 159:370, 1947.
19. Mellon, D. P. and Maley, L.: Order of Stability of Metal Complexes, Nature 161:436, 1948.
20. Stocken, L. A. and Thompson, R. H. S.: Reactions of British Antilewisite with Arsenic and Other Metals in Living Systems, Physiol. Rev., 29:168, 1949.
21. Carlsen, F. M.: Health Hazards in the Plating Shop; Some Suggestions for Their Elimination. Metal Ind., London 52:511, 1938.

22. Cronk, G. A.: Zirconium Salts in the Treatment of Rhus Toxicodendron Dermatitis, Arch. Dermatol. & Syphilol. 66:282, 1952.
23. Strauss, R. E. and Bruck, C. R.: Zirconium Pyribenzamine Cream for the Prophylaxis of Rhus Dermatitis, J. Invest. Dermatol. 20:411, 1953.
24. Foreman, H.; Vier, M.; and Magee, M.: The Metabolism of C^{14} Labelled Ethylenediamine Tetra-acetic Acid in the Rat, J. Biol. Chem. 203:1045, 1953.
25. Foreman, H. and Trujillo, T. T.: The Metabolism of C^{14} Labelled Ethylenediamine Tetra-acetic Acid in Human Beings, J. Lab. & Clin. Med. 43:566, 1954.
26. Spencer, H.; Van Kinscott, V.; Lewin, I.; and Laszlo, D.: Removal of Calcium in Man by Ethylenediamine Tetra-acetic Acid: A Metabolic Study, J. Clin. Invest. 31:1023, 1952.
27. Dudley, H. R.; Ritchie, A. C.; Schilling, A.; and Baker, W. H.: Pathologic Changes Associated with the Use of Sodium Ethylenediamine Tetra-acetate in Treatment of Hypercalcemia; Report of Two Cases with Autopsy Findings, New England J. Med. 252:331, 1955.
28. Clarke, N.; Clarke, C.; and Mosher, R.: The "In Vivo" Dissolution of Metastatic Calcium; an Approach to Atherosclerosis, Am. J. Med. Sc. 229:142, 1955.
29. Perry, H. M. and Schroeder, H. A.: Lesions resembling Vitamin B Complex Deficiency and Urinary Loss of Zinc Produced by Ethylenediamine Tetra-acetate, Am. J. Med. 22:168, 1957.
30. Seven, M. J. and Johnson, L. A.: *Chelation. A Broad Concept in Therapy.* Philadelphia, Dept. of Medicine, Hahneman Medical College, 1956

Faculty Publications

BRADLEY, S. G.: Protoplasts of *Streptomyces griseus* and *Nocardia paraguayensis*, J. Bact. 77:115, 1959.

BRADLEY, S. G.: Genetic Analysis of Segregants from Heterokaryons of *Streptomyces Coelicolor*, J. of Bact. 76:464, 1958.

BRADLEY, S. G. and ANDERSON, L.: Compatibility System Controlling Heterokaryon Formation in Streptomyces Coelicolor, Proc. Soc. Exp. Biol. & Med. 99:476, 1958.

BROZEK, J. and MORI, H.: Some Interrelations Between Somatic Roentgenographic and Densitometric Criteria of Fatness, Human Biol. 30:322, 1958.

FIELD, MARVIN F. and LICHSTEIN, HERMAN, C.: Growth Stimulating Effect of Autoclaved Glucose Media and Its Relationship to the CO_2 Requirement of Propionibacteria, J. Bact. 76:485, 1958.

FIELD, MARVIN F. and LICHSTEIN, HERMAN C.: Influence of Casein Hydrolyzates and Amino Acids on Glucose Fermentation by *Propionibacterium Freudenreichii*, J. of Bact. 76:491, 1958.

GLICK, DAVID; LICHSTEIN, HERMAN C.; FERGUSON, ROBERT B.; and TWEDT, ROBERT M.: Studies in Histochemistry LIII. Microbiological Assay in Quantitative Histo- and Cytochemistry, Proc. Soc. Exp. Biol. & Med. 99:660, 1958.

KUBICEK, W. G. and ANDERSON, W. D.: Effects of Hemorrhage and Hypoxia on Febrile Dogs and Monkeys, Am. J. Physiol. 196:163, 1959.

MURPHY, W. H.; WIENS, A. L.; and WATSON, D. W.: Impairment of Innate Resistance by Triiodothyronine; Proc. Soc. Exp. Biol. & Med. 99:213, 1958.

PUTNAM, H. D. and SCHMIDT, E. L.: Studies on the Free Amino Acid Fraction of Soils, Soil Science 87:22, 1959.

SYVERTON, J. T.: Discussion in *Poliomyelitis:* Papers and discussions presented at the Fourth International Poliomyelitis Conference, Geneva, 1957, Philadelphia, J. B. Lippincott Company, 1958, pp. 277-292.

SYVERTON, J. T. and Ross, J. D.: Growth of Human Cells of Varying Origin in Continuous Growth, Proceedings of the Fourth International Congress of Gerontology, Merano, Italy, July 14-21, 1957, Tipografia Tito Mattioli-Fidenza, 1958, Volume 1, pp. 292-298.

WEEKLY CONFERENCES OF GENERAL INTEREST

Physicians Welcome

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

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