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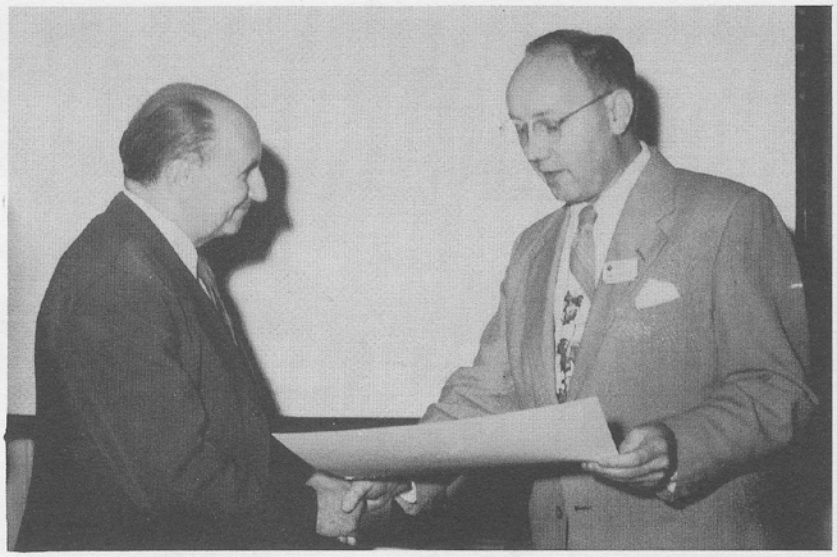

Hugh J. McDonald, President of the AACC, reading the citation for the 1953 Ernst Bischoff Award to Michael Somogyi, recipient.
(Photograph by Howard Sanders, courtesy CHEMICAL AND ENGINEERING NEWS)

THE SECRETARY REPORTS

With the first volume of "Standard Methods of Clinical Chemistry" published by Academic Press, the Association has achieved one of its main objectives. This may therefore be an appropriate time to look at some of the other plans now in view.

The International Federation of Clinical Chemistry is well on its way, having already completed the organizational phase. The Federation will not only offer opportunities for international scientific programs, but will open the way for clinical chemists of any one country to acquaint themselves with the problems and progress of their fellow chemists in other parts of the world.

One of the first projects that presented itself to the Association was that of a comprehensive journal of clinical chemistry. The need for such a specialized periodical has been obvious for some time. The financial requirements for such an undertaking have for the most part been responsible for any delays. It cannot be said at this time that the finances are now available, but efforts in that direction are being continued. As the membership of the Association expands, the problem comes nearer a solution.

A "census" of clinical chemists would contribute interesting and needed information. At the present estimates vary according to how one defines who is a clinical chemist. What is obvious, however, is that there are still many individuals who should be but are not members of the Association. And likewise there are many geographical areas that should have but do not yet have local sections.

Max M. Friedman, National Secretary

FIFTH ANNUAL MEETING, CHICAGO, ILL.

Dr. Michael Somogyi, one of the world's leading biochemists and a pioneer in the study of sugar metabolism, was presented with the 1953 Ernst Bischoff Award, at the fifth annual meeting of the AACC held at the Conrad Hilton Hotel, September 10.

The citation to Dr. Somogyi read: "For outstanding contributions to the theory and practice of clinical chemistry...a pioneer in the development and application of chemical methods to the practical problems of physiology and medicine...an independent mind with original concepts in carbohydrate metabolism...an unselfish teacher of chemists and physicians."

Dr. Somogyi has been in charge of research and chemical laboratories at the Jewish Hospital of St. Louis since 1926. He was formerly instructor in biochemistry at the School of Medicine, George Washington University, St. Louis, following years of research and teaching at Cornell and laboratories in Budapest. He was born in Austria in 1883 and received his Ph.D. in Hungary.

A pioneer in the metabolism of carbohydrates and ketone bodies, he introduced many of the methods now standard in clinical chemistry. He has been widely published on such subjects as diastases, physiology of insulin action, clinical studies of diabetes and analytical methods. He was already studying the problem of sugar metabolism in 1920, when Banting and Best showed the action of insulin to diabetes and won the Nobel Prize. Dr. Somogyi, together with P. A. Schaefer and others at Washington University, developed the most practical way to produce insulin in quantities needed in medicine. The method is still in use today.

Dr. Somogyi delivered the second Ernst Bischoff lecture at the dinner-meeting. The lecture is published completely in this issue.

The scientific sessions, held in conjunction with the Division of Biological Chemistry had excellent attendance. The Symposium on Electromigration in Stabilized Electrolytes, featured eleven papers on all phases of the subject. Dr. Hugh J. McDonald presided. Dr. Max M. Friedman was chairman of the series on Clinical Chemistry. Abstracts of all papers of the AACC scientific session are published in this issue.

ANNUAL MEETING

The Annual Meeting of the American Association of Clinical Chemists was held in the Normandy Lounge of the Conrad Hilton in Chicago on Thursday, September 10, 1953, and called to order at 5:00 P.M. by Hugh J. McDonald, president.

The proceedings of the Executive Committee held the previous evening (and published elsewhere in this issue of THE CLINICAL CHEMIST) were made known to the members.

The annual dues for the next fiscal year were approved at $7.50 for full members and $4.00 for associate members, the same as the present.

The procedure for selection of the Ernst Bischoff Award recipient was again explained to the membership, who are urged to submit nominees to the Award Committee. Details will again be presented in later issues of THE CLINICAL CHEMIST.

The volume on "Standard Methods of Clinical Chemistry" published by Academic Press was discussed from various aspects. Miriam Reiner, editor-in-chief, discussed the many difficulties encountered in the preparation of this first volume. Future volumes should be prepared with much less effort.

Harold D. Appleton reported on the progress of THE CLINICAL CHEMIST and outlined plans for future expansion of this publication.

The need for additional members and local sections was stressed. Various geographical areas seem to be ready now for the formation of local sections and await only some individual or group in the area to do the organization work.

The adoption of a code of ethics for clinical chemistry was announced and the membership was urged to seriously study this code and abide by it.

The part played by the Association in the International Federation of Clinical Chemistry and the Intersociety, as well as the close collaboration with the American Chemical Society and the American Board of Clinical Chemistry was described.

The revised resolution on professional regulation adopted by the ACS and which concerns clinical chemistry was presented to the membership without comment until further study.

The meeting was adjourned at 5:45 P.M. for the Association dinner which followed.

EXECUTIVE COMMITTEE MINUTES
September 9, 1953

The National Executive Committee met in Chicago on September 9, 1953 at 8:00 P.M. Those present included: Hugh J. McDonald, president; Monroe E. Freeman, Vice-president; Max M. Friedman, national secretary; Robert M. Hill, Joseph I. Routh, Harold D. Appleton (alternate), John G. Reinhold (alternate), and Marschelle H. Power (by invitation).

The National Treasurer submitted the following financial statement for the year July 1, 1952 to June 30, 1953:

Income	
Membership dues	$2822.50
Certificates	40.00
Directory	50.00
CLINICAL CHEMIST	518.50
Bank interest	34.12
Total	$3465.12
Expenses	
CLINICAL CHEMIST	$1932.26
Certificates	6.32
Ernst Bischoff Award	600.10
Section Allotments	336.90
Executive Committee	132.96
National Treasurer	83.26
National Secretary, Directory	184.29
Membership Committee	40.80
Total	$3316.89
Balance	$ 148.23
Carried forward, July 1, 1952	$1763.44
Bank balance, June 30, 1953	$1911.67

A check for $500.00 from the Ernst Bischoff Company towards the Award was credited to the 1951-52 income, thus adding this sum to the net operating income of 1952-53.

The appointment of Robert L. Dryer of Indianapolis as chairman of the Committee on Standards and Personnel was extended, and a sum up to $100.00 was allocated for this function.

The Committee on Membership consisting of Louis B. Dotti, chairman and Harry Sobotka, secretary, was reappointed.

The Ernst Bischoff Award Committee consisting of Otto Schales, chairman, Fritz Bischoff and Joseph H. Roe was reappointed.

Harold D. Appleton was reappointed by acclamation for a three-year term as chairman of the Editorial Board. The excellence and continued improvement of the official publication was attributed in large part to the efforts of the chairman. The Advisory Board to THE CLINICAL CHEMIST for 1953-54 shall consist of Cecelia Riegel, Joseph I. Routh, and Albert E. Sobel.

Monroe E. Freeman was appointed as representative of the Association to the International Federation of Clinical Chemistry, this appointment to become effective on January 1, 1954.

A committee consisting of the past-presidents (Max M. Friedman, Harry Sobotka, John G. Reinhold and Albert E. Sobel) was appointed to study the present constitution and bylaws of the Association and recommend changes or revision.

The Committee on Membership was requested to invite to membership into the Association those individuals who have been certified by the American Board of Clinical Chemistry but are not yet members of the Association. Ways and means were discussed for the initiation of additional local sections in the coming year.

It was moved, seconded and approved that the Executive Committee recommend to the membership that the dues for the year 1954 remain at $7.50 for full members and $4.00 for associate members.

After approval of the remaining sections of the code of ethics, this code was adopted in its entirety and will be published in an early issue of THE CLINICAL CHEMIST.

Joseph I. Routh was requested to formulate means for establishing an "employment exchange". Members will be notified in THE CLINICAL CHEMIST as to how they may in the future apply for positions or assistants.

At the invitation of the American Board of Clinical Chemistry to the Association, four names were submitted for the possible selection of one to the Board.

The future legislative policy of the Association was discussed in view of the recent Pennsylvania and New Jersey action. Any legislation which does not tend to improve the professional standing of clinical chemistry, or which requires clinical chemists to take examinations in other laboratory specialties will be opposed. The policy statement of January 19, 1950 was reaffirmed and will be published in the October, 1953 issue of THE CLINICAL CHEMIST.

The report of the Intersociety meeting held in Philadelphia on June 5, 1953 was presented to the Executive Committee. Oliver H. Gaebler, representative of the Association to the Intersociety, was praised for his efforts in behalf of clinical chemistry. A sum up to $50.00 was voted for the next year to defray expenses that may arise in this matter.

The current membership levels, full and associate, were considered adequate for the present. The proposal by some members of the Association that an additional category of "chemical technician" be added was postponed for future consideration.

The appearance of Volume 1 of "Standard methods of Clinical Chemistry" (Academic Press) published by the Association was acknowledged with much satisfaction. A vote of thanks was extended to the editor-in-chief and the contributors to this volume. The next two volumes, including one on methods in toxicology, are now in preparation.

Means for further expansion of THE CLINICAL CHEMIST, the official publication of the Association, were discussed at some length. Since original publications and editorial material could be readily available, the need for such a comprehensive journal in clinical chemistry was acknowledged, and wide circulation envisaged. The problem revolves around the financing of an expanded journal which is beyond the present capabilities of the Association's financial resources. Some aid in the form of grants and donations will be necessary for the initiation of such a project.

A vote of thanks was extended to the American Chemical Society and to the Division of Biological Chemistry for the hospitality and facilities extended to the Association during the Chicago meetings.

The sixth annual meetings of the American Association of Clinical Chemists will be held in conjunction with the 126th National Meeting of the American Chemical Society in New York City during September 12-17, 1954.

The meeting was adjourned at 11:00 P.M.

Respectfully submitted,
Max M. Friedman, National Secretary

WASHINGTON – BALTIMORE RICHMOND SECTION

The Washington-Baltimore-Richmond Section held its March meeting at The George Washington University School of Medicine on the 27th. Dr. Bernard H. Armbrecht of Georgetown University discussed the chemical aspects of porphyrinuria and Dr. Nicholas Cotsonas and Dr. Patrick Storey of Gallinger Hospital dealt with its clinical significance.

The last meeting of the current year was held May 14th at Georgetown University. At the business meeting the retiring chairman, Lt. Col. Monroe E. Freeman, summarized the growth of the group and its activities for the past year and then presented the incoming chairman, Miss Miriam Reiner of Gallinger Hospital.

The open session featured discussions on barbiturates by Dr. Leo R. Goldbaum of Walter Reed Army Medical Center and Dr. Theodore Koppanyi of Georgetown University Medical School. Abstracts of their talks are published in this issue. Following the formal program refreshments were served and a general social hour held.

THE INTERSOCIETY COMMITTEE

As reported in Vol. 4 No. 6 November 1952 OnOctober 20, 1952, representatives of six societies met in Cleveland, Ohio, to explore the possibilities of a co-operative approach to the solution of problems which they held in common. These representatives then consulted their respective governing bodies and a second meeting was held in Philadelphia, Pa., on June 5, 1953. At this meeting, a seventh society, the American Chemical Society was represented.

The societies represented and their delegates are listed below:

American Chemical Society —
Warren M. Sperry, Ph.D., Chairman, Committee on Clinical Chemistry.
B.R. Stanerson, Ph.D., Secretary of Committee on Professional Regulation.

National Association of Clinical Laboratories —
Joseph M. Chernaik, Chairman Committee on Professional Relations.

Society of American Bacteriologists—
Earle H. Spaulding, Ph.D., Chairman Committee on Certification and Problems of Personnel.

American Association of Clinical Chemists—
Oliver H. Gaebler, M.D.

Ph. D. Laboratory Section, American Public Health Association—Edmund H. Kline, Ph.D. Member of Laboratory Section Council, APHS. (Official Observer).

Conference of State and Provincial Public Health Directors —
Cleon J. Gentzkow, M.D., Ph.D. (Official Observer)

American Society of Professional Biologists—
Alfred F. Borg, Ph.D., President.
Norman C. Lafier, Ph.D., Executive Secretary (Also attended as member of Dr. Spaulding's Committee from the SAB).

The representatives present took the following action:

1. Name of the Committee.

The Committee is to be called "THE INTERSOCIETY COMMITTEE ON LABORATORY SERVICES RELATED TO HEALTH."

2. Statement of aims:

It is the object of this committee to improve the standards of laboratory services related to health. This is to be accomplished by cooperative efforts of the participating scientific societies.

Among the objectives are:

(a) To define laboratory practice as related to health,

(b) to recommend standard qualifications for personnel in laboratories related to health,

(c) to cooperate with educational institutions in developing curricula for training of personnel for laboratories related to health,

(d) to encourage professional certification of individuals in the scientific specialties practiced in laboratories related to health,

(e) to promote desirable patterns of approval of laboratories related to health,

(f) to foster career opportunities in laboratories related to health.

REAFFIRM 1950 RESOLUTION ON LEGISLATION

The National Executive Committee, meeting in Chicago last month, reaffirmed the AACC 1950 Resolution on Legislation. The resolution follows:

POLICY IN MATTERS OF LEGISLATION

Whereas,

in the public interest, it is the aim and object of the American Association of Clinical Chemists, to raise the level at which clinical chemistry is practiced in the clinical laboratory and

Whereas,

clinical chemistry is a discipline defined as "That branch of chemistry which deals with the composition of the secretions, concretions and fluids of the human body in health and disease and the chemical composition and metabolism of cells and tissues. Also the search for the presence of substances (or their derivatives) given for diagnostic or therapeutic reasons and the search for poisons (or their derivatives) are properly included in the field of clinical chemistry".

Be It Therefore RESOLVED, that,

1. We oppose any concept which defines the Practice of Clinical Chemistry as the Practice of Medicine.

2. We disapprove any regulation requiring Clinical Chemists to pass examinations in techniques other than the field of Clinical Chemistry.

3. We propose to encourage the establishment of laboratories engaged in clinical chemistry, as defined, and directed by clinical chemists. Where such separate units are not available, we hold that the procedures in Clinical Chemistry shall nevertheless be supervised and performed by those whose training and experience is adequate in the science.

Adopted by the Executive Committee of the American Association of Clinical Chemists, Inc. On January 19, 1950.

EMPLOYMENT CLEARING HOUSE

As a start for an AACC employment clearing house, Professor Joseph I. Routh, Department of Biochemistry, Iowa State University, Iowa City, Iowa, and member of the National Executive Committee of the AACC, will keep an active file of all employment opportunities. Members seeking personnel for their organizations, or those wishing to change their affiliations should consult Dr. Routh, who will then arrange for a meeting of the interested parties.

BOOK REVIEWS

STANDARD METHODS OF CLINICAL CHEMISTRY, VOL. I. American Association of Clinical Chemists, Editor—Miriam Reiner. xii, 142 pages. Academic Press Inc., 125 East 23rd Street, New York 10, N.Y. $4.50

To publish a review of the AACC's first volume on standard methods would be as if an author were invited to review his own book. We can say this much though, people that have seen the book think it a very worthwhile addition to the laboratory book collections and may well be the book to see the most use in laboratory practice.

SPECIAL CLINICAL-CHEMICAL METHODS. I. Abelin. Published by Hans Huber, Bern, and Stuttgart, 1952, 311 pages (in German). Reviewed by — A. Saifer, Jewish Sanitarium and Hospital for Chronic Diseases.

Within the confines of this paper-bound book Dr. Abelin, Director of the Medical Chemical Institute of Bern University, has compiled a series of the more unusual biochemical methods used in quantitative clinical chemistry. These include a) steroid determinations in blood and urine, b) heavy metal analysis of organic material using colorimetric and polarographic methods, c) protein determination and fractionation in both serum and cerebrospinal fluid including techniques for filter paper electrophoresis, d) sugars, 3-carbon acids and alcohols in blood and urine, e) protein flocculation methods, f) porphyrins and phosphatases, g) other miscellaneous procedures.

It is the reviewer's opinion that the methods are well chosen, clearly yet tersely presented, and surprisingly up-to-date for a text book. Since the accuracy of a clinical determination is dependent upon the procedure employed in its analysis, this book is proof of the high standards maintained in Swiss clinical laboratories. German-reading American biochemists will find it an excellent source of ready reference for a tried and tested unusual procedure.

At the end of each chapter there is an excellent bibliography of the more important papers dealing with the topic under discussion. Fifteen years ago this bibliography would have referred almost entirely to the German biochemical literature. Today, in a German textbook, more than 80% of the papers quoted are from the American, English and Scandinavian literature.

SOUTHERN CALIFORNIA SECTION

Dr. Joseph Goodman, Biochemist, Long Beach Veterans' Administration Hospital, opened the new season of section meetings with a discussion of the "Theory and Application of the Analytical Ultracentrifuge". Local members and guests met September 1, at the Los Angeles County Hospital for the address.

DIABETOGENIC EFFECTS OF INSULIN-HYPOGLYCEMIA

BY

Michael Somogyi

(From the Laboratory of the Jewish Hospital of St. Louis, St. Louis, Mo.)

The Ernest Bischoff Lecture delivered before the American Association of Clinical Chemists, September 10, 1953 at the Conrad Hilton Hotel, Chicago, Ill.

Insulin has occupied the center of my interests and has absorbed the greater part of my working hours for the past 31 years. This fact more or less determined the choice of my subject for this evening.

My first encounter with insulin took place in September 1922, when it was a newborn infant. From Toronto, its birthplace, Collip published in that year from Macleod's laboratory, a method for the preparation of insulin, but the procedure was a rather precarious one, as the end-product contained insulin only in one out of every three or four preparations, and even the yield was very small. In the same year, in teamwork with P. A. Shaffer and E. A. Doisy at Washington University, we succeeded in devising a simple method of preparation which is practicable as a commerical process, and still is used as such. The method was presented at the annual meeting of the American Society of Biological Chemists in December 1922, and in 1924 a final report on it was published in the Journal of Biological Chemistry.

Insulin challenged my attention a second time a few years later, when I moved from academic work to the field of clinical chemistry in a hospital laboratory. In the intervening years my infant acquaintance had developed into a widely employed drug, vitally essential in the treatment of diabetes. Working in a hospital, insulin confronted me with problems which can never emerge in the biochemistry department of a medical school, for these were plain clinical problems, involving the treatment of patients. I felt that they were not in the province of the chemist. But in the end situations developed which impelled me to cross the no-man's land between laboratory and practical medicine; or perhaps more correctly, between physiology and its clinical application.

I. CLINICAL OBSERVATIONS

For several years interns, many of them former students, pressed me for answers to questions they raised in connection with perplexing observations they made when treating diabetic patients. In view of my previous interest in insulin, they - somewhat naively - insisted on concrete answers from me to questions to which they could get none from any source at their disposal. The problems presented were mostly hazy and ill-defined. But in 1930, a member of the housestaff came to me with a fairly positive question. I was taught - he said - and still am being told here - that *there is a direct relationship between the dose of insulin and the amount of carbohydrate it can take care of.* This means that if a diabetic patient spills too much sugar, all I have to do is to increase the insulin dose, until glycosuria is abolished. Yet, in my second year of internship I must say that this is mere lip service. Right now I have a 29-year old severely diabetic man on the ward service who seems to defy this fundamental rule, and in fact any rule I have heard or read of. The patient suffers frequent insulin shocks and still spills large amounts of sugar. Time and again I am raising the insulin dose; now it is up at 150 units per day, and his glycosuria is as bad as ever. Sometimes it is my impression that it is worse after an increase in the insulin dose. The visiting men are unable to offer me help beyond declaring that the man is an unmanageable diabetic. I feel frustrated, I am seeking help from you. Thus the intern, a former student of mine.

His observations were not convincing, was my reply. Data on urine sugars, the amounts expressed as from 1+ to 4+, are far from quantitative, since 4+ sugar may represent concentrations from 1.5 all the way to 6 or 10 per cent. To get at the truth, I suggested, one must resort to strictly quantitative observations in clinical studies that go beyond the usual scope of clinical procedures; it is indispensable to gather well controlled quantitative data in clinical studies just the same as in any other bona fide experimental work. As an upshot of this discussion, we gathered quantitative data on this patient on four consecutive days. Giving a standardized diet, but varying the insulin doses, we ran frequent blood sugar determinations at unorthodox intervals of time, as well as quantitative determinations of the sugar excreted in the urine. The results, shown in this old chart (it is the original, drawn in 1930) were both startling and perplexing. As may be seen, the blood sugar oscillated wildly between very high hyperglycemic and very low hyperglycemic and very low hypoglycemic levels. More than that: when the insulin dose was substantially increased, hyperglycemia was augmented and, in addition, had become more persistent than with the smaller insulin doses. These paradoxical manifestations of insulin action imparted shocks not only to the patient, but also gave me a severe jolt, and I have been laboring under its effect ever since.

Although this was but a single observation, it seemed impossible to brush it aside. It cast grave doubt on the general validity of the basic principles that guides insulin therapy in diabetes. It demanded explanation. But preoccupied at the time with other activities, mainly with studies of amylase action, it was not until 193[illegible]

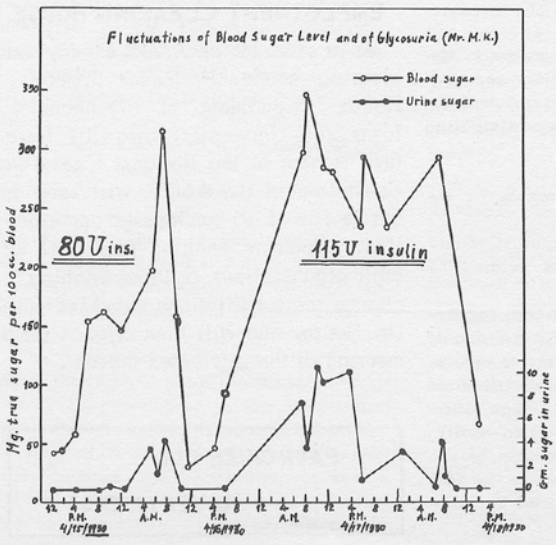

Figure I.

Showing precipitous oscillations of blood sugar level and glycosuria in an unmanageable diabetic. Hyperglycemia and glycosuria paradoxically increased when the insulin dose was increased.

that a systematic study of the problem was undertaken in our laboratory. I have enlisted in these studies the help of some eminent clinicians, foremost among them the late Albert E. Taussig, clinical professor of medicine, as well as the collaboration of the housestaffs.

Within a few weeks after launching these studies, it became evident that the single observation made in 1930 was correct: it was duplicated in every severely diabetic case that was treated with large doses of insulin. In every one of these cases blood sugar and glycosuria fluctuated between the extremes of very low and very high levels, while the insulin dose and diet were kept unchanged. Then, after about three weeks, close scrutiny of the accumulating data revealed the unmistakeable fact that blood sugar and glycosuria invariably rose to high peaks directly in the wake of hypoglycemia reactions. Then, in a few days these peaks subsided (without change in the insulin dose), and hypoglycemic reactions again set in, only to be followed with new tidal waves of hyperglycemia and glycosuria. A cause and effect relationship between the two alternating phases was unmistakable: it became evident that hypoglycemia begets hyperglycemia. In Table I is presented a limited excerpt from these early records, which shows the variations in glycosuria. Changes in the blood sugar level are shown in the next two tables. In Table II it may be seen that hypoglycemias have occurred at such periods of the day at which blood sugars are hardly ever determined in clinical practice (the usual time for such tests being in the morning before breakfast and two hours after meals). Table III represents a phase of insulin treatment that is rather typical in common practice. The patient was a girl of 20, who for years past had been treated with large doses of insulin. Before this hospitalization in 1951 she was using 80 units a day, and was transferred to a municipal hospital from a streetcar, where she was picked up unconscious from insulin shock. Under my influence members of the resident staff tried to regulate her on less insulin and, as shown in Table III, the dosage was reduced to 35 units per day. But the result was unsatisfactory, glycosuria showed great fluctuations. The fasting blood sugar, conventionally determined for guidance showed, as may be seen in Table III, an alarming increase from 183 to 388 mg. per cent during the five days between Aug. 17 and Aug. 22. On the basis of these figures, in accordance with the customary pattern of insulin treatment, for the next morning (Aug. 24) the dose was increased by 15 units. But the blood sugar had run ahead of this change, it mounted to a new high of 437 mg. per cent.

Explanation of this adverse process is simple if one is aware of the diabetogenic effect of hypoglycemia. On several occasions during the week the patient had complained of insulin reactions about 7 to 8 p.m. She felt weak, had tremors and perspired, but the physicians in charge ascribed these symptoms to neurosis, reluctant to believe that hypoglycemia could occur within three to four hours after a meal which contained about 85 gm. of carbohydrates. Hence the patient had to tide over these episodes without relief by sugar feeding.

On Aug. 23, once more, she began to develop symptoms at 7 p.m. and asked for relief. Instead, at 7:30 p.m. a blood sample was drawn for sugar determination, mainly to prove that the patient's symptoms were not caused by hypoglycemia. But as she continued to complain and clamor for relief during the ensuing two hours, at 9:30 p.m. she was at last fed orange juice and milk, directly after a second blood sample had been taken. The hypoglycemic symptoms soon disappeared. No wonder, as the laboratory reported sugar levels of 37 and 31 mg. per cent in the two blood samples.

It can be fairly inferred from this evidence that whenever she complained, actually suffering from recurrent hypoglycemic reactions also during the preceding week and that the rise in the fasting blood sugar level during that period was the aftermath of excessive insulin action, as it was in the Aug. 23-24 episode. On the basis of such clinical observations we had reached the conclusion before the end of 1935 that - *because of its diabetogenic action - hypoglycemia must be most carefully avoided in the treatment of diabetes, while using insulin in doses just sufficient to enable the patients to utilize normoral carbohydrate rations*, i.e., quantities

TABLE I.

Flare-up of Glycosuria after Hypoglycemia

Date 1935	Period of day, hours — Urine sugar, gm. 7-12	12-17	17-3	3-7	Glycosuria per 24 hrs. gm.	Insulin in 3 doses units
Oct. 25	11	8	28	0	47	70
" 26	0*	0	76	24	100	68
" 27	0	0	0**	0	0	68
" 28	0	14	0	26	40	68

* Severe hypoglycemic reaction directly before noon meal

** " " " between 21 and 22 (about 9:40 p.m.)

TABLE II.

Hypoglycemia Entails Increased Hyperglycemia

Date	Hour of day	Blood sugar mg. per 100 cc.	Insulin Time of injection 7 a.m.	4:30 p.m.	10 p.m.
Aug. 14	4:30 p.m.	117	10	14	0
	10:00 p.m.	26			
" 15	6:30 a.m.	331			
Nov. 10	11:00 p.m.	109	10	8	6
" 11	3:00 a.m.	52			
	6:45 a.m.	273			
" 13	3:00 a.m.	39	"	"	"
" 14	6:45	304			
" 18	3:00 a.m.	62	"	"	"
" 19	6:45 a.m.	374			

TABLE III.

Hypoglycemia Begets Hyperglycemia

Date 1945	Insulin units	Blood sugar mg. per cent
Aug. 17	20 - 0 - 15	183 (7 a.m.)
18	" " "	- - -
19	" " "	- - -
20	" " "	- - -
21	" " "	- - -
22	" " "	388 (7 a.m.)
23	" " "	35 (7:30 p.m.)
		31 (9:30 p.m.)
24	35 - 0 - 15	120 (1:30 a.m.)
		437 (7 a.m.)

consumed by most healthy persons. We first tried out this approach on a small group of severely diabetic men, ranging in age from 22 to 33 years, who for years had been classified as unmanageable cases and were treated with from 90 to 180 units of insulin per day.

Simple and clear as our guiding principle was, details and the know-how of its practical application involved a great deal of arduous work. It had consumed the the labors of 5½ months, until in March 1936 our first three study cases could be discharged from the hospital. But then the results were gratifying: they were able to resume a virtually normal way of life and return to work without any handicap. As ambulatory patients they received further guidance, until their glycosuria gradually decreased to a slight amount, often to nil, while their insulin dosage was being reduced to from 14 to 20 units. After this experimental group a long array of of severe cases of diabetes were treated with the same method, always with the same method, always with the same result: the greatly fluctuating glycosuria was diminished, while the large insulin doses were gradually decreased. In a few cases patients who for years had been treated with 75, 100, and even 150 units of insulin, had in a few months become aglycosuric without the need of insulin injections. An example of this *rehabilitation* process will be presented a little later in Table IV.

II. INTERPRETATION OF OBSERVED FACTS

At this juncture it was unavoidable to look for the physiologic factors which account for the diabetogenic effect of hypoglycemia. The answer came promptly, it was ready for the asking in the early literature dealing with insulin action. Walter Cannon, and Cori, among several other investigators in this country and and Europe, had shown as early as 1923 that insulin hypoglycemia induces an increased secretory activity of the adrenal medulla. Cannon and his associates demonstrated that the process is stimulated by the sympathetic nervous system, which responds sensitively to hypoglycemia. The adrenalin supply can be thus increased to such an extent that the hypoglycemic phase is followed by variable degrees of hyperglycemia. Other workers found that hormones of the anterior pituitary react to hypoglycemia in a similar manner, and Oscar Riddle, C. N. H. Long, and other investigators showed that the adrenal cortex likewise is stimulated by hypoglycemia. Recent studies of Foa and his associates indicate that the alpha cells of the pancreatic islets, which produce the blood sugar-raising hormone glucagon, also increase their activity under the impact of hypoglycemia.

It is apparent that hypoglycemia, a gravely stressful condition, sounds an alarm and mobilizes a number of compensatory factors that act in an opposite direction to insulin, and thereby inhibit the progression of hypoglycemia to a lethal degree. The quantitative relationship between the two factors can shift to a point where the action of the insulin antagonists outstrips insulin action and results in hyperglycemia by over-compensation. This is the explanation of the diabetogenic effect of insulin-hypoglycemia.

III. EXPERIMENTAL

The facts derived by other workers from experiments on laboratory animals and our own clinical observations, described in Section I, seemed to furnish a fairly secure basis for the conclusion that insulin-hypoglycemia is a diabetogenic factor. But since experimental evidence obtained on laboratory animals is not always transferable to human physiology and, furthermore, because purely empirical clinical observations did not fully satisfy us as a firm foundation for our thesis, we turned to experimental probing of its validity. We devised five different experimental approaches, all applicable to human beings as the subjects. Every one of these experiments verified our thesis and justified the new approach we recommend for the insulin therapy of diabetes. At this occasion we will have space to present only examples of two varieties of these studies.

The first experiment was performed in 1938. It involved changing a mild state of diabetes into a more severe one by adding a little excess of insulin to the dose the patient actually needed. The subject was the same patient who in 1930 furnished us with the chart given as Fig. 1, and in 1935, with the data recorded in Table I of this article. Up to 1935 he had been an invalid for several years owing to his unmanageable diabetes, but treatment as indicated in Section I of this paper, transformed him by 1938 into a mild diabetic, fully able to pursue his original trade as a tuckpointer.

As may be seen in Table IV, (a severely condensed abstract from the records of our studies on this man), by 1938 he used only 14 units of insulin per day and his glycosuria ranged between 5 and 25 gm. per 24 hours. It was in this condition that he was hospitalized for the experiments. On the same insulin and dietary regimes he had in his home life, he was under close observation before the insulin dose was increased. As may be seen, during this control week his glycosuria varied between 3 and 18 gm. per 24 hours, with 14 units of insulin. Then his insulin dose was raised by 10 units, to 24 units per day. Part of the 10 units was added to the dose given before the evening meal, part of it was injected at 11 p.m. This arrangement was designed to produce hypoglycemic intervals about midnight or shortly after, the time when he used to suffer shocks in his treatment prior to 1935.

The results of this procedure are presented as part "'4th period") of Table IV. As may be noted, in wake of the increase of the insulin dose the daily glycosuria promptly increased and went up to 63 gm. inside ten days. The minimum was 19 gm., the 10-day average 41 gm., as against 3 gm. and 18 gm. during the week of observation preceding the experiment. Simultaneously, ketonuria made

TABLE IV.

Condensed Picture of Studies on the Rehabilitation of an Unmanageable Diabetic (Mr. M.K.)

Date of observation	Glycosuria excreted per 24 hrs.		Insulin dose		Clinical condition of patient
	Range of variations	Average	Total 24 hrs.	Distribution*	
	gm.	gm.	units	units	**1st period**
Nov. 1928 to Dec. 1935		4 plus (qualit.)	80-180	(3 injections)	"Unmanageable state of diabetes; patient an invalid.
April 5, 1930		48	120	50-25-45	
					2nd period
Dec. 23 to 31, 1935	22-105	60	60	25-10-25	Beginning of rehabilitation.
					3rd period
Aug. 1 to 7, 1936	2-27	19	53	20-5-25-3	Rehabilitated to mild state of diabetes; patient resumed his normal work and way of life.
Sept. 1 to 8, 1937	0.5-30	25	20	8-0-12	
Aug. 1 to 7, 1938 (1 week before studies in hospital)	5-24	14	14	8-0-6	
Aug. 8 to 14, 1938 (Observations in hospital)	3-18	13	14	8-0-6	
					4th period
Aug. 15 - 24, 1938 (In hospital)	19-63	41	24	8-0-10-6	Experimental exacerbation of diabetes by insulin-hypoglycemia.
					5th period
Nov. 14 to 20 1938 (In hospital)	16-26	20	16	8-0-8	Restoration to state of 3rd period, with gradual further improvement.
Jan. 1 to 7, 1939	0-9.6	5	16	8-0-8	
Jan. 8 to 14, 1939	0-8	3.4	16	8-0-8	
Feb. 14 to 20, 1939	0-8	4.7	14	8-0-6	

* The first three figures in this column represent the amounts of insulin injected (subcutaneously) before each of the three meals; the fourth figure, in the year 1936, was injected at 3 A.M.; in the experimental period in 1938, it was injected at 11 P.M.

its appearance at increasingly frequent and prolonged intervals. I can confess that I was somewhat trepidated by the success of the experiment. A "mere" 10 units of extra insulin precipitated exacerbation of diabetes faster and more violently than anticipated and I hastened to terminate the experiment.

This was, of course, followed by a second course of rehabilitation. This was fully successful, so that a few months later the patient could revert to his previous 14 units of insulin. He began to have completely aglycosuric days and the maximal excretion of sugar did not exceed 8 gm. per day (see "5th period" in Table IV).

Clinical observations have indicated that it does not take marked hypoglycemic reactions to exert diabetogenic effects, but that *mild degrees of hypoglycemia, which are manifested but in very slight subjective symptoms, or even cause no noticeable symptoms at all, suffice to precipitate abnormal alimentary hyperglycemia and glycosuria.* We reproduced this fact not only on diabetic but also in healthy persons. The experimental procedure was very simple, it consisted essentially of simple glucose tolerance tests.

From Cannon's work and our own studies we have known that the blood sugar must fall at least to 60 mg. per cent in order to elicit a measurable increase in adrenalin secretion. We have found that it requires about 4 units of insulin, injected intravenously to lower the fasting blood sugar of a healthy man to this critical level. Pilot experiments have shown, furthermore, that the lowest blood sugar level, produced by 4 units, occurs about 30 minutes after the injection; and then an upward swing takes place, indicating the ascendency of the blood sugar-raising factors over insulin action.

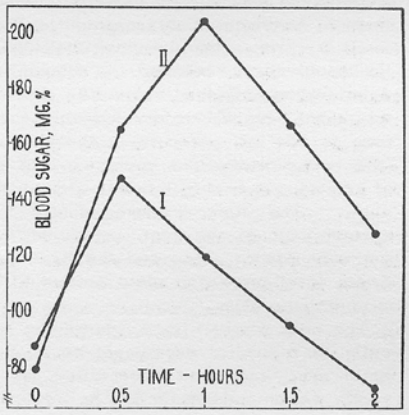

Figure II

Glucose tolerance curves showing a paradoxical action of insulin in a healthy man. Curve I was obtained after the oral administration of 100 gm. of glucose. Curve II is the result of a second test, in which 4 units of insulin were injected intravenously 45 minutes before glucose feeding. The intervening hypoglycemic interval caused deterioration of glucose tolerance.

(From The Journal of Biological Chemistry Vol. 193, No. 2, December 1951)

On the basis of these three pieces of information, glucose tolerance tests were performed in which the subjects ingested 100 gm. of glucose from 40 to 50 minutes after the intravenous injection of 4 units of insulin. Blood sugars were determined

DIABETOGENIC EFFECTS OF INSULIN – HYPOGLYCEMIA

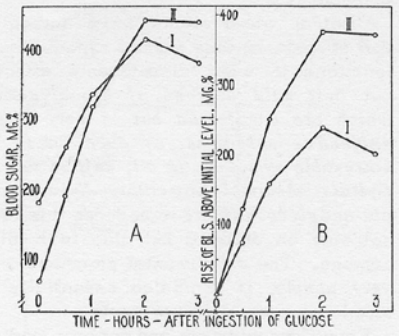

Figure III

Glucose tolerance tests in a diabetic man showing the diabetogenic effect of insulin hypoglycemia. In A, Curve I is the result of a test after the oral administration of 100 gm. of glucose; Curve II represents a test in which 7 units of insulin were injected 75 minutes before glucose feeding. B represents the same experiment in different terms (see the text).

(From The Journal of Biological Chemistry, Vol. 193, No. 2, December, 1951)

at the customary intervals after glucose feeding. As a counterpart, each subject underwent another tolerance test without injection of insulin. An example of such tests in healthy men is presented in Fig. 2. It is obvious from the graph that insullin-hypoglycemia entailed a substantial deterioration of the subject's glucose tolerance: his alimentary hyperglycemia rose to 204 mg. per cent in the venous (229 in the arterial) as against a peak of of 146 mg. per cent in the test without insulin. The urine, excreted during the second hour of the test, contained 0.5 per cent sugar. This and the hyperglycemic level present a clear picture of a mild diabetic state.

The next graph, Fig. 3, shows the results of a similar experiment on a diabetic man, under our observation for 5 years. On an appropriate diet he was consistently free of glycosuria for eleven consecutive months directly preceding this experiment. Owing to his elevated fasting blood sugar level, he required more insulin than healthy men and a longer time interval had to be allowed between insulin injection and glucose feeding. On the basis of pilot tests, he received 7 units of insulin and took his 100 gm. of glucose 75 minutes later. In 45 minutes after the injection the blood sugar came down to 35 mg. per cent, and the subject showed hypoglycemic symptoms: profuse sweating and tachycardia. When he took the glucose, 30 minutes later, his blood sugar was 67 mg. per cent.

In Fig. 3,A (left half of the chart), Curve 1 represents the conventional tolerance test, while Curve 2 shows the effect of insulin-hypoglycemia that preceded glucose feeding. It may be noted that after insulin-hypoglycemia the alimentary hyperglycemia leaped to a distinctly higher peak than in the test without insulin injection, regardless of the much lower initial blood sugar level. This difference can be better visualized in Fig. 3,B (right half of the chart), where the rise of the blood sugar above the initial level is presented instead of the actual blood sugar levels.

It is evident (and could be anticipated) that the diabetogenic effect of insulin-hypoglycemia was far more sever in the diabetic than in the healthy subject. In healthy men the disturbance was rapidly repaired, their glucose tolerance became normal by the next day. Quite different was the response of this diabetic subject: a single experimental hyperglycemia, and only of brief duration, caused such an upheaval in his regulatory system that he excreted 39 gm. of glucose from noon (the end of the test) till the next morning, on the same diet on which he had been sugar-free during the preceding eleven months. As glycosuria persisted on the following days, the patient received temporary treatment with 5 units of insulin per day one week, and - as the glycosuria decreased - 3 units for a second week. Five weeks passed before he had become sugar-free once more without insulin injections. It is noteworthy that marked ketonuria was an additional result of insulin hypoglycemia; it lasted two days following the experiment.

SUMMARY

The few examples of experimental studies I have presented here prove unequivocally, I believe, - the validity of a thesis which we originally based on clinical observations. They prove that insulin, when it is allowed to cause hypoglycemia, can greatly increase hyperglycemia and glycosuria, and even can cause ketosis. This holds true not only for severe "insulin reactions", but also for moderate degrees of hypoglycemias which manifest themselves only in mild subjective symptoms, or even may pass unnoticed.

These are physiologic laws which relentlessly assert themselves when insulin is used for the treatment of diabetes. Until the time when it will get due consideration, insulin will remain a double-edged tool which can do as much harm as good and, in some instances, the harm can out-do the good.

ABSTRACTS OF PAPERS PRESENTED AT SCIENTIFIC SESSION, 5TH ANNUAL MEETING

Scientific session was held in conjunction with the Division of Biological Chemistry, American Chemical Society

SYMPOSIUM ON ELECTROMIGRATION IN STABILIZED ELECTROLYTES

SEPARATION BY ELECTROMIGRATION IN AGAR GELS. Quintin P. Peniston, Food, Chemical and Research Laboratories, Inc., Seattle, Wash.

Various techniques have been reported for the separation of substances carrying an electric charge using systems of supporting electrolytes stabilized by means of agar gels. A number of these techniques are described with reference to their treatment of factors of importance in obtaining precision data. These factors include measurement and control of endosmosis, supporting electrolyte type and concentration, removal of electrolysis products, control of thermal gradients, and diffusion and procedures for assay and characterization of separated substances.

Some results obtained with an apparatus and technique developed in the Department of Chemistry and Chemical Engineering at the University of Washington [J. Am. Chem. Soc., *23*, 994 (1951)] show possibilities of a technique with an ultraviolet absorption method for assay of separated products. This procedure has been applied to separation of organic materials, such as low molecular weight lignin sulfonates, mixtures of phenolic substances obtained by oxidative degradation of lignin, antibiotics, mixtures of organic acids, and a study of the kinetics of the primary reactions involved in a phenol-formaldehyde condensation process. Other possibilities for agar gel electromigration analysis are indicated.

SOME CLINICAL CHEMICAL APPLICATIONS OF ELECTROMIGRATION IN PAPER. John G. Reinhold and C.A.J. von Frijtag Drabbe, Pepper Laboratory of Clinical Medicine and Harrison Department of Surgical Research, Hospital of the University of Pennsylvania, Philadelphia, Pa.

Electrophoretic separations of serum proteins of healthy individuals and of patients suffering from various diseases were made on paper at pH 8.6, using the apparatus of Grassman and Hannig. The proteins were stained with amidoblack 10 B and evaluated quantitatively on the paper strip by means of a densitometer. Findings for healthy males and females differed from those obtained by the moving boundary method in that beta-globulin was consistently lower and gamma-globulin higher. Therefore, interpretation of patterns on paper required comparison with control values obtained by the same method.

Pathological sera showed similar patterns by both methods; however, discrepancies in individual fractions were observed. Extra globulin components appeared more frequently on paper than in moving boundary patterns. Consistent results by the paper technique were obtained only if the conditions were carefully controlled. Some evidence was obtained that the results were influenced by the technique used. An example of one of the useful applications of the paper technique, the study of the effect of surgical operations on serum proteins, is described.

THE SEPARATION OF SERUM PROTEINS BY ZONE ELECTROPHORESIS. E.L. Durrum, Department of Cardiorespiratory Diseases, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington 12, D.C.

Various types of zone electrophoresis which have been used in the separation of serum proteins are considered with special reference to practical application of the techniques. Practical as well as theoretical considerations are discussed.

PAPER ELECTROPHORESIS OF THE PITUITARY HORMONES. W.F. White and *J.W. Giffee*, Jr., Fundamental Research Department, The Armour Laboratories, Chicago, Ill.

Paper electrophoretic data are shown for ACTH, TSH, FSH, STH preparations of high physiological activity. For the highly purified unhydrolyzed porcine ACTH, called Corticotropin-A [J. Am. Chem. Soc., *75*, 503 (1953)], a value of approximately 8.5 is given for the isoelectric point. Pepsin-hydrolyzed ACTH, and pepsin- and acid-hydrolyzed ACTH, show isoelectric points of 9.5 and 10.25, respectively. Attempts to eliminate the adsorption of basic proteins and peptides by the paper are discussed. A preliminary report is made on the use of paper electrophoresis in the separation and characterization of the peptides produced by the enzymatic degradation of Corticotropin-A.

ZONE ELECTROPHORESIS IN A STARCH SUPPORTING MEDIUM. Henry G. Kunkel, The Rockefeller Institute Hospital, New York 21, N.Y.

A survey of a large number of supporting media has indicated that, for the separation of serum proteins by procedures of zone electrophoresis for preparative purposes, potato starch possesses a number of advantages. No adsorption of these proteins could be detected and they shifted with the liquid volume without trailing. The starch medium was relatively easy to handle and homogeneous packing could readily be achieved either in the form of a block or in a column. By the starch block procedure as much as 5 ml. of human serum could be separated into the five main components observed with the classical Tiselius procedure and the fractions isolated. Nitrogen analyses have indicated up to 97% recovery by displacement filtration of the starch segments in short ground-glass filter columns. Certain peptides, particularly the basic type, showed reversible adsorption to the starch in aqueous buffers, and other supporting media such as glass beads and purified cellulose proved more suitable for preparation experiments in the block form. A column procedure with collection of fractions in a mechanical fraction collector after electrophoretic separation proved useful for a few specific purposes, but theoretical as well as experimental disadvantages over the block procedure were apparent.

STUDIES OF PHOSPHORUS COMPOUNDS AND OF BONE BY NEUTRON ACTIVATION PLUS ELECTRICAL MIGRATION. T.R. Sato, W.P. Norris, and H.H. Strain, Divisions of Biological and Medical Research, and Chemistry, Argonne National Laboratory, Lemont, Ill.

We have utilized neutron activation and electrical migration in moist paper to investigate the phosphorus compounds in bone. Separate samples of dried powdered rabbit bone, of bone treated with ethylenediamine, and of incinerated rabbit bone, as well as small specimens of NaH_2PO_4, $NaH_2PO_4 \cdot H_2O$, $NaH_2PO_2 \cdot H_2O$, $Na_4P_2O_7$, H_3PO_3, red P, $(NaPO_3)_3$, $(NaPO_3)_4$, $Na_5P_3O_{10}$ II, $Na_2H_2P_2O_6 \cdot 6H_2O$, $Na_2H_2P_2O_5$, and apatite were exposed to thermal neutrons for 4 and 6 weeks. These irradiated phosphorus materials were dissolved and submitted to electromigration in moist paper. An autograph prepared from this paper revealed a series of radioactive zones. As a control, a portion of each nonirradiated phosphorus compound was dissolved and also submitted to electrical migration. The paper was then dried, wrapped with aluminum foil, and irradiated with neutrons. A radioautograph of the irradiated paper revealed all the activity of each preparation in a single zone; hence each nonirradiated specimen contained a single chemical species. This principal zone, obtained by migration before irradiation, was usually the same as that of the major zone obtained by migration after irradiation of the preparation. These results indicate that the minor radioactive constituents were formed by the neutron disruption of the phosphorus-containing groups and, therefore, were not normal constituents of the substances submitted to the neutron irradiation.

The ionic species formed by the neutron irradiation of the phosphorus compounds with authentic, nonirradiated phosphorus compounds by simultaneous, electrical migration in the same sheet of paper. One of the irradiation products is hypophosphorous acid (hypophosphites). This identification supports chemical investigations of the nature of the neutron phosphate reaction recently carried out in several other laboratories.

Various phosphorus compounds have been examined in paper moistened with different acidic and basic solutions. The sequences and the separability of the zones varied with the electrolytic solution and with the treatment of the paper. The separation, the identification, and the isolation of some species were accomplished much more easily under some conditions than under others. An interesting example is the strong absorption of orthophosphate ions by untreated paper in the presence of ammonia and the weak absorption of metaphosphate, of hypophosphorous acid, and of phosphorous acid. This selective sorption of the phosphate does not occur in paper previously washed with acid and ammonia.

RE-EVALUATION OF IONTOPHORESIS IN THE FIELD OF MEDICINE. Y.T. Oester and Edward P. O'Malley, Loyola University Graduate School and Stritch School of Medicine, Department of Pharmacology, Chicago 12, Ill.

Many reports have appeared in the literature concerning the value of iontophoresis in treatment of various clinical disorders. It is our purpose to evaluate some of these uses on a rational basis in light of our findings on the effects produced by iontophoresis in experimental animals.

Our studies made use of male and female albino rats of the Sprague-Dawley strain. The iontophoresis apparatus consisted of a constant current generator as the current source. The current strength of 5 milliamperes was applied for 1 hour in all cases.

The following observations were noted in our experiments, when the appropriate polarity was used:

1. Methylene blue dye was present in the urine of the rat after appropriate iontophoresis with this dye.
2. Characteristic general pharmacodynamic responses were observed after iontophoresis with all four of the organic drugs which were used—strychnine, nicotine, picrotoxin, and d-tubocurarine.
3. The presence of P^{32}, I^{131}, Na^{24}, Ca^{45}, and labeled diiodofluorescein was demonstrated in various tissues of the rat following iontophoresis.

We believe that the combination of introduction into the tissues by electrical current followed by dissemination by way of the circulation will explain the results obtained.

DETERMINATION OF ELECTROMIGRATION MOBILITY IN PAPER-STABILIZED ELECTROLYTES. Edward P. Marbach, Hugh J. McDonald, and Robert H. Spitzer, Department of Biochemistry, Graduate School and Stritch School of Medicine, Loyola University, Chicago, Ill.

The various factors which influence the electromigration mobility in paper-stabilized electrolytes are considered. These include the effect, on the mobility of a migrant, of such factors as time, potential

ELECTROMIGRATION

gradient, pH and ionic strength of the buffer used to saturate the paper, temperature, method of supporting and wetting the paper, technique of applying the migrant including the point of application, the drying of the ionogram, possible adsorption of the migrant on the paper, and the role played by the molecular volume of the migrant. Other factors include the necessity for the separation of electrode products from the paper strip and capillary siphoning of liquid through the paper. The various ways suggested for controlling the equilibrium conditions surrounding the paper include such techniques as supporting the paper in a water-saturated helium atmosphere, the use of glass plates, etc. Also reviewed is the problem of electro-osmosis, including attempts to reduce it and to measure it. The choice of a suitable electro-osmotic indicator for a particular migrant is considered. It is shown that the distance traversed by a given indicator is related to its molecular volume and to the character of the particular paper used.

Various methods of converting mobility values obtained in paper-stabilized electrolytes to those obtained in nonstabilized electrolytes are considered. The determination of the decrease of thermodynamic activity of the migrant as a function of the weight ratio of "paper to buffer" is shown to yield a conversion factor which is theoretically sound.

SEPARATION OF INORGANIC IONS BY ELECTROCHROMATOGRAPHY. Harold H. Strain and T.R. Sato, Argonne National Laboratory, Lemont, Ill.

Differential electrical migration in moist, porous media is a convenient, economical, and widely applicable technique for the resolution of mixtures of inorganic substances. This technique facilitates the separation of charged from uncharged particles, the complete or absolute separation of positively charged particles from negatively charged particles, and the resolution of mixtures of various cations and of various anions. Mixtures separated are alkali and alkaline earth elements, the copper and tin groups, radium and its radioactive decomposition products, monovalent cations from all polyvalent cations, and alkali metal cations from the ions of virtually all other metals, some rare earths, and many heavy metals. The migration apparatus is usually a moist column or moist paper as strips, sheets, or pads with the ends attached to the electrodes or placed in the electrolytic solution bathing the electrodes.

Migrations may be unidirectional only or unidirectional followed by transverse migration in another solution. Flow of the solution transverse to the electrical migration provides batch separations and continuous separations. The separation and the sequences of the zones depend upon the concentration and composition of the mixture, the mobility and sorbability of the ions, and the electrolytic solution. For suitable electrical conductivity, the concentration of the electrolytic solution should be about 1 M for very weak electrolytes (ammonia), about 0.1 M for weak electrolytes (lactic acid), and about 0.01 M for strong electrolytes (nitric acid). For efficient separations and for the formation of uniform zones, the initial zone of the mixture should be small, and its concentration less than that of the electrolytic solution, conditions that limit the detection of the minor constituents.

ELECTRO-OSMOSIS IN ELECTROCHROMATOGRAPHY IN PAPER. Scott E. Wood and John L. Engelke, Argonne National Laboratory, Lemont, Ill.

The osmotic flow of the supporting electrolytic solution in paper as observed in electrochromatography has been studied experimentally and is discussed from a phenomenological viewpoint. This flow has been determined by measuring the movements of nonabsorbed, uncharged "indicator" substances added as spots on the paper. The supporting electrolyte was lactic acid.

Two types of apparatus were used, one in which both the electrodes and the ends of the paper dipped into reservoirs and the other in which the electrodes were fixed on the paper. In the first case the movement of the spots is of the order of 0.5 cm. per hour for an average potential gradient of 5 volts per cm. The movement increases with a decrease in the concentration of the lactic acid and appears to increase slightly with the pressure on the paper. This movement is opposed only by a hydrostatic pressure due to a difference in the levels of the solutions in two reservoirs. The movement in the second case is small. A steady state is obtained in time, after which no osmotic flow occurs. The extent of the movement increases with the wetness of the paper and with a decrease in the concentration of the lactic acid solution. The osmotic flow in this case is opposed by forces created by an unequal distribution of solution in the paper.

MOLECULAR WEIGHT DETERMINATIONS FROM ELECTROACCELERATION IN PAPER-STABILIZED ELECTROLYTES. Matthew C. Urbin and Hugh J. McDonald, Department of Biochemistry, Graduate School and Stritch School of Medicine, Loyola University, Chicago, Ill.

From a consideration of Newton's second law of motion — namely, $F = ma$ — it follows that for a given actuating force, F, the mass, m, of a moving particle could be computed if the acceleration, a, of the particle is experimentally determined. It was realized very early that a particle undergoing electromigration in a uniform electrical field would quickly reach a terminal velocity and that under these circumstances the mass could not be determined without introducing a second force, such as a magnetic field. However, if a particle were moving in a constantly decreasing or increasing electric field, conditions would be such that Newton's second law of motion could be utilized.

A technique was developed in which simultaneous crossed currents were used. Two glass plates (20 x 20 cm.) are fitted about the edges with a rubber gasket, and brought together so as to form an enclosure. Within the enclosure is suspended a sheet of filter paper (E and D, No. 613, 20 x 20 cm.) which has been immersed in a suitable buffer and allowed to drain on an absorbent paper. On each of the four sides of the glass there is placed a buffer vessel which is connected to the filter paper sheet by means of filter paper tabs (2.5 x 7.5 cm.) which overlap the square sheets by about 1.5 cm. The four vessels are then connected to electrode vessels by means of agar salt bridges. An equal potential is imposed in both directions at 90° to each other, so that migrants move at a 45° angle in a constantly decreasing electric field.

A series of 12 amino acids and 5 dinitrophenyl derivatives was studied by applying a micro quantity of a buffered solution of the migrant by means of a micropipet at the center of the square of paper. It was noticed that for the materials investigated the deceleration was constant, as might have been expected from the nature of the electric field. Therefore, it would be expected that when the reciprocal of the movement of the amino acids or their derivatives were plotted against their respective molecular weights a linear relationship would be obtained. The experimental data was found to conform to this hypothesis, when the crossed currents were used, but not when a unidirectional electric field was employed.

CLINICAL CHEMISTRY

THE BRONSTED THEORY APPLIED TO ACID-BASE BALANCE AND RESPIRATION. Arthur W. Devor, Department of Physiological Chemistry, The Ohio State University Medical College, Columbus, Ohio.

The Bronsted theory indicates a direct correlation between pH and acid-base balance. It shows an equilibrium existing between the acids, bases, and hydrogen ions. For example:

$$\text{Acid} \rightleftarrows \text{base} + \text{proton } (H^+ \text{ion})$$

$$H \cdot HCO_3^- \rightleftarrows HCO_3^- + H^+$$

The "alkaline tide" is caused by an increase in HCO_3^- base concentration. Diarrhea sometimes results in an acidosis

because of loss of HCO_3^-. Organic acids which enter the Krebs cycles do not normally give an acid reaction in the animal body, while sodium salts of these acids give an alkaline reaction because the anion of the salt accepts a hydrogen ion from $HHCO_3$ and enters the Krebs cycle cycle resulting in an increase in HCO_3^- concentration.

The idea of the proteins acting as buffers fits into the picture because they are proton (H^+) donors and acceptors thus preventing much change in pH. The strongly basic nitrogen of amino acids is readily converted into the nearly neutral amide, urea. The normal kidney can produce ammonia, which accepts hydrogen ions from acids that would otherwise use up the HCO_3^-.

During respiration the oxygen in the lungs enters the red blood cells and combines with the hemoglobin. The oxyhemoglobin produced, being a stronger acid, furnishes hydrogen ions to HCO_3^-, yielding $HHCO_3$, which in turn releases carbon dioxide. The HCO_3^- shifts into the cells and the Cl^- shifts into the plasma. As the blood passes through the tissues, the reverse process takes place because hemoglobin is a stronger base than oxyhemoglobin and the $H{\cdot}HCO_3$ formed, furnishes hydrogen ions for the stronger base.

In every case where we find a basic anion, there is a cation which is commonly called the base.

The Brönsted concept is an advancement in chemistry which offers a clear and concise explanation of acid-base balance and respiration.

MATHEMATICAL THEORY OF PERIODIC RELAPSING CATATONIA. Lewis Danziger, Milwaukee Sanitarium, Wauwatosa, Wis., and George L. Elmergreen, University of Wisconsin, Milwaukee, Wis.

Periodic relapsing catatonia is a type of mental disorder marked by fairly regular variations in the patient's condition, and by variations in the thyroid function. These cyclic changes persist, usually until death, unless the patient is given appropriate amounts of thyroid extract or thyroxin; and they return if the daily intake of thyroid falls below a level which is critical for the patient.

The following equations are presented to describe the interaction of the thyroid and pituitary glands, and to explain some of the phenomena of the disorder:

$$\frac{d\theta}{d\tau} = \frac{K_1 m \pi}{1 + m\pi} - b\theta \qquad (1)$$

$$\frac{d\pi}{d\tau} = c - \frac{K_2 n \theta}{1 + n\theta} - g\pi \qquad (2)$$

$$\pi \geq 0; \quad \pi \geq K \geq 0$$

where θ is the total level of thyroid hormone in the system at any time, t

K is the constant level of thyroid in the system maintained by daily addition by a physician

π is the level of thyrotropin in the system at any time, t

c is the rate of production of thyrotropin in the absence of thyroid inhibition

b and g are loss constants

k_1 and k_2 are constants of proportionality

m and n are the constants of the Langmuir adsorption isotherm equations.

Topological study of the equations by the method of isoclines in the $\theta - \pi$ plane, shows a mechanism in which either stability or periodic fluctuations may occur, depending on the amount of exogenous thyroid present. The theory is consistent with what is now known of the course and proper treatment of periodic relapsing catatonia.

INFLUENCE OF PARENTERAL INJECTIONS OF HIGH MOLECULAR POLYVINYLPYRROLIDONE ON SURVIVAL OF RATS RECEIVING A LETHAL DOSE OF X-RAYS. G. Podio and B. Rovatti, Department of Clinical Pathology, University of Pavia, Pavia, Italy.

Experiments previously reported showed that parenteral injections of several substances able to block the reticuloendothelial tissue, such as acacia gum, metallic ions, and some aniline dyes, increase the sensitiveness to x-ray treatment. In three groups of 25 young rats of about 150-gram body weight, was injected subcutaneously 3.5 grams per kg. of a 3.5% isotonic and isosmotic solution of high molecular polyvinylpyrrolidone (molecular weight over 50,000). A fourth group of 25 rats as control was only irradiated with 600 r (180 Kv., 15 mA., distance 90 cm., 50r per minute), which gave 100% mortality in a month. The first group was irradiated 24 hours after injection of PVP, the second group was irradiated immediately before injection of PVP, and the third group was injected 24 hours after the same x-ray treatment. This single whole-body irradiation reduced the life span of all the animals of each group treated with high molecular PVP. In the following experiments low molecular polyvinylpyrrolidone solutions, intravenously injected and more rapidly excreted through the kidney, prevented dehydration and prolonged the life span of the irradiated rats. The influence of high molecular PVP on survival of the animals receiving a lethal dose of x-rays can be interpreted as depressing the reticuloendothelial tissue which is also involved in determining the survival of the animals after radiation injury.

CHEMICAL ANALYSES OF ONCOLYTIC AND IMMUNIZING EXTRACTS OF RAT SARCOMAS. Florence B. Seibert, Eva Soto Figueroa, Elizabeth E. Miller, Mabel V. Seibert, and Margaret Reed Lewis, The Henry Phipps Institute, University of Pennsylvania, and The Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

Alcoholic extracts of rat sarcomas which were capable of causing oncolysis of sarcomas in inbred rats and of conferring immunity to further challenge by tumor transplants were analyzed and compared with extracts made in a similar manner from normal skeletal tissue from the rats bearing the tumors, from normal rats, and from rats from which tumors had been removed and in which immunity to further challenge had been demonstrated. Two different strains of rats and several of each of the four kinds of extracts were studied.

Analyses based on the solid content of the extracts showed 10 to 20% ash in the case of the tumor, and 20 to 36% in the case of the other three extracts. There was considerably less inorganic phosphorus but about the same amount of organic phosphorus in the tumor extracts as in the other extracts. There was also 3 to 5 times more desoxyribonucleic acid, 1 to 2 times less ribonucleic acid, 3 to 5 times less carbohydrate, calculated as glucose, and about half as much lactic acid in the tumor extracts as in all other extracts. The ribonucleic acid always was in excess of the desoxyribonucleic acid by about 10 to 100 times. Six to 10% of the total solids was nitrogen in all cases. A small amount of fatty acid but no cholesterol was found. The analyses of all the tumor extracts showed close agreement and the differences from the three sets of normal tissues, which also agreed closely among themselves, appeared significant.

CHEMISTRY OF EXPERIMENTAL CHLOROMA. Julius Schultz, Harry Shay, Anne Turtle, and Margot Gruenstein, Samuel S. Fels Research Institute, Temple University School of Medicine, Philadelphia 40, Pa.

The presence of verdoperoxidase and a dicarboxylic porphyrin (probably protoporphyrin) has been indicated as contributing to the characteristic green color and the red fluorescence of chloroma tissue (Abstracts, A.C.S. Meeting, September 1952, p. 46C). As a means towards investigating the possible metabolic relationship of these products, fresh tissue was homogenized in a Potter-Elvehm tube, centrifugal at low speed to remove tissue debris and then at 24000 g for 1 hour. Three layers and a green pellet formed at this speed. Each layer was examined for verdoperoxidase activity for proteins by filter paper electrophoresis and for fluorescence compounds. The pellet fluoresced red, while the ratio of enzyme activity in terms of verdoperoxidase was 1:1.5:4.5 from the top to bottom layers. Electrophoresis separated fast-moving peroxidase-active protein from the

major portions of the proteins present in each layer. The pellet, on electrophoresis, also showed a fast-moving red fluorescence zone of much greater mobility of the major portion of the proteins present. The above findings are discussed in terms of localization of these compounds in cells of chloroma tissue.

RAPID QUANTITATIVE FLAME SPECTROPHOTOMETRIC METHOD FOR URINE CALCIUM WITH A STUDY OF NORMAL AND PATHOLOGIC VALUES. Alfred D. Winer and Kenneth F. Ernst, Laboratory Service, Walter Reed Army Medical Center, Washington 12, D. C.

The application of flame spectrophotometry for the quantitative determination of calcium in biological fluids in the clinical laboratory is becoming widespread. Serum calcium has been previously described and can now be easily determined on 0.1 ml. of untreated serum in a matter of minutes. This paper presents a similar rapid method for urine calcium without prior removal of proteins or phosphates. The radiation intensity of the atomic spectral line of calcium at 422.7 mu is measured on diluted urine using an organic solvent and atomized directly into the oxygen-acetylene flame.

An intensive study of normal 24-hour urine calcium has been made and reveals a narrower range than previously described. Pathologic conditions in which the serum calcium may be normal but urine calcium markedly changed such as renal acidosis, Cushing's syndrome, metastatic carcinoma to bone, etc., have been studied for presentation.

COLORIMETRIC DETERMINATION OF CALCIUM WITH ALIZARIN AND ALIZARIN SULFONIC ACID. Samuel Natelson, Ralph Penniall, and Jean Secard, Rockford Memorial Hospital, Rockford, Ill.

The complexes calcium forms with alizarin (A) and alizarin sulfonic acid (AS) at alkaline pH were studied as a basis for calcium estimation. With alizarin in the presence of strong alkali the maximum color density of the complex is at 560 mμ, while with alizarin sulfonic acid the maximum is at 590 mμ. With alizarin sulfonic acid a reproducible curve may be obtained for calcium in an ammonium chloride-ammonium hydroxide buffer (pH 10.1 to 10.6) for from 2 to 15 micrograms. The density readings are affected by concentration of dye and pH. Magnesium, which produces a complex with similar absorption spectrum, interferes.

With alizarin advantage is taken of the fact that with alkali the calcium complex may be readily extracted with amyl or butyl alcohol, leaving the magnesium complex behind. With this procedure a straight line going through the origin is obtained from 1 to 40 micrograms. The $E^{1\%}_{1cm} = 1900$ permits the estimation of as little as 1μg. with ordinary colorimeters and the estimation of 0.1 microgram with microtechniques. To an aqueous solution containing the calcium is added alizarin dissolved in amyl alcohol. Potassium hydroxide is added to make the aqueous phase 0.3N. Mild shaking brings the calcium complex into the alcohol phase and excess alizarin into the aqueous phase. Centrifugation clarifies the alcohol layer for reading. Stronger alkali tends to emulsify the aqueous phase in the alcohol phase and is to be avoided.

MICROMETHOD FOR THE DETERMINATION OF CREATINE IN URINE BY THE JAFFE REACTION. Hertha H. Taussky, with the technical assistance of Gloria Kurzmann, Russell Sage Institute of Pathology, Department of Medicine, Cornell University Medical College and The New York Hospital, New York, N. Y.

Clark and Thompson showed the importance of pH in the quantitative conversion of creatine to creatinine and that conversion is complete at pH 2 to 2.5. Advantage was taken of this observation in the development of a simplified micromethod for the determination of creatine and creatinine in mixtures, such as urine. The method is based on the conversion of creatine to creatinine in a boiling water bath without the addition of mineral acid. The sensitivity of the method is from 10 to 120 micrograms. In brief, equal amounts of diluted urine are pipetted directly into colorimeter tubes for the determination of preformed creatinine, and into heavy walled centrifuge tubes for the conversion procedure in the water bath. The conversion of creatine to creatinine is complete in about 1.5 hours. Glucose up to 60 grams per liter does not interfere. A number of substances occurring in urine have been investigated for possible interference. A preliminary treatment of the urine is suggested to improve the specificity of Jaffe's reaction. The final color developed in the colorimeter tube is stable for at least 1 hour. The color is read in a Klett-Summerson photoelectric colorimeter with filter 54. Recovery experiments and comparisons with Benedict's method are in good agreement.

✶ PONTACYL STANDARDS FOR THE BIURET METHOD FOR TOTAL SERUM PROTEIN. Daniel Sanshuk and Monroe E. Freeman, Department of Biochemistry, Walter Reed Army Medical Center, Army Medical Service Graduate School, Washington, D. C.

The general acceptance of the biuret procedure for total serum protein has suggested the need for a standard that is more convenient, reproducible, and permanent than the "normal" pooled sera commonly used. Standards prepared with a mixture of pontacyl carmine 2B and pontacyl violet 6R have proved satisfactory. The prepared solution has an absorption curve similar to that of the biuret reagent with the same absorption peak at 540 millimicrons. Concentration curves prepared with above reagent followed Beer's law and have close agreement with the biuret protein curve when read in different instruments. The pontacyl standards are stable and permanent, and do away with the necessity of determining protein concentration of pooled sera by the laborious Kjeldahl procedure now used.

COMPOSITION OF BONE AND TEETH. RELATION TO BLOOD AND DIET. Albert Hanok and Albert Edward Sobel, Department of Biochemistry, The Jewish Hospital of Brooklyn, Brooklyn, N. Y.

An extended study repeated independently four times in 4 years was made to correlate the composition of diet, blood, tooth, and bone. Cotton rats, 16 to 18 days of age, were placed on a high calcium — low phosphorus and low calcium — high phosphorus diet for 28 days. The carbonate-phosphate ratio of the enamel and dentine of the molars and incisors and that of bone were much higher on the high-calcium diet than on the high-phosphorus diet. These changes in the carbonate to phosphate ratios of teeth and bones were related to changes in the carbonate to phosphate ratios of the blood serum.

Additional animals were placed on a caries-producing diet containing the same concentrations of calcium and phosphorus as in the preliminary diets and in addition containing added supplies of vitamins A and D. The carbonate — phosphate ratios of the bones were still higher in the high-calcium diet, but the differences between the ratios for the two diets were not as large as previously.

In comparing the results to those obtained for albino rats of the Wistar strain, the carbonate content of the bone of our strain of cotton rats is apparently less than that of the white rat. The bearing of these findings on caries susceptibility is discussed.

COMPOSITION OF BONES AND TEETH. RELATION TO CARIES SUSCEPTIBILITY. Albert E. Sobel, Albert Hanok, and James H. Shaw, Department of Biochemistry, Jewish Hospital of Brooklyn, Brooklyn, N. Y., and Harvard School of Dental Medicine, Boston, Mass.

Studies in the cotton rat were undertaken to test the hypothesis that composition of teeth is related to caries susceptibility. The particular hypothesis was that high-carbonate teeth are more caries-susceptible than low-carbonate teeth. Carbonate can be preferentially dissolved from the calcium phosphate-apatite mineral in the tooth and in addition carbonate increases the solubility of calcium phosphate.

High-carbonate teeth were produced by placing 16- to 18-day-old cotton rats on a high calcium (1.2%) — low phosphorus

(0.12%) diet for 28 days and low-carbonate teeth were produced on a low calcium (0.11%) – high phosphorus (0.87%) diet for a similar period. For the next 16 weeks the animals were fed caries-producing diets containing the same concentrations of calcium and phosphorus as before.

The average number of carious lesions for the high-carbonate group (61 rats) was 11 per rat and for the low-carbonate group (41 rats) it was 5.3 per rat. The average severity of carious lesions for the high-carbonate group was 25.2 per rat and 13.1 per rat for the low-carbonate group.

These results are in harmony with the above hypothesis.

HYPOXIA AND VITAMINS. A PRELIMINARY STUDY WITH PANTOTHENIC ACID BY TITLE. L. P. Munan and E. Munan, Department of Physiology, School of Medicine, The George Washington University, Washington, D. C.

The ability of the albino rat to survive hypoxia was measured in saline and in calcium-panthotenate-saline treated adult males of the Osborne-Mendel strain. Each animal was injected intraperitoneally either with 1 ml. of physiological saline or with an equal volume of a 1% solution of calcium pantothenate in saline at intervals of 1, 2, or 4 hours prior to induction of hypoxia. Each test and each control rat of similar body weight were enclosed in a bell jar, into the ambient air of which nitrogen gas was then introduced. Survival time in seconds was measured with a stopwatch, cessation of breathing movements serving as the index. The data as summarized in the table indicate that calcium panthotenate was without significant effect in modifying mortality following hypoxia.

Group	N	Weight, Grams (Mean & Range)	Survival Time, Minutes (mean ± S.D.)
Test	25	250 (175-312)	27.8 ± 15.8
Control	25	250 (180-312)	29.2 ± 15.7

THE ANALYSIS OF BARBITURATES IN BIOLOGICAL MATERIALS AND THE CLINICAL AND TOXICOLOGICAL INTERPRETATION OF BARBITURATE DETERMINATIONS.

Discussion by Dr. Leo R. Goldbaum of the Walter Reed Army Medical Center at the May 14th meeting of the Washington-Baltimore-Richmond section.

Dr. Goldbaum reviewed and discussed the various extraction and purification procedures, chemical and physical tests used in the identification and determination of barbiturates. The ultraviolet spectrophotometric procedure for the differentiation and quantitative determination of barbiturates from biological materials was presented along with the results of distribution studies in animal. The importance of blood level studies in barbiturate intoxication was pointed out.

THE CHEMICAL DETECTION OF BARBITURATES AND PHYSIOLOGICAL ANTAGONISTS TO BARBITURATES

by **Theodore Koppanyi, *Georgetown University Medical School***

The diagnosis of acute barbiturate poisoning is very important for subsequent therapeutic procedures. In severe acute barbiturate poisonings the most effective treatment still consists of the intravenous administration of central analeptics, such as pentylene tetrazol or picrotoxin. These drugs may produce severe poisoning or actually aggravate the patient's condition to the point of fatal outcome, if the cause of the coma is not acute barbiturate poisoning but acute opiate poisoning or coma or unconsciousness due to any cause other than overdosage with aliphatic narcotics. Since, of the aliphatic narcotics, barbiturates are the only ones in general use, the chemical detection of barbiturates is necessary before effective treatment is instituted.

The Koppanyi test, or its various modifications, will give you a simple and rapid answer to the presence or absence of barbiturates in the blood or urine. The simplest, and probably the most sensitive, of the Koppanyi tests is the lithium-micro test, which may be described as follows:

Reagents: *0.2% cobaltous acetate dissolved in absolute methyl alcohol; 0.2% lithium hydroxide dissolved in absolute methyl alcohol.*

Procedure: *Acidulated urine, blood, or gastric contents are shaken for 5 minutes in a separatory funnel with 10 volumes of chloroform, and filtered. Six cc. of the chloroform extract are divided in three equal parts A, B, and C. The cobaltous acetate reagent is added to each of the three test tubes: 0.05 cc. to A, 0.1 cc. to B, and 0.15 cc. to C. The tubes are shaken and the colors noted against a plain white background. The tubes should be observed for one minute before a final reading is made.*

The physiological antagonists to barbiturates do not form a chemically innocuous compound in vivo, nor do they speed up the metabolism or elimination of barbiturates. They act as stimulants on the same nerve centers where barbiturates act as depressors. Very likely enzyme systems are involved in this antagonism which may very well turn out to be competition for the same enzyme centers. The physiological antagonists have two important effects in barbiturate poisoning; 1. If the poisoning is not severe they can actually produce an awakening effect, after which the animals or human patients may not relapse. 2. In more severe cases these drugs do not produce an awakening but upon oft repeated administration they produce a life-saving effect. In the latter case the administration of the drug must be maintained until the return of reflex activity or consciousness.

These physiological antagonists can also be employed in the prevention of barbiturate poisoning. When dogs are given orally full anesthetic doses of combinations of pentylene tetrazol and barbiturates or picrotoxin and barbiturates they do not become anesthetized but may only show somnolence and ataxia. When supraanesthetic or fatal doses of barbiturates are administered in conjunction with pentylene tetrazol or picrotoxin, prolongation of life, and in a number of cases life-saving effects, can be clearly demonstrated. Combinations of pentylene-tetrazol and three different barbiturates are now actually on the market and only time will tell whether the general use of such combinations will result in a significant lowering of the incidents and/or mortality rate of barbiturate poisoning.

Abstract of lecture delivered before Washington-Baltimore-Richmond Section, May 14, 1953.

Colors appearing in the different tubes with different concentrations of barbital

Conc. of Barbital. mg. per cc.	0.05 cc. of each reagent	0.1 cc. of each reagent	0.15 cc. of each reagent
0.02	positive	negative	negative
0.03	positive	positive, fading in 30 seconds	fades immediately
0.04	positive	positive	fades in 30 seconds
0.05	positive	positive	fades in about 2 minutes
0.06	positive	positive	permanent for more than 2 minutes

If a permanent blue (color persisting more than 2 minutes) is secured in Tube C, and the other tubes have also shown blue, the concentration is above the range of the test. The chloroform extract should then be diluted and the test repeated. If no color is secured with the original extract, a convenient amount should be evaporated to dryness in an evaporating dish on a water-bath and the residue dissolved with chloroform. The test is repeated with this concentrated solution.

TURBIDIMETRIC METHODS*

by

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A number of routine procedures performed in the clinical laboratory depend upon the flocculation of proteins by various methods and the determination by various instruments of the degree of turbidity produced. Although simple to perform, such tests are subject to large errors unless the variables are recognized and controlled.

Two general types of instruments are employed for the estimation of turbidity. One is based upon the fact that particles in suspension act to scatter incident light in a random manner which is measured (Nephelometry), and the second is based upon the usual light absorption principles.

In nephelometric instruments the photocell is usually at right angles to the incident light source so that only the scattered light reaches it. Unfortunately, in this type of instrument, the scattered light is not in direct proportion to particle concentration alone; particle size and the wavelength of the incident light affect the results obtained.

When the particle size is less than the wavelength of light, the scattering effect increases as the particle size decreases until a maximum is attained at a size of 0.1 - 0.2 microns. If the particles are larger than 2 microns, the total Tyndall density is related to the total particle surface area. The amount of light scattered by suspended particles increases as the wavelength of the incident light decreases. The relative effects of particle size and wavelength is given in Rayleigh's law of scattering in which the ratio of scattered light to incident light is directly proportional to the cube of the particle diameter and inversely to the fourth power of the wavelength. If the particle concentration is high secondary absorption of light may also occur due to the interference between light waves from the same particles. As a generalization, it may be said that the divergence of actual from theoretical values in nephelometric measurements is so great at increased particle concentrations that its value lies in dilution ranges beyond absorption measurements.

Theoretically, the determination of optical density of a suspension by a photometer is subject to less error than the determination of the dispersed light. If the turbidity is kept low and particle size small and relatively uniform, the extinction of the light due to both absorption and scattering in passing through the medium will be approximated by the Beer-Lambert law. However, if particle size is large compared to wavelength, a divergence from the Beer-Lambert law occurs even at a relatively low turbidity because of interference between light waves scattered from the same particle. The degree of such interference is a function of particle shape and results in an increased transmission since less light is lost by scattering. If the turbidity exceeds a certain limit, an increased absorption of light occurs due to the lengthened path of the scattered light, and the silhouettes of individual particles overlap, giving a total light extinction less than expected. Whether nephelometric or absorption methods of turbidity measurements are used, the important factors to be kept in mind for satisfactory results are: small and uniform particle size, low concentrations and the proper wavelength of incident light. From a practical point, little can be done as regards small and uniform particle size other than rigid standardization of a procedure. The concentrations can be kept within desired limits by proper dilution, while the wavelength may be chosen to best suit the procedure, the blue region providing the greatest degree of sensitivity with a narrow permissible concentrange and the reverse holding in the red region.

Of the various turbidity tests carried out in clinical laboratories the thymol turbidity ranks high in importance. Unfortunately, however, the analytical agreement between laboratories on the same specimen is very poor. The causes of such differences are due to methods of standardization and insufficient care in reagent preparation. The original test of Maclagan called for a dilution of serum 1:60 with a saturated thymol solution buffered to a pH of 7.8 with barbital. The turbidity obtained was compared with Kingsbury standards which were mixtures of urotropin and hydrazine sulfate in gelatine adjusted to give a turbidity equivalent to 10-100 mg.% protein as precipitated by sulphosalicylic acid. The matched standard divided by four was considered the unit. Shank and Hoagland (J. Biol. Chem. **162**:133, 1946), as a result of difficulty in attempting to duplicate the Kingsbury standards with various albumin solutions and sulphosalicylic acid, decided to use the $BaSO_4$ standards of Wadsworth that were in use for the estimation of bacterial suspensions. Through error, their results were published on the basis of using 0.0962 normal $BaCl_2$ for the preparation of the standards rather than 0.0962 molar which agreed with the Kingsbury standards. Although the authors corrected the reprints they sent out, most of the laboratory manuals carry directions for the preparation of the standards on the basis of 0.0962 normal $BaCl_2$. Obviously, one is twice the other.

Assuming the use of the same concentration of $BaCl_2$, the $BaSO_4$ standards as prepared in different laboratories differ. The order in which the reagent are added, the temperature and the manner of mixing will influence the results. In addition various instruments respond quite differently to the same variations in the degree of turbidity. In an attempt to overcome such difficulties, other standards have been proposed such as solutions of copper sulfate, Evans blue dye and suspensions of glass. The $BaSO_4$ standards are, however still used by most laboratories.

The thymol reagent as originally prepared was a saturated solution of thymol buffered to a pH of 7.8; turbidity often developed on standing, which was usually filtered off, or the reagent was stored in the refrigerator to prevent the formation of a turbidity. Obviously, such procedures altered the concentration of thymol. De La Heurtga and Popper (J. Lab. Clin. Med. **34**: 877, 1949) showed that only moderate changes in thymol concentration would cause marked differences in serum turbidity readings and recommended the use of a 100 mgs.% concentration of thymol rather than simply a saturated solution, normally 90-117 mgs.% This appears to be a good suggestion. In addition to the effect of the thymol concentration, the pH of the completed reagent has a very marked effect on the serum thymol turbidity. A drop in the pH from 7.8 to 7.3 results in as much as a 500% increase in units at low levels and a 100% increase at elevated levels.

The changes in sera on standing will alter the results of the test. Usually such changes are of a small magnitude; a decrease of 0.2 - 0.3 unit for each 24 hours of refrigeration is usually seen. However, a change of 1-3 units may occur in either direction. The degree of change is not related to the original level.

Other frequently used turbidity procedures include the zinc sulfate procedure for gamma globulin and the estimation of protein in spinal fluid and urine.

That the zinc sulfate turbidity test is a determination of gamma globulin concentration alone is very doubtful. Although the correlation with electrophoretic patterns at low concentrations is fair, no correlation exists at elevated levels. Since the same $BaSO_4$ suspensions used in the thymol turbidity procedure are employed as standards in this test, the same errors as previously noted may occur.

The turbidimetric determination of protein in spinal fluid and urine by flocculating with sulphosalicylic acid suffers primarily from the fact that different degrees of turbidity are obtained with albumin and globulin and hence the total protein value will vary with the albumin-globulin ratio. Ten per cent trichloracetic acid has been reported to be a more satisfactory precipitant in that the turbidity is not influenced by alterations in the protein fractions. (Bossak, Rosenberg, J. Ven. Dis. 30:100, 1949)

** Review of lecture presented before the Southern California Section of the AACC, February 3, 1953, at the Hollywood Presbyterian Hospital, Los Angeles, Calif.*

REVIEW OF CURRENT LITERATURE

ELLENMAE VIERGIVER – EDITOR
CECILIA RIEGEL, C. VON FRIJTAG DRABBE, HARRY G. ANRODE

EFFECT OF PLASMA IN TOXEMIA OF PREGNANCY ON VOLUME OF NORMAL ERYTHROCYTES. Y.M. Bromberg and S.Z. Rosenberg (Hadassah Univ. Hosp., Jerusalem, Israel).

The plasma of patients suffering from toxemia of pregnancy, incubated with red blood cells of normal patients, causes an increase in mean corpuscular volume of the cells; plasma from normal or healthy pregnant women has no effect. C.R.

MEASUREMENT OF ALBUMINURIA. A comparison of β-naphthalene sulfonic acid and sulfosalicylic acid as precipitating reagents and the influence of polypeptides. G.V. Kropp and R.W. McKee (N. Eng. Deaconess Hosp., Boston, Mass.). Am. J. Clin. Path. *23*, 403-10, 1953.

A comparison of protein content of urine as determined by the Kingsbury-Clark sulfosalicylic acid procedure (1), by pptn. with β-naphthalene sulfonic acid (2), and by Kjeldahl analyses after dialysis (3) showed that (2) gave values closer to (3) than (1) did, when the methods were tested either on known amts. of protein added to urine, or on pathologic urines. Method (1) was found to give a stronger test with proteose-peptone solutions than method (2) and the authors attribute the high results with (1) to pptn. of polypeptides materials which are not pptd. in method (2). C.R.

STABILITY OF ACID PHOSPHATASE IN FROZEN SERUM. M.M. Davison. (Boston Univ. Sch. Med., Mass.). Am. J. Clin. Path. *23*, 411, 1953.

The stability of acid phosphatase as deterd. by the Gutman method is unaffected by freezing and thawing over a period of 112 days. C.R.

CHEMISTRY AND FUNCTION OF COENZYME A. F. Lipmann (Mass. Gen. Hosp., Boston). Bacteriol. Revs. *17*, 1-16, 1953.

A review with 76 references.

PAPER ELECTROPHORESIS OF PROTEINS IN CLINICAL DIAGNOSIS. J. Sternberg (Univ. Montreal). Can. Med. Assoc. J. *68*, 284-5, 1953.

The app. used in 2-dimensional paper electrophoresis is described.

DETERMINATION OF CARBON MONOXIDE IN BLOOD. P. Seifert and L. Schmieder (Univ. Heidelberg, Ger.). Deut. Z. ges. gerichtl. Med. *41*, 435-40, 1952.

The Gettler and Freimuth method spot $PdCl_2$ reduction method for the determination of CO-Hemoglobin is a simple, rapid method that can be used by clinical laboratories.

EFFECT OF EMOTIONAL STRESS ON THE BLOOD PYRUVIC ACID LEVEL. S. Gitelson and P. Tiberin (Rothschild Hadassah Univ. Hosp., Jerusalem.). Acta Endocrinol. *112*, 345-50, 1952.

Blood pyruvic acid levels rise as a result of emotional stress.

17-KETOSTEROIDS AND 11-HYDROXYCORTICOIDS. Metabolism, physiology, and clinical significance. M. Ravera (Univ. Genoa, Italy). Inform. med. (Genoa) Sez. clin. sci. *6*, 337-56, 1952. A crit. review with nearly 250 references.

CONCENTRATION OF CEREBROSPINAL FLUID IN PREPARATION FOR PAPER ELECTROPHORESIS. A simple and mild procedure. H.J. Mies (Allgem. Krankenhaus, Altona, Ger.). Klin. Wochschr. *31*, 159-61, 1953.

DETERMINATION OF PROTEIN-BOUND IODINE IN PLASMA OR SERUM. A simple and rapid method. L.W. O'Neal and E.S. Simms. (Washington Univ. Sch. Med., St. Louis, Mo.). Am. J. Clin. Path. *23*, 493-505, 1953.

Proteins are pptd. with 15% trichloracetic acid, washed, and digested with chloric acid plus chromic acid. The digest is evapd. almost to dryness, taken up in water and an aliquot added to ceric culfate-arsenious acid reagent. The loss of color due to reduction of ceric sulfate by iodide is measured spectrophotometrically. Optimal conditions of acidity, temp. and concn. of reacting substances for the colorimetric step are described. Normal range 4.0—7.3 per 100 ml. S.D. of duplicates 0.33 per 100 ml. C.R.

PROTEIN FLOCCULATION REACTIONS. A physicochemical approach. Abraham Saifer (Jewish Sanatorium and Hosp. for Chronic Diseases, Brooklyn, N.Y.) Am. J. Med. *13*, 730-43, 1952.

A critical review with 121 references.

DETERMINATION OF ISONICOTINIC ACID HYDRAZIDE IN URINE. Otto Meyer zu Schwabedissen (Univ. Freiburg i. Br., Ger.). Deut. med. Wochschr. *78*, 104-5, 1953.

Dilute urine 1:10. To 5cc. filtrate add 5cc. 25% HCl, reflux for 2 hrs., add carbon and filter. Add 1cc. Ehrlich's reagent, make up to 5cc. and read at 458mμ against a urine blank.

TETRAZOLIUM SALTS. A new tool in General and Experimental Pathology. M.M. Black, B.W. Zweifach and F.D. Speer. (Flower and Fifth Ave. Hosps., New York). Am. J. Clin. Path. *23*, 332-39, 1953.

A review. 30 references. C.R.

REGRANULATION OF BETA CELLS OF ISLETS OF LANGERHANS FOLLOWING INSULIN AND STARVATION. S.T. Nerenberg (Univ. of Minnesota, Minn.) Am. J. Clin. Path. *23*, 340-42, 1953.

The beta cells of the islets of Langerhans in rat pancreas which have disappeared as a result of insulin therapy, reappear on the 6th to 10th day after discontinuance of the insulin. In starved animals the granules reappear on the 3rd to 8th day after feeding is resumed. If extra carbohydrate is given the granules reappear earlier. The reappearance of the granules occurs even after prolonged insulin treatment (3 months). C.R.

EXCRETION AND DISTRIBUTION OF POLYVINYL PYRROLIDONE IN MAN. As determined by use of radiocarbon as a tracer. R.K. Loeffler and J. Scudder (Columbia Univ., New York). Am. J. Clin. Path, *23*, 311-21, 1953.

Polyvinyl pyrrolidone was administered to four patients using Carbon 14-labeled PVP-macrose as a tracer. Approx. 1/3 of the activity appeared in the urine in six hrs. and 2/3 was excreted in 24 hrs. Feces contd. small amts. of radioactivity. At autopsy the radioactivity was distributed throughout all tissues studied, the greatest concn. being found in kidneys, lungs, liver, spleen and lymph nodes. C.R.

THE DETERMINATION OF SERUM ALKALINE PHOSPHATASE ACTIVITY. A. Kaplan and A. Narahara. J. Lab. Clin. Med. *41*, 819-24, 1953.

A modification of the method of Gomori to make it more suitable for use in a routine laboratory. The method may be adapted to use from 0.01 to 0.50 ml. serum. H.A.

THE DETERMINATION OF SERUM ACID PHOSPHATASE ACTIVITY. A. Kaplan and A. Narahara. J. Lab. Clin. Med. *41*, 825-28, 1953.

A modification of the method of Gomori to make it more suitable for use in a routine laboratory. The method may be adapted to use from 0.05 to 0.50 ml. serum. H.A.

THE VARIABILITY OF THE SALTING-OUT CURVES OF PROTEINS OF NORMAL HUMAN PLASMA AND SERUM. E.P. Steyn-Parve and A.J. van den Hout (Univ. Utrecht, Netherlands). Biochem. et Biophys. Acta *10*, 320-5, 1953.

The salting-out curves of proteins of normal human plasma are influenced by the manner in which the blood is drawn, the anticoagulant used, the presence or absence of fibrinogen and the non-protein components of the plasma. H.A.

REVIEW OF CURRENT LITERATURE

SERIAL EXECUTION OF PAPER ELECTROPHORESIS AND DIRECT RECORDING OF CURVES WITH THE AID OF THE ELECTROCARDIOGRAPH PHOTOELECTRIC APPARATUS. H. Weicher (Stadtkrankenhaus, Darmstadt, Germ.) Klin. Wochschr. *31*, 161-4, 1953.

Description of apparatus.

A SIMPLIFIED METHOD FOR THE DETERMINATION OF AMYLASE ACTIVITY IN SERUM AND URINE USING GLYCOGEN AND THE ANTHRONE REAGENT. H. Sobel and S. Meyers (Los Angeles, Cal.). J. Lab. Clin. Med. *41*, 655-8, 1953.

0.20ml serum are added to 3ml glycogen substrate (stable about 1 month) and incubated for 15 minutes at 37°. The excess glycogen is precipitated with 3ml ethanol. 2gm Fuller's earth are added, the mixture is then shaken and centrifuged. 0.40ml of the supernate are transferred to a colorimeter tube, 1.60ml water and 4ml anthrone reagent are added. After 10 minutes the color intensity is measured with a red filter. A chart for the conversion of glycogen anthrone units to Somogyi units is given. H.A.

INFLUENCE OF THE ADDITION OF HEPARIN ON THE ELECTOPHORETIC MOBILITY OF SERUM GLOBULINS. R. Blasius and W. Seitz (Univ. Munich, Ger.). Klin. Wochschr. *30*, 905-6, 1953.

The mobility of serum globulins is accelerated, that of albumin is not affected. H.G.A.

THE EVALUATION OF THIOCYANATE IN BLOOD. J. T. Dioto; Rev. fac. cien. quim., Univ. nacl. La Plata *24*, 169-75, 1952.

A semimicro modification of Barker's method to avoid adsorption of thiocyanate on the precipitating agent. H.G.A.

A NEW METHOD FOR DETERMINATION OF CALCIUM AND MAGNESIUM IN SERUM. T. (Ishii (1st Tokyo Natl. Hosp.). Igaku to Seibutsugaku (Med. and Biol.) *24*, 9-11, 1952.

Serum is directly titrated with N-di-Na ethylenediaminetetraacetate using erichrome Black T as indicator. Ca^{++} may be precipitated with ammonium oxalate and the titration repeated to obtain concentration of Mg^{++}. Ca is obtained by difference. Results agree well with those obtained by the method of Sobel and Hanok. H.G.A.

REACTION BETWEEN CAROTENOIDS AND COPPER. S.D. Balakhovshii, N.N. Drozdova, and V.N. Fedorova. Doklady Akad. Nauk. U.S.S.R. *87*, 453-5, 1952.

The oxidation of ascorbic acid (I) is retarded if Cu and carotene are added to the system. One mole Cu (as $CuSO_4$) and 1 to 15 moles carotene per 10,000 moles I are sufficient.

VARIATIONS OF SERUM COPPER IN CANCER OF THE UTERUS. L. Rauramo and G.R. Wallgren (Univ. Helsinki, Finland). Ann. Med. Exptl. et Biol. Fenniae *30*, 259-66, 1952.

Serum Cu levels are elevated in cases of carcinoma of the uterus. Elevated values are also found in cases of benign genital neoplasms. Normal Cu values; 120± 15%: In patients with carcinoma of the uterus 178± 6%. No conclusions as to the severity of the process can be drawn from the height of the elevation.

ELECTROLYTE METABOLISM IN DIABETIC ACIDOSIS. R.G. Sprague and M.H. Power (Mayo Clinic, Rochester, Minn.). J. Am. Med. Assoc. *151*, 970-6, 1953.

It is desirable to accurately replace electrolyte losses that occur during diabetic acidosis. The use of isotonic NaCl to replace the Na and Cl loss occasionally leads to a hyperchloremia, therefore solns. containing less Cl are desirable. The P deficit may be of such magnitude that its correction may be desirable. H.A.

OCCURRENCE AND SIGNIFICANCE OF CITRIC ACID IN THE ANIMAL ORGANISM. T. Thunberg (Univ. Lund, Sweden). Physiol. Rev. *33*, 1-12, 1953.

The blood level of citric acid (I) is controlled by the parathyroid hormone. The synthesis of I from oxalacetic acid and acetic acid is accomplished by the condensing enzyme consisting of Lipmann's enzyme A and coenzyme A. Mn^{++}, Mg^{++} and ATP are required. It is suggested that I may function as a cement substance, and may play a role in cell permeability and irritability of the nervous system.

AN IMPROVED BLOOD VOLUME METHOD (EVANS BLUE DYE) UTILIZABLE EVEN IN THE PRESENCE OF HEMOLYSIS AND/OR LIPEMIA. H.A. Davis and L. Isenberg (Los Angeles, Calif.). J. Lab. Clin. Med. *41*, 789-95, 1953.

A simple method requiring the drawing of only one 11 ml. blood specimen after the injection of Evans Blue dye (T-1824) is presented. Hemolysis and/or lipemia do not interfere. Blood is drawn 10 min. after injection of the dye. 2 plasma dilutions, one with saline and one with dilute dye are made and read at 440, 620 and 710 mμ. Calculations are given. H.A.

DETERMINATION OF PBI BY DRY ASHING. H. Brown, A. M. Reingold and M. Samson (Samson Laboratories, Philadelphia, Pa.). J. Clin. Endocrinol. 13: 444-450, 1953.

A simplified procedure is presented for the determination of protein bound iodine by dry ashing. As many as twenty specimens, in duplicate, can be analyzed per day. Mercurial therapy does not lead to artefactual values as it does with distillation methods. E.V.

CLINICAL EXAMINATION OF SERUM PROTEINS, ELECTROPHORESIS, NONSPECIFIC REACTIONS, AND SELECTIVE DETERMINATIONS. R. Ardry. Ann. biol. clin. (Paris) *10*, 575-627, 1952.

A review of various techniques used for protein fractionation and the clinical application of the information derived.

A SIMPLER COLORIMETRIC METHOD FOR DETERMINING ISONICOTINIC ACID HYDRAZIDE (INAH) IN BIOLOGICAL FLUIDS. S. Aoki, I. Terai, and K. Mori (Natl. Hosp., Yamanaka.). Iryo *6*, No. 11, 33-5, 1952.

NORMAL LIMITS OF URINARY COPROPORPHYRIN EXCRETION DETERMINED BY AN IMPROVED METHOD. L. Zieve, E. Hill, S. Schwartz and C.J. Watson (Minneapolis, Minn.). J. Lab. Clin. Med. *41*, 663-9, 1953. Urinary coproporphyrin was determined by a recently developed, simple and accurate method (J. Lab. Clin. Med. *37*, 843, 1951).

The average normal values found for males were 189±59 μg per day (extremes 100-300 μg) and females 134±42 μg per day (upper limit normal 275 μg). Average values were found to be significantly higher in the fall than in the spring. H.A.

CEREBROSPINAL FLUID INORGANIC PHOSPHORUS IN NORMAL INDIVIDUALS AND IN THOSE WITH VIRAL INVOLVEMENT OF THE CENTRAL NERVOUS SYSTEM. L. Odessky, P. Rosenblatt, A.V. Bedo and L. Landau. (Brooklyn, N.Y.). J. Lab. Clin. Med. *41*, 745-53, 1953.

The authors found elevated inorganic phosphorus values in the spinal fluids of patients with CNS involvement. Using the method of Fiske and SubbaRow, values of 22 patients with no CNS involvement fell between 0.9-2.0 mg% (21 of these below 1.7 mg%, with a mean of 1.41 mg%). Elevated values (above 2.0 mg%) were found in all patients with viral involvement of the central nervous system. H.A.

A STUDY OF THE RATE OF PROTEIN SYNTHESIS IN HUMANS. I. Measurement of the urea pool and urea space. A.S. Pietro and D. Rittenberg (Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, N.Y.). J. Biol. Chem. *201*, 445-455, 1953.

Injection of N^{15}-labeled urea and measurement of its concentration in blood enables calculation of the extent to which it was diluted and in turn estimation of the size of the urea pool. Since urea is freely diffusible the total body water also may be calculated. The total body water estimated in this manner agreed closely with a figure derived by the deuterium oxide method. C.vF.D.

SKIN PIGMENTATION IN RELATION TO ADRENAL CORTICAL FUNCTION. T. C. Hall, B. H. McCracken, and G. W. Thorn (Peter Bent Brigham Hospital and Collis P. Huntington Memorial Hospital, Boston, Mass.). J. Clin. Endocrinol. 13: 243-257, 1953.

A method is presented for measuring skin pigmentation by reflectance spectrophotometry. This technic was used to study conditions affecting the color of human skin. E.V.

ADRENAL CORTICAL SECRETIONS IN RELATION TO THE REPRODUCTIVE SYSTEM OF RATS. C.R. Moore (Univ. of Chicago, Chicago, Ill.). J. Clin. Endocrinol. 13:330-368, 1953.

A comprehensive publication of the author's experimental studies concerning the problems of hormone secretion and their effects during embryonic development and early post natal stages. E.V.

TOTAL SERUM ORGANIC ACIDS. A preliminary study. H. Davis, Jr., and H.R. Jacobs. (Evanston Hosp. and Northwestern Univ. Med. Sch., Chicago, Ill.). Am. J. Clin. Path. *23*, 464-69, 1953.

A method for detern. of the concn. of serum org. acids is given as follows: Plasma is sepd. immediately from the cells. Plasma may be kept in the ice box at least one month with no change. Serum proteins are pptd. by heating at their isoelectric point. Phosphates and carbonates are removed by treatment with $Ca(OH)_2$. After filtration, the reaction is brought to pH 7.5 by the addn. of 0.05N HCl. Org. acids are then deterd. by titration to pH 3.0 with 0.05N HCl, using an electric pH meter. Results are expressed in ml. of 0.1N acid per 100 ml (mEq./l). Normal range was 4-12 mEq./l. Sera from diabetics under poor control showed wide variations from mean values; those of diabetics in good control less variation; a few patients with myocardial infraction showed serum values higher than normal. C.R.

STRUCTURE AND SHAPE OF STEROID MOLECULES IN RELATION TO BIOLOGICAL ACTIVITY. C. W. Shoppee. 2nd Congr. intern. biochim., Chim. bio- VII., Symposium biochim. des steroides (Paris) 5-12, 1952.

A review (in English). H.G.A.

THE RELATION OF WATER AND SODIUM EXCRETION TO BLOOD PRESSURE IN HUMAN SUBJECTS. D. M. Green, H. G. Wedeel, M. H. Wald and B. Learned (Evanston Hosp., Evanston, Ill.). Circulation *6*, 919-24, 1952.

The behavior of the human hypertensive subject in response to salt loading is similar to that of chronically DOCA treated animals and is not related to kidney excretory function. H.G.A.

DOES A LARGE INTAKE OF POTASSIUM MODIFY THE METABOLIC EFFECTS OF ACTH (CORTICOTROPIN) IN MAN? L.L. Bennett, G.W. Liddle and R.C. Bentinck (Departments of Physiology and Medicine and the Metabolic Unit for Research in Arthritis and Allied Diseases, University of California School of Medicine, Berkeley and San Francisco, Calif.). J. Clin. Endocrinol. 13:392-407, 1953.

The concurrent administration of large doses of potassium salts and corticotropin to arthritic patients failed to modify the anti-rheumatic effects of corticotropin. It did not prevent other effects of corticotropin such as uric acid diuresis, eosinopenia, rise in urinary nitrogen, rise in fasting blood sugar and rise in 17-ketosteroid extion. The effects upon electrolyte metabolism, however, were considerably modified. Sodium retention which occurs when corticotropin therapy is initiated was prevented or diminished when adequate amounts of potassium salts was also given. If potassium salts were administered after corticotropin therapy was underway, a marked diuresis of sodium occurred. The sodium diuresis which usually occurs when corticotropin is withdrawn may not occur if potassium salts are being given concurrently. E.V.

SYNTHESIS OF NORMAL SERUM PROTEINS AND OF ANTIBODIES. F. Haurowitz (Indiana Univ., Bloomington). 2nd Congr. intern. biochim., Chim. biol. II, Symposium biogenese des proteines (Paris) 1952, 56-61.

A review (in English). H.G.A.

SERUM PROTEIN DURING THE FIRST YEAR. G. W. Schmidt (Univ. Giessen, Germ.). Z. Kinderheilk. 71, 476-82, 1952.

The serum protein drops from 5.8 to 4.8 gm% during the first month, reaches the birth value at 5 month and rises to about 6.4 gm% at one year. Values in prematures are about 0.3 gm% lower than in full term babies of the same age. H.G.A.

A NEW METHOD FOR THE DIRECT DETERMINATION OF SERUM CHOLESTEROL. A. Zlatkis, B. Zack and A. J. Boyle. (Detroit, Mich.). J. Lab. & Clin. Med. *41*, 486, (1953).

To 3.0 ml acetic acid add 0.1 ml serum and 2 ml. of the color reagent (Ferric chloride, glacial acetic and sulfur acid). Allow to come to room temperature and read at 560 mμ. Bilirubin causes some interference if more than 0.8 mg% is present. Agreement with classical methods is said to be good. H.G.A.

THE FREE FATTY ACIDS OF BLOOD SERUM. P. Favarger, Med. et hyg. *15*, 3, 1947. H.G.A.

THE EFFECT OF AGE ON THE INTRAVENOUS GLUCOSE-TOLERANCE TEST. N. G. Shneeberg and I. Finestone (Mt. Sinai Hosp., Philadelphia, Pa.). J. Gerontol. *7*, 54-60, 1952.

Normal subjects over 40 years old (I) had a depressed response to I.V. glucose as compared to subjects 16-39 years old (II). Since some of (I) had curves similar to those of II, it is suggested that some of the presumed I studied may have been potential diabetics. H.G.A.

BLOOD SERUM MAGNESIUM IN PORTAL CIRRHOSIS AND DIABETES MELLITUS. F. L. Stutzman and D. S. Amatuzio (Minneapolis, Minn.). J. Lab. Clin. Med. *41*, 215, 1953.

The authors found serum Mg^{++} values below normal levels in patients with portal cirrhosis or diabetes. The degree of lowering seems to be proportioned to the severity of the disease process. Normal values: 1.81 ± 0.12 meg/ L.; Pathological values 1.49 ± 0.12 meg Mg^{++}/ L. H.G.A.

BROMOSULFALEIN SODIUM RETENTION EVALUATION OF HEPATIC FUNCTION IN DIABETES MELLITUS. J. Pomeranze (New York Med. Coll., New York, N.Y.) Metabolism *1*, 540-3, 1952.

Using the Mateer modification of the bromosulfalein Na retention test, it was found that 57% of diabetic patients showed an increased retention. This was found in 40% of the patients with no apparent complications and 70% with known complications. The degree of retention could be correlated with the severity of the disease as judged by difficulty of control by diet or insulin. H.G.A.

THE RELEASE OF LABELED AMINO ACIDS FROM THE PROTEINS OF RAT LIVER SLICES. M.V. Simpson (Department of Physiology, Tufts College Medical School, Boston, Mass.). J. Biol. Chem. *201*, 143-154, 1953.

The release of amino acids from protein has been studied by labeling the proteins of the intact rat with methionine-S^{35} and subsequently following its release from the proteins of liver slices. Conditions which limit the release or utilization of energy (cyanide, anaerobiosis, 2,4-dinitrophenol) have been found to depress also the liberation of labeled methionine from protein. C.vF.D.

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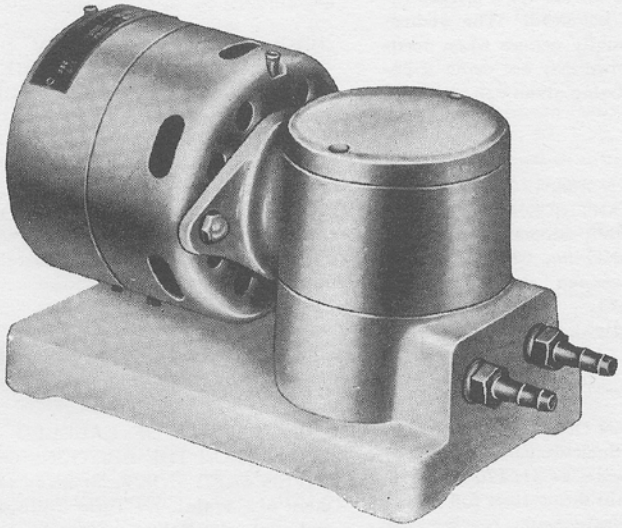

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