Report to the Editor

European Symposium on Diabetes Mellitus in Geneva November 1963

On Nov. 29 and 30, 1963, a working symposium was called together in Geneva, Switzerland, by Professor Eric Martin. The purpose of the session was to bring together various European groups working in the field of diabetes, in order to discuss and compare results in two areas of diabetes research: (1) Insulin in blood: methods, results, interpretations; (2) The early detection of diabetes and prediabetes.

The following is a list of the participants from ten countries: *Belgium*: Drs. Bellens, R.; Conard, V.; Franckson, J. R. M.; Hoet, J. J.; Hoet, J. P.; Lefebvre, P.; Malaisse, W. *Bulgaria*: Dr. Andreev, D. *Denmark*: Drs. Jersild, M.; Lundbaek, K.; Oerskov, H.; Schlichtkrull, J. *France*: Drs. Azerad, E.; Bour, H.; Darnaud, C.; Derot, M.; Lestradet, H.; Loubatieres, A. L.; Mirouze, J.; Rambert, P.; Rosselin, G.; Vague, J. *Germany*: Drs. Creutzfeldt, W.; Ditschuneit, H.; Maske, H.; Mehnert, H.; Oberdisse, K.; Pfeiffer, E. F.; Schliack, V. *Great Britain*: Drs. Butterfield, J.; Fraser, R.; Malins, J. M.; Randle, P. J.; Samaan, N.; Samols, E.; Stowers, J. M.; Taylor, K. W.; Vallance-Owen, J. *Italy*: Drs. Motta, L.; Pozza, G. *Sweden*: Dr. Cerasi, E. *Switzerland*: Drs. Bally, P.; Brueckner, R.; Bürgi, H.; Constam, G. R.; Courvoisier, B.; Curchod, B.; Fankhauser, S.; Felber, J.-P.; Froesch, R.; Jeanrenaud, B.; Labhart, A.; Martin, E.; Plattner, H.-C.; Renold, A. E.; Rilliet, B. M.; Schertenleib, F. E.; Siegenthaler, P.; Vernet, A.; Young, D. A.; Zahnd, G. R. *Turkey*: Dr. Oeker, C. *The World Health Organization* was represented by Dr. T.-S. Sze and Dr. J. Butterfield.

The working session on insulin in blood was moderated by Dr. Renold. The subject matter was discussed under four headings. The following is the moderator's interpretation of the conclusions reached:

I. *Methodology and comparison of results obtained with different methods.* It was generally apparent that presently only radioimmunoassay procedures are sufficiently practicable, accurate, and reproducible from laboratory to laboratory to warrant widespread use and application to clinical or demographic studies. This does not mean that bioassay procedures are meaningless, but that they are as yet to be considered as exclusively research tools, applicable to such inquiries as the state of insulin in blood, the effectiveness of inhibitors, the varying responsiveness of different tissues.

Among the modifications of the radioimmunoassay introduced by Yalow and Berson, the procedures discussed included the Yalow and Berson assay itself, used in a slightly modified form by Samols, a procedure developed by Ditschuneit and Pfeiffer utilizing agar gel electrophoresis for the separation of antibody-bound insulin, and the double antibody technic of Hales and Randle. Experience with the last named procedure was also reported by Felber, by Oerskov and by Cerasi. Results obtained by all of these technics agreed adequately among the various laboratories, and also with results reported in the literature with these and other variants of the radioimmunoassay. Accordingly, it would seem that this method has entered the field of semi-routine practical application, with the proviso that certain aspects need to be standardized for more widespread use. Radioinsulin of good quality should be produced regularly and predictably in one or several European centers, possibly utilizing the longer-lived I-125 rather than I-131. Better comparison between laboratories and within laboratories at different times would also very likely be achieved if a standardized source of anti-insulin guinea pig serum and anti-gamma globulin of guinea pig obtained in the rabbit were to become available. Insulin iodination procedures were discussed by Rosselin.

Among biological procedures, the net blood insulin activity present in a living organism at any one time is probably best estimated by the analysis of blood glucose disappearance after a rapid intravenous load, so fully investigated by the Brussels group represented by Conard, Franckson and Bellens. The insulin "reserve" is much better approached through prolonged intravenous or oral glucose tolerance procedures combined with the measurement of immunologically reactive insulin in the blood.

Bioassays for serum insulin-like activity presently in use are limited to those comparing the activity of serum to that of insulin standards on isolated tissues. Malaisse reported on a modification of the rat diaphragm procedure, measuring the total uptake of radioactivity by the tissue in the presence of glucose-C-14. Moody and Felber achieved a remarkable degree of sensitivity and precision using the synthesis of C-14 glycogen by the mouse diaphragm in the presence of C-14 glucose. The procedures utilizing rat adipose tissue metabolism as index of insulin-like activity are probably the simplest and most reproducible of the bioassays utilizing isolated tissues, but they are heavily beset by questions as to their specificity, particularly since they often show insulin-like activity in the serum of pancreatectomized animals. Accordingly, the procedures combining the measurement of biological activity together with ability of anti-insulin serum to suppress that activity are probably the most informative of this group. These procedures are the ones developed by Froesch and his collaborators, and by Samaan and Fraser. Moody and Felber suggested a combined immuno-bioassay utilizing C-14-glycogen synthesis by mouse diaphragm. The direct intraperitoneal comparison of the effects of insulin-like substances on both diaphragm and adipose tissue, introduced by Rafaelsen, may be of special interest in the future.

In general it may be said that antiserum-suppressible insulin activity correlates reasonably well with measurements obtained with the radioimmunoassay, although some, such as Oerskov and Lundbaek among others, do not consider the

two measurements as identical.

II. *The state of insulin in blood.* As to the state of insulin in blood, opinions still varied widely. At one end, Taylor, on the basis of extraction studies of serum and plasma, felt that it was rather likely that most, if not all, insulin was present in a form closely similar to that of extracted pancreatic insulin. Even in this case, however, a small fraction of extracted material was found which did not have physical chemical characteristics identical with that of pancreatic insulin, but the possibilities of an artefact of extraction or of a breakdown product were considered. Most workers primarily concerned with the biological insulin-like activity of serum consider it likely that a significant, perhaps a major fraction of serum insulin, is present in a form (or forms) other than that of crystalline pancreatic insulin. The biological activity of this insulin is not suppressed by anti-insulin serum (atypical insulin of Samaan, nonsuppressible insulin of Froesch). Also it is found in association with fractions of serum protein which are not characteristic of the distribution of pancreatic insulin added to serum. Finally, at the other end, the ability to elicit an immunological response to homologous pancreatic insulin in several species suggested to Renold and his collaborators that insulin identical with crystalline pancreatic insulin rarely if ever occurs in blood at all!

"Typical" or "suppressible" insulin is thought by the workers concerned to be identical with or similar to pancreatic insulin. In Samaan's view, "atypical" insulin is a modified pancreatic insulin and the modification is thought to occur primarily in the liver, resulting in an insulin activity which is no longer ultrafiltrable, whereas "typical" insulin is ultrafiltrable. In the view of Froesch and Bürgi, "nonsuppressible" insulin is also a larger molecule than insulin, as determined by gel filtration on Sephadex, but is otherwise very similar to insulin. These workers have achieved an approximately 500-fold purification of "nonsuppressible" insulin and have shown it to exert biological activity undistinguishable from insulin on adipose tissue and diaphragm, to be similar to insulin in its stability at various *p*H levels, to be extractable in acid ethanol and precipitable in ether-acetone, and to be inactivated by pretreatment with cysteine, although only in the presence of 8N urea, the latter suggesting perhaps a difference in tertiary structure. Both groups of workers find that "atypical" or "nonsuppressible" insulin is altered very little during glucose tolerance tests and is unlikely to be an acutely effective moiety of insulin activity in blood. This last mentioned characteristic distinguishes "atypical" or "nonsuppressible" insulin from the "insulin complexes" described by Antoniades, since the "insulin complexes" are said to be acutely depressed by glucose infusions and are thought of by that author as, in a sense, an inactive serum reserve form of insulin.

The possible biologic importance of the association of certain protein fractions with insulin added to serum was underlined by the observation of Zahnd that iodo-insulin not only exhibits an affinity for α-2-M globulin, demonstrated also in ultracentrifugation studies, but that incubation of serum with an anti-α-2-M serum removes a part of insulin-like activity into the precipitate.

Finally it should be mentioned that a feature hitherto common to all bioassays of serum, i.e., the recovery of excess insulin activity whenever serum fractions are tested separately, was also observed by Lynsgoe, Oerskov and Lundbaek when the fractions were tested with a radioimmunoassay procedure, although this was not confirmed by Samols.

III. *Insulin activity and immunologically detectable insulin in diabetes and prediabetes.* Studies to date continue to confirm the early observation of Lawrence and Bornstein that diabetes is not necessarily associated with abnormally low fasting levels of serum insulin activity. There was general agreement that in early diabetes the fasting values of serum insulin-like activity or immunologically detectable insulin were not, as a group, low. Higher than normal fasting levels, as a group, were reported by Ditschuneit and Pfeiffer (adipose tissue bioassay), Steinke and Renold (adipose tissue bioassay), Randle and Hales (immunoassay), and Felber (adipose tissue bioassay and immunoassay), while Oerskov and Lundbaek found elevated levels with the adipose tissue bioassay, normal levels with the immunoassay. Cerasi found normal levels with the immunoassay, Froesch with the adipose tissue bioassay.

The response to glucose administration of serum levels of insulin-like activity or immunologically detectable insulin in early diabetes has been described as abnormally high (Hales and Randle), delayed initially then high (Samols, Felber) as first described by Yalow and Berson, or delayed and rather flat (Steinke and Renold, Pfeiffer, Cerasi, Oerskov and Lynsgoe). The distribution of insulin among serum proteins, measured either as insulin-like activity or immunologically detectable insulin, appeared to favor, in diabetes, the region of the β-globulins (Pfeiffer, Lynsgoe). In prediabetes, as genetically defined, elevated levels of serum insulin-like activity may be found at a time when glucose tolerance is still normal (Steinke and Renold, Ditschuneit and Pfeiffer). In prediabetes also, these authors, as well as Cerasi, described flat response of serum insulin-like activity or immunologically measurable insulin to the intravenous administration of glucose. The observation was particularly striking in a few instances reported by Cerasi where glucose infusion was prolonged. In early juvenile diabetes Samaan observed a relative increase in "atypical" insulin activity and a relative decrease in "typical."

The general impression derived from this discussion is that of the probable presence in prediabetes and early diabetes of derangements in insulin secretion, effectiveness, or transport which are not compatible with the hypothesis of simple atrophy of the insulin reserve, at least during the early stages of diabetes. However, many differences and disagreements among workers are still present and Butterfield, as well as Fraser, emphasized the need to define more clearly the clinical state and the previous dietary habits of the groups of individuals studied. At any rate, there was always wide overlap between normal and diabetic or prediabetic groups and, in any individual case, no diagnostic value can as yet be attached to the finding of elevated serum insulin-like activity or immunologically detectable insulin. It is particularly important to define to what extent obesity is present in groups of individuals studied in this respect since elevated levels of immunologically detectable insulin in many cases of obesity are now well established.

IV. *Insulin antagonism.* The subject of insulin antagonism is, of course, related to that of serum insulin in prediabetes and diabetes, since one of the reasons for the presence of elevated levels of serum insulin in some persons with pre-

diabetes and early diabetes might be the regulatory need to offset an antagonistic activity. This antagonism might result from a primary oversupply of tissues such as muscle with substrates other than glucose, particularly fatty acids and ketone bodies. In the view of Randle and Hales, who forcefully support this point of view, the primary event is accelerated mobilization of fatty acids both from adipose tissue and from muscle stores of glycerides. The mechanism of this accelerated mobilization is as yet undefined.

Another view sees in insulin antagonism the presence in serum of substances which might compete with insulin in certain tissues, or which might have effects opposed to those of insulin on tissues. The most intensively studied of these substances is that which Vallance-Owen associates with serum albumin (synalbumin antagonist) and which he finds in the serum of diabetics and also in the serum of relatives of diabetics in a manner suggestive of dominant inheritance. Vallance-Owen is presently exploring the hypothesis that this inhibitor is related to insulin, and possibly represents the B-chain of insulin, and thus perhaps a precursor or a breakdown product of the hormone. The level of this antagonist is pituitary dependent.

Pfeiffer reported on studies in hypophysectomized and hypophysectomized-pancreatectomized dogs, and observed a decrease in serum insulin activity by about one half following hypophysectomy (adipose tissue assay) without further decline after pancreatectomy. D. A. Young emphasized the probable existence of a competitive antagonist to the effect of insulin on muscle, dependent upon the activity of the hypothalamus, since its presence appears to be regulated in the manner of a photo-periodic effect. The likely existence of regulators allowing for independent modulation of insulin effects upon muscle and adipose tissue was recognized.

Many other antagonists to insulin which have been described, such as those found in ketoacidosis, in pancreatic extracts, or in various pituitary fractions, were not discussed.

The likely co-existence of both metabolic inhibitors as in the "fatty acid cycle," as well as of competitive and possibly hormonal antagonists, seemed rather likely to many of the participants.

The opportunity for free discussion of results and interpretations as well as of methodological details was welcomed by all of the participants and it was hoped that such working sessions could be held at intervals during the coming years.

A. E. RENOLD
G. R. ZAHND

The second working day, more clinically oriented, was devoted to the early diagnosis of diabetes mellitus and was moderated by Dr. Lundbaek. The subject matter was again discussed under four headings.

I. *Laboratory procedures.* It became apparent during this day that standard conditions for most laboratory procedures are not as yet generally accepted, even for the oldest of the procedures discussed in detail, the oral glucose tolerance test. Real work on standardization acceptable to all is needed, and the hope was expressed that the World Health Organization might undertake such a task.

Oral glucose tolerance tests were discussed by Constam who prefers to use routinely the double test according to Staub-Traugott, 30 gm. of glucose being given twice. This is at variance with the recommendations of the American Diabetes Association, as well as the widely accepted standards suggested, for instance, by Conn and his collaborators. All, and in particular the chairman, Dr. Lundbaek, emphasized the importance of standard conditions, attention being paid to muscular activity before and during the test, the diet during the four days preceding the test, as well as factors such as menstruation or pregnancy. What is considered a normal diet, normal in carbohydrate content, varies greatly from country to country in Europe.

The intravenous glucose tolerance test was discussed by Vague and by Conard. The standardization of this test is already considerably advanced, thanks to the work of the Brussels group, and values obtained with the calculated glucose assimilation coefficient give a satisfactory single quantitative measure of glucose utilization. Conard recalled a gradual decrease of K with age (from 3.5 at eighteen years of age to 1.0 above sixty). Obesity is similarly associated with decreased K values. Conard also suggested the use of a constant K_2 (to distinguish it from the glucose tolerance $K = K_1$) to express decreasing glucose levels after the administration of tolbutamide. K_2 was depressed in obesity and in prediabetes. It remained normal throughout pregnancy in normal pregnant women and this was also true of intravenous glucose tolerance tests.

The intravenous tolbutamide tolerance test was also discussed by Pfeiffer who accepted 25 per cent of the initial glucose value as a minimum normal response. In prediabetes, tolbutamide tolerance tests are less sensitive than glucose tolerance tests as a means of detection, an observation which was confirmed by a number of other speakers. Lefebvre used intravenous chloropropamide for the same purpose with similar results. Mehnert recalled the risk of severe hypoglycemia and drew attention to sources of error: hepatic cirrhosis is frequently associated with abnormal glucose tolerance but normal tolbutamide tolerance, while young individuals with initially low glucose levels sometimes exhibit abnormal tolbutamide tolerance while glucose response is entirely normal.

Fraser discussed cortisone-glucose tolerance tests, particularly the procedure described by Joplin, in his group. A standard preparatory diet is given during four days, and a glucose tolerance test is carried out. Twenty milligrams of prednisone are given orally at noon, 4:00 P.M. and 8:00 P.M. on the day of the test, no food being allowed from 6:00 P.M. on. Glucosuria is measured in the urine collected from 10:00 P.M. to 6:00 A.M. the following day with blood glucose obtained at midnight and 1:00 A.M. The urinary glucose values appear to be the most valuable. Although many prediabetics may be detected in this fashion, it is clear that the test is not positive in all instances.

The possible diagnostic importance of measuring the response of free fatty acids to the administration of tolbutamide was discussed by Zahnd. Prediabetics as well as diabetics demonstrate a prolonged depression of free fatty acids following tolbutamide, whereas normal individuals exhibit a small initial depression with a major subsequent rise. This difference holds even though similar glucose curves may be present.

Mirouze discussed the usefulness of continuous recording of

blood glucose while Siegenthaler re-emphasized the frequency of abnormal glucose tolerance tests among aged individuals. The limit between normal and abnormal in the older age group is indeed difficult to establish, a conclusion which found widespread acceptance by all, including the chairman.

II. *The special case of pregnancy and prediabetes.* Malins reported on 21,540 deliveries, among which 135 babies weighing more than 10.5 pounds were found. Twenty of the mothers of these 135 infants demonstrated an immediately abnormal glucose tolerance test, half of that number being overtly diabetic. Large babies were not seen when the diabetic parent was the father. The number of stillbirths increased as the interval preceding overt diabetes decreased: thus, there were 16.7 per cent stillbirths during the four years preceding overt diabetes, while only 3.2 per cent were observed when the pregnancies preceded overt diabetes by ten years or more. During the discussion Hoet reported that according to his latest results the number of stillbirths among potential diabetic mothers decreased by a factor of ten, when insulin treatment to tolerance was instituted during pregnancy.

III. *Should prediabetes or latent diabetes be treated?* There was lively discussion of the question: Should prediabetes and latent diabetes be treated?

Loubatières reported on his experimental studies in dogs with either 90 per cent pancreatectomy or total pancreatectomy followed by the subcutaneous grafting of 20 per cent of the pancreas. Exocrine as well as endocrine function must be preserved. In a small group of animals, onset of overt diabetes seemed to be greatly delayed by treatment with sulfonylureas. Prevention of overt diabetes in these animals was only observed in the group given restricted carbohydrate.

Stowers has begun a systematic program designed to test the preventive effectiveness of chlorpropamide treatment in individuals with minor anomalies of glucose tolerance. The study so far has been carried out over eleven months and the glucose utilization index according to Duncan was improved in all treated individuals although, as yet, duration and number of observations were too limited to allow definitive conclusions. Other participants in the symposium reported on observations which, in general, seemed to indicate that glucose tolerance could be improved by treatments with sulfonylureas, while discontinuation of this therapy was immediately followed by recurrence of abnormal tolerance. Constam recalled that minor anomalies of glucose tolerance in latent diabetes may remain at this latent stage for as long as twenty-five years, even if nothing is done.

There was general agreement with the firm advice given by Oberdisse to his patients that reduction of overweight is a minimum aim of the treatment of prediabetes and latent diabetes. However, cooperation on the part of the patient is unfortunately limited either in quality or in time. Martin asked of the audience what should be curtailed: primarily carbohydrate or primarily fat in the diet? There was no general agreement in answer to this question but Loubatières, on the basis of his experiments in dogs, firmly supported the importance of carbohydrate reduction. Andreev mentioned a study which demonstrated the much greater incidence of diabetics during a detection drive among the employees of a large chocolate factory in Bulgaria as compared with the average of the country as a whole or with the employees of other factories. Schliak recalled how very definitely the incidence of diabetes decreases during periods of general caloric curtailment such as that which followed the last world war in his country, a curtailment which concerned both carbohydrates and fat, although more markedly the latter.

IV. *Systematic diabetes detection programs in Europe.* Systematic detection drives for diabetes in Europe seem clearly indicated. Schliak reported on an international study which has so far concerned two million individuals in Europe, of whom 1,600,000 were in East Germany. The systematic aspect of the survey may not have been ideal throughout and this may be the reason for the differences of prevalence found in different countries. Over the years, followups of doubtful tests will greatly improve the value of such examinations. Even so, among the 1,600,000 persons examined in East Germany, 0.33 per cent new and overt diabetics were found. The symposium was informed of the fact that the World Health Organization is presently sponsoring a sizable detection drive in Yugoslavia, for the first time in that country. Again it would be helpful if the WHO took the initiative in establishing, in collaboration with physicians interested in this subject throughout the world, standard conditions and standard tests which might be acceptable to all.

In concluding, Lundbaek came back to what seemed to be the theme of the day and, indeed, of the symposium. Standardization is the most needed element of all detection drives and of all laboratory procedures in order to obtain truly valid statistics sufficiently large to be of use. The intravenous glucose tolerance test, with the measurement of the glucose assimilation coefficient K, emerges as the quantitatively most satisfactory procedure, although it is not always practicable. What is very much needed is a large study comparing all glucose tolerance tests of a standard type with intravenous glucose tolerance tests of the type described at this symposium as well as in the literature by Conard and his group. It is quite likely that the fatty acid measurements, when they become more generally available, will provide an additional and valuable tool. Combined cortisone-tolerance tests, while helpful in some instances, do not appear to give a sufficiently definitive answer. Another general point is the importance of clearly outlining the limit between normal and abnormal in all of these cases, a differentiation which definitely must consider presence or absence of obesity, as well as age, particularly the latter. Butterfield made a plea for the recording of the precise circumstances surrounding any test, even simple urine collections and urine tests.

Another area which greatly needs standardization is terminology, and it was hoped by all that the forthcoming Congress of the International Diabetes Federation in Toronto would provide a forum for the discussion and general acceptance of terminology related to the different stages of diabetes: prediabetes; latent diabetes; potential diabetes; chemical diabetes, etc. Although Conn has greatly supported the term of prediabetes for the period which precedes the first diagnostic evidence of the disease, the term does not seem to be acceptable at present to a number of individuals, particularly among the British group. Fraser prefers the term potential diabetes. According to this terminology, between potential diabetes and overt diabetes exists the period of latent or subclinical diabetes.

B. RILLIET
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Special Articles

The Journal DIABETES *is honored to present in this issue two articles by one of America's pioneer scientists, Dr. Franklin C. McLean of the University of Chicago. His eminence in the field of metabolic research stems from his early achievements in the development of the urea clearance test, his work with Hastings and Van Slyke, and for the past twenty years his important contributions to knowledge of bone and calcium metabolism.*

The first article is a reproduction of one of his earliest works, and of great interest to our readers in its treatment of the perennial subject—glucose regulation in the light of current knowledge. It was, we believe, the first documented report of clinical blood glucose determination in the United States. Students and technicians accustomed to microchemical methods will appreciate the difficulties of biochemical investigation fifty or more years ago when it is noted that the Bertrand method as employed by Dr. McLean required 10 to 15 cc. of blood, together with time-consuming steps, for each determination.

It is also a special reward for the Editor, on the grounds of personal friendship, to present as a companion piece the provocative application of cybernetics to the same problems of glucose homeostasis by the same investigator a half century later.

The Sugar-Content of the Blood and Its Clinical Significance

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The presence of a sugar-like substance in the blood was first shown by Dobson in 1775, in a case of diabetes. Seventy years later Claude Bernard[1] showed this substance to be a constituent of normal blood, and also demonstrated that the amount of this sugar was increased by his well-known "sugar-puncture" in the floor of the fourth ventricle. Since that time the occurrence of hyperglycemia, or an increase in the amount of the sugar found in the blood in diabetes and other conditions, has been recognized, but little attempt has been made to study it clinically until within the past three or four years. Recently newer methods for the determination of the sugar-content of the blood have been devised, putting these estimations within the reach of the clinician, and our knowledge on the subject is advancing rapidly. It is the purpose of this paper to attempt to demonstrate the value of such examinations particularly in cases of glycosuria, and to show what knowledge may be derived from this procedure that will help the clinician.

The blood of a normal adult, under average conditions of diet, exercise, etc., contains a fairly constant amount of sugar, this sugar being glucose, or the same sugar found in the urine in cases of diabetes mellitus. For the blood-plasma this amount averages between 0.08 and 0.11 per cent., the upper limit being about 0.12 per cent.[2] For the total blood the figures are slightly lower, and any content above 0.11 per cent. is to be regarded as a hyperglycemia. This upper limit is rarely exceeded in health except as a result of feeding a large amount of carbohydrates at any one time, when much higher figures may be obtained in perfectly normal individuals.[3] The sugar of the blood is derived mainly from the carbohydrates of the food, the liver storing up the sugar absorbed from the intestine and holding it in the form of glycogen as a reserve, to be delivered to the blood in the form of glucose as it is needed to supply

From the Laboratory of Pharmacology, Department of Medicine, University of Oregon. Read before the Portland City and County Medical Society, Jan. 21, 1914.

[1] Bernard, Claude: Referred to in MacLeod: Diabetes: Its Pathological Physiology, London, 1913, p. 23.

[2] Renal Diabetes, editorial. THE JOURNAL A.M.A., Nov. 1, 1913, p. 1632.

[3] Jacobsen: Biochem. Ztschr., 1913, lxvi, 471: referred to in The Variations in the Content of Sugar in the Blood, editorial, THE JOURNAL A.M.A., Jan. 10, 1913, p. 131.