SUMMARY.

Among the most useful color tests for detection of formaldehyde are those in which phenylhydrazine is a principal reagent.

This paper describes two new modifications of one of these color tests. In the first potassium ferricyanide takes the place of ferric chloride, having the advantage of rapidity of operation as well as fuller development of the characteristic color.

In the second the subsidiary reagents are ammonium persulphate and sodium chloride. In both sulphuric acid instead of hydrochloric acid is used.

An original method of isolating the red coloring substance by extraction with petroleum ether or similar immiscible solvent is described, applicable to all three forms of the phenylhydrazine test No. 1 of A. Muth.

LABORATORY OF NELSON, BAKER AND CO.,
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THE USE OF INSULIN IN THE TREATMENT OF DIABETES MELLITUS.*

BY ROBERT W. KEETON.

It is proposed in this paper to discuss very briefly, from a historical viewpoint, the methods available for the treatment of diabetes prior to the introduction of insulin, the mechanism of insulin action, the clinical situations in which insulin has proven valuable, and a few questions of practical interest to the pharmacist.

DIABETIC TREATMENT PRIOR TO INSULIN THERAPY DIET RESTRICTION—FASTING.

This period extends from the time of the earliest investigators to 1919 and 1920. It was characterized by a wide variety of methods of management. The very multiplicity of these methods suggested that the key to the problem was still hidden. Out of these attempts grew some very thoroughly established clinical facts upon which all these methods were based. These may be stated:

1—Carbohydrates added to the diet of a diabetic who is showing sugar in the urine cause an increase in urinary sugar.

2—Fats added to the diet may cause the appearance of acetone in the urine or increase the quantity if it is present.

3—The diabetic gains in tolerance, if his urine can be maintained sugar-free. Conversely when sugar is persistently present in the urine the tolerance of a patient decreases.

4—Uncontrolled diabetes is a progressive disease and in the young often advances to a rapidly fatal termination.

With these facts before them the students of the subject were led, in all of their efforts to combat the disease, to the principle of diet restriction. The quantity of food fed must fall within the limits of the patient's tolerance. Van Noorden [1] was accustomed to use one or two fast days to prevent coma and he describes the immediate rapid fall of acidosis resulting. He spoke of these days quite aptly

* Read before November meeting of the Chicago Branch of the American Pharmaceutical Association.

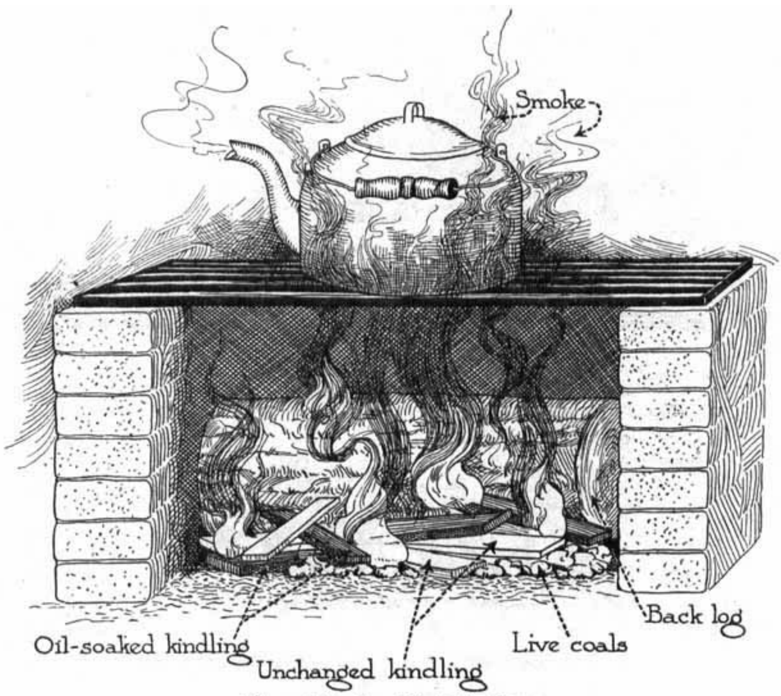
as "Metabolic Sundays." Naunyn [2] used a single "Fast Day" to clean up cases showing a resistant glycosuria. He also introduced the days, on which only green foods were served, as "Green Days." Guelpa [3], in 1909 and 1910, reported a series of 33 cases of diabetes treated successfully by fasts of three to four days' duration combined with the free use of cathartics (a bottle of Hunyadi Janos water). After desugarization he instituted a vegetable diet for two weeks, and he pointed out that such a diet continued in effect the fasting régime previously established. He further showed that he had found it necessary to repeat these periods of fasting three to four times in handling a given case over a year's time. His work failed to gain the recognition which it deserved because of the polemic on the value of purgation into which he was led. In 1915 Allen [4], working in the hospital of the Rockefeller Institute, applied this principle of fasting to a series of very severe cases. He showed that fasting was not only a safe but a decidedly practical measure for desugarization. Such fasts lasted from one to seven days. If the patient was not sugar-free at this time, he was fed up and fasted a second time. Joslin [5] has modified this procedure by first omitting the fats from the diet, then the proteins, and finally the carbohydrates. A week or ten days may be required to establish the patient in a complete fast. After desugarization the diet was built up slowly until the patient began to show sugar in the urine. This point determined his tolerance. If it was high enough to give him a maintenance diet then he would get along quite satisfactorily, otherwise he could only look forward to spending the short span of remaining years as comfortably as possible.

REPLACEMENT DIETS.

The second era of diet adjustment began with the studies of Newburgh and Marsh [6] in 1920 and 1921. They showed that a moderately severe diabetic would desugarize more readily or at least equally as readily if he were put on a very low calorific diet, which contained large quantities of fat, than if he were allowed to fast completely. In this type of diet the quantity of fat fed was often five times that of the carbohydrate. Formerly it had been considered the gravest error to use a diet in which the ratio of fat to carbohydrate was over two to one if the calories were below 1000. This teaching proved to be a veritable bombshell thrown into the ranks of those handling diabetes, the acceptance of which has waited upon a more complete understanding of the rationale of its action.

About this same time Shaffer [7] was studying the problem of acetone body formation and destruction (Ketogenesis and anti-Ketogenesis). He showed that when glucose is oxidized by hydrogen peroxide in alkaline solution aceto-acetic acid, if present, is also oxidized rapidly, whereas in the absence of glucose, aceto-acetic acid under otherwise the same conditions is rather resistant to oxidation. He concluded that one molecule of glucose accelerated the oxidation of one molecule of fatty acids. This in reality constituted the first experimental evidence of the rôle played by glucose in acidosis and furnished the first quantitative evidence on which a rational system of diet regulation might be founded.

During the spring of 1921, Woodyatt [8] attacked the problem from another angle. Prior to this time diets were planned on the basis that three distinct types of food were available and that these independently affected in certain ways the metabolism of the patient. Thus the clinician fed the patient so many grams of protein per day, but he lost sight of the transformations of the protein after it once entered the body. It had been definitely established by the studies in the nutrition laboratories that certain integral parts of the protein molecule (amino acids) are transformed into sugar, but the clinicians had not made use of this knowledge. So by a study of the available data, Woodyatt was able to construct formulas which expressed these transformations within the body. To the diabetic there are two important derivatives of food, glucose and fatty acids. Two diets apparently widely different in composition were shown to yield the same number of grams of glucose. The same findings were shown to exist with respect to fatty acids. Woodyatt was now in a position to apply to the other known laws of metabolism the studies of Shaffer with respect to fatty acid destruction and to prescribe an optimal maintenance diet, a diet on which a state of acidosis would not



Flame No. 1.—Diabetic flame.

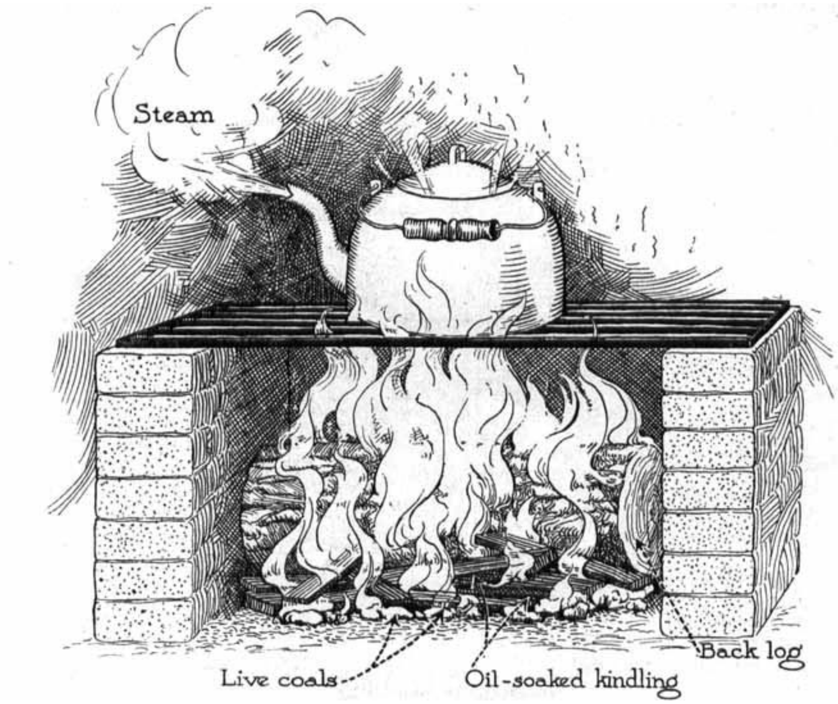
develop. The diets of Newburgh and Marsh showed much higher ratios than Shaffer's experiments would suggest and they were higher than the ratio derived clinically by Woodyatt. However they were of low caloric value and were designed to replace the period of fasting which was advocated by Allen and Joslin. Other observers tested out these diets and found that they lost their usefulness if their caloric value were increased to cover a maintenance work requirement. So Woodyatt derived the conception of a "Replacement Diet" and has defined it adequately. We need not at this time consider its definition in detail.

If a patient is fasting his metabolism is depressed but not stopped. He will derive energy from the food stored in the body tissues. Now if fasting will desugarize a patient, then the patient may be fed the same quantity of food that he would burn out of his tissue storehouse and he will desugarize equally readily.

This, then, is the reason that many of the Newburgh and Marsh diets worked well. They were replacement diets. The second period, then, of diabetic treatment was characterized by the development of fundamental conceptions of the construction of optimal maintenance diets and the development of the conception of "Replacement Diet."

MECHANISM OF INSULIN ACTION.

If we now direct our attention to the two diagrams presented we have a good conception of the way in which insulin acts. Flame No. 1 may be designated the diabetic flame. It will be noted that there are some coals of fire present which represent fuel in the process of complete combustion. The quantity of coals is small showing that in a diabetic there may be only a very small amount of food



Flame No. 2.—Normal flame.

undergoing complete oxidation. If these coals become extinguished death supervenes. On top of these are two types of kindling. One is lighter in color and is labeled "Unchanged Kindling." It is exposed to the coals and is surrounded by the flames, but it is not burning. This represents the sugar present in the tissues which has escaped oxidation and which will appear in the urine. The second type of kindling is dark in color. This is soaked in oil and is burning quite actively. The oil represents the insulin which has soaked into the glucose and has converted it into a form which will burn readily. The oil-soaked kindling therefore may be spoken of as insulin-soaked glucose or activated glucose. This fuel will undergo complete combustion and form the bed of coals. Then there is a back log which is smouldering and giving forth clouds of smoke. It is out of proportion to the coals and fire beneath it. This log represents the fats present either in the diet or body tissues. The smoke may be spoken of as the acetone bodies. In this group are included β-hydroxy-butyric acid, aceto-acetic acid, and acetone. The quantity of heat generated by such a flame is small, and so the transformation of this heat energy into steam by the kettle is small. The energy therefore which the diabetic body can develop is usually not large, and as will be seen later is very definitely limited. All forms of treatment, therefore, have as their object the development of a quantity of steam from the kettle which will prove adequate. In flame No. 2 we have the representation of a diabetic flame which has been transformed into a non-diabetic or normal one. The bed of coals is large. All the kindling (glucose) has been soaked in oil (insulin) and is burning completely. The back log (fat) is commensurate with the size of the fire beneath. It is burning cleanly without the production of smoke (acetone bodies). The energy developed through the agency of the kettle as shown by the escaping steam is abundant.

Suppose we refer to the sketch of the diabetic machine and consider what methods were available in the pre-insulin days for treating diabetes. The patient was desugarized by a period of complete fasting or by a period in which he was fed a replacement diet. In other words the sticks of kindling which were not burning and which represent the unburned sugar, present in the tissues and urine, were first removed from the fire. The back log of fat which was out of proportion to the quantity of active coals was replaced by a log of fat of smaller size. The flame was now burning cleanly and oxidizing to completion all the fuel fed. Its size had been reduced but the intensity of the flame had been increased. The patient no longer had sugar and acetone bodies appearing in the urine and accumulating in his tissues. He was in perfect balance, but he was a bed patient. The flame was not sufficiently large to furnish energy adequate for work. Next his tolerance must be established at a higher level. To do this the bed of coals was made greater by adding cautiously small sticks of kindling (sugar) until the flame was of sufficient intensity to allow an increase in the size of the back log. The kettle then boiled vigorously and the patient's tolerance was now established at the requisite level. If after the most careful management the fire could not be built to a size which would furnish adequate energy for work then the treatment of this period had reached its end. It had nothing further to offer. Expressed in the language of the clinic, we would say that when a patient's tolerance had attained its maximum, and when it was found that this tolerance was so low that an optimal work maintenance diet could not be constructed, the patient was diagnosed a hopeless diabetic. Either he must materially reduce his activities or remain permanently in the diabetic state.

With the advent of insulin theoretically a very simple means is offered for converting this smoking inefficient diabetic machine into a clean-burning normal machine producing adequate energy. The insulin, which is represented in our scheme by oil, can now be administered directly to the flame. It can be literally poured on the coals. The unburned kindling (glucose) will become soaked in it (insulin) and immediately burst into flames. The back log of fat will be ignited and the smoke will disappear. We therefore are not now limited in the size of the fire which we can build. We will build a flame at will which will produce the quantity of energy desired. Practically, however, it is not so easy to do this as it would seem from such a study.

CLINICAL SITUATION IN WHICH INSULIN HAS PROVEN VALUABLE.

1. Acidosis.—In acidosis the flame is almost extinguished by the smouldering back log of fat. There may be also a large amount of kindling (sugar) which is not burning. In this case the urine and all the tissues will contain quantities of sugar in addition to acetone bodies. This represents the usual case of acidosis. On the other hand the sugar may have been previously withdrawn from the patient and he may be in a state of profound acid intoxication and yet not show any sugar in the urine. Before insulin was available many good men treated such cases with huge doses of alkali and water by mouth. The alkali neutralized the acids. In terms of our diagram it simply decolorized or detoxicated the smoke. The water served to dilute the smoke. These measures gave the fire an opportunity to revive spontaneously. They did not correct the fundamental defect in the flame. With insulin we have a means available of stopping the formation of these acid bodies. It will be recalled that insulin changes the sugar in such a way that it will be oxidized or burned. It does not change the fats primarily. Consequently if insulin is to be effective there must be present in the organism an adequate supply of sugar. Just here the practical difficulties of handling a case of acidosis enter. If the patient is in a pre-coma stage he can then be fed sugar simultaneously with maximal insulin injections. The balance between the sugar and insulin can be followed by examination of the blood and urine for sugar at stated intervals. If the patient is in profound coma, then injections of sugar intravenously or subcutaneously may be practiced. The details of the management of such a case, which must be steered between the dangers of too much insulin with an associated hypoglycaemic reaction on the one hand and an inadequate insulin supply on the other hand, are too numerous to discuss now. One point must be clearly understood. *All coma cases cannot be revived and cannot be made to live.* The damage done to the organism through the acid intoxication and the alkali depletion may be irreparable before the patient is seen.

2. Dehydration States Resembling Coma.—An uncontrolled diabetic who is eating an unrestricted diet soon accumulates in the tissues and the body fluids quantities of sugar. The organism in attempting to rid itself of this sugar by way of the urine utilizes large amounts of water. Furthermore even the sugar which remains behind in the tissues requires for its solution appreciable quantities of water. If such a patient is examined he may be drowsy or very stuporous; a temperature of 101° to 102° may be recorded. On inspection the dry wrinkled skin is easily noted and the parched beefy tongue is observed. We realize that the patient is out of water balance. In fact he is in a state of acute dehydration which has involved the brain producing the stupor, and the muscles producing the fever. Such a case may simulate closely acidosis, but can be differentiated through the relatively high plasma reserve. Although it should properly be considered an emergency, it is not one which causes alarm. In three to four days' time under moderate daily doses of insulin the patient may be entirely normal.

3. Cases with Tolerance Inadequate for Maintenance.—These are the cases usually classified as "Very Severe Diabetes" and for which there was formerly no treatment. They vary in intensity from cases of total diabetes to those which may live for a comparatively long time but which are always showing the signs of the progress of the disease. If a patient has a residual tolerance of fifty grams of glucose per day, his tolerance may be raised by the use of insulin to 100 grams or to a quantity which will maintain him. Practically all the investigators are a unit on one point. A patient's diet should not be raised by insulin to a higher level than is necessary for the maintenance of his routine activities. Over-nourishment and excessive fattening are bad. If the patient becomes heavier and fatter his surface area is increased, his metabolism rises, and more food is required to maintain him at this level. Consequently the insulin dosage in turn must be increased. This may be classed as an economic reason for diet restriction. In the second place Allen insists that the lowering of the metabolism which comes with the maintenance of a patient on a low level diet is beneficial for his tolerance. In the third place it must be realized that a management which will keep the urine sugar-free must be one which will present the glucose and the insulin to the tissues at the same time. In other words their absorption must be simultaneous. For this reason the maintenance of a state of *a*-glycosuria is very difficult if the glucose fed is far in excess of the patient's residual tolerance.

4. The Young Diabetic.—These cases formerly were considered practically hopeless and usually became rapidly severe. The reason for this is not found in the severity of the disease *per se* but rather in a difference in the metabolism of the youthful organism. Thus a child derives a very large amount of its energy from carbohydrates. A healthy child deprived of food for a comparatively short time shows acetone in the urine. He utilizes the available carbohydrate but is not able to handle the fat. A slight infection in children is frequently associated with a surprisingly large amount of urinary acetone. In the adult these same states of inanition and infection are not accompanied by signs of incomplete fat oxidation. Either the adult has a larger available quantity of stored carbohydrate, which is improbable, or he is able to burn the fat more readily. Further, a child is an actively growing organism and requires more energy per kilo body weight to maintain himself than does an adult. These two situations can be best understood by considering three diets. A girl eight years of age requires approximately 1800 calories for maintenance and growth. In order that her urine be kept acetone-free she must have a tolerance of 150 grams of glucose. A boy twelve years of age requires 2100 to 2300 calories for development. His ability to burn fat is greater so that he can secure these additional calories with the same glucose tolerance of 150 grams. An adult man weighing 190 pounds and who has a tolerance of 150 grams of glucose can burn enough additional fat so that his diet may be raised to 2586 calories. This will enable him to do moderate work. It will be seen from these considerations that the youthful diabetic is really comparable to the situation discussed under Section 3 and for this class of cases there was no treatment prior to insulin therapy.

5. Operative Emergencies.—It is usually stated that a diabetic who is in perfect metabolic balance differs from a normal person only in the ease with which he gets out of balance. While in balance his tissues that may be damaged heal readily and all the processes of life proceed in a normal fashion. Hence it is possible to do any necessary operative procedure on a diabetic, for in insulin we have an agent through which even a complete diabetic can be placed in metabolic balance. A wide variety of operations have already been performed on diabetics. In such a list are included Drainage of Ruptured Appendix, Removal of Chronic Appendix, Tonsillectomy, Extraction of Teeth, Amputation of Leg and Arm, Supra-pubic Cystostomy, and Drainage of Pelvic Abscess. It is needless to say that operative procedures are not undertaken unless a real emergency exists or unless one is convinced that the gain will definitely outweigh the risk assumed.

6. *Rapid Control of Diabetes.*—In the pre-insulin days approximately four to six weeks were required before the patient was well controlled and before he had begun to recover his tolerance satisfactorily. With insulin this period can be shortened to two weeks.

QUESTIONS OF INTEREST TO A PHARMACIST.

1. Does insulin cure diabetes?

Insulin is a product of the pancreas and other tissues and its absence or deficiency produces a condition designated clinically as *diabetes mellitus*. There is no evidence that insulin injections into the body either stimulate the pancreas to produce more insulin or influence in any way pathological processes active in the pancreas. The injection of insulin therefore controls but does not cure diabetes.

2. Does the oral administration of insulin, or extracts of the pancreas said to contain insulin, enable the diabetic to burn more sugar?

Insulin is destroyed by both pepsin, which is secreted by the stomach, and by trypsin which is secreted by the pancreas. Oral administration of insulin in salol coated tablets or otherwise is of no value.

3. Can insulin be *satisfactorily* administered at home?

The failure of the pancreas to produce insulin is a quantitative failure. It is total in a few patients and in the others it has a widely different value. Further, the unit of insulin may have a somewhat different value in two cases of diabetes. All of these factors must be measured. The patient must also be educated to coöperate in the management of his case. Therefore a hospitalization of the patient for two weeks is necessary. During this time he can be educated and all the variables can be measured.

4. Can insulin be *safely* administered to a patient at home?

It can be given safely to any patient at home to tide him over an emergency until such a time as he can be admitted to a hospital for study and education.

5. Can a patient eat anything he desires if he takes an injection of insulin?

Here again, if one realizes that diabetes is a quantitative disease, it can be seen that even though a patient takes a large dose of insulin he may so far overstep his tolerance with the subsequent food addition that he will do his tolerance great harm. You may answer this question with an emphatic "No."

6. Will a patient have to continue the insulin injections the remainder of his life?

This depends on the severity of the disease. A patient will suffer less inconvenience from the daily injections than he will from the disease. He might just as well object to the necessity of eating three meals a day for 365 days in the year. Your answer to such an objection is—"Eat or go hungry, take your choice." So the answer is "Inject insulin or suffer from diabetes, take your choice."

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ON CHOLESTERYL PALMITATE, A CONSTITUENT OF CORPUS LUTEUM.*

BY MERRILL C. HART AND FREDERICK W. HEYL.

Cholesterol and cholesteryl esters are extremely important physiological substances and one or both are found in nearly all living tissue. The cholesteryl esters are a very labile cell constituent and vary greatly under different pathological conditions. Thus in some cases the cholesteryl esters of the human suprarenal capsule are increased 5 to 7 fold and under others they are decreased to one-tenth of their normal amount, the content of free cholesterol remaining approximately the same. An increase in the ester content is also observed in chronic kidney disease, diabetes and pregnancy and a decrease in infection.[1] The cholesteryl ester content of the suprarenal capsule is also influenced by the injection of saponin into the blood stream.

Laudau and McNee[2] showed that in man the entire cholesterol content of the adrenals is decreased in phthisis, other infections and neoplasms, while it is increased in inanition, pedatrophy and circulatory disturbances. The variations are chiefly of the esters. These experiments seem to indicate that the adrenals are a storehouse for cholesteryl esters but are not producers of cholesterol. They are regarded as intermediatory organs for cholesterol metabolism. The liver, bone marrow, lymph glands and spleen also appear to have this function.

Kondo[3] showed that 39 per cent. of the total cholesterol of the liver was in the ester form. Lapworth[4] obtained cholesterol and cholesteryl esters from kidneys, the adrenals, a dermatoid cyst and the brain. More than 99 per cent. of the brain cholesterol was found to be uncombined.

That the cholesteryl ester of the blood varies greatly was shown by the work of E. Hermann and J. Newmann[5] who found in 1 kilogram of normal female blood

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