or glass tube containing either concentrated KCL solution or agar-KCL. It is necessary that the electrode supporter and hydrogen-gas-conductor fit tightly and the salt bridge loosely in the rubber stopper, the reason for the latter being to provide an exit for the escaping hydrogen gas. The electrode (8), of a conical form, may be made by twisting a 15–25 cm long 22 gauge platinum wire (other gauges of greater diameter may be used) around the apex of a conical object, leaving at the same time an open space between the turns of the wire of from 1 to 1.5 mm and one at the apex about 2 mm in diameter. The open spaces are left for the purpose of allowing the hydrogen gas to pass through and thus bathe the entire surface area of the electrode. The length of the wire extending above the apical end of the cone-electrode should ordinarily measure about 2 cm, 0.5 cm of which is fused into the basal end of the electrode supporter, where it comes in contact with the mercury (8) enclosed therein. Connection between the electrode and the potentiometer is made by means of a copper wire (1). The hydrogen-gas-conductor, as seen in Fig. 1, forms a curvature of 180 degrees at the basal end, terminating in a point whose aperature should ordinarily be between 0.75 and 0.50 mm in diameter (10). The pointed end of the hydrogen-gas-conductor should always be situated directly opposite the center of the hollow base of the electrode, the distance between the two not exceeding 1–2 mm. Connection between the hydrogen-gas-conductor and hydrogen gas generator is made by means of a rubber tube (2). The hydrogen-gas-conductor should always be disconnected from the rubber tube (2) at the end of each determination, the basal end turned to the side of the cone-electrode by twisting, rinsed with distilled water and then returned to its proper position.

For cleaning or platinizing the electrode, the electrode supporter should be removed from the rubber stopper; this precaution is essential because of the influence of the gases, liberated during both processes, on the rubber stopper.

The short period of time required for an electrode of this type to reach equilibrium may be due to the great surface area which it exposes. The surface area of a 15 cm length of 22-gauge platinum wire electrode is about 2.2 square centimeters and that of a 25 cm length 3.75 square centimeters. A surface area between 2.2 to 3.75 square centimeters for such an electrode is considerably greater than that possessed by other types of electrodes. The shape of the electrode is also advantageous because it receives all the hydrogen gas as it is delivered by the conductor, distributing it at the same time over its entire surface by the helicoid movement of the gas. The hydrogen-gas-conductor, because of its position near to the bottom of the cell, is able to produce considerable agitation of the contents of the cell (solution) by the upward movement of the bubbles of hydrogen gas. Such an agitation is of great advantage because it brings all the parts of the solution in contact with the electrode and at the same time does not necessitate the use of a mechanical shaker.

The volume of the solution that is generally needed for the determination of the hydrogen ion concentration with the above apparatus is between 5 and 10 cc. It is possible to adapt the apparatus for smaller volumes of solution by using a smaller cell and mounting the electrode supporter and hydrogen-gas-conductor on a rubber stopper of smaller diameter. The writer does not, however, recommend the use of smaller volumes of solution than 5–10 cc., because of the rapid changes to which such smaller volumes of solution are subjected by rapid changes in the environment.

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SPECIAL ARTICLES

INACTIVATION OF INSULIN BY GLUCOSE WITH A COMMENT ON THE GENESIS OF DIABETES[1]

The inactivation of insulin by hydrogen and hydrogen sulphide and its reactivation by exposure to oxygen, recently reported from this laboratory,[2] suggested the possibility that the pancreatic hormone might be inactivated by reducing sugars, thus explaining their antidoting effects and conceivably throwing some light on the genesis of diabetes. This conjecture was very promptly turned to reality; for upon the second trial it was found that the power of insulin thus treated *in vitro* to lower the blood sugar of normal fasting rabbits was very much weakened. Further experiments in which insulin and glucose were incubated together proved even more convincing.

A fairly pure insulin A_3P_5 (meaning purified by five reprecipitations with amyl alcohol) and Merck's C. P. dextrose were used. The dosage was identical in all tests. Likewise the amount of glucose in the controls was always equal to that used in the crucial experiment. The control was accomplished by injecting the glucose solution on one side of the animal's

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[2] Allen and Murlin, Proc. Soc. Exp. Biol. and Med., 1925, XXII, 492.

body and the separately incubated insulin on the other.

Starting with a 1 per cent. solution of the dextrose, insulin was mixed with it in such concentration that by injection of one rabbit unit of the hormone not more than 10 mgm of sugar would be conveyed into the animal. By separate tests it was found that at least one gram of the same dextrose was necessary to raise the blood sugar perceptibly. Incubation was for one hour at 37–40° C. The potency of the insulin was reduced to about one third its former strength. Proceeding to 0.5 per cent. dextrose and so on down it was found that even greater inactivation (or destruction) occurred at the range 0.3 to 0.5 per cent., but that at 0.2 per cent. only slight reduction of potency occurred. A crucial point therefore lies somewhere between 0.2 and 0.3 per cent. A single experiment with its control must suffice by way of illustration. Further details will be found in a paper by R. S. Allen and the writer which will be published at an early date in the *American Journal of Physiology.*

Prep. No.		Blood sugar change mgms.	Average blood sugar change mgms.
AS_5	0.3 per cent. dextrose and insulin incubated 1 hr.	−36	
		−17	
		− 8	
		− 4	−16
		−16	
		−21	
		− 9	
AS_5C	same incubated and injected separately.	−56	
		−56	−62
		−73	

Not all grades of insulin are equally affected by sugar in this way; for Lilly's Iletin in two trials was not inactivated by 0.5 per cent. dextrose incubated one hour and three and one quarter hours, respectively. Our own crude insulin, precipitated with sodium chloride,[3] but not further purified, also is not inactivated. It contains much protein besides salt. Purified insulin is extremely sensitive to inactivation from a number of causes. Recently we lost a large batch, which seriously crippled this study, by treatment of a finely emulsified preparation with dilute HCl. This confirms the observation of Abel and his collaborators reported in a recent number of SCIENCE.[4]

To prove beyond a peradventure that excess of glucose in the circulation may inactivate insulin there present in normal amount it is only necessary to repeat the *in vitro* experiments in blood plasma. These experiments in completed form would now be available but for the accident referred to above. Preliminary results, however, bear out the hypothesis.

These observations taken in conjunction with the inactivation by hydrogen and by hydrogen sulphide furnish another link in the evidence necessary to prove that glucose and mannose antidote an overdose of insulin by their reducing property. The experiments with glucose explain also why such large doses of insulin are necessary to produce effects in animals which have previously received large injections of sugar (Gabbe,[5] Lesser,[6] Burn and Dale,[7] *et al.*). They explain why glucose given to diabetic (McCann and Hannon)[8] or normal (Higgins)[9] persons often causes a depression of the respiratory quotient, while fructose does not (Higgins; Wilder, Boothby, *et al.*);[10] for, as we have found in preliminary experiments, fructose has little, if any, inactivating effect *in vitro.*

The bearing of these facts upon the genesis of diabetes in man are obvious, especially in view of the recent demonstration by Burn and Dale, which confirms earlier work by Mann and Magath[11] that the distinction between the normal and the diabetic organism disappears with removal of the viscera (liver).

The food overstrain hypothesis of F. M. Allen has itself been much overstrained. Thousands of cases of diabetes are on record without any microscopic evidence of degeneration of the islets of Langerhans, and in order to produce such degeneration Allen was obliged to reduce the mass of pancreas in his experimental animals enormously (in physiological terms). If the liver must be present to produce the diabetic state upon removal of the pancreas we have at least a partnership instead of an individual agency to keep us normal. May it not well be that both Minkowski and Van Noorden were right, in a measure? The former set up the "failure of combustion"; the latter the "over production" of sugar theory. Banting gave us the hormone necessary to combustion, thus supporting Minkowski; *but it is destroyed by too much sugar.*

[3] Allen, Piper, Kimball and Murlin, *ibid.*, 1923, XX, 519.

[4] Abel, Geiling, Alles and Raymond, SCIENCE, 1925, August 21, LXII, 170.

[5] Gabbe, E., *Klin. Wochenschr.*, 1924, III, 1, 612.

[6] Lesser, E. J., *Biochem. Zeitschrift*, 1924, CLIII, 39.

[7] Burn and Dale, *Journ. Physiol.*, 1924, LIX, 164.

[8] McCann and Hannon, Johns Hopkins Hosp. Bull., 1923, XXXIV, 73.

[9] Higgins, H. L., *Amer. Journ. Physiol.*, 1916, XLI, 258.

[10] Wilder, Boothby, *et al.*, *Journ. Metab. Research*, 1922, II, 701.

[11] Mann and Magath, Arch. Int. Med., 1923, XXXI, 797.

What is it the liver does, the pancreas gone, to produce diabetes? Whatever else it may be we know it permits too much sugar to circulate. We know it loses glycogen under these circumstances. If its power to store glycogen were lost by injury arising from the alimentary organs themselves, would not diabetes result, if the pancreas could not maintain the balance between insulin and sugar? McCann and Hannon have described two types of severe diabetes, one which responds with a lower, the other with a higher respiratory quotient when glucose is given. The former responds to dietary treatment with low protein and balance of keto—and antiketogenic factors; the latter does not. Conceivably the former represents a primary failure of the liver, the latter primary failure of the pancreas.

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OBSERVATIONS ON KIDNEY FUNCTION IN NECTURUS MACULOSUS[1]

IN a recent publication by Wearn and Richards,[2] qualitative analyses of fluid obtained from the glomerular capsules of frogs were reported. Sugar was demonstrated in protein-free glomerular fluid at a time when the bladder urine was sugar-free. Although these observations have been generally accepted as proof of glomerular filtration and tubular reabsorption, certain objections to the finality of this proof may be raised. The evidence presented by Wearn and Richards that the fluid so collected is not merely a tubular secretion backed up into the capsule is not clear. Granting that the fluid does come through the glomerular membrane, proof has yet to be offered that the process is one of filtration.

That the method of micro-manipulation is peculiarly adapted to the study of kidney function is apparent. Technical difficulties present themselves, however, as a result of the minute size of the capsules in the frog. More favorable material would render the method less tedious and more reliable. This has been realized in Necturus maculosus. The capsules in this form are pear-shaped, and while their size varies with functional activity, their average transverse diameter is about one millimeter, their capacity being about three to four cubic millimeters. A longitudinal row of capsules lies just beneath the peritoneal covering near the medial border of the ventral aspect of the kidney.

Detailed description of the technique employed in the work outlined in this report and in further work now in progress is reserved for future publication. In brief, however, the procedure is as follows. The animal is anesthetized by immersion in .15 per cent. solution of urethane, after which the brain is pithed and the ventral surface of the kidney exposed. The animal is fastened to a board and placed on the stage of a binocular dissecting microscope. Illumination is by reflected light from a carbon arc. This arrangement does not necessitate traction on the kidney, as is the case with transillumination. The intensity of this light is ample to permit following the corpuscles through the glomerular capillaries.

The glass pipettes used for collecting glomerular fluid have an inside diameter at the tip of 30 to 50μ, and are manipulated in a microdissector of the type described by Chambers in 1922. After puncturing the capsule the glomerular fluid is withdrawn into the pipette by a mercury system which affords accurate control of the fluid column. Upon emptying the capsule the pipette is withdrawn and the fluid transferred to a capillary tube for analysis. The pipette is in the capsule for a period of from two to five minutes. In every case the bladder is completely emptied before the collection of the glomerular fluid and at the close of the experiment.

The results of the analyses of the glomerular and bladder fluid are briefly as follows: Sugar was demonstrated in the protein-free glomerular fluid at a time when the bladder urine was sugar-free. The chloride content of the glomerular fluid is markedly higher than that of the bladder urine. The blood sugar content, as determined by the Shaffer-Hartmann method, ranges from 60 to 80 milligrams per 100 cubic centimeters.

That the fluid thus obtained had not backed up from the tubules is proved by the following experiments. A minute quantity of trypan blue solution is injected into a capsule and the course of the dye followed as an indication of the direction of fluid flow in the glomerulo-tubule system. The dye is plainly seen to move out of the capsule and down the tubule.

It is evident that the glomerular fluid has entered the capsule through the glomerular membrane. The available data, however, are not adequate to permit a decision as to whether or not the process is one of filtration. Work bearing on this and various other questions of renal function is now in progress on this form. We have demonstrated that catheterization of the tubule is possible. The work has not yet progressed to the stage where a report on the tubular fluid can be made.

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[2] J. T. Wearn and A. N. Richards, "Observations on the composition of glomerular urine, with particular reference to the problem of reabsorption in the renal tubules," *Amer. Journ. Physiol.*, Vol. 71, p. 209, 1924.