6067

Absorption of Insulin by Nasal Mucous Membrane.*

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The hypodermic injection is the undesirable feature in insulin therapy, and therefore limits its usefulness. Many workers have used oral therapy, viz.: (1) Administering insulin by all enteral and respiratory routes.[1] (2) Adding to insulin: serum, defibrinated blood, bile acids, bile salts, acids, or alcohol, to prevent its destruction or inactivation by the proteolytic ferments of the gastro-intestinal tract.[2] (3) Employing substitutes which possess insulin-like properties.[3] (4) Incorporating the insulin in enteric coated capsules to make it available for absorption after passing the stomach and jejunum.[4] All these have proven to be either toxic or of dubious value.[5]

We approached the problem by altering the permeability of the

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[1] Mauriac, P., and Gandy, A., *Compt. rend. Soc. de Biol.*, 1925, **93**, 1524. Harrison, G. A., *Quart. J. Med.*, 1927, **20**, 187. Christie, C. D., and Hanzal, R. F., *J. Clin. Invest.*, 1931, **10**, 787. Peskind, S., *J. Metab. Research*, 1924, **6**, 207. Wassermeyer, H., and Schäfer, A., *Klin. Wchn.*, 1929, **8**, 210. Korbsch, J., *Klin. Wchn.*, 1925, **4**, 2327. Miller, H. R., *Arch. Int. Med.*, 1926, **38**, 779. Bernhardt, H., and Strauch, C. B., *Z. f. Klin. Med.*, 1926, **104**, 767. Lévy, M. M., and Cordier, P., *Compt. rend. Soc. de Biol.*, 1925, **95**, 248.

[2] Winters, L. B., *J. Physiol.*, 1923, **58**, 18. Bollmann, J. L., and Mann, F. C., *Am. J. Med. Sci.*, 1932, **183**, 33. Lasch, F., and Brügel, S., *Arch. f. Exp. Path. u. Pharm.*, 1927, **120**, 144. Horsters, H., and Rothmann, H., *Arch. f. Exp. Path. u. Pharm.*, 1929, **142**, 261.

[3] Watanabe, C. K., *J. Biol. Chem.*, 1918, **65**, 253. Frank, E., Nothmann, M., and Wagner, A., *Klin. Wchn.*, 1926, **5**, 2100. Von Noorden, C., *Klin. Wchn.*, 1927, **6**, 1041. Blotner, H., and Murphy, W. P., *J. Am. Med. Assn.*, 1927, **94**, 1811. Collip, J. B., *J. Biol. Chem.*, 1923, **56**, 513. Allen, F. M., *J. Am. Med. Assn.*, 1927, **89**, 1577. Stein, H. B., Longwell, B. B., and Lewis, R. C., *Arch. Int. Med.*, 1931, **48**, 313. Leclere, H., *Presse Med.*, 1928, **36**, 1634. Kahnt, K., *Med. Welt.*, 1931, **5**, 886. Nye, J., and Fitzgerald, S., *Med. J. Australia*, 1928, **2**, 626. Gunn, J., and Morrison, D., *So. African Med. J.*, 1924, **22**, 522. Geiger, E., *Fort. d. Therap.*, 1931, **7**, 257. Long, M. L., and Bischoff, F., *J. Pharm. and Exp. Therap.*, 1930, **38**, 313. Cammidge, P. J., *Brit. Med. J.*, 1925, **2**, 1216; *Compt. rendu Soc. de Biol.*, 1930, **104**, 1029. John, H. J., *J. Metab. Research*, 1922, **7**, 489.

[4] Murlin, J. R., Sutter, C. C., Allen, R. S., and Piper, H. A., *Endocrinology*, 1924, **8**, 331. Murlin, J. R., and Gaebler, O. H., *J. Biol. Chem.*, 1925, **66**, 731.

[5] Guttman, J., and Kallfelz, F., *Klin. Wchn.*, 1929, **8**, 2246.

membrane to stimulate absorption of the insulin molecule. The method was based upon the following principles: 1. The membrane should not be exposed to ferments that inactivate insulin. 2. Permeability may be increased by elevating the temperature of the membrane.[6] 3. The rate of absorption can be speeded by heating the insulin solution. 4. A "piling up" of the molecule on the membrane may be prevented by lowering the surface tension of the insulin solution. 5. All adhering materials should be mechanically washed away from the membrane. 6. The surface of the membrane should be kept at a favorable pH.

The method consisted of: (1) The nose was irrigated with normal saline, at approximately pH 4, at 40-45°C. This was immediately followed by: (2) Either a solution of Saponin was applied to the mucous membrane, or a few drops of Saponin added to the insulin solution. (3) Insulin at 50°C., was then sprayed directly into the nose with an atomizer, the stem of which had been previously heated. (4) Small plegets of cotton were then inserted into the nares to prevent the patient from blowing out the solution.

100 units of U 100 insulin were employed in all experiments. The subjects were human diabetics. The experiments were performed in the post-absorptive period, at least 14 hours after the last meal. The blood sugars were determined during control periods of 1-3 hours before the application. Blood sugars were usually determined at 5, 15, 30, 60, 120, 180, and 240 minutes after the application, by the Folin-Wu method, in duplicate. No results were acceptable where the deviation between the duplicates was greater than 2%. Urinary sugars were estimated by the Benedict quantitative method.

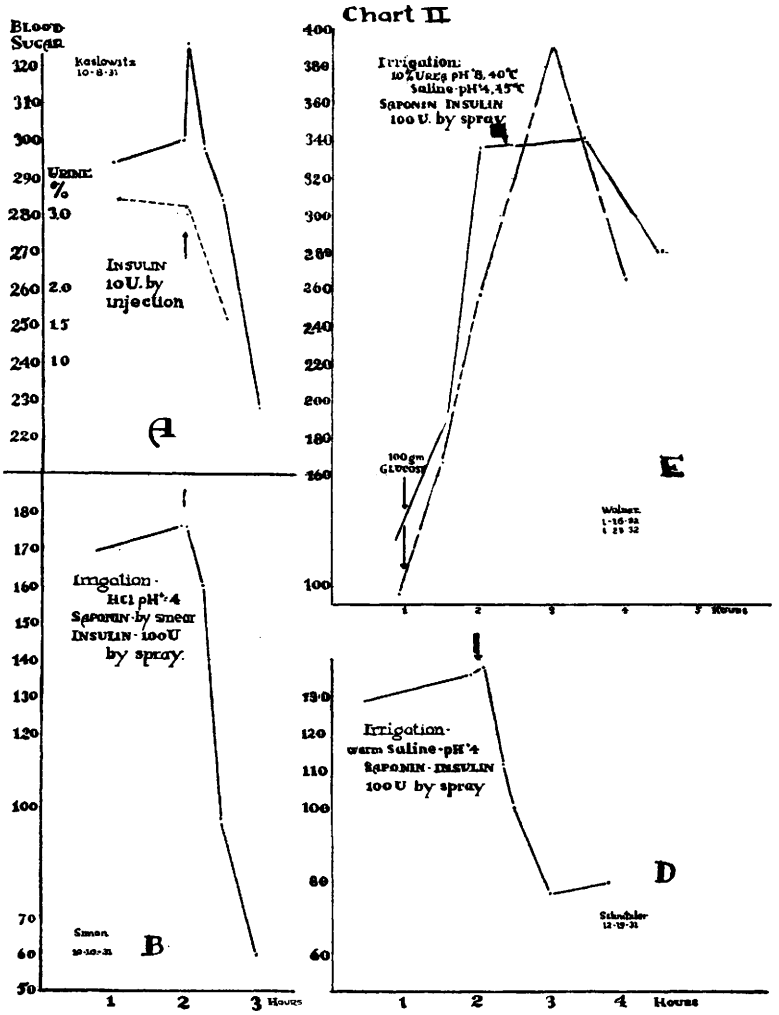
Illustrative examples of the results are given in Chart II. Ninety-six experiments were performed, with 11 failures, due either to poor irrigations, cooling of solutions, or incomplete spraying.

Mosenthal[7] reported that diabetics, if starved long enough, showed a progressive decline in blood sugar to a normal level. Macleod[8] has been confirmed in his observation that maximum depression in the blood sugar occurs within 60 minutes in the normal, and within 2 hours in the diabetic, regardless of insulin dose, or whether administered subcutaneously or intravenously. Therefore, to be sure of an insulin effect, one must know the preliminary curve

[6] Osterhout, W. J. V., Injury, Recovery and Death, in Relation to Conductivity and Permeability, Lippincott, 1922.

[7] Mosenthal, H. O., Tice-Practice of Medicine, Hagerstown, 1928, 69.

[8] Macleod, J. J. R., Carb. Metab. and Insulin, Longmans Green, 1926, 270.



A—Blood and urine sugars after the hypodermic injection of 10 units of insulin. B—Shows parallel precipitous drop in blood sugar after nasal treatment. E—After 100 gm. of glucose by mouth (broken line). One week later, same patient, 100 gm. of glucose, followed by nasal treatment. Note sudden interruption in the rise of the curve (full line). D—Is a characteristic curve produced by this method of therapy.

of the blood sugar, obtain a precipitous depression, and a maximum depression within 2 hours. Any attempt to interprete blood sugar depressions over longer periods of time subjects one to the possible criticism that it might be a starvation effect. Therefore the results of so many experiments in this field can not be adequately interpreted. Our method of nasal treatment produces curves which fulfill

every critical requirement, and strongly resemble those obtained by the subcutaneous injection of insulin.

Its clinical application is limited because this treatment produces a mild congestion in the mucous membrane of the nose, and symptoms of rhinitis. These symptoms usually last about one hour. Before this method is available for clinical use, one must establish the proper dosage. The authors are now investigating these problems.

6068

Antiurease Formation in the Hen.

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Although urease is very toxic when injected into mammals it has very little effect upon the hen unless urea is injected simultaneously. This is to be accounted for by the low urea content of hen's blood, which has been found to be about 1 mg. per 100 cc., or even less, in the 8 hens tested. From 800 to 17,000 units of urease were injected at one time, into a wing vein or directly into the heart. The white leghorn hens were observed following the injection and samples of blood and feces were analyzed for ammonia, urea, uric acid and urease. The injected urease disappeared from the blood within 4 hours after the injection and did not appear in the feces. Following injection of urease the blood urea disappeared entirely, its place being taken by ammonia. The uric acid content of hens' blood (2.0-4.0 mg. per 100 cc.) was not apparently affected by destruction of the urea, as might be expected if urea were a necessary precursor of uric acid. Examination of the feces showed that the reaction becomes slightly alkaline after the injection of urease.

Antiurease was formed in 8 hens by 4 to 10 injections of urease (each injection containing from 500 to 5,000 units) over a period of 30 to 50 days. Antiurease could be demonstrated in the blood about 14 days after the first injection. There was an incubation period of 7 days after the last injection. The amount of antiurease found in the hens, as determined by the method of Kirk and Sumner,[1] varied between 5 and 24 antiunits per cc. of serum. The chicken

[1] Kirk, J. S., and Sumner, J. B., *J. Biol. Chem.*, 1931, **94**, 21.