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Inactivation and reactivation of insulin.

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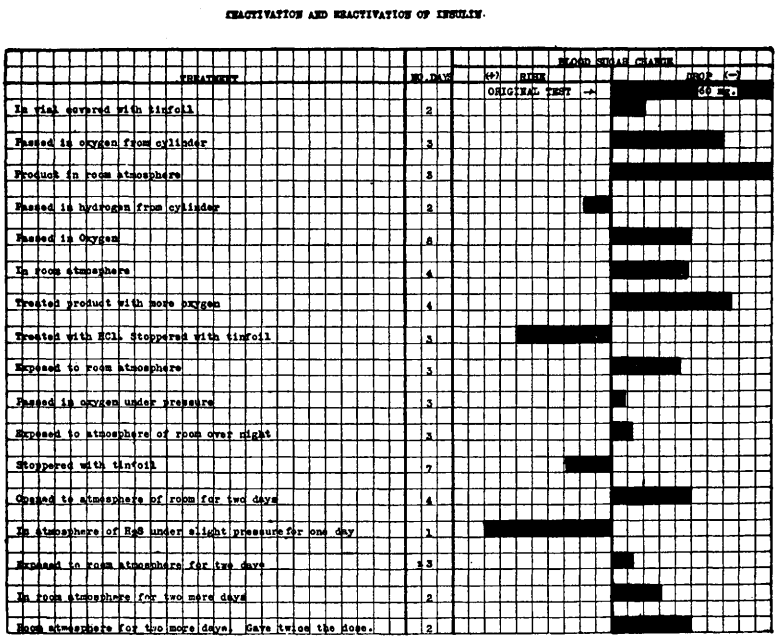
That insulin may be inactivated by treatment with reducing and oxidizing agents has been demonstrated by Shonle and Waldo.[1] They found that, when insulin was treated with a dilute solution of hydrogen peroxide or potassium permanganate, its glucopyretic property was entirely destroyed. They likewise observed that the activity was destroyed by action of such reducing agents as sodium bisulphide, sulphur dioxide, hydrogen, and stannous chloride. They were never able to recover the activity after it had once been lost by either oxidation or reduction as indicated. Dodds and Dickens[2] observed that the activity of insulin prepared by the picrate method was reduced and finally lost if subjected to formalin in various concentrations. Complete inactivation resulted from exposure to 10 per cent formalin at 37° for one hour. Destruction was much less rapid at a lower temperature. Dodds and Dickens believe that the inactivation was due to combination of formaldehyde with free NH_2 groups of the insulin protein. However, formaldehyde is a fairly good reducing agent and we suggest that the destruction of the glucopyretic property of insulin may equally well be accounted for by a reducing effect upon the insulin molecule. We have observed that fairly pure insulin products, as prepared by our acid aqueous extraction with heat, precipitation by sodium chloride, and purification by repeated reprecipitation with amyl alcohol[3] is extremely sensitive to oxidation or reduction. This was first noticed quite

[1] Shonle, H. A., and Waldo, H., *J. Biol. Chem.*, 1924, lviii, 736.

[2] Dodds, E. C., and Dickens, F., *The Brit. J. Exper. Path.*, 1924, v, 117-118.

[3] Allen, R. S., Piper, H. A., Kimball, C. P., and Murlin, John R., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 519-521.

by accident when a dried amyl alcohol precipitate was closed up in a vial, the stopper of which was covered with tinfoil. The precipitate had been dissolved in weak hydrochloric acid at an earlier stage, and it was noticed that the tinfoil became very black, due to action of the volatile acid. When the product was again tested, it was found to be almost devoid of potency. Removal of the stopper and exposure to air restored the potency. Proof that the inactivation was caused by free hydrogen was furnished by exposure to the commercial gas. A second and third time it was inactivated with hydrogen from tinfoil, and again restored by exposure to air or oxposure to oxygen under pressure. It was also inactivated by exposure to hydrogen sulphide, and then restored by exposure to air. What seems even more significant is that the reduced insulin on several occasions raised the blood sugar of rabbits very materially. Prolonged exposure to oxygen under pressure on one occasion destroyed a large part of the potency, but did not change the glucopyretic action to a glucagetic action. The chart gives the history of a single preparation.



All blood sugar tests were made in duplicate on rabbits fasted 18 hours, second blood was taken in two hours. In the chart each block of the abcissal distance represents 6 milligrams rise or fall.