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# Prolongation of Hyperglycemic Effect of Epinephrine in Rabbits by Addition of Zinc Chloride.

FRANCIS F. FOLDES.* (Introduced by L. Dienes.)

*From the Central Laboratory of the Jewish Hospital, Budapest.*

In the course of studies related to the prolongation of the action of subcutaneously injected medicines in man (Foldes[1]), it became desirable to investigate certain factors which influence the prolonging effect of the admixture of $ZnCl_2$ to these substances. The hyperglycemic response of rabbits to subcutaneous epinephrine seemed suitable for such investigation. That the addition of metal salts prolong the hyperglycemic activity of epinephrine has already been shown by Schwab[2] whose experiments served as a basis for the following observations.

*Methods and material.* Twelve rabbits, both male and female, were used for the experiments. Since the hyperglycemic response varied greatly, not only in different animals, but also in the same animal on different occasions, at least 4 animals were used for any single observation and each experiment was repeated on the same animal. An interval of at least one week was allowed to elapse between 2 epinephrine administrations on the same animal. The animals were kept in separate cages on uniform diet (oats, fresh green vegetables). Food and water were withheld for 18 hours before the experimental period.

Epinephrine hydrochloride was freshly prepared from epinephrine base on each occasion and ZnCl2 was added from a concentrated solution just before injection. The pH of the solution injected was around 5.5, its epinephrine content 0.1% and its $Zn^{++}$ concentration varied from 0.0% to 2.0%. The dose of epinephrine was 0.03 mg per kg of body weight. Larger doses were used only in a few instances, since they were apt to produce blood sugar values above 400 mg %. The determination of blood sugar values in this range, using the Hagedorn-Jensen method[3] employed in these experiments has been found difficult. The injections were made subcutaneously either in the nuchal or in the gluteal region. Care was taken to avoid intracutaneous injection of the material. For sugar determinations blood was taken in duplicate from the ear veins before and ½, 1, 2, 3, and 4 hours after the injections.

*Results.* In preliminary experiments it was found that the prolonging effect depends on the $Zn^{++}$ concentration of the injected solution and on the site of the injections. In rabbits $Zn^{++}$ concentrations below 0.4% produce no noticeable effect. As the concentration is increased the prolonging effect increases and reaches a maximum when the $Zn^{++}$ concentration is 1.2%. On further increase the hyperglycemic effect decreases and finally it ceases altogether. All this is true only if the injections are made in a region with dense subcutaneous tissue, *e.g.*, in the gluteal region. If the injections are made into the loose subcutis of the nuchal region the results are different. Here a $Zn^{++}$ concentration of 1.2% has practically no prolonging effect. This difference, depending on the site of the injection, can be seen from Fig. 1.

Studies with the solution containing $Zn^{++}$ concentration of 1.2% were made on 12 rabbits. The results are presented in Fig. 2. It can be seen from the data presented that in the first half hour following the injection of epinephrine alone, or epinephrine plus zinc, the hyperglycemic effect is practically the same. From there on, however, there is a marked difference in the behavior of the two curves. Following the injection of epinephrine plus zinc the maximum is reached

* Resident in Anesthesia in the Massachusetts General Hospital, Boston, Mass.

[1] Foldes, F. F., *J. Clin. Invest.*, 1943, **22**, 499.

[2] Schwab, H., *C. R. Acad. d. sc.*, 1937, **205**, 628.

[3] Hagedorn, H. C., and Jensen, B. N., *Bioch. Z.*, 1923, **135**, 46.

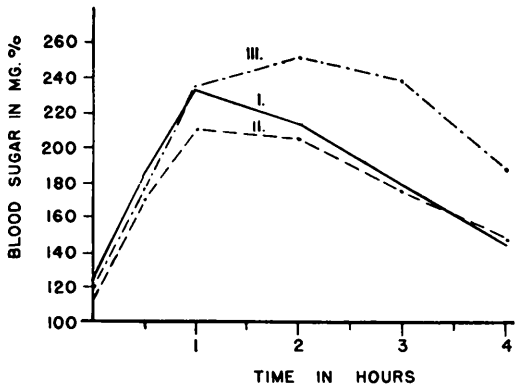


FIG. 1.

Combined blood sugar curves obtained on 4 rabbits: I. after the subcutaneous injection of 0.03 mg/kg of epinephrine alone in the nuchal region, II. after the subcutaneous injection of 0.03 mg/kg of epinephrine with 1.2% $Zn^{++}$ in the nuchal region, and III. after the subcutaneous injection of 0.03 mg/kg of epinephrine with 1.2% $Zn^{++}$ in the gluteal region.

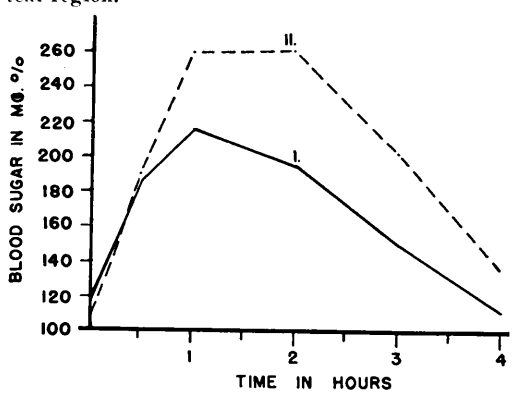


FIG. 2.

Combined blood sugar curves obtained on 12 rabbits: I. after subcutaneous injection of 0.03 mg/kg of epinephrine alone, and II. after subcutaneous injection of 0.03 mg/kg of epinephrine with 1.2% $Zn^{++}$.

later, the peak is higher, and the effect is more sustained, than after epinephrine alone. The absolute hyperglycemic effect, which can be measured by the surface area enclosed by the curve and 2 lines, one drawn through the starting point of the curve parallel with the X axis, and the other through the end point of the curve parallel to the Y axis, is 90% larger after the injection of epinephrine plus zinc, than after epinephrine alone. In a limited number of experiments similar results were obtained after the use of a 0.06 mg per kg dose. Not only was there a marked difference between the effect of this dose when administered alone or together with zinc, but the hyperglycemic effect of 0.06 mg per kg epinephrine plus zinc was superior to the hyperglycemic effect of 0.08 mg per kg epinephrine administered without zinc.

*Comment.* The experiments described corroborate the findings of Schwab in so far that a marked prolongation of the hyperglycemic effect of epinephrine has been observed after the admixture of $Zn^{++}$. Full comparison between Schwab's results and our findings cannot be made because of the lack of some data in his paper; namely, the number of rabbits used in his experiments is not stated; the dose of epinephrine and zinc injected is indicated but the concentration of the solution, which was found to be of great importance in the present work, is not given. The dose of epinephrine in his experiments was more than 8 times larger than our dose. The ratio between epinephrine and $Zn^{++}$ was 25:15 in his experiments and 1:12 in the present study. Notwithstanding the much larger dose of epinephrine used by him the hyperglycemic response (as interpreted from the curves presented in his papers) obtained was only slightly different from those obtained by us with the much smaller dose. The main difference between his curves and ours is that the maximum of the hyperglycemic effect is reached one hour later, both after the injection of epinephrine alone and epinephrine with $Zn^{++}$.

That higher blood sugar levels were attained after the injection of epinephrine plus zinc (the absorption of which is more delayed) than after the injection of epinephrine alone, on first sight seems paradoxical and therefore needs further explanation. It has been known that epinephrine is rapidly destroyed in the organism by the action of "amine oxidase" (Blaschko, *et al.*[4]) If the absorption of epinephrine is not delayed, then it enters the circulation more quickly and consequently is destroyed before it has time to mobilize liver glycogen to the fullest extent. For the development of the hyperglycemic effect, the presence of relatively small amounts of epinephrine over a longer period of time is of more importance than the pres-

[4] Blaschko, H., Richter, D., and Schlossmann, H., *J. Physiol.*, 1937, **90**, 1.

ence of larger amounts over a shorter period of time. Thus, it was found (Cori[5] *et al.*) that the continuous intravenous infusion of 0.00025 mm of epinephrine per minute produced maximal effect and that doubling or quadrupling of this amount produced no increased effect.

It is also well known (Cori[6]) that the intravenous injection of relatively large amounts of epinephrine is followed by hyperglycemia of short duration. By prolonging the absorption of epinephrine through the addition of zinc, epinephrine enters the circulation at a rate which is better for its economical utilization. This explains that epinephrine plus zinc not only has a longer lasting effect but that the maximum of this effect is also higher than when epinephrine is injected alone.

*Summary.* 1. The hyperglycemic effect of epinephrine in rabbits can be prolonged and increased by the admixture of $Zn^{++}$ (in the form of $ZnCl_2$). 2. The prolonging effect of $Zn^{++}$ depends on the concentration of the injected solution and the site of the injection. 3. Optimal results were obtained when the $Zn^{++}$ concentration of the injected solution was 1.2% and the injections were made in the subcutis of the gluteal region.

5 Cori, C. F., Cori, G. T., and Buchwald, K. N., *Am. J. Physiol.*, 1930, **93**, 273.

6 Cori, C. F., *Physiol. Rev.*, 1931, **11**, 143.

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## Relative Sensitivity of Different Phases of Growth Curve of *Bacterium salmonicida* to Alkaline Acriflavine.

WINSLOW WHITNEY SMITH. (Introduced by John F. Kessel.)

*From the Department of Agricultural Bacteriology, University of Wilsconsin, Madison, and Department of Bacteriology, University of Southern California, Los Angeles.*

The action of acriflavine solutions on *Bacterium salmonicida* has been the object of several studies[1,2,3] because of its efficacy in the prophylactic disinfection of the exterior of trout eggs to prevent the spread of *Bacterium salmonicida* infection (trout furunculosis).

None of these reports has recorded the action of organisms tested in the various phases of the growth curve. Furthermore, a search of the literature has revealed no data on the relative sensitivity to lethal agents of the growth phases of an organism grown near its optimum temperature as compared to organisms grown near the minimum.

Using a modification[3] of the method of Ruehle and Brewer[4] for testing disinfectants, *Bact. salmonicida* cultures incubated for various periods of time and at 2 different temperatures (15°C and 10°) were exposed to the action of 500 parts per million (p.p.m.) of alkaline acriflavine.* The tests of the 15° cultures were run at pH 7.7, those grown at 10° at pH 8.0. The disinfection studies were run at 10°.

Table I summarizes the tests and Fig. 1 presents the death curve of the cultures.

The cultures grown at 15° showed little variation. There was a slight but definite increase in resistance to the dye as the culture aged. A peculiarity (autoagglutination) of the test organism prevented the procurement

1 Blake, Isobel, *Fisheries, Scotland, Salmon Fish*, 1930, No. 2, His Majesty's Stationery Office, Edinburgh, 1933.

2 Gee, Lynn L., and Sarles, W. B., *J. Bact.*, 1942, **44**, 111.

3 Smith, Winslow Whitney, PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 324.

4 Ruehle, G. L. A., and Brewer, C. M., U. S. Dept. Agri., Circ. 198, 20 pp., Washington, D.C., 1931.

* Abbott Laboratories Acriflavine lot No. 539126 used in all tests.