

were autoclaved three times without interfering with the ability of the media to start and maintain the growth of the bacillus of symptomatic anthrax.

After autoclaving let the media cool, incubate for 24 to 48 hours, and the media is ready for inoculation. No titration or pH adjustment is necessary. The media keeps well; small jars containing a deep layer of it were good after having been kept at room temperature for several months.

Just why this media permits so much longer growth than others can not be stated with certainty. It is probably due in part to the high concentration of dextrose, and the presence of the calcium carbonate which neutralizes the butyric acid formed by the bacillus. That this neutralization is not complete is shown by the acid reaction of the culture filtrate, which has a pH of 5.2 to 5.8.

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Studies on the action of insulin neutralized with alkaline solutions.

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It was observed that the result of insulin injections varied with the route of administration. Using normal rabbits for our experiments, it was determined that if the injection was given intradermally the effect was more lasting than if an equal amount had been injected subcutaneously. These findings, the subject of an earlier paper, led to the question as to whether or not similar results can be obtained in the human body. Before proceeding with our studies it was found necessary to conduct comprehensive preliminary tests in animals, the results of which were important biologically and clinically. The following problems presented themselves:

(1) What pathologic changes does the intradermal injection of insulin cause in the skin?

(2) Are the changes following the intradermal injection of neutralized insulin of a different character?

(3) Is neutralized insulin less effective than the original preparation?

The tests which were to form the basis for the answer to the first two questions were made with intradermal and subcutaneous injections into normal rabbits and rats of the original preparation (iletin). The readings made at the end of $\frac{1}{2}$, 1, and $1\frac{1}{2}$ hour periods coincide with the observations of D. J. Bowie and W. L. Robinson.¹

Oedema and light degenerative processes were found especially in the tissue surrounding the subcutaneous vessels as a result of giving insulin intradermally as well as subcutaneously. Furthermore, degenerative processes in the walls of the vessels were determined, with beginning of complete thrombosis in these vessels. Some vessels showed complete thrombosis and complete destruction of greater parts of their walls. Beside these very marked pathological findings at the site of the involved vessels, there were observed degenerative changes, and even a small number of leucocytes and round cells in the surrounding tissues. The vessels not particularly injured were dilated, and contained more white cells than usual. In the epidermis special pathologic changes were missing. The changes in the subcutaneous tissues mentioned above were more marked after subcutaneous than after intradermal injections.

In a second series of similar tests in rabbits and rats, insulin was injected intradermally and subcutaneously after adding an equal amount of 1 percent sodium bicarbonate solution immediately before injection. The results of the histologic examination of these specimens were quite different. No thrombosis, no degeneration in the walls of the vessels were found, but the vessels were dilated. Small numbers of cells were found in these areas. No pathologic tissue changes were observed.

In a third series of animals injected with a solution of acetic acid of different strength, similar lesions were observed as in the first series, representing findings after the normal insulin injection.

These three series of animal tests will be discussed in detail

¹ Bowie, D. J., and Robinson, W. L., *J. Lab. and Clin. Med.*, 1923, viii, 5.

in a later paper, but it may be concluded that the main reason for the pathologic changes in the vicinity of the insulin deposit must be the small acid content of the insulin preparation which usually contains acetic acid.

The last question as to how insulin acts if neutralized immediately before injection was answered by two series of tests in normal rabbits. It was found that neutralization of insulin by sodium bicarbonate (1 percent solution) did not affect the insulin action at all. Intradermal as well as subcutaneous administration of neutral insulin did not show any decrease in its effect upon the blood sugar content. The curves of sugar determination in certain intervals were as low as those expected from the use of the original preparation.

A particular observation may be mentioned, namely that administration of neutralized insulin in some instances is followed by a greater effect than a like dose of the original acid agent. From further studies we assume that this phenomenon may be caused by the dilution of the drug. Insulin diluted by distilled water has in some instances a similar increased action.

From the result of the above studies it has been concluded that certain pathologic tissue changes in the vicinity of insulin deposits are caused by the acid content of the insulin preparation (iletin). Practically they are not very marked. They can be avoided by neutralizing insulin immediately before injection. This neutralization does not diminish the action of insulin upon the blood sugar content.