something more specific than the mere distance apart of the atoms, and that without doubt the details of atomic structure are involved.

I am indebted to my assistant, Mr. W. Koenig, for many of the readings on which these results are based.

EFFECT OF INSULIN ON THE LACTIC FERMENTATION

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A difficulty which seriously retards the progress of experimental investigations on insulin, as well as its preparation for clinical use, is that of determining even relatively the quantity present in solutions containing it. This research on its effect on the lactic fermentation was undertaken in the hope of developing a more satisfactory assay of insulin preparations than the animal-testing method affords. It was also thought that any effects observed might throw light on the mechanism of its physiological action. The research seemed promising since preliminary experiments had shown that addition of insulin considerably increased the total acid produced by the lactic fermentation as brought about by the organism Lactobacillus bulgaricus.

This research was made possible by a grant from the Carnegie Corporation of New York. We are also indebted to Eli Lilly and Company of Indianapolis for a supply of their purest insulin, to the Vitalait Laboratory of Pasadena for furnishing pure cultures of the lactic bacteria, to Miss Emma B. Mundy and Mr. John R. Adams of the Pasadena High School for bacteriological assistance, and to Messrs. G. A. Alles and A. L. Raymond of this Institute for valuable suggestions and for aid in preparing and testing the insulin preparations.

The fermentation experiments were made with mixtures that contained in 100 cc. of solution, 1 g. of glucose, 10 cc. of skimmed milk, 2 drops of a milk culture of Lactobacillus bulgaricus and varying amounts of insulin solution (or of a blank solution). The solutions were made up in glass-stoppered bottles, which were placed in a water thermostat at 32°, and which were either rotated on a horizontal shaft within the thermostat, or shaken by hand at fifteen-minute intervals for the first four hours and then occasionally till the solutions coagulated.

Two insulin samples were used, both of which were prepared from a highly concentrated aqueous solution furnished by Eli Lilly and Company. One (here called no. 1) was the precipitate obtained by adding a large

quantity of 95% ethyl alcohol; and the other (no. 2) by precipitating the alcoholic filtrate from this precipitate with ether. Both precipitates were impalpable powders, containing about 14% of nitrogen; no. 1 was light brown, and no. 2 almost snow-white.

The insulin activity of each sample was determined by finding the weight of it required, per kilo of rabbit weight, to produce in a majority of the large number of rabbits tested, the characteristic convulsions resulting from disappearance of the blood sugar; rabbits being used which had fasted for 16 to 24 hours before the tests, and which showed 110 to 120 mg. of glucose per 100 cc. of blood. The weights of samples no. 1 and no. 2 which produced this effect were found to be 0.10 mg. and 0.05 mg., respectively. Hence in sample no. 2 only half as much impurity was associated with a given quantity of insulin as in no. 1. The quantity added to 100 cc. of the fermentation mixtures was always 2.4 mg. of sample no. 1, or 1.2 mg. of sample no. 2, so that the mixtures contained about 24 times the quantity of active insulin required to produce rabbit convulsions under the above stated conditions. Before being added to the mixtures, these quantities of the samples (no. 2 being used in most cases) were dissolved in a little very dilute hydrochloric acid, and an equal volume of the same acid (called the "blank solution") was added to the control mixtures.

A fairly uniform distribution of the bacteria was secured by shaking the viscous milk culture with the skim-milk but even better checks in duplicate experiments were obtained by dropping the culture directly into the reaction bottles. Cultures were also made on a whey medium, which could be pipetted exactly, but the final accuracy obtained was no greater.

The fermentation was on the average allowed to continue for about 20 hours. The mixtures containing insulin began to coagulate about an hour sooner than the insulin-free controls. After the samples had coagulated so that the precipitates settled out quickly, the solutions were filtered; then 50 cc. portions were boiled for two minutes to expel carbon dioxide and at once titrated with 0.05 normal sodium hydroxide with the aid of phenolphthalein; from 6 to 7 cc. of this standard alkali being commonly required.

Several series of experiments made in this way showed that the mixtures containing insulin had produced, after the same interval of time, an amount of free acid greater than did the insulin-free mixtures, commonly by 20 to 25%, but occasionally by as little as 10% or by as much as 29%.

A set of experiments was made to determine directly the relative effects of the two insulin samples on the fermentation. The mixtures were so prepared as to contain the same quantity of rabbit-active insulin. Each sample increased the total acid by the same amount—namely, by about 20% more than in the mixtures which contained no insulin. This strongly

indicates that it is the insulin itself that accelerates the fermentation, and not the impurity associated with it, of which twice as much was present in one sample as in the other.

As a further control a set of experiments was made in which the effect of insulin inactivated by heating it on a boiling water-bath for 9 minutes with about 0.5 normal sodium hydroxide and then neutralizing the alkali, was compared with that of the same quantity of unheated insulin. It was found that, while the unheated insulin increased the yield of acid by 21%, the inactivated insulin gave the same result as the blank to which no insulin was added. In another set of experiments where the insulin was heated with only 0.01 normal sodium hydroxide to 100° for 15 minutes, the unheated insulin increased the acid yield by 26% and the heated insulin by 16%, over that in the blank experiment. This would indicate that the heated insulin was only partially inactivated; but it should be stated, that rabbit tests seemed to show that it had almost wholly lost its physiological activity—a result, however, that should be confirmed.

The preceding experiments were not carried out under completely sterile conditions, though precautions were taken to sterilize the flasks, water, glucose, and the skim-milk; and the insulin samples after precipitation from alcohol or ether had been immediately placed, and subsequently kept, in a vacuum desiccator. In order to make sure, however, that the effect on the fermentation was not due to bacteria introduced from the insulin sample, a solution of it was prepared and heated to 65° for 15 minutes. When plated both on nutrient agar and on lactose agar (with Andrade's indicator), it showed no growth in 48 hours; and when tested upon rabbits, it was found to have undergone little or no change in activity. Fermentation experiments made with this sterile insulin and under sterile manipulative conditions showed that it increased the yield of acid by 23%, or by about the same amount as in the preceding experiments.

A few other experiments may also be briefly mentioned. When a whey medium was substituted for the skim-milk, an increase of only 4% was observed, and the results were less concordant. When the concentration of the skim-milk and the quantity of culture were both reduced one-half, the increase in the amount of acid became negligible.

Insulin was found to increase the acid yield also when the fermentation was produced by Lactobacillus acidophilus; but in this case the increase was only about 8%.

The results of this investigation may be summed up in the statement that insulin has been proved to increase substantially (by 20–25 per cent) the quantity of acid produced by the fermentation of glucose under the influence of the organism Lactobacillus bulgaricus, and to a less extent under that of Lactobacillus acidophilus. Whether this effect is due to

stimulation of the growth of the organism, to increase in the decomposition of the glucose through co-enzyme action, or to some other cause, has not been determined. From the viewpoint also of developing a method of assaying insulin, the research is to be regarded as only a preliminary one; and it is to be continued in this direction, so as to find the best conditions for producing the maximum effect and for yielding concordant results in duplicate experiments, and to determine how the magnitude of the effect varies with the concentration of the insulin.

A NEW GROUP OF DYES FROM POISON GASES THROUGH THE 2-AMINOTHIAZOLES AS INTERMEDIATES. THE PREPARATION OF THIAZOLE DYES OF DOEBNER VIOLET TYPE[1]

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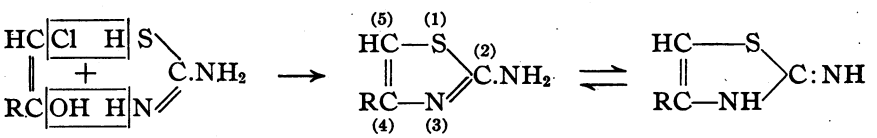
Halogenated aldehydes and ketones of the type $RCO.CH_2X \rightleftharpoons RC(OH):CHX$ (X = halogen) include some of the most powerful lachrymatory compounds known, such as the halogenated acetones and chloroacetophenone used during the war as "tear gases."

Many years ago it was discovered[2,3] that 2-aminothiazoles are obtained easily from such compounds by condensation with thioureas:



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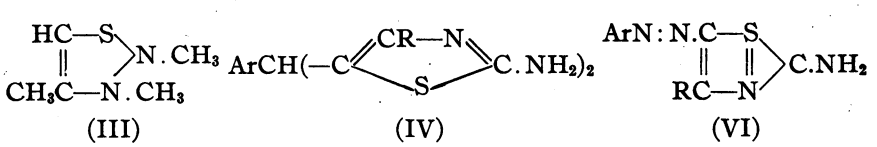

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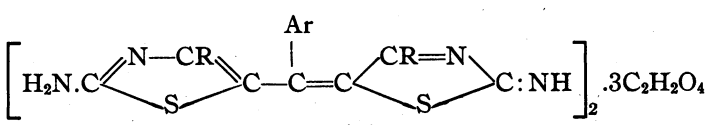

(V)