XCIH. STUDIES ON CARBOHYDRATE METABOLISM.

III. ON THE FORMATION OF AN ANTI-DIABETIC HORMONE BY THE ACTION OF A BACILLUS. (PRELIMINARY COMMUNICATION.)

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WORKING with samples of commercial yeast, it was found [Winter and Smith, 1923] that an extract could be prepared which had an effect similar to insulin when tested on normal animals and diabetic persons. Later it was found [Hutchinson, Smith and Winter, 1923] that extracts from different samples of commercial yeast differed widely in their activity. It was improbable that these differences were due to variations in the nutritional state of the yeast, and it seemed possible that the different commercial samples of yeast might contain quantities of organisms, which are responsible for the whole of the insulin-like activity. It has been found that certain yeasts in pure culture give appreciable quantities of an anti-diabetic hormone, while others are quite inactive in this respect. The possibility was not excluded, however, that there might be in commercial yeast some micro-organism, which was not a yeast, but which was capable of forming an insulin-like substance.

The following is an account of some work directed to test this possibility. The anti-diabetic hormone will be referred to as glucokinin, though it has been pointed out [Hutchinson, Smith and Winter, 1923] that the substance so named and described by Collip may be different from that which we propose to discuss.

A portion of a sample of yeast was extracted with alcohol and the activity of the extract tested on rabbits. The blood sugar determinations were made by Bang's old method. The effect on the blood sugar was to cause a moderate fall, as will be seen from the following experiment.

Rabbit weight 1·8 kg.		Blood sugar
Time		%
10·00		·14
10·10	Injected ·06 mg./g.	
12·00		·10
3·00		·12
5·30		·14

A portion of the same yeast was plated out on nutrient agar and a pure culture of a bacillus obtained. This was sown into 100 cc. peptone water containing 1 % glucose, and incubated at 37°. Rapid growth took place, and after two days alcohol was added to make the mixture 75 %. The fluid was placed in the ice-chest over-night, filtered, and concentrated to 20 cc. It was then made up to 80 % with alcohol and allowed to stand in the ice-chest for 12 hours. The supernatant fluid was then made up to 95 % with alcohol, when an appreciable precipitate was formed. Precipitation was complete in 24 hours at - 7°. The precipitate was washed with absolute alcohol, and dry ether, and dried. This powder was tested on rabbits, the standard injection being given (·06 mg./g. body weight). A marked fall in the blood sugar occurred. It appeared probable therefore that an appreciable amount of glucokinase had been formed by the action of the bacillus. Control experiments were performed in which the sterile medium was extracted with alcohol, and the resultant powder injected into a rabbit. No fall in the blood sugar occurred, though the yield of extract was similar in quantity.

Rabbit weight 1·9 kg.

Time	Blood sugar %
10·15	·12
10·30	Injected (bacillus extract)
11·45	·09
1·00	·08
3·30	·12

Rabbit weight 2 kg.

Time	Blood sugar %
10·00	·10
10·15	Injected (control)
11·30	·10
12·50	·11
2·30	·10

In the previous experiments in which the bacillus was grown in the glucose peptone water the reaction at the end of the experiment was found to be markedly acid. Since it is well known that phosphates are of importance in the metabolism of carbohydrate, the addition of phosphate was employed, both for this reason, and for the purpose of controlling the reaction of the fluid. The bacillus was sown in the following media.

I. 200 cc. peptone water containing 1 % glucose and 1 % Na_2HPO_4 .

II. The same as above, but with NaH_2PO_4 in place of the di-sodium salt.

Growth was allowed to continue for four days when the extracts were made as before. The activity was greater in the slightly alkaline medium, the following being results obtained from tests on rabbits.

Rabbit weight 2 kg. Na_2HPO_4 .

Time	Blood sugar %
11·00	·10
11·15	Injected
12·20	·08
3·00	·06
4·45	·05
6·00	·04
6·10	Convulsed
6·20	Glucose injected
6·45	Animal eating
9·00	·11

Rabbit weight 1·7 kg. NaH_2PO_4 .

Time	Blood sugar %
10·00	·09
10·15	Injected
11·30	·07
1·00	·09
3·00	·10

In another experiment in which tri-sodium phosphate was used, no growth occurred. In the above experiment in order to make the conditions com-

parable the reaction was adjusted in each case to p_H 5 before concentration *in vacuo*. Though it is uncertain whether the substance produced by the bacillus has similar properties to insulin, the known stability of insulin to acid suggested that concentration should preferably be carried out in acid solution. We have since found, however, that concentration can be carried out in a slightly alkaline medium. Some further experiments have been performed with a view to comparing the yield of active principle obtained under different conditions. The strength of glucose in the peptone water was varied from 1 to 2 %. No appreciable variation in the activity was found. In another experiment the bacillus was sown in peptone water without glucose in the presence of 1 % Na_2HPO_4 . Growth was allowed to continue for four days. Moderate activity resulted. The following shows the results of experiments with batches grown with and without glucose.

With glucose			Without glucose		
Rabbit weight 2 kg.			Rabbit weight 2.5 kg.		
Time		Blood sugar %	Time		Blood sugar %
11.00		.10	10.00		.11
11.15	Injected		10.15	Injected	
12.20		.08	11.30		.08
2.30		.06	12.30		.07
4.30		.05	2.00		.09
6.00		.04	3.30		.10
6.10	Convulsions				
6.20	Glucose				
6.35	Animal eating				
8.00		.11			

To determine the effect of aeration on the production of glucokinin, the bacillus was sown in peptone water containing 1 % glucose and di-sodium phosphate. The mixture was incubated at 37° , a stream of sterile air being drawn through the mixture for two days. Abundant growth took place. At the end of this period the action was stopped, and the usual alcoholic extract made. On testing this however on rabbits it was found that the activity was much diminished.

In view of the considerable quantities of alcohol necessary, experiments were carried out in which the medium in which the bacillus had grown was concentrated *in vacuo* without preliminary treatment with alcohol. 250 cc. of medium were concentrated to 25 cc. and made up to 80 % with alcohol. The precipitate formed was very large. When this had settled the supernatant fluid was made up to 95 % alcohol as before. The resulting precipitate contained only a small amount of active principle. Presumably the first precipitation in a large quantity of alcohol tends to remove interfering substances without causing appreciable loss of glucokinin, but when these substances are precipitated in a small quantity of 80 % alcohol the glucokinin is largely carried down with them. Further experiments are being made in order to remove these substances in the first stage by another method without the use of alcohol.

DISCUSSION.

That the convulsions caused by the injection of an extract of the medium in which the bacillus had been grown were not due to a toxic effect was shown by the fact that the animals invariably recovered as a result of injection of glucose. The effect therefore would seem to be similar to that produced by insulin or an extract of yeast with consequent hypoglycaemic convulsions. The active principle obtained by the action of the bacillus would appear to be similar to that obtained from yeast since in both cases the same delayed action is noticed. Convulsions usually occurred six hours after injection in the case of an active extract. Only one injection was necessary to cause convulsions in the case of such an extract.

The order of the yield obtained is as follows. 200 cc. of medium in which growth had taken place for four days yielded 0.6 g. of powder of which 120 mg. caused convulsions in a rabbit of 2 kilos.

We have not yet identified the organism used in these experiments. It is a short coli-form bacillus, producing acid and gas from glucose or lactose in 12 hours. It does not liquefy gelatin. Similar experiments carried out with a strain of *B. coli* (for this we are indebted to Miss M. Stephenson) showed that no active principle was formed. Experiments are in progress to determine whether other bacilli of this group can form glucokinin.

SUMMARY.

1. An insulin-like substance is formed by the action of a coli-form bacillus.
2. Injection of the extract into rabbits causes hypoglycaemic convulsions which are relieved by glucose.

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REFERENCES.

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