

assumed that approximately half the glucose derived from protein is used up in burning the ketogenic material from the  $\alpha$ -amino acids leucine, tyrosine and phenyl alanin occurring in the same protein; no allowance is made in the expression for the possible antiketogenic effect of the glycerol radicle present in the fats.

Diets high in fat were fed to a normal subject, and to arthritic patients undergoing the Pemberton<sup>1</sup> treatment. These diets were based on that suggested by Shaffer<sup>2</sup> which contained 10 per cent. of the total calories as protein, 10 per cent. as carbohydrate, and 80 per cent. as fat. The degree of acetonuria which corresponded with each diet was determined, and the results compared with the numerical values of this ratio.

From a study of these values which corresponded with a very mild degree of acetonuria it was concluded: one, that the phenomenon of ketogenesis could properly be regarded as a molecular reaction between ketogenic and antiketogenic compounds in the diet; two, that protein entered into the reaction only to the extent of the glucose which could be derived from the  $\alpha$ -amino acids contained in it; three, that the glycerol radicle of fat figured as a source of antiketogenic material only to the extent to which glucose could be derived from it; and, probably, four, that the glycerol radicle probably did figure as a source of antiketogenic material to the extent to which it could yield glucose.

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### **The glycogen content of the tissues of diabetic animals and the influence of adrenalin thereon.**

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In a series of experiments on dogs rendered diabetic by means of phlorhizin, the glycogen content of the muscles was studied immediately after the animals were killed. The muscles of thirteen animals were analyzed at the end of two days of glucosuria.

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<sup>1</sup> Pemberton, R., *Am. J. Med. Sci.*, 1917, cliii, 678.

<sup>2</sup> Shaffer, P. A., *J. Biol. Chem.*, 1921, xlvii, 449; Woodyatt, R. T., *Arch. Int. Med.*, 1921, xviii, 125.

The average glycogen content was 482 mgs. per one hundred grams of muscle. The muscles of eleven animals at the end of the third day of glucosuria contained an average of 306 mgs. per one hundred grams. At the end of the fourth day, the muscles of twelve animals contained an average of 228 mgs. of glycogen per one hundred grams. At the end of the fifth day seven animals showed an average of 155 mgs. per one hundred grams of muscle, and at the end of the seventh day two animals showed the presence of 124 and 151 mgs. per one hundred grams of muscle.

The detailed results are summarized in Table I.

TABLE I.  
GLYCOGEN CONTENT OF MUSCLE OF DIABETIC DOGS.

Days of Glucosuria.

|                 | 2   | 3   | 4   | 5   | 7   |
|-----------------|-----|-----|-----|-----|-----|
|                 | 723 | 250 | 181 | 111 | 124 |
|                 | 626 | 381 | 237 | 097 | 151 |
|                 | 573 | 337 | 226 | 080 |     |
|                 | 415 | 499 | 200 | 315 |     |
|                 | 430 | 236 | 144 | 293 |     |
|                 | 325 | 305 | 130 | 110 |     |
|                 | 295 | 121 | 120 | 105 |     |
|                 | 447 | 192 | 297 |     |     |
|                 | 435 | 356 | 282 |     |     |
|                 | 528 | 378 | 319 |     |     |
|                 | 628 | 306 | 316 |     |     |
|                 | 407 |     | 286 |     |     |
|                 | 440 |     |     |     |     |
| Average . . . . | 482 | 306 | 228 | 155 | 138 |

Throughout these periods, the animals fasted and were given phlorhizin injections by the Coolen method (one gram daily in olive oil).

These findings prove that in spite of *continuous fasting and in spite of complete diabetes, the muscle cells will hold on to a certain amount of glycogen, which we may term residual glycogen.*

In a second series of experiments, diabetic phlorhizinized dogs were given injections of three to seven milligrams of adrenalin, and were killed twenty-four hours after that. The injections were made on the second or third day of glucosuria.

The analysis of the muscles of five dogs of this series showed that absolutely no glycogen was present in them, proving that

*adrenalin will cause a complete discharge of all the glycogen from the muscle cells.*

In a third series of experiments, diabetic dogs were rendered glycogen free as in the second series. They were then given substances like glycoll and alanin, propionic and lactic acids, all of which are known to be converted quantitatively into glucose. Not in one single instance was the glucose elimination from the adrenalinized animals in any way comparable to the amounts that are excreted by animals not treated with adrenalin. In other words, either there is interference with sugar formation in the absence of the "residual" glycogen or the glucose that is formed from glucogenetic substances is utilized by these cells, and not excreted in the urine.

Table II is illustrative of this point.

TABLE II.  
INFLUENCE OF DEGLYCOGENIZATION ON EXTRA GLUCOSE FORMATION  
FROM LACTIC AND PROPIONIC ACIDS.

*Experiment 92. Phlorhizinized Dog. Twelve Hour Periods.*

| Period.   | Weight<br>in Kg. | N.   | G.    | G : N. | Extra<br>Glucose. | Remarks.   |
|-----------|------------------|------|-------|--------|-------------------|--|
| I.....    | 13.68            | 2.34 | 20.54 | 8.78   |                   | First day of phlorhizin glu-<br>cosuria.   |
| II.....   |                  | 4.29 | 42.52 | 9.92   |                   | 6 mg. adrenalin injected.  |
| III.....  | 13.23            | 6.77 | 21.86 | 3.08   |                   | 6 mg. adrenalin injected.  |
| IV.....   |                  | 6.66 | 18.66 | 2.80   |                   |  |
| V.....    | 12.83            | 7.29 | 20.59 | 2.83   |                   |  |
| VI.....   |                  | 6.04 | 22.40 | 3.71   | 4.28              | 9.0 gms. of lactic acid as<br>sodium salt dissolved in<br>24 c.c. water given sub-<br>cutaneously. |
| VII.....  | 12.20            | 5.62 | 17.97 | 3.20   |                   |  |
| VIII..... |                  | 6.47 | 20.70 | 3.20   |                   |  |
| IX.....   | 11.95            | 5.79 | 17.58 | 3.04   |                   |  |
| X.....    |                  | 4.88 | 14.72 | 3.02   |                   |  |
| XI.....   | 11.48            | 4.80 | 18.38 | 3.83   | 2.75              | 7.4 gms. propionic acid as<br>sodium salt dissolved in<br>20 c.c. water given sub-<br>cutaneously. |
| XII.....  |                  | 4.41 | 15.40 | 3.50   |                   |  |
| XIII..... |                  | 4.51 | 15.16 | 3.36   |                   |  |

At end of period XIII, ether anesthesia, animal bled to death. Glycogen in muscles 0.039, 0.038 per cent.

In this experiment the giving of adrenalin during period II is followed by a sweeping out of all the residual glycogen, as is

shown by the high G : N ratio. The giving of adrenalin during period III is followed by no more extra glucose elimination. In period VI, 9.0 grams (M/10) of lactic acid as sodium salt was administered subcutaneously. Only 4.28 grams of extra glucose were excreted in the urine, which is exactly one half of that which is usually obtained. In period XI 7.4 grams (M/10) of propionic acid were given subcutaneously. During this period only 2.75 grams of extra glucose were excreted, which is less than one third of the usual.

In 1913, Ringer and Frankel<sup>1</sup> found that the administration of acetaldehyde and propyldehyde to phlorhizinized dogs was followed by a marked drop in the formation of acetone bodies and by a rise in the glucose elimination. Explanation for the formation of sugar after propylaldehyde could be found very easily by assuming direct conversion, since it had already been established that both propylalcohol and propionic acid could give rise to glucose, but for glucose formation directly from acetaldehyde no chemical basis could be found. They therefore had to look for other channels of glucose formation after acetaldehyde administration. They suggested the possibility that the marked antiketogenetic effect of acetaldehyde and glucogenetic effect might be coupled together and that it was possible for acetaldehyde in the body to form a chemical union with either  $\beta$ -hydroxybutyric acid or acetoacetic acid, forming a compound which is glucogenetic. This hypothesis seemed to harmonize with all the facts and seemed to explain both the antiketogenetic effects of the acetaldehyde as well as the glucogenetic.

About a year later Sansum and Woodyatt<sup>2</sup> published a series of experiments in which they showed that the administering of ether or nitrous oxide by inhalation to phlorhizinized animals was followed by a marked increase in the glucose excretion, proving that the animals were not entirely glycogen free. They then treated their animals with adrenalin, to the point when they no longer excreted extra glucose, and then gave them acetaldehyde. The administration of acetaldehyde to those dogs was followed by very insignificant or no extra glucose elimination. From these

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<sup>1</sup> Ringer, A. I., and Frankel, E. M., *Jour. Biol. Chem.*, 1914, xvi, 563-579.

<sup>2</sup> Sansum, W. D., and Woodyatt, R. T., *Jour. Biol. Chem.*, 1915, xxi, 1-21.

experiments they concluded that acetaldehyde acts similarly to ether and nitrous oxide, by virtue of its hypnotic effects.

We cannot accept Sansum and Woodyatt's application of their results to acetaldehyde for a number of reasons.

I. Because in the doses given, acetaldehyde has no hypnotic effects, whereas the ether and nitrous oxide render the animals unconscious.

II. The giving of ethyl alcohol and a number of other substances to the point of complete hypnosis did not produce any extra glucose elimination, proving that it is more than hypnosis that is affecting the residual glycogen.

III. In the third series of our experiments we found that substances like glycocoll, alanin, lactic and propionic acids when administered to adrenalinized dogs yield less than one half the amount of glucose than they do ordinarily, and that a number of glucogenetic substances would never have been detected if Sansum and Woodyatt's technique were followed.

Glycogen is the most mobile food stuff that the body possesses, and if we find that after five and seven days of starvation and complete diabetes the body cells still cling to this residual glycogen, in spite of that tremendous demand for it, and still hold on to about 150 mgs. per 100 grams of muscle, it must have a different significance in the cell economy from the ordinary glycogen that moves in and out of the cell. After an animal is deglycogenized by means of adrenalin there must be established a state of "glycogen hunger." When a glucogenetic substance is given during that period we can readily conceive of that glucose being retained in part to supply that glycogen. This is how we would interpret the failure of Sansum and Woodyatt to obtain extra glucose from acetaldehyde.

In a fourth series of experiments we rendered dogs glycogen free as described by Sansum and Woodyatt and which method we have proven in the second series of these experiments actually does free the animals from glycogen. We then allowed the animals to continue fasting and be diabetic by means of phlorhizin. The animals were killed three days after the deglycogenization and the muscles were found to contain the following amounts of glycogen, 0.020, 0.033, 0.023 and 0.039 gm. per 100 grams. In one animal

which was kept for five days after deglycogenization 0.069 gram of glycogen was found per 100 grams of muscle.

These experiments prove that fasting diabetic dogs, even after they have been completely deglycogenized, possess the power of glycogen formation. Glucogenetic substances therefore can well be administered to animals without giving rise to extra glucose in the urine.

#### CONCLUSION.

I. Diabetic dogs contain glycogen in their muscles to the extent of 0.150 per cent. even after seven days of fasting and diabetes; residual glycogen.

II. This glycogen can be completely driven out by means of adrenalin.

III. Deglycogenized diabetic animals even during a period of prolonged fasting and diabetes are capable of reforming their lost residual glycogen.

IV. Failure on the part of an animal to show extra glucose elimination during the period of deglycogenization does not mean that the substance is not glucogenetic.

V. The conclusions of Sansum and Woodyatt that acetaldehyde is not glucogenetic nor antiketogenetic are objected to as invalid.

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#### Concerning antiketogenesis.

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It is a well-known fact that the withdrawal of carbohydrates from the diet of normal individuals is followed by the appearance of ketone bodies in the urine. Individuals that have interference with their power to utilize carbohydrates, as diabetics, develop degrees of ketonuria that are proportional to the severity of the disturbances in their carbohydrate metabolism. It is also established definitely that these ketone bodies are formed normally in the intermediary metabolism of fat and of certain amino acids, and that with the oxidation of carbohydrates the ketone bodies suffer oxidation. The carbohydrates therefore are known as anti-ketogenetic.