# THE CHEMISTRY OF GLUCONEOGENESIS.

## VII. CONCERNING THE FATE OF PYRUVIC ACID IN METABOLISM.[1]

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The fate of pyruvic acid in the animal body has been the subject of considerable discussion in the past two years. P. Mayer[2] administered 7 to 8 grams of pyruvic acid to normal rabbits and found that the animals, when in a good state of nutrition, developed hyperglucaemia and glucosuria, while those that had been starved, and were therefore poor in glycogen content, developed hyperglucaemia only and no glucosuria. In those experiments he found that the urine often contained albumin after pyruvic acid administration, and further found that the administration of 10 to 15 grams brought about fatal intoxication. After pyruvic acid administration, he also found lactic acid in the urine.

In a second communication,[3] which appeared after our work was far advanced, Mayer reported the influence of pyruvic acid on gluconeogenesis of phlorhizinized dogs and rabbits. In none of his experiments was there an increase in the glucose elimination after pyruvic acid administration, while in two experiments on dogs (out of four) there was a very remarkable reduction in the glucose and nitrogen elimination. The kidneys of the dog in experiment 10 were examined microscopically and the following pathological conditions were found: "kalkhaltige Cylinder in den geraden Kanälen der Papille, Trübung und geronnene Massen in den Tubuli contorti, Hämoglobinniederschläge." He then drew the conclusion that pyruvic acid is a toxic substance, which causes a

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[2] P. Mayer: *Biochem. Zeitschr.*, xl, p. 441, 1912.

[3] P. Mayer: *ibid.*, xlix, p. 486, 1913.

depression in the nitrogen and glucose elimination by decreasing the permeability of the kidneys. He offered no explanation for the failure of pyruvic acid to bring about a decrease in the permeability of the kidneys in experiments 8 and 9, in which similar amounts of pyruvic acid were given, without any appreciable change in the glucose and nitrogen eliminations. In fact, there was a slight increase in both nitrogen and glucose output in experiment 9.

In a series of experiments reported by us[4] it was found that pyruvic acid, when given to phlorhizinized dogs *per os* or subcutaneously was not a toxic substance, and that it was glucogenetic. It is true that the glucogenetic properties of pyruvic acid were not found to be very constant, but in no case did we get the drop in nitrogen and sugar output as was observed by Mayer. Simultaneously with our communication Dakin and Janney[5] reported the results of their experiments from which they came to conclusions very similar to ours. They also found that pyruvic acid was glucogenetic, and in no case did they get a drop in the nitrogen and sugar output similar to that obtained by Mayer. Results similar to Dakin and Janney's and to ours have since been reported by Cremer.[6]

In three different laboratories experiments with pyruvic acid showed that it was glucogenetic and non-toxic. Mayer was the only one who obtained two negative results and two results which show very plainly that his pyruvic acid contained something that was toxic and had a peculiar effect upon the kidneys, an effect which resembles in its microscopical lesion as well as in its functional disturbance, the results that Underhill[7] and Pearce and Ringer[8] obtained after tartaric acid administration to phlorhizinized and normal dogs.

In his third communication on this subject[9] Mayer denies the presence of tartaric acid in his pyruvic acid, and suggests that the difference in our results may be due to polymerization of our pyruvic acid during the process of neutralization.

[4] Ringer: this *Journal*, xv, p. 145, 1913.
[5] Dakin and Janney: *ibid.*, xv, p. 177, 1913.
[6] Cremer: *Berl. klin. Wochenschr.*, 1913, No. 31.
[7] Underhill: this *Journal*, xii, p. 115, 1912.
[8] Pearce and Ringer: *Journ. of Med. Research*, xxix, p. 57, 1913.
[9] P. Mayer: *Biochem. Zeitschr.*, lv, p. 1, 1913.

This explanation is not valid for two reasons: First, Dakin and Janney[10] have shown that polymerized pyruvic acid is non-glucogenetic. Second, we have, as Mayer has, observed great care in the process of neutralization. To eliminate all doubt, however, we performed one experiment in which pyruvic acid was administered subcutaneously unneutralized. As is seen from the record of the experiment, it possesses distinct glucogenetic properties, and has no toxic effect on the kidneys.

EXPERIMENT XXXIII. *Twelve-hour periods.*

| DATE Oct. 1913 | PERIOD | WEIGHT | NITROGEN | GLUCOSE | D:N | EXTRA GLUCOSE | ACETONE AND ACETO-ACETIC ACID | β-HYDROXY BUTYRIC ACID | REMARKS |
|---|---|---|---|---|---|---|---|---|---|
| 15 | XI | 11.9 | 6.40 | 24.00 | 3.75 | | 0.262 | 1.27 | |
| 15 | XII | | **6.62** | **27.56** | **4.16** | **4.7** | **0.185** | **0.72** | 8.8 gms. of pyruvic acid dissolved in 3 cc. of olive oil given subcutaneously. |
| 16 | XIII | 11.5 | **6.18** | **25.14** | **4.07** | | **0.216** | **0.76** | |

We therefore still feel convinced that Mayer's results cannot be attributed to pyruvic acid, but to some extraneous influence.

*The fate of pyruvic acid in the animal body.*

In his first communication[11] Mayer showed that after the administration of pyruvic acid, *dl-* and *d*-lactic acid appeared in the urine. Embden and Oppenheimer[12] corroborated these findings. They perfused the extirpated surviving liver of dogs with blood to which pyruvic acid as ammonium or sodium salt had been added and found an increase in the lactic acid content of the perfused blood.

In another communication[13] Embden and Oppenheimer report their experiments on the influence of pyruvic acid on the formation

[10] Dakin and Janney: *loc. cit.*

[11] P. Mayer: *loc. cit.*

[12] Embden and Oppenheimer: *Biochem. Zeitschr.*, lv, p. 337, 1913.

[13] Embden and Oppenheimer: *ibid.*, xlv, p. 186, 1912.

of aceto-acetic acid in the perfused suviving liver of dogs. Twelve experiments were performed. Five gave no increase in aceto-acetic acid, and seven gave a very marked increase. They concluded that *pyruvic acid possesses the power of yielding aceto-acetic acid, because of the intermediary formation of acetaldehyde, which undergoes aldol condensation.*

From all this, we see that pyruvic acid can give rise to lactic acid on the one hand and to acetaldehyde on the other. In this connection it is important to remember that from Embden's experiments it is evident that *acetaldehyde is not always formed from pyruvic acid.*

In our experiments we found that *in some instances pyruvic acid yielded large quantities of glucose and in others it gave almost negative results.* On examining the relationship between the glucose formation and antiketogenesis in our experiments, a remarkable fact is evident: in case of high sugar formation from pyruvic acid, there is a marked depression in the acidosis (experiments XXII, XXIII and XXV); conversely, when there is little sugar formation, there is practically no change in the acidosis. Ringer and Frankel[14] have recently shown that when acetaldehyde is administered subcutaneously to phlorhizinized dogs, it possesses the power of causing an increase in the glucose elimination and a decrease in the acidosis.

On correlating all these facts it becomes evident that *pyruvic acid possesses its glucogenetic properties because acetaldehyde and lactic acid are formed in its intermediary metabolism.*

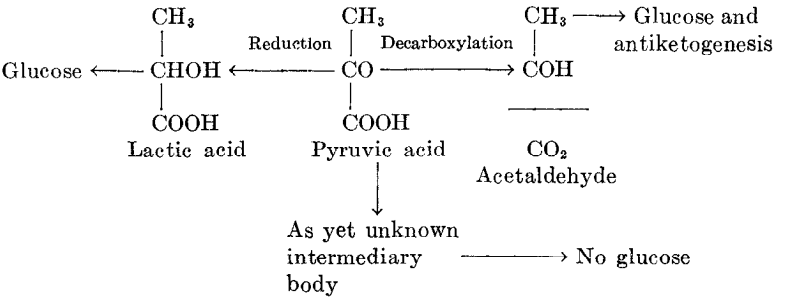
These two substances cannot possibly arise as a result of the same chemical process, and pyruvic acid must therefore be capable of following several paths of metabolism, as stated in our previous communication. The variable is not the pyruvic acid, but probably factors of equilibrium in the animal organism[15] and we believe there must exist a third possibility for the breakdown of pyruvic acid which results in no sugar formation. This would account for the very low sugar formation in experiments XXIV and XXVI of our series.[16]

[14] Ringer and Frankel: this *Journal*, xvi, p. 563, 1914.

[15] Greer, Witzemann and Woodyatt: *ibid.*, xvi, p. 455, 1914.

[16] Ringer: *ibid.*, xv, p. 152, 1913.

We may therefore formulate the fate of pyruvic acid in the animal organism by the following scheme.




Another contribution recently appeared, also dealing with the question of sugar formation from pyruvic acid.[17] Its author, Barrenscheen, perfused the extirpated liver of a phlorhizinized dog with blood to which pyruvic acid as sodium salt had been added. He found no increase in the glucose concentration of the blood after perfusion, and he therefore concluded that pyruvic acid is not a glucogenetic substance.

From what was said above it becomes evident that the method of experimentation employed by Barrenscheen, is not at all adapted for settling this question. Since it was shown that the glucogenetic properties of pyruvic acid may be very largely due to the intermediary formation of acetaldehyde, and as it was also shown that in liver perfusions acetaldehyde undergoes aldol condensation with the formation of acetone bodies, whereas in the organism as a whole it causes the formation of extra glucose, the failure of the above author to find any increase in glucose in his experiment does not in any way lend support to Mayer's conclusions.[18]

Perfusion experiments with pyruvic acid, through the liver, may, however, become instructive if simultaneous analysis be made of the lactic acid, aceto-acetic acid and glucose concentration of the blood before and after the perfusion. This will show whether lactic acid is formed in those experiments where acetaldehyde fails to be formed, or whether the two substances are formed simultaneously.

[17] Barrenscheen: *Biochem. Zeitschr.*, lviii, p. 299, 1913.

[18] The same argument is applicable to the work of Parnas and Baer: *ibid.*, xli, p. 386, 1912.