VII. THE ISOLATION OF PYRUVIC ACID FROM THE BLOOD OF VITAMIN B₁-DEFICIENT PIGEONS.

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(Received November 26th, 1935.)

THOMPSON AND JOHNSON [1935] have shown that a large increase in the amount of bisulphite-binding substances in the blood is specifically related to vitamin B₁ deficiency in the pigeon. They obtained incomplete evidence that the substance responsible for this increase is pyruvic acid. Enough pyruvic acid for analysis has now been isolated in the form of the 2:4-dinitrophenylhydrazone.

METHOD.

56 g. of blood were collected from eight vitamin B₁-deficient pigeons in head retraction. They had been dosed with glucose once according to the custom of this laboratory. About 1 ml. of 25% trichloroacetic acid was added for every 5 g. blood. The centrifugates were filtered into a large separating funnel, the precipitates were washed once with 5% trichloroacetic acid and the washings filtered into the same funnel. A 0.6% solution of 2:4-dinitrophenylhydrazine was added in the proportion of 5 ml. reagent to 20 ml. of filtrate. After standing a week, the mixture was adjusted to approximately p_H 1 with 10N NaOH, and the hydrazine and hydrazones were shaken into ethyl acetate. The ethyl acetate phase was shaken with successive portions of Na₂HPO₄ solution, first saturated, then *M*/15, until the aqueous phase was brown coloured, without any yellow tinge. The phases failed to separate sharply, presumably because of the large amounts of fat present. The alkaline solution was adjusted to approximately p_H 1 with 10N HCl, and the mixture of hydrazones and accompanying impurity of hydrazine were taken up once more in ethyl acetate. The whole procedure of shaking into Na₂HPO₄ solution, acidification and shaking into ethyl acetate was repeated until the last trace of colour could be removed by Na₂HPO₄ solution. The final solution of hydrazone in ethyl acetate was washed five times with equal volumes of *N* HCl and once with distilled water. The ethyl acetate was distilled off *in vacuo* at about 20°, leaving an oily orange residue.

The residue was extracted twice with light petroleum, dried *in vacuo* at room temperature, dissolved in a minimum volume of half-saturated Na₂CO₃ and precipitated by the addition of 10N HCl. It was twice more reprecipitated from half-saturated Na₂CO₃ solution. It was then washed twice with very small volumes of *N* HCl and once with distilled water, and dried *in vacuo* over CaCl₂ at room temperature.

The product was recrystallised three times from very small volumes of ethyl acetate and was finally recrystallised twice from ether by the addition of large volumes of light petroleum. It was dried *in vacuo* over CaCl₂ at room temperature.

The yield was 5.5 mg. of a bright yellow compound, m.p. 214° (corrected). (Found: N 21.12%. C₉H₅O₆N₄ requires N 20.88%.)

Another 55 g. of blood from 10 pigeons in head retraction yielded 7.3 mg. of hydrazone m.p. 216° (corrected). (Found: N 20.69%.)

108 g. of blood from 10 normal pigeons yielded 2.9 mg. of a 2:4-dinitrophenylhydrazone melting at 206° (corrected). There was not enough hydrazone for

recrystallisation from ethyl acetate, and most of it was lost in repeated recrystallisation from ether with light petroleum. The m.p. was never higher than 209° (corrected), but in all other properties the substance was similar to the two preparations described above. (Found: N 18.77 %.)

Analysis of 2:4-dinitrophenylhydrazones by the micro-Dumas method is liable to error, the values tending to be low unless the combustion tube is heated longer than for most compounds. The normal blood was worked up before this was realised, and the value is probably about 2.4 % low.

In these experiments 111 g. of blood from 18 vitamin B₁-deficient pigeons gave 12.8 mg. of hydrazone. This is equivalent to 3.8 mg. of pyruvic acid/100 g. of blood. Considering the inevitable losses during purification, this figure agrees satisfactorily with the figures of Table III of Thompson and Johnson [1935], who found an average of 5.65 mg. pyruvic acid/100 g. blood. In the case of normal pigeons, since the 2:4-dinitrophenylhydrazone collected was not pure, it can only be said that not more than 0.9 mg. of pyruvic acid was present in 100 g. blood.

DISCUSSION.

The assumption of Vogt-Møller [1931] that methylglyoxal is the toxic agent in vitamin B₁ deficiency has been verified to some extent by Geiger and Rosenberg [1933], who found methylglyoxal in the urine of avitaminous dogs and rats. This cannot hold for the pigeon. No trace of methylglyoxal-2:4-dinitrophenylbishydrazone was seen in working up the blood of vitamin B₁-deficient pigeons. It would have been detected if it had appeared, since it is one of the most easily identified of all hydrazones.

SUMMARY.

1. Pyruvic acid in the blood of vitamin B₁-deficient pigeons has been identified by preparation and analysis of the 2:4-dinitrophenylhydrazone.
2. In the case of normal pigeons, pyruvic acid has not been identified with certainty.

I am deeply grateful to Prof. R. A. Peters for the interest he has shown in this work.

REFERENCES.

- Geiger and Rosenberg (1933). *Klin. Woch.* **12**, 1258.
Thompson and Johnson (1935). *Biochem. J.* **29**, 694.
Vogt-Møller (1931). *Biochem. J.* **25**, 418.