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On the Alleged Oxytocic Activity of l-Carnosine.

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Since the work of McClintock and Hines,[1] it has been accepted generally that carnosine, β-alanyl-*l*-histidine, possesses oxytocic properties. They reported that this dipeptide produced a marked increase in the tonal and rhythmical contractions of strips of guinea pig uterus when such strips were immersed in Tyrode's or Ringer's solution containing the compound in a 1:2000 dilution. They stated that this action was qualitatively and quantitatively similar to that produced by histamine in dilutions of 1:100,000. The carnosine was, therefore, regarded as having one-fiftieth the oxytocic power of histamine. They furthermore reported in this study that the material isolated from muscle tissue behaved identically in all their experiments to the synthetic carnosine prepared by Baumann and Ingvaldsen[2] which had been placed at their disposal.

With the synthesis of d-carnosine[3] the possibility of studying the relationship of stereostructure to the physiological activity of carnosine was afforded. As the first step in this comparison, duVigneaud and Hunt[3] studied the effect of both optical isomers on the blood pressure. The d-carnosine in 20 times the dose of l-carnosine was found to produce no perceptible decrease in the blood pressure of cats and rabbits under amytal and luminal anesthesia, thus demonstrating that the depressor action of carnosine depends upon the spatial configuration of the molecule.

In a continuation of this comparative study it was decided to test both isomers for oxytocic activity. Much to our surprise, however, when l-carnosine was tried on the isolated virgin guinea pig uterus no oxytocic action could be detected. It was likewise found that the d-isomer was inert in this respect.

The method employed in testing for oxytocic activity was that outlined in the United States Pharmacopoeia for the assay of posterior pituitary extracts. Histamine was used as a reference standard.

[1] McClintock, J. T., and Hines, H. M., PROC. SOC. EXP. BIOL. AND MED., 1925, **22**, 515.

[2] Baumann, L., and Ingvaldsen, T., *J. Biol. Chem.*, 1918, **35**, 263.

[3] duVigneaud, V., and Hunt, M., *J. Biol. Chem.*, 1936, **115**, 93.

The concentrations of histamine which we considered effective, that is, capable of producing significant but submaximal contractions of the isolated uteri proved to be about one part per 10 million. This dose was one one-hundredth of that used by McClintock and Hines. The concentration they employed was far greater than that which we found necessary to invoke maximal contractions.

The l-carnosine used in these experiments was synthesized by the method of Sifferd and duVigneaud,[4] while the d-carnosine was prepared according to the directions of duVigneaud and Hunt.[3] The compounds possessed the physical constants reported in these papers.

Initially, we tried an amount of l-carnosine 50 times as great as the submaximal dose of histamine. This amount of carnosine, corresponding to a concentration of 1:300,000, proved to be without effect on the muscle strip. We then determined the least amount of histamine which would produce a perceptible contraction. This corresponded to a concentration of 1:150,000,000. We then found that carnosine in one thousand times this concentration was wholly without effect. In further efforts to determine whether we could get carnosine to stimulate the uterine strip under any conditions, we tested it repeatedly with different uterine strips in varying concentrations as high as 1:2000 without any perceptible effect on the tissue as far as an increase in tonus or rhythm was concerned. It was equally ineffective following various concentrations of histamine from 1:150,000,000 to as high as 1:100,000. It was also ineffective when posterior pituitary was used instead of histamine to establish the responsiveness of the tissue strips.

As a final step in this investigation, a highly purified sample of carnosine isolated from natural sources was tested for oxytocic activity. This sample had been isolated from fresh sheep muscle by a combination of the methods of Dietrich[5] and Gulewitsch and Amiradzibi[6] and is the sample utilized by Sifferd and duVigneaud in proving the identity of their synthetic product with naturally occurring carnosine.[4] No response was obtained with this sample in 2 trials at a concentration of 1:100,000, which corresponded in this experiment to one hundred times the submaximal dose of histamine.

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4 Sifferd, R. H., and duVigneaud, V., *J. Biol. Chem.*, 1935, **108**, 753.

5 Dietrich, M., *Z. physiol. Chem.*, 1914, **92**, 212.

6 Gulewitsch, W., and Amiradzibi, S., *Ber. Deut. chem. Ges.*, 1900, **33**, 1902.