sugar levels was 8 mg%, hence we have added 8 to the readings for CO treated animals.

The control hyperglycemic average was 129±6.9 mg%. In the CO treated it was 132 mg%, but after addition of the initial difference it becomes 140 mg%. This difference is well beyond the range of normal variation.

Conclusions. 1. Acute carbon monoxide asphyxia raises the blood sugar in direct proportion to the percentage of COHb induced. 2. Repeated exposure to carbon monoxide seems not to change the insulin tolerance significantly but to reduce the dextrose tolerance below the limits of normal variation. It reduces the fasting blood sugar 6-8 mg below that of untreated litter mates.

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Spatial Configuration and Preparation of Canavanine.

J. F. CADDEN. (Introduced by V. du Vigneaud.)

From the Department of Obstetrics and Gynecology, Cornell Medical College and New York Hospital, New York City.

Canavanine is one of the few amino acids whose spatial configuration has not yet been established. Since this compound, found to exist in a free form in the Jack Bean, is a product of nature it would be of interest to establish its spatial configuration by the method of Lutz and Jirgensons.[1] This method is based on the observation of these workers that the naturally occurring amino acids show a change in specific rotation toward the positive with increasing amount of HCl.

Optical rotation measurements were carried out on 0.1 M canavanine solutions with varying molecular equivalents of HCl and NaOH. In Fig. 1 is shown the curve thus obtained. From this one readily sees that there is a decided shift toward the positive with increasing amount of HCl. Dextrorotatory canavanine therefore should be designated as 1(+) canavanine.

Experimental. Preparation of Canavanine. Jack Bean meal is extracted several times with 50% alcohol (by weight). The extracts are concentrated to remove the alcohol and then made up to a volume approximately one-tenth the volume of the extracts with 5% sulfuric acid. To this acid solution is now added sufficient flavianic acid to precipitate the canavanine. The solution is warmed gently to redissolve

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[1] Lutz, O., and Jirgensons, B., *Ber. chem. Ges.*, 1930, **63**, 448; 1931, **64**, 1221.

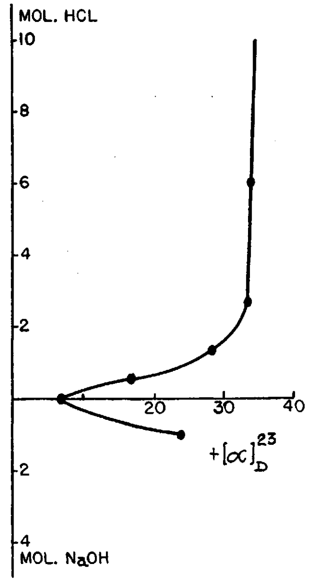

FIG. 1.

The specific rotation of 0.1 M canavanine solutions containing different molecular equivalents of HCl and NaOH.

the precipitate thus formed and then allowed to cool spontaneously. After standing overnight the precipitate is filtered off and washed with cold water. The precipitate is now heated with several portions of 95% alcohol. The alcoholic solutions are combined and allowed to stand overnight. After filtering off the precipitate the filtrate is evaporated under reduced pressure and then on standing a yellow flavianate settles out. The canavanine flavianate thus obtained decomposes at 218-220° C.

Canavanine flavianate is now dissolved in hot water, barium hydroxide added until the solution is definitely alkaline to phenolphthalein. The mixture is allowed to cool, the insoluble barium flavianate filtered off and washed several times with hot water. The filtrate is made acid to congo red with sulfuric acid. To the mixture is added a small amount of norit and after stirring for a few minutes the barium sulfate is removed by centrifugation and filtration. The water clear solution is concentrated under reduced pressure to a small volume. This is added to alcohol with vigorous stirring. The crystals thus obtained are dried by air and then placed in a desiccator. The sulfate decomposes at 172° C.

Canavanine is prepared by adding to a solution of the sulfate exactly the required amount of barium hydroxide, removing the barium sulfate and concentrating the filtrate to a small volume. To the concentrated solution is added alcohol until the solution becomes quite cloudy. On standing in the icebox canavanine crystallizes out. The crystals are washed with alcohol and placed in a desiccator. M. P. 171° C. (decomposition). Although this is a lower decomposition temperature than that reported by Kitagawa[2] and co-workers and by Gulland and Morris[3] who found the free base to decompose at 182° C., a sample kindly furnished me by Dr. Kitagawa behaved in the same manner in my hands as the samples prepared by me.

The values found for carbon and hydrogen 34.14% and 7.01% respectively agree closely with the theoretical values 34.08% and 6.87%. The value found for nitrogen 31.21% was somewhat lower than the theoretical value of 31.81%. It may be noted that the preparation used by Gulland and Morris in their study of the structure of canavanine gave a value of 30.7% for nitrogen. Kitagawa has reported values similar to that obtained by me. However the low nitrogen value does not perceptibly affect the data presented in this report.

Conclusions. The spatial configuration of dextrorotatory canavanine has been determined by the method of Lutz and Jirgensons. It was found to be in agreement with that of the other naturally occurring amino acids and should therefore be designated as l(+) canavanine. A simplified procedure for the purification of the canavanine flavianate prepared from Jack Bean has been described.

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Effect of Subtotal Nephrectomy upon Estrous Cycle of the Albino Rat.

J. T. DIAZ. (Introduced by John C. Burch.)

From the Department of Obstetrics and Gynecology, Vanderbilt University School of Medicine, Nashville, Tenn.

In a previous study of experimental hypertension in the rat,[1] it was found that subtotal nephrectomy was followed by a progressive increase in blood pressure over a period of 3 to 4 months. Daily vaginal smears from a few females that had developed hypertension as a result of this operation revealed marked abnormalities in the

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[2] Kitagawa, M., *J. Biochem.* (Japan), 1937, **25**, 23.

[3] Gulland, J. M., and Morris, C., *J. Chem. Soc.*, 1935, 763.

[1] Diaz, J. T., and Levy, S. E., *Am. J. Physiol.*, 1939, **125**, 586.