higher content of ethyl chaulmoograte; (2) iodizing ethyl chaulmoograte with 0.5% iodine has little effect on toxicity; (3) addition of 4% creosote increases the toxicity, commensurate with the amount of creosote present; (4) the degree of unsaturation of crude ethyl chaulmoograte is not a sufficiently sensitive index of toxicity to be reliable; and (5) if subcutaneously administered ethyl chaulmoograte exerts its toxic effect through liberation of Na chaulmoograte into the circulation, the summation of rates of hydrolysis and diffusion cannot much exceed that causing a disappearance of 2 millimols per Mol of the injected ester per hour, since Na chaulmoograte kills rats in intravenous acute doses[3] of 0.1-0.125 gm./kg.

Intensive treatment of leprous humans with ethyl chaulmoograte may give rise to a sterile tetanus in rare instances, perhaps related to the type of terminal convulsions seen in rats after administration of a single lethal dose. Conceivably, then, from this observation and the autopsy findings reported in Table I, death may occur from respiratory failure caused either by multiple emboli in the lungs or by prolonged central depression following a relatively short period of excitation, or from reduction of diffusible calcium in the plasma through formation of Ca dichaulmoograte, leading to tetany. No conclusions can be drawn here as to the importance of these and other factors in producing types of injury seen on chronic administration of chaulmoograte[4] nor as to the possible relative therapeutic rank of the different preparations, aside from an application of this acute toxicity data as a rough index of the absolute amount of ethyl chaulmoograte present.

7650 C

Attempts to Isolate Dihydroxy-pyrrol-Alanine from Gelatin Hydrolysates.

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Van Slyke and Hiller[1] and Van Slyke and Robson[2] have reported the presence in gelatin of a new amino acid which is precipitable by phosphotungstic acid. On the basis of the elementary analysis of

[3] Emerson, G. A., Anderson, H. H., and Leake, C. D., *Arch. Internat. de Pharmacodyn. et de Therapie*, 1934, **48**, 247.

[4] Frazier, C. N., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 44.

the copper salt, a positive test for a pyrrol group, and the ratio of amino to total nitrogen, the product was considered as being probably dihydroxy-pyrrol-alanine. According to the criteria which have been suggested by Vickery and Schmidt,[3] the evidence brought out by Van Slyke and his coworkers is not sufficient to consider this substance as one of the accepted amino acids. The experiments herein described deal with a number of attempts to isolate and identify the product described by Van Slyke and his coworkers.

Three lots of about 1 kilo each of a good grade of gelatin were treated essentially as described by Van Slyke and his coworkers except that the bulk of the arginine was first removed by means of flavianic acid. On decomposing the phosphotungstic acid precipitate a product was obtained which was extremely soluble in water but no evidence was obtained to the effect that this substance was dihydroxy-pyrrol-alanine. In the first experiment there was present in this fraction 884 mg. of nitrogen of which 340 mg. were liberated by treatment with nitrous acid in 3 minutes and 374 mg. in 30 minutes. Flavianic acid gave no precipitate with a fairly concentrated solution even on standing for 24 hours at 0°. The substance represented by the amino nitrogen was almost quantitatively precipitated by rufianic acid and by picrolonic acid. The rufianate was amorphous and decomposed without melting. The picrolonate was crystalline and fractional recrystallization from water and dilute alcohol resolved it into at least 3 fractions which differed widely in melting point, solubility, and somewhat in crystal form. None of these fractions could be isolated in pure form.

In the second experiment it was likewise not found possible to isolate a pure product. The evidence pointed to the belief that the product was a mixture which contained a considerable amount of peptids since the amino nitrogen increased over 50% when the substance was hydrolyzed in a sealed tube with 6 N HCl at 120-140° for 4 hours.

In the third experiment a kilo of Coignet's gold label gelatin was hydrolyzed by boiling 24 hours with 8 normal sulfuric acid. The bulk of the sulfuric acid was removed by addition of $Ca(OH)_2$ and the remainder with $Ba(OH)_2$. The small amount of $Ca(OH)_2$ in solution was precipitated by means of oxalic acid. The bulk of the arginine was precipitated with flavianic acid. In order to eliminate

[1] Van Slyke, D. D., and Hiller, A., *Proc. Nat. Acad. Sci.*, 1925, **7**, 185.

[2] Van Slyke, D. D., and Robson, W., PROC. SOC. EXP. BIOL. AND MED., 1925, **23**, 23.

[3] Vickery, H. B., and Schmidt, C. L. A., *Chem. Rev.*, 1931, **3**, 169.

the possibility that the substance sought had been carried down by the crude arginine flavianate, the latter substance was recrystallized from 2 liters of 9% H_2SO_4 and the mother liquor was added to the main solution. The excess flavianic acid contained in the main solution was extracted with butyl alcohol. Since the latter solvent had extracted a considerable amount of amino nitrogen which could not be removed by washing with 10% H_2SO_4, it was evaporated to dryness *in vacuo* and the residue was taken up in a small amount of hot water. The flavianic acid was precipitated by the addition of HCl, the solution was diluted with 5% H_2SO_4, and the bases which had been extracted along with the flavianic acid were precipitated with phosphotungstic acid. The precipitate was washed and added to the bulk of the precipitate which was obtained by the addition of phosphotungstic acid to the main solution. The precipitated phosphotungstates were decomposed with $Ba(OH)_2$, histidine and the small amounts of arginine remaining in the solution were precipitated with silver sulfate and $Ba(OH)_2$ and the lysine was removed as the picrate in accordance with the procedure described by Vickery and Leavenworth[4] and Vickery and Block.[5] The alcoholic filtrate from the lysine picrate was evaporated to dryness and the residue was dissolved in water to which about 15 cc. of H_2SO_4 were added. The picric acid was extracted with toluene. The diluted solution was again treated with phosphotungstic acid.

There was present in the solution from the decomposition of this second phosphotungstate precipitate 1.655 gm. of nitrogen of which 480 mg. represented amino nitrogen. The solid material in this solution was fractionated with absolute alcohol. The alcohol soluble portion contained 1.360 gm. of nitrogen of which 391 mg. were in the form of amino nitrogen, while the alcohol insoluble fraction contained 139 mg. of nitrogen of which 40 mg. represented amino nitrogen. Further fractionation and identification of these two products are shown in Diagram I. A few comments on the diagram deserve consideration.

The precipitation of the reineckate, the decomposition of the precipitate, and removal of the inorganic ions were carried out in accordance with the procedure of Kapfhamer and Eck.[5] The reinecke precipitate from the alcohol soluble fraction contained a considerable amount of arginine whose removal by the silver method was not wholly successful. This necessitated treatment with flavianic

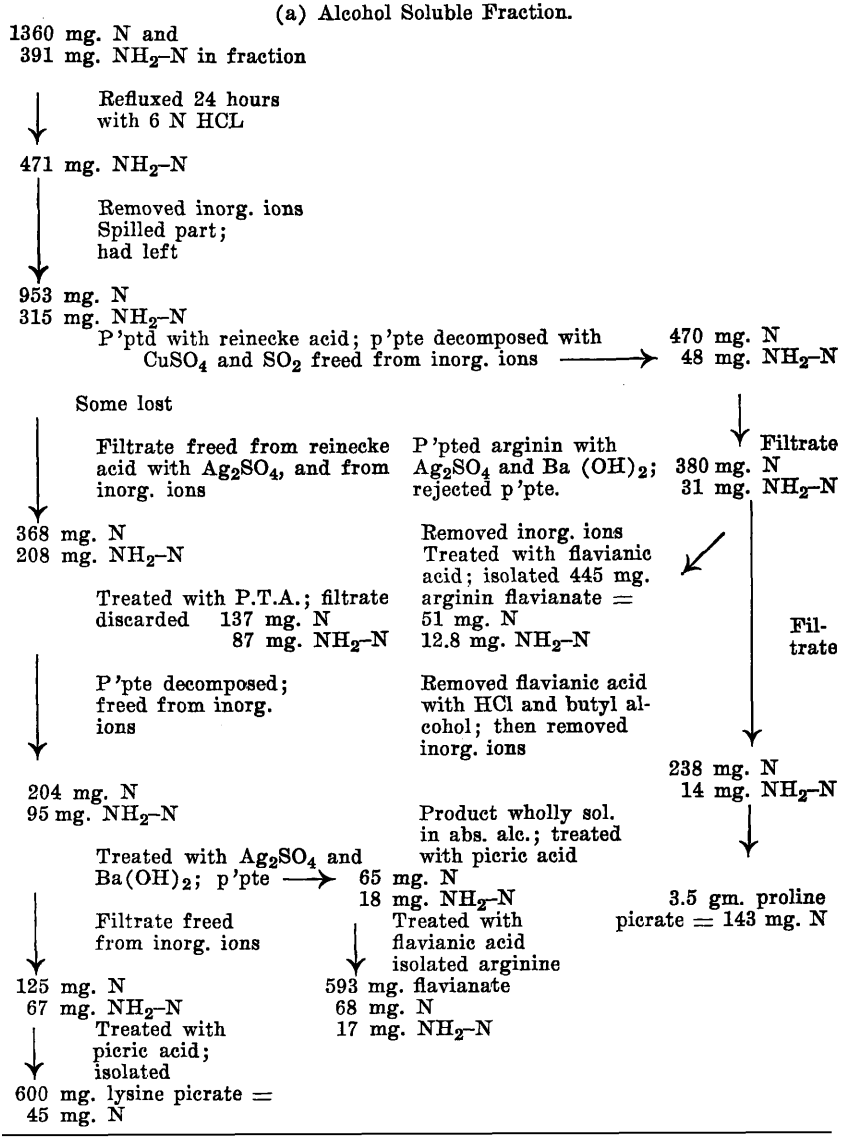
[4] Vickery, H. B., and Leavenworth, C. S., *J. Biol. Chem.*, 1929, **83**, 532. Vickery, H. B., and Block, R. J., *J. Biol. Chem.*, 1931, **93**, 105.

[5] Kapfhamer, J., and Eck, R., *Z. physiol. Chem.*, 1927, **170**, 294.

acid. To test the purity of the arginine flavianate it was recrystallized from dilute H_2SO_4 and then boiled with water to decompose any arginine diflavianate. The nitrogen values of the purified product corresponded very closely to that calculated for arginine flavianate. The proline picrate contained practically no amino nitrogen. It melted at 149°. Towne[6] reports 148°, while Kapfhamer

DIAGRAM I.

(a) Alcohol Soluble Fraction.



[6] Towne, B. W., *Biochem. J.*, 1928, **22**, 1083.

(b) Alcohol Insoluble Fraction

139 mg. N
40 mg. NH_2–N in fraction

↓ Hydrolyzed by boiling 24 hours with 6 N HCl; humus and inorg. ions removed

138 mg. N
54 mg. NH_2–N

↓ Treated with reinecke acid; filtrate freed from inorg. ions → 16 mg. N, 11 mg. NH_2–N. This gave no p'pte with P.T.A. and was discarded.
P'pte decomposed, freed from inorg. ions

114 mg. N
26 mg. NH_2–N

Treated with picric acid; isolated hydroxy-proline picrate

1238 mg. hydroxy-proline picrate = 48 mg. N plus 418 mg. oily picrate contg. 2% NH_2–N (apparently not homogeneous)

and Eck found 154°. The nitrogen content was 16.18%. The first crop of lysine picrate contained 18.47% of nitrogen and exploded at 257°. The hydroxyproline picrate, after recrystallization from water and then from an alcohol ether mixture, melted at 180°. Kapfhamer and Eck give 188°. The nitrogen content was 15.6%. It was practically free from amino nitrogen.

It is evident from the data presented that no substance corresponding to the empirical composition of dihydroxy-pyrrol-alanine was isolated in the 3 experiments. The fraction in which this compound should have been present contained some peptids. After hydrolysis, in addition to arginine and lysine, considerable amounts of proline and hydroxyproline were isolated. This is not surprising since both of these amino acids are in part precipitated by phosphotungstic acid. Since both the reineckate and the picrate of these 2 amino acids are relatively soluble, the amounts present in the hydrolysate were doubtless considerably greater than were isolated. The discarded filtrate which contained 137 mg. nitrogen of which 87 mg. were in the form of amino nitrogen probably contained either peptids or unprecipitated hexone bases in addition to some monoamino acids. If dihydroxy-pyrrol-alanine was present in any of the fractions of the gelatin hydrolysate, the amount must have been relatively small.

It should be mentioned that the empirical formula of dihydroxy-pyrrol-alanine differs from that of glycyl-hydroxyproline only by having two more hydrogen atoms. On hydrolysis there should be no increase in amino nitrogen. It is not impossible that this peptid may have been mistaken for an apparently new amino acid and

especially since the pyrrol test is not a specific test for this group. Gelatin is known to be difficultly hydrolysable. While the present experiments do not wholly deny the existence of the amino acid described by Van Slyke and his associates, they nevertheless indicate that more decisive proof must be advanced before the substance can be considered as an accepted amino acid.

7651 C

Necessary Concurrence of Thyroid in the Marked Adrenal Cortical Hypertrophy Following Beef Anterior Pituitary Implants.

MORVYTH MC QUEEN-WILLIAMS. (Introduced by Herbert McLean Evans).

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Emery and Atwell[1] showed that the administration to normal animals of extracts of whole sheep pituitaries provoked a marked increase in adrenal weight due predominantly if not exclusively to the hypertrophy of the cortex. Independently, we had obtained the same effect in rats from implants of beef anterior pituitary tissue. The present communication reports a striking diminution in this effect when a preceding ablation of the thyroids has been performed.

Fresh beef hypophyses were washed in alcohol before dissection, and to eliminate all chance of infection, both merthiolate (1:10,000) and hexylresorcinol (1:13,000) were added to many of the batches immediately after grinding. Of 214 adult male and female rats, some of which were unilaterally adrenalectomized, 41 had been previously hypophysectomized, 69 including many first thyroidectomized received pituitary implants, 67 including thyroidectomized were left untreated, and a group of 37 normals was sacrificed and right and left adrenals weighed separately.

Results. 1. Implants of adequate amounts of finely ground beef hypophyses, whether freshly prepared or stored at 0°C. for several months, induced marked adrenal hypertrophy in every case in which the animal possessed intact thyroids, but had little or no effect on thyroidectomized rats (Tables I and II).* 2. Two grams of an-

[1] Emery, F. E., and Atwell, W. J., *Anat. Rec.*, 1933, **58**, 17.

* In 37 *normal* male rats (64 to 82 days) the *left* adrenals averaged 18.3 mg., the right 16.0 mg.; only once did the right exceed the left. Consequently, in all the experiments only *left* unilateral adrenalectomies were performed.

Autopsies of unilaterally adrenalectomized rats revealed that the *right* adrenal always exceeded the left, extirpated 5 days to 4 months previously. More extended study on this compensatory hypertrophy is contemplated.