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Time Factor in Retention of Nitrogen After Intravenous Injection of a Mixture of Amino-Acids.*

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As reported previously,[1] an acid hydrolysate of casein containing no tryptophane did not produce nitrogen balance when given intravenously. If tryptophane (and methionine) were added, nitrogen balance was achieved at once. In the course of these studies it was noted that if the tryptophane were injected several hours after the basic incomplete mixture its favorable effect on the retention of nitrogen is lost and that a negative nitrogen balance occurs, almost as if no tryptophane had been given. This observation was repeated and confirmed in several experiments which are described herein. Van Slyke, Cullen and McLean[2] found that the first amino-acids reaching the liver are deaminized and the nitrogen excreted very rapidly and that they do not await further absorption of additional amino-acids without which synthesis is impossible. They noted that this phenomenon, wasteful as it seems, occurred even when there was a great need of nitrogen because of starvation. A perhaps analogous time factor in the retention of nitrogen by carbohydrate was recently observed by Larson and Chaikoff.[3] In normal dogs they found that carbohydrates induced a retention of nitrogen from protein feeding only when the sugar was given not more than 4 hours before or 4 hours after the protein was ingested. The greatest retention occurred when the 2 were given simultaneously.

Three pairs of experiments were carried out; the data herein reported are from one of them, typical of all. Two dogs were observed simultaneously and nitrogen studies started only after a preliminary period of starvation and after the daily urinary output had reached a uniform level. Previous to the collection period the animals were started on twice daily gavage feedings. These consisted of 12½% sucrose solution containing sodium, potassium, calcium, and magne-

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1 Elman, Robert, PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 610.

2 Van Slyke, D. D., Cullen, G. E., and McLean, F. C., as quoted in Peters and Van Slyke, *Quantitative Clinical Chemistry*, 1931, Vol. 1, p. 394.

3 Larson, P. S., and Chaikoff, I. L., *J. Nutrit.*, 1937, **13**, 287.

TABLE I.

Day	Urine			Daily Avg				Remarks
	Vol., cc	Total N, g	Creat. N, g	Creat. N g	N Intake g	N Output g	N Balance g	
1	600	1.40	.072					Dog 04
2	680	1.43	.068					Wt 6.8 kg
3	670	.87	.068	.070	0	1.23	—1.23	
4	1020	1.77	.069					"Delayed" Injection
5	1160	2.56	.081					
6	940	2.18	.064	.071	1.50	2.17	— .67	
7	640	.76	.064					Recovery
8	900	1.07	.089					
9	720	.93	.072	.075	0	.92	— .92	
10	820	1.38	.081					Complete mixture
11	960	1.55	.071					injected
12	820	1.30	.064	.072	1.50	1.42	+ .08	
13	910	.72	.057					Recovery
14	700	1.09	.082					Wt 6.2 kg
15	750	.99	.074	.071	0	.93	— .93	
1	660	1.23	.058					Dog B70
2	780	1.34	.064					Wt 7.6 kg
3	660	.76	.056	.059	0	1.11	—1.11	
4	1220	1.18	.072					Complete mixture
5	1180	1.51	.082					injected
6	1280	1.07	.070	.075	1.50	1.25	+ .25	
7	880	.99	.072					Recovery
8	940	1.47	.084					
9	980	.68	.067	.075	0	1.05	—1.05	
10	1320	1.53	.086					"Delayed" injection
11	1180	1.57	.073					
12	1000	1.73	.069	.075	1.50	1.61	— .11	
13	840	.97	.070					Recovery
14	780	.78	.065					Wt 7.4 kg
15	730	.90	.075	.070	0	.88	— .88	

sium chloride (*i. e.,* Ringer's solution) and also an adequate amount of Vitamin B concentrate (Labco). This mixture yielded about 50 calories per kilogram and contained but an insignificant amount of nitrogen. Observations were made in 3-day periods as indicated in Table I. The amino-acids† were always given as a 10% solution with 10% glucose; about 10 to 15 cc were given each hour until the entire daily dose was injected. When the tryptophane (and methionine) were "delayed" they were injected 6 hours after the last injection of the basic mixture. The dose of tryptophane and methionine was 2% each by weight of the total amount of amino-acids previously injected. The period of observation was 15 days; 3 days each for preliminary data, for the "complete" injection, for recovery, for the "delayed" injection, and for the second recovery. The sequence was reversed in the second dog so that when the first one was receiving the "complete" mixture, the other was receiving the "delayed" injection. Kjeldahl determinations were used for the determination of nitrogen and the alkali picric acid method for creatinine.

From the data reported herein it is seen that positive nitrogen balance occurs only when the "complete" mixture is given at once. If tryptophane is injected 6 hours after the rest, this did not occur. It would seem from the present findings that the absence of one essential amino-acid, *i. e.,* tryptophane, at the time all the others are present in the blood and tissue prevents retention of nitrogen and presumably delays or even prevents synthesis of amino-acids to protein. These observations would seem to indicate that for the most efficient utilization of amino-acids they should be presented to the tissues at the same time, thus introducing a time factor in the biological value of amino-acids dependent normally on the rate of digestion and absorption from the intestinal tract. This time factor may play a rôle in regeneration of protein in any condition wherein absorption from the intestinal tract is delayed for one reason or another. It has been noted by Groen,[4] for example, that in the human with various diseases, notably Vitamin B deficiency, absorption of glucose is impaired. Cori[5] has discussed the fact that the general condition of an animal has a pronounced influence on the rate of intestinal absorption and that when 2 substances are fed simultaneously, the rate of absorption of each was reduced. This may be of great significance in view of the findings of Chase and Lewis[6] in white rats that there are

† The acid hydrolysate of casein was generously supplied by Mead Johnson & Co. Tryptophane and methionine were obtained from Eastman Kodak Co.

4 Groen, J., *N. E. J. Med.,* 1938, **218**, 247.

5 Cori, C. F., *Physiol. Rev.,* 1931, **11**, 144.

6 Chase, B. W., and Lewis, H. B., *J. Biochem.,* 1934, **106**, 315.

great differences in the rate of absorption of amino-acids from the intestinal tract; leucines, essential amino-acids, for example, were one-third as absorbable as glycine, a non-essential.

Summary. The injection of tryptophane (and methionine) 6 hours after the injection of an incomplete mixture of amino-acids lacking only tryptophane failed to induce positive nitrogen balance, whereas the injection of tryptophane (and methionine) simultaneously succeeded in doing so. It is inferred that retention of nitrogen is facilitated when all of a complete mixture of amino-acids is presented to the tissues at the same time.

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Inhibitory Effects of Adrenalin on Autonomic Function.

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Hoskins and Rawley[1] and Chu and Hsu[2] showed that adrenalin inhibits reflex sympathetic responses in the vascular system. The present study was undertaken to investigate the applicability of these observations to other autonomic reflexes.

In cats under urethane and chloralosane anesthesia the sympathetic nerve to the pupil and nictitating membrane of one eye was separated from the vagus and severed in the neck. Blood pressure and nictitating membranes were recorded by kymograph, and the 2 pupils and a galvanometer indicating the galvanic (sweating) reactions of the foot pads were photographed. Brachial plexus, sciatic and splanchnic nerves were freed, cut, and arranged for stimulation of their central ends. The autonomic responses of the respective pupils and nictitating membranes with and without sympathetic supply, the galvanic (sweating) reactions and blood pressure changes are compared (1) during secretion of adrenine, (2) under the influence of adrenalin perfusion, and (3) after adrenalectomy.

The kymographic and photographic records show that during infusion of adrenalin (1:100,000 to 1:250,000, 1-3 cc/min) sympathetic reflex responses decrease. This is evident from the decreased reflex responses of the normally innervated pupil and nictitating

1 Hoskins, R. G., and Rowley, W. N., *Am. J. Cliniol.*, 1915, **37**, 471.

2 Chu, L. W., and Hsu, F. Y., *Quart. J. Exp. Physiol.*, 1938, **27**, 307.