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Effect of Parenteral Amino Acids (Hydrolyzed Casein) on Growth of Rats.*

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(Introduced by Evarts A. Graham.)

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Humans and experimental animals may be placed in positive nitrogen balance by the intravenous administration of a protein hydrolysate.[1-3] Moreover, casein digests given parenterally are utilized for the manufacture of serum proteins in dogs made hypoproteinemic by dietary means,[4] or by plasmapheresis.[5] Experiments are presented herein showing that growth is promoted when protein hydrolysates are injected subcutaneously as the sole source of nitrogen in the diet (with the exception of very small amounts in the vitamin supplements).

Methods. The hydrolysate used (Amigen) was prepared by Mead Johnson and Company, it is an enzymatic digest of casein and pork pancreas, containing two-thirds amino acids and the rest in the form of dipeptides. Five per cent solutions of Amigen were made in distilled water and the pH adjusted to 7.4 with sodium hydroxide. The solutions were then sterilized by passing them through a Berkefeld filter. When given parenterally the solution was injected slowly into the subcutaneous tissues of the back of the rat through a 31 gauge needle.

The non-protein (basal) portion of the diet was given by mouth. It is described by Miller, Kemmerer, Cox and Barnes,[6] and was kindly furnished by them. It was shown by these authors not to support growth; however, they found good growth occurred when 15% Amigen was added thereto.

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3 Shohl, A. F., Butler, A. M., Blackfan, K. D., and MacLachlan, E., *J. Pediat.,* 1939, **15**, 469.

4 Elman, R., Sachar, L. A., Horvitz, A., and Wolf, H., *Arch. Surg.,* in press.

5 Madden, S. C., Zeldis, L. J., Hengerer, A. D., Miller, L. L., Rowe, A. P., Turner, A. P., and Whipple, G. H., *J. Exp. Med.,* 1941, **73**, 727.

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Newly weaned rats of the Anheuser-Busch strain were used; the duration of each experiment was 7 days. The amount of solution injected each day was 12 to 16 cc (0.6 to 0.8 g of Amigen) and was divided into 5 equal doses given 1½ to 2 hours apart. Weights were taken each morning just before the first injection, *i.e.,* 10 to 12 hours after the last injection of the previous day. No edema was noted after this interval.

Experiment I. The rats in this experiment were divided into 3 groups. Group A consisted of 3 rats which received the stated dose of Amigen parenterally, Group B of 5 rats which received the same amount of Amigen by mouth. Each group was offered the basal diet in amount to make up 85% of the total diet. Group B consumed all of its food invariably, in contrast to the injected rats, which ate very little of their basal diet. Thus Groups A and B received the same amount of Amigen, but varying amounts of the basal diet. Group C received the basal diet alone.

Experiment II. The rats in this experiment were divided into 2 groups. Group A was composed of 2 rats which received the stated dose of Amigen parenterally, Group B, of 3 rats, which received in the same manner and in similar amount a 5% solution of glucose in physiological saline solution. Both groups were offered the basal diet by mouth ad libitum. As in Group A of Experiment I the injected rats of both groups failed to consume all of their basal diet. The purpose of this experiment was to determine the effect of injected fluid itself upon the weight of the rats, *i.e.* to rule out the possibility that any gain in weight might be due to subcutaneous edema produced by the injection.

Experiment III. In this experiment, the method of paired feedings was used in order to remove differences due to variations in the amount of basal diet consumed. Two groups of rats were used; both received the stated amount of Amigen, Group A parenterally, Group B by mouth. In Group B the same amount of basal diet was fed by mouth as the injected rats of Group A had consumed the previous day. There were 4 rats in the first group and 2 in the second group. Weight changes on the day in which the rats had the same diet were then compared. In this experiment, the daily parenteral dose was divided into only 3, instead of 5, injections.

Findings and comment. The results (shown graphically in the figure) indicate that some growth may be achieved on a diet, the sole source of nitrogen of which is given parenterally in the form of a solution of hydrolyzed protein. Thus, in 3 experiments, each for a period of 7 days, gains in weight (as measured from the time in-

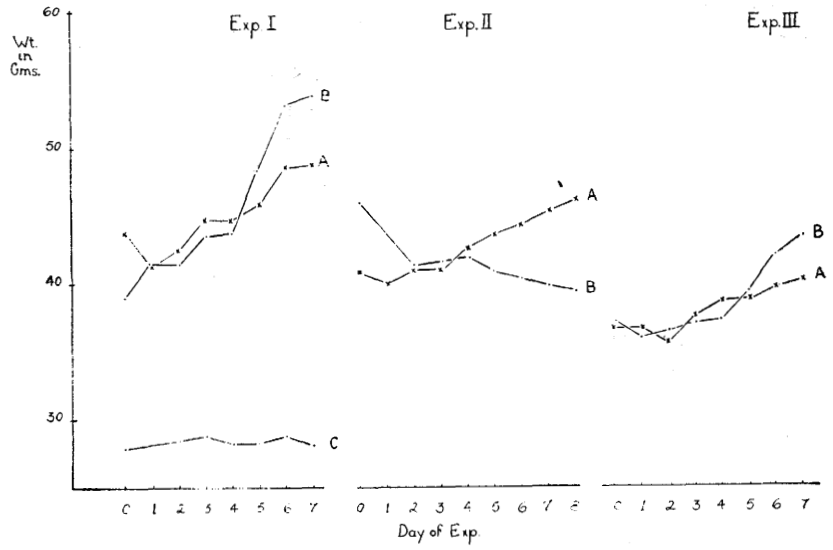

FIG. 1.

jections were begun to the time they were discontinued) were obtained, varying from 9 to 11% (Curves A). While it is true that rats receiving Amigen by mouth in Experiment I, (Curve B) gained more in weight, this was probably due to the fact that they consumed more of the non-protein part of the diet than the injected animals. It is noteworthy that even for the small amounts of Amigen injected, a large amount of fluid is required as a vehicle; in many instances, the daily volume equalled approximately half of the body weight. This of itself may have so influenced the animals that they failed to consume the whole of their allotted basal diet. The problem of hypodermoclysis is due to the necessity of giving the solution in an isotonic form, thus requiring large volumes of fluid to make up the large amount of protein needed for growth in rats. For example, to give what would be an adequate protein intake (3 g daily), 60 cc of solution would have to be injected, or nearly twice body weight of the rats. The discrepancy in the amount of basal diet consumed was controlled in the paired feeding experiment performed in Experiment III; the growth curves were similar, whether the Amigen was given by mouth or parenterally, although actually not very different from the uncontrolled Experiment I. The failure of rats injected with glucose in saline to gain weight, the absence of clinically obvious edema, and the long interval which was allowed to elapse between the last injection and the taking of the weight all indicate that the observed gain in weight was due to growth and not to accumulation of fluid.

Summary. Growth of rats was obtained when the sole source of nitrogen in the diet was provided by parenterally injected casein digest.

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Development of Tolerance to Gold Salts in Rats.

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The minimum lethal dose of gold sodium thiosulphate intramuscularly in rats was found to be approximately 35 mg/kg. The rats which survived this dose were given repeated doses of 35 mg/kg every other day and then tested for the maximum tolerated dose of gold sodium thiosulphate, as compared to normal rats (Table I).

TABLE I.
Tolerated Doses of Gold Sodium Thiosulphate in Normal and Tolerant Rats.

Dose, mg/kg	Normal rats		Tolerant rats*	
	Survived, No.	Fatal, No.	Survived, No.	Fatal, No.
25	7	0		
40	1	3		
50	1	21		
100	1	31	7	0
150			3	0
200			0	3
250			0	3
300			0	2

*This group received 4 doses of 35 mg/kg every other day and was tested on the second day after the last chronic dose.

Whereas normal rats tolerated less than 40 mg/kg, animals that had received 35 mg/kg of gold sodium thiosulphate intramuscularly every other day for 4 doses tolerated 150 mg/kg, an increase of over 200%.

The relation of the size and number of doses to the development of tolerance is shown in Table II. The criterion of tolerance formation was survival following the injection of 100 mg/kg of gold sodium thiosulphate, a dose that is practically invariably fatal in normal rats. 35 mg/kg were given every other day to 36 rats, and the test dose of 100 mg/kg was given after a varying number of doses. As few as 2 chronic doses were found to be sufficient to cause the formation of tolerance to gold sodium thiosulphate, although not