treated normal animals or in castrated animals treated by daily subcutaneous injections of the same amount of testosterone. 2. In the dosage used, growth of the prostatic-seminal vesicle complex in rats treated by local application of testosterone to the penis is similar to that obtained by subcutaneous injection. 3. As measured by gross testicular weights, the testes are not appreciably inhibited by local application of testosterone to the penis, in the dosage used.

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Intravenous Injection of Amino Acids on Glucose Utilization Rate of Hypophysectomized and Insulin-Treated Rabbits.

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The high utilization rate[1] of the hypophysectomized rabbit makes it an excellent preparation for the study of the factors influencing carbohydrate metabolism. We attempted to determine if, and to what extent this sugar requirement could be substituted by amino acids. For this we carried out the determination of the glucose utilization rate in fasted hypophysectomized rabbits by the method previously described,[1] *i. e.*, by determining the rate at which glucose must be injected intravenously to maintain the blood sugar at a constant normal level. Having established this rate, we measured the effect of injecting a solution of amino acids,* containing 1.0% nitrogen (Frederick Stearns and Co.). In the first animal studied it was found that the glucose injection rate necessary to maintain a normal blood sugar level was markedly decreased during the injection period of the amino acids. We found the lowered glucose utilization period persisted for some 24 hours after the stoppage of

[1] Greeley, P. O., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1070.

* We wish to express our appreciation to the Frederick Stearns and Company Laboratories for the supplies of amino acids used in this work. We are indebted to Doctor Melville Sahyun, Director of Biochemical Research of that Company, for the constitution of the preparation. He states, "The amino acids are prepared by hydrolysis of casein in sulphuric acid and the subsequent removal of sulphate, ammonia, calcium and phosphate; also the separation of the non-hydrolyzable fraction by adsorption so that the final preparation consists of the pure amino acids from casein hydrolysate." The distribution of amino acids in the mixture is probably similar to that of hydrolyzed casein from which it was prepared.

amino acid injection. This could not be explained as a substitution and led us to the investigation of the effect of intravenously injected amino acids on glucose utilization rate.

Four hypophysectomized rabbits were used. First, the maximum glucose utilization rate was determined as described previously.[1] We were unable to explain the variation in this maximum rate in different rabbits. Then the glucose utilization rate was determined for the next period, about 2 hours in duration, during which the solution of amino acids was injected. These rabbits were observed up to 48 hours following this injection period. (Table I.)

TABLE I.
Hypophysectomized Rabbits.

Rabbit No.	Glucose per hr: Before injecting amino acids g	Glucose per hr: After injecting amino acids g	Amino acid solution injected cm³	Hr followed after injecting amino acids	Glucose spared g	Glucose equivalent of amino acids injected g
1	1.23	0.360	77	25	21.8	3.9
J.	1.39	0.375	120	48	48.8	6.1
G.	0.284	0.10	37	48	8.8	1.87
F.	0.720	0.119	31	48	28.8	1.57

The results (Table I) show a marked effect of the amino acids on the glucose utilization. That this is not due to any large degree to substitution follows from a qualitative examination of the figures. In the sixth column is given the amount of glucose "spared" by the amino acid injection. That is the difference between the glucose which would have been used had the animal maintained the maximum rate (column 2) and the amount actually used (column 3) during the after period (column 5). These amounts of glucose are quite large and could not have come from the conversion of the amount of amino acids given, to glucose. The true glucose-nitrogen ratio for casein (the amino acids were essentially those of casein) has not been determined. The excretory G:N ratio for casein in the phloridzin dog[2] is 3.06. Drury, Bergman and Greeley[3] stated the "true" G:N ratio for body protein of the phloridzinized dog as 6.04 as compared to the "excretory"† G:N ratio of 3.65. If we assume

[2] Janney, N. W., *Biol. Chem.*, 1915, **20**, 321.

[3] Drury, D. R., Bergman, H. C., and Greeley, P. O., *Am. J. Physiol.*, 1936, **117**, 323.

† The classical G:N ratio (here referred to as "excretory") considers only that glucose which is excreted by the kidneys and does not include that glucose formed from protein which is oxidized by the tissues. The total or "true" G:N ratio includes both the glucose excreted and that oxidized by the tissues.

the same relationship to exist between "excretory" and "true" G:N ratios for casein we would have 5.07 g of glucose formed for each gram of protein nitrogen. In column 7 is given the possible glucose that might come from the amino acids figured from this ratio. Close calculation is not necessary, for one could assume that the entire amount of the amino acids be converted to glucose and still be unable to account for the change in glucose utilization of the rabbits in this way.

We conclude that this effect is not substitution from another observation. To 2 hypophysectomized rabbits, after attaining their maximal glucose utilization rate, we administered 40 cc amino acids by stomach and followed them thereafter. In one case the utilization rate before the giving of the amino acids was 390 mg per hour; for the 6 hours after the amino acid feeding the rate was 146 mg per hour and during the next 13 hours 308 per hour. The comparable figures for the other animal were 680 mg, 627 mg, and 500 mg. These results suggest very little if any of the specific action obtained by intravenous injection, and can rather be explained as a substitution effect. The gastro-intestinal tract should be the route by which maximal substitution should be effected since the amino acids after absorption are largely taken up by the liver and converted very rapidly to glucose. As shown by Van Slyke and Meyer[4] only a small fraction of the fed amino acids gets into the general circulation and to the other tissues.

Having established this effect in hypophysectomized rabbits we next attempted to determine whether amino acids had a similar effect on rabbits with a high glucose utilization rate from another cause, insulin.‡ Two male 3-day-fasted rabbits of the same weight (2 kg) were both given an injection of 2.5 units insulin intravenously and each hour thereafter 1 unit subcutaneously for 10 hours. One of these was given 40 cc amino acid solution (containing 2.5 g amino acids). Both were given just enough glucose intravenously to maintain them at a normal blood sugar level. At the end of 11 hours the control rabbit had required 11.3 g glucose whereas the amino-acid-injected rabbit had required 5.9 g. The difference, 5.4 g, could not all have come from amino acids since if we assumed even a 100% conversion of amino acids to glucose it would give only 2.5 g. This experiment was repeated on two 2-day fasted male rabbits of the same weight (2.1 kg). Everything was

[4] Van Slyke, D. D., and Meyer, G. M., *Biol. Chem.*, 1913, **16**, 231.

‡ We wish to express our appreciation to the Eli Lilly Company for the insulin used in these experiments and to the Abbott Laboratories for the nembutal used in this work.

carried out as in the previous experiment except that the hourly insulin was given I.V. and the experiment continued for 15 hours. At the end of this time the control had required 22.2 g sugar, the amino acid rabbit 11.4 g, a difference of 10.8 g glucose compared to 2.5 g amino acids injected.

There can be no question of the action of this preparation of amino acids on the glucose utilization rates of hypophysectomized, and of insulin-injected rabbits. We are continuing our work in the attempt to determine what constituents are responsible for this action. Jacobs[5] found that cysteine diminishes the hypoglycemic action of insulin and believes this to be due to a specific action of the sulphydryl group. Our results could hardly be explained on this basis since the mixture we used contained no cysteine and very little cystine (0.3 to 0.5% of the total amino acid content).

Summary. A mixture of amino acids when injected intravenously into rabbits with high glucose utilization rates (from hypophysectomy, and from insulin) markedly reduced this glucose requirement.

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Effect of Vitamin B_1 and Vitamin B_2 Complex on the Loss of Weight Produced in Rats by Experimental Hyperthyroidism.

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Himwich, Goldfarb and Cowgill[1] reported that an increased amount of undifferentiated vitamin B_1 is needed during experimental hyperthyroidism, and Sure and Buchanan[2] found that vitamin B_1 has an antithyrogenic action. Later Drill[3] found that a large amount of yeast fed to rats receiving thyroxin will prevent a loss of liver glycogen. In this investigation a study was made on the effect of vitamin B_1 and of a yeast concentrate on the loss of weight produced by experimental hyperthyroidism.

Adult rats, weighing about 250 g, were all fed diet No. 8 *ad libitum*. This diet consisted of: salts, 4; cod liver oil, 4; Crisco,

5 Jacobs, H. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 305.

1 Himwich, H. E., Goldfarb, W., and Cowgill, G. R., *Am. J. Physiol.*, 1932, **99**, 689.

2 Sure, B., and Buchanan, K. S., *J. Nutrition*, 1937, **13**, 513.

3 Drill, V. A., *J. Nutrition*, 1937, **14**, 355.