

bourne(13) that survival of chick embryos infected with influenza B virus may be prolonged or shortened depending on time of injection of cortisone which was shown to suppress inflammatory response.

In our study, the protective effect is manifested primarily in a limited prolongation of survival time although occasional embryos will survive to time of hatching. Living *E. coli* seem to be more effective in this regard than endotoxin. Preliminary observations based on hemagglutinin titrations and infectivity determinations of allantoic fluid from protected and control eggs indicate that the protective effect is associated with a temporary suppression of virus levels in allantoic fluid. Therefore, whatever mechanisms may be involved in the widely demonstrated endotoxin provoked alterations of resistance, they are available, at least in part, to the developing chick embryo which should be a valuable tool in further study of these phenomena.

Summary. Phases of enhanced resistance and susceptibility to Newcastle disease virus were induced in chick embryos by infection with *E. coli*. These effects were duplicated by treatment of embryos with *E. coli* endotoxin at appropriate times.

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Effects of Various Amino Acids Upon Glucose Uptake of Rat Epididymal Adipose Tissue.* (26377)

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Insulin-like activity, as measured by glucose uptake of the rat diaphragm, has been stated to be increased by addition of the amino acids, hydroxyproline and lysine, in low concentrations to the incubation medium

(1). Insulin-like or anti-insulin effects of various amino acids were measured in a bio-assay system utilizing glucose uptake by Wistar rat epididymal adipose tissue(2). This bio-assay method has been shown to be sensitive to as little as 1-10 microunits insulin/ml.

Methods. This method of insulin bio-assay has been described and analyzed in detail previously(3). In this study, 35 to 60 mg segments of epididymal adipose tissue

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TABLE I. Relationship of Amino Acids at 10^{-3} M Concentration to Glucose Uptake.

Amino acid	No. of determinations	Difference in glucose uptake (mg glucose/g fat)		Significance of amino acid mean uptake compared with control mean uptake, P
		Mean	S.E.M. ($\pm$)	
Arginine	9	-1.47	.221	<.001
Glutamic acid	10	-.94	.37	>.02, <.05
Histidine	9	-1.16	.32	<.01
Isoleucine	10	-.94	.51	>.05, <.10
Leucine	72	-.77	.28	<.01
Lysine	25	+.22	.45	>.6
Proline	10	-.59	.31	>.05, <.10
Tyrosine	5	-2.53	.23	<.001

were excised from 100-180 g male Wistar rats. The tissue was added to beakers containing 2.5 ml of Krebs-bicarbonate buffer, glucose, 0.2% serum albumin, and L-amino acids or insulin. In some instances an L-amino acid and insulin were added simultaneously. Non-insulin controls were present in each set of determinations. Incubation proceeded in a Dubnoff metabolic shaking incubator for 5 hours, at 36.5°C , under 95% CO_2 . After deproteinization by the Somogyi method, residual glucose was determined on 1 ml aliquots of incubating medium by the Glucostat method. Glucose uptake was calculated as mg of glucose per g of adipose tissue. Various concentrations of insulin and amino acids, individually or in combination, were tested this way and compared with appropriate controls.

The differences between glucose uptake in the presence of an amino acid and the corresponding mean control uptake represent

effective glucose uptake of the individual amino acid. These results, calculated as mean difference of uptake, are expressed as milligrams of glucose per gram of fat tissue. Statistical significance of these differences were ascertained with the Student "t" method.

Results and discussion. The amino acids tested at 10^{-2} – 10^{-3} M concentration exhibited inhibition of glucose uptake by the fat pad. Six amino acids exhibited statistically significant inhibition of glucose uptake. These were arginine, glutamic acid, histidine, leucine, lysine, and tyrosine. Two amino acids, isoleucine and proline, exhibited slight inhibition at 10^{-3} M concentration, but this was statistically not significant (Table I).

In all determinations involving more than one concentration of the amino acid tested, it was invariably found that a decrease in amino acid concentration diminishes the inhibitory action of the amino acid (Table II).

TABLE II. Relationship of Dilution of Amino Acid to Glucose Uptake.

Amino acid	No. of determinations	Difference in glucose uptake (mg glucose/g fat)		Significance of amino acid mean uptake compared with control mean uptake, P
		Mean	S.E.M. ($\pm$)	
10^{-2} M Glutamic acid	10	-2.16	.42	<.001
10^{-3} M " "	10	-.94	.37	>.02, <.05
10^{-3} M Isoleucine	10	-.94	.51	>.05, <.10
10^{-7} M " "	4	-.34	.39	>.4, <.5
10^{-2} M Leucine	15	-2.68	.55	<.001
10^{-3} M " "	72	-.77	.28	<.01
10^{-5} M " "	32	+.49	.33	>.1, <.2
10^{-7} M " "	28	+.53	.27	>.05, <.10
10^{-2} M Lysine	15	-1.91	.53	<.01
10^{-3} M " "	25	+.22	.45	>.6

Preliminary observations were made utilizing a combination of leucine, at 10^{-3} M concentration, and insulin at 100 micro-units/ml. Coincidentally, separate determinations for leucine at 10^{-3} M concentration and insulin at 100 micro-units were obtained. Individually, there was a significant increase in uptake with insulin and a significant decrease in uptake with leucine. The combination of insulin and leucine demonstrated a significant increase in glucose uptake above the non-insulin control while being associated with a significant decrease in glucose uptake as compared with the insulin control. In 21 determinations, the mean difference in glucose uptake of the insulin-leucine combination compared with the non-insulin control was $+ 2.58$ mg glucose/g adipose tissue ($p < .001$). When the combination was compared with the insulin control, the mean difference in glucose uptake was -2.04 mg glucose/g adipose tissue ($p < .001$).

A pH effect was considered as a possible factor in the inhibitory action of leucine upon glucose uptake. Determinations of pH per-

formed upon leucine samples were identical with those of control samples, thereby eliminating pH changes as significantly related to the inhibitory action of leucine.

Previous unpublished studies, utilizing an incubation time of 2 hours, demonstrated statistically significant inhibition of glucose uptake by adipose tissue in the presence of certain amino acids. The magnitude of the effects tended to be less than appeared in the present study utilizing longer incubation times.

Summary. Six of 8 amino acids tested at concentrations of 10^{-2} – 10^{-3} M demonstrated significant inhibition of glucose uptake by adipose tissue. This inhibitory action was diminished as amino acid concentration was further decreased.

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The Value of Certain Steroidal Sapogenins in Rations of Fattening Lambs and Cattle. (26378)

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The growth-promoting action for ruminants of diethylstilbestrol and certain other estrogens is well known. However, because of the estrogenic activity of these compounds, there must be close control of the quantities given in practical feeding operations. Combinations of estrogens with androgens or progesterone have been used as growth stimulants, the estrogenic action being diminished by the other hormone so that anabolic action predominates. However, it would be desirable to have available a single, non-estrogenic

compound with substantial anabolic action (1).

There are structural similarities between natural animal steroid hormones and certain plant steroids. These plant products are not known to have estrogenic activity, nor have they been evaluated for effect on ruminant productive performance. The present report provides data on growth rate, feed efficiency and carcass quality of fattening steers and lambs fed plant steroidal sapogenins, saponins, and a natural source of unsolubilized sapogenins. The products tested were smilagenin and smilagenin saponin isolated from *Agave lecheguilla*, ground *Agave lecheguilla*

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