female rats. However, recent observations suggest that male rats have a high incidence of certain types of cardiac lesions (to be reported later).

There are interesting strain differences in appearance of both coronary and general arteriosclerotic lesions. To date, selected strains were more susceptible than others. This is suggestive of the same type of situation existing in the human disease, which is known to have a predilection for certain families.

The intimal basophilia observed in some coronary arteries remains to be studied further. We hope, by histochemical means, to demonstrate whether this is calcium or mucopolysaccharide, or perhaps both existing in close association. Moon(6) has described intimal accumlation of acid mucopolysaccharide as one of the earliest changes in human arteriosclerosis.

Stress† would appear to be an important determinant in coronary lesions produced in rats. Three factors appear to be required: repeated breeding, unilateral nephrectomy, and administration of ACTH. It is our supposition that repeated breeding puts stress on the adrenal glands and that this stress is further heightened by administration of repeated doses of ACTH. Possibly, the unilateral nephrectomy intensifies action of ACTH. The parallelism between experimental results and those reported by Friedman *et al.*, in humans(7) is interesting and suggestive.

Summary. 1) Severe coronary arteriosclerosis has been produced in female rats (a) bred repeatedly, (b) subjected to unilateral nephrectomy, and (c) treated with ACTH. The coronary lesions in these animals resemble those seen in man. Morphologically, the coronary arteriopathy is varied, *i.e.*, intimal hyperplasia, deposition of basophilic material in intimal and medial layers (possibly acid mucopolysaccharides), elastosis, and mural swelling leading to complete obliteration of the lumen. Coronary and mural thrombi occur frequently. 2) No fat or salt is added to the diet. Serum cholesterol levels are normal. The possible role of "stress" in induction of this type of coronary artery disease has been discussed.

† We use the word "stress' in the broad sense as described by Selye.

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Effect of Intravenous Amino Acids on Plasma Non-Esterified Fatty Acids.* (24702)

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Plasma levels of non-esterified fatty acids (NEFA) seem determined largely by rate at which adipose tissue releases these substances. The following operations result in decrease in concentration of circulating NEFA: Carbohydrate administration(1,2), insulin injection (1), tolbutamide administration(3), and glucagon injection(4). In contrast, increased plasma NEFA concentrations have been ob-

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served after fasting(1,5), in uncontrolled diabetes mellitus(6), after epinephrine injection (1,2,4), and after growth hormone administration(7). Fat ingestion does not appear to induce appreciable changes in level of circulating NEFA(1,2). These results suggest that rate of NEFA release from adipose cells is related to glucose utilization(8). Although effect of carbohydrate administration on NEFA metabolism has been widely studied, the effect of protein on NEFA has received little consideration. Gordon(5) mentioned that concentration of plasma NEFA decreases following ingestion, separately, of α L-alanine, L-glutamic acid, and L-leucine, but to a lesser degree than the decrease produced by glucose. Since parenteral, as well as oral, administration of amino acids lowers peripheral venous glucose concentration(9), an investigation was made of the effect of intravenous amino acids on concentration of plasma NEFA and blood glucose.

Methods. Eight healthy adult volunteers served as subjects. Each was studied at rest in postabsorptive state. Venous blood samples were obtained from one antecubital vein without stasis and, simultaneously, capillary blood was pipetted from finger tip, following cutaneous puncture. Fasting samples and samples following infusions of 0.9% saline solution were obtained. Six subjects received 500 ml infusion of 10% protein hydrolysate† over a 2-hour period, and blood samples were obtained at ½-hourly intervals during infusion. Two subjects were given 400 ml infusion of 5% L-arginine monohydrochloride‡ in water in a 1½ hour period, and blood samples collected as described above. Capillary (arterial(10)) and venous blood glucose were measured by the Nelson modification of Somogyi procedure(11). NEFA were determined by Dole's method(1), except that constant boiling hydrochloric acid was used for standardizing the alkali used in titration. All determinations were done in duplicate. Probabilities were estimated by use of Student's test of "t"(12).

† Amigen (glucose-free), a pancreatic hydrolysate of casein was supplied by Mead Johnson and Co.

‡ Supplied by Cutter Labs.

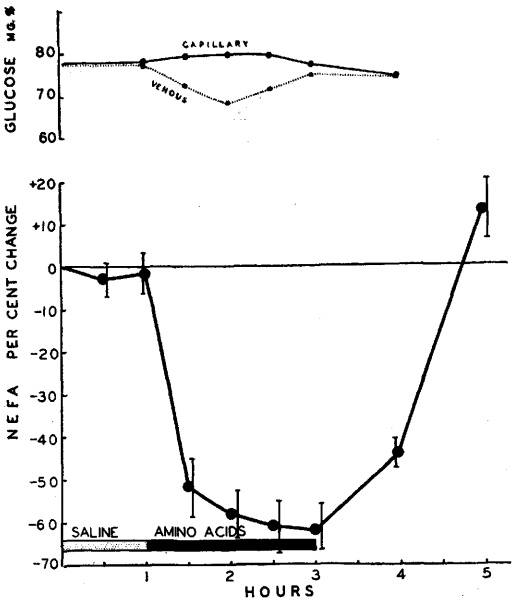

FIG. 1. Changes in mean levels of plasma NEFA (% of control) and blood glucose (capillary and venous mg %) during protein hydrolysate infusion in 6 normal subjects. Vertical bars represent one stand. error.

Results. Continued fasting for 2 hours was associated with slight elevations of NEFA levels as previously reported(1,2). Saline infusions produced no significant change in NEFA levels. In all subjects, administration of protein hydrolysate induced precipitous falls in NEFA concentration (Fig. 1). The major drop in NEFA occurred within 30 minutes after start of infusion. NEFA levels continued to fall at less rapid rate during next 90 minutes of infusion. NEFA levels tended to return toward baseline values within 60 to 90 minutes after cessation of amino acid infusion, and complete return to fasting levels occurred within 2 hours. Similar prompt decreases in NEFA concentration occurred in the 2 subjects receiving L-arginine (Fig. 2).

Concurrently with fall in NEFA levels, venous blood glucose fell significantly during the first 90 minutes of protein hydrolysate infusion. During this time capillary-venous (C-V) glucose differences were significantly increased (Table I). A progressive return to preinfusion levels of glucose C-V difference, and venous glucose concentration occurred in one-hour period following end of infusion.

TABLE I. Changes in Venous Blood Glucose and Capillary-Venous (C-V) Glucose Difference (mg %) during Infusion of 500 ml of 10% Protein Hydrolysate.

Min. after start of infusion	Mean diff. from fasting venous blood glucose	Stand. error of observed diff. between means	p	Mean C-V glucose diff.	Stand. error of diff. between means of C-V glucose	p
Fasting				− 0.9*		
30	−5.6	2.8	approx. .100	+ 7	1.2	<.005
60	−9.8	2.0	<.005	+11.5	2.7	>.005 <.010
90	−6.5	1.8	>.010 <.025	+ 8.4	1.8	approx. .005
120	−3.0*			+ 2.2*		
180	" *			0 *		

* Not significant.

During the same time, levels of NEFA tended to rise toward baseline values (Fig. 1). Increase in arteriovenous glucose difference was also observed in 2 subjects receiving arginine infusion. Values for glucose C-V differences at 30 and 60 minutes, respectively, after start of infusion were 13 and 4 mg % in the first subject, and 11 and 6 mg % in the second subject. A rise occurred in both arterial and venous glucose concentrations (Fig. 2).

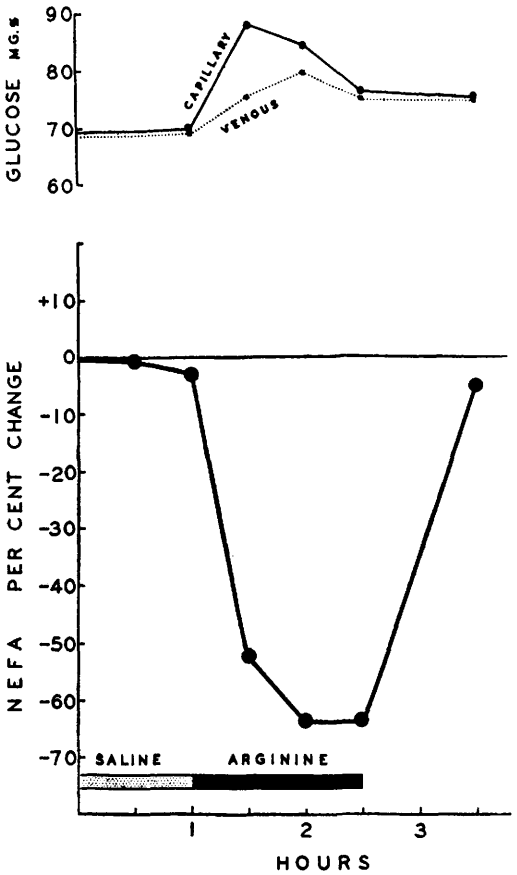

FIG. 2. Changes in mean levels of plasma NEFA (% of control) and blood glucose (capillary and venous mg %) during L-arginine monohydrochloride infusion in 2 normal subjects.

Discussion. Previous studies have suggested that rate of NEFA release from adipose tissue tends to vary inversely with concurrent rate of glucose utilization in body. Our experiments show clearly that parenteral administration of an amino acid mixture (protein hydrolysate) and administration of a single amino acid, L-arginine, were associated with prompt and dramatic fall in plasma NEFA concentration (Fig. 1, 2). This decrease in NEFA levels was sustained throughout period of infusion, but was followed by return to pre-infusion values within 90-120 minutes after infusion was ended.

The results can be considered in relation to several possible explanations. First, amino acids may directly inhibit NEFA release from adipose cells. The effect of amino acids on *in vitro* release of NEFA apparently has not been reported. A second explanation might be that administration of amino acids promotes glucose utilization, and thereby indirectly inhibits NEFA release. This might be accomplished in one of several ways: Amino acid administration might stimulate insulin release from the pancreas. Evidence of a fall in venous glucose levels and of increased glucose utilization following administration of amino acids has been reported(9,13). Since the amino acid mixture used contained an ap-

preciable quantity of glucogenic amino acids, it is also possible that rapid conversion of these amino acids to glucose by the liver could account for the changes that occurred in NEFA.

Glucose C-V differences and venous glucose levels were measured concurrently with plasma NEFA in our study. The results show that C-V glucose differences increased significantly during administration of amino acid mixture with concomitant fall in venous glucose. Capillary glucose levels remained virtually unchanged; thus it is difficult to attribute NEFA changes to amino acid-induced hyperglycemia. Moreover, Gordon(5) reported fall in NEFA when nonglucogenic amino acid, leucine, was fed. During administration of the glucogenic amino acid, arginine, C-V glucose differences also increased, but this time, associated with a rise in capillary and venous glucose levels. This observation is in agreement with reports that response of blood glucose to amino acids differs with individual amino acids administered(14). Since blood flow through the forearm was not measured during experiments, the conclusion that rate of glucose uptake by tissues of forearm increased, must remain tentative. However, when added to observation that venous blood sugar level fell significantly during administration of amino acid mixture, these results suggest that at least some of the NEFA-lowering effects of parenterally administered amino acids occurred because of an induced increase in rate of glucose utilization.

Summary. (1) The effect of infusing an amino acid mixture (protein hydrolysate) on plasma NEFA, venous glucose level, and capillary-venous glucose difference, was studied in 6 healthy volunteer subjects. In every instance, plasma NEFA levels fell precipitously during amino acid infusion, and capillary-venous glucose differences increased concurrently. Similar results were obtained when a single amino acid, L-arginine, was infused in 2 normal subjects. (2) The results suggest that NEFA-lowering effects of parenterally administered amino acids may have been mediated entirely or in part *via* an induced increase in rate of glucose utilization.

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Absence of Intestinal Viral Flora in Infants During Epidemic of *Escherichia coli* Gastroenteritis. (24703)

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A large number of instances have been recorded in which specific strains of *Escherichia coli* have been isolated from most or all cases in outbreaks of infant diarrhea(1-6). Several

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