Our findings support a hypothesis(1,2, 14,15), based on a decrease in gastric mucus, to explain the appearance of steroid-induced ulcers. Corticosteroids would render the mucosa more sensitive to the digestive and irritative properties of gastric juice by weakening the mucus barrier. In this respect, it is significant that the diminution in mucus in the stomach itself (Fig. 5) took place before ulcers developed, which favors the concept of an etiologic role of mucus in ulcerogenesis. The low acidity of gastric juice after prednisolone overshadows the widely accepted theory that ulcers are necessarily related to hyperacidity.

Summary. Prednisolone, administered subcutaneously to rats, produced gastric ulcers and reduced the mucus of gastric juice and tissue. Determination of hexosamine was used as a measure of mucus. Ulcers were limited to the corpus and never appeared in the antrum. The antrum was found to contain twice as much mucus as the corpus. Gastric juice acidity was markedly reduced. These data favor a hypothesis ascribing a protecting role to mucus in the natural defense of the gastric mucosa against ulcerogenic agents. Steroid ulcers may be explained by a diminution of gastric mucus.

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In vivo and *in vitro* Effect of Glucagon on DL-Leucine-1-C^{14} Incorporation Into Protein of Rat Pancreas.* (28729)

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It has been found(1,2) that administration of glucagon to rats and rabbits results in a depletion of zymogen granules of acinar cells of the pancreas. Neither adr nalectomy, hypophysectomy, atropinizatio nor fasting had any effect on this action

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lucagon. Since glucagon did not affect rate f secretion of amylase by the pancreas, it eemed likely that the synthesis of zymogen ranules was inhibited. Salter *et al*(3) and zzo and Glasser(4) have shown that gluca- on administration decreases blood amino acid vels in intact animals, and Glasser and Izzo 5) have reported a similar effect in adrenal- ctomized rats. The present studies on in- orporation of DL-leucine-1-C^{14} into total rotein and zymogen granule fractions of ancreas indicate that glucagon does not ave a direct effect on protein synthesis in ıe pancreas, but produces an inhibition that ; secondary to lowering of essential amino cid levels in the blood.

Materials and methods. In vivo *studies.* Iale Wistar rats weighing between 150 and 70 g were used. Each rat was injected i.v. ·ith 7 μC of DL-leucine-1-C^{14} 45 minutes efore being killed. The rats were given a ubcutaneous injection of 0.45 mg of gluca- on† in an alkaline solution either 2 or 5 ours before killing the animals.

The zymogen granule fraction of the pan- reas was isolated using the method of Siekeitz and Palade(6). The protein of the pancreas and zymogen granule fraction was precipitated in trichloroacetic acid (TCA) and lated for counting in a Picker proportional as flow counter according to the method described by Siekevitz(7). All counts were corected for self-absorption by use of a standrd curve. Protein of the zymogen granule raction was measured by the method of owry *et al*(8). The non-protein fraction of he serum was obtained by extracting with % TCA.

In vitro *studies.* Paired studies on segnents of the pancreas consisting of histoogically intact cells from the same rat were erformed in 3 ml of a modification of the ncubation medium described by Campagne 9) for maximal synthesis of rat pancreatic rotein *in vitro*. The medium for control aninals consisted of Krebs-bicorbonate buffer at H 7.4 containing fumarate, asparagine, and glutamine, 7 non-essential amino acids, 11 amino acids essential for maximal synthesis of rat pancreatic protein, 7.5 mg of glucose and 0.1 μC of DL-leucine-1-C^{14} with a specific activity of 6.0 mC per mg. The amino acids were each at least 10 times the concentration found in normal human serum as measured by the method of Stein and Moore (10). Twenty μg of kanomycin sulfate were added to each stock solution of amino acids and small aliquots frozen for daily use. The modifications for the experimental groups will be described.

After addition of approximately 100 mg of pancreas, each incubation bottle was gassed with 95% O_2-5% CO_2 for 5 minutes and then placed in a Dubnoff shaker for 45 minutes, 2 hours or 5 hours at 37°C. At the end of each incubation period the specific activity of the protein of the pancreas was determined.

One group of rats was injected subcutaneously with 0.45 mg of glucagon and serum collected 2 hours later. The alpha-amino nitrogen concentration was determined by the method of Frame *et al*(11). The sera of the glucagon-treated group with the lowest α-amino nitrogen levels were pooled and the sera of saline-injected controls with the highest α-amino nitrogen concentration were pooled and the α-amino nitrogen determinations repeated. Paired studies using 2 ml of pooled serum and 0.1 μC of DL-leucine-1-C^{14} were performed, incubating the pancreas segments for 5 hours at which time the specific activity of the protein was determined.

Results. In vivo *studies.* A 47% decrease ($P<0.01$) was found in specific activity of the protein of the pancreas 2 hours after administration of glucagon when compared with control values (Table I). However, no significant difference was found in the radioactivity of the non-protein portion of the serum of the same animals.

The specific activity of the protein of the zymogen granule fraction of glucagon-treated rats was 56% less than controls. This difference was statistically significant ($P<0.01$) (Table I).

A 30% decrease ($P<0.01$) was found in

† Crystallin glucagon lot No. 258-2348-54-2 was indly supplied by Dr. Mary Root, Eli Lilly & Co., ndianapolis, Ind.

TABLE I. Effect of Glucagon on DL-leucine-1-C^{14} Incorporation into Protein of Pancreas and Zymogen Granule Fraction of Pancreas of Rats.

Time, hr	Tissue	Group	No. of rats	Protein, S.A.* ± SEM	% change	P
2	Pancreas†	Control	9	164 ± 14		
		Glucagon	9	87 ± 17	−47%	.01
2	Zymogen granules‡	Control	9	270 ± 68		
		Glucagon	9	123 ± 24	−56%	.01
5	Pancreas‡	Control	6	76 ± 5.8		
		Glucagon	6	53 ± 4.4	−30%	.01

* Specific activity measured in cpm per mg of protein.
† Specific activity of DL-leucine-1-C^{14} was 15.6 mC per mmole.
‡ Specific activity of DL-leucine-1-C^{14} was 6.45 mC per mmole.

specific activity of the protein of the pancreas 5 hours after glucagon administration when compared to controls (Table I). As in the 2-hour group, no significant difference was found in the radioactivity of the non-protein portion of the serum of these rats.

In vitro *studies*. The specific activity of pancreatic protein continued to rise during 5 hours of incubation *in vitro* (Fig. 1). When the concentration of essential amino acids was decreased to 50% of control level, the protein specific activity was significantly lower at each time interval tested. The results indicate that amino acid incorporation into protein continued throughout the period studied.

Lowering all amino acids or only the essential amino acids to 10% of the control concentration resulted in a significant lessening ($P<0.01$) of the DL-leucine-1-C^{14} incorporation into pancreatic protein during a 45-minute incubation (Table II). Lowering all the essential amino acids except leucine

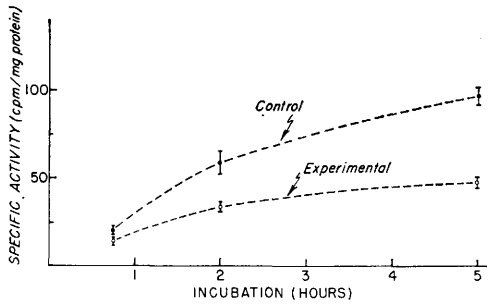

FIG. 1. Effect of lowering essential amino acid concentration to 50% of control on specific activity of pancreatic protein. Each vertical bar indicates ± standard error of mean for 4-12 determinations (Table II).

TABLE II. *In vitro* Studies on DL-leucine-1-C^{14}* Incorporation into Pancreatic Protein.

Time of incubation	Factor varied	% of control conc	Pairs of studies	Mean difference in S.A.† ± S.E.D.	P
45 min	All amino acids	10	4	−14.5 ± 1.8	.01
"	Non-essential amino acids	10	3	+ 1.4 ± 1.4 (N.S.)‡	
"	Essential amino acids	10	6	− 8.1 ± 1.2	.01
"	*Idem*	50	11	− 5.9 ± 1.6	.01
"	"	150	8	+ 5.6 ± 3.2 (N.S.)	
"	Glucagon *in vitro*	—	4	+ .5 ± 4.9 (N.S.)	
2 hr	Essential amino acids	50	12	−22.8 ± 6.5	.01
"	*Idem*	150	8	+ 5.0 ± 3.4 (N.S.)	
"	Glucagon *in vitro*	—	4	+ 9.2 ± 6.9 (N.S.)	
5 hr	Essential amino acids	50	4	−49.0 ± 6.7	.01
"	*Idem*	150	4	+30.0 ± 6.0 (N.S.)	
"	Glucagon *in vitro*	—	10	+11.0 ± 7.9 (N.S.)	
"	Total serum amino acids after glucagon treatment of rats	71	5	−17.6 ± 4.6	.02

* Specific activity of DL-leucine-1-C^{14} was 6.0 mC per mmole.
† Specific activity measured in cpm per mg of protein.
‡ Not significant.

to 10% of control concentration and maintaining the amount of DL-leucine-1-C^{14} constant resulted in a significant decrease ($P<0.01$) in the radioactive leucine incorporation into protein. Lowering only the essential amino acid concentrations to 50% of the control level also produced a significant decrease ($P<0.01$) in radioactive leucine incorporation (Table II). No significant change in incorporation was found when glucagon was added to the medium. Neither lowering the non-essential amino acids to 10% of control level nor raising the essential amino acids to 150% of the control resulted in a significant change of DL-leucine-1-C^{14} incorporation (Table II). Similar results were observed after 2 and 5 hours of incubation (Table II). In all of these experiments a constant ratio of radioactive leucine to non-radioactive leucine was administered.

The alpha-amino nitrogen concentration of the serum of glucagon-treated rats was 5.25 mg per 100 ml, which was 71% of control value. There was significantly less incorporation ($P < 0.02$) of leucine-1-C^{14} into the protein of pancreatic segments incubated for 5 hours in the serum of glucagon-treated rats than in paired segments incubated in control serum (Table II).

Discussion. It has been demonstrated(1,2) that the rate of secretion of pancreatic digestive enzymes is not affected by glucagon, and there is no histological evidence that glucagon causes an increased catabolism of zymogen granules. Thus, the histological depletion of zymogen granules which parallels the chemical decrease in amylase concentration after glucagon treatment of animals is probably attributable to a decreased rate of formation. The present finding that treatment of rats with glucagon lowers the specific activity of protein in the pancreas and in the zymogen granules of the pancreas after administration of radioactive leucine supports the concept that there is a decrease in rate of protein synthesis.

Although glucagon has been reported to lower the total amino acid level of serum in animals(3,4,5), there was no significant decrease in the level of DL-leucine-1-C^{14} in glucagon-treated rats as compared with control animals, as judged by the radioactivity of the non-protein fraction of the serum. If glucagon lowers serum amino acid levels through stimulation of gluconeogenesis in the liver, as suggested by the studies of Izzo and Glasser(4) and Miller(12), then the concentration of leucine in the serum should not be affected since leucine is not converted to glucose in the liver(13,14).

The results of the *in vitro* studies indicate that the decreased synthesis of pancreatic protein following glucagon administration is not a result of direct action of the hormone on the pancreas, but is more likely secondary to the lowering of essential amino acid concentrations in the serum. No significant effect was found on the incorporation of the DL-leucine-1-C^{14} into the protein of the pancreas when glucagon was added to the incubation medium for any of the time periods studied (Table II). The data reveal that the incorporation of radioactive leucine into protein of the pancreas is sensitive to changes in concentration of essential amino acids. Decreasing the concentration of the essential amino acids resulted in a significant decrease in the radioactive leucine incorporation into the pancreatic protein after incubation (Table II). The inhibitory effect of the decreased essential amino acid levels increased in magnitude with time (Fig. 1). Lowering of the concentration of non-essential amino acids had no effect on pancreatic protein synthesis. Increasing the essential amino acid concentration above the initial level did not significantly increase the incorporation of leucine into pancreatic protein since the system was designed to yield a maximal rate of pancreatic protein synthesis (Table II).

The concept that glucagon inhibits synthesis of pancreatic protein by lowering blood amino acid levels is further substantiated by the finding that pancreatic tissue incubated in serum from glucagon-treated rats showed less incorporation of radioactive leucine than tissue incubated in control serum. The total α-amino nitrogen level in pooled serum of glucagon-treated rats was 29% lower than in control serum. In contrast to glucagon, it has

been found that cortisone causes an increase in blood amino nitrogen levels(4) and an increase in the zymogen granulation of the pancreas(1,2,15).

From these data and those obtained previously(1,2) it may be stated: 1) Glucagon administration *in vivo* causes a decrease in protein synthesis of the pancreas, and in formation of zymogen granules, but does not alter the rate of exocrine secretion; this produces a histological and chemical depletion of the secretory digestive enzymes in the acinar cells. 2) This action of glucagon is secondary to a lowering of essential amino acid levels in the serum.

Summary. *In vivo* studies measuring DL-leucine-1-C^{14} incorporation into pancreatic protein helped confirm earlier histological and chemical observations that glucagon administration to rats and rabbits inhibited synthesis of pancreatic protein. *In vitro* studies revealed that glucagon had no direct effect on DL-leucine-1-C^{14} incorporation into protein of segments of rat pancreas. Incubation of pancreas in medium with lowered essential amino acid levels or in serum from glucagon-treated rats with lowered amino acid levels resulted in decreased incorporation of radioactive leucine into pancreatic protein. Neither lowering non-essential amino acids nor adding glucagon to incubation medium had any effect. It was suggested that glucagon administration *in vivo* causes a decrease in protein synthesis of the pancreas and that this action of glucagon is secondary to the lowering of essential amino acid levels in the serum.

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Graft-Host Response in Murine Radiation Chimeras.* (28730)

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A problem in bone marrow therapy of lethally irradiated mice is the "secondary disease" mortalities arising from a graft-host immune reaction(1,2). In an attempt to influence these delayed deaths, we administered hemopoietic tissues of strain (A) mice to newborn strain (B) animals. The bone marrow from (B) animals was later injected into lethally X-irradiated (A) animals. Seventeen out of 28 animals receiving bone marrow from donors neonatally treated with host tissue survived 90 days, whereas 9 out of 29 receiving bone marrow from normal

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