gest that a soluble, nonimmunogenic form of self-antigen might have a role. Such a nonimmunogenic self-antigen could produce tolerance by occupying specific lymphocyte receptors and preventing their activation by immunogenic self-antigen. Tolerance to basic protein would, therefore, depend upon a balance between the concentration of the immunogenic and nonimmunogenic forms of the antigen. This concept is supported by the finding of Teitelbaum and co-workers that allergic encephalomyelitis can be suppressed, or even cured, by injecting animals with a low-molecular-weight basic copolymer of amino acids (*7*). The copolymer was observed to cross-react with lymphocytes previously sensitized to encephalitogenic basic protein (*23*). Hence it is conceivable that the copolymer suppresses encephalomyelitis by binding to specific lymphocyte receptors and preventing recognition of immunogenic basic protein. Our findings implicating nonimmunogenic self-antigen in the regulation of autosensitization against fibroblast antigens (*15, 16*) would suggest that a nonimmunogenic fragment of basic protein may exist in vivo and prevent activation of lymphocytes capable of recognizing immunogenic basic protein.

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17. The Lewis rats, originally obtained from Microbiological Associates (Baltimore), were continually inbred and were tested for homozygosity by Dr. A. Meshorer of the Animal Breeding Center of this institute. Rats aged 4 to 6 weeks were used for the experiments.
18. The central nervous system antigen was prepared by homogenizing with a glass piston the cerebellums and spinal cords of 20 rats in about 20 ml of phosphate-buffered saline at room temperature. The particulate matter was removed by centrifuging the homogenate for 20 minutes at 3000 rev/min. The supernatant fluid was collected and lyophilized. The protein concentration was determined by the biuret reaction. The extract was dissolved in water for use in experiments.
19. Central nervous system antigen (1.2 mg of protein in 0.2 ml of H_2O) was emulsified with 0.2 ml of complete Freund's adjuvant (Difco, Detroit) and injected into the hind foot pads of six Lewis rats. The animals received in the dorsum of the hind feet 0.1 ml of pertussis vaccine (Rafa, Jerusalem) containing 24×10^6 microorganisms (H. C. Rauch, personal communication). Within 11 to 21 days all of the six Lewis rats developed paralysis. Histological lesions diagnostic of encephalomyelitis were observed in all the animals.
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Arachidonic Acid Causes Sudden Death in Rabbits

Abstract. *Injection of sodium arachidonate (1.4 milligrams per kilogram) into the marginal ear veins of rabbits caused death within 3 minutes. Histological examination showed platelet thrombi in the microvasculature of the lungs. Rabbits were protected from the lethal effects of arachidonic acid by pretreatment with aspirin. Fatty acids closely related to arachidonic acid did not cause death.*

The fact that arachidonic acid, the essential fatty acid precursor of prostaglandins E_2 and $F_{2\alpha}$, aggregates platelets in human platelet–rich plasma (*1*) suggested that it might induce platelet aggregation in vivo. To test this hypothesis, we injected solutions of sodium arachidonate (*2*) into the marginal ear veins of New Zealand rabbits weighing 2 to 3 kg (*3*). The following results were obtained: one animal died 2 minutes after an injection of 6 mg/kg (*4*); 15 animals injected with 1.4 mg/kg all died between 15 seconds and 3 minutes later, most succumbing at about 2 minutes; two animals injected with 1 mg/kg died rapidly and two so injected did not, although the latter two exhibited rapid respiration and gasping 45 seconds after the injection; three animals injected with 0.7 mg/kg did not die but showed rapid respiration; of five animals injected with 0.5 mg/kg, four survived and showed no gross toxic effects, while one died in 2 minutes. Five of those rabbits that had survived doses lower than 1.4 mg/kg were challenged with 1.4 mg/kg on a later date and all died within 2 minutes. None of eight controls (*5*) showed any of the signs observed in the rabbits injected with arachidonic acid. An approximate

Table 1. Prevention of gross toxic and lethal effects of arachidonic acid by aspirin: response of rabbit to intravenous injection of sodium arachidonate (1.4 mg/kg).

Rabbit	Time after intraperitoneal administration of aspirin or vehicle					
	2 hours	1 day	2 days	3 days	4 days	7 to 9 days
No. 1* (control, no aspirin)	Death in 2 min					
No. 2	No effect	No effect	Death in 2 min			
No. 3	No effect	Rapid respiration at 1 min	Rapid respiration at 2 min	Death in 2 min		
No. 4	No effect	No effect	No effect	No effect	Death in 2.4 min	
No. 5	No effect	No effect	No effect	No effect	No effect	Rapid respiration

* This control animal received an intraperitoneal injection of the vehicle. The previously mentioned 15 animals that died after injection of 1.4 mg of sodium arachidonate per kilogram may also be considered as controls.

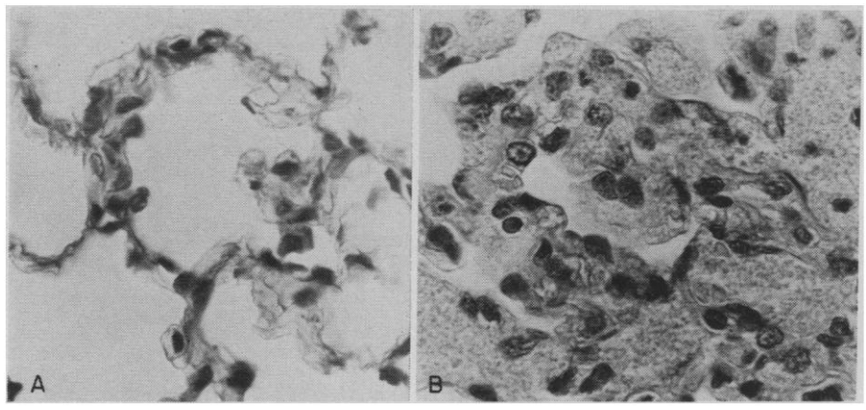

Fig. 1. (A) Photomicrograph of section of lung tissue taken from control rabbit that had received sodium carbonate vehicle. No platelet thrombi are seen. (B) Photomicrograph of section of lung tissue of a rabbit that died 2 minutes after injection of sodium arachidonate (1.4 mg/kg) into an ear vein. Massive platelet thrombi are seen. (Hematoxylin and eosin; × 500)

LD_{50} (dose lethal to 50 percent of animals) calculated from the above data was 1.0 mg/kg (*6*).

Blood samples were taken by cardiac puncture from several animals immediately after respiration ceased. They were full of platelet aggregates, and accurate platelet counts could not be obtained.

Specimens of lungs, heart, kidney, liver, pancreas, and brain were obtained immediately post-mortem and prepared for microscopic examination (*7*). All animals killed by arachidonic acid showed platelet aggregates in the vessels of the microcirculation of the lungs. The degree of platelet aggregation was variable and often was great enough in many vessels to completely occlude their lumina (Fig. 1). The microvasculature of other organs in these animals never exhibited such platelet thrombi. Four of the control animals were killed (*5*). Their pulmonary microcirculation was either clear or showed an occasional small platelet aggregate (Fig. 1).

To see whether sudden death was a specific effect of arachidonic acid, the sodium salts of five closely related fatty acids (*8*) were each injected into two rabbits at doses four times the LD_{50} of arachidonic acid. None of these animals showed any signs of toxicity.

Since ingested aspirin has been shown to inhibit both platelet aggregation and prostaglandin production for several days in humans (*9*), we pretreated four rabbits with aspirin (*10*) to see whether it would protect rabbits from a lethal dose of arachidonic acid. As seen in Table 1, these rabbits were indeed protected, the protective effect persisting for 2 to 4 days in three rabbits. The fourth rabbit appeared to be protected for more than 9 days. However, this rabbit may have been able to tolerate arachidonic acid at 1.4 mg/kg without aspirin.

The platelet thrombi showed no gross evidence of fibrin, suggesting that the blood coagulation system was not involved. To verify this, a rabbit was given heparin (*11*) and then injected with arachidonic acid (1.4 mg/kg). This rabbit also died in 2 minutes with platelet aggregates in the small arterial vessels of the lungs.

The cause of death in these animals appears to be blockade of the microcirculation of the lungs by platelet aggregates. However, other factors may be involved, such as bronchoconstriction caused by the production of prostaglandin $F_{2\alpha}$ by platelets (*1*) or by lung tissue, possibly in response to the particulate nature of the aggregates (*12*).

The present data, along with our previous findings relating platelet prostaglandin synthesis to blood clotting (*13*) and platelet aggregation (*14*), suggest that arachidonic acid may be a key compound in hemostasis and thrombosis. It is found in ester linkage in phospholipids, cholesteryl esters, and triglycerides in blood and other tissues. The unesterified form also may be bound to albumin in plasma. Release of arachidonic acid from any of these sources would trigger platelet aggregation and prostaglandin synthesis by making the prostaglandin precursor available. For example, it could be released from platelet phospholipids by the sequential action of phospholipase A_1 and a lysophospholipase, enzymes known to be present in human platelets (*15*). An essential role of other naturally occurring aggregating agents such as collagen, adenosine diphosphate, and thrombin may be to trigger phospholipase activity causing release of arachidonic acid. That normal hemostasis depends on platelet prostaglandin synthesis is suggested by the fact that aspirin prolongs the bleeding time in humans (*16*) and inhibits platelet aggregation and prostaglandin synthesis (*8*).

Pathological states in which arachidonic acid is not available to platelets may result in a bleeding diathesis. On the other hand, the sudden, local release of large amounts of arachidonic acid could result in thrombosis leading to disabling and fatal thrombotic diseases such as pulmonary embolism, myocardial infarction, and stroke.

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2. Sodium arachidonate solutions were prepared by dissolving arachidonic acid (> 99 percent pure, Hormel Institute, Austin, Minn.) in 100 m*M* Na_2CO_3. Final dilutions for injection were made in sterile 0.9 percent sodium chloride solution (Travenol Laboratories, Inc., Deerfield, Ill.). The sodium arachidonate was contained in 1 ml and was injected at the rate of 1 ml/min.
3. In a study unrelated to platelet aggregation [W. L. Bloom, *Metabolism* **6**, 777 (1967)] it was observed that three rabbits died immediately after the injection of large amounts of arachidonic acid. The significance of this finding was not discussed.
4. Less than 1 minute after injection of a lethal dose of arachidonic acid the animals began to exhibit obvious respiratory difficulties including rapid breathing and flaring of the nostrils. This was soon followed by bulging of the eyes with extreme dilation of the pupils, convulsive seizures, loss of corneal reflex, and cessation of respiration. When breathing stopped, the chest cavity was rapidly opened and either cardiac arrest or ventricular fibrillation was noted.
5. Control animals were injected with either sodium carbonate solution diluted in saline or with saline solution alone.
6. The LD_{50} was calculated by the method of J. T. Litchfield and F. Wilcoxon [*J. Pharmacol. Exp. Ther.* **96**, 99 (1949)].
7. The tissue was fixed in 10 percent neutral formalin and the wet tissue was blocked in a uniform manner. The paraffin sections were cut at 5 μm. Stained in hematoxylin and eosin and phosphotungstic acid–hematoxylin stain.
8. The other fatty acids tested were linoleic acid; 8,11,14-eicosatrienoic acid; 11,14,17-eicosatrienoic acid; 5,8,11,14,17-eicosapentaenoic acid (all > 99 percent pure, Hormel Institute, Austin, Minn.); 4,7,10,13,16,19-docosahexaenoic acid (> 90 percent pure, Nuchek, Elysian, Minn.). Sodium salts and dilutions of each were prepared for a sodium arachidonate.
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10. Acetylsalicylic acid (Merck, Inc., Rahway,

N.J.) was freshly dissolved in 0.3*M* sodium acetate solution and injected intraperitoneally at a dose of 13 mg/kg, 1 to 2 hours before the challenging dose of arachidonic acid.

11. Heparin (1000 units per milliliter, Liquaemin sodium "10," Organon, Inc., West Orange, N.J.) was injected intravenously to give an immediate concentration of about 10 units per milliliter of circulating blood.
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Selective Pancreatic Enzyme Secretion due to a New Peptide Called Chymodenin

Abstract. *Chymodenin, a peptide newly isolated from porcine duodenum, elicited a rapid threefold increase in secretion of chymotrypsinogen while increasing secretion of total protein by only about 40 percent; lipase secretion was unaffected. The pancreas appears able, under chymodenin stimulation, to rapidly alter the transport of an individual enzyme as opposed to producing en masse secretion of enzyme.*

Digestion in higher organisms is the result of enzymatic hydrolysis of a wide variety of chemical linkages present in the ingested meal, including selected peptide bonds, glycosidic linkages, and esters of fatty acids. The major source of digestive enzymes, the exocrine pancreas, apparently exerts control over specific enzymes over the long term in response to specific diets (*1*, *2*). It is not clear, however, whether the pancreas may in the short term (that is, within the digestive period of a single meal) alter the rate of secretion of individual enzymes selectively, reflecting intestinal digestive demands. Rapid selective secretion of protein by pancreatic tissue was proposed nearly a century ago (*3*) and appears to occur in other tissues, such as the pituitary gland. Recent work from this laboratory demonstrated that the pancreas responds to a specific stimulus—the presence of lysine in the intestine—by rapidly altering the relative amounts of enzymes transported into the secreted fluid (*4*). We have suspected that if short-term selective secretion of enzyme from the pancreas were to play a role in the regulation of digestive catalysis, then the regulation would in all likelihood involve changes in the transport pattern for specific enzymes (expressed within minutes) followed by longer term compensatory alterations in enzyme synthesis.

We examined the effect of intravenous administration of a newly isolated peptide, purified from porcine duodenum, upon the secretion of enzyme from the pancreas of the anesthetized rabbit in situ. We report here that administration of the new peptide caused dramatically increased secretion of chymotrypsinogen (ChTg) within 15 minutes, whereas lipase output remained unchanged and only a modest increase in protein output was observed. We have named this peptide chymodenin, after the enzyme which secretion it stimulates and its tissue of origin.

The starting material for the chymodenin purification was a peptide-containing extract of porcine duodenum that had been separated from cholecystokinin-pancreozymin (CCK-PZ) and secretin after extraction with acidic methanol followed by precipitation with KOH as described by Mutt (*5*). This fraction of the extract had previously been shown to contain peptides which in low concentrations were capable of releasing enzymes from isolated rat pancreatic zymogen granules in vitro (*6*). The fraction was further purified to yield an apparently homogeneous peptide by sequential ion exchange and gel-filtration chromatography on SP-Sephadex C-25, eluted in an ammonium bicarbonate gradient; on carboxymethyl cellulose in an ammonium bicarbonate gradient; by equilibrium chromatography on QAE-Sephadex A-25 in sodium pyrophosphate; and finally by gel filtration on Sephadex G-75. Following this purification, the peptide was homogeneous as judged by polyacrylamide disc gel electrophoresis in both acidic and sodium dodecyl sulfate gel systems. It differed in amino acid composition and electrophoretic mobility from any of the known gastrointestinal hormones and had an apparent molecular weight of about 5000 determined both by minimal amino acid composition and gel-filtration chromatography. It showed no secretin or CCK-PZ activity in the cat even in milligram doses. Details of the purification and chemistry will be presented elsewhere (*7*).

Male albino New Zealand rabbits (weighing 1.7 to 2.5 kg) were anesthetized with Dial with urethane [0.8 ml per kilogram of body weight (Ciba Pharmaceutical Co.)]. After cannulation of the pancreatic duct and an initial 1-hour stabilization period, secretion was collected for sequential 20-minute periods, and all collected samples were analyzed for protein, ChTg, lipase, and fluid output. Chymodenin was administered intravenously at a dose of 10 μg in 1.0 ml of 0.9 percent NaCl per animal; controls received an equal volume of 0.9 percent saline. Injections of either chymodenin or saline were made in each animal at 2 hours and again at 4 hours after cannulation. Animals were treated exclusively with either chymodenin (nine animals) or saline (nine animals). Samples taken during the three periods in the hour immediately following each injection were used as the treated samples. Protein, ChTg, and lipase were measured as described elsewhere (*8*), and fluid output was measured by weight.

Preliminary experimental results indicated that unstimulated output amounts of lipase, ChTg, and protein were highly variable from time to time and from animal to animal. This variability, common in studies of this nature and apparently caused by endogenous unspecified influences on pancreatic output, frequently introduces difficulty in statistically distinguishing effects due to treatment. Thus data collected from glands in a potentially "responsive" state (unstimulated) are not usually separated from data derived from the same glands during periods of endogenously stimulated secretion. In the present study, preliminary observations indicated that lipase secretion, although variable as a function of time and animal, was unaffected by the peptide, and therefore a high lipase output in any sample collection period was taken to indicate substantial endogenous stimulation of enzyme secretion during that period. To identify periods of active endogenous secretory stimulation, we calculated the mean lipase outputs for the control group and the group treated