Effects of Acute Experimental Pancreatitis on Insulin Metabolism in the Dog

Application of a New Equilibrium Infusion Technic

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SUMMARY

Experimental pancreatitis has been induced in dogs by instillation of bile into the pancreatic duct. An equilibrium infusion technic with I-131-insulin has been employed to evaluate the subsequent changes in the plasma levels of immunoreactive insulin (IRI). Evidence for acute intravascular insulinolysis (due to a putative release of pancreatic proteases and/or peptidases into the circulation) could not be demonstrated. Changes compatible with modest, late attenuation in the rates of insulin degradation were observed in some studies. However, in all experiments, the induction of pancreatitis effected prompt increases in total plasma IRI which exceeded concurrent changes in the "steady-state" concentration of infused I-131-insulin. Thus, the elevations of plasma IRI were greater than could be ascribed to alterations in insulin degradation or distribution. Direct analysis of pancreatic venous effluent indicated that the "extra" IRI originated, at least in part, from a purge of preformed pancreatic insulin or immunologically reactive insulin-like material. It displayed the same immunological reactivity on serial dilution as IRI released in response to conventional hyperglycemia. Consideration of the concomitant changes in plasma glucose and FFA during acute pancreatitis suggested that insulin-like effects may be greater in the liver than the periphery. DIABETES 17:437-43, July, 1968.

The metabolism of insulin during acute pancreatitis has not been studied previously, although certain diabetes-like features, such as hyperglycemia,[1] hyperlipemia,[2] and occasional ketoacidosis[1] may occur. The present experiments were designed to test several potentially disruptive actions upon insulin homeostasis: First, in view of the known release of preformed exocrine principles from the pancreas, e.g., amylase, lipase, trypsin, etc., the possibility of a comparable purge of stored insulin was explored. Secondly, in view of the recent demonstration that pancreatic tissue contains enzymes capable of cleaving insulin,[3] and that plasma proteolytic activity is increased during pancreatitis,[4,5] the possibility of acute intravascular degradation of circulating insulin was examined. Mirsky, Perisutti and Davis[6] have already shown that other peptide hormones, such as glucagon and ACTH, may be destroyed *within* the circulation as a consequence of activated proteolysis.

In our efforts, experimental pancreatitis was induced in dogs, and a new infusion technic was employed to investigate the acute interactions between the release, distribution and degradation of insulin. The present manuscript delineates the feasibility of this approach, and its application to correlate the metabolism of insulin with the changes in plasma glucose and FFA. Preliminary results have already been presented in abstract form.[7]

MATERIALS AND METHODS

(1) *Preparative technics.* Female dogs (18-23 kg.), fasted for sixteen hours, were anesthetized with pentobarbital sodium, and the main pancreatic duct was cannulated after ligation of the accessory pancreatic duct. A saline drip was delivered into the left femoral vein, and pulse and blood pressure were monitored via a cannula in the left femoral artery. The right femoral artery was cannulated for sampling peripheral arterial blood. In some experiments, an additional catheter was introduced into the pancreatico-duodenal or the portal vein to sample the pancreatic venous effluent.[8] The venous catheters and the cannula from the pancreatic duct were exteriorized, and the abdominal cavity was closed.

The saline drip was continued for one hour after

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preparative surgery to permit return to "steady-state" conditions. Thereafter, a single priming injection of I-131-insulin was administered intravenously, and a constant infusion of I-131-insulin (1 ml./min. via Bowman pump) was substituted for the saline. Each milliliter of infusate contained 1.0 mU. of beef insulin, labeled with 0.13-0.32 μc I-131,* and 2 mg. albumin; the prime consisted of an amount of I-131-insulin equivalent to two hours' infusion. For some studies, the infusate was supplemented with glucose (100 mg./ml.) to obtund the endogenous production of glucose by a constant exogenous delivery.

Two hours after the start of I-131-insulin infusion, i.e., "0 time," acute pancreatitis was induced by introducing 5 to 20 ml. of dog gallbladder bile into the cannulated pancreatic duct. The infusion was continued while observations were secured for two to six and one-half more hours. At the end of each experiment, animals were sacrificed; and severity of the pancreatitis was graded by direct observation and histological examination of formalin-fixed sections.

(2) *Analytical procedures.* Blood specimens were collected into chilled heparinized tubes and centrifuged immediately at 4° C. Aliquots of plasma or infusate were counted in well-type scintillation detectors to quantify the total cpm of I-131/ml. The proportion of total radioactivity consisting of intact I-131-insulin was estimated by buffer-flow chromatography,[9,10] and the concentration of I-131-insulin (cpm/ml.) was thus derived. Absolute values for I-131-insulin (μU./ml.) were calculated on the basis of the specific activity of the infusate.

Separate aliquots of plasma were frozen at —18° C. and subsequently analyzed for glucose,[11,12] FFA,[13] amylase,[14] and total protein.[15] Total immunoreactive insulin in plasma and infusate (IRI) was estimated by double antibody precipitation.[16,17] Frozen plasmas were stored sufficiently long to render the residual counts from the infused radioactivity negligible during immunoassay. Human insulin was employed to construct 13-point standard curves so that the immunoassay values for total IRI in dog plasma represent "human insulin equivalents."[18]

RESULTS

(1) *Establishment of pancreatitis.* In confirmation of others,[19] instillation of bile into the pancreatic duct elicited prompt increases in plasma amylase to values exceeding control by 6,000 to 23,000 U./100 ml. plasma at the end of six hours (figures 1-3). The increments were greater with 20 ml. than with 5 or 10 ml. of bile. In most instances, a small fall of blood pressure occurred immediately and persisted thereafter; shock was never observed (figures 1-3). The concentration of total protein in plasma remained constant or diminished slightly. The absence of hemoconcentration was consistent with the lack of major hemodynamic changes.

Inspection of the peritoneal cavity at the end of each experiment disclosed gross pancreatic inflammation for two to three inches on either side of the pancreatic duct. Histological examination revealed dilatation of canaliculi with extravasation of bile into glandular areas, and necrosis, hemorrhage, and infiltration by polymorphonuclear cells. Damage was greatest in the central portions of the pancreas where the main duct bifurcates.

(2) *Effects of pancreatitis on the metabolism of infused I-131-insulin:* In the present experiments, intact I-131-insulin accounted for an average of 39.1 per cent (range 25.8 -51.6 per cent) of the total circulating radioactivity after priming injections and two hours of I-131-insulin infusion. It also accounted for an average of 54.7 per cent (range 14.0-93.0 per cent) of the total immunoreactive insulin in plasma (IRI) at this time (figures 1-3). The variable percentile contribution of infused I-131-insulin to total IRI can be ascribed to differences in the endogenous rates of insulin secretion prior to the induction of pancreatitis.

Preliminary control studies in seventeen dogs[20,21] indicated that two hours of infusion following priming injection sufficed to establish "steady-state" levels of circulating I-131-insulin: Mean plasma concentrations of intact I-131-insulin at the end of three, four and five hours of continuing infusion averaged 99.9 per cent (range: 84.6 to 130.7 per cent), 98.3 per cent (range: 68.8 to 122.5 per cent) and 100.8 per cent (range: 72.2 to 128.2 per cent) of the two-hour values respectively. Analyses of the pancreas of control animals indicated that the infused I-131-insulin had not exchanged with pancreatic insulin stores.[20,21]

Six technically satisfactory experiments (Dogs A-E) were obtained in which bile was instilled after two hours of I-131-insulin infusion, and arterial blood was monitored for the subsequent 5.5 to 6.5 hrs. Dogs A and B received 5 ml. bile; Dogs C and D, 10 ml.; and Dogs E and F, 20 ml. In four of these studies (Dogs A, B, D and F), the I-131-insulin was infused without glucose; the infusate was supplemented with glucose in

*Purchased from Abbott Laboratories, North Chicago, Illinois.

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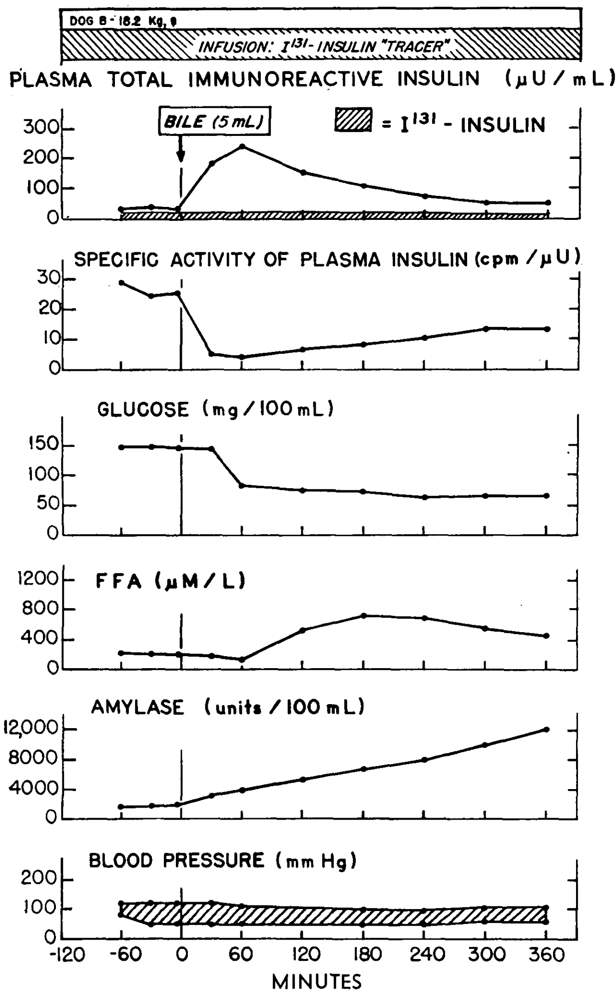
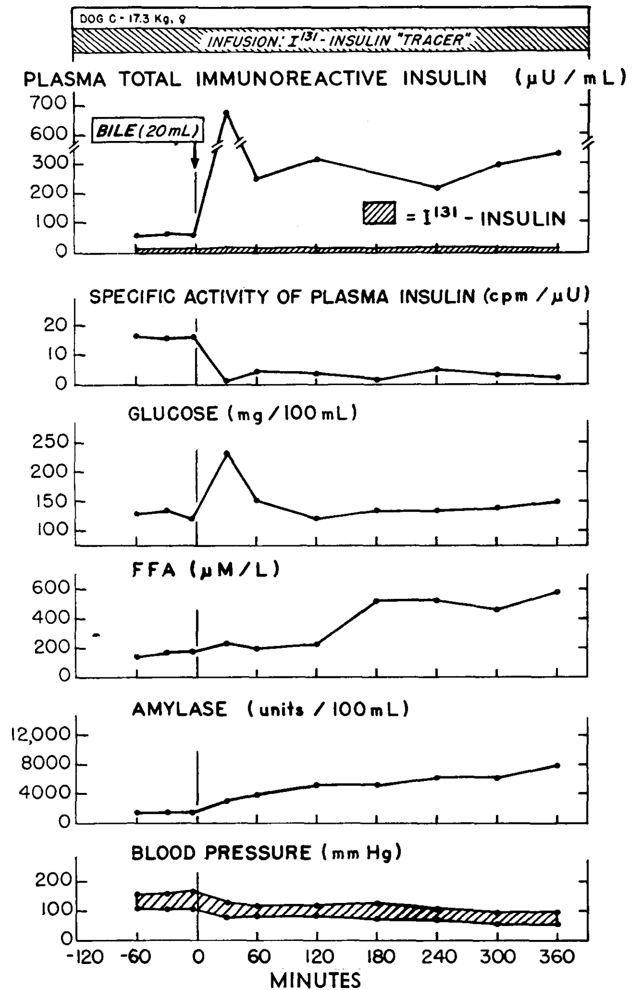


HEMODYNAMIC AND METABOLIC CHANGES DURING BILE PANCREATITIS IN THE DOG (DOG B)

FIG. 1. Tracer amounts of I-131-labeled insulin were infused in saline solution without supplemental glucose at constant rate (1.0 mU./min.) for eight hours (—120 to +360 minutes). Plasma specimens were secured from the femoral artery as indicated above. Bile was introduced into the main pancreatic duct at 0 minutes (i.e. "0 time"). Contributions of intact I-131-insulin to Plasma Total Immunoreactive Insulin (IRI) are depicted by the cross-hatched area. On the basis of the specific activity of the infused I-131-insulin, circulating I-131-insulin accounted for 24.6 μU./ml. at "0 time" and progressively fell to 19.4 μU./ml. during the subsequent six hours. For estimation of the specific activity of plasma IRI, the observed cpm/ml. of intact I-131-insulin at "0 time" were adjusted to 1,000 cpm/ml. All other radioactive assays were adjusted accordingly to facilitate comparison of specific activities in the experiments of figures 1-3.



HEMODYNAMIC AND METABOLIC CHANGES DURING BILE PANCREATITIS IN THE DOG (DOG C)

FIG. 2. Experimental details as in figure 1 except for the inclusion of glucose (100 mg./ml.) in the infusate. Intact I-131-insulin accounted for 16.4 μU./ml. of the Plasma Total Immunoreactive Insulin at "0 time" and 19.6 μU./ml. six hours later (e.g. the cross-hatched area).

the other two experiments (Dogs C and E). Representative results from animals B, C and E are shown in figures 1, 2 and 3 respectively.

Instillation of bile did not cause significant acute reductions of circulating I-131-insulin in any instance (figures 1-3). Thus, induction of pancreatitis was not accompanied by manifest evidence of acute intravascular insulinolysis. In three of the six animals (Dogs B, C and D), fluctuations in the plasma concentrations of I-131-insulin did not exceed ranges encountered in control studies: Levels fell 21.1 per cent and 12.4 per cent below two-hour values in Dogs B (figure 1) and D respectively, and rose 19.5 per cent in Dog C (figure 2). On the other hand, during 5.5 to 6.5 hrs. after administration of bile, circulating I-131-insulin slowly and progressively rose 62.0 per cent in Dog E (figure

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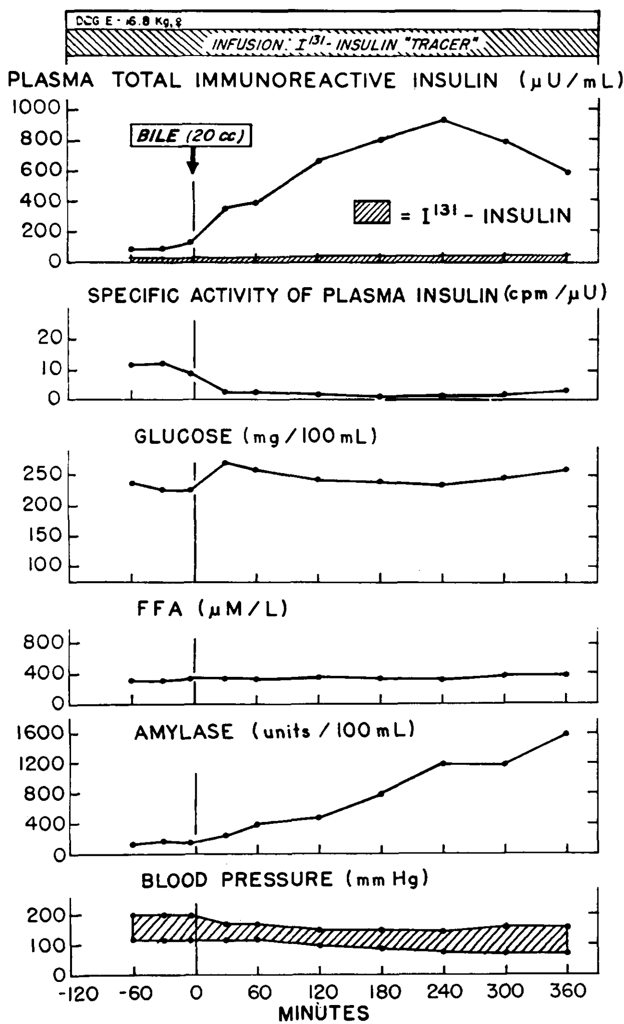

FIG. 3. Experimental details as in figure 1 except for the inclusion of glucose (100 mg./ml.) in the infusate. Intact I-131-insulin accounted for 16.6 μU./ml. of the Plasma Total Immunoreactive Insulin at "0 time" and progressively rose to 26.9 μU./ml. during the subsequent six hours (e.g. the cross-hatched area).

3), 91.8 per cent in Dog A and 140.9 per cent in Dog F. Since the concentration of plasma proteins did not increase concurrently, these mounting levels of I-131-insulin, during continuing infusion, presumably were not due to hemoconcentration and contraction of the virtual volume for insulin distribution, i.e., the "insulin space." Instead, they probably resulted from modest reductions in the rates of insulin degradation by tissues.

(3) *Effects of pancreatitis on total immunoreactive insulin.* Concentrations of IRI in plasma increased two to ten-fold *within less than sixty minutes* following induction of pancreatitis (figures 1-3). The increases were sustained for the subsequent 5.0 to 6.0 hrs. in all animals except B (figure 1) and invariably exceeded the concurrent excursions in the plasma concentrations of I-131-insulin. Thus, the specific radioactivity of plasma IRI (cpm./μU.) was depressed in all experiments (figures 1-3). The attenuated specific radioactivity indicated that all the augmentation of plasma IRI could not be ascribed to a contraction of "insulin space" nor to a retardation of insulin degradation and that some "extra" IRI must have originated via a release of insulin from pancreatic stores.

To document this directly, pancreatico-duodenal or portal venous effluent was sampled in five additional studies. The immediate increases in plasma IRI and the reductions in IRI specific radioactivity were substantially greater in the pancreatico-duodenal vein (figure 4) or portal vein (table 1) than in the femoral artery. Moreover, these increases persisted despite return of plasma glucose to the same or to lower values than at "0 time" (figure 4, table 1). Gradients for IRI concentrations between peripheral and portal blood were narrowed after induction of pancreatitis in one study (table 1), a finding which could be consistent with diminished hepatic extraction and degradation of insulin following experimental pancreatitis.

In one experiment, intravenous glucose (0.5 gm./kg.) was administered acutely two hours preceding the bile (table 1) so that the IRI released by glucose and by pancreatitis could be compared. Immunoassays for IRI in serial dilutions of portal venous plasma are shown in figure 5. Equivalent initial concentrations of plasma IRI yielded identical dilution curves. Parallel slopes were also obtained on serial dilutions of peripheral plasma. Thus, within the limitations of the dilution technic[16,17,22,23] immunological differences could not be demonstrated between the circulating IRI which appears in the two situations.

(4) *Acute effects of pancreatitis on plasma glucose and FFA.* In most experiments, transient increases of blood sugar occurred during the first thirty minutes following the introduction of bile (figures 2-4; table 1). Plasma glucose subsequently fell to lower levels than at "0 time" in all except one (figure 4) of the experiments in which saline was infused without glucose. Contrariwise, in the two experiments in which infusions were supplemented with glucose (figures 2 and 3), blood sugar did not decline below "0 time" values.

All dogs except E (figure 3) exhibited a slow

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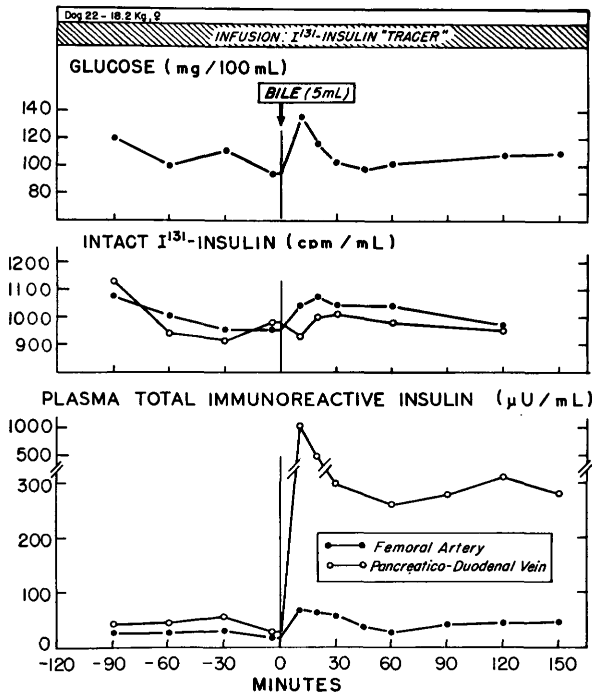
TABLE 1

Release of IRI in response to glucose vs. bile pancreatitis

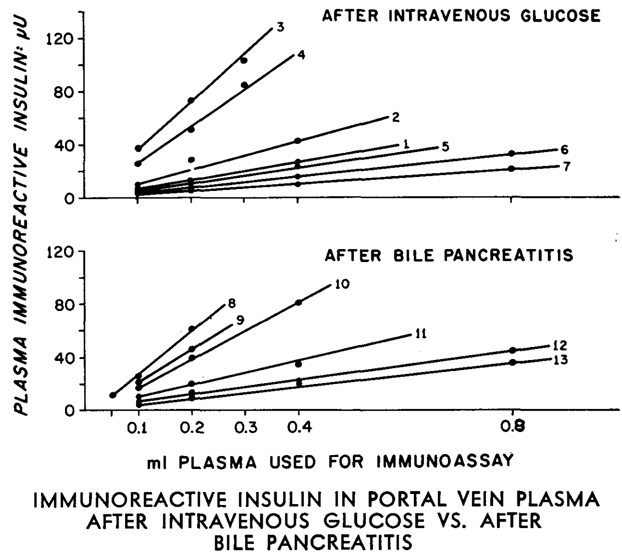
Spec #	Time (Min.)	Femoral artery Glucose* (mg./100 ml.)	Femoral artery Amylase* (U./100 ml.)	Femoral artery IRI* (μU./ml.)	Portal vein IRI* (μU./ml.)
1	—5	114	2,000	11.7	68.6
		Inject Intravenous Glucose 0.5 gm./kg.			
2	+10	255		57	115
3	+20	221		82	356
4	+30	147	2,286	77	261
5	+45	111		22	55
6	+60	102		12	39
7	+120	90	3,200	6	26
		Inject 5 ml. Bile into Pancreatic Duct			
8	+10	99		189	266
9	+20	83		199	242
10	+30	69	5,333	177	182
11	+60	54	6,400	98	103
12	+90	58		58	61
13	+120	56	10,000	42	47
14	+150	56	12,000	35	58

*Values represent concentrations in plasma.



EFFECTS OF BILE PANCREATITIS ON INSULIN IN THE PANCREATIC VENOUS EFFLUENT AND PERIPHERAL BLOOD (DOG 22)

FIG. 4. Plasma specimens were secured simultaneously from the femoral artery and pancreatico-duodenal vein during sustaining infusion of I-131-insulin in saline without supplemental glucose. Values for intact I-131-insulin are expressed on the basis of cpm/ml. plasma. Five milliliter gallbladder bile were introduced into the pancreatic duct at "0 time."



IMMUNOREACTIVE INSULIN IN PORTAL VEIN PLASMA AFTER INTRAVENOUS GLUCOSE VS. AFTER BILE PANCREATITIS

FIG. 5. Each specimen of portal vein plasma was analyzed by immunoassay in multiple dilutions. The numbers refer to the parent specimens as shown in table I.

progressive rise in plasma FFA (figures 1 and 2) despite the persistent anesthesia and the augmentation of plasma IRI.

DISCUSSION

The above studies constitute the first application of infusion technics to evaluate disruptive challenges to insulin homeostasis. We have employed infusions of

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I-131-insulin to facilitate interpretation of changes in the concentration of plasma IRI. The underlying assumption is that excursions in circulating I-131-insulin during equilibrium infusions may afford an index of alterations in the distribution and/or destruction of endogenous IRI when disequilibrating events are introduced. Although it has not been proven that endogenous and I-131-labeled IRI are degraded at exactly the same rate, any putative differences would not vitiate the qualitative interpretations to which this report has been confined.

Our experiences justify certain conclusions concerning the metabolism of insulin during experimental pancreatitis. The fact that pancreatic insult, sufficient to cause histological damage and elevations of plasma amylase, did not effect meaningful reductions in the plasma concentration of infused I-131-insulin would suggest that *acute intravascular insulinolysis,* via liberated proteases and/or peptidases, does not occur to a significant extent. Precipitous cleavage of insulin within the circulation should not have been masked by the late, and slowly progressive retardation of tissue insulin degradation which we observed in some experiments. On the other hand, the invariable prompt rise in the total IRI of plasma after pancreatitis (in excess of the concurrent changes in the concentration of I-131-insulin in peripheral blood as well as in pancreatic venous effluent) would indicate that some release of preformed insulin from the pancreas occurs.*

The prolonged persistence of this "extra" IRI excludes mediation via the transient hyperglycemia of early pancreatitis. Hence, other mechanisms must be invoked. Loubatières has postulated that some discharge of insulin may be triggered by increased pressure within the pancreatic duct.[26] Clearly, this constitutes one possibility. Direct stimulation of β cells (via circulating kinins, released glucagon,[27] or other substances of gastrointestinal origin[28]) constitutes another. For example, the transitory rise in blood sugar which we observed could reflect some liberation of glucagon. Similarly, continuing release of trypsin could sustain the elaboration of insulin by digesting the additional amino acid sequence off stored proinsulin.[29] Indeed proinsulin itself (or other immunoreactive precursors) might be released under such circumstances. However, it seems likely that most of the IRI was purged from pancreatic stores as a direct consequence of the cellular disruption which may occur in acute pancreatitis.

The "extra" IRI exhibits the same immunological properties on serial dilution as IRI secreted in response to hyperglycemia. Whether it also possesses the biological potency of postglucose IRI remains to be demonstrated. Our limited data do not justify definitive conclusions. However, certain speculations are tempting on the basis of the different effects of pancreatitis upon blood sugar in those experiments in which only saline was infused (so that all circulating glucose was derived from endogenous production) vis-a-vis the two experiments in which glucose was infused (so that most circulating glucose was of exogenous origin, and blood sugar values were influenced principally by rates of glucose utilization in the periphery). In all the former studies except one (figure 4), blood sugar fell *below* "o time" values during the acute pancreatitis. On the other hand, blood sugar remained constant in the two experiments with glucose infusions. The differences suggest that acute pancreatitis may affect the production of glucose in the liver more than the utilization of glucose in the periphery.

Several possible explanations warrant consideration. Conceivably, the increased plasma IRI of acute pancreatitis could represent "true" or native insulin which restrained glucose output from the liver[30] but did not promote glucose utilization in the periphery due to the antagonistic effects of intracellular lipolysis, vasoactive peptides, neurohumoral activity, or cardiovascular collapse. Alternatively, the "extra" IRI could represent an immunologically reactive storage or precursor form of insulin, such as proinsulin,[29] or even some degradation and/or polymerization product with immunological reactivity and attenuated hormonal potency.[31,32] The lowering of blood sugar could then be due, perhaps, to insulin-like capabilities in the liver but not the periphery. Finally, the fall in blood sugar in the experiments with saline might merely connote attenuation of splanchnic blood flow by direct hemodynamic effects of the

*Although "IRI" has been demonstrated in bile,[24,25] simple calculations indicate that the increase in plasma IRI cannot be ascribed to the material which we introduced into the pancreatic duct. For example, if one assumes a virtual volume for insulin distribution of 30 per cent of body weight, i.e., approximately six liters in the dogs which we employed, and IRI concentrations in gallbladder bile averaging 102 μU./ml. in the dog,[24] (or even 1,780 μU./ml., as in the rabbit[25]) instillation of as much as 20 ml. of bile could not have increased plasma IRI levels by more than 0.3-5.9 μU./ml. $\left(\frac{102 \times 20}{6{,}000} \text{ or } \frac{1{,}780 \times 20}{6{,}000}\right)$. Indeed, even if one assumed that all the insulin remained confined to the blood volume, i.e., approximately one liter in the dogs which we employed, maximal increases in IRI would not exceed 2.0-35.6 μU./ml.

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pancreatitis independently of any hormonal actions of the "extra" IRI. Differentiation cannot be made from the available information, and studies directed towards clarification are in progress.

Nonetheless, some extrapolations to acute pancreatitis in man may not be premature. If preformed insulin is purged from pancreatic stores early in the clinical as in the experimental situation, and if pancreatic inflammation precludes adequate subsequent repletion, diminished insulinogenic reserve could ensue. The latter could contribute to some of the diabetes-like features that may be present when the patient with acute pancreatitis seeks medical help.

ACKNOWLEDGMENT

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