The Effects of Glucagon on Sodium and Water Unidirectional Fluxes in the Dog Intestine

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by

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I would like to thank the members of my committe for their thoughtful criticism of this thesis and especially my advisor, Dr. Mailman, for his guidance and counseling. Special thanks are given to my wife, Joanie, and parents whose good wishes were with me always.

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Abstract

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ABSTRACT

Glucagon has been shown to decrease absorption of salt and water from chronic canine intestinal loops, but to increase absorption from rat small intestine in acute experiments. The purpose of this investigation was to determine if glucagon could acutely alter ileal salt and/or water absorption by the canine ileum, and, if so, to determine if the mechanism of action was through cardiovascular changes, or through peripheral glucose or insulin release.

Absorptive site and total blood flows and resistances, unidirectional sodium (^{22}Na) and water ($^{3}H_2O$) fluxes, and blood pressures were determined from a canine ileal segment perfused with saline following the infusion of intraarterial glucagon, intravenous insulin and/or glucose, or intraarterial histamine.

Glucagon infused into a mesenteric artery not supplying the perfused intestinal segment significantly decreased absorptive and secretory sodium and water fluxes, whereas direct infusion into the mesenteric artery significantly increased these fluxes. Absorptive site resistance and blood flow were more affected by glucagon than total resistance and total blood flow in the entire segment. Increased blood flow and/or in-

creased cellular metabolism are possible explanations of the ileal response to intraarterial glucagon. Insulin had little apparent effect although induced hyperglycemia, or a peripheral effect of it, increased sodium secretory fluxes. Intraarterial histamine also had little effect on the Na and H_2O fluxes or on intestinal blood flow.

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INTRODUCTION

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The transfer of charged and uncharged solutes and solvents is one of the most important functions of the gastrointestinal tract. The complex microcirculation and epithelial structure of the intestine are specialized for transport just as other transporting epithelia have their special features involved with transport.

Absorption by certain transporting epithelia, especially kidney and toad bladder, has been shown to be regulated by certain hormones (24,48). In contrast, salt and water absorption by the small intestine does not seem to be sensitive to hormonal control. For example, aldosterone, which increases Na transport, or antidiuretic hormone, which increases H_2O transport across kidney and toad bladder (24,48), have little effect on small intestinal transport of Na and H_2O (12, 22,23). However, certain splanchnic hormones, although in some cases only at high doses, do affect intestinal salt and H_2O transport. Glucagon (10 μg/min), vasoactive intestinal polypeptide, and gastric inhibitory polypeptide at high doses and pentagastrin at doses submaximal for gastric acid secretion all increased salt and H_2O secretion from Thiry-Vella loops of canine jejenum and ileum (4). In this same study, cholecystokinin octapeptide and secretin had little effect or

decreased secretion slightly (4). However, in acute experiments using perfused canine intestinal segments, CCK, at physiologic doses, reduced absorption (8). Acute experiments in rats however, showed that glucagon (4-256 μg/kg) increased intestinal absorption of salt and water, but that secretin reduced absorption (34). Pentagastrin and CCK had no acute effect on salt and H_2O transport by rat intestine (35). In other studies, gastrin or pentagastrin, reduced absorption in everted hamster ileum (27), canine Thiry-Vella loops (28), and dog jejenum and ileum (9,76). Therefore, there seems to be some uncertainty concerning the effects of gastrointestinal hormones possibly due to different responses in different species or to the type of intestinal preparation employed. Insulin has also been shown to decrease Na transport by everted rat gut (2). In the above studies, considering glucagon only, glucagon increased intestinal absorption of salt and H_2O in rats, but decreased it in dogs. These opposite effects of glucagon on intestinal absorption in dog and rat suggests that glucagon can have several effects, some of which decrease intestinal absorption and that these were quantitatively more important in the dog studies (3,4) and other effects which increase intestinal transport and that these were quantitatively more important in the rat studies (34). The effect of glucagon on intestinal salt and H_2O absorption and its mechanism

of action were the objects of interest of this study.

Glucagon has several effects in the body including vasodilation (47,69), increasing blood levels of glucose (25), and increasing insulin release (61). Of particular interest in this study was the possibility that glucagon could affect intestinal salt and water transport through vasodilator effect of the cardiovascular system. Vasodilation could alter Starling forces and in turn these could cause a change in intestinal absorption.

Starling forces, namely capillary hydrostatic pressure and colloid osmotic pressure, have recently been found to have effects on salt and water transport across kidney (15) and frog skin (50). These effects of Starling forces are exerted both under physiologic conditions, *in vivo* (41) and these effects can be also demonstrated *in vitro* (73). Effects of hydrostatic pressure on intestinal transport have been demonstrated *in vitro* (33,74), but there is only indirect evidence that, *in vivo*, intestinal absorption can be affected by Starling forces. Saline infusion reduces intestinal absorption (or increases secretion), but these effects of saline infusion on intestinal transport have been attributed to either hormonal effects (55) or to the effects of physical forces (36). Occlusion of the venous drainage from the gut also increases Na secretion into the lumen (67). However, if Starling forces are affecting intestinal transport, these forces must be exerted at the absorptive site and little work has been

done relating absorptive site blood flow or pressure to transport processes.

Winne and co-workers have shown that an increase in total blood flow through the rat intestine was associated with an increased mucosal blood flow and also the increased absorption of a number of passively absorbed substances (51,75). Dobson and co-workers have made similar studies in the cow rumen and have come to similar conclusions (20,21). Therefore, increased absorptive site blood flow may increase passive lumen-to-blood transport at the same time an accompanying increase in capillary hydrostatic pressure could increase the passive blood-to-lumen fluxes and the net effect on transport would partly depend on the magnitude of these two effects. Hydrostatic and colloid osmotic Starling forces at the capillary level are known to influence molecular and ion transfer across transporting epithelial tissues (16,17,19). Net transport across epithelia is quantitatively equal to the absorptive flux (out of the lumen) minus the secretory flux (into the lumen) (71,72) of a particular species. Secretory fluxes are generally attributed to the driving effects of physical forces (63). Absorptive fluxes across certain tissues of certain species are primarily dependent on and can influence the level of epithelial cellular metabolism (29,63).

Hormonal control of gut transport has not been

studied very much in relation to absorptive site blood flow where capillary-mucosal exchange actually takes place. In the intestine, the net effect of a vasoactive agent is a function of its effect on the simultaneous activity of vascular smooth muscle (37), local metabolism (65), and gut visceral muscle tone (7,32). Therefore, depending on the dose, the same vasoactive agents can either increase or decrease total intestinal blood flow. Blood flow through the whole intestine occurs through four parallel pathways, namely through the muscularis, submucosa, crypts, and villi (26) and these separate pathways may be subject to independent regulation. Therefore, total blood flow may not parallel intestinal absorptive site blood flow. A direct cardiovascular effect on intestinal transport would therefore have to be related to absorptive site blood flow. Norepinephrine, in doses which decrease total flow, does not alter the relative fraction of blood perfusing the mucosa, submucosa, or muscularis (60). Reduced vascular perfusion pressure will reduce total blood flow, but has little effect on flow through the villi (6).

Vasoactive agents can also exert indirect cardiovascular effects since changes in blood pressure sensed by the baroreceptors would cause a compensatory cardiovascular response through the sympathetic nervous system. The magnitude of the effect of the vasoactive agent and the magnitude of the sympathetic effect would

then tend to counteract each other. Different vascular beds could respond differently depending on their sensitivity to the vasoactive agent as compared to the sympathetic nervous system.

The increase in blood glucose caused by glucagon could supply more nutrient to the mucosal cell and would increase active transport if glucose delivery were rate limiting for mucosal cell metabolism. Plasma insulin, which is increased by glucagon, could also affect cell metabolism or mucosal cell transport (2). Insulin also reduces blood pressure and may have indirect effects on intestinal transport through the mechanisms described above (1). Glucagon (59,69) and histamine (47) are both vasodilators and have effects on the gastrointestinal vasculature. Insulin has no, or little effect on intestinal vasculature though it does have effects in the splanchnic area (45,56). Glucagon and insulin are involved in the homeostatic control of circulating glucose.

The question arises as to whether hormonal effects in the gastrointestinal area are due to vascular changes, direct effects on cellular and local metabolism, or both. A hormone might also exert indirect effects on absorption via its activities in peripheral tissues. The following research was undertaken to determine the effect of the naturally occurring splanchnic polypeptide glucagon on sodium and water unidirectional fluxes and on microcirculatory parameters.

STATEMENT OF THE PROBLEM

STATEMENT OF THE PROBLEM

There is a paucity of information concerning intestinal blood flow and its influence on absorption. Humoral agents can exert vascular or metabolic (or both) controls on absorptive processes. In particular, this investigation was initiated to ascertain the effects of the naturally occurring splanchnic hormone, glucagon, on ileal salt and water absorption and mechanisms by which the hormone might exert its effects. The effects of glucagon could be exerted through glucagon vasodilator activity, either directly on the intestine or through peripheral vasodilation, or through glucagon's effect to increase peripheral glucose or insulin. These possibilities were examined in this study.

METHODS AND MATERIALS

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ANIMALS

Mongrel dogs (14.5 to 29.1 kg) of both sexes were dewormed with Vermiplex (Pittman-Moore, Dallas, Texas) and maintained one week before use. Dogs were allowed food and water ad libitum but were fasted twenty-four hours before the experiment. The dogs were anesthetized with sodium pentobarbitol (25 mg/kg) intravenously.

SURGICAL PROCEDURES

An endotracheal tube was placed into the trachea of the dog to facilitate breathing while under anesthesia. The right femoral artery and femoral vein were exposed and both were catheterized with 14 gauge catheters (C. R. Bard, Murray Hill, N. J.). The arterial catheter was connected to a mercury manometer.

An eight inch midline laparotomy was made by cautery (B-2, Liebel-Flarsheim, Cincinnati, Ohio). An ileal segment of gut was isolated distal to the terminus of the ileocolic artery. The ends of the gut segment were cannulated for perfusion and collection of the perfusate. The gut lumen was rinsed with approximately 500 ml of isotonic saline (37° C) until the effluent was clear. An intestinal venous branch draining the segment was cannulated with a polyethylene cannula (PE 90) and connected to a saline manometer.

The gut segment was returned to the abdominal cavity with care taken not to bend the cannulae. The intestinal perfusion cannula was connected through a heating condenser to a reservoir of isotonic saline containing radioactive ^{22}Na- sodium chloride, ^{14}C- inulin, and ^{3}H- water (New England Nuclear, Boston, Mass.). The temperature of the perfused solution was measured by an electronic thermometer (Tri-R, Model TOL, Jamaica, N. Y.) and maintained at 36-38° C. The radioactive solution was perfused through the segment by a rotary pump (Cole Parmer, 7014-2, Chicago, Ill.). An equilibration period of 60 min preceded nine 20 min sampling periods. Anesthesia was maintained by intravenous injections of 0.5 ml of sodium pentobarbitol (50 mg/ml) as required to maintain a strong blink reflex.

SURGICAL PROCEDURES (SPECIAL)

GLUCAGON

An intestinal arterial branch of the perfused or an adjacent segment was cannulated with polyethylene tubing (PE 90) and then connected to a syringe pump (Instrumentation Specialties Inc., Model 180, Lincoln, Neb.) filled with isotonic saline which was infused into the artery (0.3 ml/min). Experimental animals had infusions directly into the artery perfusing the segment (direct infusion). Control animals had an intestinal artery cannulated which did not perfuse the segment and infusion was therefore indirect

(indirect infusion). After equilibration, the first three sampling periods were carried out during isotonic saline intraarterial infusion; the second three periods were carried out during infusion of 0.05 μg/kg/min glucagon (E. Lilly, Indianapolis, Ind.) in isotonic saline intraarterially; the third three periods were carried out during infusion of 0.5 μg/kg/min glucagon in isotonic saline intraarterially. Ten minutes of equilibration separated the first and second, and the second and third groups of periods. Other experiments in this laboratory, carried out in a manner similar to these experiments, demonstrated that there were no significant changes with time in any of the parameters measured for up to 3.5 hrs in control animals (38). Control experiments in three animals corroborated the lack of change due to time alone (data not included). Hence, there is no effect of time on gut transport or blood flow. At the end of each experiment, placement of the intraarterial catheter was corroborated by dye injection.

INSULIN

After the first three sampling periods a single dose of insulin (E. Lilly, Indianapolis, Ind.), 0.1 U/kg, was given via the femoral vein. The injection was immediately followed by a femoral intravenous infusion of dextrose at 0.2 gm/min for one hour. At the end of one hour the dextrose infusion was stopped and another single dose of insulin, 0.1 U/kg, was given. The same procedure was followed

in control animals except that they were given doses of heat denatured insulin (by heating in boiling isotonic saline for 60 min) before and after a 0.2 gm/min dextrose infusion.

HISTAMINE

An intestinal arterial branch of the perfused segment was cannulated with polyethylene tubing (PE 90) and then connected to a peristaltic pump filled with isotonic saline which was infused into the artery during the equilibration hour and the first three twenty minute periods. Histamine hydrochloride (Sigma, St. Louis, Mo.) was then infused into the intestinal arterial catheter at doses of 0.1, 1.0, 5.87, or 52.8 μg/kg/min for one hour. One animal was used for each dose. At the end of each experiment, placement of the intraarterial catheter was checked by dye injection.

SAMPLING

The gut effluent was collected for twenty minute periods for three control periods and six subsequent experimental periods. Mesenteric blood samples were obtained by allowing blood to drip from the exposed end of the intestinal venous cannula. Blood pressure values were observed on the mercury and saline manometers at the end of each period. Hematocrit values were obtained for both venous and arterial samples. Blood samples were centrifuged and the plasma retained. At the end of each experiment the gut segment was

removed, drained, and weighed. All appropriate measurements are expressed per gram of wet gut weight.

ANALYTICAL DETERMINATIONS AND CALCULATIONS

A one hundred microliter sample was used to determine sodium and potassium concentrations by flame photometry (Eppendorf, Hamburg, Germany). Another one hundred microliter sample was used for a coulombmetric titration with a chloridometer (Buchler Cotlove, Fort Lee, N. J.) to determine chloride concentration.

One ml samples of gut effluent and plasma were utilized for counting ^{22}Na- sodium chloride, ^{14}C- inulin, and ^{3}H- water. The samples were dissolved in 10 ml of scintillation cocktail containing 3.33 ml Triton X-100 (Beckman, Fullerton, Cal.), 6.66 ml toluene (Kodak, Rochester, N. Y.), and 0.042 ml Liquifluor (New England Nuclear, Boston, Mass.). Isotope counting was carried out on a Beckman LS-150 Liquid Scintillation Counter. Compensation was made for quenching and spillover using quench curves generated by mixtures of plasma and hemolyzed red blood cells.

The clearance of tritiated H_2O provides an estimate of absorptive site blood flow (9,51,75) and can also be used to estimate total blood flow by an application of the Fick principle (44). If arterial pressure and mesenteric vein pressure are also known capillary pressure can be calculated by the method of Pappenheimer (52).

Unidirectional sodium and water fluxes were calculated from gut and plasma samples by the method of Berger and Steele (5). Total blood flow to the segment was calculated on the basis of the Fick principle as the clearance of ^3H_2O using modifications of the methods of Dobson (20,21) and Winne (51,75):

$$\text{Total Tritium Clearance} = \frac{\text{Tritium Absorbed}}{(\text{Arterial Tritium})-(\text{Venous Tritium})},$$

$$\text{Absorptive Site Tritium Clearance} = \frac{\text{Tritium Absorbed}}{(\text{Average Lumenal Tritium})}.$$

These tritium clearance values are multiplied by a factor of 1/1-(.23)(Hematocrit), which corrects for the volume of distribution of ^3H_2O in the blood (44), to obtain values for total and absorptive site blood flows. Resistances are calculated as

$$\text{Resistance} = \frac{(\text{Arterial Pressure})-(\text{Venous Pressure})}{(\text{Clearance of Tritium})}.$$

Capillary pressures were determined according to the relationship developed by Pappenheimer (52) in which

$$\text{Capillary Pressure} = \frac{(\text{Art. Pressure})(\text{Post R/Pre R})+(\text{Ven. Pressure})}{1 + (\text{Post R/Pre R})},$$

where Post R and Pre R designate post-capillary resistance and pre-capillary resistance respectively.

Statistical analysis was by paired t-test, within each group in which the differences between control and experimental periods were calculated, and by unpaired t-test, between the differences of the control and experimental animals.

RESULTS

TABLE I

CONTROL VALUES PRECEDING A RESPONSE TO NATURALLY OCCURRING SPLANCHNIC AGENTS*

PARAMETER	GLUCAGON (N=13)	INSULIN (N=9)	HISTAMINE (N=4)
Net Na absorbed (μeq/gm·min)	.829±.100	1.58±.130	1.22±.156
Na secretory flux (μeq/gm·min)	1.46±.181	1.14±.089	1.42±.165
Na absorptive flux (μeq/gm·min)	2.29±.167	2.72±.138	2.64±.181
Net water absorbed (μl/gm·min)	6.88±.612	10.1±.853	8.69±1.07
Water secretory flux (μl/gm·min)	16.6±1.62	15.5±1.23	17.4±1.14
Water absorptive flux (μl/gm·min)	23.5±1.55	25.6±1.46	26.1±1.93
Arterial pressure (mm Hg)	112.±3.43	114.±3.77	115.±4.32
Mesenteric venous pressure (mm Hg)	10.7±.905	8.93±.383	9.01±.413
Total blood flow (ml/gm·min)	.441±.034	.547±.051	.451±.092
Absorptive site blood flow (μl/gm·min)	25.0±1.90	27.8±1.61	28.5±2.02
Total resistance (mm Hg/ml/gm·min)	232.±35.6	200.±28.2	243.±51.1
Absorptive site resistance	4085±371.	3791±230.	3708±430.

* These values represent the control values from which the changes given in the figures were calculated.

RESULTS

The effects on intestinal fluxes and the cardiovascular parameters of two doses of glucagon were observed: 0.05 μg/kg/min and 0.5 μg/kg/min. Six control animals had glucagon infused into an intestinal artery not supplying the perfused ileal segment (indirect infusion) and seven experimental animals had glucagon infused directly into the artery supplying the segment (direct infusion). The effects of intravenous single doses of insulin (0.1 U/kg), first with a concomitant dextrose infusion (0.2 g/min) and then without a dextrose infusion, were tested in five dogs. Four animals were given the dextrose infusion, and doses of heat denatured insulin.

Table I gives the control period values, i.e. the average values for the 60 min before the infusions were started, for the three groups of dogs employed in these experiments: those infused with glucagon, insulin and/or glucose, and histamine hydrochloride. All values given in the graphs represent the mean (± SEM) difference between the average value of the control periods and the average value of the periods during the infusion, paired for each individual animal.

The net sodium flux (fig. I) was not significantly changed from control levels by direct glucagon infusion

Figure I. Changes from control periods in net sodium flux, sodium secretory flux, and sodium absorptive flux in canine ileum at 0.05 and 0.5 μg/kg/min glucagon infusion directly into the mesenteric artery of a perfused segment (N=7) or indirectly into an adjacent segment (N=6). Significant differences between control and experimental periods are shown within each group (asterisks inside bars) and between directly perfused and indirectly perfused animals (asterisks at the bottom of each graph). *, **, and *** designate significant differences at the 5%, 1%, and 0.1% levels, respectively.

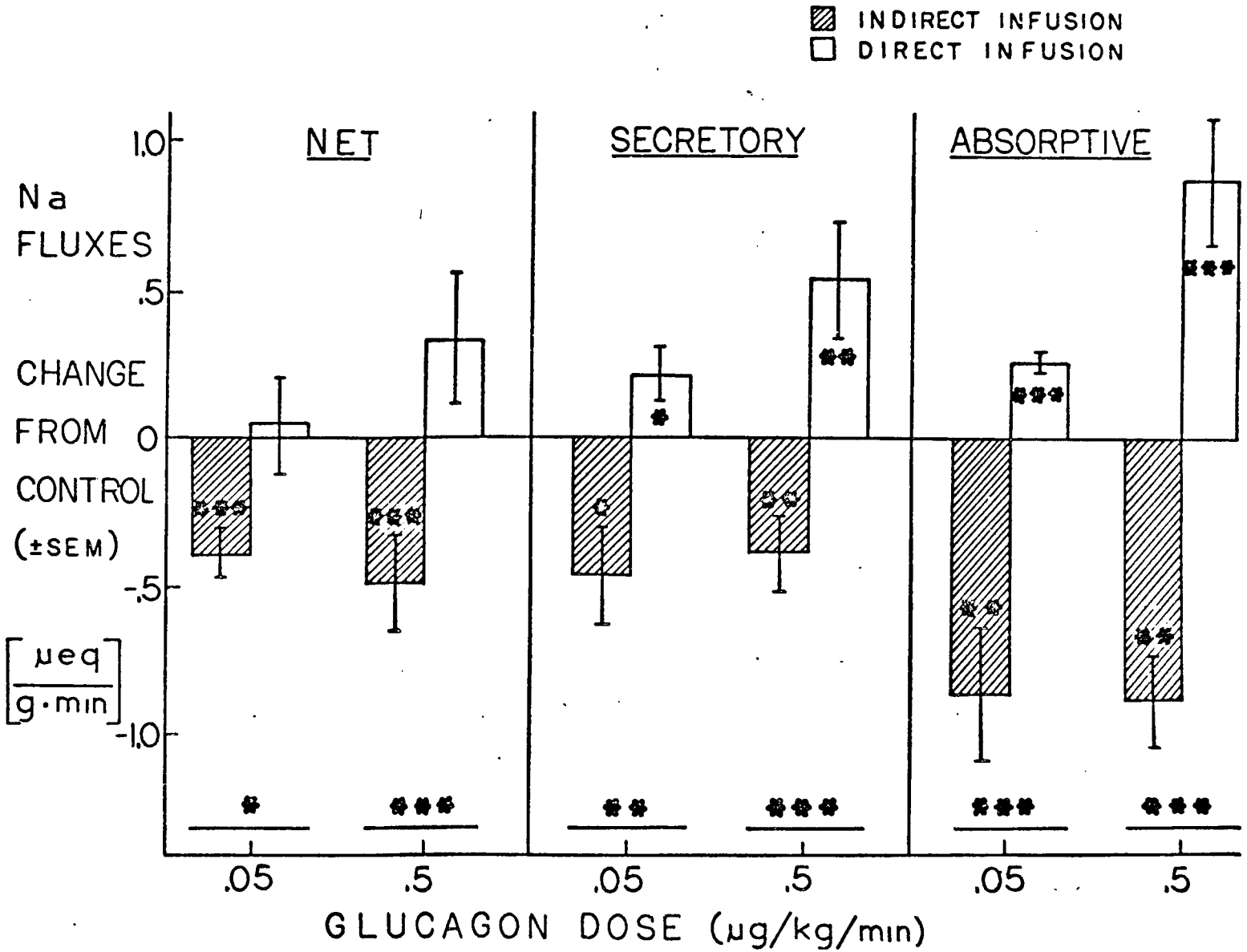
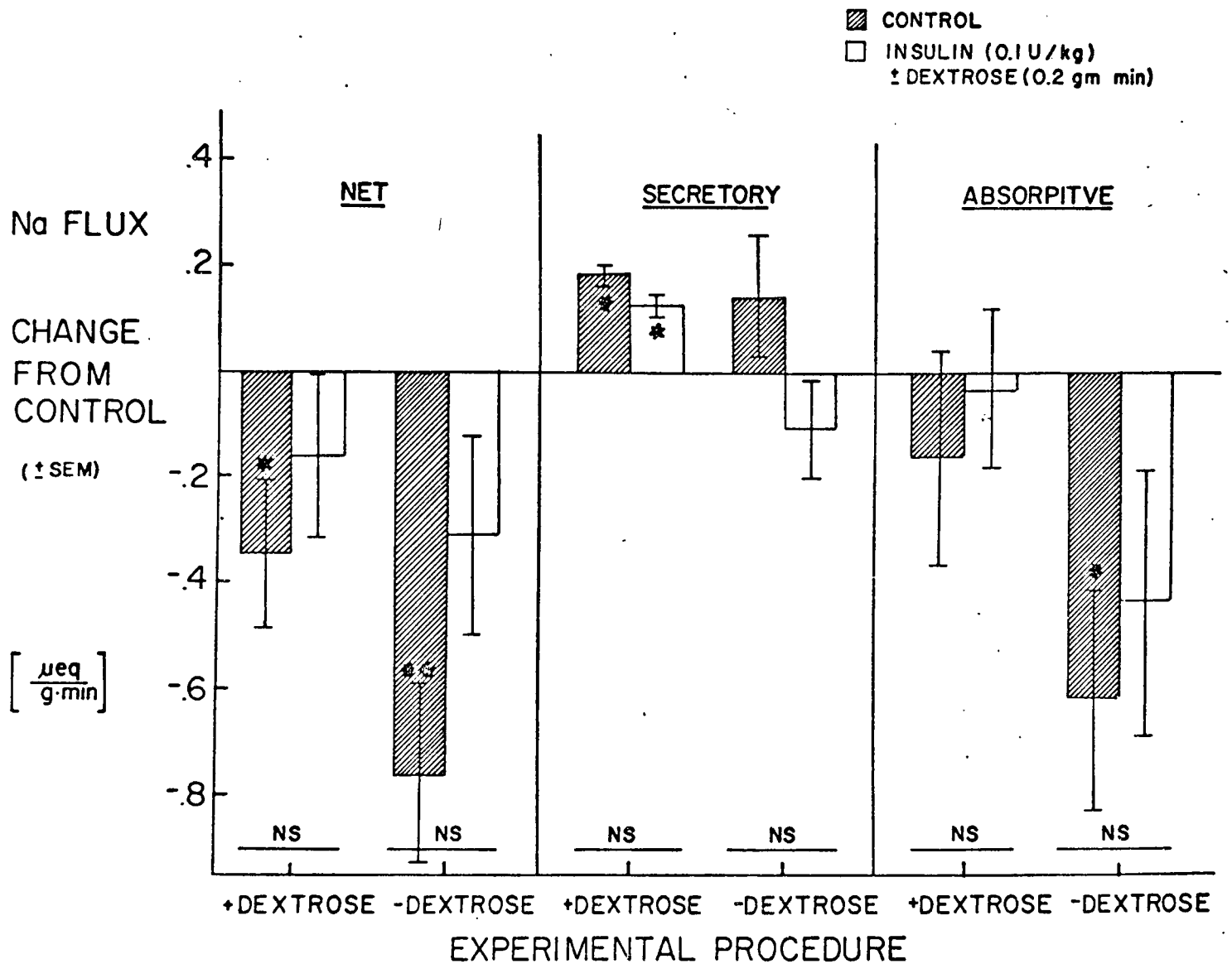

INDIRECT INFUSION
DIRECT INFUSION
NET
SECRETORY
ABSORPTIVE
1.0
.5
0
-.5
-1.0
Na FLUXES
CHANGE FROM CONTROL (±SEM)
[μeq / g·min]
.05
.5
.05
.5
.05
.5
GLUCAGON DOSE (μg/kg/min)

Figure II. Changes from control periods in net sodium flux, sodium secretory flux, and sodium absorptive flux in canine ileum following insulin (0.1 U/kg) with or without subsequent dextrose infusion (0.2 g/min) (N=5). Control animals were given heat denatured insulin plus dextrose (N=4). Significant differences between control and experimental periods are shown within each group (asterisks inside bars) and between control and experimental animals (asterisks at the bottom of each graph). *, **, and *** designate significant differences at the 5%, 1%, and 0.1% levels, respectively.


CONTROL
INSULIN (0.1 U/kg)
± DEXTROSE (0.2 gm min)
Na FLUX
CHANGE FROM CONTROL
(± SEM)
[μeq / g·min]
.4
.2
-.2
-.4
-.6
-.8
NET
SECRETORY
ABSORPITVE
NS
+DEXTROSE
-DEXTROSE
EXPERIMENTAL PROCEDURE

at either dose, but it was significantly decreased ($P < .001$), compared to control, by indirect glucagon infusion at both doses. The net sodium flux was significantly increased by direct glucagon infusion, as compared to indirect infusion, at both the lower ($P < .05$) and the higher ($P < .001$) doses of glucagon. Secretory sodium flux (fig. I) was significantly decreased as compared to control doses at both the lower ($P < .05$) and the higher ($P < .01$) doses of indirect glucagon infusion; absorptive sodium flux (fig. I) also decreased significantly ($P < .01$) at both the lower and the higher doses of indirext glucagon infusion. Absorptive sodium fluxes were increased significantly ($P < .001$) as compared to control at both doses of direct glucagon infusion; secretory sodium flux increased significantly at the lower ($P < .05$) and the higher ($P < .01$) doses of direct glucagon infusion. Both the absorptive and the secretory sodium flux changes were significantly greater ($P < .01$) during direct glucagon infusion when compared to indirect infusion.

Net sodium absorption (fig. II) decreased significantly from control levels both during ($P < .05$) and after ($P < .01$) the dextrose infusion. Net sodium absorption also decreased below control period values, but not significantly, in animals infused with dextrose plus insulin. The sodium secretory flux (fig. II) was significantly increased ($P < .05$) during the dextrose infusion, but returned to control levels after the dextrose infusion was stopped. The sodium secretory flux increased significantly ($P < .05$) during the dextrose

Figure III. Changes from control periods in net water flux, water secretory flux, and water absorptive flux in canine ileum at 0.05 and 0.5 μg/kg/min glucagon infusion directly into the mesenteric artery of a perfused segment (N=7) or indirectly into an adjacent segment (N=6). Significant differences between control and experimental periods are shown within each group (asterisks inside bars) and between directly perfused and indirectly perfused animals (asterisks at the bottom of each graph). *, **, and *** designate significant differences at the 5%, 1%, and 0.1% levels, respectively.

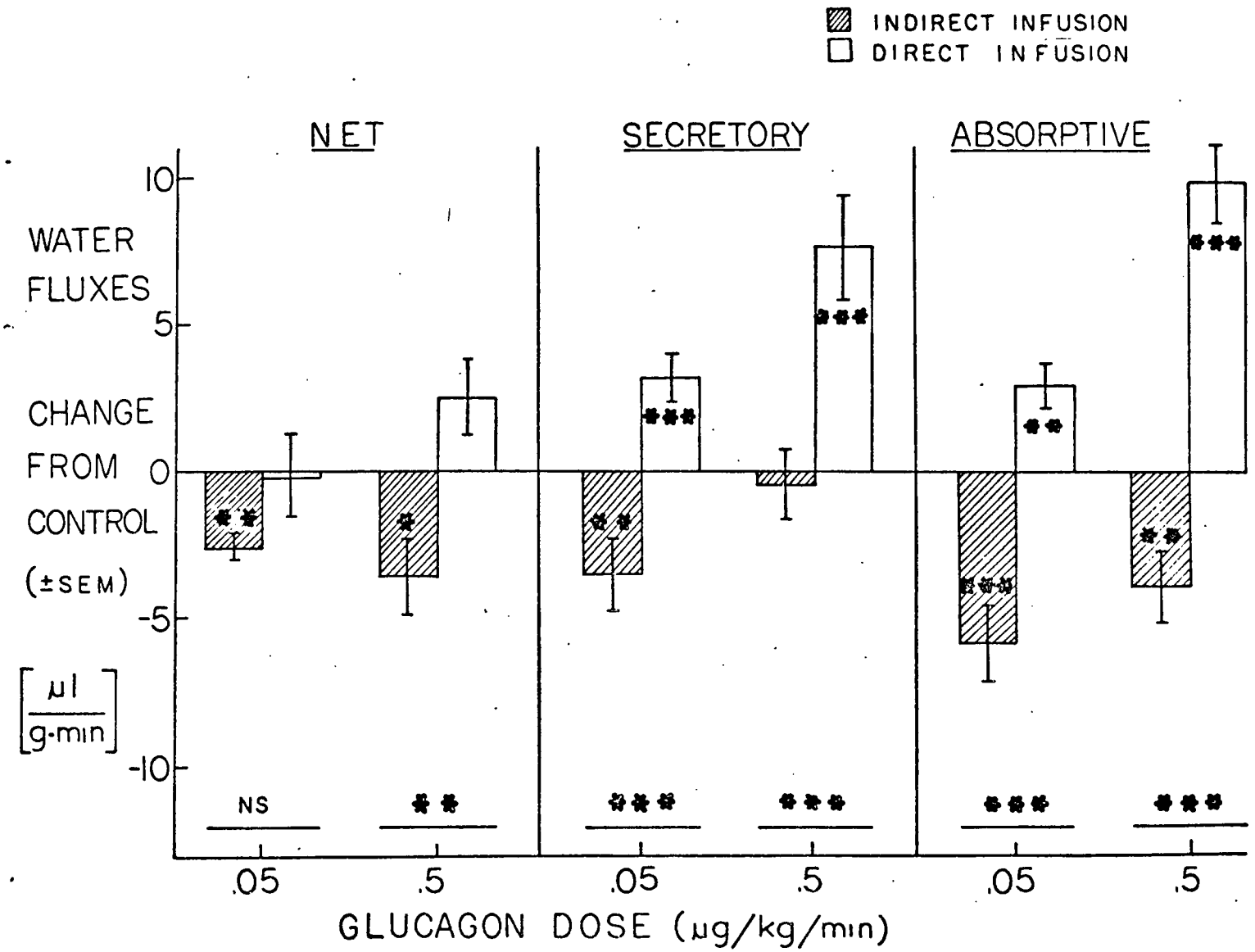
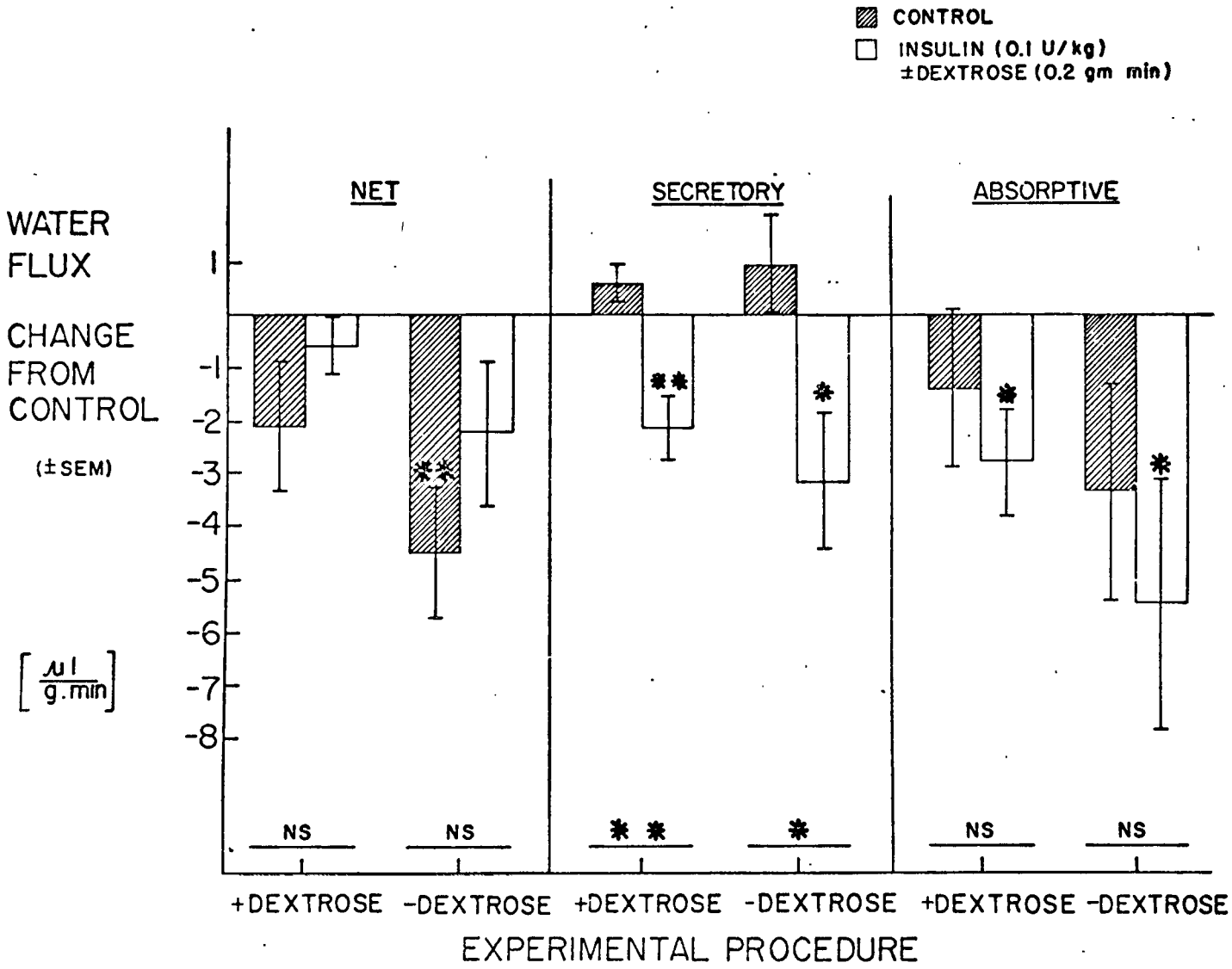

INDIRECT INFUSION
DIRECT INFUSION
NET
SECRETORY
ABSORPTIVE
10
5
0
-5
-10
WATER FLUXES
CHANGE FROM CONTROL (±SEM)
[µl / g·min]
NS
.05
.5
.05
.5
.05
.5
GLUCAGON DOSE (µg/kg/min)

Figure IV. Changes from control periods in net water flux, water secretory flux, and water absorptive flux in canine ileum following insulin (0.1 U/kg) with or without a subsequent dextrose infusion (0.2 g/min) (N=5). Control animals were given heat denatured insulin plus dextrose (N=4). Significant differences between control and experimental periods are shown within each group (asterisks inside bars) and between control and experimental animals (asterisks at the bottom of each graph). *, **, and *** designate significant differences at the 5%, 1%, and 0.1% levels, respectively.


CONTROL
INSULIN (0.1 U/kg)
±DEXTROSE (0.2 gm min)
NET
SECRETORY
ABSORPTIVE
WATER FLUX
CHANGE FROM CONTROL
(±SEM)
[µl / g.min]
1
-1
-2
-3
-4
-5
-6
-7
-8
NS
NS
**
*
NS
NS
+DEXTROSE
-DEXTROSE
+DEXTROSE
-DEXTROSE
+DEXTROSE
-DEXTROSE
EXPERIMENTAL PROCEDURE

plus insulin infusion and then returned to control levels after the dextrose infusion was stopped. The sodium absorptive flux (fig. II) was significantly decreased after the dextrose infusion was stopped.

Net water absorption (fig, III) decreased significantly below control period levels during both the lower ($P < .01$) and the higher ($P < .05$) doses of indirect glucagon infusion, but net water absorption was not significantly changed by direct glucagon infusion. The increase in net water absorption was significantly greater during direxT glucagon as compared to indirect glucagon infusion only at the higher ($P < .01$) glucagon dose. Secretory water flux (fig. III) decreased significantly ($P < .01$) as compared to the control periods at the lower dose of indirect glucagon infusion; absorptive water fluxes (fig. III) decreased significantly at both the lower ($P < .001$) and higher ($P < .01$) dose of indirect glucagon infusion. Both the secretory water fluxes and the absorptive water flux at the higher dose of direct glucagon infusion increased significantly ($P < .001$) above control period levels. At the lower glucagon dose, absorptive water flux was also increased significantly ($P < .01$) during direct infusion. The changes of both the abosrptive water fluxes and the secretory water fluxes are significantly greater during direct glucagon infusion as compared to indirect glucagon infusion ($P < .001$).

Net water absorption (fig. IV) decreased significantly ($P < .01$) in the hour after dextrose infusion had stopped.

Figure V. Changes from control periods in total blood flow and absorptive site blood flow in canine ileum at 0.05 and 0.5 μg/kg/min glucagon infusion directly into the mesenteric artery of a perfused segment (N=7) or indirectly into an adjacent segment (N=6). Significant differences between control and experimental periods are shown within each group (asterisks inside bars) and between directly perfused and indirectly perfused animals (asterisks at the bottom of each graph). *, **, and *** designate significant differences at the 5%, 1%, and 0.1% levels, respectively.

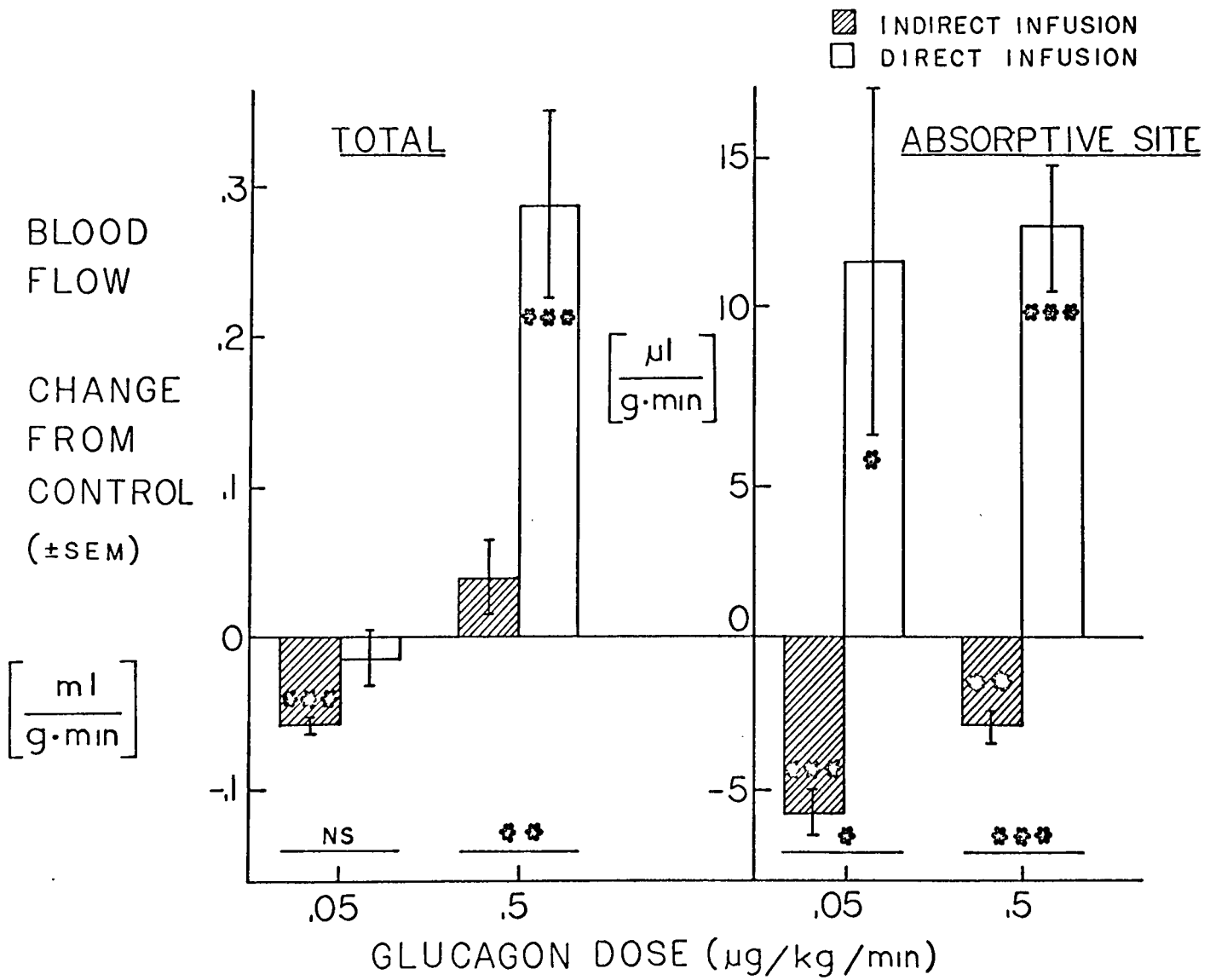
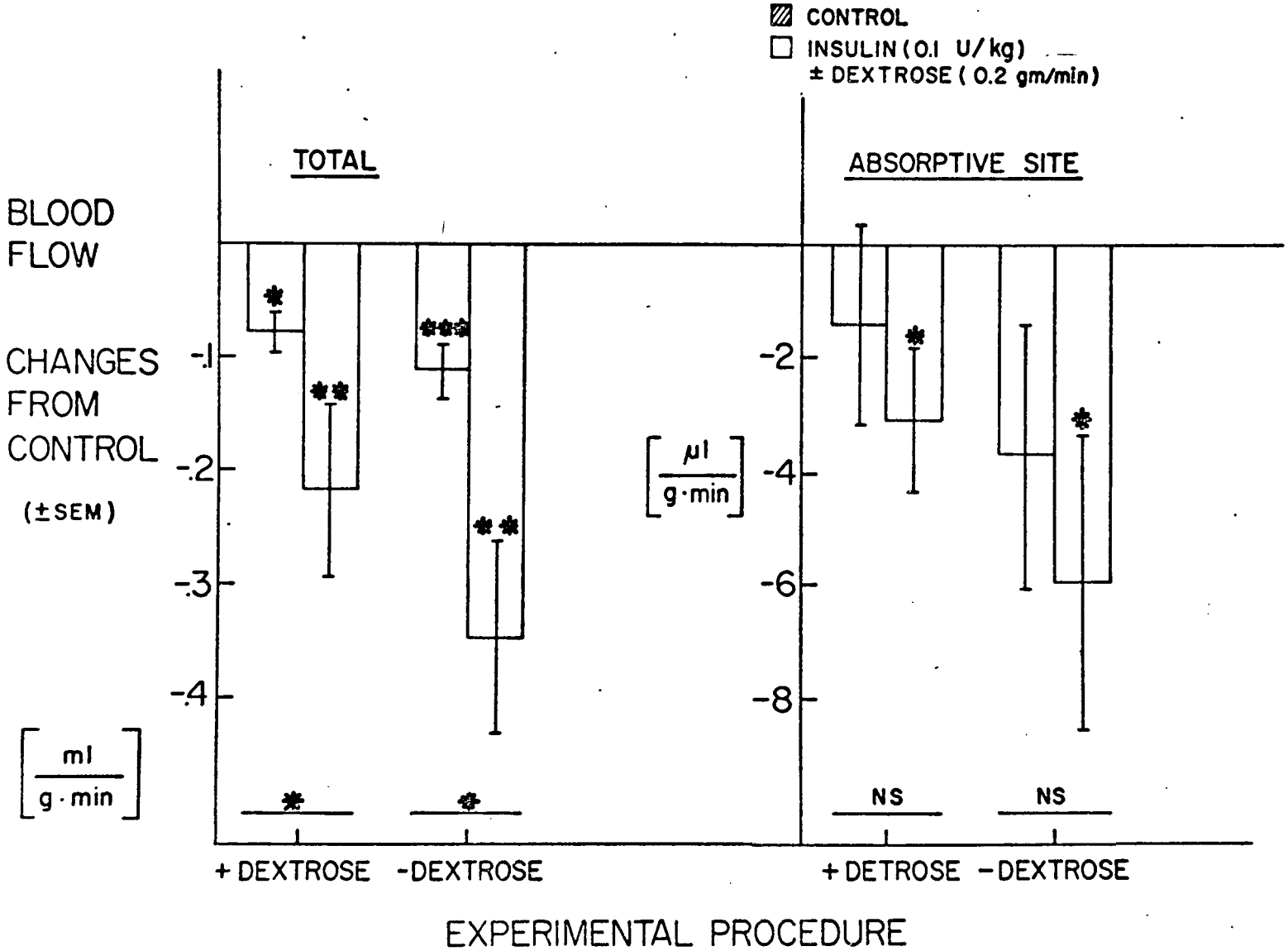

INDIRECT INFUSION
DIRECT INFUSION
TOTAL
ABSORPTIVE SITE
BLOOD FLOW
CHANGE FROM CONTROL
(±SEM)
[ml / g·min]
[µl / g·min]
.3
.2
.1
0
-.1
15
10
5
0
-5
NS
.05
.5
.05
.5
GLUCAGON DOSE (µg/kg/min)

Figure VI. Changes from control periods in total blood flow and absorptive site blood flow in canine ileum following insulin (0.1 U/kg) with or without a subsequent dextrose infusion (0.2 g/min) (N=5). Control animals were given heat denatured insulin plus dextrose (N=4). Significant differences between control and experimental periods are shown within each group (asterisks inside bars) and between control and experimental animals (asterisks at the bottom of each graph). *, **, and *** designate significant differences at the 5%, 1%, and 0.1% levels, respectively.


CONTROL
INSULIN (0.1 U/kg)
± DEXTROSE (0.2 gm/min)
TOTAL
ABSORPTIVE SITE
BLOOD FLOW
CHANGES FROM CONTROL
(±SEM)
[ml / g·min]
[µl / g·min]
-1
-2
-3
-4
-2
-4
-6
-8
NS
NS
+ DEXTROSE
-DEXTROSE
+ DETROSE
-DEXTROSE
EXPERIMENTAL PROCEDURE

The water secretory flux (fig. IV) decreased significantly following insulin injection both during ($P < .01$) and after ($P < .05$) the dextrose infusion. Water secretory fluxes were significantly lowered by insulin as compared to the animals injected with denatured insulin both during ($P < .01$) and after ($P < .05$) the dextrose infusion. The water absorptive flux (fig. IV) decreased significantly ($P < .05$) following insulin injection both during and after the dextrose infusion.

Changes in total tritium clearance (fig. V), an indicator of total blood flow to the segment, were significantly decreased by indirect glucagon infusion at the lower dose and increased ($P < .001$) at the higher direct glucagon dose and were significantly greater ($P < .01$) as compared to indirect infusion. Absorptive site tritium clearance (fig. V), an indicator of absorptive site blood flow, decreased significantly at the lower indirect glucagon dose ($P < .001$) and higher indirect glucagon dose. Absorptive site tritium clearance increased significantly during direct glucagon infusion at both the lower ($P < .05$) and the higher ($P < .001$) glucagon doses. The changes in absorptive site blood flow were significantly larger during direct infusion at both the lower ($P < .05$) and the higher ($P < .001$) glucagon doses as compared to indirect glucagon infusion.

Total blood flow to the gut segment (fig. VI) decreased significantly both during ($P < .05$) and after ($P < .001$) the

Figure VII. Changes from control periods in systemic arterial pressure and mesenteric venous pressure in canine ileum at 0.05 and 0.5 μg/kg/min glucagon infusion directly into the mesenteric artery of a perfused segment (N=7) or indirectly into an adjacent segment (N=6). Significant differences between control and experimental periods are shown within each group (asterisks inside bars) and between directly perfused and indirectly perfused animals (asterisks at the bottom of each graph). *, **, and *** designate significant differences at the 5%, 1%, and 0.1% levels, respectively.

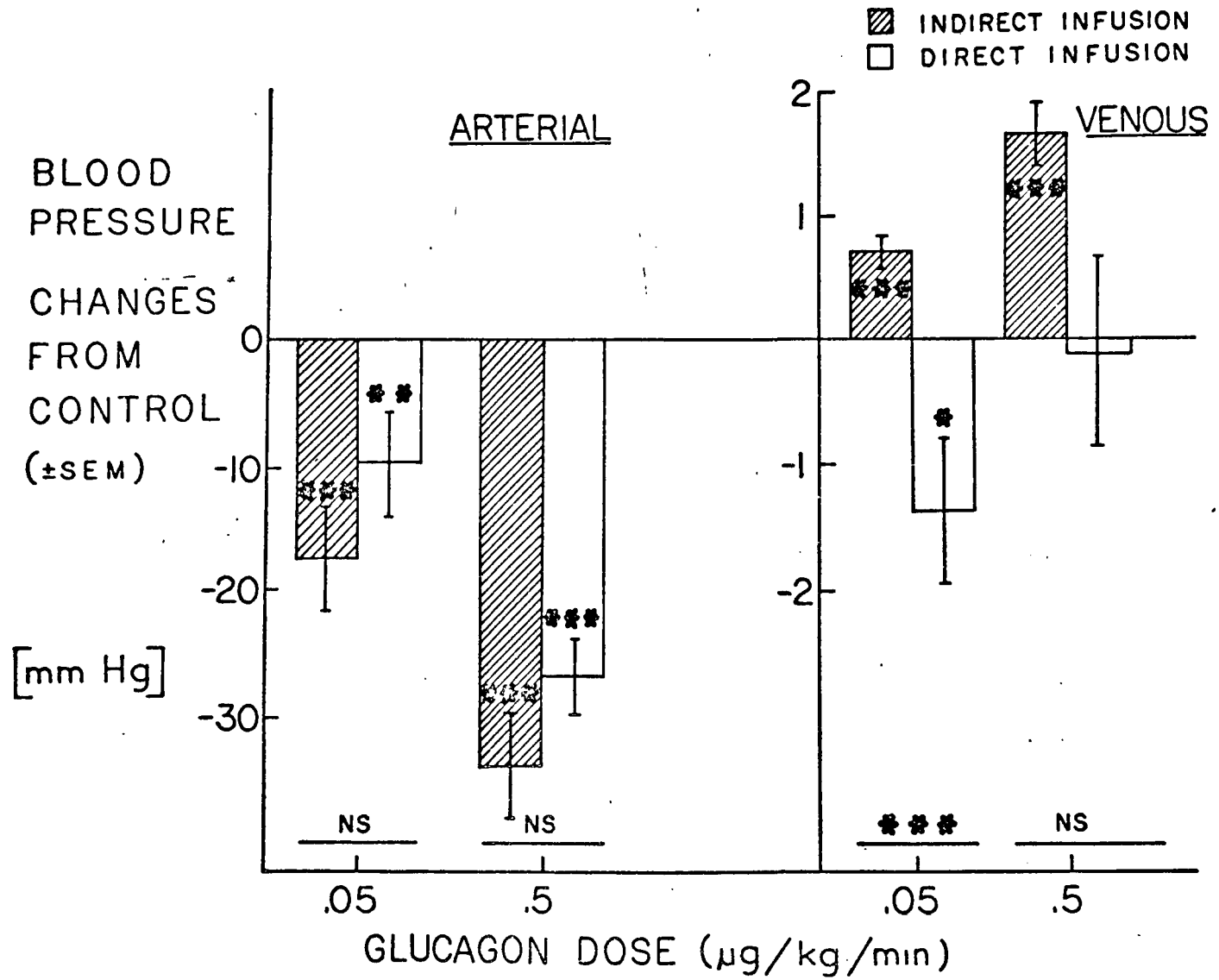
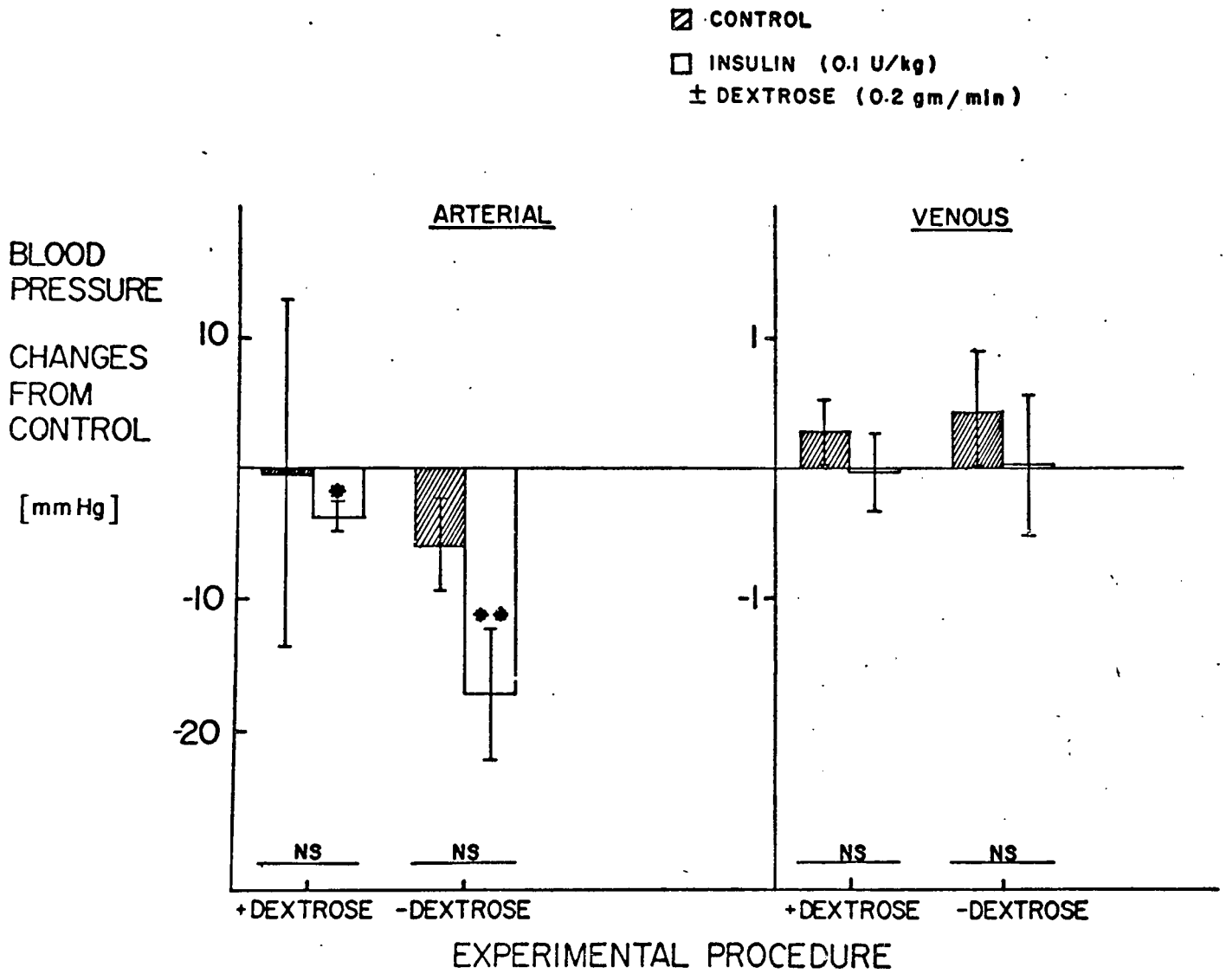

INDIRECT INFUSION
DIRECT INFUSION
ARTERIAL
VENOUS
BLOOD PRESSURE CHANGES FROM CONTROL (±SEM)
[mm Hg]
0
-10
-20
-30
2
1
-1
-2
NS
NS
NS
.05
.5
.05
.5
GLUCAGON DOSE (µg/kg/min)

Figure VIII. Changes from control periods in systemic arterial pressure and mesenteric venous pressure following insulin (0.1 U/kg) with or with out a subsequent dextrose infusion (0.2 g/min) (N=5). Control animals were given heat denatured insulin (N=4). Significant differences between control and experimental periods are shown within each group (asterisks inside bars) and between control and experimental animals (asterisks at the bottom of each graph). *, **, and *** designate significant differences at the 5%, 1%, and 0.1% levels, respectively.


CONTROL
INSULIN (0.1 U/kg)
± DEXTROSE (0.2 gm/min)
ARTERIAL
VENOUS
BLOOD PRESSURE
CHANGES FROM CONTROL
[mm Hg]
10
-10
-20
1
-1
NS
NS
NS
NS
+DEXTROSE
-DEXTROSE
+DEXTROSE
-DEXTROSE
EXPERIMENTAL PROCEDURE

dextrose infusion; following the insulin injection, total blood flow also decreased significantly ($P < .01$) both during and after the dextrose infusion. Total blood flow decreased significantly ($P < .01$) more following insulin injection when compared to animals not given insulin both during and after dextrose infusion. Absorptive site blood flow (fig. VI) decreased slightly during and after dextrose infusion and decreased significantly ($P < .05$) following insulin injection both during and after dextrose infusion.

The systemic arterial pressure (fig. VII) during both direct and indirect glucagon infusion decreased significantly at both doses of glucagon as compared to control period values, but the changes during direct and indirect glucagon infusion were not significantly different from each other.

Arterial pressure (fig. VIII) decreased following the insulin dose during the dextrose infusion ($P < .05$); the decreased arterial pressure also was significant ($P < .01$) following insulin after the dextrose infusion ceased. There was no effect on arterial pressure during or after dextrose infusion alone.

Mesenteric venous pressure (fig. VII) increased significantly ($P < .001$) during both doses of indirect glucagon infusion. Mesenteric venous pressure decreased significantly ($P < .05$) at the lower direct glucagon dose, but returned

Figure IX. Changes from control periods in total resistance and absorptive site resistance in canine ileum at 0.05 and 0.5 μg/kg/min glucagon infusion directly into the mesenteric artery of a perfused segment (N=7) or indirectly into an adjacent segment (N=6). Significant differences between control and experimental periods are shown within each group (asterisks inside bars) and between directly perfused and indirectly perfused animals (asterisks at the bottom of each graph). *, **, and *** designate significant differences at the 5%, 1%, and 0.1% levels, respectively.

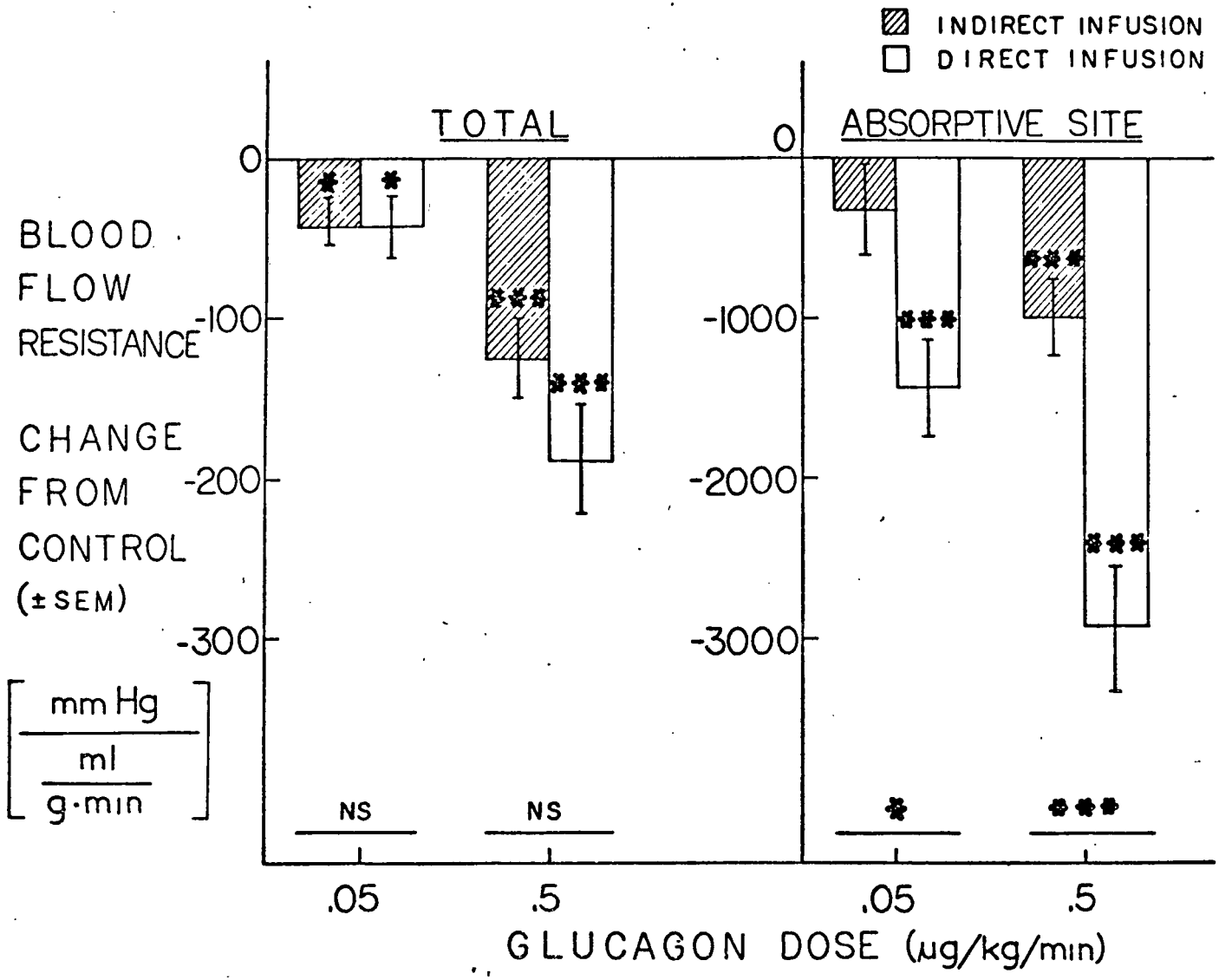
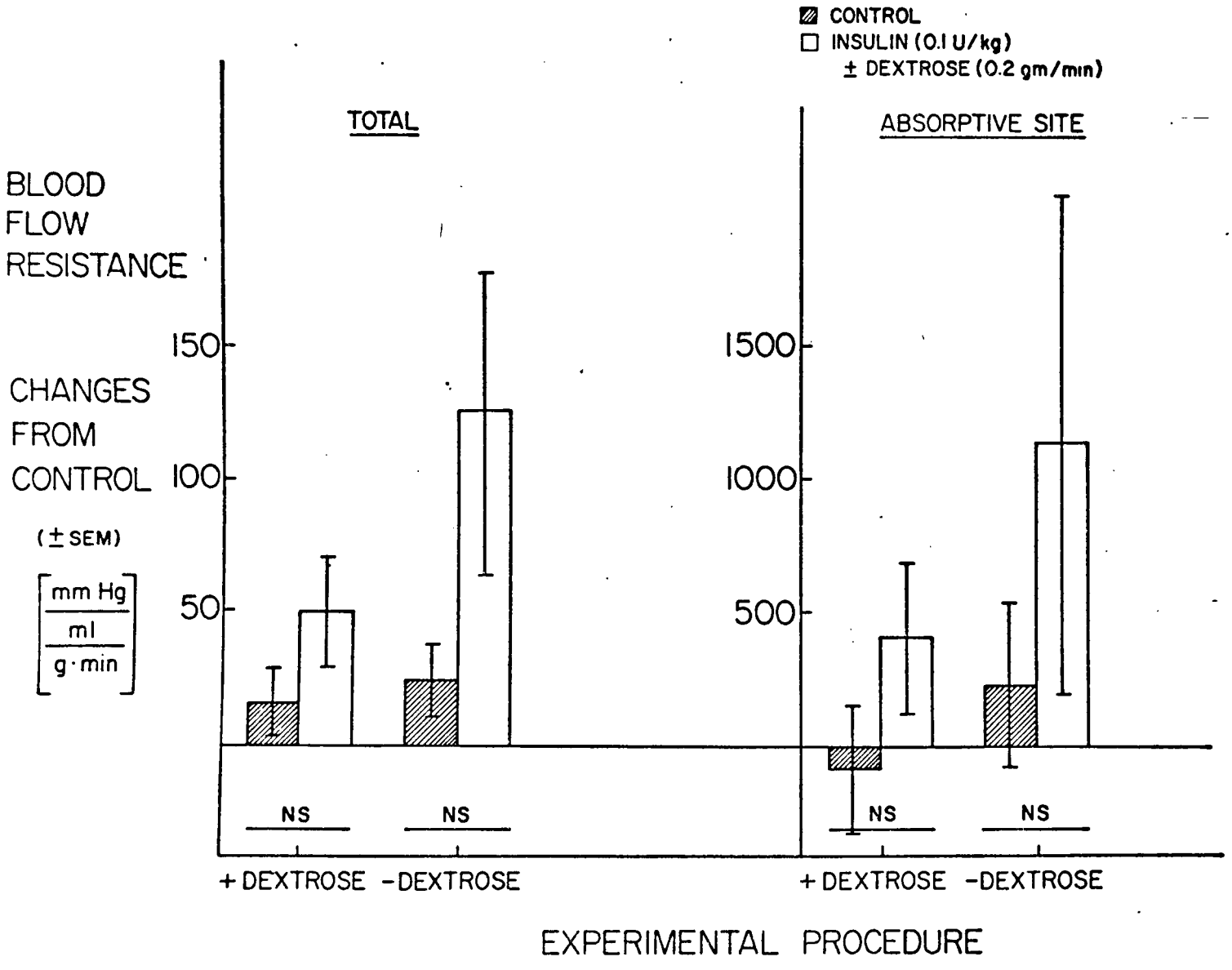

INDIRECT INFUSION
DIRECT INFUSION
TOTAL
ABSORPTIVE SITE
0
-100
-200
-300
0
-1000
-2000
-3000
BLOOD FLOW RESISTANCE
CHANGE FROM CONTROL (± SEM)
[mm Hg / ml / g·min]
NS
NS
.05
.5
.05
.5
GLUCAGON DOSE (µg/kg/min)

Figure X. Changes from control periods in total resistance and absorptive site resistance in canine ileum following insulin (0.1 U/kg) with or without subsequent dextrose infusion (0.2 g/min) (N=5). Control animals were given heat denatured insulin plus dextrose (N=4). Significant differences between control and experimental periods are shown within each group (asterisks inside bars) and between control and experimental animals (asterisks at the bottom of each graph). *, **, and *** designate significant differences at the 5%, 1%, and 0.1% levels, respectively.


CONTROL
INSULIN (0.1 U/kg)
± DEXTROSE (0.2 gm/min)
TOTAL
ABSORPTIVE SITE
BLOOD FLOW RESISTANCE
CHANGES FROM CONTROL
(± SEM)
mm Hg / ml / g·min
150
100
50
1500
1000
500
NS
NS
NS
NS
+ DEXTROSE
- DEXTROSE
+ DEXTROSE
- DEXTROSE
EXPERIMENTAL PROCEDURE

to control levels at the higher dose. Mesenteric venous pressure changes were significantly lower ($P < .001$) during direct glucagon infusion at the lower dose when compared with indirect infusion.

Mesenteric venous pressures (fig. VIII) were unchanged during insulin plus dextrose or dextrose alone or following stoppage of the dextrose infusion with or without insulin.

The total intestinal blood flow resistance (fig. IX) decreased significantly at the lower ($P < .05$) and the higher ($P < .001$) glucagon doses for both direct and indirect infusion. The changes in these values were not significantly different for direct infusion as compared to indirect infusion. Absorptive site resistance (fig. IX) decreased significantly ($P < .001$) at both doses of directly infused glucagon and for the higher indirectly infused glucagon dose. Absorptive site resistance was significantly lower during direct infusion than during indirect glucagon infusion at the lower dose ($P < .05$) and at the higher dose ($P < .001$).

The total resistance of the intestinal segment (fig. X) did not change during glucose infusion alone, but significantly increased ($P < .05$) during the insulin plus dextrose infusion. Absorptive site resistance (fig. X) did not change significantly after insulin without dextrose infusion nor during or after dextrose infusion without insulin.

Figure XI. Capillary pressures (assuming a constant post-capillary resistance) versus sodium and water fluxes at control values, and during a 0.05 and 0.5 μg/kg/min glucagon infusion. o, △, and □ designate values for control, and indirect glucagon infusion at the lower dose, and the higher dose, respectively. •, ▲, and ■ designate values for control, and direct glucagon infusion at the lower dose, and the higher dose, respectively.

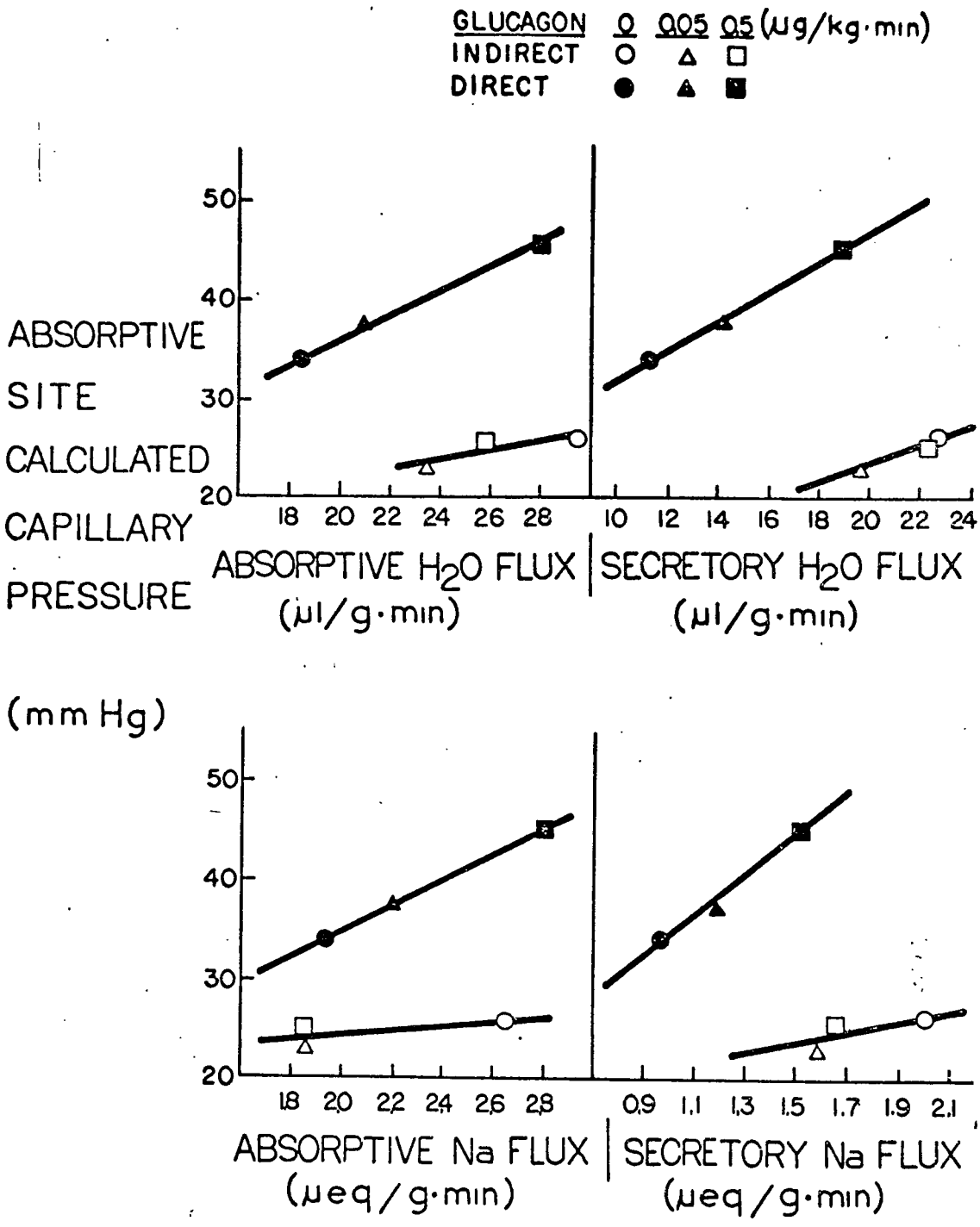

GLUCAGON 0 0.05 0.5 (μg/kg·min)
INDIRECT
DIRECT
ABSORPTIVE SITE CALCULATED CAPILLARY PRESSURE (mm Hg)
50
40
30
20
18 20 22 24 26 28
10 12 14 16 18 20 22 24
ABSORPTIVE H2O FLUX (μl/g·min)
SECRETORY H2O FLUX (μl/g·min)
50
40
30
20
18 20 22 24 26 28
0.9 1.1 1.3 1.5 1.7 1.9 2.1
ABSORPTIVE Na FLUX (μeq/g·min)
SECRETORY Na FLUX (μeq/g·min)

TABLE II

CHANGES FROM CONTROL PERIOD VALUES IN RESPONSE TO INTRAARTERIAL HISTAMINE-HCl

PARAMETER	DOSE OF HISTAMINE-HCl* (μg/kg/min) 0.1	1.0	5.87	52.8
Net Na absorbed (μeq/gm·min)	-.075	.180	.040	-.346
Na secretory flux (μeq/gm·min)	.023	.024	-.175	.168
Na absorptive flux (μeq/gm·min)	-.050	-.070	-.140	-.177
Net water flux (μl/gm·min)	-.720	.890	-.440	2.35
Water secretory flux (μl/gm·min)	-.080	-3.96	-1.90	.420
Water absorptive flux (μl/gm·min)	-.810	-3.10	-2.20	-1.93
Arterial pressure (mm Hg)	-23.	-18.	-1.	-25.
Mesenteric venous pressure (mm Hg)	1.80	2.06	1.09	1.58
Total blood flow (ml/gm·min)	.640	-.129	.010	-.178
Absorptive site blood flow (μl/gm·min)	-1.30	-4.20	-4.00	-2.31
Total resistance (mm Hg/ml/gm·min)	24.	71.	-3.	215.
Absorptive site resistance (mm Hg/ml/gm·min)	-1420.	-14.	211.	360.

* The indicated doses of histamine-HCl were infused into an artery supplying a length of canine ileum from which the parameters were measured

Capillary blood pressure (assuming a constant post capillary resistance (49))was observed to be linearly related to the secretory and absorptive sodium and water fluxes (fig. XI) during both direct and indirect glucagon infusion.

The effects of intraarterial histamine were examined with four dogs (Table II). The fluxes of sodium and water into the lumen were not consistently changed by histamine infusion. The absorptvie fluxes of sodium and water were decreased at all tested doses of histamine, as were the absorptive site blood flow and arterial pressure, while venous pressure increased. Inconsistent effects were seen in the measurements of total blood flow to the segment.

DISCUSSION

DISCUSSION

Glucagon, at high rates of intravenous infusion, has been shown to increase the absorption of ions and water from rat small intestine (34), but to decrease absorption in canine ileal Thiry-Vella loops (3). Decreased absorption of salt and water in the experiments presented herein was observed in control animals during indirect glucagon infusion. The indirect infusion was equivalent to an iv infusion since the intestinal segment received glucagon only after glucagon had passed through the general circulation. The effects of glucagon infused directly into the circulation of the intestinal segment were opposite to the effects of an indirect infusion. The observed changes between the direct and indirect glucagon infusion are generally opposite, but as the indirect glucagon infusion dose increases, the observed effects approach those obtained by direct infusion. The direct infusion of glucagon increased transport by increasing unidirectional absorptive fluxes relatively more than increasing secretory fluxes. Glucagon might mediate its actions on sodium and water unidirectional fluxes through a direct effect on cellular metabolism *via* intracellular cAMP (68), through the initiation of hyperglycemia (25), or *via* compensatory insulin secretion (61), or through a direct effect on the vasculature (68).

Cholera toxin, prostaglandin E_1 and E_2 stimulate active salt secretion (40,54) *via* a cAMP dependent process without affecting non-electrolyte absorptive fluxes (10) in the intestinal epithelium. The vasodilatory effects of beta receptors have been attributed to stimulation of adenyl cyclase in vascular smooth muscle and a subsequent increase in intracellular cAMP (57). Increased glycogenolysis and gluconeogenesis, two of the major effects of glucagon, reflect an adenyl cyclase mediated increase in hepatic intracellular cAMP levels. However, it is unlikely that changes in epithelial intracellular cAMP might mediate changes in absorption (10) due to glucagon because glucagon, *per se*, has no effect on mucosal adenyl cyclase activity (40).

Peripheral effects of glucagon include direct stimulation of insulin secretion (61) and hyperglycemia (25). Insulin infusion and hyperglycemia did not have the same effects as an indirect glucagon infusion. However, some parameters are changed in the same direction by indirect glucagon infusion, insulin injection and dextrose infusion so that the latter two factors may contribute to the effects of an indirect glucagon infusion. Thus, insulin with or without a concomitant dextrose infusion mimics certain of the effects of an indirect glucagon infusion, namely, a decrease in net and absorptive water fluxes, a decrease in total and absorptive site blood flows, and a decrease in arterial blood pressure. Hyperglycemia decreased net sodium absorption as did an indirect glucagon infusion, but hyperglycemia did so by increasing the sodium secretory flux

whereas the indirect glucagon infusion decreased the absorptive sodium flux relatively more than the secretory sodium flux. Net water absorption and total blood flow are significantly decreased by both hyperglycemia and an indirect glucagon infusion although the decrease in net water absorption becomes significant only in the hour after the dextrose infusion had stopped.

The absorptive water fluxes parallel absorptive site blood flow, following insulin injection, during dextrose plus insulin injection, and during glucagon infusion, substantiating the view that appearance of $^{3}H_2O$ into the vasculature is largely blood flow limited (20,21,51,75).

Induced hyperglycemia, or peripheral effects of it, increased the sodium secretory fluxes in animals infused with dextrose or dextrose plus insulin in spite of decreased absorptive site blood flow. Insulin, however, has been found to have no effect on mucosal epithelial adenyl cyclase activity (40) suggesting that the increased sodium secretory fluxes may be mediated by a direct or indirect effect of hyperglycemia.

Insulin decreases sodium and water absorption (2,30) in man and rat; no significant changes in sodium fluxes due to insulin alone were observed in our experiments.

The net effect of a vasoactive agent is a function of its direct effect on the simultaneous activity of vascular smooth muscle, cellular and local metabolism, and

gut visceral muscle tone (32) or an indirect effect through its actions on peripheral tissues.

The decrease in arterial blood pressure was the same for both directly and indirectly infused glucagon since blood pressure would depend on the total body effect. Folkow et al. (26) have determined that the vascular supply to the gut is divided into at least four distinct components. Blood flows through the fat tissue in the mesentery, muscularis, submucosa, and mucosa, which in turn is subdivided into separate flows to the crypts and villi. With a decrease in blood pressure alone, villous plasma flow remains relatively constant while the total blood flow to the intestine decreases (6). Total blood flows were not significantly decreased with either a direct or an indirect glucagon infusion except for a small decrease at the lower dose of glucagon in control animals. However, absorptive site blood flows were significantly increased with a direct infusion and signicantly decreased with an indirect infusion at the lower glucagon dose, suggesting that the observed significant changes in blood flows were not entirely due to changes in arterial pressure.

Increased gut tone, in response to injected or secreted insulin during or after dextrose infusion might cause the significant decreases in total blood flow (42), since aortic smooth muscle tissue in vitro is unresponsive to insulin (49). Absorptive site blood flow also significantly decreases in response to insulin suggesting that the changes in flows

are not completely pressure mediated (6). Changes in blood flows during the induced hyperglycemia could be mediated by the decreased arterial pressure since absorptive site blood flow is unchanged but total blood flow is significantly decreased.

The positive inotropic and positive chronotropic effects of high glucagon doses (57,68), considered with its ability to increase splanchnic blood flow (47), and selectively vasodilate mesenteric arteries after hemorrhage (69) suggest that the observed significant decreases in systemic arterial pressure, absorptive site resistance, and total resistance were due to vasodilation of vascular smooth muscle in both the gut and other portions of the body. The effect of glucagon was relatively more specific for the absorptive site than the total gut segment as seen by the significant differences between direct and indirect infusion on decreased absorptive site resistance but not on total resistance. The differences in venous pressures also indicate a selective effect on the gut segment. Hepatic effects are the same for both direct and indirect infusion. Glucagon might exert a direct vasodilating effect on absorptive site vascular smooth muscle as increased levels of intracellular cAMP vasodilate mesenteric arteries (66).

To determine the effect of progressive vasodilation, intraarterial histamine hydrochloride, a vasodilator, was administered in an attempt to induce dose related changes in sodium and water unidirectional fluxes. Vasodilation

induced by histamine yielded effects similar to those of an indirect glucagon infusion in that both substances decrease sodium and water absorptive fluxes, arterial pressure, and absorptive site blood flow and increase venous pressure. As has been observed by others (64,70), histamine does not cause consistent changes in total intestinal blood flow. The above changes may therefore be mediated through the effects of peripheral vasodilation caused by glucagon and a consequent vasoconstriction mediated through the sympathetic nervous system.

Histamine seems to have effects on transport processes, but the data did not allow any conclusions as to the relationship between passive sodium and water movement and cardiovascular effects.

Curran et al. (13,14) have proposed a model, based on non-equilibrium thermodynamics and the Staverman reflection coefficient, which satisfies many observations of membrane water transport. The mechanism utilizes two membranes in series with different reflection coefficients. Active solute transport occurs across the first membrane (having a high Staverman coefficient) causing osmotic fluid movement into the middle compartment. The increased hydrostatic pressure in the middle compartment then causes passive solute and solvent transfer across the second membrane (with a low Staverman coefficient).

Diamond's hypothesis of local osmosis (16,17,19) proposed that long extracellular pathways could explain isotonic water transport. Channel length, channel radius, channel permeability, transport site, and the solute transport rate are significant determinants of transport characteristics as proposed by Diamond (18). The transport model proposed by Diamond differs from that of Curran in that it assumes standing gradients within the second compartment. The energy utilized during water absorption is that required for operation of the sodium and chloride transport systems. Water movement is generally considered passive with its direction and magnitude being determined by osmotic and hydrostatic pressure gradients (11) generated by active sodium transport and Starling forces. Anatomical analogies to the three compartment, double membrane (barrier) hypotheses of Curran and Diamond are present in the intestine. The three compartments are possibly the cell interior, the lateral and subepithelial spaces, and the capillary lumen; the two membranes (barriers) might be the membrane around the epithelial cell and the capillary walls, basal openings of intercellular spaces, or serosal tissue. Quantitative predictions of mucosal (lumenal) membrane effects on salt and water transport have not been proposed; however, asymmetry of structure between brush border and serosal (and lateral space) membranes with respect to solute transport is a necessary feature (53). Eighty five percent of the total intestinal tissue conductance has been attributed to ionic diffusion through a transepithelial

extracellular shunt pathway (58).

Hypotheses (18,62,75) based on the above models have been proposed in order to explain the effects of Starling forces on unidirectional fluxes across epithelia. These hypotheses are based on the presence of the Starling driving forces at the capillary level which affect the movement of water and solutes through hydraulically conductive pathways between or through the cells. The results of these experiments are partially consistent with these hypotheses. The vasodilation produced by direct glucagon infusion (which results in increased absorptive site blood flow and decreased absorptive site resistance) would allow relatively more of the arterial pressure to be transmitted to the capillaries, and thus would tend to increase secretory sodium and water fluxes. This tendency however would be reduced by the simultaneous decrease in arterial pressure and in the case of the direct glucagon infusion, by the decreased venous pressure. The net effects on capillary pressure and their relationship to unidirectional fluxes were illustrated in figure XI.

The increased unidirectional fluxes in response to a direct glucagon infusion could be explained by an increased absorptive site blood flow, or increased capillary pressure. The plot of capillary pressures versus unidirectional fluxes shows that a direct and indirect glucagon infusion do not have points on the same line or lines with the same

slope, although within each group the fluxes are proportional to capillary pressure. This suggests that the changes in unidirectional fluxes cannot be explained solely by changes in capillary pressures. The increased absorptive site blood flows could increase $J_{secretory}$ by increasing convection or passive diffusion (16-19,63) and increase $J_{absorptive}$ by increasing the supply of oxygen and nutrients (29,31,63). The clearance of ^3H_2O into the vasculature is blood flow limited, as found by others (20,21,51, 75) since the decreased water flux is associated with decreased absorptive site blood flow during indirect glucagon infusion. The unidirectional sodium and water secretory and absorptive fluxes were observed to increase or decrease in parallel to absorptive site blood flow. Increased absorptive site blood flow may be a reflection of increased capillary surface area (46). This would permit a greater area for both absorption and secretion and this seems to be the mechanism most consistent with the results.

This mechanism is viewed as follows. Absorptive site vasoconstriction or vasodilation is caused by decreases or increases, respectively, in the number of open capillaries which in turn decreases or increases the capillary surface area available for exchange between the blood and intestinal lumen. Capillary surface area would be directly proportional to both the absorptive and secretory fluxes for any given set of conditions. Superimposed on the effect of capillary surface area would be any additional effects on cap-

illary pressure, and cell transport.

In summary, glucagon causes vasodilitation both peripherally and in the small intestine. Vasodilitation at the intestinal absorptive site is more pronounced than in the remainder of the intestine. Peripheral vasodilitation caused by indirect glucagon infusion causes decreased arterial blood pressure which in turn causes intestinal vasoconstriction through sympathetic activity. Glucagon infusion, directly into the intestinal arterial supply can overcome this indirect vasoconstriction. Intestinal absorptive site vasodilation increases the unidirectional secretory and absorptive fluxes of both Na and H_2O. Intestinal vasoconstriction produces the opposite effects. Insulin injection, glucose infusion and peripheral vasodilation by means of histamine reinforce some of the effects produced by indirect glucagon infusion, probably also through effects on intestinal blood flow.

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