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Glucagon and the Circulation

ALFRED E. FARAH

Sterling Drug Inc., Sterling-Winthrop Research Institute, Rensselaer, New York

I. Introduction

A HYPERGLYCEMIC effect of various insulin preparations was already observed by Banting and Best in 1921 when they were conducting their celebrated studies with insulin [see Best (37) and Collip (84)]. In 1923, Murlin et al. (334) were able to separate a hyperglycemic factor from insulin, a factor they called "glucagon," the mobilizer of glucose. Additional evidence for a pancreatic hyperglycemic factor was obtained by Bürger and Brandt (67). The variability of the hyperglycemic effect of insulin preparations (289) raised doubts whether this factor was a hormone, rather than an accidental contaminant. However, the classical studies of Foa (142) elucidated the physiological importance of endogenous glucagon, and this was followed by the important findings of Sutherland and coworkers (474) that glucagon acted via the formation of 3′,5′-cyclic adenosine monophosphate (cAMP). Furthermore, a major contribution to the physiology of glucagon was the introduction of the method of immunoassay by Yallow and Berson (522), as adapted for glucagon by Unger et al. (497).

Glucagon was isolated in pure form by Staub et al. (449), and its structure and amino acid sequence was described by Bromer et al. (59). Total synthesis was accomplished by Wünsch and coworkers, and a review

of the synthesis of this hormone has been published by Wünsch and Weinges (521).

Some of the earliest observations on the effects of commercial insulin on the heart were made by Visscher and Müller (507a). They observed an increase in heart rate and improved contractility of the dog heart-lung preparation (HLP) with some insulin preparations, while other preparations were inactive. Tada (478) and Trendelenburg (492) observed the epinephrine-like effects of insulin preparations on isolated intestinal strips, and the suggestion was made that this phenomenon could be developed into a bioassay for insulin that would be far less tedious than the standard bioassay based on the blood-sugar-lowering properties of insulin in rabbits. However, Farah (133) observed that there was no correlation between the intestinal smooth muscle and blood sugar effects, and it was concluded that some commercial amorphous insulin preparations contained a contaminant that had epinephrine-like properties. This was further substantiated by the effects of crystalline and amorphous insulin preparation on the cardiac activity in the HLP of the dog. Crystalline insulin, which had no effects on the intestinal strips, was not a positive chronotropic and/or inotropic agent in the HLP, while amorphous insulin preparations increased heart rate and contractility of the heart in direct proportion to their effects on the isolated intestinal smooth muscle (133). These data thus suggested that certain amorphous insulin preparations contained an impurity that had sympathomimetic effects.

After the isolation and crystallization of glucagon, Eli Lilly and Company made available crystalline and some amorphous glucagon preparations. All these preparations had inotropic and chronotropic effects on the isolated heart, which on a molar basis exceeded those observed with epinephrine (136). These early observations on the HLP were followed by extensive animal studies by Glick et al. (166), Lucchesi (284), Lucchesi et al. (286), Regan et al. (391), and Whitehouse and James (510), which were later extended to clinical studies by Parmley and Sonnenblick (363).

The effects of glucagon on renal function were first observed by Staub et al. (450), and the relation of glucagon to diabetic glomerular changes was observed by Mogensen (323). The importance of glucagon in the glomerular vascular changes observed in diabetes has been extensively studied by Seyer-Hansen (426) and Cortes et al. (91), and it is likely that these glomerular changes are independent of cAMP formation.

The natriuresis of fasting and the antinatriuresis of carbohydrate refeeding were described by Benedict (32), Bloom (46), and Gamble et al. (160). The relation of this natriuresis to glucagon was observed by Unger et al. (496), who demonstrated an increased glucagon and reduced insulin concentration in the blood of starving patients.

The hormone glucagon is secreted by the pancreatic A cells and the oxyntic mucosa of the stomach (332, 355). The glucagon-containing cells are best shown by histochemical reactions for indoles since the glucagon molecule is relatively rich in tryptophan (73). In the human and most mammalian islets, the A cells are randomly distributed and are rich in highly electron-dense particles approximately 400 mμ in diameter, which have been identified as the glucagon-containing intracellular bodies.

Glucagon has a molecular weight of 3485 and is a linear oligopeptide consisting of 29 amino acid residues with an N-terminal histidine and a C-terminal threonine. The C-terminal portion of the molecule probably contains the immunological determinant for the production of the specific antibody for pancreatic glucagon, while the N-terminal portion probably determines the production of the less specific antibody which reacts with glucagon and several glucagon degradation products or biosynthetic precursors. Human, porcine, and bovine glucagon have identical amino acid compositions and have similar physiological and immunological characteristics. The complete structural integrity of glucagon molecules is essential for both its biological and immunological characteristics.

Several biologically active peptides, such as secretin, the vasoactive intestinal peptide, and the gastrointestinal inhibitory polypeptide, have homologous amino acid sequences with glucagon (61, 109b, 213). All of these polypeptides have biological activities related to glucagon and have been shown to have inotropic and chronotropic effects on the heart (75, 411, 414) and to increase the cardiac concentration of cAMP (74).

Glucagon has been used as a cardiotonic, a vasodilator, and an inhibitor of gastrointestinal smooth muscle. It has also been used in the treatment of pancreatitis, gallstones, gastric bleeding, and a variety of other conditions.

This review will specifically deal with the cardiovascular-renal effects of pancreatic glucagon. For general discussions of glucagon physiology and biochemistry, the interested reader is referred to the reviews by Foa, Bajaj, and Foa (143), Lefebvre (261), Lefebvre and Unger (264), and Unger and Orci (498, 499).

II. Effect of Glucagon on the General Circulation

Glick (165), Glick et al. (166), and Lucchesi (284) have observed no change, a slight reduction, or an increase in the systemic blood pressure of anesthetized dogs when glucagon was administered intravenously. The main changes produced by glucagon were on the heart, and here contractility and heart rate were increased in a dose-dependent manner. In heart failure, the positive inotropic effect of glucagon will decrease the sympathetic tone by decreasing the reflex sympathetic stimulation as a result of the improvement in the cardiac activity and the concomitant improvement in the blood flow to various organs (58). Another factor is the increased release

of epinephrine from the adrenal medulla caused by glucagon. Thus, Scian et al. (424) have shown that in an isolated perfused adrenal gland preparation in dogs, glucagon had a marked stimulatory effect on both epinephrine and norepinephrine secretion. Sarcione et al. (416) have shown that a single intravenous injection of glucagon increased the glucose, lactate, and epinephrine concentrations of peripheral blood. Furthermore, Lefebvre (259) observed that adrenalectomy eliminated the antiphlogistic effects of glucagon, as well as the inhibitory effects of glucagon, on the spontaneous contractions of the rat uterus (112). Glucagon reduced the epinephrine content of the adrenal medulla (263). In a similar manner, Fasth and Hultén (138) and Kock (233) have shown that adrenalectomy in cats reduced the effects of glucagon on the motility of the colon. The ability of glucagon to release epinephrine has actually been used as a test for pheochromocytoma (257, 262).

These findings could complicate interpretations of the observed effects in the intact animal since many cardiovascular effects of glucagon resemble those of epinephrine. However, the observation that glucagon had a positive inotropic effect and reduced blood pressure and peripheral resistance in the presence of the beta-blocker propranalol in the anesthetized dog shows that these effects are not primarily due to the released epinephrine and norepinephrine, although they could have contributed to the effects of glucagon on the general circulation.

In intact dogs, glucagon increased the cardiac force and reduced peripheral resistance (see table 1). Under conditions where severe heart failure was induced by means of a continuous infusion of pentobarbital, glucagon increased the depressed blood pressure and increased the cardiac output and the maximum rate of increase in intraventricular pressure (dp/dt). In experimental myocardial infarction in dogs, glucagon increased the depressed arterial pressure (297, 385) and decreased the peripheral vascular resistance. In man, peripheral vascular resistance was reported to be decreased in some studies, while in others mean arterial pressure and peripheral vascular resistance were unchanged (116). [For reviews, see Murtagh et al. (335) and Kones and Phillips (243).] However, when glucagon had an effect on cardiac output, it usually decreased peripheral resistance.

A. Peripheral Vascular Effects

Some of the early studies indicated a marked increase in hepatic blood flow due to glucagon administration (141, 432). On the other hand, blood flow through skeletal muscle of dog and man increased only slightly (166, 335), thus indicating regional circulatory effects of glucagon. This was substantiated by the findings of Ross (412) that intraarterial injections of glucagon in cats dilated the mesenteric vascular bed, produced minimal effects in the renal and femoral circulation, and the hepatic arterial bed was constricted by glucagon. Tibblin et al. (487, 488), Kock et al. (234–236), Madden et al. (294), and Hulstaert et al. (203) have confirmed and extended these effects of glucagon in anesthetized dogs on regional vascular beds by means of electromagnetic flowmeter measurements. Glucagon increased aortic, superior mesenteric, and renal blood flow, while splenic and femoral blood flow did not increase significantly. Further studies by Hulstaert et al. (203) have shown that glucagon given intravenously decreased blood pressure and increased superior mesenteric and hepatic artery blood flow substantially, and as a result, total liver and portal blood flow increased to a much greater extent than hepatic arterial flow. Kazmers et al. (219, 220) confirmed and expanded these findings and have shown that in anesthetized dogs glucagon infusions increased superior mesenteric artery flow to a greater extent than cardiac output. The nutrient capillary circulation, as well as flow through arteriovenous shunts, was increased significantly by glucagon infusions. However, nutrient capillary flow was proportionately far greater than flow through the shunts. In these experi-

TABLE 1

Infusion of glucagon, 0.25 μg/min in 0.25 ml of 3% glucose into left renal artery of a female dog weighing 16.8 kg and anesthetized with pentobarbital (unpublished)

Time (min)	RPF,* (ml/min)		GFR (ml/min)		Na^+ (mEq)		K^+ (mEq)		Cl (mEq)	
	R	L	R	L	R	L	R	L	R	L
0–15	52.0	61.4	18.4	20.1	12.4	14.2	9.0	10.5	3.9	4.2
15–30	57.6	58.8	19.6	18.4	12.0	11.9	8.6	9.4	3.3	4.0
30–45	60.3	57.4	18.0	19.1	13.6	12.3	8.1	8.6	3.0	3.8
45–50	Infusion of 0.25 μg glucagon into left renal artery									
50–65	58.0	63.6	19.4	20.6	14.0	14.8	8.1	11.2	3.6	6.5
65–80	61.2	58.4	19.1	21.4	12.2	21.2	8.8	26.4	3.2	10.2
80–95	64.3	60.1	17.8	20.9	12.0	36.8	9.3	22.9	3.8	11.8
95–110	60.1	58.6	18.9	18.6	14.9	41.7	8.2	25.1	3.0	12.9
110–125	57.2	59.9	19.0	19.0	23.0	48.6	8.8	27.3	2.8	11.2
125–140	59.4	61.2	17.3	19.6	24.2	42.9	8.6	24.9	3.6	10.9
140–150	Infusion of 3% glucose, 0.25 ml/min									
150–165	50.2	58.0	18.1	18.8	10.5	26.8	8.0	15.9	3.0	6.3
165–180	61.8	64.2	18.9	19.7	11.9	11.1	8.8	10.5	3.6	5.0
180–195	55.9	60.1	18.0	20.8	9.6	8.2	9.1	11.0	3.2	3.6

* Abbreviations used are: RPF, renal plasma flow; GFR, glomerular filtration rate; R, right kidney; L, left kidney.

ments, femoral blood flow decreased in spite of an increased cardiac output and decrease in total peripheral resistance. Thus, the major effects of glucagon on blood flow in dogs was to redistribute blood to the splanchnic area at the detriment of blood flow through the hind limbs.

Species differences in the response of regional blood flow to glucagon have been observed. In the dog, the major increase was observed in portal blood flow due to an increase in mesenteric flow, although hepatic arterial blood flow also increased (27, 203, 234, 235, 432). Similar findings have been observed in monkeys (56). In the cat, portal vein flow was increased, while the hepatic arterial bed was constricted (245, 412). In rats (350) and man (141, 512), total hepatic blood flow was increased by glucagon; however, no differential hepatic blood flow determinations have been reported. In the pig, the increase in hepatic blood flow was exclusively due to an increase in hepatic arterial blood flow, and superior mesenteric blood flow was decreased (280).

Kock et al. (236) and Richardson and Withrington (399, 400) observed that vasoconstrictor effects of sympathetic stimulation, norepinephrine, angiotensin, and vasopressin on the hepatic vascular bed were reduced by the administration of glucagon. It has been suggested that this antagonism may have physiological implications in which glucagon would protect the liver vasculature from a variety of circulating vasoconstrictor agents (399). However, the amount of glucagon infused in these experiments would produce glucagon concentrations in hepatic arterial blood, which would exceed the glucagon concentration observed in normal man (350 pg/ml), or under pathological conditions (400 to 1500 pg/ml) (437).

By using microsphere, Bond and Levitt (50) determined blood flow to various portions of the gastrointestinal tract. The effect of glucagon was to increase the blood flow to all layers of the stomach and bowels. In pentagastrin-stimulated mucosa, both acid secretion and aminopyrine clearance (a measure of mucosal blood flow) were increased. Under these conditions, glucagon given subcutaneously decreased both acid secretion and aminopyrine clearance, indicating a reduction in blood flow (279). This is in contrast to the findings of Bond and Levitt (50), where mucosal blood flow was increased by glucagon. It is thus possible that the tone of the small vessels may determine the response to glucagon. Thus, in the constricted vascular smooth muscle, glucagon relaxed the vessels, whereas in the fully dilated vessels, glucagon constricted the vessels.

B. Pulmonary Circulation

There have been conflicting reports concerning the action of glucagon on the pulmonary circulation and either no change, an increase, or a decrease in pulmonary arterial pressure has been reported (106, 116, 335, 464). However, in the abnormal pulmonary circulation as seen in newborn calves, glucagon lowered the high pulmonary arterial pressure and pulmonary vascular resistance (230). In calves with hypoxic pulmonary vasoconstriction, glucagon induced a rapid reduction in pulmonary arterial pressure and resistance (41). It is likely that the positive inotropic effect of glucagon reduced left auricular pressure in the diseased animal and thus decreased pulmonary arterial pressure.

In human heart failure, Murtagh et al. (335) also observed a reduction of pulmonary arterial pressure after glucagon injections; however, Diamond et al. (106) could not confirm this. It is our experience that in the HLP of the dog, glucagon will increase pulmonary arterial pressure and cardiac output when the basal pulmonary arterial pressure and left auricular pressure are near normal levels. In the heart failure preparation where both left auricular and pulmonary arterial pressures were high, glucagon reduced both these pressures significantly. Since reflex changes cannot occur in the preparation, it is most likely that the marked improvement in cardiac contractility and reduction in left auricular pressure produced by glucagon were important factors in the reduction of pulmonary arterial pressure. Similar observations have been made by Turnheim and Kraupp (494) with several catecholamines and related substances.

C. Action of Glucagon on Vascular Smooth Muscle

As discussed previously, glucagon reduced vascular resistance in several organs, including the splanchnic, hepatic, and renal beds. A limited number of studies have determined the effects of glucagon on isolated vascular smooth muscle. Gagnon et al. (156) determined the effects of glucagon on rat and rabbit aortic strips, rabbit renal artery, and rabbit anterior mesenteric vein and inferior vena cava. Of these preparations, only the rat aorta and rabbit renal arterial strips responded to glucagon by relaxation of norepinephrine contracted isolated vessels. These investigators have shown that the rabbit renal arterial strips contracted by means of norepinephrine could be relaxed by isoprenaline, histamine, adenosine, adenosine mono-, di-, and triphosphate, cAMP, and glucagon, while cyclic guanosine monophosphate (cGMP) had no effect on this isolated strip. The glucagon-induced relaxation of the renal artery was not blocked by H_1-antihistaminics or alpha- and beta-adrenergic blockers, while the phosphodiesterase inhibitors, papaverine and aminophylline, potentiated these relaxant effects of glucagon. A low concentration of indomethacin (5 μg/ml) had some potentiating action on the glucagon-induced relaxation. In a second paper, Gagnon et al. (157) have studied the effects of four phosphodiesterase inhibitors, all of which potentiated the relaxing effects of glucagon on renal artery strips. Of these phosphodiesterase inhibitors, 3-isobutylmethylxanthine (IBMX) produced the greatest potentiation. cAMP relaxation was potentiated by papaverine and indomethacin and was inhibited by theophylline and IBMX. The calcium antagonist, verapamil, inhibited the relaxer ef-

fects of both glucagon and cAMP. These relaxer effects of verapamil could be counteracted by increasing the concentration of calcium ion in the perfusion fluid, whereas those of glucagon and cAMP were not affected by variations in the extracellular Ca^{++} concentration. These results suggested that glucagon produced its vasodilation by blocking Ca^{++} extrusion from the smooth muscle cell, or increased calcium sequestration intracellularly, possibly by a cAMP-dependent mechanism.

The role of cAMP in the relaxant effects of glucagon on vascular smooth muscle has been postulated (156, 157). Agents that presumably change vascular tone via a change in the intracellular cAMP content should fulfill the following criteria set forth by Sutherland et al. (474): 1) It should have an effect on adenylate cyclase and/or phosphodiesterase activity of the vascular tissue. 2) It should change the cAMP content of vascular tissue with a time course consistent with a triggering action for the tissue response. 3) Its effects on the tissue should be potentiated by drugs that inhibit tissue phosphodiesterase. 4) The effects should be mimicked by the addition of cAMP or derivatives of cAMP. Of all these conditions, the available evidence with glucagon supports criteria 3 and 4. No data concerning the effects of glucagon on cAMP content, or activation of adenylate cyclase, or phosphodiesterase in smooth muscle have been reported. The data of Gagnon et al. (156, 157) thus only suggest that glucagon may relax smooth muscle via cAMP production and its action is different from the relaxation produced by the calcium blockers.

The role of cyclic nucleotides in smooth muscle relaxation due to adrenergic beta-stimulation is reasonably well established (337, 338). Acetylcholine, the ionophore A23187, adenosine tri- and diphosphate, thrombin, and arachidonic acid depend, at least for a part of their vascular muscle-relaxing effects, on the presence of a functional endothelium (102, 154, 155). This cell type, which can be maintained in culture, has a hormonally sensitive cyclic nucleotide system (66). The relation of glucagon-induced relaxation of vascular smooth muscle to endothelial effects of glucagon has not been determined.

D. Glucagon and Kidney Function

The early observations by Staub et al. (450) that relatively large doses of glucagon increased renal sodium, potassium chloride, iodate, and phosphate excretion, have been confirmed in several species, including man. [For a review, see Katz and Lindheimer (216).]

Elrick et al. (121), Dalle et al. (98), Birge and Avioli (38), and Johannesen et al. (211) concluded from experiments on humans that glucagon had a direct effect on tubular electrolyte reabsorption since electrolyte excretion could not be consistently related to an increase in glomerular filtration rate, urine flow, or increase in plasma glucose concentration or excretion. In support of a tubular action of glucagon, Pullman et al. (384) observed that glucagon, when injected into a renal artery of a dog, produced a bilateral increase in glomerular filtration rate (GFR) and filtration fraction (FF), but produced a unilateral increase in ion excretion (Na, Cl, Ca, Mg) (table 1). The differential effects of glucagon on the infused kidney were most marked on sodium excretion and less so on the excretion of the other ions studied. Potassium excretion was not significantly different in the infused or control kidney. Based on these observations, Pullman et al. (384) concluded that glucagon exerted a direct effect on tubular reabsorption of sodium, chloride, and other ions (table 1). However, Stowe and Hook (460) have claimed that either intravenous or intrarenal infusion of glucagon increased renal blood flow without increasing glomerular filtration. Manitol diuresis or reserpine pretreatment increased renal blood flow and reduced the response to glucagon. These investigators suggested that most of the effects of glucagon on ion transport were due to the renal vasodilator effects of glucagon. However, they were careful to point out that these findings did not rule out a direct effect of glucagon on renal sodium transport.

Similar experiments conducted by Levy (273) and Levy and Starr (275a) have shown that glucagon caused an increase in glomerular filtration rate and sodium load which could explain the observed natriuresis. Renal denervation, thyroparathyroidectomy, cholinergic and gamma- and beta-adrenergic blockade, as well as dopaminergic and histaminergic blockade did not prevent glucagon diuresis. These effects of glucagon on GFR and sodium excretion were observed in chronic caval dogs with ascites (274, 275a) and in several experimental preparations in which renal blood flow had been curtailed. These investigators concluded that glucagon natriuresis is determined largely by the increased GFR and concomitant increase in sodium load; however, a direct tubular effect of glucagon could not be ruled out by these data. Levy (273) injected secretin, a peptide related to glucagon, which had no effect on kidney hemodynamics or electrolyte excretion, nor did it affect the glucagon-induced increase in the GFR, but partially inhibited the glucagon-induced natriuresis, possibly by preventing the direct renal tubular effects of glucagon. Levy and Starr (275a) reported that glucagon had no effect on the tubular fluid-plasma insulin ratio of fluid collected from the proximal tubule by micropuncture technique, thus indicating that glucagon did not act on the proximal tubule. Ueda et al. (495) have determined the effects of glucagon on GFR and have concluded that this increase was due to a selective dilation of the afferent arteriole, thus increasing filtration pressure in the glomeruli without having to invoke changes in glomerular permeability. By using radiological techniques, Danford (99) observed that glucagon caused a vasodilation of the renal arterial bed with improved visualization of the small vessels and an increase in renal blood flow. Direct dilator effects of glucagon on isolated renal arteries have been reported by

Gagnon et al. (157), and all these data make it likely that glucagon in relatively large doses produced a dilation of the renal vascular tree with a concomitant increase in blood flow and glomerular filtration and an increase in ion excretion. However, the data of Birge and Avioli (38), Dalle et al. (98), Elrick et al. (121), and Pullman et al. (384) suggest that besides the vascular effects, glucagon has a direct action on tubular electrolyte excretion.

Recent observations by Kirschenbaum and Zawada (229) have confirmed and extended the results of Pullman et al. (384). They have shown that the infusion of 0.2 μg of glucagon per minute into the renal artery did not increase GFR, but induced a natriuretic effect. This effect on sodium excretion could be blocked by a prostaglandin synthetase inhibitor. A larger dose of glucagon (1 μg/min), when infused into the renal artery, increased both GFR and sodium excretion, and indomethacin blocked the effects on sodium excretion, but did not modify the increase in GFR. Olsen (353) observed that indomethacin had no influence on the general blood pressure, heart rate, and renal blood flow effects of glucagon, but reduced the glucagon-induced sodium, potassium, chloride, and water excretion by the dog kidney. It is thus likely that the alleged renal tubular effects of glucagon are mediated by the production of a prostaglandin which increased urinary sodium potassium and chloride excretion. Prostaglandins (PGE, PGA and PGI_2), when injected into the renal arteries of dogs, increased renal blood flow and water, sodium, and potassium excretion with minimal changes in glomerular filtration (188, 309, 310, 525).

Direct renal tubular effects of prostaglandins have been demonstrated, and the interaction of prostaglandins with the antidiuretic hormone has been documented. Vasopressin stimulated the production of prostaglandins in the renal medulla (29, 215), and prostaglandins inhibited the vasopressin effects on water reabsorption in the isolated toad bladder (356) and in the rat and man (34, 228, 288, 457). Inhibition of prostaglandin synthesis enhanced the effects of the antidiuretic hormone (34, 228, 288). [For reviews, see Morel et al. (328) and Ausiello and Orloff (16).]

Glucagon can stimulate the formation of cAMP in renal tissue. Thus, Marcus and Aurbach (299), Melson et al. (313), and Popovtzer and Wald (379) have demonstrated the increased cAMP production by glucagon in isolated renal cortical tubules and slices of the rat, and Mulvehill et al. (331) and Kim et al. (225) have demonstrated similar effects of glucagon in human renal tissue. Bailly et al. (21) isolated segments of renal tubules from rats and determined the glucagon-sensitive adenylate cyclase activity in portions of the renal tubules. The most sensitive portions of the tubule were the medullary and cortical ascending thick limbs of Henle followed by the early distal convoluted tubules, the cortical collecting tubules, and the medullary collecting tubules. The proximal tubules and the thin segments of the loop did not respond to glucagon. cAMP has been demonstrated in isolated glomeruli, but acute effects of glucagon on cAMP production could not be seen, although histamine and serotonin markedly elevated the cAMP content of glomeruli (111). It has been suggested that these latter two substances can contribute to the inflammatory reaction in kidney tissue and thus could participate in the process of glomerular injury (111).

All of the above findings suggest that in sufficiently large doses glucagon had an effect on the renal vasculature, as well as an effect on the renal tubular membranes. However, it is unlikely that glucagon plays a significant role on renal electrolyte excretion under normal conditions since Forrest et al. (144) and Sherwin et al. (431) have not been able to demonstrate a significant change in renal electrolyte water excretion when plasma-glucagon levels were increased within physiological limits.

1. Diabetes. In both experimental and human diabetes the end results of diabetes are frequently severe impairment of kidney function. In the earlier stages, increased GFR and renal plasma flow (RPF) are observed and later stages are characterized by intercapillary glomerulosclerosis in which modular eosinophilic deposits are formed within the glomerular tufts.

In diabetics without ketoacidosis receiving insulin, the infusion of small amounts of glucagon increased the GFR, RPF, and glucagon plasma concentration to values observed in poorly controlled diabetics (109a, 365). These investigators have shown that glucagon induced a dilation of the glomerular arterioles, thus increasing GFR. Glucagon caused a more marked increase in RPF and GFR in diabetics than in nondiabetics (364). This is possibly caused by the abnormal glucagon:insulin ratio since insulin administration readily corrected this ratio and reduced both GFR and RPF to approximately normal levels.

In chronic insulin-dependent diabetes, the glomeruli of the kidneys are hypertrophied and show an increase in the thickness of basement membranes, in the volume of capillary tufts and the lumen of the capillaries (358, 359). A similar increase in glomerular size has been demonstrated in streptozotocin-induced diabetes in rats (177, 427). Concomitant with these anatomical changes, the increased glomerular filtration rate has been observed in human diabetics (323, 324, 325, 364, 365) and experimentally induced diabetes (71).

The major biochemical changes observed in diabetic glomeruli were an increased RNA content and rate of synthesis in the renal cortex, as well as an increased rate of incorporation of orotate (92, 94). These changes are similar to those seen following renal hypertrophy (92, 427), and these biochemical changes in diabetic rat kidneys are exaggerated following unilateral nephrectomy and the resultant hypertrophy of the remaining kidney (92). It is known that unilateral nephrectomy accelerates the diabetic glomerular changes in diabetic rats (451) and possibly also in man (110, 307).

Control of the diabetes with insulin will correct or prevent the renal and glomerular hypertrophy and the increase in glomerular filtration rate seen in insulin-dependent human diabetics (324) and will also reverse the metabolic changes in the renal cortex of diabetic rats (91, 426). Cortes et al. (91) determined the incorporation of orotate into isolated glomeruli and whole renal cortex in streptozotocin-induced diabetes in rats. In these rats, glomerular RNA was increased, and the rate of orotate incorporation into RNA and total nucleotides was increased as compared with control subjects. Insulin infusions that did not influence the plasma glucose concentration, but reduced the hyperglucagonemia, reduced the increase in RNA and orotate incorporation, and addition of glucagon to the insulin infusion increased glomerular RNA and orotate incorporation. The incorporation of orotate into glomeruli showed a positive correlation with plasma glucagon concentration, but not with plasma glucose or insulin concentration. A similar correlation of plasma glucagon with the RNA content and orotate metabolism was also demonstrated in cortical renal tissue. When labelled adenine was infused into control and diabetic rats, no difference in the rate of incorporation of adenine could be demonstrated.

The negative results obtained with adenine incorporation when glomerular RNA was increased show that the rate of RNA synthesis and adenine incorporation do not correlate. It is possible that the increased RNA content of glomeruli could be due to a decreased rate of catabolism of this RNA. The changes seen in compensatory hypertrophy of the kidney and the renal changes in diabetes are similar (93, 427, 489). This observation suggests that the glomerular changes observed in glomeruli of diabetic animals are an early manifestation of glomerular hypertrophy.

Relatively small amounts of insulin that had no effect on plasma glucose reversed glomerular filtration and the biochemical glomerular changes and the hyperglucagonemia of diabetic animals. Plasma glucose and insulin concentration did not, while plasma glucagon concentration correlated with the changes in orotate incorporation into glomerular RNA. As previously discussed, patients and rats with uncontrolled diabetes show an increase in glomerular filtration, which is possibly related to the hyperglucagonemia observed in these diabetic animals. Chronic orotate administration produced an increase in uridine triphosphate in the normal kidney (92, 94, 113), and glucagon increased this synthesis (252). Cortes et al. (91) propose that hyperglucagonemia, independently of insulin and glucose plasma concentrations, may be a factor in the development of the diabetic glomerular basement changes. This effect of glucagon could be mediated by its effects on uracil nucleotide metabolism, rather than on glomerular cAMP, especially so since acute effects of glucagon do not increase glomerular cAMP (111).

2. *Natriuresis of Fasting.* This was first described by Benedict in 1915 (32) and Gamble et al. in 1923 (160) and is another condition in which glucagon has been implicated as the causative agent of increased sodium and potassium excretion. During complete starvation of obese patients, the electrolyte loss reached its peak on the third and fourth fasting days and reverted gradually to control levels on days 7 to 10 (46, 47, 443, 504). Refeeding with glucose during the height of the starvation diuresis promptly reverted the natriuresis to an antidiuresis (46, 162, 193). In the study of Veverbrants and Arky (504), a constant sodium intake was instituted, and the results showed a parallel sodium and potassium, but not a water diuresis during the fasting period. When carbohydrate was given after fasting, it markedly reduced sodium and potassium loss. Fat refeeding, on the other hand, enhanced the sodium excretion, while protein refeeding delayed the onset of the antinatriuresis. [For reviews, see Kolanowski (237–239).]

The relation of glucagon to this starvation natriuresis was suggested by the parallelism of the sodium loss to the increase in the plasma-glucagon concentration (242, 417, 442). During starvation, Unger et al. (496) observed an increase in the plasma concentration of glucagon and a reduction in the insulin concentration. These observations have been confirmed and extended (5, 300, 417), and a good correlation between glucagon concentration and sodium loss, both during the starvation and refeeding periods, could be established. However, simultaneously with the increased glucagon concentration a host of other changes occur during starvation, thus insulin concentration changes, ketonemia and ketonuria become prominent, and ammonia excretion is increased during the phase when natriuresis is suppressed (433); glucose refeeding, as well as glucagon administration, has effects on ketone body formation and excretion which could thus affect urinary electrolyte excretion.

The effects of fasting on the natriuresis was not related to aldosterone secretion since during the fast when sodium excretion was increased, aldosterone secretion rate was increased, while during the period when sodium excretion was declining, aldosterone secretion rate was reduced (442). Plasma renin activity during the fast decreased, and, as the fast continued, plasma renin activity increased. During carbohydrate refeeding, while aldosterone secretion was decreasing, plasma renin activity increased. Thus, during fasting and refeeding, aldosterone and renin activity were dissociated (55). Furthermore, Gersing and Bloom (162) have shown that the aldosterone blocking agent spironolactone increased the saluretic effect of starvation and had no effect on the antisaluresis produced by glucose refeeding. However, Boulter et al. (55) were unable to confirm this, and in their experiments spironolactone administration prevented the glucose refeeding antinatriuresis. They concluded that mineralocorticoid activity, especially in the proximal and distal tubular apparatus, probably plays an important role in starvation natriuresis and the glucose

refeeding phenomenon. Further studies have shown that during starvation the renal tubules are refractory to mineralocorticoids (349, 442), and glucagon administration could produce a condition of refractoriness to mineralocorticosteroids (349). These workers concluded that since glucagon had this antimineralocorticoid effect, glucagon was the causative agent for starvation diuresis. However, Kolanowski et al. (241) and Kolanowski (237) have shown that the renal sensitivity of aldosterone in the fasted state is intact and, contrary to expectations from a blockade of tubular mineralocorticoid effects, glucagon actually increases potassium excretion as reported by many investigators and reviewed by Kolanowski (238–240). Furthermore, since glucagon is a diuretic it could overcome the mineralocorticoid sodium retention by a mechanism unrelated to mineralocorticoid activity. Other diuretics, such as mercurials, can overcome the mineralocorticoid-induced sodium retention (134). It is thus less likely that starvation diuresis is caused by a desensitization of the renal tubule to aldosterone.

A probable explanation for starvation natriuresis has been proposed by Sigler (433), who has tested the hypothesis that starvation ntriuresis is due to the metabolically generated anions (keto acids). He observed that ammonium loss lagged behind the increased organic acid and phosphate excretion and thus had to be covered by increased cation excretion. Sodium loss was considerable and exceeded chloride loss. During glucose refeeding, sodium excretion fell and seemed to correlate with the reduced organic acid anion excretion and a lag in the reduction of ammonium ion excretion. It is of interest that simultaneous glucose refeeding and glucagon administration during starvation did not prevent the expected reduction in ketonemia, but blocked the reduction of ketone bodies and sodium excretion in the urine (240, 241), which should have followed glucose administration. These data of Sigler support the hypothesis that the organic anion generation and excretion are the major causes of starvation cation loss.

A relation of glucagon to the organic anion excretion has been suggested by Kolanowski et al. (241) and Kolanowski (240). Infusion of glucagon during an episode of starvation did not change the plasma concentration of ketone bodies, but enhanced the ketonuria (12, 300). Since glomerular filtration was unchanged, it was postulated that in ketogenic states glucagon could increase ketone body excretion by reducing the tubular reabsorption of these ketoacids, thus explaining the effects of glucagon during starvation and refeeding.

3. Renin Secretion. Renin secretion due to glucagon was observed in rat isolated perfused kidneys (501) and in man (140). In dogs, Olsen (353) and Holdaas et al. (196) could only demonstrate a small or inconsistent response of plasma renin activity following glucagon administration. Indomethacin, which blocked the glucagon-induced natriuresis, did not change in a consistent way glucagon-induced plasma renin activity (353). However, Ueda et al. (495) have shown that in anesthetized dogs glucagon in a dose of 1 μg/kg/min increased plasma renin activity and increased renin secretion rate, which was potentiated by pretreatment with theophylline and was not blocked by propranalol. Since glucagon can increase cAMP production, it has been suggested that cAMP is responsible for renin effects of glucagon. Renin release can be stimulated by cAMP and dibuteryl cAMP, both in vitro and in vivo (351, 524). Thus, the effectiveness of cAMP and the potentiation by a phosphodiesterase inhibitor of the effects of glucagon on renin release suggest that cAMP production in juxtaglomerular region is the cause of renin release. Indomethacin did not affect this release, and it is thus probable that renin release was not mediated via prostaglandin production.

4. Effect of Glucagon on Renal Ion Excretion. A. POTASSIUM. After glucagon administration, there is a transient increase in the plasma K^+ concentration followed by a prolonged hypokalemia (119, 159).

The hyperkalemia is not due entirely to the hepatic glycogenolysis since the hyperkalemia precedes the increase in hepatic vein glucose concentration (287). Ellis and Becket (118) have shown that in cats, where liver glycogen had been depleted by means of phloridzin, a hyperkalemic response can be elicited with both glucagon and epinephrine. Furthermore, with repeated doses of glucagon, the glucose response was blunted, while the plasma K^+ increase was unchanged. Thus, it is likely that glucagon-induced hyperkalemia is independent of glycogenolysis. Preceding the release of glucose from the liver, there was an increase in the hepatic vein potassium concentration, and it is likely that this potassium was derived from the liver (95, 439). This increase was soon superseded by a decrease in the plasma K^+ concentration, which was probably due to the uptake of K^+ by a variety of tissues, including smooth and striated muscle, and was related to the increase in insulin release produced by glucagon. In diabetics, glucagon produced a greater hyperpotassemia than in normal subjects, possibly because of the lack of insulin response to glucagon (101, 305). It is thus likely that the hyperkalemia is due to glucagon, while the hypokalemia is due to insulin-induced uptake of K^+ by various tissues.

It is unlikely that the hypotassemia is related to the kaluresis following glucagon injections, since the amount lost in the urine cannot explain the degree of hypopotassemia observed.

B. CALCIUM AND PHOSPHORUS EXCRETION. Glucagon injections increased both calcium and phosphate ion excretion (18, 38, 121, 384, 450), and this was associated with a decrease in the plasma calcium and potassium concentration. The reductions in plasma phosphate concentrations were attributed to increased phosphate excretion and utilization for glycogen breakdown (104), or to the increased peripheral utilization of glucose caused by the glucagon-induced insulin secretion (51). However,

as DeVenanzi (104) has shown, glucagon can cause a hypophosphatemia in the absence of hyperglycemia and insulin release, and the total amount of calcium and phosphorous excreted in the urine could not explain the degree of hypophosphatemia and hypocalcemia. The observation by Avioli et al. (18) that nephrectomy did not prevent the glucagon-induced hypocalcemia suggested that glucagon acts on bone by decreasing the rate of calcium and phosphate release similar to the effects produced by calcitonin. Since total thyroidectomy in the dog (18) and rabbit (370) prevented the glucagon-induced reduction in plasma calcium and phosphate, it was suggested that glucagon caused the release of calcitonin from the thyroid gland. Similar studies in rats indicated that in this species, glucagon effects on calcium were not blocked by thyroidectomy, but were inhibited when both the thyroid and thymus glands were removed (24). Since aberrant thyroid tissue can be found in rat thymus glands (15), it is reasonable to conclude that the hypocalcemic effects of glucagon are dependent on the presence of the thyroid gland.

Stern and Bell (455) and Care et al. (70) have demonstrated the release of calcitonin when thyroid slices were exposed to relatively low concentrations of glucagon (5 ng/ml). The relation between calcitonin release and cAMP has been demonstrated by Care et al. (70), Avioli (17), and Bell (31). These data suggest that glucagon, by activating the cyclase system, caused the release of calcitonin, which, in turn by its action on bone, caused the decrease in the plasma phosphate and calcium concentration. However, a direct effect of glucagon on bone has not been excluded since Stern and Bell (455) have shown that glucagon inhibits bone resorption when stimulated by parathyroid hormone or cAMP.

Glucagon increased renal phosphate and calcium excretion, and this effect has been observed when glucagon was given either intravenously or intraarterially. When given into the renal artery, glucagon caused an increase in phosphate excretion on the infused side with lesser effects on the opposite kidney. These findings suggest that the changes in phosphate excretion are due to an effect of glucagon on tubular phosphate reabsorption since glomerular filtration changes are most likely not operative under the conditions of these experiments.

All these findings indicate that glucagon can affect bone metabolism as well as renal excretion of calcium and phosphate. However, it is questionable that these glucagon effects have physiological implications since the concentrations needed to produce these changes far exceeded the physiological or pathological concentrations observed in intact animals [see Saudek et al. (417) and Sherwin et al. (431)].

C. ZINC EXCRETION. Urinary excretion of zinc is usually low in normal man, but is markedly increased in diabetes mellitus (371), starvation (443), and trauma (14). All these conditions have in common an increase in the plasma-glucagon concentration relative to insulin. Victory et al. (505) have shown that glucagon infusions at a rate of 5 ng/kg/min significantly increased urinary zinc excretion. The changes in zinc excretion correlated with the changes in glomerular filtration rate, although direct tubular effects of glucagon on zinc reabsorption and secretion cannot be excluded.

Direct and indirect renal effects of glucagon on electrolyte excretion and hemodynamics have to be considered. The effects of glucagon on renal blood flow may be related to an increased cAMP concentration in vascular smooth muscle of the renal arterial bed. The increased glomerular filtration rate observed was probably a result of the increase in blood flow, although a dilation of the afferent glomerular capillaries has been postulated. The increased glomerular filtration rate, hypertrophy, and thickening of the glomerular basement membranes seen in diabetes and renal hypertrophy are possibly related to an increased plasma glucagon:insulin ratio. These effects are not related to cAMP, but may be due to the internalization of glucagon and binding to a nuclear receptor site which affects RNA and protein synthesis.

The renal tubular effects of glucagon are caused by an increase in glomerular filtration rate and by an effect on the tubular reabsorption of electrolytes. The increase in sodium, chloride, and potassium excretion may be related to the production of cAMP in the thick limb of Henle and the distal tubules. These cAMP effects on renal electrolyte excretion are probably mediated by prostaglandins. Renin excretion was increased by glucagon, and it is postulated that cAMP is the mediator of this reaction.

Glucagon hyperkalemia is a transitory process since insulin release will counteract this effect and produce a hypokalemia. The kaluresis produced by glucagon does not explain the extent of hypokalemia, and a major factor here is the insulin-induced K^+ uptake by muscle and liver. Glucagon plays a role in starvation diuresis and is probably related to a decrease in the tubular reabsorption of keto-acids produced during starvation. The excess anion excretion caused by glucagon will thus increase sodium and potassium excretion.

III. Effect of Glucagon on Mechanical and Electrophysiological Properties of the Heart

A. Effects on Rate and Contractility of the Heart

Glucagon increased the rate and the contractile force of the heart in several species. In the dog HLP, the effects of glucagon were dependent on the state of the heart. In the nonfailing heart, glucagon produced an increase in heart rate with minimal changes in cardiac output and auricular pressure. In the failing heart, an increase in heart rate was accompanied by a marked increase in cardiac output and a reduction in the cardiac size (136). Furthermore, glucagon improved the relation between auricular pressure and cardiac output and improved the sufficiency index. The minimal effective concentration of glucagon was about 5×10^{-9} M, and such

a concentration was about as effective as a similar concentration of epinephrine. The effects of glucagon on rate and contractility of the isolated and the intact heart have been reported by Boder and Johnson (49), Glick et al. (166), Lucchesi (284), Matsuura et al. (306), Regan et al., (391), and Whitehouse and James (510). Moura and Simpkins (330) observed an increase in rate and contractility, as well as an increase in cAMP in cultured heart cells.

In the isolated auricular and ventricular muscle of dog, rat, and cat, 10^{-8} M glucagon produced minimal effects, while 10^{-6} M produced maximal effects on both heart rate and contractile force. Since rate changes can influence contractile force, Marsiglia et al. (302) kept the heart rate constant and still observed the increased contractile force. Similar findings were reported by Simaan and Fawaz (435), who reduced the rate effects of glucagon with veratramine without significantly changing the effect on cardiac contractility. As would be expected, glucagon increased the dp/dt, both in the dog (166) and rat heart (291).

Time to peak tension and relaxation time are both decreased by catecholamines, and this effect has been related to cAMP production and its effects on calcium entry and release and the increase in the rate of calcium sequestration by the endoplasmic reticulum (217, 311, 395, 396). Greeff (173) and MacLeod et al. (291) observed a reduction of time to peak tension with glucagon, whereas Glick et al. (166) and Spilker (445) did not observe this decrease. Gaide et al. (158) and Marcus et al. (298) have shown that glucagon alone did not significantly change time to peak or relaxation time, but did so in the presence of a phosphodiesterase inhibitor, which also potentiated the contractile force.

In our experience, the basal rate of stimulation, as well as the degree of increase in contractile force, are factors contributing to the effect on the time to peak and relaxation time. With slow basal heart rates (15/min) and increases of contractility exceeding 50%, this effect on time to peak tension is seen with glucagon; with high basal rates (120/min), this effect becomes more difficult to observe even though contractility may increase more than 50%.

Farah and Tuttle (136) did not observe the effects of glucagon in the intact dog. However, Fricke et al. (148), Glick et al. (166), Hammer et al. (178), Lucchesi et al. (286), Regan et al. (391), and Whitehouse and James (510) observed in intact dogs that glucagon in a dose of 0.5 to 16 mg/kg increased heart rate and contractile force and reduced ventricular and diastolic pressure and increased dp/dt in the ventricle. This effect of glucagon lasted about 25 to 35 minutes, and repeated doses of glucagon did not show a decreased response.

Dose response relations have been published by Chiba (76) and Kimura et al. (226) in isolated cardiac tissue and by Lydtin et al. (290), Smitherman et al. (437), and Tarnow et al. (484) in human subjects and anesthetized dogs. In the isolated tissue, the maximal increase in heart rate per incremental increase in contractile force was greater for glucagon than norepinephrine (76). Both heart rate and contractile changes tended to decline with time in the isolated heart preparation, and the further addition of glucagon did not affect this decline. The rate of decline was dose-dependent and was greatest with the higher doses (10^{-6} M, unpublished). This is probably a manifestation of desensitization and will be discussed later. In intact dogs, the heart rate increase lasted 15 to 30 minutes, and repeated doses produced the same effect (284). Tarnow et al. (484) injected 10, 20, 40, and 80 μg/kg of glucagon, and some of their results are summarized in tables 2 and 3. It is clear that all parameters determined increased with the increase in dosage of glucagon.

In man, Kones and Phillips (243), Lydtin et al. (290), and Smitherman et al. (437) have determined a dose-related effect of glucagon on heart rate, ejection fraction, systolic volumes, and plasma glucose and insulin concentrations. The doses of glucagon required to produce hemodynamic effects produced glucagon-plasma concentration, which would not be observed either under normal or pathological conditions. Thus, it is unlikely that glucagon plays any significant physiological or pathological role on the control of cardiac function.

B. Effect of Glucagon on Electrical Properties of the Heart

When glucagon (0.5 to 30 μg/ml) was added to isolated Purkinje fibers, no change in the resting potential was observed. However, in spontaneously beating fibers, glucagon increased the rate of discharge (383, 398). The rate of rise of the fast sodium potential, expressed as maximum rate of change of zero-phase action potential (dv/dt), was unchanged (383, 456). In human cardiac tissue obtained from patients during surgical procedures, Prasad (380) observed that glucagon increased conduction velocity and membrane responsiveness and increased the rate of rise of the Zero-phase of the action potential, but had no effect on the refractory period, the duration, or the amplitude of the action potential. Stewart et al. (456) did not observe any changes with glucagon in normal canine Purkinje tissue; however, in quinidine-treated tissue where dv/dt was reduced and the refractory period and the action potential were prolonged, glucagon shortened the action potential and refractory period, returned dv/dt toward normal levels, and thus improved conduction. Edmands et al. (117), Pruett et al. (383), Spilker (445), and Stewart et al. (456) observed no significant change in the duration or amplitude of the action potential; however, Spilker (445) described a slight prolongation of the action potential of calf Purkinje fibers, while Reynold et al. (398) observed a decrease in phases II and III of rat atrial and ventricular septal tissue after addition of glucagon. Species differences and the various sources of cardiac tissue, as well as the state of this tissue, probably explain the divergent results reported in

TABLE 2

*Effect of glucagon (80 μg/kg) on the hemodynamics of the anesthetized dog (average values taken from nine dogs weighing 29 to 35 kg; anesthesia used was halothane-nitrous oxide)**

Time (min)	Heart rate (beats/min)	Cardiac output (ml/min/kg)	dp/dt (mm Hg/sec)	Left ventricular end Diastolic Pressure (mm Hg)	Stroke volume (ml/kg)	Blood Pressure (mm Hg)	Peripheral resistance, mm Hg (ml/kg/min)	Coronary blood flow (ml/100 gm/min)	Oxygen consumption (ml/100 gm/min)
Control	80 ± 4	88 ± 5	2119 ± 224	7.9 ± 0.9	1.12 ± 0.1	107 ± 5	1.19 ± 0.08	88 ± 8	11.3 ± 1
1	109 ± 8†	131 ± 10†	2935 ± 239†	6.9 ± 0.8†	1.24 ± 0.13	102 ± 7	0.77 ± 0.06†	131 ± 9	14.3 ± 1.7
5	121 ± 10†	126 ± 11†	2910 ± 205‡	5.6 ± 0.7†	1.08 ± 0.12	103 ± 7	0.822 ± 0.07†	129 ± 11	16.9 ± 1.6
10	117 ± 10†	111 ± 9†	2544 ± 181†	5.6 ± 0.8†	1.0 ± 0.13	98 ± 6†	0.88 ± 0.07†	119 ± 12	15.7 ± 1.7
20	98 ± 6†	97 ± 6†	2219 ± 241†	6.8 ± 1.0†	1.03 ± 0.12	101 ± 4	1.02 ± 0.05†	103 ± 10	13.2 ± 1.5

* Data from V.J. Tarnow et al., Arzneim.-Forsch. **25**: 1906–1910, 1975.
† *P* value less than 0.05.
‡ *P* value less than 0.01.

TABLE 3

*Effect of glucagon on maximal hemodynamic changes observed in nine anesthetized dogs**

Maximal change	Glucagon (μg/kg)			
	10	20	40	80
Heart rate/min	+11	+13	+24	+41
Arterial pressure, mm Hg	−4	−3	−5	−9
Cardiac output, ml/min/kg	+18	+22	+34	+43
Stroke volume, ml/kg	+0.06	+0.14	+0.13	+0.12
Left ventricular end diastolic pressure, mm Hg	−0.5	−1.1	−2.1	−2.3
dp/dt, mm Hg/sec	+244	+371	+542	+816
Coronary flow, ml/100 gm/min	+16	+20	+29	+43
Mv O_2, ml/100 gm/min	+1.4	+2.1	+3.6	+5.6

* V.J. Tarnow et al., Arnzneim.-Forsch, **25**: 1906–1910, 1975.

the literature. It is likely that in tissue where the action potential has been depressed, either by an antiarrhythmic agent (456) or by disease (380), glucagon can show effects on the various phases of the action potential and can restore some of these toward normal values. Prasad (381) and Prasad and Weckworth (382) reversed quinidine- and procainamide-induced cardiac toxicity and arrhythmias with glucagon. However, here the increased heart rate may have played a role in overcoming the drug-induced arrhythmia.

In depolarized Purkinje fibers (22 mM K^+), glucagon could not initiate the slow current, nor did it change the slow action potential initiated by a catecholamine (444). In the intact heart where sinus rate and atrioventricular conduction were partially blocked by a calcium blocker (verapamil or D600), glucagon or calcium ion could not reverse these effects, while a catecholamine readily restored the rate and atrioventricular conduction (526). It is thus possible that glucagon may not act via the slow calcium channels. Table 4 depicts the effects of glucagon (kindly supplied by Eli Lilly and Company) on dog papillary muscle and trabecular tissue. Crystalline glucagon in a concentration of 5×10^{-6} M increased the overshoot and phase 2 of the action potential and had no significant effects on the refractory period, the maximal rate of rise of the Zero-potential (dv/dt), or the resting potential. In the presence of a 20 mM K^+, the depolarized muscle did not respond to glucagon (2.5×10^{-6} M; 1×10^{-5} M), although these muscles responded well to the addition of epinephrine. When the action potential was depressed by the antiarrhythmic drug Norpace, glucagon in a concentration 5×10^{-6} M increased the overshoot and phase 2 of the action potential, but had no effect on the resting potential dv/dt, total duration of the action potential, or the refractory period (unpublished).

Whitsitt and Lucchesi (511) observed that glucagon could antagonize the propranalol-induced decrease in atrioventricular conduction velocity, and Iijima et al. (206) and Steiner et al. (453) recorded His bundle action potential and noted that glucagon reduced the time interval between impulse artifact and response. In several conditions in which auriculoventricular heart block was produced, glucagon improved auriculoventricular conduction. Thus, when the auricles were driven at a high rate, a second degree of heart block could be observed that was improved when glucagon was administered (256, 281, 453).

Atrioventricular pacemaker activity can be started either by crushing or by inhibiting the sinus node by means of the injection of a cholinergic agent. Lucchesi et al. (286) and Urthaler et al. (500) have shown that glucagon will increase the rate of discharge of the atrioventricular node, and this effect of glucagon was not blocked by propranalol (286).

Idioventricular rhythm was produced either by vagal stimulation or injection of formalin into the atrioventricular node. Vagal stimulation, which produced an inhibition in the sinus and atrioventricular node, after a time will show the ventricular escape phenomenon, which is due to an idioventricular rhythm. Steiner et al. (453) were unable to show an effect of glucagon on the vagal escape time. However, experiments of Wilkerson et al. (516) have shown that glucagon can increase the idioventricular rhythm and reduce the escape time if one takes into account the rate dependent suppression of this rhythm.

TABLE 4

Effect of glucagon on intracellular potential of dog papillary muscle and trabeculae; average values from seven preparations

	Glucagon concentration			
	Control	1 μg/ml	5 μg/ml	10 μg/ml
Resting potential, mv	83.4 ± 1.6	84.1 ± 1.0	83.8 ± .9	85.0 ± 1.4
Action potential amplitude, mv	103.4 ± 2.0	108 ± 1.6	107 ± 1.4	110 ± 2.1
V_{max} (dv/dt)	274 ± 11	270 ± 18	263 ± 14	271 ± 10
Duration of 50% repolarization, msec	162 ± 11	156 ± 8	148 ± 16	149 ± 12
Refractory period, msec	190 ± 14	182 ± 10	180 ± 7	190 ± 9
Overshoot, mv	20.4 ± 1.6	24.0 ± 2*	26.2 ± .8*	26.7 ± .6*
Phase 2 amplitude, mv	94.9 ± 1.6	100.2 ± 1.0	105 ± 0.8*	106 ± 1.1*

* Significant at 0.01 level.

In another method where atrioventricular block was produced by injecting formalin into the atrioventricular node, glucagon did not increase the idioventricular rhythm during the acute phase of this procedure, but in the chronic preparation, glucagon increased the rate of discharge of the idioventricular focus (100, 205, 520). All of these findings are supported by the observation that in the isolated Purkinje fiber glucagon increased the spontaneous discharge rate (382, 456). Thus, it can be concluded that glucagon can increase the discharge rate from all the areas of the heart that can beat spontaneously.

C. *Effect of Glucagon on Cardiac Arrhythmias*

Glucagon has been used in the treatment of heart failure where the presence or a susceptibility to cardiac arrhythmias is a frequent complication. Thus, the arrhythmogenic potential of glucagon has been studied in a variety of experimental preparations and in human patients.

Since glucagon improves auriculoventricular conduction, its effects on experimentally produced auricular fibrillation and flutter have been studied. Curry et al. (96) and Hawthorne and Hinds (183) have shown that glucagon did not increase the auricular flutter rate in dogs, but increased the ventricular rate significantly. Lipski et al. (281) and Steiner et al. (453) observed that the production of second degree heart block by driving the auricle at a high rate could be overcome by the administration of glucagon. Unpublished experiments with auricular flutter preparations prepared by the Rosenblueth and Garcia-Ramos (409) method, confirm the above findings, and here 200 μg/kg glucagon barely increased auricular flutter rate, but increased ventricular rate significantly, changing a 2:1 auricular rhythm to one approaching 2:1.6 rhythm. This effect of glucagon was dose-dependent and lasted 15 to 30 minutes. Similar results were observed in a limited number of preparations where auricular fibrillation was induced with the local application of aconitine on the left auricular appendage. All these findings can be readily explained by the improved atrioventricular conduction produced by glucagon. Similar changes in human auricular arrhythmias have been observed in human patients (243, 363, 415).

The ventricular irregularities following coronary artery occlusion are a major clinical problem, and the arrhythmogenic or antiarrhythmogenic properties of an inotropic agent become a major concern. Lucchesi et al. (286) administered small amounts of glucagon and did not observe any increase in the heart rate or ventricular irregularities that were produced by ligating a coronary artery in a dog by the two-step method of Harris (180). In these dogs, a small dose of epinephrine produced a severe ventricular tachycardia. In the Harris coronary ligation, the ventricular irregularities seen between 8 and 48 hours postinfarction are superseded after 3 to 6 days by a preponderance of sinus rhythm; however, these preparations are highly susceptible to ventricular arrhythmias and fibrillation, especially when sympathetic stimulation occurs. In such cardiac preparations, Lucchesi et al. (286) gave relatively small amounts of glucagon (4 to 12 μg/kg), and their results clearly show that glucagon did not cause the appearance of or increase the already existing ventricular tachycardia. Their results indicate a slight reduction in the ventricular irregularities following glucagon administration, whereas the injection of a small dose of epinephrine produced a severe ventricular tachycardia. Madan (292) and Madan et al. (293) and Singh et al. (436) also used the Harris infarction model and injected relatively large doses of glucagon (30 to 100 μg/kg) and have shown a striking reduction in the ventricular arrhythmia and, in several instances, were able to produce a reversal of the idioventricular rhythm to a sinus rhythm. Manchester et al. (297) have demonstrated the beneficial effects of glucagon in experimentally produced myocardial infarction and shock.

Another type of cardiac irregularity can be produced by injecting toxic doses of digitalis glycosides. These ventricular irregularities were reversed by glucagon to a sinus rhythm (82, 292). However, Singh et al. (436) reported that glucagon did not reverse the ouabain-induced ventricular irregularities in dogs and in fact increased these irregularities and the heart rate.

In all these preparations glucagon increased the heart rate, and the reduction in ventricular irregularities observed by some could be due to the overdrive effect of the increased rate (103, 174, 322). In our own experience in the dog HLP, early cardiac irregularities induced by

ouabain were partially or completely reversed by 200 to 500 μg of glucagon per liter of blood, and also produced a marked increase in the sinus rate. Late ventricular irregularities concomitant with atrioventricular dissociation responded poorly to glucagon, and the ventricular tachycardia could not be influenced, although auricular rate was increased significantly. Opie et al. (354) have proposed the hypothesis that cAMP may be a contributing cause to the arrhythmias produced by beta-adrenergic agents in the ischemic heart model. It is likely that glucagon also increased cAMP content of the heart, but was not arrhythmogenic and may even have antiarrhythmogenic properties. Thus, the correlation of irregularity production and cAMP content may not be a general principle. All these findings support the conclusion that glucagon in positive inotropic doses is not arrhythmogenic and may actually have antiarrhythmogenic effects. The mechanism of this antiarrhythmogenic effect is probably partially due to the overdrive effects of the increased heart rate produced by glucagon, although a direct membrane effect on ventricular tissue cannot be excluded (456). Another factor in intact animals could be the insulin release following glucagon adminstration, which could increase the uptake of potassium by the heart, and this could influence the discharge frequency and excitability of the heart (137).

D. Effect of Glucagon on Coronary Blood Flow and Cardiac Oxygen Consumption

Since glucagon increases cardiac rate, it will increase cardiac oxygen consumption in the nonfailing heart (326, 391). Bache et al. (19), Gorman et al. (172), Marsiglia et al. (302), and Sharma et al. (428) stimulated the nonfailing heart at a constant rate, and here glucagon increased cardiac blood flow and oxygen consumption, although heart rate was kept constant. The observed increase in contractile force of the ventricle is the likely cause of this increase in oxygen consumption, and both coronary blood flow and oxygen consumption increases were dose-dependent (484). By using a modified HLP, Simaan and Fawaz (435) have shown that maximal effects of glucagon on cardiac contractility were attained when 50 μg of glucagon per minute were infused into the dog heart-HLP. Increasing the rate of infusion of glucagon only increased the heart rate and oxygen consumption without changing the work of the heart. When sinus rate changes due to glucagon were reduced by the administration of veratramine, it decreased the incremental changes of both heart rate and oxygen consumption without a change in the stroke work of the heart. Thus, the increased heart rate produced by glucagon may have been responsible for some of the increase in cardiac oxygen consumption.

Glucagon decreased free fatty acid extraction and increased lactate production and glucose uptake by the heart. None of the metabolic effects of glucagon were blocked by previous reserpine administration.

The reduction of blood pressure, coronary blood flow, and cardiac oxygen consumption due to hemorrhage can be restored by the administration of glucagon (297, 487). In man, similar results to those observed in dogs were reported by Goldschlager et al. (170) and Manchester et al. (296).

In nonbeating hearts, glucagon did not increase coronary blood flow or oxygen consumption (167, 168). Thus, the changes in rate and contractility produced by glucagon must have been an important cause of these changes. In a similar vein, Moir and Nayler (326) concluded that the reduction in coronary resistance produced by glucagon was due to the chronotropic and inotropic effects of this hormone. The catecholamines, which also increase the heart rate and contractility of the heart, increase coronary blood flow and cardiac oxygen consumption; however, these increases are greater with the catecholamines, possibly because of the more marked effects on contractile force and the increased free fatty acid uptake by the heart (65, 319–321).

Because of the increase in heart rate and contractile force and the increase in oxygen demand of the nonfailing heart, the effects of glucagon on the cardiac lesion following coronary occlusion predictably would produce deleterious results. Lekven et al. (265), Maroko et al. (301), and Shell and Sobel (429) have shown that following coronary artery occlusion, glucagon increased the height of the ST-segment, but this was statistically less than the observed effects of isoproterenol. However, under heart failure conditions, glucagon could either produce no deleterious effect, or even improve the metabolic status of the heart because of its positive inotropic effect and the concomitant reduction in cardiac volume and oxygen demand per unit of work performed (441).

E. Factors Influencing Effects of Glucagon on the Heart

1. Species Differences. Farah and Tuttle (136) observed positive inotropic effects in isolated auricles of dogs, cats, rats, and guinea pigs, while rabbit auricular tissue did not respond to glucagon. These species differences have been reviewed previously by Farah (135).

It is of interest to note that in the guinea pig heart the auricles responded normally, while ventricular muscle responded minimally to glucagon when rate and contractile and cAMP responses were used as criteria (291, 408). On the other hand, Henry et al. (187) found that glucagon increased the contractile force of the guinea pig heart without increasing its cAMP content. Since sinus rate of the guinea pig heart was increased, it is possible that the increase in contractile force observed by Henry et al. (187) could be due to rate effects on the contractile force of the guinea pig ventricle (291, 408). Spilker (445) studied the effects of rate of stimulation on the inotropic effect of glucagon and reported that at rates above 24 beats per minute, contractile force increases due to glucagon were rate-dependent.

2. Heart Failure. The response to glucagon in human

heart failure was quite variable, and this variability was especially apparent when chronic heart failure was studied (243). In general, the severity of the heart failure determined the hemodynamic response of the patient to glucagon. Thus, classes III and IV heart failure responded significantly less than the milder heart failures (13, 509).

In the dog HLP, a variety of acute heart failures were studied, and their responsiveness to glucagon was determined. Heart failure produced by pentobarbital or spontaneous heart failure responded well to glucagon, even when heart failure was severe. However, heart failure produced by the metabolic inhibitors sodium azide or dinitrophenol responded to a lesser extent than the spontaneous or pentobarbital failure, especially when heart failure was severe [Farah (135)]. Similar decreased responses to ouabain in azide and dinitrophenol failure have been reported by Gruhzit and Farah (175). The same studies indicated that the severity of the heart failure determined the degree of cardiac improvement. Thus, in severe heart failure, the response to glucagon, epinephrine, and ouabain was less than in either mild or medium degree heart failure.

Isolated papillary muscle obtained from cats where the light pulmonary artery had been chronically banded did not respond to glucagon when inotropism or cAMP increases were used as criteria (169, 272). Similar results were reported when human heart tissue was studied. Thus, the inotropic or cAMP response was much weaker in tissue obtained from failing human hearts, as compared with tissue obtained from nonfailing hearts (171, 362, 509). Strauer (461, 462) repeated those experiments, and his data show a small increase in contractile response to a maximally effective dose of glucagon, which seems to be a much weaker response than has been observed in isolated cardiac tissue obtained from normal human hearts.

Nobel-Allen et al. (347) and Winokur et al. (518) determined glucagon positive inotropic effects in cat hearts where a pulmonary artery was constricted for a period of time. They claim that the responses of these hearts were about the same as those observed in normal cat hearts. It is difficult to reconcile all of these conflicting data; however, in some of these studies the severity of chronicity of the heart failure was not taken into account. Newman (342, 343) determined the effects of glucagon, beta-adrenergic agents, and calcium ion on a volume heart failure induced by joining the vena cava to the aorta of dogs. During the acute stages of this failure, the dogs responded to all three groups of inotropic agents. During the chronic phase, there was a reduction of the inotropic response to all of these agents, although blood pressure and heart rate responses did not show these differences.

When heart failure was induced by anoxia, the recovery of this heart failure was enhanced in the presence of glucagon, although it did not significantly change the rate of decline of the contractile force during the anoxic phase (68, 420). The improved recovery in the presence of glucagon could be due to the increased glycolysis and adenosine triphosphate (ATP) production which could maintain the integrity of the tissue during the anoxic period.

In a study by Nakano and Moore (336), chronic heart failure was induced by repeated administration of ethanol, and here glucagon produced a weaker response in the chronic than in acute heart failure.

From all these findings, it is clear that the type of heart failure, its severity, as well as its chronicity, are determining factors in the positive inotropic response of the heart to glucagon. The heart failures induced by azide and dinitrophenol interfere with energy production, and it is these types of heart failure that respond poorly to the inotropic action of glucagon. It is possible that in chronic heart failure in man, energy supply and/or oxygen supply to cardiac muscle may be defective, thus, the variation in response of human heart failure to glucagon.

3. Age. The response to some cardioactive agents increases with the age of the fetus and often precedes the innervation of the heart. The inotropic or chronotropic response to catecholamines develops progressively and runs parallel with the increase in the adenylate cyclase (85, 97, 378). The response to glucagon appears late in the developmental period of the heart and responsiveness of the heart to glucagon may become apparent only after birth (78, 151, 515). Species variations in the responsiveness of heart to glucagon have been described by Wildenthal (513). Thus, mouse hearts respond to glucagon during the 17th to 18th day of gestation, whereas rat hearts respond only after birth of the fetus. The response observed was an increase in heart rate, which was not accompanied by an increase in the cAMP content of the heart. In a similar vein, Ahumada et al. (6) observed that heart strips from fetal or newborn lambs did not show an increase in contractility, nor an increase in cAMP following the administration of glucagon. Cardiac tissue from adult sheep showed an increase in both contractile force and cAMP. Responsiveness of the cyclase of fetal and newborn lamb cardiac tissue was observed when fluoride was added to the tissue and adenylate cyclase of liver tissue was already demonstrable in fetal and newborn animals (78). The response of human fetal adenylate cyclase in cardiac tissue was studied by Menon et al. (314) and Dail and Palmer (97), who observed a basal- and fluoride-sensitive adenyl cyclase activity in cardiac tissue as early as the 8th to 9th weeks of gestation. However, glucagon sensitivity could be observed only after the 17th week. It is thus likely that in heart tissue the adenylate cyclase activity develops at an earlier time than the glucagon receptor.

4. Hypertension. The sensitivity of heart muscle obtained from spontaneously hypertensive rats to adrenergic agents was reduced, and the binding of [^{3}H]dihydroxyprenalol by these hearts was also reduced (153, 277). Similar observations have been reported for glucagon.

Largis et al. (255) and Staneva-Stoycheva and Bogoslovova (448) have shown that the enlarged heart obtained from spontaneously hypertensive rats had a reduced inotropic response to glucagon. Dhalla et al. (105) and Chatelain et al. (74) extended these findings and observed a reduced glucagon response of the cardiac adenyl cyclase system, whereas the fluoride and the guanylnucleotide responses were not changed. This possibly indicated that in these hearts the number of glucagon receptors was reduced (74). The difference between hearts from spontaneously hypertensive rats and from normal control subjects was not due to the hypertrophy of the heart since no such differences in glucagon sensitivity were observed in hypertrophied hearts obtained from Goldblatt hypertensive rats (74).

5. *Adrenergic Blocking Agents.* The early experiments with glucagon showed that the mixed beta-blocker agonist dichloroisoproterenol (DCI) was an inhibitor of the inotropic and chronotropic effects of glucagon on the heart (136, 284, 391, 510). However, later observations by Glick et al. (166), Lucchesi (284), Spilker (445), and Peterson et al. (369) have shown that the more specific beta-blocking agent propranolol did not counteract either the chronotropic or inotropic effects of glucagon. This discrepancy was explained by Lucchesi (284), who has shown that the inhibition produced by DCI could be blocked by propranolol. Theophylline and tyramine, which are inotropic and chronotropic agents, will block the effects of glucagon on the heart. Thus, glucagon, although it has many properties similar to those of catecholamines, must have a receptor distinct from the one responsible for the action of catecholamines.

6. *Phosphodiesterase Inhibitors.* According to Marcus et al. (298), theophylline increases the positive inotropic effect of glucagon, while Antonaccio and Lucchesi (11) have concluded that this phosphodiesterase inhibitor did not potentiate the effects of glucagon on the heart. Brunt and McNeill (62) have demonstrated a small increase in the glucagon-induced contractile response. The findings on cAMP concentrations clearly show that theophylline increased the effects of glucagon on the cardiac content of this adenine derivative (62). Wildenthal and Wakeland (515) were unable to demonstrate an effect of a phosphodiesterase inhibitor (RO 7-2956) on the chronotropic effect of glucagon in the fetal mouse heart. In our experience, theophylline, which potentiated the effects of epinephrine on dog heart muscle preparations, did not significantly increase the effects of glucagon. Thus, a discrepancy between inotropic and the cAMP effects of glucagon on the heart has to be considered.

IV. Glucagon Receptor and Adenylate Cyclase

The production of cAMP by glucagon has been demonstrated in a variety of cells, including cardiac muscle cells [For recent reviews, see Rodbell (404) and Ross and Gilman (410).]

In most cells and membrane preparations, a basal activity of the cyclase exists that can be stimulated by a variety of hormones, hormonal analogues, guanosine triphosphate and its analogues, sodium fluoride, cholera toxin, other bacterial toxins, calmodulin, and a variety of phospholipids.

There are three membrane entities that have been postulated to transform the hormone signal on the cell surface to the intracellular production of cAMP from ATP. These entities are the receptor (R), the catalytic unit that converts ATP to cAMP (C) and a regulatory component (N) that binds guanosine triphosphate (GTP) and acts as a coupler between the receptor and the catalytic unit (C). [For reviews, see Birnbaumer and Iyengar (39), Rodbell (404), and Ross and Gilman (410).]

It has been suggested that the receptor and catalytic unit of adenylate cyclase can undergo lateral migration in the membrane. When glucagon binds to the receptor, it interacts with the N and C components of the adenyl cyclase to form a multicomponent complex, which spans the cell membrane. This is generally known as the mobile receptor model, and a great deal of data suggests that this model is basically valid and explains the activation of the cyclase by occupancy of a receptor (33, 227).

The binding of a hormone to a receptor besides increasing the mobility of the receptor also causes its aggregation on the membrane followed by internalization of the hormone receptor complex by a process of endostosis. This process of hormone internalization may explain the growth-promoting effects of glucagon since glucagon can bind to the nuclear membrane (30, 227). The intracellular hormone-receptor complex may be degraded by lysosomes, or the receptor may be recycled to the cell membrane, thus regenerating surface receptors, [for a review, see King and Cuatrecasas (227)].

A. Glucagon Receptor

The binding of ^{125}I-labelled glucagon to tissue membranes has been demonstrated. In the presence of optimal conditions, the relationship between receptor occupancy of glucagon and the response (production of cAMP) follows a hyperbolic relation. As little as 10% occupation of the receptor by glucagon produced nearly maximal activity (406).

Klein et al. (231) and Levey et al. (270) have studied isolated cardiac membrane preparations and have shown that glucagon is bound to particulate and solubilized fractions. This binding of glucagon was not dependent on GTP or phosphatides, and labelled glucagon could be replaced from the binding site by unlabelled glucagon. Glucagon binding was sensitive to both pH changes and temperature. Binding and activation of the adenyl cyclase by glucagon were related; however, here again maximal effects on the cyclase could be attained when less than 5% of the maximal binding sites had been occupied.

Interaction of agonists and antagonists with beta-adrenergic receptors are different since antagonists seem only to occupy the receptor, while antagonists occupy

the receptor and cause a change in the receptor, which activates the adenylate cyclase system. Differences in temperature sensitivity of agonist and antagonist binding indicate that agonist binding is temperature sensitive, while antagonist binding is relatively insensitive to temperature changes (327). Most drug and hormone-binding studies have been conducted in isolated membrane fragment preparations. Data published have shown that differences in results are obtained when intact cells are compared to isolated membranes (207, 327, 486). Thus, Terasaki and Brooker (486) observed that in intact cells, a maximal increase in cAMP was observed when an immeasurably small fraction of the receptors was occupied. Srikant et al. (446) have shown that in animals treated chronically with glucagon, the receptors were reduced to one third, although the cyclase response to glucagon was unchanged.

Such data raise questions concerning the nature of such binding sites, and it has been argued that the majority of the glucagon binding sites are not true receptors and that many of the binding sites determined by the in vitro technology are nonspecific and are probably not related to either cAMP formation or inotropic effects (40). A different explanation of these findings has been proposed, namely, that the glucagon-binding receptors are in excess of those required for maximal activity of the cAMP system. It is not possible to resolve this dilemma at this time; however, the early experiments with beta-adrenergic receptors should suggest caution in the interpretation of these findings with regard to the functional significance of glucagon binding.

A highly purified glucagon binding protein was isolated from a liver membrane preparation by Giorgio et al. (164). This protein had a molecular weight of 190,000 and had a high specificity for glucagon, although it also bound a small amount of insulin. Johnson et al. (212) used ^{125}I-labelled glucagon bound to the receptor of liver tissue. It was crosslinked to the receptor by treating the membrane with hydroxysuccinimidyl-*p*-azidobenzoate. This crosslinked material was isolated on a gel as 63,000 dalton protein. Unlabelled glucagon added previously to this crosslinking reaction abolished the appearance of the labelled glucagon on the membrane protein. Furthermore, guanine nucleotides that reduce the amount of bound glucagon also reduced the amount of the crosslinked ^{125}I-glucagon. These findings suggest that the receptor from liver has a molecular weight of 63,000. By using target analysis, Houslay (199) and Houslay et al. (201) have determined the size of the glucagon receptor from liver and its component parts. Theoretically, the presence of Mg^{++} and ATP would measure the functional size of the catalytic unit, while activation by a guanine nucleotide or fluoride will measure the complex of the catalytic and regulatory unit. In the presence of glucagon, all three units should be linked, giving the total size of the R-N-C-complex. The smallest size was 100,000 daltons obtained in the presence of Mg ATP and possibly represents the catalytic unit. Activation by flouride or guanosine nucleotide gave a 240,000 dalton unit. In the presence of glucagon, the target size increased to 340,000 daltons, thus indicating a 100,000 dalton value for the receptor. It is of interest that Nielsen et al. (344) have determined similar values for the various components in erythrocyte membranes. However, the data obtained by Martin et al. (303) do not totally agree with the above values and differences in technique and enzyme material could explain these discrepancies. Target analysis of proteins and complex enzyme systems is influenced by many factors, and interpretation may be both difficult and debatable.

Levey et al. (270) have prepared a soluble adenylate cyclase complex from cardiac tissue, and molecular weight measurements in this relatively crude preparation gave values of 100,000 to 200,000 daltons. Fractionation of this material produced a 26,000- and a 100,000-dalton fraction.

When target analysis was applied to the resting state of the receptor-adenylate cyclase system, a molecular weight greater than 6 million was determined (344). This suggested that in the ground state the enzyme consisted of aggregates of receptor-regulator complexes (R-N). Thus, activation of the receptor by glucagon could cause first a dissociation of this R-N aggregate. The individual R-N aggregates could then react with the catalytic unit. This interpretation would be compatible with the findings obtained with high energy electron target analysis. Such a model has been proposed by Levey (268) and Levey et al. (270), in which they suggest that the binding of glucagon to the cardiac receptor will cause a dissociation at the receptor site, which then could react with the catalytic site.

B. Receptor Coupling

Early studies by Rodbell (403) and Rodbell et al. (407) have shown that GTP-activated adenyl cyclase in isolated membranes probably binds to the regulatory N unit. This N unit acts as a GTPase to form GDP from GTP. NaF, which also stimulates the cyclase system, interferes with the effects of GTP, and it has been postulated that both act via a common site on N. Another activator of the adenyl cyclase system is cholera toxin, which acts by inhibiting the GTPase of N and thus enhances the effects of GTP (72). Cholera toxin enhances the effects of hormones (250) and inhibits the stimulatory effects of NaF on the cyclase system. Another activator of this system is adenosine, and it is possible that the N-unit is a multicomponent molecule that mediates the effects of a variety of agents that affect the cAMP-generating system.

In the presence of GTP, the binding of glucagon and catecholamines to liver membranes was reduced and reached a steady state very rapidly, and the binding ran parallel to the activation of the adenyl cyclase (279). Rodbell (404) suggested that GTP binding to the N-unit

alters the receptor from a tight binding one-to-one of lower affinity. These findings have suggested that hormone binding to the high affinty receptor will cause the binding of GTP to the N-unit, which in turn will convert the high to the low affinity receptor and bind to the catalytic C-unit and turn on cAMP synthesis. The conversion of the high to the low affinity state of the receptor is a crucial reaction for activation of the cyclase since antagonists that bind to the high affinity receptor do not activate the cyclase, and their binding to the receptor is not influenced by the addition of GTP (278). One must thus conclude that antagonist binding studies may not represent the true state in which an agonist is bound to the receptor.

The N-unit is found in human red blood cell membranes, but receptor (R) and catalytic units are in very low concentration (345). The lymphoma variant A-C lacks the catalytic unit N, and, on addition of red blood cell membranes containing N, the variant A-C behaves like the wild type cell and responds to agonists by producing cAMP.

Several other variants of cells have been studied. One variant contains N-units and receptor, is not activated by the adrenergic hormone, but is activated by NaF. It is likely that in this strain N and R are uncoupled, and thus no receptor activation of the cyclase system occurs. Another variant cell type contains the adenylate cyclase system, but cannot respond to the addition of hormone, or GTP analogues, although the nucleotide alters the binding of agonist to the beta-adrenergic receptor. Here the defect seems to lie in the linkage between N and the catalytic unit (404). These data support a multicomplex concept for adenyl cyclase activation.

Further evidence that the receptor and the N- and C-units are independent units was obtained by hybridization techniques. After irreversibly inactivating the catalytic activity of a membrane that contained a beta-adrenergic receptor, the inactivated membrane was fused to another cell membrane that did not contain a receptor. This produced a functional unit that generated cAMP on addition of a beta-adrenergic agent (357, 423). Similar studies with the glucagon receptor have been reported by Schramm (422), thus supporting the concept that the glucagon receptor and the cyclase units are distinct and separate entities.

C. N-Unit

The regulatory compound can be extracted from membranes. It is a water-soluble protein having rodlike structure and is probably associated with the cytosol side of the membrane. It is inactivated by sulfhydryl reagents and thus cannot couple with the catalytic unit (447, 459). Studies have shown that basal-, as well as NaF-stimulated adenyl cyclase activity in liver membranes was inactivated by iodoacetamide, and glucagon markedly stimulated the rate of this inactivation (459). Glucagon in the presence of a labelled sulfhydryl reagent led to the labelling of a glycoprotein (458). Williams and Lefkowitz (517) have shown that catecholamines also enhance the reactivity of the sulfhydryl group of the N-unit. Rodbell et al. (405) have observed that the sulfhydryl reagents also inhibit the guanyl nucleotide stimulation, as well as the binding of glucagon to the liver membranes. The marked increase in reactivity to iodoacetamide caused by the addition of glucagon, supports the idea that sulfhydryl groups play an important role in the glucagon activation of the adenylate cyclase system. It is probable that glucagon binding to the receptor increases the sensitivity of the N-unit to the sulfhydryl reagent by inducing a conformational change in the N-units protein, which bind the nucleotide and sodium fluoride. Recent observations by Suen et al. (472) have shown that sulfhydryl reagents, as well as the reducing agent dithiothreitol, decreased the binding of dopamine to nervous tissue membranes, but dithiothreitol had no effect on the binding of the antagonist spiroperidol. The inhibition of agonist binding by dithiothreitol seemed to have a specificity for a dopamine binding site, and the effects of the reducing agent could be reversed by hydrogen peroxide. These findings suggest that the oxidation-reduction state of the receptor may play a role in the conformational state of the receptor.

Similar studies conducted on the beta-adrenergic receptor have shown that sulfhydryl groups regulate the interaction of the receptor to regulatory unit N (283, 502, 503). Suen et al. (471) have shown that N-ethyl maleimide in dog brain membrane fraction had no effect on the basal cAMP production, but inhibited dopamine stimulated activity, as well as sodium fluoride and GTP adenyl cyclase activity. However, the nonreversible analogues Gpp(NH•)p and GTPyS protected against the enzyme inactivation by sulfhydryl reagents. This suggests that the sulfhydryl reagent competes for the guanine nucleotide binding site on the N-unit of the cyclase. Confirmation of such a concept was provided by Korner et al. (244), who have suggested that the guanine nucleotide binding protein exposed a specific sulfhydryl group upon interaction of the agonist with the beta-adrenergic receptor. The observation of Suen et al. (472) that N-ethylmaleimide decreased the binding of the agonist dopamine, but not of the antagonist spiroperidol, suggests that the activation of the receptor by an agonist causes the appearance of sulfhydryl groups in the N-unit essential for binding GTP, while the antagonists do not activate the appearance of the sulfhydryl group in the N-unit and thus do not activate the cyclase system (244).

The N-unit from liver has been highly purified by Northrup et al. (348), and the molecular weight has been estimated at 130,000 daltons, both by hydrodynamic evaluation and by target analysis. This protein consists of three units with molecular weights of 35,000, 43,000, and 53,000 daltons. The 43,000- and 53,000-dalton proteins are labelled when exposed to radioactive cholera toxin. The 35,000-dalton protein does not react with

GTP, and it has been postulated that this protein modulates the cholera-toxin-labelled proteins and regulates their interaction with the receptor and the catalytic unit (404). The R-N complex is probably a stable structure and part of the N-units are bound to the R complex and, on activation, another fraction will be bound to the C-unit. Another possibility is that free N-C-units do not exist, and N combines with C only after receptor occupancy with an agonist, and the R-N complex is formed.

D. Catalytic Unit (C)

This unit combines with Mg ATP or Mn ATP with equal V_{max} and K_m. Little is known about the physical properties of the catalytic unit since it has not been prepared in purified form.

A molecular weight of about 230,000 daltons has been suggested by Schlegel et al. (421) and Stengel and Hanoune (454). It probably binds ATP in conjunction with Mg or Mn, although binding of these divalent ions have also been observed on the N-unit. Treatment of liver membranes with phospholipase A_2, or a phospholipase C, diminished the effects of glucagon on the adenylate cyclase, as well as the affinity of the receptor (251, 413). Such treatment also reduced the activation of cAMP production by GTP and its ability to modify the binding of glucagon to the receptor. The polyone antibiotic filipin and amphotericin B caused a marked reduction in the glucagon and GTP-induced stimulation of cAMP production. These findings suggest that the phospholipids are involved in the binding of R to N and N to C, but filipin did not influence the binding of glucagon to the receptor (108). These results are in agreement with the concept of a multiunit receptor-adenylate cyclase system (199, 404) and that lipids play an important role in the structure of the receptor, as well as the coupling of the R-, N-, and C-units. In a membrane preparation, benzyl alcohol increased the cAMP production in the presence of glucagon, GTP, and fluoride. This increase in activity correlated with changes in fluidity of the membrane. These membrane fluidity changes thus would allow greater lateral movement of the receptor in the membrane bilayer, and this could produce more collisions of the receptor with the cyclase system (107, 200).

Detergent- or phospholipase-treated solubilized enzymes lose activity, which can be restored by the addition of various types of phospholipids (304, 483). Rubalcava and Rodbell (413) have shown that treatment of liver membranes with phospholipase C abolished the stimulation of the adenylate cyclase system by glucagon without inhibiting sodium fluoride stimulation. Furthermore, Kempen et al. (224), Pohl et al. (377), and Rethy et al. (393) have restored adenyl cyclase activity in detergent-treated membranes with a variety of phospholipids, especially the acidic ones. Levey (266–268) and Levey and Klein (271) have shown that with cardiac membranes, glucagon, and histamine effects on adenyl cyclase were restored with phosphatidylserine. Norepinephrine effects were not restored with phosphatidylserine, but were restored on addition of phosphatidylinositol. These interesting findings suggest that specific phospholipids may be involved in either the receptor binding, or the activation of the adenyl cyclase by glucagon.

Further evidence for phospholipid involvement in the membrane activation by several hormones was described by Hokin and Hokin (194, 195), who described an increase in phosphatidylinositol (PI) turnover following the addition of acetyl choline to pancreatic tissue. More recently, Axelrod and his associates have described the relation of the regulation of beta-adrenergic agonists by the enzymatic methylation of phosphatidylethanolamine to form phosphatidylcholine. This synthesis involves the transmethylation by two methyl-transferases and probably influences the fluidity of the membrane, as well as the number of beta-adrenergic receptors (189–192, 463).

Lo and Levey (282) reported that glucagon mediated the incorporation of labelled orthophosphate into phosphatidylserine and phosphatidylethanolamine, but not into phosphatidylcholine. Dibuteryl cAMP did not increase this incorporation of phosphate into phospholipids.

Many agonists that stimulate cell activity by increasing Ca^{++} entry also induce an increase in the turnover rate of phospholipids. The increased turnover rate of phosphoinositol (the PI response) is the best known and has been observed in a variety of cells (161, 194, 195).

PI, one of the minor membrane phospholipids, is found in both the plasma and endoplasmic membranes and is hydrolyzed to 1,2-diacylglycerol by a PI phosphohydrolase or a PI phosphodiesterase. It is this diacylglycerol that is essential for the activation of a calcium-dependent kinase, which may be a signal for the control of membrane phosphorylation and is independent of cAMP. [See Takai et al. (480).]

It is of interest that PI can be phosphorylated by a specific kinase to form di- (DPI) and triphosphoinositides (TPI). These inositides are strong calcium binders and could be related to membrane-bound calcium. Hydrolysis of these inositol phosphates would release membrane-bound calcium. This could change the ionic permeability of the membrane and trigger further release of intracellular calcium and thus increase the intracellular Ca^{++} concentration (36, 317, 318). Michell (315, 316) has discussed the possibility that the interconversion of the phosphorylated inositides may be important in the generation of action potentials (36). It is possible that the di- and triphosphoinositides may have functions separate from those of the monophosphoinositide.

In smooth muscle of the iris, Abdel-Latif and Akhtar (1) and Abdel-Latif et al. (2, 3) have shown that acetylcholine, as well as norepinephrine, increased the breakdown of TPI, and these effects were blocked by atropine and phentolamine, respectively. Stimulation of the sympathetic nerves also increases the breakdown of TPI, and Ca^{++} was required for both the TPI breakdown, as

well as the increased turnover rate, but the turnover rate of phosphoinositol was not Ca^{++}-sensitive.

Based on these studies, Akhtar and Abdel-Latif (7) suggest that Ca^{++} behaves like a second messenger, and intracellular calcium causes the breakdown of membrane TPI and also acts in the stimulus-contraction coupling of muscle. Torda (490) has reported that cAMP increased the activity of diphosphoinositide kinase, which phosphorylates DPI to TPI.

All the above data suggest that phosphoinositide, and possibly other phospholipids, play a role in membrane depolarization, as well as in stimulus-contraction coupling. Further research in this area of membrane biochemistry and pharmacology may lead us to an understanding of the cAMP-dependent and cAMP-independent phenomena related to both cardiac and smooth muscle activity.

Takai et al. (479–481) have related PI turnover rate to the control of a protein phosphorylation, which requires the absolute presence of Ca^{++} and a phospholipid for catalytic activity, while cyclic nucleotides have no effect on this protein kinase. The protein kinase is found in an inactive form in tissues and, in the presence of calcium, the enzyme will attach to the membrane, and phosphatidylserine is an activating factor.

A hormonal stimulus will induce the hydrolysis of PI to give an unsaturated diacylglycerol, which increases the affinity of the kinase for Ca^{++} and for the phospholipid. This novel activated protein kinase [C kinase of Takai et al. (479)] can phosphorylate several regulatory enzymes and proteins and probably influences multiple membrane functions (480). Gaut and Huggins (161) have shown that epinephrine increases the turnover rate of the cardiac phospholipids and the phosphoinositide complex. This increased turnover rate was found in the phosphodiester linkage of monophosphoinositide. Studies with various tissues have shown that a stimulus will initiate the cleavage of the phosphodiester linkage by a mechanism similar to that of phospholipase C producing a diacyl-glycerol and inositol phosphate (114, 223, 315). The phospholipid involved contains arachidonic acid, and it is possible that this diacylglycerol may behave like a second messenger by activating the Ca^{++}-sensitive C-kinase.

A second possible activator of this C-kinase was a Ca^{++}-dependent protease (176), which is found in many tissues. Mellgren (312) has shown that a protease occurs in the heart, which is activated by a 10^{-5} M Ca^{++} concentration and occurs in other tissues as well. It is thus possible that a protease activation of C-kinase may also play a role in the transmission of receptor-induced activation of a cell membrane. These interesting preliminary findings suggest that the lipid-protein interactions described may play a role at least in some types of receptor function. The ubiquitous connection between surface activation and calcium in cardiac and smooth muscle suggests that this mechanism of activating phosphorylation via the C-kinase could play a role, especially in those instances where cAMP production does not occur. However, it also could be operative in a cAMP-producing activation and may be responsible for the phosphorylation of proteins not phosphorylated by cAMP.

E. Glucagon and cAMP Formation in the Heart

Glucagon effects on the heart should fulfill the Sutherland criteria (402) if its action is via the production of cAMP.

Glucagon stimulated the formation of cAMP in both cellular preparations, as well as the intact heart (60, 126, 127, 253, 254, 258, 269, 333, 401). The effects of glucagon on cardiac contractility and on cAMP production were not blocked by beta-blockers. Mayer et al. (308) and Øye and Langslet (360) found that cAMP production did not precede the contractile effects of glucagon; however, the limited sensitivity of the method for determining cAMP may have played a role in their inability to determine early changes in the cAMP content of the heart. Brunt and McNeill (62), as well as MacLeod et al. (291), have shown an increase in cAMP which paralleled the increase in the contractile force changes produced by glucagon in rat hearts. Furthermore, Brunt and McNeill (62) have shown that the phosphodiesterase inhibitor theophylline increased the glucagon-induced cAMP content of the heart. However, in these experiments, theophylline had only a very slight effect on increasing the effects of glucagon on contractility of the heart. Henry et al. (186, 187) described a serious discrepancy between contractile and cAMP effects in the guinea pig heart. In their experiments, glucagon increased the contractile force of both the guinea pig and rat hearts, but did not increase the cAMP in the guinea pig heart. In broken heart cell preparations, glucagon increased the cAMP content of the rat heart preparation, but not in the guinea pig heart. This problem was restudied by MacLeod et al. (291) and Rodgers et al. (408), who have shown that auricular muscle from guinea pigs responded to glucagon by an increase in rate, contractility, and cAMP. On the other hand, guinea pig ventricular muscle did not respond to glucagon, either by a contractile change or by an increase in cAMP. In the rat heart, MacLeod et al. (291) and Rodgers et al. (408) have confirmed the findings of Henry et al. (186) and have shown a glucagon-induced increase in contractile force and an increase in cAMP content of both auricular and ventricular muscle of the rat. These authors have also shown that a slight increase in contractility of the guinea pig heart seen after glucagon is probably caused by the increase in heart rate produced by glucagon. Frequency-force relations in the guinea pig ventricular muscle have been observed by MacLeod et al. (291) and Rodgers et al. (408), and it is possible that the latter effect caused the increase in contractility observed by Henry et al. (186, 187), and thus was not related to a direct effect of glucagon on guinea pig ventricular muscle.

In cat cardiac tissue, an increase in contractility and increase in the cAMP content have been reported by Gold et al. (169), Levey and Epstein (269), and Levey et al. (272). In the dog heart, glucagon increased contractile force; however, to our knowledge, no reports on the effects of cAMP content have been published.

In the mouse heart, increases in rate due to glucagon have been observed, although no increase in cAMP has been observed (79, 506, 514). It is possible that similar to the guinea pig heart, the mouse auricle responds, while the ventricle does not respond to glucagon. Since cAMP was determined in the whole heart, the nonresponsive ventricle was the predominant tissue in the chemical determination. In fetal hearts of mice (514, 515), rats (78, 514), and sheep (6), glucagon did not increase either the rate, contractility or cAMP content of the fetal hearts.

In experimentally produced chronic heart failure in cats, Levey and Epstein (269) and Levey et al. (272) have shown a lack of response of these hearts to glucagon when either contractility or cAMP changes were measured. However, Nobel-Allen et al. (347) have shown that in hypertrophied failing cat hearts the contractile response to glucagon was similar to that seen in normal cats. Unfortunately, no cardiac cAMP values were reported.

In heart tissue obtained from human heart failure patients, Goldstein et al. (171), Prasad (380), and Strauer (461, 462) were unable to show glucagon effects either on the contractile force or the cAMP content, while tissues obtained from nonfailing hearts responded to glucagon by an increase in both the contractile force and cAMP content.

As has been described, glucagon decreased the time to peak tension and relaxation time and raised phase 2 of the intracellular action potential. All these effects are similar to those observed with epinephrine. The effects of cAMP on cardiac muscle are difficult to demonstrate since this drug does not penetrate readily through the sarcolemmal membrane. However, Kukovetz and Pöch (248) and Kukovetz et al. (249) have shown that cAMP increased contractility of the heart in the presence of a phosphodiesterase inhibitor. Furthermore, dibutylyl cAMP gave more consistent results, probably because it penetrates the membrane and is broken down to buteryl cAMP intracellularly. In skinned cardiac muscle, cAMP increased the rate of contraction and relaxation produced by calcium ion (130). A convincing finding is that iontophoresis of cAMP into myocardial cells caused a rise in phase 2 of the action potential and in the rate of discharge of Purkinje fibers (493). Furthermore, Vogel and Sperelakis (507b) and Li and Sperelakis (275b) have shown that microiontrophoresis of cAMP into depolarized Purkinje fiber cells induced the slow action potential in a dose-dependent manner. These observations suggest that glucagon and cAMP effects on the heart have many similarities and thus fulfill the last criterion set forth by Sutherland. However, it is possible that cAMP is not the sole messenger for glucagon action, and glucagon may activate sarcolemmal calcium channels independent of cAMP. Since phosphorylation of calcium channels by cAMP and calcium entry are so closely coupled, it is difficult to distinguish between the above mechanisms.

The hormone sensitive adenylate cyclase activity is increased by the addition of guanine nucleotides (406, 407) and seems to play a regulatory role in a variety of tissues, including cardiac tissue. Fricke et al. (149) have studied the kinetics of activation of cardiac adenylate cyclase by GTP and glucagon. The addition of both glucagon and GTP increased cardiac cAMP synergistically. The apparent K_m value was not significantly changed by either glucagon or GTP; however, V_{max} values increased synergistically in the presence of both these compounds.

To equate a rise in total cardiac cAMP concentration with a change in cardiac contractility may produce erroneous conclusions since compartmentalization of cAMP and protein kinase have been described. [For reviews, see Brunton et al. (64), Corbin et al. (89), Earp and Steiner (115), and Terasaki and Brooker (485).] Terasaki and Brooker (485) have shown that in rat atrial tissue cAMP can be determined in both free and bound forms. The bound form is found in both the soluble and particulate fractions and addition of a beta-agonist increased the cAMP content of the free form. Immunochemical methods have shown that the intensity of cyclic nucleotide fluorescence and the localization within the cell changed significantly after stimulation of the cell with a hormone (452). Furthermore, Keely (221) has shown that PGE_1 and epinephrine increase cAMP and protein kinase, but only epinephrine activates phosphorylase in the perfused rat heart. In adipocytes, Honeyman et al. (198) have shown that phosphorylase and lipase can be activated independently by either uretonin or isoproterenol (INE). Similar data have been published by Brunton et al. (63) and Hayes et al. (184), who have compared the effects of prostacycline E_1 and INE in the perfused hearts of several species. Thus, INE increased the ventricular pressure development, cAMP content, soluble protein kinase activity, phosphorylase kinase, phosphorylase, glycogen synthetase, and troponin I phosphate. PGE_1, although it increased cAMP content and the soluble protein kinase, had no effect on pressure development, troponin phosphate, glycogen synthetase, phosphorylase, or phosphorylase kinase. An examination of the subcellar distribution in cardiac tissue was conducted by Corbin et al. (89) and Brunton et al. (64), and INE increased contractile force, phosphorylase, the soluble and particular cAMP and the soluble protein kinase, but reduced the particulate protein kinase by about 30%, possibly by a translocation of the particulate protein kinase to the soluble fraction. PGE_1, on the other hand, increased the soluble cAMP and soluble protein kinase, but had no effect on contractility, the particulate cAMP,

or particulate protein kinase. These data indicate that particulate cAMP and the translation of particulate protein kinase activity are related to phosphorylase activity and probably to the inotropic effect of the catecholamine (64, 185).

The data presented show that correlations between cAMP concentrations and cardiac functions must be interpreted with caution, especially so when negative correlations are considered. In general, the Sutherland postulates have been fulfilled for glucagon actions on the heart and with very few exceptions the effects of glucagon on rate and contractility of the heart correlate with the increase in the cardiac cAMP concentration.

V. Glucagon Effects on Cardiac Carbohydrate and Lipid Metabolism

Glucagon effects on cardiac metabolism are in general similar to the changes observed with catecholamines (90, 247, 361). Glucagon may act via cAMP; thus, glycolysis was increased by increasing breakdown of glycogen and the inhibition of glycogen synthesis. Since glucagon has a positive inotropic effect, the work of the heart will increase and thus cause an increase in cardiac oxygen consumption, the citrate cycle turnover rate, lipolysis and beta-oxidation of lipids. Increase in work of the normal heart will result in minimal increases in carbohydrate metabolism, especially if insulin is available in maximally effective concentrations. Thus, with glucagon one observes some increase in carbohydrate metabolism and a marked increase in lipid oxidation. This will sustain the normal levels of phosphocreatine and ATP in the heart.

In the glycolytic path, hexokinase, phosphofructokinase, and pyruvatekinase are essentially irreversible reactions and thus can act as possible control sites for glycolysis. Glucagon, by increasing the breakdown of glycogen, will increase the supply of glucose-1-phosphate, glucose-6-phosphate, fructose-1-6-phosphate, and the output of lactate (386). The activation of glycogenolysis by glucagon is initiated by the formation of cAMP, which activates the cAMP-dependent kinase. This kinase converts a low activity to a high activity phosphorylase kinase by a phosphorylation reaction, which requires ATP. However, phosphorylase kinase can also be activated by calcium levels of the order of 10^{-7} M, which are found intracellularly. Phosphorylase kinase in the presence of ATP will phosphorylate the inactive phosphorylase *b* to the active *a* form. Phosphorylase *a* will then form glucose phosphate from glycogen, which will enter the glycolytic pathway. Further control of glycolysis occurs via a protein phosphatase, which converted the phosphorylase *a* to the *b* form and is activated by ATP and is inhibited by AMP and inorganic phosphate (470).

Skeletal and cardiac muscle glycogen synthetase was phosphorylated in the presence of cyclic AMP to an inactive form. There the major phosphorylation of the synthetase occurred at a trypsin-sensitive or COOH terminal domain, while insulin, which also activated this enzyme caused a specific dephosphorylation of the trypsin insensitive or NH_2 terminal domain. This finding suggests that insulin does not act via an inhibition of the cAMP-dependent protein kinase (430, 438).

In liver tissue, glucagon via cAMP-dependent protein kinase increased the phosphorylation of pyruvate kinase, phosphofructokinase, and fructose-1-6-bisphosphatase. The phosphorylation of pyruvate kinase caused the inhibition of the conversion of phosphoenolpyruvate to pyruvate and increased the binding of ATP and alanine, both of which were inhibitors of this enzyme by decreasing the affinity of its substrate phosphoenolpyruvate (125, 246, 372). It is not known whether a similar mechanism exists in heart muscle.

Phosphofructokinase, which converts fructose-6-phosphate to fructose-1-6-phosphate, is one of the major regulating sites in the glycolytic path. Muscle phosphofructokinase was inhibited by Mg ATP, and citrate and was activated by fructose-6-phosphate, fructose-1-6-diphosphate ADP, 5′-AMP inorganic phosphate, and NH_4. However, increased rates of glycolysis and activation of phosphofructokinase can occur in the absence of changes in ATP, AMP, and inorganic phosphate (340). Thus, other control mechanisms besides substrate and product concentrations are probably operative. The addition of glucagon or cAMP to rat hepatocytes caused a reduction in the concentration of fructose-1-6-bisphosphate, and an inhibition of phosphofructokinase activity and phosphorylation of the enzyme (214, 372–374). It has been shown that the phosphorylation of phosphofructokinase was not related to the activity of the enzyme (81). However, a powerful activator of this enzyme was isolated from liver tissue and synthetized and was shown to be fructose-2-6-bisphosphate (373). This compound was a highly active allosteric activator of phosphofructokinase, which could overcome ATP inhibition. The mechanism of this glucagon inhibition of phosphofructokinase activity could be due to an enhanced degradation of fructose-2-6-bisphosphate. An enzyme that catalyzes the dephosphorylation of fructose-2-6-bisphosphate has been isolated from liver and required Mg^{++} and ATP. This enzyme was inactivated in the presence of the catalytic subunit of the cAMP-dependent protein kinase. El-Maghrabi et al. (120) and Pilkis et al. (374) suggest that the mechanism by which glucagon inhibits the formation of fructose-2-6-bisphosphate may involve the phosphorylation of the 6-phosphofructo-2-kinase by cAMP-dependent kinase. All these findings have been made in liver tissue, and it is clear that in this organ the inhibition of phosphofructokinase by glucagon would inhibit glycolysis and promote gluconeogenesis. It would be of interest to see whether these reactions are also operative in cardiac muscle and whether they are affected by glucagon, work, hypoxia, anoxia, diabetes, and cardiac ischemia.

Since glucagon increases the work of the heart, it will

increase oxygen consumption and turnover rate of the citrate cycle and the beta-oxidation of fatty acids. This stimulatory effect is presumably due to a decline in cardiac ATP and an increase in ADP and AMP. Fossel et al. (145) and Morgan (329) have used phosphorus nuclear magnetic resonance spectroscopy to show that during diastole of an isolated heart, ATP and phosphocreatine was high, while during the course of systole, both phosphocreatine and ATP decreased. This suggested that during systole glycolysis would be increased, and actually it was shown that inorganic phosphate, sugar phosphates, and nicotinamide adenine dinucleotide (NAD^+) were highest during systole and lowest during diastole. These changes were blunted when pyruvate was added to the glucose-containing perfusate, suggesting that when glucose is the sole substrate, glucose oxidation is probably limited by the transport of reducing equivalents into mitochondria (232). This limitation was not seen when pyruvate was added. Other studies have shown that the addition of fatty acids to the perfusion fluid helps to sustain a higher concentration of high energy phosphate compounds than when glucose is the sole substrate in the perfusion fluid (341). These observations suggest that addition of pyruvate or fatty acid to the perfusion fluid of isolated hearts may prolong the life span of these preparations.

The above findings show that fatty acid oxidation is essential during an increased work load on the heart and the release of free fatty acids from the heart and adipose tissue are thus important for sustaining an increased work load of this organ. The increased fatty acid oxidation and the resulting accumulation of citrate during the glucagon-induced positive inotropic effect would thus counteract and limit the direct effects of glucagon on phosphorylase by reducing the passage of substrate via phosphofructokinase, which is inhibited by citrate.

cAMP is an important regulator of lipolysis (132). In general, one can state that all agents that increased lipolysis also increase cAMP in adipocytes. Thus, catecholamines, glucagon, cholera toxin, thyroid hormone, growth hormone, ACTH, LH, and methylxanthines all increase lipolysis, as well as cAMP in adipocytes. Insulin, on the other hand, inhibits lipolysis in adipose tissue. In the intact animal, glucagon effects will be modified by the interaction of glucose and insulin and significantly influence glucagon-induced lipidemia. Other substances that inhibit lipolysis are PGE, adenosine, and alpha$_2$-adrenergic agonists.

Protein kinase activity was increased by cAMP, both in adipocytes and in muscle, and, in the presence of ATP, lipase activity was increased. This increased lipase activity is probably due to the transfer of a phosphate from ATP to the lipase, and this activation of triglyceride lipase could be readily reversed by a Mg^{++}-dependent lipase phosphatase. [For a recent review, see Fain (132).] The intravenous administration of glucagon produced a rapid release of insulin, which inhibited lipolysis produced by glucagon (260). This inhibitory effect of insulin on lipolysis probably explains the transient course of the lipidemia in normal man, especially so since in insulin-dependent diabetics, glucagon produced a marked increase in plasma glycerol concentration (an index of lipolysis), while similar doses in normal man produced a reduction in the plasma glycerol concentration (276, 419). It is thus likely that in man insulin release, as well as glucagon-induced adrenergic stimulation, may play a role in modifying lipolysis due to glucagon (260).

In contrast to catecholamines, glucagon produced a slight lipidemia in intact normal dogs. Furthermore, glucagon had only minimal effects on the free fatty acid uptake of the heart, which was in marked contrast to the effects of catecholamines that increased the uptake of fatty acids (65, 319). This uptake of free fatty acids has been related to the extra oxygen consumption of the heart following the stimulation with isoproterenol and may be related to the cardiac irregularities frequently observed following adrenergic stimulation. The minimal effects of glucagon on fatty acid uptake may explain the low arrhythmogenic potential of this polypeptide.

An increased contractility will require the increased consumption of fatty acids. This is in line with the findings of Jesmok et al. (210), who observed that inotropic agents, such as ouabain, epinephrine, and glucagon, increased the release of glycerol from the heart. Glycerol release should be an index of triglyceride utilization by the heart and was probably caused by the increased contractility and work performance of the heart.

Triglycerols and other lipids are transported in body fluids by lipoproteins of different densities that contain a variety of apoproteins. These complexes solubilize the hydrophobic lipids and transport these lipids to the various organs. Chylomicrons, the largest of the lipoproteins, and VLDL (very low density lipoproteins) transport dietary and liver triglycerides and other lipids to peripheral tissues. Located in the capillaries of adipose and other peripheral tissues are lipoprotein lipases (clearing factor), which act on the chylomicron triglycerides and VLDL to release free fatty acids [see Tan (482)]. The free fatty acids can be taken up by various tissues and will be oxidized or resynthesized to triglycerides. A reciprocal relationship between the hormone-sensitive triglyceride lipase and lipoprotein lipase of peripheral tissues has been observed. Thus, lipoprotein lipase from adipose tissue is reduced in the fed state, while hormone-sensitive triglyceride lipase activity is increased. On refeeding, the adipose tissue lipoprotein lipase activity was increased, and triglyceride lipase activity was inhibited. It is possible that lipoprotein lipase activity in adipose tissue is more sensitive to product inhibition than is the triglyceride lipase. Thus, the available data suggest that lipolytic agents inhibit adipose tissue lipoprotein lipase, possibly by product inhibition, although direct effects of the lipolytic agents on the

enzyme complex have not been excluded. [For reviews, see Fain (132), Friedman et al. (150), Nilsson-Ehle (346), and Olivecrona et al. (352).]

Lipoprotein lipase is found in heart muscle, and this enzyme is released into the perfusion fluid when the heart is perfused with heparin (53). Cardiac lipoprotein lipase changes observed during a variety of physiological changes are frequently in the opposite direction of those observed in peripheral fat tissue (54, 387). Thus, cold exposure or starvation that decreased adipose tissue lipoprotein lipase, increased cardiac lipoprotein lipase activity. Furthermore, Alousi and Mallov (10) have shown that cardiac lipoprotein lipase is increased by the chronic administration of epinephrine and thyroid hormone, and both procedures are known to decrease lipoprotein lipase activity in epididymal fat tissue. Feeding experiments have shown that both a high glucose or a fat intake depress cardiac lipoprotein lipase (10), and refeeding experiments of starved rats conducted by Borensztajn et al. (52) have shown a reduction in cardiac tissue and an increase in adipose tissue lipoprotein lipase.

Glucagon had minimal effects on cardiac lipoprotein lipase in starved rats, but it increased lipoprotein lipase activity in adipose tissue. However, when the starved rats were refed with glucose, glucagon inhibited the expected decrease in cardiac lipoprotein lipase activity (52). Oscai (358) administered 0.5 to 0.10 μg of glucagon per rat. The high dose of glucagon increased the level of heparin nonreleasable lipoprotein lipase of heart tissue and reduced the cardiac concentrations of free fatty acids and glycerol esters. On the other hand, low doses of glucagon resulted in a reduction of heparin nonreleasable lipoprotein lipase activity and increased the concentration of free fatty acids. It is quite possible that glucagon caused insulin release from the pancreas and thus counteracted the effects of the low dose of glucagon.

The main function of adipose tissue is storage of lipids, which can be released when energy requirements of peripheral tissues are increased. The heart, on the other hand, is mainly an energy-consuming organ and has relatively low lipid storage capabilities. Thus, the major source of fatty acids for the heart will have to be derived from plasma lipids. To accomplish this efficiently, the cardiac lipoprotein lipase is possibly less sensitive to inhibition by the fatty acids released by the hormone-sensitive triglyceride lipase, thus producing a constant supply of fatty acids to the heart. Glucagon, by increasing cardiac work, will cause an increase in fatty acid oxidation. The fatty acids will be supplied by triglyceride hydrolysis by the hormone-sensitive lipase and by the increased activity of the cardiac lipoprotein lipase, which is increased directly by glucagon, as well as indirectly by the increased cardiac work.

VI. Glucagon and Protein Phosphorylation

When glucagon combines with its receptor, it stimulates adenyl cyclase to form cAMP from ATP. cAMP then combines with inactive cAMP-dependent kinase to form the active form of this enzyme. The active form is the catalyst that in the presence of Mg^{++} and ATP phosphorylates a variety of proteins. There are at least two types (types I and II) of cAMP-dependent protein kinases; however, the mechanism of activation by cAMP seems to have similar specificities and rate constants and shows slight differences in molecular weight and rate of dissociation. [For reviews, see Beavo and Mumby (28), Barany and Barany (23), Krebs and Beavo (246), and Stull and Mayer (470).] In bovine and guinea pig cardiac muscle, the type II enzyme predominates (88); however, rat and mouse ventricles and human atria had about equal amounts of those isoenzymes. About 30% to 50% of the activity of cAMP-dependent protein kinase was found in the particulate fraction, and this fraction is most probably of type II, while type I isoenzyme predominated in the soluble fraction. This suggests compartmentalization of this enzyme system (470). Keely et al (222) have shown that glucagon, as well as epinephrine, increased the activity of the cAMP-dependent kinase in heart muscle at a rate proportional to the increase in the concentration of cAMP. Thus, the in vivo data support the in vitro findings that cAMP production activates the cAMP-dependent kinase under the influence of hormonal stimuli.

The cAMP-dependent kinase in the presence of ATP can phosphorylate a variety of proteins, and it is desirable to demonstrate such a phosphorylation under physiological conditions.

cAMP kinase will phosphorylate cardiac membrane fractions rich in sarcolemmal, as well as endoplasmic reticulum. In both membrane preparations, cAMP kinase will phosphorylate proteins of an apparent molecular weight of 20,000 to 24,000, which are distinct from the Ca^{++}-sensitive ATPase (molecular weight, 95,000) found in these membrane preparations. This phosphorylated protein has been called phospholamban (217, 475–477), and in cardiac tissue, it is an insoluble membrane protein found in both sarcolemmal and endoplasmic reticulum enriched membranes. The presence of phospholamban in both sarcolemma and endoplasmic reticulum probably plays in important role in the regulation of calcium fluxes into and out of these membranes. Phosphorylated sarcolemmal protein was formed in the presence of ATP and cAMP protein kinase, and this increased calcium binding by these membranes (202, 473, 519). The similarities between sarcolemmal and sarcoplasmic reticulum cAMP-dependent protein kinases, as well as calcium accumulation, suggest that these membranes have many properties in common. However, all these membrane preparations are not pure, and thus results must be interpreted with caution.

The phosphorylation of cardiac sarcoplasmic reticulum enriched microsomal preparation has been shown to occur, both in vitro (475–477) and in vivo (139). The latter authors obtained microsomal preparations from

isoproterenol-treated hearts and hearts from control subjects and have shown that isoproterenol administration increased the calcium binding of these sarcoplasmic preparations over control preparations.

Reversibility of these membrane phosphorylations could be controlled by inhibitors of phosphorylation and/or phosphatases. Two relatively low molecular weight, heat-stable inhibitors of cAMP-dependent protein kinase have been isolated from many tissues (28). These inhibitors were found to be specific since they did not inhibit cAMP-dependent protein kinase. Walsh and Ashby (508) have reported that this inhibitory activity could be modulated by hormones; however, it is by no means clear whether this inhibitory substance plays a physiological role in cardiac or smooth muscle.

Dephosphorylation of the membrane protein is accomplished by specific and nonspecific protein phosphatases, which reverse the effects of the protein kinase. The specificity of at least some protein phosphatases will be essential for the fine tuning of muscle contraction, and this could be attained either by substrate specific protein phosphatases, or by compartmentalization of a nonspecific enzyme in the vicinity of the substrate. [For a review, see Brunton et al. (64) and Stull (465).]

Several phosphorylation reactions involving protein kinases and specific muscle proteins have been described. Thus, troponin I obtained from skeletal muscle was phosphorylated by cAMP-dependent protein kinase (20, 22, 124). However, the physiological importance of this is not clear since neither electrical or catecholamine stimulation of intact muscle seemed to affect the degree of phosphorylation of troponin (468).

Purified troponin from cardiac muscle was phosphorylated by cAMP-dependent protein kinase (83, 388, 389). This phosphorylation occurred in cardiac troponin I and could also be demonstrated in the isolated heart exposed to epinephrine (440). The results suggest that cAMP produced a rapid phosphorylation of troponin I in cardiac muscle. Most of the radioactive phosphate incorporated was into troponin I, although other components were also phosphorylated and this suggests that cAMP protein kinase may not be the only kinase responsible for the phosphorylation of troponin (467). England (122) was first to show that the inotropic state produced with epinephrine was associated with the phosphorylation of troponin I. However, in a later publication, England (123) has shown that on reversing the isoproterenol-induced inotropic state by washing, the phosphorylation of troponin I was not reversed, thus indicating that troponin I phosphorylation is not always correlated with the inotropic state. Furthermore, Ezrailson et al. (129) have shown that ouabain, increased frequency, and increased calcium concentration did not increase the phosphorylation of troponin I, although a positive inotropic response was produced in the perfused hearts. England (123) also perfused hearts with glucagon, which increased the cAMP content, but there was no good correlation between the inotropic effect and troponin phosphorylation. The inotropic response preceded the troponin phosphorylation, and relative to the increase in cAMP, the extent of troponin phosphorylation was relatively small. The phosphorylation of troponin decreased the Ca^{++} sensitivity of actomyosin ATPase, and it has been postulated that troponin phosphorylation is related to the reduced relaxation time of cardiac muscle produced by catecholamines. However, even this point correlates poorly since the experiments of England (123) have shown that the troponin phosphorylation persists after the catecholamine effects on the contractile force have been reversed by washing.

Myosin of muscle consists of two heavy chains and two smaller polypeptides known as light chains. Perrie et al. (367, 368) have shown that the light chain of myosin existed in phosphorylated and nonphosphorylated forms, which could be interconverted by a phosphorylase kinase and phosphatase. In a later publication, Pires et al. (375) demonstrated a distinct protein kinase with specificity for the myosin light chain, which was different from the cAMP-dependent protein kinase. This myosin light chain kinase was dependent on the presence of calcium and another protein known as the calcium-dependent regulator protein or calmodulin, which also regulated the activity of brain cyclic nucleotide phosphodiesterase and adenylate cyclase.

Reddy et al. (390) reported that cAMP-dependent protein kinase catalyzed the incorporation of phosphate into the myosin light chain. However, this finding was not confirmed (466) and probably was related to the propensity of cAMP protein kinase to phosphorylate denatured proteins (69).

Frearson et al. (147) claimed that epinephrine infusions decreased the phosphate content of the myosin light chain isolated from rabbit heart; however, this finding has not been confirmed by Holroyde et al. (197) and Stull et al. (469). [For a review, see Stull (469).] It is thus likely that in cardiac muscle, cAMP production will not affect myosin light chain phosphorylation, and it is difficult to assign a functional role to phosphorylation of cardiac myosin light chains.

With smooth muscle, the evidence is good that the phosphorylation of the myosin light chain plays an important role in the contraction of this type of muscle. The evidence is consistent with the view that Ca^{++}, calmodulin, and a myosin light chain kinase are essential for the phosphorylation of the myosin light chain. [See Conti and Adelstein (86, 87).] Phosphorylation of this light chain allows interaction of myosin with actin to stimulate ATPase activity and contractility of smooth muscle. Thus, removal of calcium from the bathing solution resulted in a reduction of the phosphate content of the myosin light chain in carotid arteries of the pig (26). Aksoy and Murphy (8) have demonstrated a temporal relation between the phosphorylation of the myosin light chain and force generation in intact carotid arteries.

Contraction of arterial smooth muscle with high potassium concentrations caused an increase in the phosphate content of the myosin light chain, which preceded the contraction of the muscle. Washing the muscle caused relaxation and a rapid reduction in the phosphate content of the myosin light chain.

Contractions of smooth muscle produced by a variety of agents were counteracted by phenothiazines. This is possibly due to the inhibition of the Ca^{++} calmodulin activation of the myosin light chain kinase, which catalyzed the phosphorylation of smooth muscle myosin light chains.

Coronary artery cAMP content was increased in the presence of adrenergic agents (425), and Silver et al. (434) have shown that the activity of the cAMP-dependent protein kinase was increased in these arteries. Smooth muscle myosin light chain kinase can be phosphorylated by cAMP-dependent protein kinase, which markedly decreased the affinity of Ca^{++} calmodulin to the myosin light chain kinase. This will reduce the phosphorylation of the light chain, which in turn will reduce the ATPase activity and contraction of smooth muscle actomyosin (4, 87). The phosphorylation of the myosin light chain kinase in the presence of beta-adrenergic agonists is a possible explanation for the relaxation of smooth muscle produced by these agents. Glucagon relaxes smooth muscle of arteries and may activate the cyclase to produce cAMP. Thus, a possible mechanism of this relaxing effect of glucagon on smooth muscle may be the phosphorylation of the myosin light chain kinase by cAMP-dependent kinase.

Krebs and Beavo (246) have proposed four criteria for establishing a physiological role for phosphorylation and dephosphorylation of various enzymes and proteins: 1) Show that the purified enzyme or protein can be phosphorylated at a significant rate by a protein kinase and can also be dephosphorylated by a phosphoprotein phosphatase. 2) Correlated functional changes with changes in phosphate content of the protein or enzyme. 3) Show that the protein or enzyme can be phosphorylated and dephosphorylated under in vivo conditions, which correlate with the functional changes. 4) Correlation of the levels of protein kinase and phosphoprotein phosphatase activity with the levels of phosphorylation of the protein or enzyme.

These criteria have not been satisfied with cardiac and striated muscle protein phosphorylation. The data obtained with smooth muscle fulfill most of these criteria, and it is thus possible that beta-adrenergic and glucagon-induced relaxation of vascular smooth muscle may be related to the phosphorylation and dephosphorylation of the myosin light chain kinase of smooth muscle.

VII. Glucagon, Calcium Ions, and Cardiac Contractility

The relation between ATP and the effects of calcium ion transport was first described by Hasselbach and Makinose (181, 182). These investigators observed that an ATP-requiring calcium sequestering mechanism inhibited the muscle protein contractions by lowering the Ca^{++} concentration in the medium that caused the release of bound Ca^{++} from the muscle protein. It is well-known that cAMP stimulates the uptake of Ca^{++} by the sarcoplasmic reticulum and plasma-membrane-enriched fraction (475, 476). [For reviews, see Hui et al. (202) and Katz (217).] This reaction was mediated by a cAMP-dependent protein kinase that catalyzed phospholamban (218). It is the phosphorylated phosphalamban that increased the Ca^{++} turnover rate in the membrane preparation. It is likely that both passive and active Ca^{++} movements across the sarcolemmal and sarcoplasmic membranes are at least partially regulated by cAMP (131). Calcium ion controls the overshoot and the phase 2 of the cardiac action potential and is also responsible for the so-called slow potential that can be observed in partially dipolarized cardiac tissue (394–397). This slow potential requires calcium or a calcium substitute. The slow action potential is blocked by a variety of calcium blockers, and it is probable that this calcium-dependent current is important for coupling the action potential to the contraction process (444).

Since cAMP would tend to increase Ca^{++} uptake, as well as release from the sarcoplasmic reticulum and possibly the plasma membrane (202), glucagon should increase both the contractility and relaxation of cardiac muscle. The data presented have shown that under appropriate conditions glucagon will increase the rate of rise of the contractile response, will decrease the time to peak tension, and increase the rate of relaxation (130). Keely et al. (222) have shown that glucagon increased cAMP levels and protein kinase activity, whereas Entman et al. (126) observed that glucagon increased Ca^{++} accumulation in a cardiac microsomal fraction, which was not blocked by a beta-adrenergic blocking agent. [For further details, see Katz (217).] The increased contractile response to glucagon was accompanied by an increase in the exchange of cardiac Ca^{++} (339), and Frangakis and McDaniel (146) have shown that glucagon, as well as cAMP, increased the uptake of radiolabelled calcium by isolated cardiac cells. Barritt and Spiel (25) have studied the intracellular calcium distribution and have concluded that glucagon increased the rate of transfer of rapidly exchanging calcium fraction and increased the quantity of an intracellular exchangeable calcium compartment, which included mitochondria. Mitochondrial uptake of Ca^{++} was increased by glucagon (57, 152); however, the significance of this with regard to the effects of glucagon on muscle contraction is not clear.

Blinks and his coworkers have developed an elegant technique for measuring free intracellular calcium (9, 43, 45). They introduced a calcium-binding protein, aequorin, into the cell. When aqeuorin binds calcium ion, it emits a light signal, which can be recorded and is

roughly related to the Ca^{++} concentration. With this methodology, Blinks and his coworkers have shown that several inotropic drugs will increase the contractile force and the aequorin signal, thus indicating an increase in the intracellular calcium ion concentration. Glucagon increased the light energy of the aequorin signal, decreased the time to peak light and peak tension, and increased the rate of decline of the light signal (44). The decline in the light signal is possibly related to the rate of Ca^{++} sequestration by the endoplasmic reticulum.

Since glucagon increased cAMP content of cardiac tissue, the changes in light intensity and calcium exchange are possibly related to the cAMP-induced phosphorylation of the plasma and reticular membranes (217). Calcium activation in heart muscle independent of cAMP has been demonstrated in cardiac muscle by Blinks et al. (43, 44). Thus, rate changes have profound effects on both the contractile force and intracellular Ca^{++} concentration, but are not accompanied by increases in the concentration of cAMP.

VIII. Desensitization to Glucagon

The exposure of an isolated cell membrane or isolated tissue to a hormone is frequently followed by a reduction of the response to a second dose. Repeated in vivo administration of beta-adrenergic agonists to asthma patients has caused a reduction in beta-agonist stimulation of leukocytes (163), and in rats repeated administration of a beta-agonist produced a reduced cAMP response and receptor binding in lung membranes (418). This phenomenon, known as tachyphylaxis or desensitization, is dependent upon the type of tissue and agonist, the concentration, time of exposure to the agonist, the composition of the medium, and requires ATP. A great deal of work on the desensitization of isolated cells and cell membranes has been published, and the interested reader is referred to reviews by Iyengar and Birnbaumer (208) and Perkins et al. (366). The refractoriness of cells could be caused by several specific phenomena. Thus, a short exposure to catecholamines produced an uncoupling of the receptor from the adenyl cyclase producing unit and resulted in an agonist-specific, but reversible loss of responsiveness. In the beta-adrenergic system, it has been shown that desensitization is accompanied by the inability of a Mg^{++} and guanine nucleotide to induce a high affinity complex with the agonist. Pretreatment of S49 cells with isoproterenol resulted in a reduction in the affinity of the agonist for the beta-adrenergic receptor in the presence of Mg^{++} and guanosine nucleotide.

With prolonged exposure to catecholamines, a slowly reversible nonspecific response to agents that increases cAMP has also been reported. This exposure reduced cAMP production by the beta-agonist, as well as to PGE_1. This nonspecific desensitization is probably related to a marked decrease in cAMP production, rather than an increase in cAMP inactivation by phosphodiesterase (80).

In some types of cells, increased destruction of cAMP by phosphodiesterase probably contributes to this nonspecific desensitization phenomenon.

Desensitization by glucagon in isolated plasma membranes requires ATP and Mg^{++} ions (209), and it has been suggested that a phosphorylation reaction is involved in this desensitization, especially so since a phosphoprotein phosphatase could reverse such a desensitization reaction (204).

Studies by Harden et al. (179) have shown that desensitized receptors, when placed on sucrose density gradient, will show two distinct peaks, while a similar procedure from control receptors showed only one peak. The appearance and disappearance of the new type receptor followed the changes in beta-adrenergic-stimulated cAMP production. By using bullfrog red blood cells, Chuang and Costa (77) reported that incubation of these cells with isoproterenol resulted in the detection of a soluble beta-receptor fraction, whereas in control cells the receptor was insoluble. All the above data suggest that agonist interaction with the receptor induced changes in the structure of the receptor protein.

Recently, Hirata et al. (190, 191) and Mallorga et al. (295) have shown that desensitization in astrocytoma cells with isoproterenol could be blocked with quinacrine. The desensitization was accompanied by increased activity of a phospholipase A_2 and an increase in the turnover rate of phosphatidylcholine, an increase in the release of arachidonic acid and a decrease in the number of beta-adrenergic receptors. These authors have suggested that the activity of phospholipase A_2, which is blocked by quinacrine, may play a role in the desensitization phenomenon. Torda et al. (491) and Yamaguchi et al. (523) have shown that forced immobilization or repeated administration of isoproterenol to rats reduced the number of beta-receptors in the heart and spleen. This effect was accompanied by a reduction of the chronotropic and pressor responses in these rats. This reduction in responsiveness was blocked by the phospholipase A inhibitor, quinacrine. It has been suggested that desensitization could be related to the distribution and synthesis of phospholipids. The phospholipase A_2 could interfere with the cAMP synthesis via the production of lysophosphatidic acids, which could reduce the responsiveness of the cell to a beta-agonist. In relation to these findings, Berridge (35) has shown that in blowfly salivary glands, 5-hydroxytryptamine (5-HT) caused an increase in calcium transport which showed a typical desensitization following the addition of a second dose of 5-HT. This desensitization could be completely reversed by the addition of inositol.

It is apparent that a variety of mechanisms for desensitization have been proposed. These are probably not exclusive, and different mechanisms may be operative in different cells and with different receptor activators.

Decreased glycogenolysism of the liver to a second dose of glucagon in the whole animal and in isolated cells

and slices of liver (42, 376, 392) has been demonstrated. [For opposite results, see Srikant et al. (446).] This reduction in response to glucagon had specificity since it did not reduce the response to a catecholamine, nor did a second dose of glucagon affect the release of K^+ from liver tissue (118). There was no reduction in receptor binding, nor was the desensitization due to an increased destruction or loss of cAMP, and the membrane preparation responded normally to the addition of fluoride ion. Uncoupling of the receptor has been suggested as a possible mechanism, and a phosphorylation reaction requiring either ATP or GTP has been invoked (48, 128, 209).

In heart tissue, glucagon produced a desensitization to a second dose of the inotropic and chronotropic responses (136, 291, 408). On the other hand, in the intact dog or cat, repeated injections of glucagon given at about hourly intervals did not show any significant sign of reduction in the response of cardiac contractile force or heart rate. No specific studies on the mechanism of desensitization of the heart or smooth muscle to glucagon have been reported.

IX. SUMMARY

Glucagon is a vasodilator substance that reduces blood pressure via a decreased vascular resistance in the splanchnic and hepatic vasculature. Species differences in the response of various vascular beds to glucagon have been documented.

In the kidney, glucagon in relatively large doses increased renal plasma flow, glomerular filtration, and electrolyte excretion. It has been shown that intraarterial injection of glucagon into the renal artery can produce an increase in electrolyte excretion on the side that received an injection with minimal or no changes in glomerular filtration. This indicated a direct tubular effect of this polypeptide. This effect may be related to the increased glomerular filtration observed in poorly controlled diabetics where insulin concentrations are low and glucagon concentrations are high. The tubular effects of glucagon are probably mediated via cAMP and prostaglandin formation in renal tubular cells, especially the ascending limbs of Henle and collecting ducts. Glucagon increases the RNA concentration in glomerular tissue, and this effect is probably independent of cAMP. The latter effect of glucagon has been related to the glomerular enlargement and membrane thickening observed in poorly controlled insulin-dependent diabetics. Starvation natriuresis has been related to increased concentrations of glucagon in blood. The likely mechanism is that glucagon increased the renal excretion of organic acids, possibly by inhibiting the renal tubular reabsorption of these acids. Little is known concerning the effects of glucagon on the cAMP content of vascular smooth muscle. Indirect evidence suggests that such effects may be mediated via the production of cAMP. If this can be established, it would be likely that the glucagon-induced vasodilation is due to a cAMP-dependent phosphorylation of the myosin light chain kinase. This kinase shows reduced sensitivity to the Ca^{++} calmodulin complex when it is phosphorylated by the cAMP-dependent kinase and thus may produce relaxation of smooth muscle.

In cardiac muscle, glucagon produced positive inotropic and chronotropic effects. These effects show species differences and in some species activate only the auricle with minimal effects of ventricular muscle. The effects of glucagon in general resemble those of a beta-adrenergic agent; however, glucagon seems to be nonarrhythmogenic in a variety of cardiac preparations and its effects are not blocked by propranolol. In some of these experimental conditions the chronotropic effects of glucagon play an important role in the antiarrhythmogenic effects, although direct cardiac membrane effects have been postulated.

Several factors can modify the inotropic effects of glucagon. Thus, the severity, type, and chronicity of heart failure play a role in determining the cardiac response to glucagon. Glucagon effects on the heart are not blocked by pure beta-adrenergic blocking agents, but are blocked by mixed adrenergic agonist-antagonist agents.

In heart muscle glucagon can increase cAMP concentration and will increase intracellular Ca^{++} concentration via the cAMP-dependent and independent protein kinases.

A glucagon receptor has been described, and its relation to cAMP formation has been discussed. It is clearly different from the alpha- or beta-adrenergic receptors. However, the mechanism of cAMP formation produced by glucagon and beta-adrenergic agents seems to be quite similar. Glucagon probably caused the phosphorylation of endoplasmic and sarcolemmal membrane proteins via the phosphorylation of the low molecular protein "phospholamban." The phosphorylation of phospholamban may be related to an increased release, as well as uptake, of calcium by the endoplasmic reticulum. It is unlikely that the phosphorylation of troponin I of the thin filament of cardiac actomyosin can explain the increased contractility and relaxation rate produced by glucagon. In a similar vein the phosphorylation of the myosin light chain does not seem to be related to the cardiac effects of glucagon on contractile force.

Repeated administration of glucagon to the intact cat did not produce tachyphylaxis of the cardiac effects, although marked desensitization to glucagon of isolated cardiac tissue has been observed. Little is known concerning the mechanism of this desensitization.

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