ABSTRACT

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HISTAMINE RELEASE BY CORTISONE INDUCED HYPERGLYCEMIA

The purpose of this work was to determine in guinea-pigs whether or not a single injection of cortisone caused a detectable change in plasma histamine level, and whether or not this effect was a direct action of cortisone on histamine metabolism.

A single dose of cortisone caused a significant increase in plasma histamine level. Cortisone also increased the level of blood glucose. When cortisone-induced hyperglycemia was abolished by the injection of insulin, no rise in the level of plasma histamine occurred. The increase in plasma histamine level following a single cortisone injection could be duplicated by the administration of glucose. These results suggest that the increase in plasma histamine concentration induced by cortisone was mediated by the hyperglycemic effect of cortisone.

The chronic administration of cortisone or glucose over a period of 10 days showed that both substances decreased tissue histamine. Histamine depletion by cortisone was greater than that caused by glucose and could be partially blocked by daily injections of insulin.

HISTAMINE RELEASE BY CORTISONE INDUCED HYPERGLYCEMIA

by

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INTRODUCTION

It was reported earlier that prolonged administration of cortisone to guinea-pigs resulted in a fall in levels of tissue histamine, while no decrease in histamine content was found if treatment was maintained for less than 10 days (Kovacs, 1965). These findings were confirmed by Hicks (1965) who reported that prolonged administration of cortisone or dexamethasone over 14 to 28 days produced a gradual fall in tissue histamine levels, whereas no change in tissue histamine content occurred if single doses of cortisone, dexamethasone or fludrocortisone were given.

It has been suggested that the lowered tissue histamine content following prolonged glucocorticoid administration might explain in part some of the therapeutic and side effects of these drugs (Telford and West, 1960; Halpern, 1964; Zachariae, 1964; Kovacs, 1965; Hicks, 1965; Heisler and Kovacs, 1967). However, the mechanism of the therapeutic effect which occurs already hours after steroid therapy has been instituted is not known.

It was thought that although a single injection of cortisone does not cause detectable changes in tissue histamine levels of the guinea-pig, histamine release might

still be involved, which could then be reflected in the plasma. Plasma glucose levels following cortisone administration were also determined, since it has often been stated that the relative effectiveness of glucocorticoids in allergic diseases parallels their effectiveness in regulating carbohydrate metabolism (West, 1959; Telford and West, 1960), and according to Glenn (1961), the hyperglycemic effect of cortisone is the first measurable metabolic change.

The results presented here show that in the guinea-pig an increase in plasma histamine concentration occurred following a single dose of cortisone. This increase was mediated by the hyperglycemic effect of cortisone and could be duplicated by glucose.

I. HISTORICAL REVIEW

1. THE ACTION OF CORTISONE ON HISTAMINE METABOLISM

A. Histamine and the Adrenal Cortex

Considerable evidence has accumulated in the past 20 years to indicate a strong connection between adrenal steroids and histamine metabolism. Although the adrenal cortical hormones are not in pharmacological terms, true 'antihistamine', they do under various conditions increase the resistance of laboratory animals to histamine toxicity.

A functional relationship between histamine and the adrenal cortex was postulated by Dale in 1920, when he found that after adrenalectomy cats became about five times more sensitive to intravenous injections of histamine. A similar result was subsequently obtained by other investigators using different species, such as the rat (Voegtlin and Dyer, 1925; Scott, 1927), guinea-pig Banting and Gairns, 1926) and mice (Halpern and Wood, 1950a; Muñoz and Schuchardt, 1953, 1954). Halpern and co-workers (1952a, 1952b) reported that adrenalectomy increased histamine toxicity fifty-fold in mice, and treatment with cortisone and adrenaline (both of which alone only had a

slight restorative effect) brought the resistance of the adrenalectomized animals up to the normal level. Promethazine also produced a similar effect (Halpern and Wood, 1950b). Selye (1949) and Halpern (1956) showed that the increased susceptibility of adrenalectomized rats to histamine could be counteracted by cortisone treatment.

These findings prompted a search for a more specific relationship between the adrenal glands and histamine. In 1940, Karady et al found that the histaminase activities of rat tissues were markedly inhibited by adrenalectomy, and that these effects were partially prevented by administration of adrenal cortical extracts. In guinea-pigs, Kahlson et al (1953) also reported a loss of tissue histaminase activity following removal of the adrenal glands.

As adrenalectomy inhibits the inactivation of histamine in the body, the amounts of histamine in the tissues may be raised: this theory has been investigated in rats by many workers and increases in the histamine contents of the tissues of this species have been found. The original observations were made in 1941 by Rose and Browne who found that histamine levels of certain tissues were increased in adrenalectomized rats. This effect was more pronounced if the animals were maintained on water rather

than saline. Similar findings have been reported by Marshall (1943). Hicks and West (1958a) found little difference in tissue histamine levels of adrenalectomized rats maintained on saline but confirmed the marked increase in tissue histamine levels in animals maintained on water. Bartlet and Lockett (1959) in addition to showing an increase in tissue histamine levels of adrenalectomized rats, found that restoration of skin histamine following depletion by Compound 48/80 was accelerated by adrenalectomy. Telford and West (1961a) also reported an increase in tissue histamine levels in adrenalectomized rats. In guinea-pigs, Kovacs (1965) found an increase in pulmonary and skin histamine levels following the administration of metyrapone. The increases in the histamine contents of rat tissues after adrenalectomy are likely to be the result of increases in the histamine binding capacity of the tissues rather than to changes in histaminase activity as has been suggested by the work of Schayer (1955, 1956a), who reported that after adrenalectomy the skin and lungs have a greater ability to bind C^{14}-histamine formed <u>in vitro</u> from C^{14}-histidine.

B. Cortisone and Histamine Metabolism

a) Effect of Cortisone on Reaccumulation and Binding Histamine

Goth et al (1951) showed that injection of Tween 20 in dogs resulted in a drop in blood pressure and a second injection 24-48 hours later again produced a hypotensive effect. However, if the dogs were pretreated with cortisone, only the first injection of Tween 20 was effective in lowering the blood pressure, the second was not. This was interpreted as an inhibiting effect of cortisone on the reaccumulation of histamine.

Studies of Halpern and his co-workers (Halpern, 1956; Halpern and Briot, 1956) have shown that cortisone can interfere with the biosynthesis of histamine upon prolonged treatment. Halpern (1964) reviewed several of his experiments in which tissue histamine content was lowered in the rat by the administration of a histamine releaser, such as Compound 48/80 or 1935L. Concurrent cortisone treatment in experimental animals, similarly depleted, revealed that the restoration of histamine to normal levels, after the treatment ceased, was delayed. This confirmed Goth's suggestion (1951) that cortisone retards the restoration process of tissue histamine levels

after depletion by histamine releasers. Schayer and co-workers (Schayer et al, 1954; Schayer, 1959), utilizing radioactively labelled C^{14}-histidine, were also able to show the inhibitory effect cortisone has on the rate of binding of newly formed histamine. The effect was also shown in vitro (Schayer et al, 1955).

b) Effect of Cortisone on Histidine Decarboxylase Activity

The inhibitory effect of glucocorticoids on binding of C^{14}-histamine was extended to the study of their effects on histidine decarboxylase activity.

Schayer (1956b) reported that histamine formation by rat lung was reduced by treatment with cortisone and increased by adrenalectomy. This effect was interpreted by the author as an effect on histidine decarboxylase activity. Histidine decarboxylase activity in the mouse lung was similarly affected (Schayer, 1963). Schayer (1966), in reviewing the literature, stated that although the adrenal cortex influenced the rate of formation and binding of new histamine in rat tissues, only in the lung, and possibly muscle, could the effect be attributed to changes in the activity of histidine decarboxylase.

Telford and West (1961b) studied the action of glucocorticoids on the histidine decarboxylase activity of the rat liver and stomach. They found that liver histidine decarboxylase activity was suppressed while that of the pyloric stomach was increased. Exposure of rats to cold produced a similar reduction in liver histidine decarboxylase activity and increase in the stomach. Schayer (1960, 1963) found that cold increased the enzyme activity in the mouse skin, and guinea-pig lung, whereas the histidine decarboxylase activity of rat lung was reduced to a value below normal. The suppression of histidine decarboxylase activity of some rat tissues in moderate stress was attributed to glucocorticoid release and reflects the pronounced response of this species to these hormones (Schayer, 1966).

c) Effect of Cortisone on Tissue Histamine Levels

Hicks and West (1958b), Cass and Marshall (1962), showed that treatment with glucocorticoids in the rat, caused a significant fall in histamine levels in many tissues. Telford and West (1960) have reported that chronic administration of glucocorticoids depleted some rat tissues (duodenum, jejunum, ileum) and increased histamine content in others (notably the fundic and pyloric stomach).

There exists a species difference in regard to the effect of cortisone on gastric histamine levels of rats and guinea-pigs. Following glucocorticoid administration, Telford and West (1960) reported elevated histamine levels in the stomach of the rat. Schayer (1956) found an increase in histamine binding in the rat stomach after glucocorticoid treatment; he also reported that glucocorticoids increased histamine formation in vitro (Schayer, 1956) and in vivo (Schayer, 1959) in the rat pyloric stomach. Studies on guinea-pigs by Heisler and Kovacs (1967) showed that cortisone administration for 10 days produced a significant decrease in the histamine level of the intact stomach and one cortisone injection in pylorus ligated guinea-pigs already produced a fall in gastric histamine levels.

An effect of cortisone on tissue histamine levels in the guinea-pig was reported by Kovacs (1965), who found that on repeated administration for at least 10 days, a significant fall in the average histamine content of guinea-pig tissues could be achieved. Greatest decrease in histamine content occurred in the lung and liver. Similar findings were reported by Hicks (1965), who also found that prolonged treatment with cortisone over a period of 14-28 days produced a gradual fall in tissue histamine

levels. He suggested that the fall in tissue histamine levels produced by cortisone may be a result of removal of histamine from tissues by normal turnover processes with accompanying inhibition of replenishment.

d) Effect of Cortisone on Blood Histamine Levels

Code and Mitchell (1953) demonstrated that administration of cortisone reduced the concentration of whole blood histamine levels in the guinea-pig, dog, and human beings.

Rose and Browne (1940) reported a decrease in whole blood histamine levels of patients during surgical shock and post-traumatic shock. This effect of shock on blood histamine was interpreted as the result of a decreased number of circulating basophil leucocytes in response to an increased secretion of adrenocortical hormones. Code and Mitchell (1957) and Noah and Brand (1957) had shown that administration of cortisone lowered the whole blood histamine level and the basophil cells in a parallel manner. The same was found by Mitchell and Cass (1959) and Lindell and Westling (1966).

Rose et al (1951) and Grob (1952) reported a transitory increase in the total output of histamine in the urine when cortisone was given to patients who had various

allergic diseases. An increased urinary excretion of free histamine in healthy subjects was found by Mitchell and Code (1954); Mitchell et al (1954). Dieckhoff (1957a) found a regular and relatively pronounced increase in the urinary histamine levels upon the administration of cortisone to children with asthma and eczema, but found (Dieckhoff, 1957b) no clearcut changes in urinary histamine output in the healthy child during treatment with cortisone.

2. THE EFFECT OF GLUCOCORTICOIDS ON CARBOHYDRATE METABOLISM

A. Carbohydrate Metabolism and the Adrenal Gland

The effect of the adrenal glands on carbohydrate metabolism was noted in 1908, when Bierry and Malloizel reported that after adrenalectomy in dogs, the blood sugar might decrease one-half to one-fifth of the original level. Porges (1909) confirmed this observation and Schwartz (1910) noted the disappearance of glycogen from the liver of the rat following the removal of the adrenal glands. Kuriyama (1918) reported that adrenalectomized rats could maintain liver glycogen provided they took food normally. After fasting, however, difficulty in storing glycogen was noted.

Rogoff and Stewart (1926) and Hartman et al (1927) believed that the reduction in blood sugar level of the adrenalectomized dog and cat was a terminal effect. It was also thought to be due to or exaggerated by the trauma of adrenalectomy (Banting and Gairns, 1926).

In 1926, Zwemer showed that the oral administration of glucose to adrenalectomized cats prolonged their survival to some 220 hours, which is about 4 times longer than that of untreated controls. Cori and Cori (1927) reported that after fasting, the liver glycogen of the adrenalectomized rats disappeared, and a low blood sugar was present. Muscle

glycogen level was unaffected. These authors also found that the adrenalectomized animals could form glycogen when they were fed glucose.

Data gathered from man and dog with adrenal insufficiency favoured, for some time, the view that electrolyte regulation was the primary and prepotent concern of the adrenal cortex (Lucas, 1926; Rogoff and Stewart, 1926; Hastings and Compere, 1931; Loeb, 1932; Harrop et al, 1933) and the lowering of the blood sugar and glycogen levels was ascribed to the poor food intake due to anorexia. It was concluded that the carbohydrate disturbances following adrenalectomy were only secondary to the electrolyte shifts (Loeb, 1933; Harrop et al, 1935; Allers and Kendall, 1937).

Britton and Silvette and colleagues (Britton, 1930; Britton and Silvette, 1934; 1937; Britton et al, 1938) studied carbohydrate changes after adrenalectomy, and the effects of treatment with cortical extracts in many species. They emphasized that the disturbances in carbohydrate levels (the blood sugar and glycogen changes) were more apparent than the shifts in water, sodium and potassium distribution. These authors found that the liver glycogen was depleted and some degree of hypoglycemia was present in adrenalectomized rats, guinea-pigs, marmots, cats, dogs and donkeys (Britton and Silvette, 1931, 1932a, 1934, 1937, 1938; Britton et al, 1938). Animals with low liver glycogen

levels did not show the usual hyperglycemia following emotional stress or adrenaline injection (Britton and Silvette, 1934, 1938). Treatment with cortical extract was found to cause an increase in blood sugar and glycogen deposition in normal as well as adrenalectomized animals (Britton and Silvette, 1931, 1932b, 1938), whereas treatment with salts had no effect on blood sugar levels. As a result of these studies it was suggested that the cause of death after adrenalectomy was predominately a failure in carbohydrate metabolism.

B. Metabolic Effects of Glucocorticoids

a) Maintenance of Body Stores of Carbohydrates by Cortisone

During fasting, in intact animals, the maintenance of normal blood sugar values is due to the ability to manufacture glucose from non-carbohydrate sources. In normal animals, fasting causes a mobilization of fat and protein. The fatty acids are either used directly, in many tissues, or transported to the liver and transformed, in part, to "ketone bodies", which are then readily utilized for the production of energy. The amino acids, resulting from protein mobilization, are deaminated

in the liver and their keto-residues are, for the most part, transformed to glucose-6-phosphate, the immediate precursor of blood glucose (Levine and Goldstein, 1964).

The classic experiments of Long et al (1940) showed that adrenal insufficiency in animals is characterized by low blood glucose levels during fasting, depletion of liver but not muscle glycogen, decreased excretion of urinary nitrogen, high standard respiratory quotient and increased sensitivity to insulin. Similar findings in patients with Addison's disease were reported by Thorn et al (1940). In both cases, the metabolic disturbances could be reversed by the administration of cortisone.

Long and colleagues (Long et al, 1940; Long, 1942) showed that administration of cortisone and related glucocorticoids permitted the fasting, adrenalectomized animals to maintain body carbohydrate stores in the face of continued utilization, i.e., these steroids promote gluconeogenesis. Similar findings have been reported by Ingle (1942), Swingle and Remington (1944). This conclusion was strengthened by the fact that the administration of glucocorticoids during fasting led to an increased nitrogen excretion in the urine (Long, 1942; Ingle, 1944, 1949).

The influence of glucocorticoids in clinical and experimental diabetes has been studied by Long and Lukens (1936), Bloomfield (1939), Wells (1940), Wells and Kendall (1940) and Ingle (1944). They showed that the removal of the adrenal glands from the depancreatized, phlorhizinized or alloxanized animal sharply diminished the degree of glycosuria, the level of blood sugar and the extent of nitrogen excretion during fasting. Ketosis was also decreased or abolished, and death in hypoglycemia occurred, if the fasting period was prolonged. On the other hand, glucocorticoid administration rendered the adrenalectomized animal severely glycosuric even when fasting; and aggravated the degree of glycosuria and hyperglycemia of fed diabetic or diabetic, adrenalectomized preparations. In all such instances nitrogen excretion was increased (Wells and Kendall, 1940; Wells, 1940; Ingle, 1944).

b) Effect of Cortisone on Enzymatic Activities

Early studies on the influence of cortisone on enzyme systems have been reported by Verzar (1952), and Dorfman (1952).

Cohen (1951) reported that cortisone administration in mice and human beings resulted in an increase in -

serum glucuronidase activity. Kochakian and Robertson (1951) found that the liver and kidney arginase activity of mice is elevated after the injection of cortisone. Umbreit (1951) noted that the low activities of liver amino acids oxidase and kidney proline oxidase of adrenalectomized rats could be restored to normal by the administration of cortisone. An inhibitory effect of cortisone upon the activity of hyaluronidase in vivo both in animals and man has been demonstrated by Opsahl (1951). Umbreit (1950) pointed out that in the majority of studies, the rates of the various enzymatic reactions tended to be diminished or unchanged after adrenalectomy and to be increased after prior treatment with cortisone. He emphasized that no effect of cortisone upon enzyme systems has been clearly demonstrated in the absence of the intact cell. This fact suggested the possibility that cortisone acts by controlling the access of substrates or cofactors to the enzyme within the cell rather than directly acting on the enzyme itself (Thorn et al, 1954).

Recent studies on the action of cortisone in changing the activities of several enzyme systems involved in carbohydrate metabolism have not been able to clarify, whether these changes are primary as a result of the effect of cortisone on the enzyme system itself, or secondary

because of changes in substrate concentrations occurring from changes in metabolism at another level. Analysis of key rate limiting enzymatic processes suggested that glucose-6-phosphatase and alanine α-ketoglutarate transaminase might occupy pivotal positions insofar as glucocorticoid effects on carbohydrate metabolism are concerned (Ashmore and Weber, 1959; Nichol, 1961). Both were increased by cortisone or hydrocortisone, and decreased by adrenalectomy (Nichol, 1961). Pyruvic acid had also been demonstrated to occupy a key position in carbohydrate metabolism. Frawley and Shelley (1961) have shown that cortisone increased production and decreased removal of pyruvic acid. Landau et al (1962) reported that cortisone increased the conversion of CO_2 and pyruvate into glucose and glycogen. Since pyruvic acid can be formed from alanine by the action of alanine α-ketoglutarate transaminase, it was suggested that the increase in activity of this enzyme may be important in cortisone-induced gluconeogenesis from protein (Nichol, 1961).

In addition, it had been shown that certain enzymes necessary for aerobic glycolysis could be inhibited, whereas others involved in the pentose shunt (Huggins and Yao, 1959), gluconeogenesis (Nichol, 1961), and glycogenesis

(Rosen et al, 1959) could be stimulated by cortisone. Cortisone may uncouple oxidative phosphorylation, inhibit adenosine-triphosphatase (ATP) formation (Kerppola, 1960); inhibit cytochrome oxidase (Kerppola and Pitkanen, 1960); affect NADPH-NAD transhydrogenations (Talalay and Williams-Ashman, 1960); and inhibit the oxidation of NADH to NAD (Yielding and Tomkins, 1959), all of which may be involved in the effects of glucocorticoids on carbohydrate metabolism.

c) Effect of Cortisone on Carbohydrate Utilization

Ingle (1949) reported that, in addition to its effect on gluconeogenesis, cortisone might inhibit some phase of carbohydrate utilization. This view had been supported by the following evidences: the observation that cortisone caused a fall in the respiratory quotient in animals (Long et al, 1940) and human beings (Thorn et al, 1940), the finding that cortisone suppressed the tolerance of eviscerated rat for intravenous administrated glucose (Ingle et al, 1947).

Welt and Wilhelmi (1950) observed that there was a decreased conversion of carbohydrate to fat in the cortisone-fed, intact animal. This effect was confirmed

by Brady et al (1951). Using rats, in in vitro experiments, these authors reported that the incorporation of C^{14}-labelled acetate by liver slices into long-chain fatty acids was inhibited by the previous injection of cortisone. They concluded that the inhibitory effect of cortisone in the conversion of carbohydrate to fat might take place at a point other than the initial reaction involving glucose uptake by the tissues, i.e., the hexokinase reactions. The site of these effects was suggested to be in the lipogenetic system of the fat cell (Welt and Wilhelmi, 1950; Brady et al, 1951).

The role of glucocorticoids in the peripheral utilization of glucose was studied by Ashmore et al (1956). They suggested that glucocorticoids reduced peripheral utilization of glucose by decreasing the conversion of glucose to fatty acids. Skinner and Madison (1959) studied the permissive role of cortisone in the inhibition of hepatic insulin binding during operative stress and cited the reduction of binding of insulin to cells as a possible way in which glucocorticoids reduced peripheral utilization of glucose.

In the experiments of Glenn et al (1961), on the action of hydrocortisone in fasted adrenalectomized rats, results were obtained that indicated the reduction in the

rate of peripheral oxidation of glucose as the cause of the increased liver glycogen following hydrocortisone administration. Further data obtained by these authors also suggested that hydrocortisone blocked oxidative metabolism of glucose at a point after its entry to the cell, probably due to the inhibition of one or more intracellular enzymatic processes, with resulting accumulation of the intermediate metabolite.

d) Effect of Cortisone on Protein Metabolism

The ability of cortisone to stimulate gluconeogenesis from protein has been implicated in the influence of that hormone on carbohydrate metabolism. There is general agreement that cortisone and related glucocorticoids are essential for the mobilization of protein and amino acids from tissue stores.

Long (1942) and Ingle (1944, 1949) showed that the administration of glucocorticoids during fasting leads to an increased nitrogen excretion in the urine. Hoberman (1950) and Clark (1950) in studying the effect of cortisone upon the fate of injected isotopic glycine in adrenalectomized animals, reported both a stimulation of protein catabolism and an inhibition of anabolism. The previous finding of a

lessened accumulation of amino acids in the plasma of hepatectomized-adrenalectomized rats (Bondy, 1949) likewise showed the antianabolic action of the 11, 17-oxysteroids, such as cortisone. The observation (Cagan et al, 1950) that cortisone will restore to normal the diminished activity of liver amino acid oxidase in adrenalectomized animals, indicates that cortisone might act in part at the amino acid level of nitrogen metabolism.

Further support for the view that glucocorticoids influence gluconeogenesis from protein had been provided by Welt et al (1952), who showed that cortisone-fed rats given a constant infusion of C^{14}-labelled glucose increased liver glycogen levels from some source other than body glucose.

The effect of glucocorticoids on protein mobilization and gluconeogenesis is not exerted uniformly on all tissues of the body. In the animal or man given large doses of glucocorticoids, the major losses of proteins occur in the lymphatic nodes, the thymus, the spleen, the subcutaneous, interstitial and bony tissues, then later in the skeletal muscle fibres (Albright et al, 1941; Albright, 1942-1943; Dougherty and White, 1943; Dougherty et al, 1944; Baker, 1951). The epidermis, the liver, kidneys, heart and brain are not affected measurably (Baker, 1950, 1951; Baker et al, 1951).

Although the effect of glucocorticoids on protein mobilization and gluconeogenesis have been thoroughly studied, as yet it cannot be stated categorically whether the glucocorticoids enhance mobilization and proteolysis, or exert their influence by retarding protein formation from amino acids.

e) Effect of Cortisone on Lipid Metabolism

Levin and Farber (1950) showed that livers of fasting adrenalectomized rats contained considerably smaller quantities of neutral fat than those of intact animals, and pretreatment with cortisone prevented this disturbance. The administration of large doses of cortisone was found to cause intense lipemia and fatty livers in intact rabbits (Kobernick and More, 1950; Pierce and Bloom, 1952) and slight increases in neutral fat in the livers of normal rats (Levin and Farber, 1950). These observations have been interpreted in the past as indicating a low rate of fat mobilization and catabolism in the adrenalectomized animal that could be reversed by cortisone. Stoerk and Porter (1950) found that partially starved adrenalectomized rats had less neutral fat in both liver and carcass than did intact animals on isocaloric diets.

This finding of a low body fat in adrenalectomized animals indicated either a diminished liponeogenesis or an increased catabolism of fat. Welt and Wilhelmi (1950) from a study of the uptake of deuterium into liver and carcass fat by adrenalectomized rats on high-carbohydrate fat-free diets, concluded that adrenalectomy was followed by an increased rate of lipogenesis from carbohydrate.

That cortisone may accelerate gluconeogenesis from fat had been suggested by Kinsell et al (1952) who demonstrated that in diabetic patients maintained on carbohydrate-free diet, cortisone may produce a degree of glycosuria in excess of that readily explicable on the basis of gluconeogenesis from protein alone.

In the normal person, a great proportion of fatty acids is synthesised from glucose by the fat cells and is stored as triglycerides (Cahill, 1961). Hausberger (1958), Winegrad and Renold (1958) had shown that lipogenesis is increased and regulated primarily by the amount of insulin in the blood, and is inhibited by cortisone.

3. THE INTERACTIONS OF CARBOHYDRATE WITH HISTAMINE RELEASE

A. The Permissive Action of Glucose

The mast cells in guinea-pigs and rats had been shown to undergo degranulation and release histamine on exposure of the tissue to histamine releasers (Paton, 1958) or of the sensitized tissue to antigens (Mota, 1958; Hogberg and Uvnäs, 1957, 1958).

Numerous recent reports have confirmed Parrot's (1942) observation that anaphylactic histamine release is an energy requiring process. The significance of energy yielding substrates and high-energy compounds in the release of histamine had been studied by Diamant and Uvnäs (1961) and Diamant (1962). They studied the role of glucose on histamine release from rat mast cells, and guinea-pig lung tissue. These authors showed that histamine release from these tissues was markedly reduced under anoxic conditions in the absence of glucose, when exposed to Compound 48/80 or antigen-antibody reactions. The anoxic inhibition was found to be reversed by glucose. This was interpreted as an indication that mast cell degranulation and subsequent histamine release are energy-requiring processes. In the absence of oxygen, glycolysis furnishes the energy for this process. Westerholm (1960)

also showed that the depression of histamine release from cat skin by Compound 48/80 under anoxia was abolished by glucose.

A species difference between the rat and guinea-pig with respect to histamine release from the lung was reported by Chakravarty (1960). He showed in in vitro experiments, that in guinea-pig lung glucose partially reverses the anoxic inhibition of anaphylactic histamine release. Moussatché and Provoust-Danon (1961) showed that the enhancing effect of glucose on antigen-induced histamine release was correlated with the glycolytic activity of the tissue. They further observed that in the presence of mono-iodoacetate, glucose did not enhance anaphylactic histamine release under nitrogen anoxia.

The above data suggested that glucose is necessary, probably as an energy source, for making mast cell degranulation and histamine release possible, particularly under anoxic conditions. This effect was referred to by Goth (1967) as the permissive action of glucose.

B. The Inhibitory Effect of Glucose

a) Effect on Rat Anaphylactoid Reactions

Dextran, ovomucoid and yeast mannan (a polymer of mannose and zymosan) have all been shown to produce an anaphylactoid reaction when injected intraperitoneally into the rats (Voorhees *et al*, 1951; Bombara and Morabito, 1961). The anaphylactoid reaction caused by dextran, like anaphylaxis, is accompanied by a discharge of endogenous histamine (Goth *et al*, 1957).

The study of the correlation between sugars and histamine was triggered in 1957 by two independent observations. Goth *et al* (1957) showed that dextran induced anaphylactoid reactions and subsequent histamine release was inhibited in rats with high blood sugar levels. Adamkiewicz and Langlois (1957) observed that insulin rapidly reversed the resistance of diabetic rats to dextran.

Goth had demonstrated both *in vivo* (1957) and *in vitro* (1960), that the inhibition of the dextran anaphylactoid reaction by glucose is accompanied by the inhibition of the discharge of endogenous histamine. It was shown subsequently that 2-deoxyglucose could inhibit histamine release induced by dextran or ovomucoid *in vivo* and *in vitro* (Goth, 1959; Goth and Knoohuizen, 1962).

In rats, the inhibitory effects of various sugars are exerted against large molecular compounds which are either polysaccharides or contain a carbohydrate moiety. Beraldo et al (1962) and Poyser and West (1965) produced capillary leakage in rats by topical injection of the following polysaccharides: dextran (Beraldo et al, 1962), yeast mannan, ovomucoid (Poyser and West, 1965). Capillary leakages produced by these agents were shown to be inhibited when the blood sugar level was elevated, as in diabetes (Beraldo et al, 1962) or when the polysaccharides were mixed with mono- or disaccharides identical with the sugar present in the polysaccharides, or related to them by stereochemical features (Poyser and West, 1965). Bonaccorsi and West (1963) showed, and this was confirmed by Poyser and West (1965), that a combination of mepyramine and methysergide completely inhibited the intradermal responses to dextran and ovomucoid, suggesting that histamine and 5-hydroxytryptamine were involved at some stage of the capillary responses to the polysaccharides.

The inhibition of the anaphylactoid reaction observed when glucose and dextran were simultaneously injected into rats (Adamkiewicz and Adamkiewicz, 1960) suggested that the inhibitory effect of glucose occurs at the site of the reaction, blocking the disruption of mast

cells. This hypothesis was supported by the results of in vitro experiments, in which mast cell disruption and histamine release by dextran were inhibited by glucose (Beraldo et al, 1962). Since dextrans are polymers of glucose, it was suggested that the mechanism of the inhibition is probably competitive in nature. The studies of Dias Da Silva and Lemos Fernandes (1965) on the inhibition of dextran-induced histamine release from peritoneal cells by glucose further supported the hypothesis of 'competitive inhibition'. These studies suggested the presence of 'dextran receptors' in the rat mast cells and that competition between dextran and glucose is for a sugar carrier.

Goth (1967) summarized the inhibitory effect of sugars on histamine release in the rat as follows: mast cells appear to have receptors for dextran and related compounds which by means of a heat-susceptible mechanism activate degranulation and histamine release. Glucose and related sugars may block such mast cell reactions by competing with the polymeric compounds for receptors in the mast cells.

b) Effect on Immune Reactions

With regard to the anaphylactic reaction, it had been shown (Adamkiewicz et al, 1964) that

following a fixed dose of horse serum or egg white in rats sensitized to these antigenic materials, the mortality from anaphylactic shock was 50-60% in hypoglycemic animals, but there was no mortality in normal glycemic animals. Alloxan diabetes have also been shown to protect 50% of rats against anaphylaxis produced by horse serum plus the B. pertussis adjuvant (Thompson, 1961), or produced by B. pertussis alone (Ganley, 1962). This protection can be reversed by the administration of insulin. Aggravation of anaphylactic shock by insulin in rats had been reported by Sanyal et al (1959). They concluded from their experiments that, "insulin does not effect the formation of antibodies but renders the animal more sensitive to the antigen-antibody reaction, or to the product of the reaction". Analogous conclusions had previously been reached by Flashman on the aggravating effect of adrenalectomy (1926) and by Molomut on the aggravating effect of hypophysectomy (1939). All these studies were performed in the rats with hypoglycemia.

Adamkiewicz studied the correlation between the state of glycemia and immune responses. He stated in 1963, that the intensity of experimental and clinical immune responses was correlated with the degree of glycemia of the reacting subject. Hyperglycemias resulting from

sugars, cortisol, adrenaline, or from diabetes inhibit the anaphylactoid reactions, anaphylaxis, and the tuberculin reaction; but potentiate infections. On the other hand, hypoglycemias resulting from fasting, insulin and adrenalectomy potentiate the anaphylactoid reactions, anaphylaxis, and the tuberculin reaction; but inhibit infections. As a working hypothesis, he proposed that hyperglycemia inhibits certain antigen-antibody reactions which could result in an inhibition of hypersensitivity. Further studies of Adamkiewicz et al (1964), demonstrated that when the hypoglycemic state induced by fasting or by insulin was neutralized by administration of glucose the susceptibility of rats to anaphylactic shock was reduced. Changes in the glycemic state was brought about at a time when synthesis of antibodies was completed, suggesting that the modifying effect of glycemic states on anaphylactic shock was due to mechanism other than antibody synthesis. Studies on mice (Adamkiewicz and Sacra, 1966) showed a similar correlation between immune reactions and different blood sugar levels. When injected with a fixed overdose of rabbit antimouse or antirat-erythrocyte serum, the mortality of the mice from the resultant erythrocytolytic syndrome was 90% in hypoglycemic animals; 50% in normoglycemic animals, while there was no

mortality in hyperglycemic animals.

c) Effect on Histamine releasers and Exogenous Histamine

The administration of a fixed dose of the histamine releaser Compound 48/80 (4.5 mg/kg, ip) resulted in 100% mortality in hypoglycemic, 50% in normoglycemic, and 12% in hyperglycemic rats (Sacra and Adamkiewicz, 1965). However, there was no significant difference between hypo-, normo-, or hyperglycemic rats in the amount of endogenous histamine released. Besides discharging histamine, this compound also liberates a "slow-reacting substance" (Paton, 1951), a lipase (Ho et al, 1966) and, probably, other toxic substances (Adamkiewicz and Sacra, 1967). According to the latter authors, although glucose could have modified the toxicity of these released substances, the possibility of its antagonizing histamine itself had to be further explored.

To this end, experiments involving histamine induced capillary leakage were performed in rats (Adamkiewicz and Sacra, 1965). It was found that the capillary leakage induced by intradermal injections of histamine was almost completely inhibited if histamine was injected between the second and fourth hour after oral administration of d-glucose.

The inhibition of the effects of exogenous histamine by sugars has also been demonstrated. (i) Adamkiewicz and Sacra (1964) reported that the blood pressure fall in dogs after histamine injection (100 µg/kg, iv) was reduced by about 50% when the blood glucose levels were increased to 300 mg%. (ii) The <u>in vitro</u> contraction of the guinea-pig ileum, following addition of histamine to the bath (2×10^{-7} mg/ml), was inhibited by 10 to 95% as the glucose concentration in the bath was increased from 100 to 500 mg%. (Adamkiewicz and Sacra, 1964) (iii) The total gastric acid secretion, following a fixed dose of histamine (10 mg/kg, iv) was inhibited by 70% in hyperglycemic rats as compared to normoglycemic rats (Adamkiewicz and Sacra, 1966). Equimolar sucrose was also inhibitory in this test, while urea and NaCl were not.

Hirschowitz and Sachs (1965), using 2-deoxyglucose as the stimulant of gastric secretion, obtained a 95% inhibition by preloading dogs with glucose or mannose.

In summarizing their findings, Adamkiewicz and Sacra (1967) stated that the increase of blood glucose levels above the physiological range results in the inhibition of certain anaphylactic and anaphylactoid reactions, the immune erythrocytolytic syndrome and the actions of Compound 48/80, and exogenous histamine. Conversely, low blood sugar levels increase the susceptibility of the animals to most of these conditions.

II. EXPERIMENTAL WORK

1. MATERIALS AND METHODS

A. Materials

a) Glassware

All the syringes, needles and test-tubes used in the determination of plasma glucose were cleaned with detergent (Biodegradable Sparkleen from Fisher Scientific Co.) only. They were rinsed with distilled water and dried before use.

All other glassware was cleaned with acid dichromate mixture or Sparkleen, rinsed with distilled water and dried in a steam cabinet before use.

b) Chemicals

Atropine Sulphate, supplied by Merck, Sharpe and Dohme Ltd..

Benzoic Acid (certified reagent), supplied by Fisher Scientific Co..

Cadmium Sulphate (certified reagent), supplied by Fisher Scientific Co..

Calcium Chloride (certified reagent), supplied by Fisher Scientific Co..

"Desicote", supplied by Beckman Instruments Incorporated.

Dextrose (certified reagent), supplied by Fisher Scientific Co..

Ether (anhydrous), supplied by Fisher Scientific Co..

Glucose Oxidase reagent (Glucostat), supplied by Worthington Biochemical Corporation.

Heparin Sodium, 10,000 International (USP) Units, supplied by Connaught Medical Research Laboratories.

Histamine Dihydrochloride, supplied by Fisher Scientific Co..

Hydrochloric Acid Solution - N/2 (certified reagent), supplied by Fisher Scientific Co..

Magnesium Chloride (certified reagent), supplied by Fisher Scientific Co..

Phosphate Buffer Solution - 0.2M (Meyers-Free-Rosinske) Ph 7.2, supplied by Fisher Scientific Co..

Potassium Chloride (certified reagent), supplied by Fisher Scientific Co..

Sodium Chloride (certified reagent), supplied by Fisher Scientific Co..

Sodium Hydroxide - N/2 (certified reagent), supplied by Fisher Scientific Co..

Sodium Phosphate (certified reagent), supplied by Fisher Scientific Co..

Sulfuric Acid - N/2 (certified reagent), supplied by Fisher Scientific Co..

Trichloroacetic Acid, 100% w/v, (certified reagent), supplied by Fisher Scientific Co..

c) Agents Used in Animal Treatment

Cortisone Acetate	Cortone injections, obtained from Merck, Sharpe and Dohme Ltd..
Dextrose (certified reagent)	supplied by Fisher Scientific Co..
Ethyl Chloride Spray (for anesthesia)	supplied by British Drug Houses Ltd..
Insulin Toronto (crystalline zinc insulin)	supplied by Connaught Medical Research Laboratories.
Phenoxybenzamine Hydrochloride	supplied by Smith, Kline and French Ltd..
Pronethalol	supplied by Ayerst, McKenna and Harrison Ltd..

d) Animals

A multicolored short-haired variety of guinea-pigs has been used. They were obtained from the Quebec Breeders Association. Only male guinea-pigs, weighing 300-320 g at the start of the experiments, were employed. All animals were fed with a diet of Purina Guinea-Pig Chow, water and hay ad libitum.

Three animals were kept in each cage consisting of a raised bottom of wide wire mesh (whenever fasted), in order to prevent coprophagy.

B. Methods

a) Glucose Oxidase Method for the Determination of Plasma Glucose Levels

The glucose oxidase method, which is highly specific, is based upon the release of hydrogen peroxide from glucose during its oxidation by glucose oxidase. The peroxide is detected by a suitable indicator in the presence of horseradish peroxidase.

$$\text{Glucose} + O_2 + H_2O \xrightarrow[\text{Oxidase}]{\text{Glucose}} H_2O_2 + \text{Gluconic Acid}$$

$$H_2O_2 + \text{Chromogenic Oxygen acceptor} \xrightarrow{\text{Peroxidase}} \text{Oxidized Chromogen}$$

[o-tolidine / o-dianisidine] [blue, orange, etc.]

The glucose oxidase method employed in this study was carried out according to the procedure described by Saifer and Gerstenfeld (1958).

i) Preparation of Glassware

Centrifuge tubes (Sorvall, 15 ml), syringes (5 ml) and needles (20 gauge) were heparinized and siliconized before use.

The centrifuge tubes were filled to the brim with Desicote solution at room temperature. After 3 minutes, the Desicote solution was poured back in its original container and the tubes were dried in a steam cabinet at 60°C. The dried siliconized tubes were then cooled down to room temperature, and 0.1 ml of a solution of sodium heparin containing 1,000 µg/ml (147 USP units/ml) was added to each tube. The tubes were again dried in a steam cabinet at 60°C.

The syringes and needles were siliconized in the same way; heparinized by carefully rinsing with 0.1 ml of the above mentioned sodium heparin solution, and dried before use.

ii) Preparation of the Glucose Oxidase Reagent

The reagent, Glucostat, is a coupled enzyme system suitable for the colorimetric, specific determination of glucose. It contains glucose oxidase, horseradish peroxidases, and phosphate buffer in one vial (glucose reagent) and o-dianisidine in a second vial (Chromogen). The reagents were prepared as suggested by Saifer and

Gerstenfeld (1958). The glucose reagent (Glucostat x 4) was dissolved in 64 ml of distilled water, and the Chromogen x 4 was dissolved in 126 ml of distilled water.

The two solutions were initially stored frozen in separate containers and melted at room temperature when used. However, stored in this way, it was found that the stability of the enzymes did not last for more than a few days. This was probably due to the constant melting and refreezing of the enzymes.

To increase the stability of the enzymes, the 64 ml of dissolved Glucostat was divided into ten volumes of 6.4 ml each and stored frozen separately in 10 ml brown bottles. The 126 ml of Chromogen was also divided into ten volumes of 12.6 ml, and stored frozen separately in dark bottles. In this way, the enzymes were kept stable for at least two weeks.

Immediately before use, one bottle each of the enzymes was melted at room temperature. They were then mixed, together with 1 ml of 0.2M phosphate buffer solution, giving a volume of 20 ml. This volume was sufficient for ten glucose determinations.

iii) Procedure

Blood obtained from guinea-pigs by cardiac puncture under ethyl chloride anesthesia, was carefully transferred into heparinized-siliconized centrifuge tubes.

The tubes were immediately centrifuged in a Sorvall SS-3 Automatic Superspeed Centrifuge at a speed of 3,000 RPM for 15 minutes. The plasma obtained was transferred into clean dry tubes with pasteur pipettes.

Only 0.1 ml plasma was needed for each glucose determination, the remaining plasma was used for the determination of histamine. Each glucose determination was carried out in duplicate. To each 0.1 ml plasma, 7.0 ml of a 0.25% cadmium sulphate and 1.0 ml of a 0.12N sodium hydroxide were added. These were mixed by carefully inverting the tube. The mixture was then left at room temperature for 10 minutes. Then it was mixed again and centrifuged at 3,000 RPM for 15 minutes. Clear supernatants were decanted into Klett test-tubes in such a way as to avoid carrying over particles of the precipitate. To each tube of supernatant 2 ml of the glucose oxidase reagent was added, mixed and incubated at 37°C for 60 minutes. After incubation, 1.0 ml of a 0.5N sulfuric acid was added to each tube, mixed and left at room temperature for 5 minutes. The tubes were then read in a Klett-Summerson colorimeter with a No. 42 filter against a distilled water blank prepared in a similar manner. Internal standards in duplicate were prepared simultaneously with each set of unknowns by carrying known amounts of

glucose in 0.1% benzoic acid through the procedure. Glucose concentrations were proportional to the colorimetric readings (optical density) in the 60 to 270 mg% range.

iv) The Standard Curve

The standard curve was obtained by studying the variations of known glucose concentrations on the optical density. Glucose concentrations from 60 mg% to 300 mg% were freshly prepared from a 1% glucose stock solution in 0.1% benzoic acid. The effect of variation of glucose concentrations on the optical density was read in a Klett-Summerson colorimeter, with a No. 42 filter. All readings were made in duplicate and the colorimeter was calibrated with a distilled water reagent blank. The scale readings (optical density) were proportional to glucose concentrations.

It was found by plotting the different glucose concentrations (mg%) against optical density (units) that the curve was linear for concentrations from 60 to 270 mg% (Figure 1).

b) Methods for Extracting Histamine from Tissues and Plasma

For histamine extraction from tissues a method

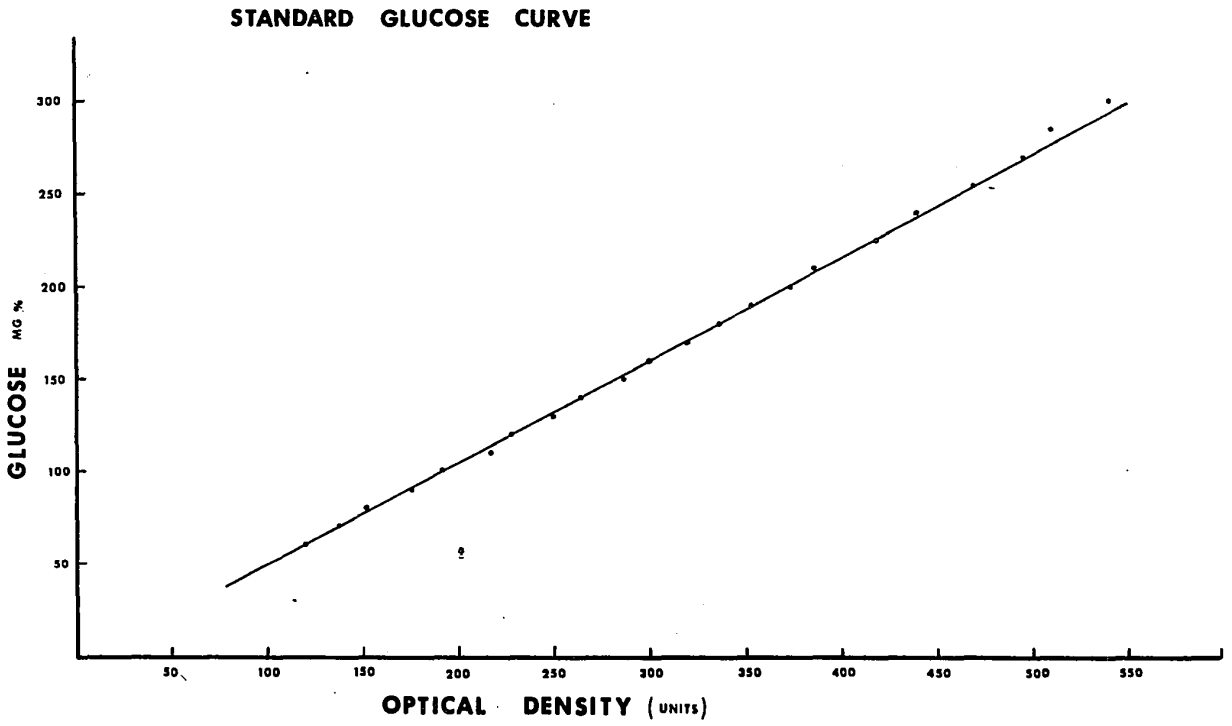

Figure 1: Standard glucose curve obtained by studying the effect of various glucose concentrations (mg%) on the optical density (Units).

similar to that described by Riley and West (1953) was used. This procedure was in good agreement with the modified Barsoum-Gaddum-Code method (Code and McIntire, 1956).

Before the experiment, the guinea-pigs were fasted for 16 hours; water was given at all times. They were sacrificed by a blow to the back of the neck and immediately exsanguinated by severing the carotid arteries. Excision of the tissues for extraction then followed.

The lungs were removed first, rinsed in distilled water, dried on filter paper and weighed. Lungs exceeding 0.8% of the body weight were considered pathological and were not used. The other organs for extraction were placed separately in physiological saline solution. All these organs were prepared for extraction (dried and weighed) within one hour of their removal from the body.

Organs to be studied were minced individually in Petri dishes with scalpels (blade No. 20) and placed separately into 50 ml homogenizing flasks. For each gram of fresh tissue, 2 ml of 10% trichloroacetic acid was added to the flask. The tissue was then homogenized for 15 minutes in a Virtis 45 Homogenizer at a speed of 45,000 RPM. The tissue homogenate was then centrifuged for

15 minutes in a Sorvall SS-3 Automatic Superspeed Centrifuge at a speed of 6,000 RPM. After centrifugation, the clear supernatant was extracted with 20 ml of ether (anhydrous) in order to remove fat and excess trichloroacetic acid. The ether was washed twice with 5 ml of distilled water in order to remove any histamine which have been taken up by the ether. The latter was combined with the trichloroacetic acid fraction and boiled for 5 minutes. The extract was then left to cool down to room temperature and stored in the refrigerator. At this point, the pH of the extract was 2.0 - 2.5. Immediately before use, the extract was neutralized to pH 7.2 - 7.5, with 1N NaOH solution.

Extraction of histamine from plasma: guinea-pigs were anesthetised with ethyl chloride. Blood was drawn by cardiac puncture (ventricle) and placed into heparinized-siliconized centrifuge tubes. After centrifugation at 3,000 RPM for 15 minutes, each plasma was transferred into a clean dry tube with a pasteur pipette and extracted for histamine immediately. The plasma volume was recorded and 1 ml of 10% trichloroacetic acid was added to each ml of plasma. They were mixed by inverting the tube and left at room temperature for 5 minutes. After centrifugation for 15 minutes at 3,000 RPM, the supernatant was extracted with 2 ml of ether; the ether washed twice with 0.5 ml of

distilled water; the combined watery extracts boiled for 3 minutes and stored in the refrigerator for a maximum of 3 days. The plasma extract was neutralized with 0.1N NaOH immediately before use.

c) Histamine Bioassay

Histamine determinations were carried out on the isolated guinea-pig ileum preparation, a method introduced by Guggenheim and Loeffler (1916).

i) Apparatus

An organ-bath was used. The capacity of the organ-bath was 10 ml, and the temperature of the water bath surrounding it was maintained at 35°C by means of a thermostat. The Tyrode solution passed into the 10 ml bath through two glass coils, thus maintaining a constant temperature of the solution. The Tyrode solution in the bath was kept well oxygenated by a mixture of 5% CO_2 and 95% O_2; the current was adjusted so as to produce 30-40 bubbles per minute. Contractions of the isolated ileum preparation were recorded on a smoked drum by a frontal writing lever.

ii) Physiological Solution

Tyrode solution was used. This was made up freshly before use. Twenty litres were prepared at a time, as follows:

A 20 litre bottle was half-filled with distilled water and the following reagents were added one at a time and thoroughly mixed:

- 160 g of sodium chloride
- 20 g of sodium hydrocarbonate (dissolved separately)
- 20 g of dextrose
- 40 ml of a 10% calcium chloride solution
- 40 ml of a 10% potassium chloride solution
- 20 ml of a 5% sodium phosphate solution
- 10 ml of a 40% magnesium chloride solution

The volume was then made up to 20 litres with distilled water. Atropine sulphate in a concentration of 5×10^{-7} g/ml was also added to eliminate spontaneous activity of the ileum strip.

All solutions used in making the Tyrode solution were prepared from stock solutions. The 10% solution of calcium chloride was stored in the refrigerator. Fresh stock solutions were prepared each month.

iii) Preparation of the Ileum Strip

Only male guinea-pigs weighing between 280-320 g were used. The animal was sacrificed by a blow to the back of the neck and exsanguinated by severing the carotid arteries. The abdomen was opened through a midline incision and a piece of terminal ileum approximately 10 cm long was removed. The inside of the ileum was carefully washed three to four times with Tyrode solution using a 10 ml volumetric pipette. Care was taken to avoid stretching of the ileum strip. The strip was then placed in a Petri dish containing Tyrode solution. A section 2.5-3 cm long was used for the bioassay procedure. The lower end of the strip was attached to a platinum hook on the oxygen inlet tube and placed near the bottom of the 10 ml bath. The upper end was attached by means of a piece of thread to the writing lever. The lever was balanced with plasticine and adjusted for best efficiency and sensitivity. The magnification was approximately 1:3. The oxygen-carbon dioxide mixture was then turned on and the preparation was well aerated throughout the experiment. The ileum strip was left for 15-20 minutes to become adjusted to its new environment prior to the experiment.

iv) Preparation of the Histamine Standard

Throughout this study histamine was expressed in terms of the base. Histamine dihydrochloride was used as the standard. A stock solution containing 100 µg/ml of histamine base was prepared by dissolving 16.5 mg of the salt in 100 ml of distilled water, and stored in the refrigerator. A fresh stock solution was prepared every two weeks. From the stock solution, concentrations of 0.5 µg/ml, 0.1 µg/ml, and 0.05 µg/ml were freshly prepared for the bioassay.

v) The Bioassay Procedure

Fifteen-twenty minutes after the preparation had been set up, a dose of the standard histamine solution was introduced into the bath with a 1 ml tuberculin syringe via a 23 gauge needle. The dose was chosen so as to give a satisfactory contraction (4-5 cm in length) and over which small differences in the amount given produced the greatest and most consistent changes in the length of the ileum. The dose used was usually 0.1 µg (0.2 ml of a 0.5 µg/ml solution). The ileum was stimulated every two minutes until it was standardized (same dose giving the same magnitude of contraction). Following each dose, the histamine was left in contact with the ileum for 20 seconds.

The ileum was then washed twice by filling, emptying and refilling the bath. Bath volume was maintained at a constant level.

After standardization of the ileum strip, the first dose of the tissue extract was introduced into the bath. Only a small amount was given, to obtain an approximate indication of the histamine concentration of the extract. It was left in contact with the ileum for 20 seconds and then washed out. If necessary, the extract was diluted with Tyrode solution for subsequent testing. The next response at the two minutes interval was obtained with the standard histamine dose. Injections with the standard solution were given into the bath until the responses were of the same height as the ones obtained just before the tissue extract was given. Then a second dose of the tissue extract was introduced into the bath. The aim was to match the heights of the contractions that were obtained by the standard histamine dose with an appropriate dose of the tissue extract. When this was found and tested twice, it was concluded that the histamine present in the volume of tissue extract given was equal to the amount of histamine present in the standard dose (Figure 2). Since the concentration of the histamine standard was known, the concentration of histamine in the tissue extract could be calculated in knowledge of the total volume of the extract

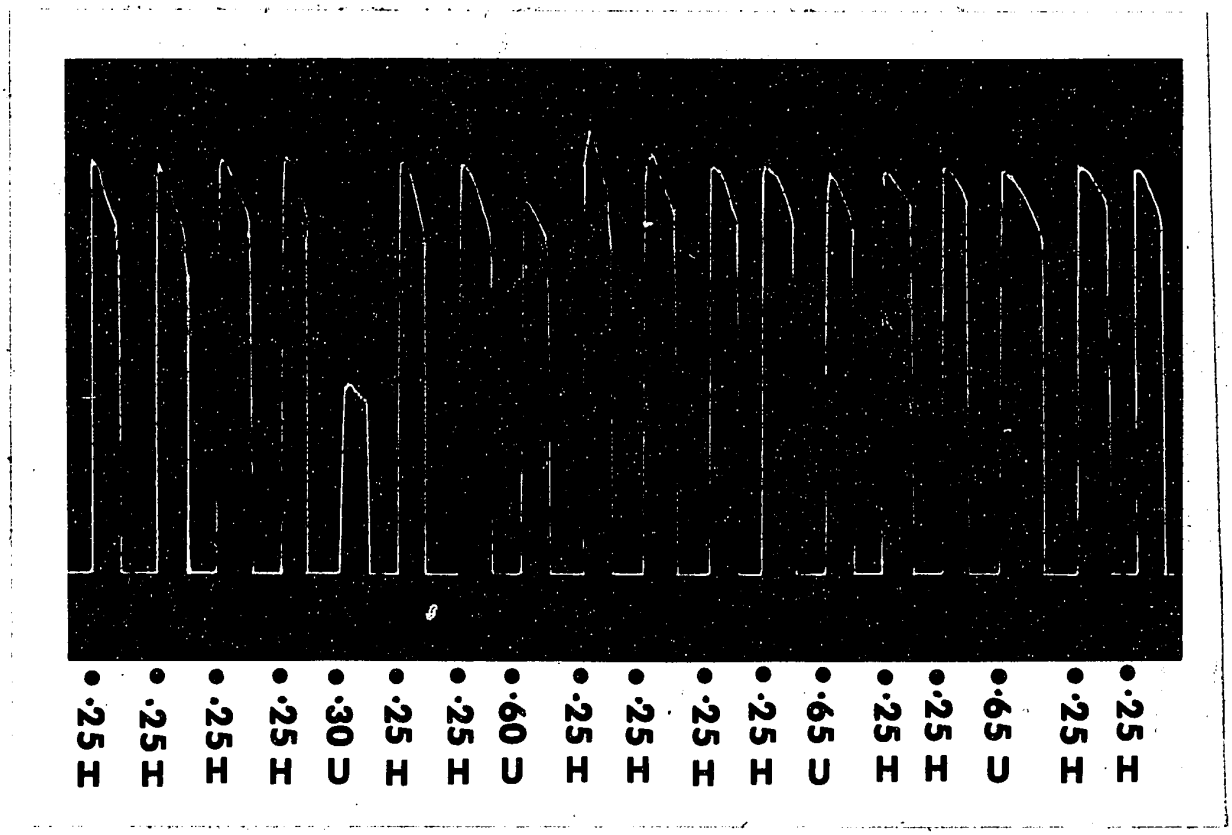

Figure 2: Tracing obtained during an assay of tissue histamine on the guinea-pig ileum preparation.

H = Histamine standard; figures in µg of histamine base.

U = Tissue extract prepared from guinea-pig lung, expressed in ml.

From this assay 0.65 ml of U was considered to contain 0.25 µg of histamine.

and the organ weight involved.

$$\text{Tissue histamine concentration } \mu g/g = \frac{\text{Total volume of extract (ml) x Amount of Standard Histamine } (\mu g)}{\text{Volume of extract used (ml) x Weight of tissue (g)}}$$

In rare cases when exact matching of contractions exerted by the standard histamine dose and the tissue extract could not be established, two doses of the extract were considered, one which gave a somewhat higher contraction and one that gave a somewhat lower contraction than the standard. The average of these two volumes was used when calculating the histamine concentration of the tissue extract.

The histamine concentration in guinea-pig plasma is low. Due to the fact that plasma was available in small quantities only, the bioassay procedure for determining histamine concentration in plasma was modified as follows:

After standardizing the ileum strip in the usual way, a volume of 0.5 or 1 ml of the plasma extract was injected into the bath. This was followed by the addition of standard doses of histamine in order to obtain the height of the original contractions. Thereafter a chosen

volume of the standard histamine solution was given to the bath in an attempt to match the contraction produced by the plasma extract. After finding the right volume, this volume was again matched with the plasma extract (Figure 3). Thus, the determination of plasma histamine differed from that of tissue histamine in that in the latter, a volume of the extract was chosen which gave the same contraction as the standard dose of histamine; while in the former case a volume of the standard solution was chosen to match the contraction obtained from a fixed dose of the plasma extract.

$$\text{Plasma histamine content } \mu g/ml = \frac{\text{Total volume of plasma extract (ml)} \times \text{Amount of standard histamine } (\mu g)}{\text{Original plasma volume (ml)} \times \text{Volume of plasma extract used (ml)}}$$

Contractions of the ileum produced with the tissue or plasma extracts were blocked by the previous addition of 10^{-8} g/ml of promethazine hydrochloride, thus confirming that the contractions were caused by histamine present in these extracts and were not due to any other smooth muscle stimulants present in them.

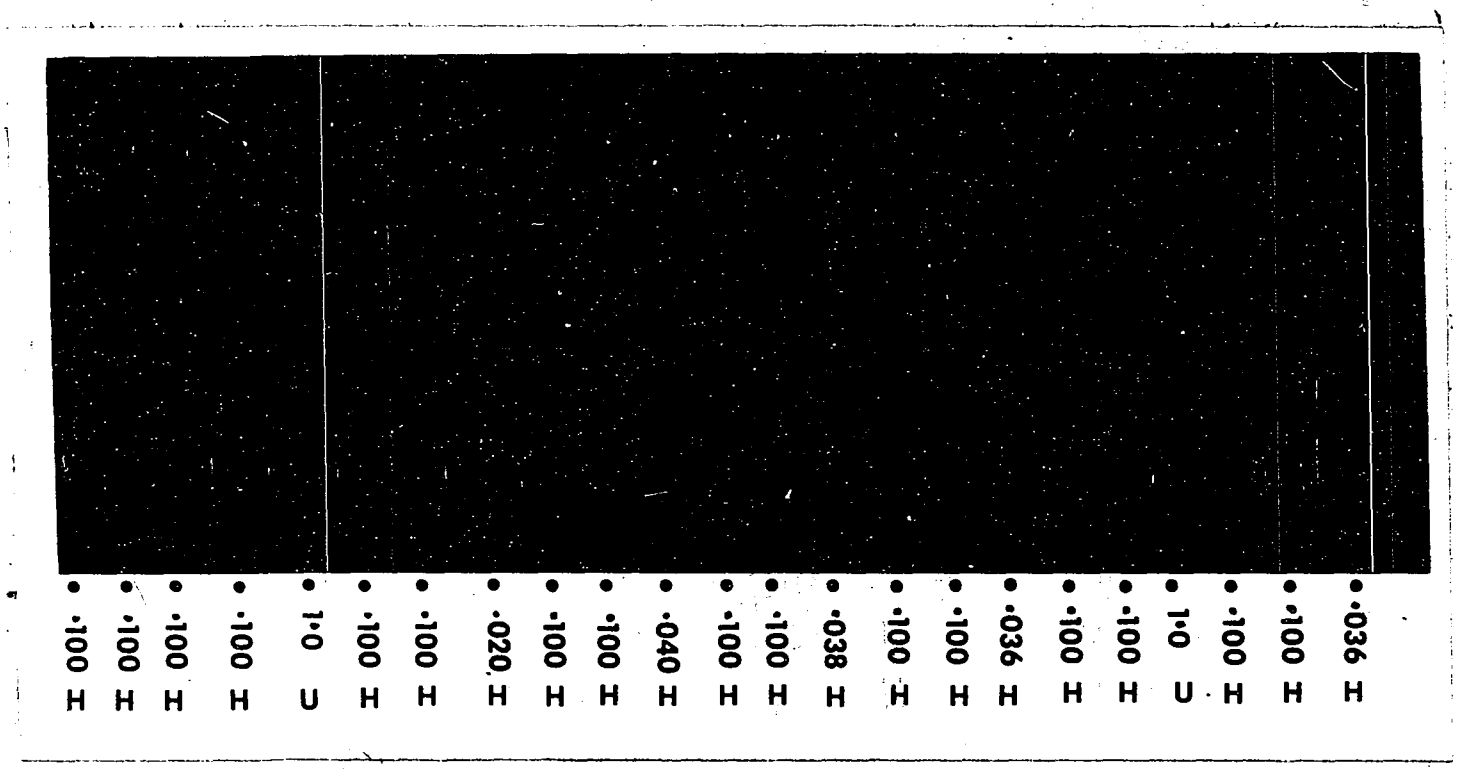

Figure 3: Tracing obtained during an assay of plasma histamine on the guinea-pig ileum preparation.

H = Histamine standard; figures in µg of histamine base

U = Plasma extract, expressed in ml

From this assay 1.0 ml of U was considered to contain 0.036 µg of histamine.

2. RESULTS

A. Normal Values

a) Acute Experiments

The control values obtained for plasma glucose and plasma histamine levels were recorded from male guinea-pigs, weighing between 300-320g at the start of the experiments. Since it was found in preliminary experiments that the plasma glucose and histamine levels remained fairly constant in each individual animal studied, and were not altered by saline injection, subsequent control and experimental values were determined in the same animal before and after treatment. A period of at least 5 days was allowed for the guinea-pigs to recover following control determinations. Animals which did not gain weight normally were excluded. All determinations were made on the fasted animals.

b) Chronic Experiments

In chronic experiments, control and experimental plasma glucose and histamine values were also performed on the same animal. Control values for tissue histamine levels were obtained by injecting equal volumes of 0.9% physiological saline solution to untreated guinea-pigs

simultaneously with the treated animals. Since results for tissue histamine levels from control guinea-pigs receiving saline solution were always in the same range, one set of control data is used throughout the chronic experiments. All determinations were made on fasted animals.

B. Effect of a Single Injection of Cortisone on Plasma Glucose and Plasma Histamine Levels

Thirty-nine guinea-pigs received 100 mg/kg cortisone acetate each, using a 50 mg/ml suspension. Injections were made subcutaneously. Plasma glucose and plasma histamine concentrations were determined at different time intervals following cortisone injection, using from 4 to 7 guinea-pigs each time. Results were compared with control values from the same guinea-pigs. Lung histamine determinations were also made and compared with saline treated controls.

Urinary glucose tests (with "Combistix" reagent strips), made immediately preceeding sacrifice, were negative.

The values obtained for plasma glucose levels, plasma and tissue histamine levels are shown in Table 1.

Table 1

THE EFFECT OF CORTISONE (100 mg/kg) ON THE PLASMA GLUCOSE AND HISTAMINE LEVELS OF GUINEA-PIGS

Injections were given subcutaneously. Values are means and standard errors. Histamine concentrations are expressed in terms of the base. Figures in parentheses represent percentage increase compared to control values.

No. of Animals	Time of expt. min.	Plasma Glucose mg%		P	Plasma Histamine γg/ml		P	Lung Histamine μg/g
		Initial	After Cortisone		Initial	After Cortisone		
4	30	121 ± 2.87	124 ± 1.48 (2.5)	N.S.	35 ± 3.2	36 ± 4.48 (2.8)	N.S.	19.7 ± 2.35
7	50	122 ± 2.55	154 ± 3.65 (26.2)	<0.001	37 ± 2.89	39 ± 2.36 (5.4)	N.S.	18.2 ± 1.0
6	80	128 ± 2.63	142 ± 3.7 (10.9)	<0.02	42 ± 1.45	54 ± 3.46 (28.5)	<0.01	19.9 ± 0.83
6	140	130 ± 4.09	133 ± 2.57 (2.3)	N.S.	32 ± 3.37	102 ± 24.15 (219)	<0.02	18.1 ± 2.45
6	200	124 ± 4.65	123 ± 2.77	N.S.	41 ± 1.76	98 ± 11.52 (139)	<0.001	18.7 ± 2.14
6	260	126 ± 1.99	127 ± 2.16 (0.7)	N.S.	38 ± 5.00	44 ± 4.40 (15.7)	N.S.	16.3 ± 0.11
4	320	126 ± 2.91	127 ± 2.76 (0.7)	N.S.	34 ± 4.53	37 ± 5.76 (8.8)	N.S.	19.4 ± 3.71

Maximal increase in plasma glucose concentrations was obtained at 50 minutes following the single injection of cortisone (an increase of 26.2% as compared to control value). Plasma glucose levels remained elevated until about 140 minutes following injection.

Plasma histamine levels, expressed in nanograms per ml, started to increase after 50 minutes, reached maximal levels at 140 minutes (an increase of 219%) and were nearly back to normal at 320 minutes following treatment. Lung histamine levels were normal.

These results showed that a single injection of cortisone caused a significant increase in plasma histamine levels which was clearly preceded by hyperglycemia (Figure 4).

C. Effect of a Single Dose of Cortisone followed by Insulin on Plasma Glucose and Plasma Histamine Levels

The object of this experiment was to determine whether the cortisone-induced hyperglycemia could be responsible for the increase in plasma histamine concentration which followed it.

Preliminary experiments had shown that 6 Units/kg insulin given subcutaneously 30 minutes after cortisone could block the cortisone-induced hyperglycemia which

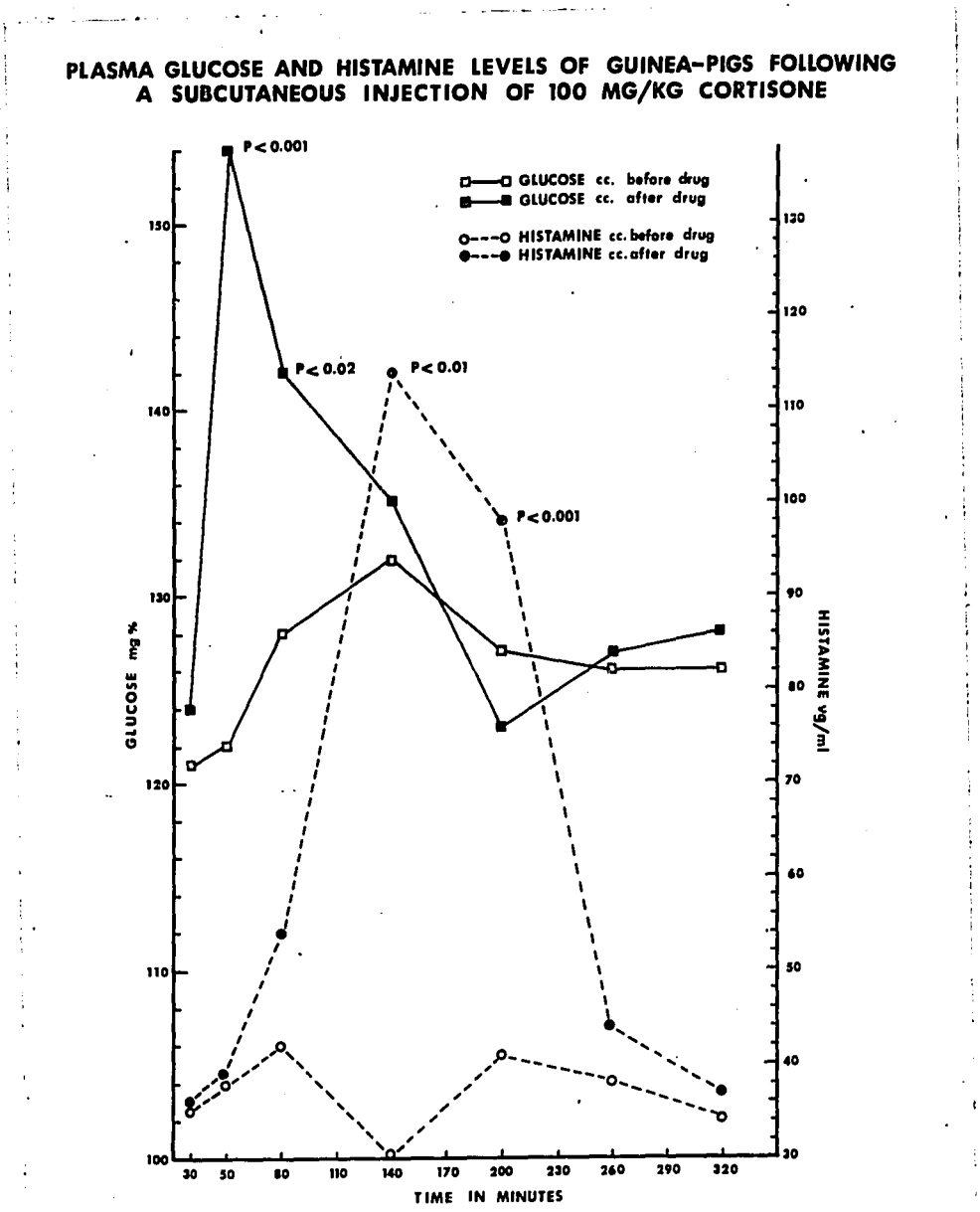

Figure 4: Plasma glucose and histamine levels of guinea-pigs following a single injection of 100 mg/kg of cortisone acetate. Each point plotted at a given time is the mean of results obtained from 4-7 guinea-pigs before and after cortisone administration.

- Control glucose levels mg%
- Control histamine levels γg/ml
- Glucose levels after cortisone mg%
- Histamine levels after cortisone γg/ml

normally occurred at 50 minutes without producing hypoglycemia.

Animals were fasted for 16 hours before treatment. Nine guinea-pigs received a single injection of 100 mg/kg cortisone acetate each, given subcutaneously, followed 30 minutes later with a subcutaneous injection of 6 Units/kg insulin. Seven guinea-pigs received a single subcutaneous injection of 100 mg/kg cortisone acetate each, followed 60 minutes later with a subcutaneous injection of 6 Units/kg insulin.

From values obtained from these experiments, see Table 2, it can be seen that if cortisone-induced hyperglycemia was blocked by the injection of insulin given 30 minutes after cortisone, no plasma histamine increase was observed at 140 minutes, at a time when cortisone alone produced peak plasma histamine levels.

However, when insulin was given 60 minutes after cortisone injection, at a time when hyperglycemia has already developed, the increase in plasma histamine levels was not blocked. A significant increase in plasma histamine levels occurred at 140 minutes and 180 minutes following cortisone administration, which correlates well with data obtained with cortisone alone.

Table 2

EFFECT OF CORTISONE (100 mg/kg) FOLLOWED BY INSULIN (6 U/kg) ON PLASMA GLUCOSE AND HISTAMINE LEVELS OF GUINEA-PIGS

All injections were administered subcutaneously. Values are means and standard errors. Histamine concentrations are expressed in terms of the base.

*P < 0.02 **P < 0.01

Treatment	No. of Animals	Time of Expt. min.	Plasma Glucose mg%		Plasma Histamine γg/ml	
			Initial	After drugs	Initial	After drugs
Cortisone + Insulin 30 min. later	6	50	123 ± 9.28	124 ± 9.51	68.5 ± 18.00	68.5 ± 18.99
As above	3	140	125 ± 4.85	39 ± 6.11	29 ± 1.53	23 ± 3.00
Cortisone + Insulin 60 min. later	3	140	123 ± 3.85	42 ± 4.62	32 ± 3.18	109 ± 17.9**
As above	4	180	130 ± 4.31	35 ± 2.74	28 ± 5.84	116 ± 25.3*

D. Effect of a Single Injection of Glucose on Plasma Glucose and Plasma Histamine Levels

Experiments with cortisone and insulin showed that if cortisone-induced hyperglycemia was abolished by insulin, no increase in plasma histamine levels occurred. This indicated that the increase in blood sugar level might be involved in the chain of events which led to histamine increase in the plasma. These experiments with glucose were performed to clarify whether or not this is so and to see if the increase in plasma histamine following a single injection of cortisone could be duplicated with a single injection of glucose.

Animals fasted for 16 hours received 2.5 g/kg glucose each, using a 30% solution. Injections were made subcutaneously. Twenty minutes were allowed for absorption and experiments were performed at different time intervals following injection. Urinary glucose was also tested with "Combistix" reagent strips, and results were negative.

From the data obtained, Table 3, it can be seen that, similarly to a single injection of cortisone, a single injection of glucose also produced a significant increase in plasma histamine concentrations. Again, hyperglycemia

Table 3

THE EFFECT OF GLUCOSE ON PLASMA GLUCOSE AND HISTAMINE LEVELS OF GUINEA-PIGS

Injections were given subcutaneously. Values are means and standard errors. Histamine concentrations are expressed in terms of the base. Figures in parentheses represent percentage increase compared to control values.

Dose of Glucose g/kg	No. of Animals	Time of expt. min.	Plasma Glucose mg/%		P	Plasma Histamine γg/ml		P
			Initial	After glucose		Initial	After glucose	
2.5	4	30	120 ± 2.73	416 ± 13.14 (247)	<0.001	31 ± 1.45	45 ± 7.83 (45)	N.S.
2.5	4	140	116 ± 7.64	214 ± 27.38 (86)	<0.02	37 ± 9.99	100 ± 12.51 (170)	<0.01
2.5	4	260	124 ± 3.24	146 ± 1.47 (17.7)	<0.001	32 ± 2.15	66 ± 4.53 (106)	<0.001
2.5	3	380	121 ± 4.51	136 ± 2.31 (12.3)	<0.05	34 ± 3.79	48 ± 8.25 (41)	N.S.
5.0	8	140	141 ± 5.24	462 ± 37.66 (228)	<0.001	42 ± 7.29	187 ± 8.39 (345)	<0.001
5.0	6	260	132 ± 4.67	239 ± 42.43 (81)	<0.05	35 ± 1.48	106 ± 9.99 (203)	<0.001

appeared to precede the increase in plasma histamine levels. At 30 minutes, when the plasma glucose level had reached a 247% increase (as compared to control value), the change in plasma histamine level was not significant. However, at 140 minutes, when the plasma glucose level started to decrease, the plasma histamine level reached a significant peak value with a 170% increase, as compared to control value.

Experiments with a higher dose of glucose, 5.0 g/kg, showed a dose response for histamine release (Table 3). The administration of a higher dose of glucose resulted not only in a higher plasma glucose level but also in a higher plasma histamine level than the lower dose.

E. Effect of Adrenergic Blocking Agents on Cortisone Induced Hyperglycemia

These experiments were carried out to ascertain that the cortisone-induced hyperglycemia is due to the action of cortisone and not to the release of adrenaline.

Animals were fasted for 16 hours. Six guinea-pigs received 100 mg/kg of cortisone acetate, given subcutaneously. A second group of 3 animals was pretreated with 5 mg/kg pronethalol, intraperitoneally,

followed 5 minutes later with a subcutaneous injection of 100 mg/kg of cortisone acetate. A third group of 3 guinea-pigs was pretreated with 5 mg/kg phenoxybenzamine, given intraperitoneally, and followed 5 minutes later with a subcutaneous injection of 100 mg/kg of cortisone acetate. The last group of 4 animals was pretreated with both pronethalol and phenoxybenzamine, 5 mg/kg each, given intraperitoneally 5 minutes apart, followed 5 minutes later with a subcutaneous injection of cortisone, 100 mg/kg. Experiments were performed 50 minutes after the cortisone administration.

Results shown in Table 4 indicated that adrenaline did not seem to be responsible for the hyperglycemia caused by cortisone, since cortisone alone or in conjunction with the adrenergic blocking agents gave the same percentage increase in plasma glucose levels, 26.0 and 27.1% respectively.

Table 4

THE EFFECT OF ADRENERGIC BLOCKING AGENTS ON CORTISONE INDUCED HYPERGLYCEMIA

Plasma glucose concentrations were determined 50 min. after cortisone (100 mg/kg s.c.) injection.

Treatment	No. of Animals	Plasma glucose mg%		Increase %
		Control	Treated	
Cortisone	6	122 ± 2.56	154 ± 3.66	26.0
Pronethalol (5 mg/kg i.p.) + Cortisone 5 min. later	3	122 ± 3.69	146 ± 2.35	19.6
Phenoxybenzamine (5 mg/kg i.p.) + Cortisone 5 min. later	3	115 ± 2.73	151 ± 6.66	31.3
Phenoxybenzamine + Pronethalol 5 min. later + Cortisone 5 min. after pronethalol	3	118 ± 2.00	150 ± 2.66	27.1

F. Effect of a Prolonged Cortisone Treatment on Plasma Glucose and Plasma Histamine Levels

Guinea-pigs received 100 mg/kg cortisone acetate daily, using a 50 mg/ml suspension. Injections were made subcutaneously, once in the morning, for a period of 10 consecutive days. Towards the end of the treatment, weight and water consumption were seen to increase abnormally, probably as a result of salt retention caused by the administration of cortisone. The animals were fasted 16 hours prior to the last administration. Urinary glucose was tested with "Combistix" reagent strips immediately before the experiment, and results showed variation from "trace" to negative. Experiments were performed at different time intervals following the last dose of cortisone.

Table 5 shows the results obtained from these chronic experiments. Maximal increase in plasma glucose levels was obtained at 80 minutes following the last dose of cortisone injection, and was returned to normal by 200 minutes. Plasma histamine concentrations started to increase after 50 minutes and reached a peak value at 80 minutes (204.3% increase as compared to control data). Plasma histamine levels remained elevated and were back to normal values by 24 hours after the last dose of cortisone (Figure 5).

Table 5

THE EFFECT OF PROLONGED ADMINISTRATION OF CORTISONE (100 mg/kg) ON THE PLASMA GLUCOSE AND HISTAMINE LEVELS OF GUINEA-PIGS

Animals were injected subcutaneously once daily for 10 days. Values are means and standard errors. Histamine concentrations are expressed in terms of the base. Figures in parentheses represent percentage increase compared to control values.

No. of Animals	Time of expt. min.	Plasma Glucose mg%		P	Plasma Histamine µg/ml		P	Lung Histamine µg/g	P
		Initial	After Cortisone		Initial	After Cortisone			
4	30	118 ± 3.31	124 ± 3.12	N.S.	36.5 ± 3.28	38 ± 3.90	N.S.	-	
4	50	122 ± 1.94	166 ± 14.9	<0.05	27 ± 2.74	29 ± 2.56	N.S.	-	
3	80	127 ± 5.21	175 ± 25.7	N.S.	23 ± 5.49	70 ± 12.42 (204.3)	<0.05	12.4 ± 1.09	<0.001
5	200	121 ± 2.29	120 ± 3.09	N.S.	30 ± 4.81	91 ± 3.31 (203.3)	<0.001	10.9 ± 0.48	<0.001
6	320	123 ± 1.68	123.6 ± 1.87	N.S.	28 ± 6.89	83 ± 8.35 (196.4)	<0.05	8.9 ± 0.40	<0.001

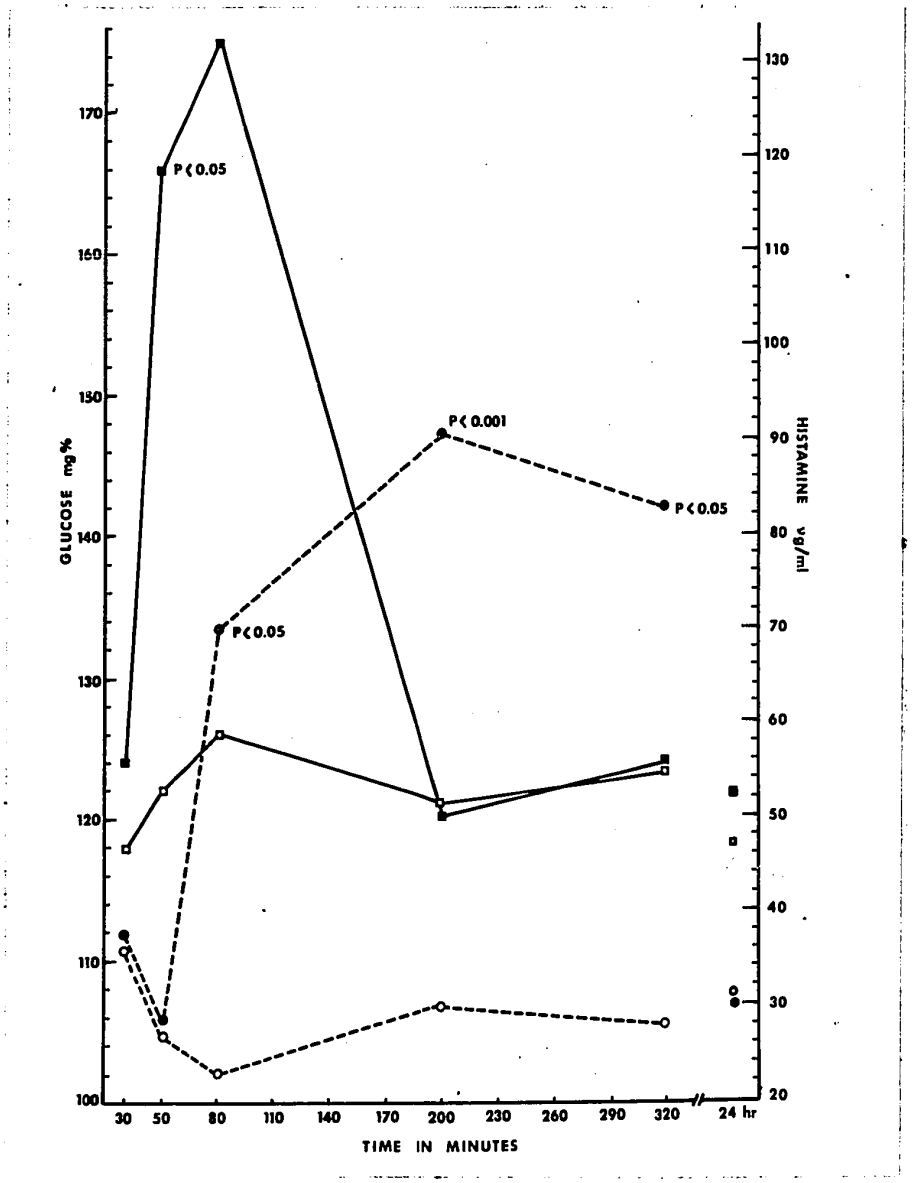

Figure 5: Plasma glucose and histamine levels of guinea-pigs following treatment with 100 mg/kg of cortisone acetate for 10 days. Each point plotted at a given time is the mean of results obtained from 3-5 guinea-pigs before treatment and after the last dose of cortisone.

□—□ Control glucose levels mg%

○----○ Control histamine levels γg/ml

■—■ Glucose levels after cortisone mg%

●----● Histamine levels after cortisone γg/ml

Lung histamine levels were significantly decreased at all the time intervals studied.

Data obtained from these experiments indicated that on prolonged cortisone administration, the effect on plasma glucose levels, plasma and tissue histamine levels was different from those obtained with a single injection of cortisone. Prolonged cortisone treatment caused a higher glucose level and hyperglycemia was more prolonged.

Also, prolonged cortisone administration caused a significant but smaller increase in plasma histamine levels than the single injection. However, this increase seemed to be more sustained, lasting longer. At 320 minutes there was still a significant increase in plasma histamine levels, whereas, the plasma histamine level was only slightly increased at this time, following the single cortisone injection.

G. Effect of Cortisone, Glucose, and Cortisone with Insulin on the Histamine Content of Guinea-pig's Plasma and Organs

Six guinea-pigs received 100 mg/kg cortisone acetate, given subcutaneously once daily every morning for 10 consecutive days. Seven animals were given 2.5 g/kg glucose solution, also given subcutaneously once daily for 10 consecutive days. A group of 4 animals received 100 mg/kg cortisone acetate followed 30 minutes later with a subcutaneous dose of 6 Units/kg insulin, for 10 days. Another group of 4 animals was given physiological saline solution simultaneously with the treated animals. These animals served as controls in the determination of tissue histamine levels. Experiments were performed 4 hours after the last treatment, and plasma and tissue histamine levels were determined. The animals were fasted for 16 hours prior to the last drug administration. Urinary glucose was tested in the glucose and cortisone treated animals. Only 2 out of the 7 animals treated with glucose showed a slightly positive reaction.

As can be seen from Table 6, following multiple doses of cortisone, plasma histamine levels were highly elevated ($p<0.001$), and the histamine content of the lung, liver and heart was significantly decreased.

Treatment for 10 days with glucose also produced a significant increase in plasma histamine levels and a significant decrease in lung, liver and heart histamine levels. However, the changes in histamine content were less marked than those obtained with cortisone alone.

The experiment with cortisone and insulin for 10 days, showed that on prolonged administration insulin could only partially block the effects of cortisone on histamine concentrations. There was no increase, but a 15.4% decrease, in plasma histamine concentrations, and virtually no decrease in lung histamine content. However, the liver and heart histamine levels were still decreased as compared to control values, although the decrease was less than that obtained by cortisone alone.

Table 6

PLASMA AND TISSUE HISTAMINE LEVELS FOLLOWING TREATMENT WITH CORTISONE, GLUCOSE AND CORTISONE WITH INSULIN

Animals were injected subcutaneously once daily for 10 days and sacrificed 4 hours after the last injection. Values are means and standard errors. Histamine concentrations are expressed in terms of the base.

Treatment	No. of Animals	Plasma Histamine γg/ml		P	Tissue Histamine Content μg/g					
		Control	Treated		Lung	P	Liver	P	Heart	P
Saline	4	27 ± 2.00	28 ± 4.20	N.S.	21.0 ± 0.86		2.06 ± 0.16		6.00 ± 0.02	
Cortisone Acetate 100 mg/kg	6	28 ± 6.89	83 ± 8.35	<0.001	8.9 ± 0.40	<0.001	0.91 ± 0.06	<0.001	3.42 ± 0.21	<0.001
Glucose 2.5 mg/kg	7	29 ± 1.46	73 ± 3.62	<0.001	13.6 ± 0.70	<0.001	1.09 ± 0.04	<0.001	4.72 ± 0.37	<0.01
Cortisone + Insulin 6 U/kg 30 min later	4	39 ± 1.58	33 ± 3.24	N.S.	18.5 ± 1.94	N.S.	1.19 ± 0.03	<0.01	4.34 ± 0.31	<0.01

III. DISCUSSION

Individual plasma histamine levels on repeated determinations remained remarkably stable in our guinea-pigs, which confirms previous observations (Code and MacDonald, 1937; Rose and Browne, 1940; Emmelin, 1945) that the histamine concentration of blood, while varying within fairly wide limits in a species, is sustained at a rather constant level in one and the same individual. For this reason, both the control and experimental plasma histamine concentrations were determined in the same animal.

The results of the present experiments show that hyperglycemia brought about by one or several injections of cortisone lead to an increase in plasma histamine levels of the guinea-pig. Further, the significant fall in tissue histamine levels brought about by cortisone on prolonged administration was partly abolished if the hyperglycemic effect of cortisone was prevented. There are data in the literature reporting a fall in blood histamine levels of the normal subjects following cortisone administration (Code and Mitchell, 1953; Kelemen and Bikich, 1956). In these studies, histamine determinations were carried out on the whole blood, only once, at a time when the number of circulating eosinophil and basophil cells

was significantly reduced. The 24 hours output of free histamine was found to be increased in the urine of healthy subjects (Mitchell and Code, 1954).

Schayer et al (1954, 1956) found that glucocorticoid treatment decreased the rate of binding of new histamine in rat tissues and Hicks (1965) suggested that the fall in tissue histamine levels of guinea-pigs seen after repeated injections of cortisone is related to the prevention of replenishment of tissue histamine following normal turnover processes. This effect of cortisone seems to be relected in these experiments when cortisone in conjunction with insulin has been administered repeatedly. The minimal decrease in pulmonary histamine levels under these circumstances might reflect, that in tissue which has a high histamine content (Schayer et al, 1956) or is rich in mast cells (Kahlson et al, 1963) a histamine molecule once formed is firmly held and has a long intracellular lifetime. However, glucocorticoids were also found to interfere with the biosynthesis of histamine (Halpern, 1956) and with the normal activity of mast cells (Asboe-Hansen, 1952). It appears that glucocorticoids affect various aspects of histamine metabolism, and their hyperglycemic effect might be an important step in a complex chain of reactions.

The increase in plasma histamine concentration induced by cortisone could be duplicated by glucose. On prolonged administration glucose reduced tissue histamine levels as well, although the decrease was less than that obtained by cortisone.

Carbohydrates have two known interactions with histamine release. On one hand, glucose is necessary, probably as an energy source, for making mast cell degranulation and histamine release possible under anoxic conditions (Diamant and Uvnäs, 1961; Diamant, 1962). On the other hand, various carbohydrates inhibit the dextran anaphylactoid reaction in rats (Goth et al, 1957; Beraldo et al, 1962) probably by competing with dextran for receptors in the mast cell (Dias da Silva and Lemos Fernandes, 1965). The results described in this thesis suggest a third type of interaction between glucose and histamine: in the intact guinea-pig glucose acts as a histamine releaser.

There are various data in the literature indicating a beneficial effect of increased blood glucose levels under certain experimental conditions. Long (1956) noted that in guinea-pigs, insulin hypoglycemia was associated with a marked sensitivity to tuberculin, while hyperglycemia was associated with a decrease in sensitivity.

Ganley (1962) found that B. Pertussis sensitized mice, rendered diabetic with alloxan, were less susceptible to the toxicity of histamine and the action of anaphylaxis than control sensitized animals. Adamkiewicz and Sacra (1966) reported in a review of their findings that the increase of blood glucose levels above the physiological range resulted in the inhibition of certain anaphylactic and anaphylactoid reactions, the immune erythrocytolytic syndrome and the actions of exogenous histamine. Capillary leakage induced by topical histamine injection was almost completely inhibited when histamine was injected between the second and fourth hour after glucose administration. Similarly, if blood glucose levels of dogs were increased, the blood pressure fall after histamine injection was reduced by about 50%. Unfortunately in none of these studies were plasma histamine concentrations determined.

From the experiments performed to date, it is not possible to draw any conclusion concerning the significance, if any, of an increase in plasma histamine levels following the administration of cortisone or glucose. However, a decrease in histamine sensitivity of blood vessels after previous injection of histamine has been reported by several investigators (Phemister and Handy, 1927; Epstein, 1932; Von Euler, 1938; Anrep et al, 1939).

Emmelin reported in 1945 that the more the plasma histamine concentration was raised in a cat by means of slow infusion histamine, the smaller the fall in blood pressure became following a constant dose of histamine intravenously injected. Histamine sensitivity was depressed only as long as the histamine content of plasma was increased. The rapid transfusion of blood from cats with high histamine levels into untreated cats caused bronchial constriction, contraction of the gut and a fall in blood pressure in the recipient. In other experiments of Emmelin (1945) the histamine concentration of plasma could be raised to high levels without marked fall in blood pressure, provided that the high level was established successively. He suggested that the effector cells can adapt themselves to high histamine concentrations.

There are also clinical observations in the literature which seem to suggest the possibility that an inverse relationship might exist between blood histamine levels and histamine toxicity. In general, patients with urticaria have low total blood histamine levels (Rose, 1941; Rorsman, 1961; Beall, 1963). Rose and Browne (1940) found a decrease in blood histamine levels during surgical shock, and Rose reported in 1940, that the histamine content of the blood of patients with angioneurotic edema

was at a very low level during an attack as compared to the level found during the quiescent phase. He suggested (1941) that "symptoms of histamine intoxication are associated with a decrease in the blood histamine rather than in increase of this substance in the blood".

IV. SUMMARY

(1) Preliminary experiments showed that plasma glucose and plasma histamine levels remained fairly constant in each individual guinea-pig.

(2) Plasma glucose and plasma histamine levels were determined in male guinea-pigs before and after treatment with various agents.

(3) In guinea-pigs treated with a single injection of 100 mg/kg cortisone acetate, given subcutaneously, maximal plasma glucose level was obtained 50 minutes following the injection.

(4) Plasma histamine level increased significantly at 140 minutes following a subcutaneous injection of 100 mg/kg cortisone acetate. This increase was clearly preceded by hyperglycemia.

(5) Subcutaneous injection of 6 Units/kg insulin, given 30 minutes after the subcutaneous injection of cortisone acetate (100 mg/kg), was found to block the hyperglycemia induced by cortisone in 50 minutes, without producing hypoglycemia.

(6) No increase in plasma histamine level was observed when the cortisone induced hyperglycemia was blocked by the subcutaneous injection of 6 Units/kg insulin, given 30 minutes after cortisone treatment.

(7) Injection of insulin (6 Units/kg, subcutaneously) 60 minutes after the treatment with cortisone (100 mg/kg, subcutaneously), did not block the increase in plasma histamine level.

(8) The increase in plasma histamine concentration following a single injection of cortisone acetate (100 mg/kg, subcutaneously), could be duplicated by a single injection of glucose (2.5 g/kg, subcutaneously). The increase in plasma histamine level following the single injection of glucose was also preceded by hyperglycemia.

(9) A dose response for histamine release in the plasma was observed when treatment with a subcutaneous injection of 5.0 g/kg glucose, produced a higher plasma histamine level, than a lower dose.

(10) Pretreatment with pronethalol (5 mg/kg, intraperitoneally), or phenoxybenzamine (5 mg/kg, intraperitoneally), or both together, did not block the cortisone induced hyperglycemia.

(11) Administration of cortisone acetate (100 mg/kg, subcutaneously) once daily for 10 consecutive days, produced a longer and more sustained increase in plasma histamine level than a single injection.

(12) Cortisone treatment (100 mg/kg, subcutaneously) for 10 consecutive days also produced a significant decrease in lung, liver and heart histamine content.

(13) Administration of glucose (2.5 g/kg, subcutaneously) once daily for 10 consecutive days also produced a significant increase in plasma histamine level and a significant decrease in tissue histamine content. These effects were less marked than those produced with cortisone.

(14) On prolonged administration insulin only partially blocked the changes in histamine levels brought about by cortisone. There was no increase in plasma histamine level, but there was still a decrease in histamine content in some of the tissues studied.

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