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HISTAMINE RELEASE BY CORTISONE INDUCED HYPERGLYCAEMIA

BY

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It has been reported that prolonged administration of cortisone to guinea-pigs resulted in a fall in levels of histamine in the tissue although no decrease in histamine contents was found if treatment was not maintained for at least 10 days (Kovacs, 1965). These findings were confirmed by Hicks (1965) who reported that administration of cortisone or dexamethasone over 14 or 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.

Although a single injection of cortisone does not cause detectable changes in tissue histamine levels of the guinea-pig, it might still release histamine and this should be reflected in the plasma histamine level. Experiments were therefore designed to determine plasma histamine levels after administration of cortisone. Plasma glucose levels were also determined, because according to Glenn, Bowman & Bayer (1961) hyperglycaemia is the first measurable metabolic change produced by cortisone.

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

METHODS

Animals

Male guinea-pigs weighing 300–320 g at the start of the experiments were used. They were of the multi-coloured short-haired variety, obtained from the Quebec Breeders Association (Canada). The guinea-pigs were fed on a diet of Purina guinea-pig chow, water and hay *ad libitum*. Before experiments the animals were fasted for 16 hr in cages with wide mesh wire bottoms; water was allowed at all times.

Preparation of plasma

Each tube and syringe was siliconized with Desicote solution (Beckman Instruments Inc.) and heparinized with 0.1 ml. of a 0.1% sodium heparin solution. Blood was obtained from guinea-pigs by cardiac puncture (ventricle) under brief ethylchloride anaesthesia. The syringe was filled with minimal traction on the plunger, the blood was immediately centrifuged at 1,500 rev/min and the plasma removed with Pasteur pipettes. Preparation of the plasma for glucose and histamine determinations was started within 30 min.

Determination of plasma glucose levels

The glucose oxidase method, as described by Saifer & Gerstenfeld (1958) was used. The reagent Glucostat was obtained from Worthington Biochemical Corporation. Glucose concentrations were measured in duplicate, using a Klett–Summerson colorimeter with a No. 42 filter. Internal standards in duplicate were run simultaneously with each set of unknowns.

Histamine extraction

The extraction of histamine from guinea-pig organs was carried out by the method described previously (Kovacs, 1965). Plasma histamine was extracted essentially by the same method, with the following slight modifications. To each ml. of plasma, 1 ml. of 10% trichloracetic acid was added. The tube was inverted several times and left at room temperature for 5 min. after centrifugation, the clear supernatant fluid was separated and extracted with 2 ml. of ether. The ether was washed twice with 0.5 ml. of distilled water. The watery fractions were combined, boiled for 3 min and neutralized with 0.1 N sodium hydroxide immediately before use.

Histamine assays

Histamine assays were carried out on the guinea-pig isolated ileum preparation, using a 10 ml. organ bath and Tyrode solution with atropine sulphate 5×10^{-7} g/ml. The temperature of the bath was kept at 35° C by means of a thermostat. The Tyrode solution in the bath was oxygenated by a mixture of 5% carbon dioxide and 95% oxygen. All histamine values refer to the base. Tests with promethazine hydrochloride 10^{-8} g/ml. confirmed that the activity recorded was caused by histamine.

Treatments

Subcutaneous injections of the following substances were given: cortisone acetate, 0.2 ml./100 g body weight of a 50 mg/ml. suspension (Cortone; Merck, Sharp & Dohme); insulin injection, 0.15 ml./100 g body weight of a 40 u./ml. solution diluted ten times with distilled water (Insulin-Toronto; Connaught Medical Research Laboratories; the preparation is a colourless solution of zinc-insulin crystals); glucose, 0.83 ml./100 g body weight and 1.66 ml./100 g body weight of a 30% solution in distilled water (Dextrose; Fisher Scientific Co.); saline, 0.83 ml./100 g body weight of a 5.4% solution in distilled water (sodium chloride, Fisher Scientific Co.); and calcium chloride, 0.83 ml./100 g body weight of a 1.22% solution in distilled water (calcium chloride, anhydrous, Fisher Scientific Co.). Details of dosage and times of administration are given under RESULTS. Because levels of plasma histamine and glucose were found to remain fairly constant in individual guinea-pigs and were not altered by a saline injection, each guinea-pig served as its own control in experiments in which only levels of plasma histamine were studied. A period of at least 5 days was allowed for the guinea-pigs to recover after control determinations of plasma histamine levels. Animals which did not gain weight normally were excluded.

Statistical analysis

For statistical evaluation of the results Student's *t* test was used.

RESULTS

Effect of a single injection of cortisone on plasma glucose and plasma histamine levels

Forty-two guinea-pigs were injected subcutaneously with cortisone acetate (100 mg/kg). Plasma glucose and plasma histamine levels were determined at different times after the injection, using six guinea-pigs each time. Results were compared with control values from the same animals and are shown in Fig. 1. There was maximal increase in glucose levels 50 min after administration of cortisone and glucose levels were elevated until about 150 min after injection. Plasma histamine levels started to increase 50 min after

the cortisone injection, reached maximal levels at 140 min and were elevated for more than 260 min. There was a significant increase in plasma histamine levels which was clearly preceded by hyperglycaemia.

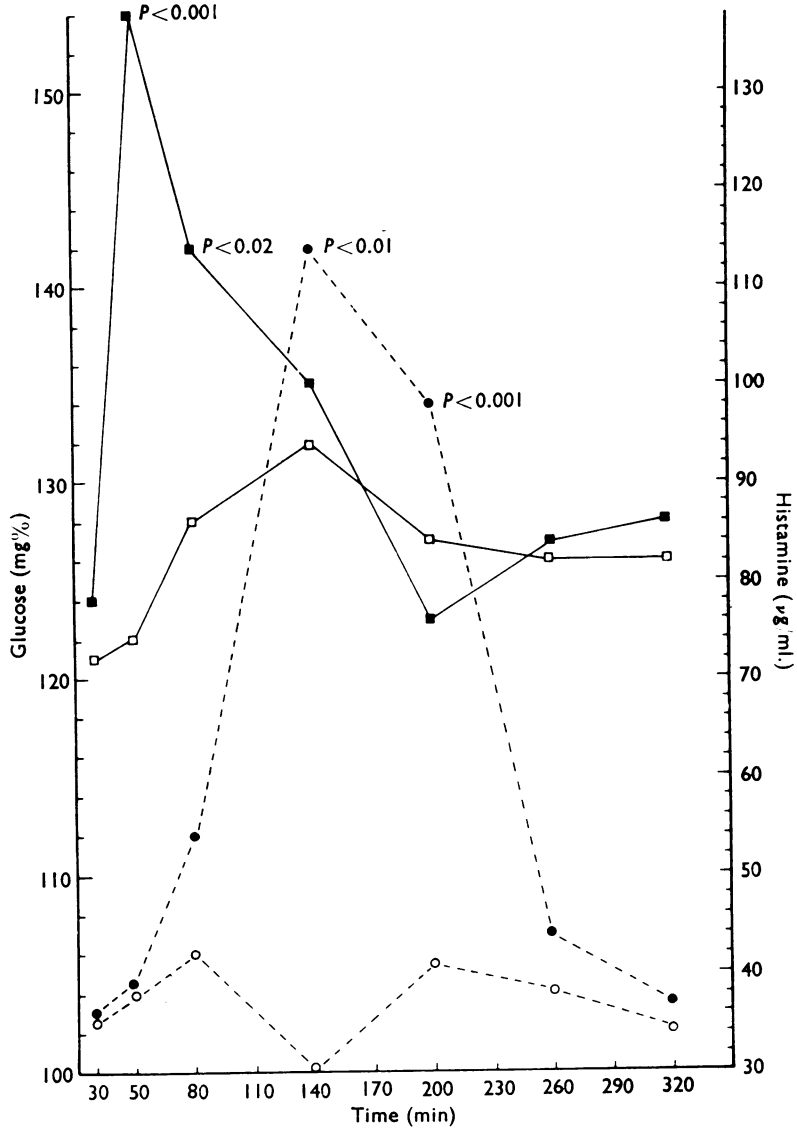

Fig. 1. Plasma glucose and histamine levels of guinea-pigs following a single subcutaneous injection of cortisone acetate 100 mg/kg. Each point represents the mean of results from six experiments. The points plotted at a given time were obtained from the same six guinea-pigs. The lines join results obtained with different groups of six guinea-pigs. □——□, Control glucose levels; ○ - - - ○, control histamine levels; ■——■, glucose levels after cortisone; ● - - - ●, histamine levels after cortisone.

Effect of a single dose of cortisone, given in conjunction with insulin, on plasma glucose and plasma histamine levels

Nine guinea-pigs were treated with cortisone acetate (100 mg/kg) and received insulin 6 u./kg 30 min later. Plasma glucose and plasma histamine levels were determined 50 or 140 min after injection of cortisone. As can be seen from Table 1, if the hyperglycaemia induced by cortisone was abolished by insulin, no increase in plasma histamine levels was observed at 140 min, at the time when cortisone alone produced peak plasma histamine levels. It can also be seen from Table 1 that if insulin was administered 60 min after the cortisone injection, at a time when hyperglycaemia had already developed, the increase in plasma histamine levels still occurred.

TABLE 1

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

All cortisone acetate and insulin injections were administered subcutaneously. Values are means and standard errors. Histamine concentrations are expressed in terms of the base. N.S.=Not significant.

Treatment	No. of animals	Time after cortisone (min)	Plasma glucose (mg %)		Plasma histamine (γg/ml.)		*P*
			Control	Treated	Control	Treated	
Cortisone, insulin 30 min later	6	50	123±9·28	124±9·51	68·5±18·00	68·5±18·99	N.S.
As above	3	140	125±4·85	39±6·11	29·0± 1·53	23·0± 3·00	N.S.
Cortisone, insulin 60 min later	3	140	123±3·85	42±4·62	32·0± 3·18	109·0±17·90	<0·01
As above	4	180	130±4·31	35±2·74	28·0± 5·84	116·0±25·30	<0·02

Effect of a single dose of glucose on plasma histamine levels

Because hyperglycaemia always preceded the increase in plasma histamine levels after injection of cortisone and there was no increase in plasma histamine levels if the hyperglycaemic effect of cortisone was prevented by insulin, it was of interest to assess the effect of glucose on plasma histamine levels. Twenty-nine guinea-pigs were injected subcutaneously with glucose 2.5 g/kg or 5 g/kg and their plasma glucose and plasma histamine levels were determined at different times after injection. Results are shown in Table 2. It can be seen that hyperglycaemia *per se* led to an increase in plasma histamine levels. A significant increase in plasma histamine levels occurred at 140 and 260 min after the injection of glucose. Hyperglycaemia preceded the increase in plasma histamine concentration ; that is, 30 min after an injection of glucose 2.5 g/kg, plasma glucose levels were maximal while plasma histamine levels just started to rise. It can also be seen from the data that a higher dose of glucose produced not only higher plasma glucose levels but also higher plasma histamine levels than were obtained with a lower dose. The subcutaneous injection of hypertonic saline or isotonic calcium chloride solution did not lead to any increase in plasma glucose and plasma histamine levels at 140 min following the injection.

Effect of repeated injections of cortisone on plasma glucose and plasma histamine levels

Twenty-four guinea-pigs were treated for 10 days with daily doses of cortisone acetate 100 mg/kg. At different times after the last injection, the plasma glucose and plasma histamine levels of four guinea-pigs were determined and compared with the control

values of the same animals. Results are shown in Fig. 2. Peak plasma glucose levels were higher than those obtained with a single injection of cortisone. Maximal plasma histamine levels were lower than those produced by a single injection of cortisone but maintained for longer; 320 min after the last injection, histamine levels were still markedly elevated but they returned to normal 24 hr after the last cortisone injection.

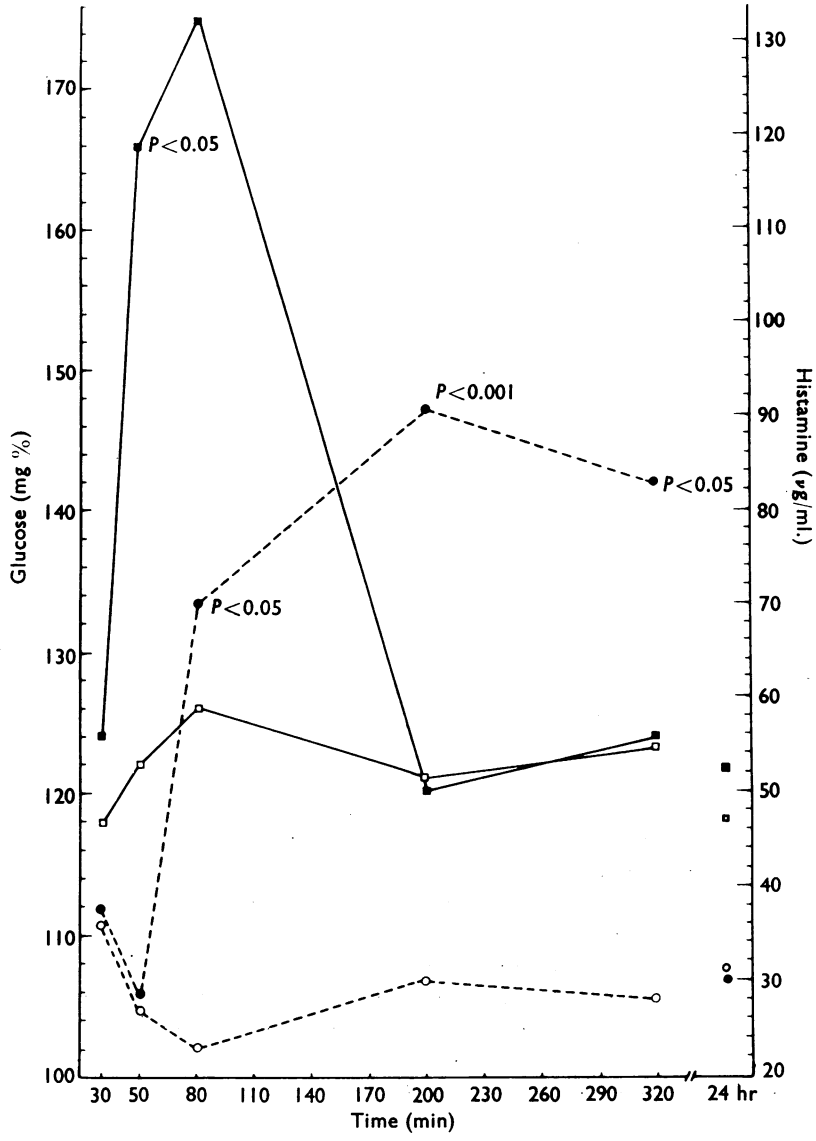

Fig. 2. Plasma glucose and histamine levels of guinea-pigs following treatment with cortisone acetate 100 mg/kg for 10 days. Each point represents the mean of results from four experiments. The points plotted at a given time were obtained from the same four guinea-pigs. The lines join results obtained with different groups of four guinea-pigs. □——□, Control glucose levels; ○---○, control histamine levels; ■——■, glucose levels after cortisone; ●---●, histamine levels after cortisone.

TABLE 2

EFFECT OF GLUCOSE ON PLASMA HISTAMINE LEVELS OF GUINEA-PIGS

Injections of glucose, saline and calcium chloride were given subcutaneously. Values are means and standard errors Histamine concentrations are expressed in terms of the base. Figures in parentheses represent the percentage increase relative to control values. N.S.=Not significant.

Treatment and dose	No. of animals	Time (min)	Plasma glucose (mg %)			Plasma histamine (γg/ml.)		
			Control	Treated	*P*	Control	Treated	*P*
Control I: NaCl 0·50 g/kg	6	140	125±2·62	124± 3·00	N.S.	38±2·31	37± 2·21	N.S.
Control II: $CaCl_2$ 0·10 g/kg	5	140	123±1·93	123± 1·32	N.S.	37±3·13	37± 4·56	N.S.
Glucose 2·50 g/kg	4	30	120±2·73	416±13·14 (247)	<0·001	31±1·45	45± 7·83 (45)	N.S.
Glucose 2·50 g/kg	4	140	116±7·64	214±27·38 (86)	<0·02	37±9·99	100±12·51 (170)	<0·01
Glucose 2·50 g/kg	4	260	124±3·24	146± 1·47 (17·7)	<0·001	32±2·15	66±4·53 (106)	<0·001
Glucose 2·50 g/kg	3	380	121±4·51	136± 2·31 (12·3)	<0·05	34±3·79	48± 8·25 (41)	N.S.
Glucose 5·00 g/kg	8	140	141±5·24	462±37·66 (228)	<0·001	42±7·29	187± 8·39 (345)	<0·001
Glucose 5·00 g/kg	6	260	132±4·67	239±42·43 (81)	<0·05	35±1·48	106± 9·99 (203)	<0·001

TABLE 3

HISTAMINE CONTENTS OF GUINEA-PIG PLASMA AND ORGANS FOLLOWING TREATMENT WITH CORTISONE, GLUCOSE, OR CORTISONE WITH INSULIN

Animals were injected with cortisone acetate, glucose, or cortisone acetate with insulin subcutaneously once daily for 10 days and killed 4 hr after the last injection. Values are means and standard errors. Histamine concentrations are expressed in terms of the base. N.S.=Not significant.

		Histamine content (γg/g) in								
		Plasma (γg/ml.)								
Treatment	No. of animals	Control	Treated	*P*	Lung	*P*	Liver	*P*	Heart	*P*
Saline	4	27·0±2·00	28·0±4·20	N.S.	21·0±0·86	N.S.	2·06±0·16	N.S.	6·00±0·02	N.S.
Cortisone 100 mg/kg	6	28·0±6·89	83·0±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 g/kg	7	29·0±1·46	73·0±3·62	<0·001	13·6±0·70	<0·001	1·09±0·04	<0·001	4·72±0·37	<0·001
Cortisone+insulin 6 u./kg 30 min later	4	39·0±1·58	33·0±3·24	N.S.	18·5±1·94	N.S.	1·19±0·03	<0·01	4·34±0·31	<0·01

Effect of a prolonged treatment with cortisone, glucose or cortisone in conjunction with insulin on plasma and tissue histamine levels

Twenty-one guinea-pigs were treated once daily for 10 days with cortisone acetate (100 mg/kg) or glucose (2.5 g/kg) or cortisone acetate (100 mg/kg) followed 30 min later by insulin (6 u./kg) or saline. Animals were killed 4 hr after the last injection and their plasma and tissue histamine levels were determined. Table 3 shows that after the administration of multiple doses of cortisone, plasma histamine levels rose and tissue histamine levels were reduced. Multiple doses of glucose also produced an increase in plasma histamine levels and a decrease in tissue histamine levels, although the effect was somewhat less marked than that of cortisone. If insulin was administered after each dose of cortisone, no significant change occurred in plasma histamine levels and virtually no decrease in pulmonary histamine levels was found. There was, however, still a decrease in histamine contents of the liver and heart, though the decrease was less than that obtained by cortisone treatment alone.

DISCUSSION

Individual plasma histamine levels on repeated determinations remained remarkably constant in our guinea-pigs, which confirms previous observations (Code & MacDonald, 1937; Rose & 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 the same individual. For this reason, levels of histamine were determined in the same animal before and after treatment and the former was used as the control value.

A fall in blood histamine levels has been reported in normal human subjects after administration of cortisone (Code & Mitchell, 1953; Kelemen & Bikich, 1956). In these studies, however, histamine determinations were carried out only once on whole blood when the number of circulating eosinophil and basophil cells was significantly reduced. The 24 hr output of free histamine, however, was found to be increased in the urine of healthy human subjects after administration of cortisone (Mitchell & Code, 1954).

Schayer, Smiley & Davis (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 seems to be only partly responsible for the effect of cortisone on the level of histamine, reflected in our experiments when cortisone in conjunction with insulin has been repeatedly administered. The minimal decrease in pulmonary histamine levels in these circumstances might reflect that in tissues which have a high histamine content (Schayer *et al.*, 1956) or are rich in mast cells (Kahlson, Rosengren & Thunberg, 1963) a histamine molecule once formed is firmly held and has a long intracellular lifetime. Glucocorticoids, however, were also found to interfere with the biosynthesis of histamine (Halpern, 1956) and with the normal activity of mast cells (Asboe-Hansen, 1952). It seems that glucocorticoids affect various aspects of histamine metabolism and their hyperglycaemic effect might be an important step in a complex chain of reactions.

The increase in plasma histamine level induced by cortisone can be duplicated by glucose. In prolonged administration glucose reduced tissue histamine levels as well, though the decrease was less than that obtained with 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 release of histamine possible under anoxic conditions (Diamant & Uvnäs, 1961 ; Diamant, 1962). On the other hand, various carbohydrates inhibit the dextran anaphylactoid reaction in rats (Goth, Nash, Nagler & Holman, 1957 ; Beraldo, Dias da Silva & Lemos Fernandes, 1962) probably by competing with dextran for receptors in the mast cell (Dias da Silva & Lemos Fernandes, 1965). The results described in this paper suggest a third type of interaction between glucose and histamine—that is, glucose acts as a histamine releaser, at least in the guinea-pig.

There are various data in the literature which imply that increased levels of blood glucose are beneficial in certain experimental conditions. Long (1956) noted that in guinea-pigs insulin hypoglycaemia is associated with a marked sensitivity to tuberculin while hyperglycaemia is associated with a decrease in sensitivity. Ganley (1962) found that mice sensitized with *B. pertussis* and rendered diabetic with alloxan were less susceptible to the toxicity of histamine than control sensitized mice. Adamkiewitz & Sacra (1967) reported 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, for example, was almost completely inhibited when histamine was injected between the second and fourth hour after administration of glucose. Similarly, if the blood glucose levels of dogs were increased, the fall in blood pressure after injection of histamine 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 about the significance of an increase in plasma histamine levels after the administration of cortisone or glucose. A decrease in the sensitivity of blood vessels to histamine after previous injection of histamine has, however, been reported by several investigators (Phemister & Handy, 1927 ; Epstein, 1932 ; v. Euler, 1938 ; Anrep, Barsoum, Talaat & Wieninger, 1939 ; Emmelin, 1945).

There are also clinical observations in the literature which seem to suggest the possibility that an inverse relationship exists between blood histamine levels and histamine toxicity (Rose, 1940 ; Rose & Browne, 1940 ; Rose, 1941a, b ; Rorsman, 1961 ; Beall, 1963).

SUMMARY

1. Plasma glucose and plasma histamine levels of guinea-pigs were determined before and at different time intervals after a subcutaneous injection of cortisone acetate 100 mg/kg.

2. A single injection of cortisone brought about a significant increase in the plasma histamine level, which was always preceded by hyperglycaemia.

3. If cortisone was prevented from producing a hyperglycaemia by an injection of insulin, no increase in the plasma histamine level was observed. If insulin was administered at a time when hyperglycaemia induced by cortisone had already developed, the increase in the plasma histamine levels was not blocked.

4. The increase in the plasma histamine level after a single cortisone injection could be duplicated by a subcutaneous injection of glucose 2.5 g/kg or 5 g/kg.

5. The results indicate that the increase in plasma histamine concentration induced by a single injection of cortisone is mediated by the hyperglycaemic effect of cortisone.

6. The daily administration of a single dose of cortisone or glucose for 10 days caused a release of histamine from the lung, liver and heart. This could be partly blocked by insulin.

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