Blood Pyruvate in Cats after Hydrocortisone Hemi-Succinate

The concentration of pyruvate in the blood has been found to be raised in Cushing's syndrome[1,2] and in people given anti-inflammatory steroids. Such steroids had usually been given for days or months when the observations were made but an effect has been observed 8 h after a first dose of cortisone or from 4 to 8 h after adrenocorticotrophic hormone[3]. The difference in the blood pyruvate concentrations of normals and of those who had received steroids was increased if glucose was given[4].

In animals gluco-corticoids raised the blood glucose before the liver glycogen[5]. Recently an increase in blood lactate was also found to occur relatively rapidly while the blood pyruvate, after injection of pyruvate into the peritoneal cavity, was higher than in controls when hydrocortisone had been given 5 h before[6]. It seemed worth while to determine whether a single dose of a glucocorticoid could raise the blood pyruvate and how quickly it could do it.

Methods. Blood was drawn from conscious cats, weight 2–2.5 kg, by way of a polythene catheter (internal diameter 0.5 or 1.0 mm) previously inserted into the superior vena cava via an external jugular vein during general anaesthesia. Clotting in the cannula was prevented by filling it with heparin-saline. No experiment was done less than two days after cannulation. When adrenalectomy was performed both glands were removed at one operation by a trans-abdominal route. Adrenalectomised cats were given parenteral hydrocortisone injections and intravenous saline infusions for the first three to four days after operation and then saline to drink as the only addition to the usual diet of milk, meat, and cat food. At least 48 h passed after an injection of hydrocortisone before an experiment was done. Cats were deprived of food and drink for $4^1/_2$ h before an experiment.

Hydrocortisone was given intravenously as the sodium hemi-succinate derivative ('Efcortelan', Glaxo), 100 mg of steroid (0.28 m*M*) dissolved in 2 ml of water. Control injections were 2 ml normal saline (twice), 2 ml of 0.1 *M* phosphate buffer, pH 7.4 (once), 20 mg sodium bicarbonate in 2 ml water (twice), and 45 mg sodium succinate in 2 ml water (thrice). An adrenalectomized cat was used in two of the control experiments.

Blood pyruvate was measured by a chicromatograph method[7] as the 2:4-dinitrophenylhydrazone derivative, separated from other carbonyl compounds in an acid system[8]. 30% of the derivative of oxaloacetate decomposes to that of pyruvate in this system but in no chromatogram did the primary band of oxaloacetate appear. Blood glucose was measured by the glucose oxidase method[9] and blood lactate by the method of BARKER and SUMMERSON[10]. About 1 ml of blood was used for each determination of pyruvate. 14 ml of blood was the most drawn during one experiment.

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Tab. I. Venous blood pyruvate in normal and adrenalectomized cats

	Blood pyruvate ± S.E.M., mg/100 ml	
	Before injections	Before injections and after control injections
Normal	0.53 ± 0.02 (21)	0.50 ± 0.01 (56)
Adrenalectomized	0.43 ± 0.03 (12)	0.45 ± 0.02 (20)
Probability of difference	$t = 2.24$ $p = 0.05 - 0.02$	$t = 2.32$ $p = 0.05 - 0.02$

Tab. III. Blood glucose after hydrocortisone hemisuccinate in 5 normal cats

Time after injection (min)	15	30	60
Rise in blood glucose mg/100 ml ± S.E.M.	− 2.6 ± 2.5	+ 2.4 ± 3.1	+ 6.6 ± 3.2

Tab. II. Venous blood pyruvate after hydrocortisone hemi-succinate (the number of observations for each value is in brackets)

Type of animal	Solution injected	Blood pyruvate ± S.E.M., mg/100 ml Time after test injection, min							
		− 10	0	5	10	15	20	30	60
Normal	Control	0.53 ± 0.03 (5)	0.53 ± 0.02 (6)	0.53 ± 0.04 (4)	0.43 ± 0.04 (5)	0.53 ± 0.03 (6)	0.50 ± 0.04 (5)	0.51 ± 0.04 (6)	0.53 ± 0.06 (6)
Normal	Hydrocortisone	0.58 ± 0.07 (4)	0.53 ± 0.03 (5)	0.72 ± 0.02 (2)	0.84 ± 0.06 (4)	0.86 ± 0.11 (6)	0.71 ± 0.06 (4)	0.69 ± 0.11 (6)	0.59 ± 0.05 (5)
Adrenal-ectomized	Hydrocortisone	0.39 ± 0.04 (3)	0.48 ± 0.04 (6)	0.65 ± 0.05 (3)	0.81 ± 0.07 (4)	0.82 ± 0.13 (6)	0.74 ± 0.17 (3)	0.67 ± 0.08 (6)	0.50 ± 0.06 (5)
		Significance of differences							
Normal + Hydrocortisone v. Normal before injection (Table I, column 1)				$t = 3.32$ $p =$ 0.01–0.001	$t = 6.40$ $p = < 0.001$	$t = 4.94$ $p = < 0.001$	$t = 3.83$ $p = < 0.001$	$t = 2.62$ $p =$ 0.02–0.01	
Adrenalectomized + Hydrocortisone v. Adrenalectomized before injection (Table I, column I)				$t = 3.45$ $p =$ 0.01–0.001	$t = 6.14$ $p = < 0.001$	$t = 3.96$ $p =$ 0.01–0.001	$t = 3.35$ $p =$ 0.01–0.001	$t = 3.38$ $p =$ 0.01–0.001	

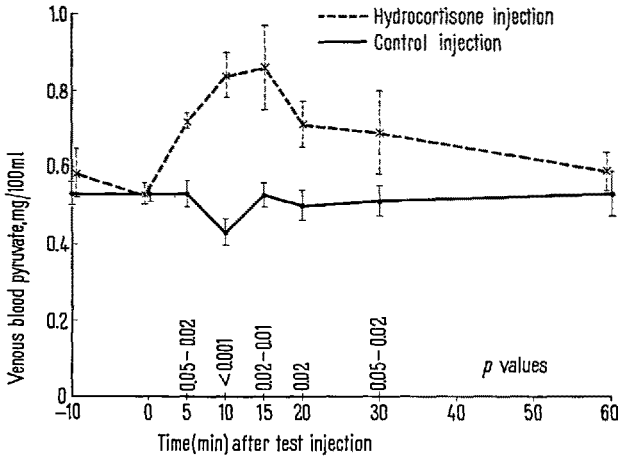

Fig. 1. Blood pyruvate in normal cats after hydrocortisone hemisuccinate. Values and standard errors shown. p values given for differences of means.

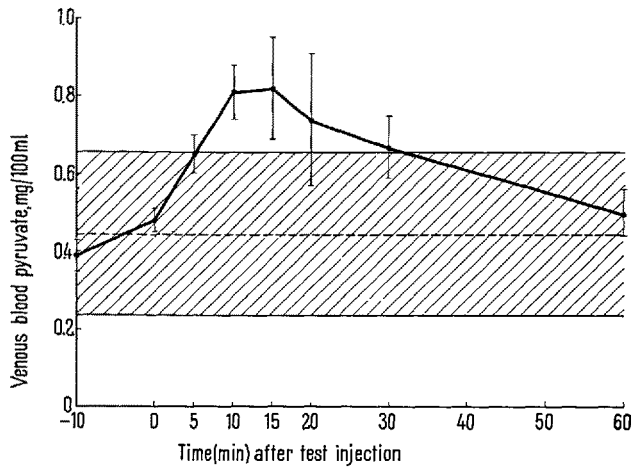

Fig. 2. Blood pyruvate in adrenalectomized cats after hydrocortisone hemisuccinate. Dotted line is mean of all values in adrenalectomized cats not given hydrocortisone, with shaded area $\pm$ 2 $\times$ standard deviation. Bars show standard error of mean values.

Results. The pyruvate concentration in venous blood was higher in normal animals than in the adrenalectomized (Table I), as previously reported[11]. No significant rise of blood pyruvate occurred after any of the control injections. After hydrocortisone hemi-succinate was injected blood pyruvate rose rapidly in both normal (Figure 1) and adrenalectomised (Figure 2) cats. In Table II are the values from which the Figures are drawn and the significance of the difference between values before and after hydrocortisone.

The rise of pyruvate preceded that in blood glucose (Table III). In three experiments the blood lactate was measured. A small rise occurred after hydrocortisone so the rise in pyruvate did not occur at the expense of lactate.

Discussion. It has recently been suggested that glucocorticoids produce effects before their actions on carbohydrate metabolism have became apparent[12]. A feature of the present results is that a metabolic effect of hydrocortisone was observed in the conscious cat, not long starved, 10 min after its intra-venous administration in a large dose. The dose is large by physiological or even medicinal standards; nevertheless it is comparable on the basis of body weight with the doses used by LONG, KATZIN, and FRY[5] in their classic study of the effect of steroids on carbohydrate metabolism and by others in subsequent experiments both *in vivo* and *in vitro*.

The explanation of the rise in pyruvate is unknown, as is the tissue most concerned. Some possibilities are:

(1) That hydrocortisone in the concentration used acted as a non-specific stimulus different from that of any of the control solutions. If so, the hormones of the adrenal medulla could not have been involved in the observed response since it was as apparent in adrenalectomized as in normal cats.

(2) That metabolism of the succinate part of the injected molecules in the presence of hydrocortisone caused the rise of blood pyruvate. But an equimolecular amount of non-esterified succinate caused no rise of blood pyruvate, even when injected into a normal cat given large parenteral injections of hydrocortisone for the previous four days. Also, blood oxaloacetate was normal within 5 min of the injection of an equivalent amount of oxaloacetate. Moreover, when a gluco-corticoid not esterified with succinate was injected intravenously (20 mg of prednisolone-21-phosphate, 'Predsol', Glaxo (twice) and 115 mg of hydrocortisone-21-phosphate (once)), there again occurred a rapid rise in blood pyruvate, similar to that observed after hydrocortisone hemi-succinate, and again preceding the rise in blood glucose[13].

(3) That the large dose given revealed an early effect of hydrocortisone on metabolic processes or tissue permeabilities. So large a dose might have been necessary because an index as crude as the blood pyruvate concentration was used. Changes in protein or amino-acid metabolism have been suggested to explain the effect of steroids on carbohydrate metabolism[14]; it may be noted that as quick a rise in blood pyruvate occurred after intravenous alanine as after hydrocortisone[13].

This last of the three possibilities is the one favoured here.

Zusammenfassung. Hydrocortison-Na-hemi-Succinat (100 mg des Steroids) lebenden, $4^1/_2$ h fastenden Katzen injiziert, ergab sofortigen Anstieg der Pyruvat-Konzentrationen im Blut mit nachfolgendem Blutzuckeranstieg.

Injektionen von Kontrollösungen ergaben keine Reaktion. Nach Steroidgaben erfolgte sowohl bei normalen wie adrenolektomierten Tieren ebenfalls Anstieg.

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