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# A RAPID RISE IN BLOOD PYRUVATE IN CATS GIVEN LARGE DOSES OF HYDROCORTISONE

BY

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In Scandinavia, a little over 10 years ago, the concentration of pyruvate in blood was found to be increased in patients with Cushing's syndrome (Kerppola, 1953) and in patients treated with cortisone or corticotrophin for various inflammations (Lövgren, 1952; Gitelson, 1954). This latter group had usually been treated for weeks or months before the blood pyruvate was measured; however, increase in pyruvate was observed 8 hr after treatment with cortisone or corticotrophin had been begun, but more than one dose had always been given by this time. The findings in Cushing's syndrome were later confirmed (Frawley, 1955). An abnormally large increase in blood pyruvate after oral glucose in Cushing's syndrome had also been reported (Hills, Power & Wilder, 1952), and this was confirmed by Henneman & Bunker (1957).

These results are not easily explained by the usual beliefs that the glucocorticoids increase hepatic gluconeogenesis and perhaps also decrease the extrahepatic utilization of glucose. Experiments were done, therefore, to investigate the effect of a single injection of a glucocorticoid upon the blood pyruvate. A preliminary report has already been published (Hockaday, 1962).

## METHODS

Blood was drawn from, and injections were made into, conscious cats without disturbance through a polyethylene catheter, previously inserted aseptically during diethyl ether anaesthesia, usually into the superior vena cava or innominate vein (but also elsewhere, as described below) of animals of either sex, weighing 2 to 4 kg, fasted for 5 hr. Some animals had been adrenalectomized bilaterally by the transabdominal route; an experiment was never done less than 5 days after adrenalectomy or less than 5 days after intramuscular injections of hydrocortisone which were sometimes given to adrenalectomized cats on the days immediately after adrenalectomy. No steroid other than hydrocortisone was so injected. In experiments on the legs, during anaesthesia throughout with intravenous thiopentone sodium, one femoral artery was catheterized and tied off so that arterial blood could be drawn, while a tributary of the femoral vein on the other side was also catheterized so that femoral venous blood could be sampled without obstruction of the main vessel. Urine was obtained through a polyethylene catheter passed up the urethra into the bladder of male cats, again during thiopentone anaesthesia. Evisceration was performed by removal of the alimentary tract from the stomach to the rectum, together with the spleen, liver and gall bladder. At the end of the operation, glucose (290 mg/kg body weight) was injected intravenously and observations were begun 1 hr later.

Blood pyruvate was measured as its 2,4-dinitrophenylhydrazone derivatives after purification by paper chromatography in an acid system (Hockaday, 1961). This two-phase system was a 10 : 10 : 17 : 3 mixture of light petroleum (boiling point 100 to 120° C), toluene, acetic acid and water. The chromatograms

were developed in the descending manner, with the nonpolar phase mobile, after equilibration in the chromatography tank for at least 2 hr. The two isomers of pyruvate have closely similar $R_F$ values (about 0.44) in this system (Bush & Hockaday, 1962), and formed a single band, best located on the dried chromatogram by inspection with ultraviolet light. The hydrazone was eluted from the relevant zone (cut from the whole chromatogram) in apparatus described by Bush (1961), with a filtered solution of 60 ml. of ethanol, 40 ml. of ethyl acetate and 1 ml. of 5% aqueous metaphosphoric acid as solvent. The extinction coefficient of the eluted 2,4-dinitrophenylhydrazone dissolved in a standard volume of solvent was read at 390 mμ on a Unicam SP 600 spectrophotometer.

Blood for pyruvate estimation was transferred directly into chilled 5% metaphosphoric acid solution and its amount determined by weight. Urine was used directly without protein precipitation. The preparation and initial extraction of the 2,4-dinitrophenylhydrazone derivative were by usual methods (Barrenscheen & Dreguss, 1931; Friedemann & Haugen, 1943; Abdel-Tawab, Broda & Kellner, 1959).

This method gave an average recovery of 76% of sodium pyruvate (3 to 21 μg as pyruvate) added to the mixture of blood and metaphosphoric acid. No correction for this partial recovery is made in the reported results. The deviation from their mean value of estimations on duplicate samples of blood drawn at the same time was 3.7±0.4% (mean and standard error).

Blood glucose was estimated by the enzymatic method of Huggett & Nixon (1957) and blood lactate by the method of Barker & Summerson (1941).

Hydrocortisone was injected as its sodium hemisuccinate ester (Efcortelan, Glaxo) or as its phosphate, and prednisolone as its disodium phosphate (Predsol, Glaxo). Dexamethasone was injected intravenously as its phosphate, and aldosterone was given unesterified.

## RESULTS

Intravenous injection of 20 or 25 mg of hydrocortisone was not followed by any change in the pyruvate concentration in central venous blood in cats (Tables 1, 2). However, 100 mg was followed by an increase in pyruvate (Table 1), and 50 mg by a small rise. After 100 mg the maximum concentration occurred as soon as 10 min after the injection, while

TABLE 1

VENOUS BLOOD PYRUVATE LEVELS IN NORMAL CATS AFTER INTRAVENOUS INJECTION OF HYDROCORTISONE HEMISUCCINATE

| | | Blood pyruvate (mg/100 ml.) | | | | |
|---|---|---|---|---|---|---|
| | | | Change at min after hydrocortisone | | | |
| Experiment | Hydrocortisone dose (mg) | Initial | 10 | 15 | 30 | 60 |
| 1 | 25 | 0·56 | 0·03 | 0·06 | −0·01 | −0·07 |
| 2 | 25 | 0·61 | −0·02 | −0·04 | −0·09 | −0·04 |
| 3 | 25 | 0·49 | 0·08 | 0·06 | 0·12 | 0·04 |
| 4 | 50 | 0·50 | 0·21 | 0·13 | 0·05 | 0·09 |
| 5 | 50 | 0·57 | 0·11 | 0·22 | 0·17 | −0·09 |
| 6 | 50 | 0·48 | 0·20 | 0·34 | 0·12 | −0·05 |
| 7 | 50 | 0·62 | 0·18 | 0·39 | 0·22 | −0·09 |
| 8 | 100 | 0·54 | 0·15 | 0·09 | −0·04 | −0·04 |
| 9 | 100 | 0·43 | — | 0·50 | 0·05 | 0·10 |
| 10 | 100 | 0·62 | 0·38 | 0·08 | 0·21 | 0·10 |
| 11 | 100 | 0·50 | 0·35 | 0·88 | 0·65 | −0·06 |
| 12 | 100 | 0·54 | 0·27 | 0·19 | 0·05 | 0·11 |
| 13 | 100 | 0·73 | — | 0·04 | −0·08 | — |
| 14 | 100 | 0·40 | 1·49 | 1·00 | — | 0·60 |
| 15 | 100 | 0·75 | 1·09 | 1·19 | — | 0·25 |
| 16 | 100 | 0·59 | 0·94 | 1·07 | — | 0·16 |
| | Mean for expts. 8–16 | 0·57 | 0·67 | 0·56 | 0·14 | 0·15 |
| | Standard error | ±0·04 | ±0·19 | ±0·16 | ±0·11 | ±0·07 |

TABLE 2

BLOOD PYRUVATE LEVELS IN CATS AFTER INJECTION OF STEROIDS OTHER THAN HYDROCORTISONE HEMISUCCINATE

| Steroid | Dose (mg) | Adrenal glands | Blood pyruvate (mg/100 ml.) Initial | Change at min after steroid 10 | 15 | 20 | 30 | 60 |
|---|---|---|---|---|---|---|---|---|
| Hydrocortisone phosphate | 20 | Present | 0·61 | 0·16 | 0·27 | 0·12 | −0·08 | −0·01 |
| | 20 | Present | 0·58 | −0·04 | −0·02 | −0·04 | 0·01 | 0·01 |
| | 50 | Removed | 0·64 | 0·32 | −0·05 | −0·13 | 0·07 | −0·07 |
| | 100 | Present | 0·63 | 0·67 | 0·60 | 0·58 | 0·26 | 0·04 |
| Prednisolone phosphate | 20 | Present | 0·41 | 0·49 | 0·17 | 0·14 | 0·34 | 0·16 |
| | 20 | Present | 0·51 | 0·20 | 0·28 | 0·31 | 0·43 | 0·44 |
| Dexamethasone phosphate | 10 | Removed | 0·35 | 0·03 | 0·03 | 0 | 0·04 | 0·52 |
| Aldosterone | 2 | Present | 0·46 | 0·06 | 0·01 | 0 | 0·12 | 0·01 |

30 and 60 min afterwards the pyruvate concentration had returned to values not significantly greater than the initial value. The injection of solutions of various sodium salts (including 45 mg of the succinate) in ten experiments was followed by an increase of blood pyruvate concentration of 0.18 mg/100 ml. at the greatest, while at no time was there a significant change in the mean value for the ten experiments from the resting value.

An increase in pyruvate concentration was seen after hydrocortisone phosphate, and after prednisolone phosphate at a dose of steroid as low as 20 mg, an amount approximately equivalent in anti-inflammatory effect to 100 mg of hydrocortisone (Table 2).

Aldosterone (2 mg) or dexamethasone phosphate (10 mg) had no such effect in the 30 min after their injection. Increase in pyruvate after 100 mg of hydrocortisone also occurred after an 18-hr fast, in cats anaesthetized with thiopentone, and when the steroid was injected subcutaneously (Table 3).

The increase in pyruvate occurred in blood from the inferior vena cava (Table 3) as well as from the superior vena cava. When femoral arterial and venous blood from anaes-

TABLE 3

MISCELLANEOUS MEASUREMENTS OF BLOOD PYRUVATE

HC = Hydrocortisone hemisuccinate. Pred = Prednisolone phosphate. Except where otherwise stated, blood was taken from the superior vena cava

| Experiment | Blood pyruvate (mg/100 ml.) Initial | Change at min after test 10 | 15 | 20 | 30 | 45 | 60 |
|---|---|---|---|---|---|---|---|
| HC (100 mg) after 18-hr fast | 0·63 | 0·27 | −0·05 | 0 | −0·10 | 0·01 | — |
| HC (100 mg) subcutaneously | 0·36 | 0·19 | 0·36 | 0·72 | 0·54 | 0·28 | 0·24 |
| HC (100 mg) subcutaneously | 0·75 | — | −0·08 | — | 0·15 | 0·10 | −0·21 |
| *Pentothal anaesthesia* | | | | | | | |
| (*a*) HC (100 mg) | 0·26 | 0·27 | 0·39 | 0·29 | 0·18 | 0·24 | 0·15 |
| (*b*) HC (50 mg) | 0·41 | 0·59 | — | 0·31 | — | — | −0·02 |
| (*c*) Pred (20 mg) | 0·28 | — | 0·39 | — | 0·23 | — | 0·17 |
| *Blood from inferior vena cava* | | | | | | | |
| (*a*) HC (100 mg) | 0·59 | 0·25 | 0·39 | — | 0·12 | — | −0·04 |
| (*b*) HC (100 mg) | 0·52 | 0·48 | 0·39 | — | 0·17 | — | 0·05 |
| *Cats which retched after* | | | | | | | |
| (*a*) Carbachol (25 μg) subcutaneously | 0·60 | 0·05 | — | 0·10 | 0·07 | 0·11 | — |
| (*b*) Pharyngeal stimulation | 0·57 | 0·04 | −0·06 | — | — | — | — |

thetized cats was examined, hydrocortisone injection was followed by an increase in the pyruvate concentrations in both. The increase was greater in venous than in arterial blood (Table 4).

Sometimes the injection of steroid caused the cat to retch for 0.5 to 1 min. Such a reaction would not seem the cause of the pyruvate rise because this occurred equally in animals that did not retch, while cats that retched after subcutaneous injection of 25 $\mu$g of carbachol or pharyngeal stimulation showed no increase in blood pyruvate (Table 3).

TABLE 4

ARTERIOVENOUS DIFFERENCE IN PYRUVATE CONCENTRATION ACROSS A LEG AFTER INTRAVENOUS HYDROCORTISONE (100 mg) OR SODIUM PHOSPHATE TO NORMAL CATS

| Injected | Time after injection (min) | Pyruvate concentration | |
|---|---|---|---|
| | | Arterial (mg/100 ml.) | Venous minus arterial (mg/100 ml.) |
| Hydrocortisone | 0 | 0·42 | −0·01 |
| | 10 | 0·41 | 0·17 |
| | 15 | 1·18 | 0·17 |
| | 60 | 0·71 | 0·01 |
| Hydrocortisone | 0 | 0·47 | 0·02 |
| | 10 | 0·47 | 0·11 |
| | 15 | 0·69 | 0·17 |
| | 60 | 0·60 | 0·19 |
| Hydrocortisone | 0 | 0·54 | −0·01 |
| | 10 | 0·59 | 0·14 |
| | 15 | 0·70 | 0·21 |
| | 60 | 0·59 | 0·03 |
| Sodium phosphate | 0 | 0·56 | −0·02 |
| | 10 | 0·59 | −0·03 |
| | 15 | 0·54 | 0·01 |
| | 60 | 0·48 | 0·02 |

TABLE 5

BLOOD LACTATE AND LACTATE/PYRUVATE RATIOS AFTER INTRAVENOUS HYDROCORTISONE OR CONTROL SOLUTION

| Expt. | Injection | Adrenal glands | Blood lactate (L) (mg/100 ml.) and lactate/pyruvate ratio (L/P) at intervals (min) after injection | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0 | | 15 | | 60 | |
| | | | L | L/P | L | L/P | L | L/P |
| 1 | Hydrocortisone (100 mg) | Present | 9·8 | 16·6 | 30·6 | 18·4 | 10·8 | 14·4 |
| 2 | Hydrocortisone (100 mg) | Present | 20·7 | 27·6 | 22·3 | 11·5 | 20·1 | 20·2 |
| 3 | Hydrocortisone (100 mg) | Removed | 11·9 | 23·8 | 15·2 | 12·1 | 8·8 | 20·0 |
| 4 | Hydrocortisone (50 mg) | Present | 8·6 | 15·6 | 11·5 | 15·1 | 9·0 | 17·0 |
| 5 | Sodium phosphate | Present | 7·9 | 18·6 | 11·0 | 19·4 | 10·1 | 24·4 |
| 6 | Sodium phosphate | Present | 12·4 | 17·8 | 12·1 | 17·7 | 9·0 | 19·1 |

The rise in pyruvate was accompanied by neither a fall in blood lactate concentration (Table 5) nor a reduction in the small amount of pyruvate excreted in the urine (Table 6).

Injection of hydrocortisone into adrenalectomized cats was followed by an increase in pyruvate concentration similar to that seen with intact animals (Table 7). In both normal and adrenalectomized animals the increase in pyruvate preceded any increase in glucose.

TABLE 6

URINARY EXCRETION OF PYRUVATE AFTER GLUCOCORTICOID ADMINISTRATION

Urinary flow and pyruvate values refer to the periods indicated

| | | Interval (min) from time of injection | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | −50 | −30 | −10 | 0 | 10 | 20 | 55 |
| *Expt.* 1: | *Hydrocortisone hemisuccinate* (100 *mg*) | | | | | | | |
| | Blood pyruvate (mg/100 ml.) | 0·71 | 0·53 | 0·43 | 0·41 | 1·10 | 0·72 | 0·39 |
| | Urinary flow (mg/min) | 97 | | 19 | | 322 | 103 | |
| | Urinary pyruvate (mg/100 ml.) | 0·68 | | 0·79 | | 0·44 | 0·53 | |
| | Urinary pyruvate (μg/min) | 0·66 | | 0·15 | | 1·42 | 0·55 | |
| | | −60 | −25 | 0 | | 15 | 35 | 65 |
| *Expt.* 2: | *Prednisolone* (20 *mg*) | | | | | | | |
| | Blood pyruvate (mg/100 ml.) | 0·27 | 0·32 | 0·28 | | 0·76 | 0·51 | 0·45 |
| | Urinary flow (mg/min) | 59 | | 52 | | 73 | 53 | |
| | Urinary pyruvate (mg/100 ml.) | 0·71 | | 1·23 | | 0·61 | 0·73 | |
| | Urinary pyruvate (μg/min) | 0·42 | | 0·64 | | 0·44 | 0·39 | |

TABLE 7

BLOOD PYRUVATE IN ADRENALECTOMIZED CATS AFTER 100 mg OF HYDROCORTISONE HEMISUCCINATE INTRAVENOUSLY

| | Blood pyruvate (mg/100 ml.) | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Change at min after hydrocortisone | | | | | |
| | Initial | 5 | 10 | 15 | 20 | 30 | 60 |
| | 0·50 | 0·25 | 0·27 | 0·20 | — | 0·08 | −0·18 |
| | 0·53 | 0·04 | 0·36 | −0·05 | — | 0·09 | 0·15 |
| | 0·52 | — | — | 0·91 | — | 0·52 | 0 |
| | 0·46 | 0·18 | 0·18 | 0·29 | 0·19 | 0·12 | 0·04 |
| | 0·49 | — | — | 0·31 | 0·58 | 0·23 | — |
| | 0·28 | — | 0·68 | 0·44 | 0·24 | 0·18 | 0·22 |
| Mean | 0·46 | | 0·37 | 0·35 | | 0·20 | 0·05 |
| Standard error | 0·04 | | 0·11 | 0·13 | | 0·07 | 0·07 |

TABLE 8

BLOOD PYRUVATE AND GLUCOSE CONCENTRATIONS IN ACUTELY EVISCERATED CATS AFTER 100 mg OF HYDROCORTISONE INTRAVENOUSLY

| | | Blood pyruvate (P) and glucose (G) (mg/100 ml.) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Change at min from hydrocortisone | | | | | | | |
| | Adrenals before | Initial | | −10 | | 10 | | 20 | | 30 | |
| Expt. | evisceration | P | G | P | G | P | G | P | G | P | G |
| 1 | Present | 2·53 | 118 | 0·39 | 9 | 0·67 | −22 | 1·06 | −34 | 0·93 | −46 |
| 2 | Present | 0·84 | 107 | 0·21 | 20 | 1·06 | −27 | 0·76 | −40 | — | — |
| 3 | Present | 1·36 | 72 | 0·08 | 11 | 0·45 | −19 | 0·36 | −38 | 0·22 | −47 |
| 4 | Removed | 1·03 | 58 | — | — | — | −4 | 0 84 | −24 | 0·01 | −29 |

No rise in blood glucose concentration had occurred 30 min after injection of hydrocortisone whereas small rises were seen after 60 min. Increase of pyruvate also occurred after hydrocortisone injection into acutely eviscerated cats (Table 8), one of which had been previously adrenalectomized.

## DISCUSSION

The present results show that a single intravenous injection of a large amount of hydrocortisone (100 or 50 mg) is rapidly followed by a transitory increase in the concentration of pyruvate in venous and arterial blood, and that this increase precedes any change in blood glucose concentration. The dose used may be compared with the estimated daily secretion of hydrocortisone and corticosterone by the adrenal glands of the cat (3.5 to 8.5 mg/kg body weight; Bush, 1952). 100 mg of hydrocortisone has been injected intravenously into children less than a year old in the treatment of the adrenogenital syndrome.

The increase in pyruvate could represent its " under-utilization," "over-production," or both. The liver is a major site of pyruvate utilization, but acute removal of this organ, together with the intestines, does not prevent or exaggerate the effect. The arteriovenous difference measurements also show that an extrahepatic effect occurs, and that there is " over-production " of pyruvate.

This " over-production " might result from a change in membrane permeability, but the breakdown of complex molecules, such as glycogen or triglyceride, is another possibility. Verzár & Wenner (1948a, b) claimed a glycogenolytic action for a number of steroids when these were incubated *in vitro* with rat diaphragm, and found desoxycorticosterone acetate to be the most potent. The steroid concentration had to be 5 mg/100 ml. or more. Verzár & Montigel (1942) had previously shown that adrenal steroids in high concentration increased the phosphorylation of glycogen by rat muscle *in vitro*.

Other evidence for a glycogenolytic action of corticoids concerns the liver. Glenn (personal communication) found that 20 to 40 mg of hydrocortisone hemisuccinate injected into the portal vein decreased the liver glycogen of rats previously fed a high glucose diet. Also, 30 to 80 mg of hydrocortisone given intraperitoneally decreased liver glycogen in rats previously injected with glucose. The results of both Glenn, Bowman, Bayer & Meyer (1961), and Hilz, Tarnowski & Arend (1963) suggest that a decrease in liver glycogen during the first hour after injection of smaller doses of hydrocortisone into rats precedes the well-established increase in glycogen.

However, the pyruvate increase might result from a change in fat metabolism such as has been suggested to occur within 15 min of intra-arterial injection of hydrocortisone into the human forearm (Jenkins, Lowe & Titterington, 1964).

## SUMMARY

1. Intravenous injection of a large dose (100 or 50 mg) of hydrocortisone (as hemisuccinate or phosphate) into conscious cats was followed by a rapid increase in venous and arterial blood pyruvate concentrations. This increase occurred before any increase in blood glucose concentration. The pyruvate also increased after prednisolone (20 mg) but not after various sodium salts (including succinate).

2. The increase in pyruvate was neither associated with a fall in blood lactate concentration nor with a reduction in urinary pyruvate excretion.

3. There was a greater increase of pyruvate level in femoral venous than in femoral arterial blood.

4. The increase in pyruvate occurred in both chronically adrenalectomized and acutely eviscerated cats, and in normal cats anaesthetized with thiopentone sodium.

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