RESEARCH PAPERS

EFFECTS OF SALICYLATE ADMINISTRATION ON REPUTED INDICES OF ADRENAL CORTICAL ACTIVITY IN THE RAT

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Received May 15, 1961

Changes in the urinary sodium and potassium concentration and 17-ketosteroid excretion patterns of rats treated with large doses of salicylate have been compared with those seen in animals given a standard pituitary-adrenal stimulus (exposure to cold). The significance of the general lack of correlation of responses to these two forms of treatment is discussed in the light of the frequently accepted theory that salicylates stimulate the adrenal cortex.

THE claim of Hetzel and Hine (1951) that salicylates stimulate the adrenal gland by way of the pituitary to increased release of cortical hormones has been supported by many observations in experimental animals and man. In a recent review Smith (1959) emphasises the general acceptability of the evidence, disputing only that the antirheumatic effects of salicylate are mediated via the adrenal cortex.

The conclusions of the early workers in this field were based on adrenal ascorbic acid or cholesterol depletion in rats after heavy dosage with salicylate (Robinson, 1951; van Cauwenberge, 1951) and the absence of these responses in the hypophysectomised animal (van Cauwenberge, 1951). More recently, conclusive evidence of increased levels of circulating plasma 17-hydroxycorticosteroids after treatment with salicylate has been obtained for rats (Done, Ely and Kelley, 1958; Roskam, 1957; van Cauwenberge, 1954); dogs (Done, Ely and Kelley, 1958); guinea-pigs (Good, Done, Ely and Kelley, 1957); and man (Done, Ely and Kelley, 1955; Roskam, 1956).

The increased plasma corticosteroid response to massive salicylate dosage is not paralleled in the urinary steroid metabolites where there is considerable disparity in reports of levels after salicylate administration. While increase in urinary excretion of reducing corticoids is reported by van Cauwenberge and Huesghem (1952), they found no consistent response in 17-ketosteroid output of salicylate-treated adults with rheumatic diseases. Pellegrini and Sala (1952) and Roskam (1956) report similar findings. Other workers confirm the absence of a consistent urinary increase after salicylate treatment in either 17-ketosteroids (Böe and Stöa, 1953; Bonati, Bertolani and Lorenzini, 1951; Henly, 1952; van Cauwenberge and Huesghem, 1951); the 17-hydroxycorticosteroids (Smith, Gray and Lunnon, 1954), or reducing steroids (Böe and Stöa, 1953). In fact Done and co-workers (1955, 1958) report consistent depression of steroid excretion during salicylate treatment.

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Until recently there appear to have been no detailed studies of urinary sodium and potassium levels after salicylate administration, and yet the sodium : potassium ratio of the urine is known as a sensitive index of adrenal mineralocorticoid activity (Hetzel, McSwiney, Mills and Prunty, 1956; Simpson and Tait, 1955). However, in 1959, Hetzel, Charnock and Lander reported low sodium and high potassium levels in the urine of human subjects within a few hr. after administration of 5 g. of sodium salicylate, a finding which is in close accord with our own preliminary observations in man (Blane, 1957).

The plasma and urinary electrolyte changes resulting from acute cold exposure have been shown in rats to be compatible with a theory of increased adrenal activity in both normal (Munday and Blane, 1960, 1961) and hypothermic animals (Munday, Blane, Chin and Machell, 1958). In the normal rat so stressed there is also a marked increase in the short-term (12–36 hr.) output of 17-ketosteroids (Munday and Blane, 1960). It was decided therefore to test the value of the same criteria in the assessment of salicylate as a pituitary-adrenal stimulant. The response to salicylate of the urinary sodium and potassium levels, and the 17-ketosteroid excretion was studied in rats.

Experimental Methods

Male rats of the Hooded strain were used and during the 12-hr. experimental periods were housed in metabolism cages constructed to avoid dilution or contamination of urine by drinking fluid.

Normally rats were supplied with a standard cube diet and water. Solid food however was withheld during the test periods to avoid urine contamination and instead a 5 per cent glucose in 0·85 per cent saline solution was available *ad libitum*. This short-term change of diet has been shown to be without effect on plasma electrolyte and 17-ketosteroid levels (Munday and Blane, 1960).

Techniques used in the measurement of urinary electrolytes and 17-ketosteroids were the same as have been described previously (Munday and Blane, 1960).

Both adrenal glands were removed from rats of 100 to 150 g. under pentobarbitone anaesthesia. Until required they were maintained in draught-free cages and supplied with 1 per cent saline and rat cake. On this régime their plasma electrolyte levels remained within the normal range. Careful macroscopic examination at autopsy confirmed the completeness of adrenalectomy in all animals.

Acetylsalicylic acid was given to rats by stomach-tube as 1·0 ml. of 6 per cent solution in 2 per cent sodium carbonate. Since the intact animals used in most experiments weighed between 300 and 350 g. the dose of 60 mg. per rat corresponded to something less than 20 mg. per 100 g. weight. Proportionately reduced doses were used for the smaller adrenalectomised rats. Other groups of rats were given sodium salicylate instead of acetylsalicylic acid at an increased dose (30 g./100 g.) corresponding to the reported lessened therapeutic activity of the sodium salt. The "control" animals received a 1·0 ml. placebo of sodium bicarbonate

solution containing an amount of sodium equivalent to that given to the "experimental" rats in their salicylate solution.

RESULTS

Rats supplied with glucose in saline produced large volumes of clean urine in the 12-hr. test period and the urinary sodium and potassium

TABLE I

EFFECTS OF ACETYLSALICYLIC ACID, SODIUM SALICYLATE AND EXPOSURE TO COLD ON THE 12-HR. VALUES OF URINARY SODIUM, POTASSIUM AND 17-KETOSTEROID EXCRETION IN MALE RATS

	Controls	Acetylsalicylic acid	Sodium salicylate	Cold exposure (0° C.)
Na	150·7 ± 0·56 (12)	141·1 ± 0·63 (12)	143·9 ± 1·42 (12)	137·6 ± 0·89 (10)
K	13·78 ± 0·62 (12)	34·25 ± 2·78 (12)	38·30 ± 2·19 (12)	15·89 ± 0·27 (10)
Na:K	11·21 ± 0·52 (12)	4·37 ± 0·33 (12)	3·86 ± 0·19 (12)	8·68 ± 0·14 (10)
17-KS	53·9 ± 1·45 (18)	26·2 ± 1·02 (8)	23·6 ± 1·11 (8)	62·5 ± 2·14 (8)

Na and K concentrations as m-equiv., 17-KS as μg./12 hr./rat, numbers of animals in parenthesis means ± standard errors.

concentrations as well as the 17-ketosteroid excretion were found to vary within very narrow limits under these conditions (Table I).

Urine collected in the immediate 12-hr. period after administration of either salicylate showed a much depressed Na:K ratio by virtue of a fall in sodium level and rise in potassium (Table I). The directional similarity of these electrolyte changes to those obtained on cold exposure

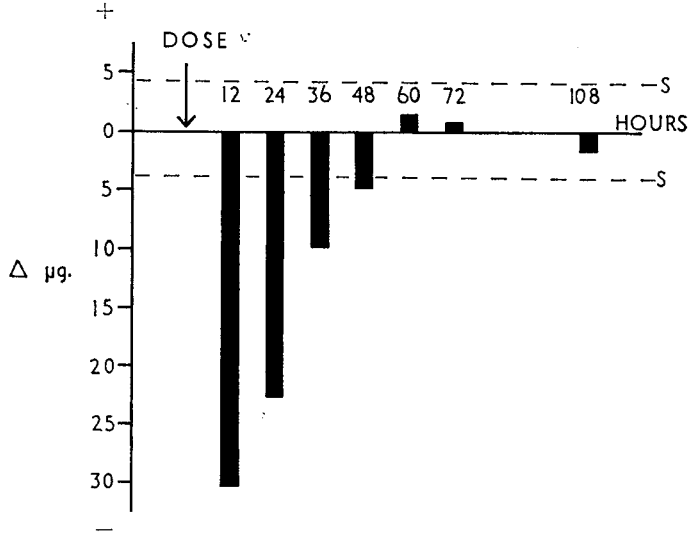

FIG. 1. Typical effect of a single dose of sodium salicylate on the urinary 17-ketosteroid excretion of a group of 8 male rats. Base line = mean value of 52·9 μg./12 hr./rat from 16 rats with S the standard deviation. Columns represent the mean change in 17-ketosteroid excretion from the control level (△ μg.) measured for the whole group of salicylate-treated animals at 12 hr. intervals. These mean differences at 12, 24 and 36 hr. are highly significant with $P < 0{\cdot}005$. Dose at zero time = 30 mg./100 g. rat.

is also illustrated in Table I and supports the contention of earlier authors that salicylate stimulates the pituitary-adrenal axis.

In direct contrast to the increased 17-ketosteroid excretion on short-term cold exposure, the 17-ketosteroid excretion of rats treated with acetylsalicylic acid or sodium salicylate was significantly diminished (Table I). Longer term experiments provided conclusive evidence that the urine 17-ketosteroid excretion of rats is greatly reduced during the first 12 hr. after dosing with salicylate and that there is a gradual return to normal over the following 36 hr. (Fig. 1).

Bilaterally adrenalectomised rats were given sodium salicylate and their urinary sodium and potassium levels determined after 12 hr. (Table II). In the adrenalectomised control animals the régime on which they

TABLE II

EFFECTS OF SODIUM SALICYLATE ON URINARY SODIUM AND POTASSIUM CONCENTRATION OF ADRENALECTOMISED RATS

	Na	K	Na/K
Adrenalectomised untreated ..	160·2 ± 2·62 (14)	14·1 ± 1·01 (14)	11·68 ± 0·72 (14)
Adrenalectomised treated with sodium salicylate	190·1 ± 6·25 (14)	49·7 ± 4·00 (14)	3·94 ± 0·23 (14)

Na and K concentrations as m-equiv., numbers of animals in parenthesis, means ± standard errors.

were maintained caused only a slight elevation of both sodium and potassium urine concentrations and the Na : K ratio was within the normal range. On treatment with salicylate there was, by contrast with intact animals, a marked rise in the urinary sodium level. However, the potassium concentrations rose at the same time to such high levels that the Na:K ratio was as low in these adrenalectomised as in the intact animals.

DISCUSSION

It has been established earlier that the urinary sodium and potassium changes in rats caused by exposure to cold are dependent on the integrity of the adrenal gland (Munday and Blane, 1960). That an essentially similar change in electrolyte pattern should have been observed in man receiving heavy doses of salicylate (Hetzel, Charnock and Lander, 1959) and is now shown in intact rats appears to further support the long-established hypothesis that salicylates have an effect in stimulating the pituitary-adrenal axis. There are difficulties in the way of accepting this interpretation.

In all salicylate-treated intact animals, loss of potassium was massive compared to that seen in cold-stressed rats and furthermore potassium excretion was also greatly increased in adrenalectomised rats receiving salicylate. It appeared therefore that potassium excretion may be affected by salicylate without the intervention of adrenal corticosteroids. Guest, Rapoport and Roscoe (1945) found the potassium loss in men treated with acetylsalicylic acid or sodium salicylate to be secondary to an alkalosis caused by hypernoea, and it is noteworthy that the rats in our experiments were frequently observed to be hyperventilating after dosage

with salicylate. The high urinary potassium in salicylate treated rats may represent—in part at least—a loss of fixed base consequent on respiratory alkalosis. A further possible alternative explanation is suggested by the *in vitro* work of Hicklin (1959) and Manchester, Randle and Smith (1958) which has yielded some evidence that salicylate may act directly to bring about potassium loss from tissues through interference with the energy supplies for active transport of this ion. Hence, for the present, potassium loss in salicylate-treated subjects and the Na:K ratio must be rejected as reliable indices of adrenal activity.

Sodium excretion on the other hand was reduced in intact salicylate-treated rats and raised in adrenalectomised animals receiving the same treatment. This could be interpreted to suggest that a sodium-retaining factor is released from the adrenal cortex in increased amounts by salicylate and that urinary sodium concentration may provide an adequate indication of the level of adrenal activity in these circumstances, at least for mineralocorticoid output.

The reduced urinary steroid excretion in subjects receiving salicylate presents another problem and should not immediately be taken as an indication that adrenal activity is depressed. Done, Ely and Kelley (1958) have found consistently low urinary 17-hydroxycorticosteroid and 17-ketosteroid levels in human subjects and animals treated with salicylate at a time when plasma corticosteroid levels were consistently high. Similar plasma changes did not occur in hypophysectomised or adrenalectomised experimental animals. In considering mechanisms to account for the low levels of urinary steroid metabolites while plasma corticosteroid levels are high it is of interest that salicylate may be secreted in a significant proportion as the glucuronide (Kapp and Coburn, 1944). Steroids too are mainly excreted as conjugates, and combination with glucuronic acid in the liver is known as a major pathway (Dorfman and Ungar, 1954). Salicylate might therefore act as a competitive inhibitor of corticosteroid excretion in a manner comparable to that already demonstrated for *N*-acetyl-*p*-aminophenol (Corte and Johnson, 1958). In this instance there would be a concentration of circulating corticosteroid while the steroid-glucuronide levels in the urine, including the 17-ketosteroid fraction, would fall.

The demonstration of Na:K ratios as low in adrenalectomised as in intact rats treated with salicylate, and the dramatic fall in 17-ketosteroid excretion of salicylate-treated rats shows clearly the inadequancy of these indices in the assessment of salicylate as a pituitary-adrenal stimulant. Sodium alone followed an excretion pattern that paralleled that seen in animals given a standard stress. Further detailed studies are required in this field with particular attention paid to the effects of salicylate on plasma corticosteroid levels, having regard to the possibility that these may be raised only because renal clearance of steroids is inhibited.

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