SOME EFFECTS OF SODIUM SALICYLATE ON MUSCLE METABOLISM

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Reid, Macdougall, and Andrews (1957) have demonstrated anew that aspirin (acetylsalicylic acid) is capable of reducing hyperglycaemia, glycosuria, and ketonaemia in some diabetic patients. In the rat, both aspirin and sodium salicylate are capable of reducing glycosuria and hyperglycaemia in diabetes induced by partial pancreatectomy with forced feeding (Ingle, 1950), alloxan (Smith, Meade, and Bornstein, 1952), or cortisone (Smith, 1952). The mechanism of this action of salicylate is not known. Salicylate is unlikely to act in diabetes by reducing gluconeogenesis, for in normal rats salicylate treatment produces negative nitrogen balance (Winters and Morrill, 1955), and it does not reduce the negative nitrogen balance of diabetes induced in rats with cortisone (Smith, 1952).

Smith and Jeffrey (1956) found that salicylate slightly inhibited the uptake of glucose by the isolated rat diaphragm, an observation which would seem to indicate that salicylate does not increase the utilization of glucose by muscle. However, the experiments of Smith and Jeffrey were carried out with diaphragm incubated in a phosphate-buffered medium. Randle (1956) and Randle and G. H. Smith (unpublished) have observed that oxygen lack and a number of compounds which interfere with the oxidative production of energy-rich phosphate promote the uptake of glucose by diaphragm incubated in a medium buffered with bicarbonate, but not in a medium buffered with phosphate. Since salicylate is also believed to interfere with the oxidative production of energy-rich phosphate (Meade, 1954; Smith and Jeffrey, 1956; Brody, 1956; Penniall, 1956), it seemed important to reinvestigate the effect of salicylate on the uptake of glucose by diaphragm in a medium buffered with bicarbonate.

When isolated rat diaphragm is incubated in the presence of a ^{14}C -labelled amino-acid, ^{14}C is incorporated into the protein of the diaphragm (Sinex, MacMullen, and Hastings, 1952; Krahle, 1953; Manchester and Young, unpublished). Since salicylate treatment produces negative nitrogen balance in the intact rat it was of interest also to investigate its influence on the incorporation of ^{14}C -amino-acids into the protein of the isolated diaphragm.

Methods

Diaphragm.—The preparation and incubation of diaphragm were as described by Randle (1956).

Media.—Bicarbonate-buffered medium (Gey and Gey, 1936), gassed with $\text{O}_2 + \text{CO}_2$ (95:5), pH 7.4 at 37°C . Phosphate-buffered medium (Krebs-Ringer phosphate, after Umbreit, Burris, and Stauffer, 1949), gassed with O_2 , pH 7.4 at 37°C . Glucose was added to give a concentration of 2.5 mg./ml. The radioactive amino-acids [^{14}C]glycine and

uniformly labelled L-[^{14}C]glutamic acid and L-[^{14}C]lysine were present at a concentration of about $0.1\ \mu\text{mol./ml.}$ and a specific activity of about $50\ \mu\text{C./mg.}$ Sodium salicylate was added to yield a concentration of $5 \times 10^{-3}\text{M.}$ Crystalline insulin (Boots) was dissolved in N/300 HCl to 20 units/ml. and diluted with medium to 0.1 unit/ml.

Glucose uptake was measured by the disappearance of glucose from the medium when one hemidiaphragm was incubated in each flask for 1 hour in 1 ml. of medium. Glucose was estimated either by the Somogyi (1945) modification of Nelson's method or by the method of Huggett and Nixon (1957). Glucose uptake was calculated as milligrams of glucose disappearing from the medium per gramme of wet diaphragm per hour of incubation. Potassium loss from the diaphragm was measured by the change in potassium content of the medium during incubation. Potassium was estimated by flame photometry. Potassium loss was calculated as micro-equivalents of potassium added to the medium per gramme of wet diaphragm per hour of incubation.

Total muscle water was determined by weighing the diaphragm wet and after freeze-drying.

Thiosulphate, glucose, and xylose spaces were determined with the whole diaphragm preparation of Kipnis and Cori (1957), a preparation in which muscle attachments to rib and vertebral column are intact. The diaphragm was incubated for one hour in 10 ml. of medium containing (for glucose and thiosulphate space) glucose 2.5 mg./ml. and sodium thiosulphate 20 mEq/l., or (for xylose space) D-xylose 1.5 mg./ml. and glucose 1 mg./ml. At the end of incubation glucose or thiosulphate or xylose was determined in diaphragm muscle and medium. The space for thiosulphate, xylose, or glucose was calculated from the formula (space = quantity in muscle/concentration in medium). Muscle extracts and medium were deproteinized with tungstic acid for thiosulphate determination and by the method of Somogyi (1945) for glucose or xylose determinations. Thiosulphate was determined in muscle extracts or medium by the method of Newman, Gilman, and Philips (1946), glucose by the method of Huggett and Nixon (1957), and xylose by the method of Roe and Rice (1948).

Uptake of ^{14}C into Diaphragm Protein.—Two hemidiaphragms were incubated together in 2 ml. of medium in each flask for two hours. After incubation the diaphragm was homogenized in trichloroacetic acid (T.C.A.) and the precipitate collected and heated in T.C.A. for 15 minutes at 90°C . The insoluble material was treated twice with 0.4 N NaOH and the dissolved protein then reprecipitated from the NaOH extract with T.C.A., washed with ethanol-ether (50:50), ether, and dried in a vacuum desiccator. The dried sample of protein was ground in a mortar, collected on a 1 sq. cm. plastic planchette, and counted as an infinitely thick sample (Popják, 1950).

Results

Salicylate and Glucose Uptake.—In a medium buffered with bicarbonate, sodium salicylate ($5 \times 10^{-3}\text{M.}$) added *in vitro* increases the uptake of glucose by isolated diaphragms from intact rats (Table I). The concentration of salicylate used is about the same as the blood level of salicylate in the diabetics treated with aspirin by Reid *et al.* (1957). Sodium *p*-hydroxybenzoate, unlike salicylate (*o*-hydroxybenzoate), does not increase glucose uptake.

In a phosphate-buffered medium sodium salicylate does not stimulate glucose uptake. This confirms the earlier observation of Smith and Jeffrey (1956).

Salicylate and the Glucose and Xylose Spaces.—Sodium salicylate *in vitro* does not significantly alter the total water or the thiosulphate space of the isolated rat diaphragm. It may therefore be inferred that salicylate does not alter the volumes of intracellular and extracellular water. The addition of insulin is without effect on the total water content but leads to a small reduction in the thiosulphate space. Free glucose has an entirely extracellular distribution in the diaphragm in the presence or absence of insulin or salicylate,

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since in no instance does the glucose space exceed the thio-sulphate space. In the same way D-xylose has a principally extracellular distribution in the absence of salicylate or insulin. When insulin or salicylate is present, however, D-xylose acquires a partially intracellular distribution and comes into equilibrium with about 40% of intracellular water (Table II).

Salicylate and Potassium Loss.—The presence of sodium salicylate *in vitro* leads to a greatly increased loss of potassium from the isolated rat diaphragm during incubation; neither sodium *p*-hydroxybenzoate nor insulin significantly affects the loss of potassium (Table I).

Salicylate and Incorporation of Amino-acids into Protein.—In the absence of salicylate, ^{14}C -labelled glycine, glutamic acid, and lysine are incorporated into protein of the isolated rat diaphragm. In the presence of salicylate ^{14}C incorporation into protein is very greatly reduced (Table III). On the other hand, when insulin is added *in vitro* incorporation of ^{14}C is substantially increased (Krahl, 1953; Manchester and Young, unpublished).

Discussion

These observations show that sodium salicylate increases glucose uptake and potassium loss by the isolated rat diaphragm and decreases incorporation into protein of ^{14}C -labelled glycine, lysine, and glutamic acid. On the other hand, sodium *p*-hydroxybenzoate does not stimulate glucose uptake (or potassium release), and this may be correlated with the fact that sodium *p*-hydroxybenzoate, unlike sodium *o*-hydroxybenzoate (salicylate), does not raise the B.M.R. *in vivo* (Cochran, 1954). The incorporation of amino-acids into protein and the maintenance of high intracellular

potassium are processes known to be dependent upon the availability of high-energy phosphate, and the influence of salicylate upon these processes would be in keeping with the view that it interferes with the production of high-energy phosphate by oxidative mechanisms (uncouples oxidative phosphorylation). The diminution of incorporation of amino-acids into protein of the isolated rat diaphragm in the presence of salicylate is in accord with the observation that salicylate therapy promotes negative nitrogen balance in the intact animal. The fact that salicylate promotes glucose uptake by diaphragm in a bicarbonate-buffered medium would support the suggestion of Reid *et al.* (1957) that salicylate stimulates metabolism in peripheral tissues—that is, muscle.

This observation suggests that salicylate may reduce glycosuria and hyperglycaemia in diabetes by promoting the utilization of glucose by muscle. The finding by Reid *et al.* (1957) that salicylate raises the B.M.R. in diabetic as well as in non-diabetic subjects is in keeping with this idea. The limiting step in the utilization of glucose by muscle is thought to be the rate of entry of glucose into the muscle cell. Park, Bornstein and Post (1955), Park and Johnson (1955), and Levine and Goldstein (1955) believe that insulin accelerates the utilization of glucose by muscle by increasing the rate of entry of the sugar into the muscle cell. Park *et al.* (1955) have obtained evidence that insulin accelerates the uptake of glucose by the isolated rat diaphragm *in vitro* by this mechanism, and in keeping with this view is the observation that insulin increases the volume of distribution of the non-utilizable sugar D-xylose in the water of the isolated diaphragm (Kipnis and Cori, 1957). It is likely that the volume of distribution of glucose in the water of the isolated diaphragm is not increased by insulin under these

TABLE I.—Influence of Sodium Salicylate ($5 \times 10^{-3}\text{M}$) and of Insulin (0.1 unit/ml.) on Uptake of Glucose and Release of Potassium by the Isolated Rat Diaphragm *in vitro*

Experiment No.	Addition to Medium	Glucose Uptake. Mean $\pm$ S.E. of Mean. mg. Glucose/g. of Wet Diaphragm/Hour of Incubation	Potassium Loss. Mean $\pm$ S.E. of Mean. $\mu\text{equiv. Potassium/g. of Wet Diaphragm/Hour of Incubation}$
<i>Bicarbonate-buffered Medium</i>			
1a	No addition	2.5 ± 0.20 (14)	8 ± 1.2 (14)
1b	Sodium salicylate ($5 \times 10^{-3}\text{M}$)	4.0 ± 0.28 (14) } $P < 0.001$	31 ± 1.5 (14) } $P < 0.001$
1c	Sodium <i>p</i> -hydroxybenzoate ($5 \times 10^{-3}\text{M}$)	2.0 ± 0.21 (6) } $P < 0.001$	8 ± 0.9 (6) } $P < 0.001$
2a	No addition	2.3 ± 0.17 (6) } $P < 0.001$	8 ± 0.9 (6) } $P < 0.05$
2b	Insulin (0.1 unit/ml.)	9.1 ± 0.24 (6) } $P < 0.001$	5 ± 0.9 (6) } $P < 0.05$
<i>Phosphate-buffered Medium</i>			
3a	No addition	3.2 ± 0.28 (6)	17 ± 0.6 (6) } $P < 0.001$
3b	Sodium salicylate ($5 \times 10^{-3}\text{M}$)	2.8 ± 0.13 (6)	42 ± 0.9 (6) } $P < 0.001$

The value of P is given when the difference between means is significant. Number of observations is given in parentheses. Values derived from the incubation of hemidiaphragms.

TABLE II.—Influence of Sodium Salicylate ($5 \times 10^{-3}\text{M}$) and of Insulin (0.1 unit/ml.) on the Total Water Content and the Thiosulphate, Glucose, and Xylose Spaces of the Isolated Rat Diaphragm Incubated in a Bicarbonate-buffered Medium

Experiment No.	Addition to Medium	Total Muscle Water $\mu\text{l. Water/100 mg. of Wet Diaphragm. Mean} \pm \text{S.E. of Mean}$	Thiosulphate Space $\mu\text{l. Water/100 mg. of Wet Diaphragm. Mean} \pm \text{S.E. of Mean}$	Glucose Space $\mu\text{l. Water/100 mg. of Wet Diaphragm. Mean} \pm \text{S.E. of Mean}$	Xylose Space $\mu\text{l. Water/100 mg. of Wet Diaphragm. Mean} \pm \text{S.E. of Mean}$	Glucose Uptake mg. Glucose/Hour of Incubation
1a	No addition	74 ± 0.5 (6)	22 ± 1.3 (6)	17 ± 2.0 (6)	26 ± 0.6 (10)	1.8 (6)
1b	Sodium salicylate ($5 \times 10^{-3}\text{M}$)	75 ± 0.6 (6)	25 ± 1.3 (6)	18 ± 2.2 (6)	44 ± 3.0 (3) } $P < 0.001$	3.8 (6)
2a	No addition	74 ± 0.9 (5)	21 ± 1.0 (5) } $P < 0.05$	16 ± 0.9 (5)	26 ± 0.6 (10) } $P < 0.001$	4.4 (5)
2b	Insulin (0.1 unit/ml.)	75 ± 0.5 (5)	17 ± 1.2 (5) } $P < 0.05$	14 ± 1.4 (5)	48 ± 1.0 (4) } $P < 0.001$	6.2 (5)

The value of P is given when the difference between means is significant. Number of observations is given in parentheses. The diaphragm preparation used in these experiments consisted of rib, vertebra, intercostal muscle, and the diaphragm with its attachments to these structures intact. Uptake of glucose under these conditions is not therefore by the diaphragm alone.

TABLE III.—Influence of Sodium Salicylate ($5 \times 10^{-3}\text{M}$) on Incorporation of ^{14}C from ^{14}C -labelled Glycine, Lysine, and Glutamic Acid into Protein of the Isolated Rat Diaphragm *in vitro*

Addition to Medium	Incorporation into Protein, Mean $\pm$ S.E. of Mean, Counts/min./Protein Sample, in the Presence of		
	Glycine	Lysine	Glutamic Acid
No addition	448 ± 18 (4)	$1,399 \pm 36$ (4)	193 ± 13 (4)
Sodium salicylate ($5 \times 10^{-3}\text{M}$)	204 ± 11 (4) } $P < 0.001$	676 ± 26 (4) } $P < 0.001$	92 ± 14 (4) } $P < 0.01$

The value of P is given for the difference between means. Number of observations is given in parentheses.

conditions because the potential rate of intracellular utilization of glucose exceeds the rate of entry of glucose into the muscle cell (Park *et al.*, 1955). Salicylate, like insulin, increases glucose uptake, expands the xylose space, but does not alter the glucose space. We conclude, therefore, that the action of salicylate in increasing glucose uptake includes acceleration of the rate of entry of glucose into the muscle cell.

The observation that oxygen lack or substances such as cyanide, 2:4-dinitrophenol, and arsenite which inhibit oxidation or uncouple oxidative phosphorylation increase the uptake of glucose by the isolated rat diaphragm (Randle and Smith, 1957) has led to the suggestion that the entry of glucose into the muscle cells of this preparation is normally restrained by an active process (Randle and Smith, 1957). The present observations with salicylate lend support to this idea.

Although both insulin and salicylate increase the glucose uptake of isolated diaphragm their actions on the diaphragm with respect to potassium release and incorporation of amino-acids into protein differ greatly. Salicylate promotes loss of potassium from the muscle and diminishes the incorporation of amino-acids into protein, whereas insulin stimulates amino-acid incorporation and does not promote loss of potassium. Nevertheless both insulin and salicylate may accelerate glucose uptake by interfering with some restraint on glucose entry involving energy-rich phosphate; salicylate, by restricting the oxidative production of energy-rich phosphate; and insulin, not by interfering with the production of energy-rich phosphate, but perhaps by interfering specifically with the availability of energy-rich phosphate to a process concerned with the regulation of glucose entry. Such an action of insulin would neither be incompatible with its ability to stimulate incorporation of amino-acids into protein nor would it interfere with the maintenance of high intracellular potassium.

We attach more significance to the stimulating effect of salicylate on glucose uptake in bicarbonate-buffered medium than to the lack of this effect in a phosphate-buffered medium, because bicarbonate-buffered medium more closely resembles extracellular fluid in ionic composition. Furthermore, phosphate-buffered media are known to be unsuitable for incubation of tissues such as diaphragm which exhibit a high rate of glycolysis (Dixon, 1934), and phosphate-buffered media contain an abnormally high concentration of phosphate, an ion which in high concentration is itself capable of influencing glucose uptake (Viltee, Deane, and Hastings, 1949).

Summary

Sodium salicylate, like insulin, increases the uptake of glucose by isolated rat diaphragm, and enables the non-utilizable sugar D-xylose to enter the muscle cells. Neither insulin nor sodium salicylate enables free glucose to accumulate within the cells of isolated diaphragm, presumably because the potential rate of glucose utilization with the cell exceeds the rate of entry of glucose into the cell. The effect of insulin on isolated diaphragm differs from that of salicylate in that salicylate promotes loss of potassium and diminishes incorporation of ^{14}C -labelled amino-acids into the protein, whereas insulin does not promote potassium loss and increases incorporation of labelled amino-acids into protein. The uptake of glucose into the cells of isolated diaphragm appears to be restrained by an active process, dependent upon the oxidative production of energy-rich phosphate. It is suggested that salicylate promotes uptake of glucose by inhibiting the production of energy-rich phosphate and that insulin promotes glucose uptake, more specifically, by limiting the availability of energy-rich phosphate to a process concerned with the regulation of the entry of glucose into the cell.

It is suggested that the antidiabetic action of salicylate results, at least in part, from its effect in increasing the utilization of glucose by muscle.

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EFFECT OF "NACTON" IN PATIENTS WITH DUODENAL ULCER

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One of the mainstays of treatment of patients with duodenal ulcer is the reduction of the acidity of the gastric contents. This is usually attempted by counteracting the acid physico-chemically with antacids. In addition, atropine and drugs with an atropine-like action have long been used to block excitation of the acid-secreting parietal cells. Generally these drugs have two disadvantages in that their action in reducing gastric secretion runs parallel with their side-effects, such as drying of the mouth and interference with visual accommodation, and their effect is capricious even in the hands of experienced workers (Kirsner and Palmer, 1953).

Investigation

The present paper describes the use of "nacton" (1-methyl-2-pyrrolidyl) methyl benzilate methyl methosulphate; I.S. 499), a compound with an atropine-like action of long duration, which has given regular reduction of gastric secretion in patients with duodenal or gastric ulcer at dose levels that did not produce noticeable side-effects. The chemical (Doyle *et al.*, 1958) and pharmacological (Acrod *et al.*, 1957) details and the results of a preliminary study in man (Douthwaite *et al.*, 1957) have been published.