that were optimum for amino acid incorporation were determined.

3. Evidence is produced that the amino acid incorporation into protein of mitochondria was not caused by contaminating microsomes or bacteria.

4. The incorporation of amino acids into mitochondrial proteins was stimulated by preincubation of the mitochondria with ribonuclease.

5. The rate of incorporation of amino acids was shown to be correlated with the efficiency of the production of energy by oxidative phosphorylation.

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The Effect of Salicylate on Adenosine-Triphosphatase Activity of Rat-Liver Mitochondria

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Although salicylic acid and its derivatives were first used in clinical medicine more than 80 years ago (Maclagen, 1876), very little is known of the mechanism by which they exert their wide variety of therapeutic and toxic effects. When salicylates are given in therapeutic dosage to the whole animal they produce a rapid but transient increase in metabolic rate (Austen, Rubini, Meroney & Wolff, 1958; Hetzel, Charnock & Lander, 1959). Salicylates can uncouple the oxidative phosphorylation of isolated mitochondria in concentrations that are within the range obtained in salicylate

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therapy (Brody, 1956; Penniall, 1958; Jeffery & Smith, 1959), and this effect has often been suggested as the mechanism of salicylate action *in vivo* (Reid, 1958; Adams & Cobb, 1958).

Falcone (1959) has suggested that salicylates uncouple oxidative phosphorylation by stimulation of mitochondrial adenosine-triphosphatase activity, which has long been thought not to be the action of a single enzyme (Potter, Siekevitz & Simonson, 1953). Myers & Slater (1957) claimed that mitochondrial adenosine-triphosphatase activity exhibits four separate pH optima. Further, several studies have related the increased activity of the so-called 'latent' adenosine-triphosphatase activity of mitochondria to disruptive changes in the structure of these organelles (Witter, Watson & Cottone, 1955). It is well known that this activity increases during either the aging process or in conjunction with such procedures as freezing and thawing, and that aged mitochondria show an increased dependence on the availability of Mg^{2+} ions (Klemperer, 1957; Myers & Slater, 1957). Salicylate has been shown to affect magnesium metabolism *in vivo* (Charnock, Opit & Hetzel, 1961).

Tests were therefore made of the effects of salicylate and Mg^{2+} ions on mitochondrial adenosine-triphosphatase activity, both in fresh and aged preparations, at the four pH 'optima' (6·3, 7·4, 8·5 and 9·4) described by Myers & Slater (1957), and the effects were compared with that of 2,4-dinitrophenol. In addition, examinations were made of the adenosine-triphosphatase activity of mitochondria isolated from salicylate-poisoned rats, and of the effect of exposing mitochondria to a relatively high concentration of salicylate before determination of adenosine-triphosphatase activity.

EXPERIMENTAL

Mitochondria were isolated according to Schneider (1948) from the livers of inbred black-and-white rats weighing 200–300 g. Particular care was taken that the temperature did not rise above 1° at any time during the preparation. The homogenization medium was 0·44M-sucrose containing EDTA (mM) adjusted to pH 6·8 by the addition of potassium hydroxide. The mitochondrial pellet was washed once in homogenization medium and finally suspended in 0·25M-sucrose so that 10 ml. of suspension contained mitochondria derived from 1 g. of fresh liver tissue.

A representative value for the yield of mitochondria/g. of tissue was obtained by weighing one pellet from each batch of mitochondria prepared. The water content of this mitochondrial pellet was then determined gravimetrically by drying to constant weight at 105°.

A portion of each batch of mitochondria was set aside to age under standard conditions (18 hr. at 4°).

Those experiments which examined the effect of pre-treatment *in vitro* of mitochondria by salicylate were conducted, after separation of the pellet, by resuspension of the mitochondria for 15 min. at 0° in 5 ml. of 0·44M-sucrose containing sodium salicylate (5 mM). To control these experiments some pellets were immediately examined for enzyme activity and others were suspended in sucrose alone. After this procedure the mitochondria were quickly resedimented and the supernatant fraction was removed as completely as possible by inversion of the tubes, draining, and drying the sides of the tubes with absorbent tissue. The mitochondria were finally resuspended in 0·25M-sucrose as before.

The supernatant fractions obtained from the pretreatment experiments were centrifuged at 22 000*g* for 30 min. to sediment any residual mitochondria and then tested for adenosine-triphosphatase activity.

The basic medium for the determination of adenosine-triphosphatase activity was 2 ml. of an aqueous solution, containing potassium chloride (0·075M), sucrose (0·05M) and ATP (disodium salt) (Sigma Chemical Co.) (2 mM), which was adjusted to pH 6·3, 7·4, 8·5 or 9·4 with tris–acetic acid buffer (0·05M) as described by Myers & Slater (1957). To this mixture magnesium chloride, sodium salicylate or 2,4-dinitrophenol was added, in 0·1 ml. of solution, either alone or in combination, to give final concentrations of 1, 5 and 0·1 mM respectively. To these mixtures were added 0·1 ml. portions of the final suspension of either fresh or aged mitochondria, so that the 'enzyme' concentration was well within the range of linear relationship shown to exist between mitochondrial dry wt./ml. of solution and 'enzyme' activity (Kielley & Kielley, 1951; Myers & Slater, 1957).

The mitochondria were then incubated at 23° whilst subjected to gentle mixing by rotation of the vessels. After a short period of equilibration, zero-time vessels were removed and the remaining vessels incubated for 30 min. The reactions were stopped by the immediate addition of 2 ml. of 10% (w/v) trichloroacetic acid.

After centrifuging the deproteinized mixtures, a portion of the supernatant was assayed for inorganic phosphorus by the method of Taussky & Shorr (1953). The results are expressed as μg.atoms of inorganic phosphorus liberated/mg. dry wt. of mitochondria/hr.

Measurement of oxidative phosphorylation by mitochondria was conducted in a conventional Warburg apparatus at 30° for 30 min. by the method of Umbreit, Burris & Stauffer (1957). The 3 ml. of incubation mixture contained 50 μmoles of phosphate as potassium phosphate buffer (pH 6·8), 24 μmoles of potassium chloride, 30 μmoles of glucose, 2·5 μmoles of ATP, 30 μmoles of potassium fluoride, and 25 μmoles of substrate (an aqueous solution of either α-oxoglutaric acid or succinic acid, neutralized to pH 6·8 with potassium hydroxide). Potassium malonate (25 μmoles) was added when α-oxoglutarate was the substrate.

The phosphate-acceptor system was 30 units of hexokinase (Sigma Type III) in 0·2 ml. of 1% (w/v) glucose. Mitochondria equivalent to 0·5 g. of fresh liver tissue were added last to each vessel, in 0·5 ml. of 0·25M-sucrose.

When salicylate was given to rats it was administered twice daily by stomach tube, in a dose of 30 mg./100 g. of body wt., for at least 5 days or until signs of salicylate toxicity were evident. Both the potassium and sodium salts of salicylic acid were used in aqueous solution without

pH adjustment. Plasma salicylate was estimated by the method of Trinder (1954).

All the reagents were of analytical grade unless otherwise specified.

RESULTS

Mitochondrial adenosine-triphosphatase activity. The adenosine-triphosphatase activity determined in a system without Mg^{2+} ions, sodium salicylate or 2,4-dinitrophenol is referred to as basic activity at a particular pH. The shape of the curve formed by joining the activities determined under similar conditions but at four different pH values is referred to as the pattern of activity.

Effect of pH. The mean basic activity (μg.atoms of inorganic phosphorus liberated/mg. dry wt. of mitochondria/hr.) of fresh mitochondrial preparations increased from 0·53 at pH 6·3 to 0·76 at pH 7·4 or 8·5 and to 1·33 at pH 9·4 (Table 1). Myers & Slater (1957) also found increasing adenosine-triphosphatase activity with increasing pH.

When the preparations were left at 4° for 18 hr., the adenosine-triphosphatase activity (μg.atoms of inorganic phosphorus liberated/mg. dry wt. of mitochondria/hr.) at pH 6·3 was 0·43 (only 81 % of the corresponding activity of fresh mitochondria); at pH 7·4 and pH 9·4 the activity was reduced to 0·54 and 0·65 respectively (71 % and 49 % respectively of the corresponding activity of fresh mitochondria), and at pH 8·5 the activity was increased to 0·87 (114 % of the corresponding activity of fresh mitochondria) (Table 1).

The maximum basic activity was at pH 9·4 in fresh preparations, but at pH 8·5 in aged mitochondria. The patterns of activity obtained in five individual experiments were identical.

Effect of 5 mM-*sodium salicylate.* With fresh mitochondria, 5 mM-sodium salicylate increased the adenosine-triphosphatase activity by 180 % at pH 6·3 and by nearly 100 % at pH 7·4 and 8·5 (Table 1). At pH 9·4 there was a small but consistent inhibition of adenosine-triphosphatase activity (−20 %).

The addition of 5 mM-sodium salicylate to aged preparations of mitochondria was without effect on the activity of adenosine triphosphatase at any pH examined (Table 1).

Effect of mM-Mg^{2+} *ion.* The addition of Mg^{2+} ions (as mM-magnesium chloride) to fresh mitochondria increased the adenosine-triphosphatase activity irrespective of the pH, but the effect decreased from 260 % above the basic activity at pH 6·3 to 140, 150 and 85 % at pH 7·4, 8·5 and 9·4 respectively (Table 1).

The adenosine-triphosphatase activity of aged mitochondria was markedly increased by the addition of mM-Mg^{2+} ion to the incubation medium. The increases in activity were 710, 555, 268 and 220 % above the basic activity at pH 6·3, 7·4, 8·5 and 9·4 respectively (Table 1).

The stimulating effect of Mg^{2+} ions on the adenosine-triphosphatase activity of both fresh and aged mitochondria declined with increasing pH. The effect was greater in aged mitochondria where the activities stimulated by Mg^{2+} ions were greater at pH 6·3, 7·4 and 8·5 than those of corresponding fresh preparations.

Combined effect of 5 mM-*sodium salicylate and* mM-Mg^{2+} *ion.* At pH values below 8·5 the simultaneous addition of 5 mM-sodium salicylate and mM-Mg^{2+} ion to fresh mitochondria increased the adenosine-triphosphatase activity above that obtained with either substance alone; at pH 9·4 the adenosine-triphosphatase activity was intermediate between those produced by the addition of either agent alone (Table 1).

When added simultaneously to aged mitochondria, sodium salicylate reduced the adenosine-triphosphatase activity, stimulated by Mg^{2+} ions,

Table 1. *Effect of* pH, 5 mM-*sodium salicylate and* 0·1 mM-Mg^{2+} *ion on adenosine-triphosphatase activity of fresh and aged mitochondria*

The reaction medium and experimental conditions are described in the text. The results are the mean of five experiments and the figures in parentheses represent the percentage stimulation above the basic activity determined without additions.

	Adenosine-triphosphatase activity (μg.atoms of P liberated/mg. dry wt. of mitochondria/hr.)							
	Fresh mitochondria				Aged mitochondria			
pH	6·3	7·4	8·5	9·4	6·3	7·4	8·5	9·4
Additions								
None	0·53	0·76	0·76	1·33	0·43	0·54	0·87	0·65
Salicylate	1·46 (+180 %)	1·45 (+90 %)	1·56 (+105 %)	1·03 (−20 %)	0·46	0·51	0·78	0·60
Mg^{2+} ions	1·82 (+260 %)	1·85 (+140 %)	1·93 (+150 %)	2·45 (+85 %)	3·39 (+710 %)	3·54 (+555 %)	3·13 (+260 %)	2·09 (+220 %)
Mg^{2+} ions + salicylate	2·08 (+290 %)	2·38 (+210 %)	2·33 (+210 %)	1·63 (+20 %)	2·62 (+525 %)	3·16 (+470 %)	3·34 (+300 %)	2·06 (+220 %)

at pH 6·3 and 7·4, but the activity at pH 8·5 was increased by 40% and that at pH 9·4 was unchanged (Table 1). This differs from the effect with fresh preparations.

Effect of 0·1 mM-2,4-*dinitrophenol.* In three experiments with fresh mitochondria the pH maximum of the basic activity was again at pH 9·4. The addition of 0·1 mM-2,4-dinitrophenol increased the adenosine-triphosphatase activity by 960, 410, 130 and 80% at pH 6·3, 7·4, 8·5 and 9·4 respectively (Table 2). Myers & Slater (1957) also observed a decreased effect of 2,4-dinitrophenol with increasing pH. The 2,4-dinitrophenol-stimulated adenosine-triphosphatase activities are much greater than those produced by 5 mM-sodium salicylate.

When added to aged preparations of mitochondria, with a basic-activity pH maximum at 8·5, 0·1 mM-2,4-dinitrophenol was without effect at pH 6·3, but the adenosine-triphosphatase activity at pH 7·4, 8·5 and 9·4 was increased by 150, 260 and 640% respectively (Table 2). The effect of 2,4-dinitrophenol decreases with pH in fresh but increases with pH in aged mitochondrial preparations.

Adenosine-triphosphatase activity of liver mitochondria isolated from salicylate-poisoned rats. Rats were given sodium salicylate by stomach tube until they exhibited symptoms of salicylate poisoning (see Experimental section).

The patterns of adenosine-triphosphatase activity in both fresh and aged mitochondrial preparations from the livers of salicylate-poisoned rats (Table 3) were similar to those from untreated rats. The quantitative values of enzyme activity were lower than those found with mitochondrial preparations obtained from untreated rats, but statistical analysis of the data failed to reveal any significant difference between the groups.

Effect of suspending mitochondria at 0° *in* 0·44 M-*sucrose with and without* 5 mM-*sodium salicylate.* After suspension of fresh mitochondria in 0·44 M-sucrose for 15 min. at 0°, the basic adenosine-triphosphatase activity at pH 6·3 was unchanged, but the activities measured at pH 7·4, 8·5 and 9·4 were increased by 17, 40 and 52% respectively. Maximal activity was at pH 9·4, as in fresh untreated mitochondria. This treatment did not significantly alter the adenosine-triphosphatase activities of aged mitochondria. Maximal activity was again at pH 8·5 (Table 4).

The suspension of fresh preparations of mitochondria in 0·44 M-sucrose containing sodium

Table 2. *Effect of* 0·1 mM-2,4-*dinitrophenol on the adenosine-triphosphatase activity of fresh and aged mitochondria*

The experimental conditions were identical with those of Table 1 and are described in the text. The results are the mean of three experiments and the figures in parentheses represent the percentage stimulation produced by 2,4-dinitrophenol in the absence of Mg^{2+} ions.

	Adenosine-triphosphatase activity (μg.atoms of P liberated/mg. dry wt. of mitochondria/hr.)							
	Fresh mitochondria				Aged mitochondria			
pH	6·3	7·4	8·5	9·4	6·3	7·4	8·5	9·4
Additions								
None	0·46	0·92	1·82	2·96	0·15	0·05	1·01	0·15
2,4-Dinitrophenol	4·86 (+960%)	4·64 (+405%)	4·17 (+130%)	5·47 (+85%)	0·15	0·81 (+150%)	3·60 (+260%)	1·11 (+640%)

Table 3. *Adenosine-triphosphatase activity of fresh and aged liver mitochondria from rats poisoned with sodium salicylate*

The rats were given 30 mg. of sodium salicylate/100 g. body weight daily. Mitochondria were then prepared from the livers of these rats when evidence of salicylate-poisoning was apparent. The reaction medium and experimental conditions are described in the text. The results are the mean of two experiments.

	Adenosine-triphosphatase activity (μg.atoms of P liberated/mg. dry wt. of mitochondria/hr.)							
	Fresh mitochondria				Aged mitochondria			
pH	6·3	7·4	8·5	9·4	6·3	7·4	8·5	9·4
Additions								
None	0·30	0·38	0·45	0·67	0·12	0·20	0·30	0·33
Salicylate	0·55	0·73	0·68	0·52	0·10	0·15	0·30	0·26
Mg^{2+} ions	1·10	1·45	1·45	1·52	2·00	2·73	2·47	1·83
Mg^{2+} ions + salicylate	1·02	1·58	1·68	1·30	1·50	2·65	2·45	1·63

Table 4. *Effect on adenosine-triphosphatase activity of mitochondria after suspension at 0° in 0·44 M-sucrose with and without 5 mM-salicylate*

Before the assay of enzyme activity liver mitochondria were suspended in either 0·44 M-sucrose or 0·44 M-sucrose containing sodium salicylate (5 mM). After 15 min. at 0°, the mitochondria were sedimented and resuspended in 0·25 M-sucrose. The reaction medium and experimental conditions are described in the text. The results are the mean of two experiments. No additions were made to the reaction media.

	Adenosine-triphosphatase activity (μg.atoms of P liberated/mg. dry wt. of mitochondria/hr.)							
	Fresh mitochondria				Aged mitochondria			
pH	6·3	7·4	8·5	9·4	6·3	7·4	8·5	9·4
Additions								
None	0·55	1·04	1·30	2·32	0·48	0·81	1·16	0·88
Sucrose	0·61	1·22	1·83	3·54	0·53	0·90	1·04	0·66
Sucrose + salicylate	0·67	0·74	1·77	2·99	0·38	0·66	1·11	0·42

salicylate (5 mM) did not significantly alter the adenosine-triphosphatase activity at pH 6·3. The activity at pH 7·4 was reduced below that of either untreated mitochondria or those suspended in sucrose alone. The activities at pH 8·5 and 9·4 were greater than those of untreated mitochondria, but less than those of mitochondria suspended in sucrose alone. Maximal activity was at pH 9·4, as in fresh untreated mitochondria. An unchanged pattern of activity with a maximum at pH 8·5 was found with aged preparations of mitochondria (Table 4). Generally, the addition of sodium salicylate (5 mM) to the suspension medium resulted in less change from the control adenosine-triphosphatase activities than did suspension in 0·44 M-sucrose alone.

The supernatant fractions obtained from these experiments were also assayed for adenosine-triphosphatase activity. Maximal activity was found at pH 8·5 in every experiment and the presence of 5 mM-sodium salicylate in some fractions had little effect. This is identical with the adenosine-triphosphatase activity of aged mitochondrial preparations.

Mitochondrial oxidative phosphorylation. Each batch of mitochondria, including those prepared from the livers of salicylate-poisoned rats, was examined for oxidative-phosphorylation efficiency as well as for adenosine-triphosphatase activity. In seven experiments mitochondria from untreated rats carried out the oxidative phosphorylation of α-oxoglutaric acid and of succinic acid with mean P:O ratios of 3·0 and 1·5 respectively. Mitochondria from salicylate-poisoned rats (eight experiments) gave corresponding mean P:O ratios of 3·2 and 1·6 respectively.

The plasma of the salicylate-treated group of rats, assayed immediately before removal of liver tissue, contained 32 mg. of salicylic acid/100 ml. (mean value).

DISCUSSION

The pattern of basic adenosine-triphosphatase activity with fresh mitochondria is as reported by Myers & Slater (1957). The uniform increase in adenosine-triphosphatase activity with pH in these preparations may be thought to reflect the effect of pH on the substrate for the reaction, but the same pH effect is not seen with aged mitochondria, where the activity at pH 8·5 is greater than at pH 9·4.

In the absence of added Mg^{2+} ions, 2,4-dinitrophenol (0·1 mM) considerably increased adenosine-triphosphatase activity in fresh mitochondria, although this effect decreased with increasing pH, as reported by Myers & Slater (1957). This agent also increased adenosine-triphosphatase activity in aged mitochondria where, in contrast with fresh preparations, the effect increased with increasing pH. As the mitochondria were the only variable component of these test systems, this observation precludes an effect of pH solely on the ionic species of the activator, a possibility previously considered by Myers & Slater (1957), Cooper (1960) and Hemker & Hülsmann (1961).

The lower 2,4-dinitrophenol-stimulated adenosine-triphosphatase activity of aged mitochondria compared with that of fresh preparations is presumably related to the well-known increased requirement for Mg^{2+} ions by these preparations (Klemperer, 1957). Cooper (1960) suggested that this effect is due to a loss of 'bound' Mg^{2+} ions from the mitochondria during the aging process, and that, when this loss is overcome by the establishment of a high external concentration of Mg^{2+} ions *in vitro*, the enzyme activity is increased. This mechanism alone would not lead to Mg^{2+}-ion-stimulated adenosine-triphosphatase activities in aged mitochondria which, in our experiments, exceeded those of corresponding fresh preparations.

However, if the permeability of the mitochondria is also increased by aging, so that the substrate for the reaction (external ATP) can react more readily with the enzyme system, then the observation of not only restored but increased activity may be explained.

The effect of salicylate (5 mM) differed from that of 2,4-dinitrophenol (0·1 mM), not only in the degree of stimulation of fresh-mitochondrial-enzyme activity, which was much lower although the concentration of salicylate was 50-fold greater than that of 2,4-dinitrophenol, but also in that it did not stimulate activity in aged mitochondria at any pH examined. This suggests that, unlike the stimulatory effect of 2,4-dinitrophenol, that of salicylate can only be produced with a high degree of membrane integrity, which is only found with freshly prepared mitochondria.

The patterns of enzyme activity obtained in the presence of both salicylate and Mg^{2+} ions with fresh and aged mitochondria indicate an independence of action by these agents which would be compatible with different sites of enzyme action and is consistent with the postulate that enhancement by salicylate of adenosine-triphosphatase activity requires an 'intact' membrane whereas that produced by Mg^{2+} ions does not.

Pretreatment of fresh mitochondria with sodium salicylate (5 mM) at 0° produced little change in the normal pattern of adenosine-triphosphatase activity, and the activity was not inhibited, in contrast with the effect of the addition of sodium salicylate (5 mM) to the incubation medium. This suggests that the effect of salicylate is readily reversible, and accounts for the normal oxidative-phosphorylation efficiency of mitochondria isolated from the livers of salicylate-poisoned rats.

These findings offer an explanation, in terms of mitochondrial permeability, of the mechanism by which salicylate stimulates mitochondrial adenosine-triphosphatase activity *in vitro*, and may also explain the uncoupling effect of salicylate on the transfer of phosphate during the oxidation of α-oxoglutarate by isolated mitochondria (Charnock, Opit & Hetzel, 1962). Further investigation of the physiological significance of the action of salicylate on the mitochondrial membrane seems warranted.

SUMMARY

1. The adenosine-triphosphatase-activity patterns of fresh and aged rat-liver mitochondria were different at pH 6·3, 7·4, 8·5 and 9·4. Maximum activity was at pH 9·4 in fresh, but at pH 8·5 in aged mitochondria. It is suggested that the different activities found in fresh mitochondria with changing pH are at least in part due to changes in the permeability of the mitochondria to adenosine triphosphate.

2. 2,4-Dinitrophenol (0·1 mM) greatly stimulated the adenosine-triphosphatase activity of fresh mitochondria in the absence of Mg^{2+} ions. This effect decreased with increasing pH. With aged mitochondria in the absence of Mg^{2+} ions, the effect of 2,4-dinitrophenol was reduced, but here the stimulation increased with increasing pH.

3. Sodium salicylate (5 mM) stimulated adenosine-triphosphatase activity in fresh mitochondria only. As with the effect with 2,4-dinitrophenol, maximum activation occurred at pH 6·3, least at pH 9·4. This activation is separate from that produced by Mg^{2+} ion (mM) and is cumulative with it.

4. Sodium salicylate (5 mM) did not effect the adenosine-triphosphatase activity of aged mitochondria, which showed much enhancement of activity by Mg^{2+} ions. Salicylate produced some inhibition of the Mg^{2+}-ion-stimulated adenosine-triphosphatase activity of aged mitochondria.

5. Mitochondria from salicylate-poisoned rats showed the same pattern of adenosine-triphosphatase activity at four pH values, and the same degree of efficiency of oxidative phosphorylation, as that found with mitochondria from untreated rats.

6. Because stimulation by salicylate of adenosine-triphosphatase activity occurs only with fresh mitochondria, it is suggested that the mitochondrial 'membrane' is the site of salicylate action. An increase in the permeability of this membrane to adenosine triphosphate in the presence of salicylate could explain the stimulation of adenosine-triphosphatase activity by this drug *in vitro*.

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An Evaluation of the Effect of Salicylate on Oxidative Phosphorylation in Rat-Liver Mitochondria

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The antipyretic, anti-inflammatory and analgesic properties of the salicylates are well known, although intensive investigation of the pharmacology of these agents has failed to demonstrate their mechanism of action. An uncoupling action of salicylate on oxidative phosphorylation processes in isolated mitochondria *in vitro* has been well documented (Brody, 1956; Penniall, 1958; Jeffery & Smith, 1959); this action appears to be compatible with many of the effects of salicylate on the whole animal (Hetzel, Charnock & Lander, 1959; Charnock, Opit & Hetzel, 1961), and has been proposed as the physiological mechanism of action of the drug (Smith, 1955; Reid, 1958).

During a study of the effect of salicylate on mitochondrial adenosine-triphosphatase activity (Charnock & Opit, 1962), it was found that mitochondria with unchanged properties of oxidative phosphorylation and adenosine-triphosphatase activity could be isolated from rats poisoned with salicylate *in vivo*.

Therefore an assessment of the physiological significance of salicylate-induced uncoupling of mitochondrial oxidative phosphorylation seemed warranted. The results of an examination of both this effect and of the permeability of mitochondria to salicylate *in vitro* and *in vivo* are reported here.

EXPERIMENTAL

Oxidative phosphorylation. Young actively growing male laboratory rats weighing 200–250 g. were used, to avoid the decrease in phosphorylation rates associated with liver tissue of older animals (Weinbach & Garbus, 1956, 1959). Sodium salicylate (30 mg. of salicylate radical/100 g. body weight/day) was given by stomach tube until symptoms of salicylate poisoning, e.g. hyperventilation, body wastage and haemorrhage, were evident. One-third of the dose was given at 9.00 a.m. each day, and the remainder 10 hr. later.

Liver tissue was homogenized in 0·44M-sucrose, containing EDTA (1 mM), adjusted to pH 6·8 with potassium hydroxide.

Mitochondria were isolated from this mixture by the method of Hogeboom, Schneider & Pallade (1948). The temperature was maintained below 1° at all stages in the preparation. The mitochondria were washed once with 0·44M-sucrose and the pellets were finally suspended in ice-cold 0·25M-sucrose. Mitochondria derived from 0·5 g. of wet liver were added last to each test vessel, which contained (final vol. 3 ml.): 50 μmoles of inorganic phosphorus as phosphate buffer, pH 6·8; 24 μmoles of potassium chloride; 30 μmoles of magnesium sulphate; 2·5 μmoles of ATP (sodium salt) (Sigma Chemical Co.); 30 μmoles of glucose; 30 μmoles of potassium fluoride; 25 μmoles of an aqueous soln. of α-oxoglutaric acid, β-hydroxybutyric acid or succinic acid as substrate. The free acids were neutralized to pH 6·8 with potassium hydroxide. Potassium malonate (pH 6·8) (25 μmoles) was added to inhibit succinoxidase activity when α-oxoglutarate was the substrate. The phosphate-acceptor system, which was tipped from the side arm of the vessels after temperature equilibration, was 0·2 ml. of 1% (w/v) glucose containing 30 units of hexokinase (Sigma Type III). The centre well of each Warburg vessel contained 0·2 ml. of 5M-potassium hydroxide. After incubation for 30 min. at 30° in air, the reaction was stopped by the addition of 0·2 ml. of 10N-sulphuric acid. The esterification of inorganic phosphorus was determined indirectly by the loss of inorganic phosphorus from the medium, with the method of Fiske & Subbarow (1925) as modified by Taussky & Shorr (1953).

When the effect of the addition of sodium salicylate *in vitro* was examined, a final concentration of 5 mM was

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