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# INHIBITION OF HEPATIC LIPOGENESIS BY SALICYLATE

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SUMMARY

Salicylate has been found to be an inhibitor of fatty acid synthesis in isolated rat hepatocytes. Half-maximal inhibition of fatty acid synthesis occurs at approximately 2 mM. The inhibitory effect of salicylate on fatty acid synthesis is not relieved by the addition of acetate, suggesting that salicylate inhibits the conversion of acetate into fatty acids. Acetyl-CoA carboxylase activity in homogenates of hepatocytes is not influenced by previous exposure of the intact cells to salicylate. Partially purified acetyl-CoA carboxylase, isolated and assayed in the absence of citrate, is markedly inhibited by salicylate. However, in the presence of 0.5 mM citrate, which is the concentration of this metabolite in the cytosol of the liver cell, salicylate activates the enzyme. Upon treatment of acetyl-CoA carboxylase with salicylate (in the absence or presence of citrate), followed by separation of enzyme and effector on a Sephadex G-25 column, the enzyme activity is enhanced as compared to the salicylate-free control, demonstrating that the inhibitory effect of salicylate (in the absence of citrate) is reversible, but not the stimulatory effect (in the presence of citrate). Salicylate inhibition of fatty acid synthesis by hepatocytes is not rapidly reversible; hepatocytes preincubated with salicylate followed by a wash procedure (centrifugation and resuspension) still show depressed rates of fatty acid synthesis from acetate upon further incubation.

Salicylate was found to prevent pyruvate accumulation in hepatocyte suspensions observed in the absence of this compound; salicylate even induces the disappearance of pyruvate and lactate initially present in the cell suspension. This suggests that salicylate activates pyruvate and lactate con-

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sumption, which is most likely related to the well-known fact that salicylate uncouples oxidative phosphorylation. The latter action of the drug will stimulate citric acid-cycle activity. This causes an inhibition of fatty acid and cholesterol synthesis since acetyl units will be specifically channelled into the citric acid cycle and not into the lipogenic pathway.

It is concluded that part of the inhibitory effect of salicylate on fatty acid biosynthesis is exerted at (a) step(s) in the conversion of acetate into fatty acids, acetyl-CoA carboxylase not being a target of this compound. In addition, salicylate prevents that pyruvate, generated by glycolysis, enters the lipogenic pathway. The latter effect of salicylate would also explain the observed inhibition of cholesterol synthesis by this compound.

## INTRODUCTION

Injection of salicylate into rats has been shown to inhibit the incorporation of labelled acetate into hepatic fatty acids [1]. This inhibitory effect of salicylate on fatty acid synthesis has also been demonstrated with supernatant fractions of rat-liver homogenates [2—4]. In such fractions salicylate inhibits the incorporation of [1-$^{14}$C]acetate and [1-$^{14}$C]acetyl-CoA into fatty acids, but not that of [2-$^{14}$C]malonyl-CoA [2,4]. These observations suggest that at least part of this inhibition is exerted at the level of acetyl-CoA carboxylase, the enzyme considered to catalyse the rate-limiting step in hepatic fatty acid synthesis. Furthermore, administration of salicylate to intact rats lowers the activity of hepatic acetyl-CoA carboxylase [5]. Likewise, acetyl-CoA carboxylase in a partially purified supernatant fraction of rat liver is inhibited by salicylate [5]. It has recently been demonstrated that the effect of salicylate on acetyl-CoA carboxylase is quite complex. Preincubation of rat-liver acetyl-CoA carboxylase with salicylate results in activation of the enzyme, whereas the presence of salicylate in the actual enzyme assay has an inhibitory effect [6].

Upon in-vivo administration of salicylate (cf. ref. 1) it is not clear whether the drug acts directly on the liver cell or that the observed effects result from changes in the hormonal and metabolic milieu bathing the liver. In rat-liver homogenates (cf. ref. 2—4) the structural integrity of the cell is destroyed. In contrast, isolated liver cells do not have these serious drawbacks. Thus, we have studied the effect of salicylate on fatty acid and cholesterol synthesis by intact hepatocytes. Experiments are described which attempt to correlate the salicylate-induced inhibition of fatty acid synthesis by hepatocytes and the effects of salicylate on acetyl-CoA carboxylase.

## METHODS AND MATERIALS

### *Isolation and incubation of hepatocytes*

Hepatocytes were isolated from male Wistar rats (200—250 g) by the method of Seglen [7] with modifications described previously [8]. The rats

were meal-fed a stock, pelleted diet between 04.00 h and 07.00 h by an automatic rat feeding machine. They were sacrificed at 09.00 h.

Isolated hepatocytes were suspended in Krebs-Henseleit bicarbonate buffer (pH 7.4) supplemented with 3.5% bovine serum albumin (defatted and dialysed), 10 mM glucose and other additions as indicated. In previous studies [8] these cells had been shown to be hormone-sensitive with regard to the rate of de novo fatty acid synthesis, indicating that the hepatocytes are suitable for metabolic studies [9]. Incubations were carried out at 37°C in a metabolic shaker (90 strokes/min) in 25-ml Erlenmeyer flasks that initially contained about 120 mg wet weight of cells (equivalent to about 18 mg of cellular protein) in a volume of 3 ml. At the indicated times these vessels were sampled. During incubation, flasks were continuously gassed with 95% oxygen, 5% carbon dioxide.

*Lipogenesis by hepatocytes*

Rates of fatty acid synthesis from $^3H_2O$ or [1-$^{14}$C]acetate were estimated as described by Harris [10].

To determine the reversibility of salicylate inhibition of fatty acid synthesis, cells were preincubated with the drug. After 30 min at 37°C the cells were washed twice by centrifugation at room temperature for 1 min at 100 *g* and the pellet (intact hepatocytes) was resuspended in salicylate-free bicarbonate buffer (10 mM glucose, 3.5% albumin) and further incubated with [1-$^{14}$C]acetate as described above.

In experiments on cholesterogenesis, the non-saponifiable fraction of total lipids was subjected to thin-layer chromatography after the addition of carrier cholesterol. Chromatography was performed on silica G with petroleum ether (b.p. 40—60°C)/diethyl ether/acetic acid (80 : 20 : 2, v/v/v) as developing agent. The silica containing the cholesterol was scraped from the plate, mixed with scintillation liquid, and counted for radioactivity.

*Acetyl-CoA carboxylase activity in hepatocytes*

Acetyl-CoA carboxylase activity was measured in homogenates of cells previously incubated at 37°C with various additions. Aliquots (2-ml) of the cell suspension were centrifuged at room temperature for 1 min at 100 *g*. The pellet (intact hepatocytes) was resuspended in 0.3 ml buffer containing 50 mM potassium phosphate (pH 7.0), 3 mM β-mercaptoethanol, and 1% (v/v) Triton X-100. In an aliquot of this mixture acetyl-CoA carboxylase activity was determined by the [$^{14}$C]bicarbonate-fixation assay exactly as previously described [11].

*Partial purification of acetyl-CoA carboxylase*

Rat liver acetyl-CoA carboxylase was prepared in the absence of citrate by a modification and extension of the procedure of Margolis and Baum [12]. The citrate-free isolation was initiated by precipitating the enzyme at 100 000 *g* in the presence of $MgCl_2$ (4 mM) and 1% (v/v) Triton X-100

[13]. The enzyme was extracted from the pellet with 50 mM Tris—HCl (pH 7.5) and 3 mM β-mercaptoethanol.

*Assay of partially purified acetyl-CoA carboxylase*

Acetyl-CoA carboxylase (0.05 mg protein) was preincubated for 30 min at 37°C in 0.1 ml of a medium containing 50 mM Tris—HCl (pH 7.5), 3 mM β-mercaptoethanol, 1 mM EGTA and, where indicated, 0.5 mM potassium citrate and 10 mM sodium salicylate. After this preincubation the assay of enzyme activity by the fixation of [$^{14}$C]bicarbonate into acid-stable material (malonyl-CoA) was performed exactly as described [11]. Reaction conditions were chosen so that the fixation of $^{14}$C-label was proportional to time and enzyme concentration.

To assess the reversibility of the salicylate effect on acetyl-CoA carboxylase activity, the enzyme was preincubated with salicylate for 30 min at 37°C as described above. Subsequently, enzyme and effector were separated on a Sephadex G-25 column ($h$ = 7.5 cm, $\phi$ = 0.5 cm). The column was equilibrated and eluted with a solution identical to the enzyme-assay preincubation medium (described above). Carboxylase activity in the eluate was determined by adding assay mixture as described [11].

*Lactate and pyruvate determination*

Lactate and pyruvate were determined spectrophotometrically in neutralized perchloric extracts of hepatocyte suspensions as described [14].

*Sources of materials*

All radioactive chemicals were purchased from the Radiochemical Centre, Amersham. Acetyl-CoA, bovine serum albumin (fraction V), collagenase (type 1) and nucleotides from Sigma; silica G from Merck; L-lactate from Serva; other chemicals from Baker. Glucagon was kindly supplied by Dr. Walter Shaw, Lilly Research Laboratories, Indianapolis.

## RESULTS

*Salicylate and lipogenesis by isolated liver cells*

Bicarbonate-buffered suspensions of hepatocytes were incubated in the presence of 10 mM glucose as the sole exogenous carbon source. The synthesis of fatty acids by the cells was monitored by the incorporation of $^{3}$H from $^{3}H_2O$ into fatty acids. The $^{3}H_2O$ method is considered the most reliable method available to assess overall rates of lipogenesis [15]. Figure 1 shows the incorporation of $^{3}H_2O$ into fatty acids as a function of incubation time. Salicylate (5 mM) drastically inhibits fatty acid synthesis by isolated hepatocytes. Figure 2 illustrates that at a salicylate concentration of approximately 2 mM fatty acid synthesis is decreased to 50% of the control value.

The salicylate-induced inhibition of fatty acid synthesis is not relieved b exogenous acetate (Table I). This suggests that at least part of the inhibitory action of salicylate on lipogenesis is localized at (a) step(s) in the conversion of acetate into fatty acids (cf. ref. 11).

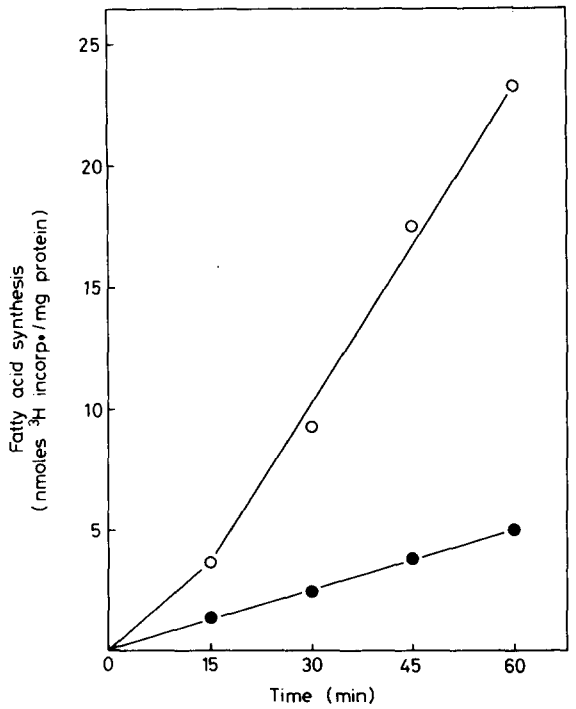


Fig. 1. Time course of $^3H_2O$ incorporation into fatty acids by isolated hepatocytes. Hepatocytes were incubated with 0.3 mCi/ml $^3H_2O$. Control (○); salicylate, 5 mM (●).

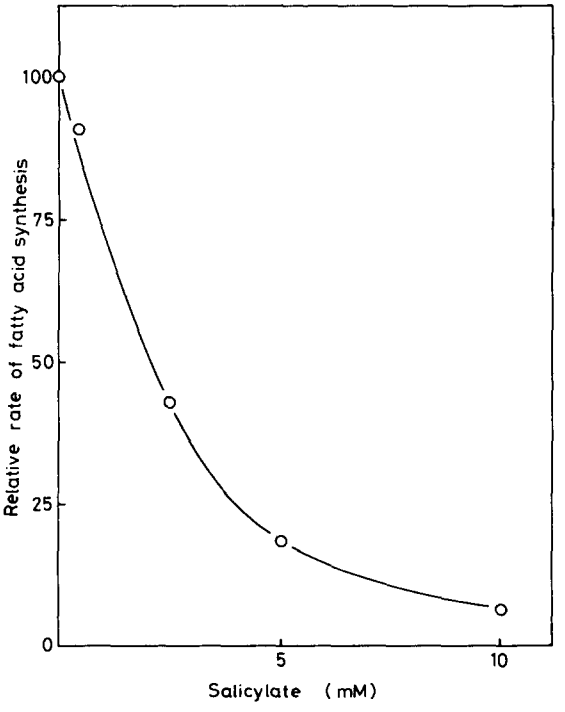


Fig. 2. Influence of salicylate on fatty acid synthesis by isolated hepatocytes. Hepatocytes were incubated for 1 h with 0.3 mCi/ml $^3H_2O$.

TABLE I

EFFECT OF ACETATE ON THE INHIBITION BY SALICYLATE OF FATTY ACID AND CHOLESTEROL SYNTHESIS BY ISOLATED RAT HEPATOCYTES

Results are expressed as means ± S.D. for 3 different incubations. Hepatocytes were incubated with 10 mM glucose and the indicated additions for 1 h. To monitor lipogenesis $^3H_2O$ (1 mCi/ml) or [1-$^{14}$C]acetate (0.3 Ci/mol) was added to the incubations. For the effect of salicylate (Student's *t*-test): $*P < 0.01$.

| Additions | (nmoles/mg protein/h) | | | |
|---|---|---|---|---|
| | Fatty acid synthesis | Cholesterol synthesis | Fatty acid synthesis | Cholesterol synthesis |
| | by $^3H_2O$ incorporation | | by [1-$^{14}$C]acetate incorporation | |
| None | 34.2 ± 0.9 | 2.7 ± 0.1 | | |
| Salicylate (5 mM) | 5.2 ± 0.6* | 2.0 ± 0.1* | | |
| Acetate (10 mM) | 57.4 ± 2.3 | 2.1 ± 0.1 | 20.8 ± 1.6 | 3.9 ± 0.1 |
| Salicylate + acetate | 10.7 ± 1.0* | 2.0 ± 0.1 | 4.3 ± 0.2* | 2.5 ± 0.2* |

Klimov et al. [3,4] have suggested that salicylate inhibits hepatic cholesterol synthesis. These authors demonstrated that salicylate inhibits the incorporation of [1-$^{14}$C]acetate and [1-$^{14}$C]acetyl-CoA into non-saponifiable lipids by supernatant fractions of rat-liver homogenates. However, the non-saponifiable lipids constitute a mixture of ill-defined composition. Table I shows that salicylate inhibits cholesterol synthesis by intact liver cells as measured by the incorporation of $^3H_2O$ or [1-$^{14}$C]acetate into cholesterol. Half-maximal inhibition of cholesterogenesis occurs at approximately 7.5 mM salicylate [16]. Acetate inhibits the incorporation of $^3H_2O$ into cholesterol (Table I), an effect observed earlier [17]. In the presence of acetate, salicylate does not further inhibit incorporation of $^3H$ into cholesterol.

*Salicylate and acetyl-CoA carboxylase activity*

The data presented so far (Table I, Figs. 1 and 2) are in agreement with reports [2,4,5] that salicylate inhibits acetyl-CoA carboxylase. However, as Table II documents, treatment of intact hepatocytes with salicylate does not alter the activity of acetyl-CoA carboxylase as measured in homogenates of these cells. This suggests that either the drug does not affect acetyl-CoA carboxylase within the intact hepatocyte or that a possible effect is not preserved upon cell disruption. In comparison, lactate increases and glucagon decreases the activity of acetyl-CoA carboxylase measured in homogenates of isolated hepatocytes previously exposed to these compounds (Table II), indicating that alterations in the activity of acetyl-CoA carboxylase within the intact cell can survive disruption of the cell. In these hepatocytes fatty acid synthesis is depressed by glucagon [8] and enhanced by lactate [17].

If a possible effect of salicylate on acetyl-CoA carboxylase is lost during

TABLE II

EFFECTS OF SALICYLATE, LACTATE AND GLUCAGON ON THE ACTIVITY OF ACETYL-CoA CARBOXYLASE IN ISOLATED RAT HEPATOCYTES

Hepatocytes were incubated for 30 min with 10 mM glucose and the indicated additions. Afterwards, enzyme activity was measured in cellular homogenates. Results are expressed as means ± S.E. for 3 different incubations. A mUnit of enzyme activity is equivalent to 1.0 nmol $H^{14}CO_3^-$ fixed per min at 37°C under the conditions of the assay, corrected for acetyl-CoA-independent bicarbonate fixation. Versus control (Student's *t*-test): $*P < 0.01$. Identical experiments, except for the salicylate data, as reported earlier [11].

| Additions | Acetyl-CoA carboxylase activity (mUnits/mg protein) |
|---|---|
| None | 2.10 ± 0.03 |
| Salicylate (10 mM) | 2.24 ± 0.07 |
| L-lactate (10 mM) | 2.93 ± 0.05* |
| Glucagon (10 nM) | 1.80 ± 0.07 |

the homogenization procedure, the effect may be observed with an in-vitro acetyl-CoA carboxylase assay system. Table III illustrates that salicylate drastically inhibits partially purified acetyl-CoA carboxylase if the preincubation and assay of the enzyme are conducted in the absence of added citrate. However, in the presence of 0.5 mM citrate, which is the citrate concentration observed in the cytosol of hepatocytes from meal-fed rats [18], salicylate slightly, but significantly activates the carboxylase (Table III).

To simulate the procedure for the assay of acetyl-CoA carboxylase activity in isolated hepatocytes, the partially purified carboxylase preparation was treated with effectors, followed by the separation of enzyme and effector. The stimulatory effect of citrate is still observed when the enzyme is assayed in the eluate of the Sephadex G-25 column (Table IV). Likewise, the stimula-

TABLE III

EFFECTS OF SALICYLATE AND CITRATE ON PARTIALLY PURIFIED ACETYL-CoA CARBOXYLASE ACTIVITY

The partially purified enzyme was incubated with the indicated additions for 30 min at 37°C prior to the assay of enzyme activity. A mUnit of acetyl-CoA carboxylase activity is equivalent to 1.0 nmol $H^{14}CO_3^-$ fixed per min at 37°C under the conditions of the assay. Results are expressed as means ± S.D. for 3 different incubations. Versus control (Student's *t*-test): $*P < 0.01$.

| Additions | Acetyl-CoA carboxylase activity (mUnits/mg protein) |
|---|---|
| None | 221 ± 17 |
| Salicylate (10 mM) | 89 ± 9* |
| Citrate (0.5 mM) | 1006 ± 26 |
| Salicylate + citrate | 1222 ± 33* |

TABLE IV

EFFECT OF SALICYLATE PRETREATMENT ON PARTIALLY PURIFIED ACETYL-CoA CARBOXYLASE ACTIVITY AND ON FATTY ACID SYNTHESIS BY ISOLATED CELLS

Partially purified acetyl-CoA carboxylase was preincubated for 30 min in the presence of the indicated additions. Enzyme activity was assayed in the eluate from a Sephadex G-25 column. Hepatocytes were incubated for 30 min with or without 10 mM salicylate. Cells were washed twice by centrifugation and resuspension of the pellet and incubated for 30 min in a salicylate-free buffer with 10 mM [1-$^{14}$C]acetate (0.3 Ci/mol). Results are expressed as means ± S.D. for 3 different incubations. Versus control (Student's *t*-test): *$P < 0.01$.

| Additions | Acetyl-CoA carboxylase activity (mUnits/mg protein) | Fatty acid synthesis (nmol/mg protein/h) |
|---|---|---|
| None | 296 ± 5 | 33.0 ± 1.2 |
| Salicylate (10 mM) | 459 ± 12* | 23.4 ± 0.4* |
| Citrate (0.5 mM) | 809 ± 60 | |
| Salicylate + citrate | 1162 ± 22* | |

tory effect of salicylate on carboxylase activity (when added together with 0.5 mM citrate) is persistent after the separation of salicylate and citrate from the enzyme (Table IV). Interestingly, the inhibitory effect of salicylate on acetyl-CoA carboxylase in the absence of citrate (Table III) is changed into a stimulatory effect upon recovery of the enzyme in the eluate of the column (Table IV). A satisfactory explanation for this observation can not be offered at present.

Salicylate inhibition of fatty acid synthesis by isolated hepatocytes is not rapidly reversible. Hepatocytes preincubated with 10 mM salicylate for

TABLE V

EFFECT OF SALICYLATE ON PYRUVATE AND LACTATE LEVELS IN HEPATOCYTE SUSPENSIONS

Hepatocytes were incubated for 60 min with 10 mM glucose in the absence or presence of 10 mM salicylate. Pyruvate and lactate were measured in the total hepatocyte suspension. Results represent the average (and range) of two separate incubations.

| Incubation time (min) | Pyruvate (nmol/mg protein) | | Lactate (nmol/mg protein) | |
|---|---|---|---|---|
| | Control | Salicylate | Control | Salicylate |
| 0 | 23 (23,22) | — | 106 (109,103) | — |
| 60 | 36 (36,35) | 11 (12,10) | 100 (101,99) | 41 (41,40) |

30 min followed by washing twice by means of centrifugation and resuspension of the pellet (intact hepatocytes) still display depressed rates of [1-$^{14}$C]-acetate incorporation into fatty acids upon further incubation (Table IV).

*Salicylate and pyruvate accumulation*

As can be appreciated from Table V, salicylate drastically inhibits pyruvate accumulation by isolated hepatocytes. Moreover, in the presence of salicylate pyruvate and lactate levels in hepatocyte suspensions are markedly lower than those observed at the beginning of the incubation period. This suggests that salicylate inhibits glycolysis and/or activates the degradation of pyruvate and lactate.

## DISCUSSION

It is clear from the present study that salicylate inhibits fatty acid biosynthesis by isolated rat hepatocytes. At least part of the inhibitory action of salicylate is exerted at a step of the pathway between acetate and fatty acids. Exogenous acetate fails to relieve salicylate inhibition of fatty acid synthesis (measured by $^{3}H_2O$ incorporation) and the drug significantly inhibits [1-$^{14}$C]acetate incorporation into fatty acids. A possible site of inhibition is acetyl-CoA carboxylase, generally considered to be the rate-limiting enzyme in fatty acid biosynthesis. In cell-free extracts of rat liver, salicylate inhibits the incorporation of [1-$^{14}$C]acetate and [1-$^{14}$C]acetyl-CoA into fatty acids, but the incorporation of [2-$^{14}$C]malonyl-CoA is not affected [2,4]. Contrary to what would be expected, the activity of acetyl-CoA carboxylase in homogenates of salicylate-treated hepatocytes is not significantly different from the control value (Table II). However, it is feasible that a possible effect of the drug on acetyl-CoA carboxylase is not preserved through the homogenization and dilution steps which take place prior to measuring the activity of the enzyme. In contrast, the effects of lactate and glucagon are persistent upon cell disruption (cf. Table II). Klimov et al. [5] have demonstrated that salicylate administration to intact rats will lower the activity of hepatic acetyl-CoA carboxylase. In these experiments [5] salicylate was injected intraperitoneally 14 h prior to the assay of carboxylase activity. Since the half-life of rat-liver acetyl-CoA carboxylase ranges from 1 day to 3 days in various metabolic conditions [19], these investigations belong in the category of long-term control of enzyme activity. Possibly, salicylate affects the synthesis and/or degradation of acetyl-CoA carboxylase. In the present study, experiments did not last longer than 1 h and, therefore, only effects on the catalytic properties of a constant number of carboxylase molecules are expected (short-term control).

Partially purified acetyl-CoA carboxylase is drastically inhibited by salicylate provided that no citrate is added (Table III). In the presence of 0.5 mM citrate salicylate significantly activates acetyl-CoA carboxylase (Table III). Dular and Dakshinamurti [6] have also reported that under certain conditions salicylate may activate rat liver acetyl-CoA carboxylase.

However, these authors used a carboxylase preparation containing the unphysiological concentration of 20 mM citrate and, therefore, their enzyme cannot be compared with the enzyme used in the present study. The inhibition of acetyl-CoA carboxylase by salicylate found in the absence of citrate is consistent with the salicylate-induced inhibition of fatty acid synthesis from acetate by the cells. The cytoplasmic concentation of citrate in liver cells from meal-fed rats is about 0.5 mM [18]. In the presence of this concentration of citrate the drug activates the carboxylase (Table III). This effect is still present when the enzyme is assayed in the eluate of a Sephadex G-25 column (Table IV). In apparent contrast to this observation, hepatocytes preincubated with salicylate and reisolated afterwards still display depressed rates of fatty acid synthesis from acetate (Table IV). It seems likely, however, that washing the cells will not remove salicylate located intracellularly.

The present study demonstrates that the short-term inhibitory effect of salicylate on de novo fatty acid synthesis in the intact hepatocyte does not appear to be exerted at the acetyl-CoA carboxylase reaction as has been suggested previously [2—5]. Exposure of hepatocytes to salicylate does not affect the activity of acetyl-CoA carboxylase (Table II). In the presence of physiological citrate concentrations salicylate has a stimulatory effect on the activity of partially purified acetyl-CoA carboxylase (Table III); an effect that is preserved upon separation of the enzyme from its effectors (Table IV).

Since glycolysis furnishes acetyl units for fatty acid synthesis, the effect of salicylate on the accumulation of the glycolytic endproducts pyruvate and lactate was determined. Salicylate not only inhibits pyruvate accumulation but even lowers pyruvate and lactate levels in hepatocyte suspensions as compared to those at the beginning of the incubation period when the drug was added (Table V). This suggests that salicylate stimulates the degradation of pyruvate and lactate by other pathways than that of fatty acid synthesis. It seems likely that salicylate-mediated uncoupling of oxidative phosphorylation reactions as observed in isolated mitochondria [20,21] is responsible for this observation. This action of salicylate explains the observed increased citric acid-cycle activity in the liver after administration of the drug to intact rats [1].

Uncoupling of oxidative phosphorylation reactions by salicylate would explain the observed inhibition of fatty acid synthesis by this compound. First, the salicylate-induced uncoupling of oxidative phosphorylation will lower the content of ATP in the liver cell (cf. ref. 22). ATP is necessary for the generation of acetyl-CoA in the cytosolic space by the acetyl-CoA synthetase and citrate cleavage enzyme catalysed reactions. ATP is also needed for the acetyl-CoA carboxylase reaction. Secondly, uncoupling of oxidative phosphorylation increases citric acid-cycle activity (cf. ref. 1), which will prevent acetyl units generated by glycolysis from entering the fatty acid and cholesterol biosynthetic pathway. It should be mentioned that salicylate uncouples oxidative phosphorylation reactions in respiring isolated mitochondria [20,21] at concentrations (1—2 mM) encountered

therapeutically. Serum levels in this order of magnitude are required to give maximal symptomatic control in the treatment of acute rheumatic fever [23].

In summary, the present study unequivocally demonstrates that salicylate inhibits lipogenesis by isolated hepatocytes. This inhibitory action is not due to a direct inhibition (alteration of the enzyme's structure) of acetyl-CoA carboxylase, but within the intact cell salicylate may have an indirect inhibitory effect (through a decrease in cellular ATP levels) on acetyl-CoA carboxylase. The latter effect would not survive cell disruption. In addition, the drug may have an inhibitory effect on other sites in the conversion of acetate into fatty acids. Furthermore, salicylate prevents the incorporation of lactate and pyruvate into fatty acids.

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