However, in the case of gibberellic acid, which has so large an effect on intact peas and only a small effect on excised sections (*11*), this investigation has failed to show any specific promotion of GA_3 action by any extract or additive tested. These facts suggest that the analysis of accessory growth factors, such as the "calines" proposed by Went (*12*), has continuing pertinence in the study of plant growth (*13*).

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Action of Salicylate on Metabolism of Acetate-2-C^{14} in the Rat

Salicylates have been shown to uncouple oxidative phosphorylation reactions in mitochondrial preparations (*1*), and this action may explain the increased oxygen consumption observed after the administration of salicylates to man (*2*) and to the rat (*3*). It may also be related to some of the effects of salicylates on carbohydrate metabolism in animals and isolated tissues. Thus, although an increased rate of glycogenolysis due to release of adrenaline is an important factor (*4*) in the depletion of glycogen caused by salicylate in the normal rat (*5*), impairment of glycogenesis due to an inadequate production of energy-rich phosphate bonds may also be concerned, particularly since salicylate diminishes glycogen synthesis in isolated rat-liver slices (*6*). The hypoglycaemic action of salicylate in the alloxan-diabetic rat (*7*) may also be interpreted as resulting from defective carbohydrate synthesis.

Table 1. Effect of salicylate on the incorporation of C^{14} in the liver glycogen and on the excretion of $C^{14}O_2$ and $C^{12}O_2$ in the breath of rats given acetate-2-C^{14}. Results are expressed as means plus or minus standard deviation. The significance of the differences between the means of the control and salicylate groups has been analyzed by the *t* test, and values of *P* are included.

Rats (No.)	Liver glycogen d.p.m./mg. (disintegration/min mg)	Total CO_2 [(0–60 min) mg/min]	$C^{14}O_2$ Cumulative % injected dose (0–60 min)	Specific activity of total CO_2 (μc/g) C/10 μc injection		
				10 min	30 min	60 min
			Control			
6	5193 ± 1640	7.32 ± 0.45	32.94 ± 2.01	2.43 ± 0.20	3.48 ± 0.37	2.28 ± 0.19
			Salicylate (500 mg/kg)			
4	20 ± 12.5 $P = 0.01$	14.70 ± 1.52 $P = 0.01$	52.27 ± 3.93 $P = 0.01$	1.87 ± 0.24 $P = 0.2$	3.50 ± 0.35 $P = 0.9$	1.42 ± 0.17 $P = 0.05$
			Salicylate (250 mg/kg)			
4	60 ± 18 $P = 0.01$	9.25 ± 0.40 $P = 0.02$	48.22 ± 4.33 $P = 0.02$	2.05 ± 0.28 $P = 0.4$	4.26 ± 0.41 $P = 0.3$	2.17 ± 0.13 $P = 0.8$

The effect of salicylate on the appearance of C^{14} in the liver glycogen and expired CO_2 of rats given acetate-2-C^{14} has been studied (*8*). Male rats of the Long-Evans strain, weight 200 to 250 g, were fasted for 24 hours and given 3 millimoles of sodium lactate by stomach tube at the beginning of the experiment. Thirty minutes later they received sodium salicylate by intraperitoneal injection; acetate-2-C^{14}, approximately 10 μc per rat, was administered by the same route after a further 30 minute interval. The radioactivity and CO_2 content of the breath were measured for 1 hour by the continuous recording equipment described by Tolbert, Kirk, and Baker (*9*). The rats were then killed by an intraperitoneal injection of Nembutal, the liver was excised, and the liver glycogen was isolated by the method of Marks and Feigelson (*10*) and purified to constant specific activity according to the directions of Stetten and Boxer (*11*).

The results, given in Table 1, show that salicylate, in a dose of either 250 mg or 500 mg/kg of body weight, inhibited the incorporation of C^{14} into liver glycogen after the injection of the labeled acetate. The higher dose of salicylate caused significant increases in both the $C^{12}O_2$ and the $C^{14}O_2$ but did not change the specific activity of the total CO_2. A similar but less marked pattern was observed with the lower salicylate dose.

The major pathway by which acetate carbons are incorporated into liver glycogen is via acetyl-coenzyme A, the Krebs cycle, decarboxylation of oxaloacetate to give phosphopyruvate, and the modified reversal of the Embden-Meyerhof scheme of glycolysis (*12*) and energy-rich phosphate bonds are necessary at various intermediate steps. The virtual absence of radioactivity in the liver glycogen of the salicylated animals, therefore, is consistent with the view that salicylate impairs carbohydrate synthesis by interfering with the production of energy-rich phosphate bonds. It has been emphasized by Weinman, Strisower, and Chaikoff (*12*) that the mere demonstration of incorporation of isotope into glycogen does not necessarily mean that a net synthesis of glycogen from a labeled fatty acid has occurred. These workers consider that glycogen synthesis from acetate is made possible only by an additional (that is, nonacetate) influx of Krebs cycle intermediates into the cycle. The administration of lactate to the animals in the present work could provide such an influx and make possible the net increase of glycogen via the synthetic reactions outlined above.

The increased excretion of $C^{12}O_2$ and $C^{14}O_2$ in the breath of the salicylated rats may be a direct result of the well-known action of salicylate in causing hyperventilation by stimulation of the respiratory center. However, a contributory factor may be an increased substrate breakdown as a consequence of inefficient phosphorylation mechanisms.

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