for the guinea pig by employing normal, high-fat, and lactose diets. The average growth rate of the groups receiving Solka-floc was slightly better in all cases. Guinea pigs grew very well on diets containing 12% corn oil (7.7 g/day) and at a rate of 4.9 g/day with 24% corn oil diets using Solka-floc as the bulk. Guinea pigs fed diets with lactose as the sole carbohydrate grew at a rate of about 2.0 g/day. Substitution of sucrose for half of the lactose increased growth to over twice the rate obtained with lactose alone.

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Hydrolysis of Succinylmonocholine by a Liver Esterase.* (21941)

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Succinyldicholine (SDC), a short-acting muscle relaxant(1), has replaced to a considerable extent the older relaxants d-tubocurarine and decamethonium since its introduction in 1949(2). Little systematic work, however, has yet been reported on its mode of breakdown in the body. It is known(3) that there is little excretion of unchanged SDC after intravenous injection and that, *in vitro*, serum cholinesterase hydrolyses SDC to succinylmonocholine (SMC) and choline(4,5). This enzyme also hydrolyses SMC to succinic acid and choline, but at a much lower rate(5,6). In many patients with a low serum-cholinesterase level, a prolonged response to SDC is encountered(7,8) but it is also known that a similar abnormal prolongation of the period of relaxation after SDC administration can occur in subjects with normal serum-cholinesterase levels(9,10). It seems possible that this lack of correlation between serum-cholinesterase level and duration of relaxation may be due to the presence of another esterase which is also capable of hydrolysing SDC. It has also been suggested(11) that SMC, which itself has slight muscle-relaxant properties (12), accumulates in the body after SDC administration and potentiates the action of SDC. Since the hydrolysis of SMC by serum cholinesterase is slow, especially in the presence of SDC(5), SMC might be expected to accumulate under these conditions unless there is another esterase in the body which is also capable of hydrolysing SMC.

It was therefore decided to determine whether esterases hydrolysing SDC or SMC are present in other tissues. The present communication concerns such an enzyme which is present in liver and which is distinct from liver cholinesterase. This enzyme catalyzes the hydrolysis of SMC but is without action on SDC.

Materials and methods. Samples of succinyldicholine dichloride and succinylmonocholine iodide were kindly supplied by Burroughs-Wellcome & Co. The rats used were a local hooded strain (170-220 g). The animals were stunned and exsanguinated, and the tissues removed and placed in cracked ice. Homogenates (17% in water) were prepared in Potter-Elvehjem type homogenizers at 0-5°C. The rates of enzymatic and non-enzymatic ester hydrolysis were followed in

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TABLE I. Hydrolysis of SMC and SDC by Rat Liver Homogenate.

Substrate	Rate of hydrolysis, μl CO_2/hr above endogenous: Without enzyme	With 170 mg homogenized rat liver	With 340 mg homogenized rat liver
.01*M* SMC	0	55	97
.02*M* "	1	54	110
.01*M* SDC	14	33	29
.02*M* "	28	58	58

the conventional Warburg apparatus by measurement of the CO_2 evolution from a 0.025 *M* sodium bicarbonate medium under anaerobic conditions at 37°C. The initial concentration of ester was normally 0.01 *M*, the total volume 3.0 ml and the vessels were gassed with 93% N_2-7% CO_2 for 20 minutes. The substrates were tipped in from the sidearms and after a further period of 10 minutes an initial reading was taken. Readings were then taken every 10 minutes for a period of one hour. Liver homogenate was separated into sub-cellular fractions according to the method of Schneider (13).

Results. Rat liver homogenate increases the rate of production of CO_2 from SDC and, to a greater extent, from SMC. Unlike the hydrolysis by serum, this liver-catalysed hydrolysis was not inhibited by 10^{-3} *M* eserine or by 10^{-4} tetraethylpyrophosphate (TEPP). However, a study of the kinetics of SDC hydrolysis showed that the rate of hydrolysis was not increased when the concentration of liver homogenate was doubled, but that the rate was doubled by doubling the concentration of SDC (from 0.01 *M* to 0.02 *M*). With SMC, however, doubling the enzyme concentration doubled the rate of hydrolysis but increasing the substrate concentration from 0.01 *M* to 0.02 *M* gave no increase in activity. Typical results are given in Table I. Since SDC has an appreciable rate of non-enzymatic hydrolysis under the given experimental conditions, the enzymatic hydrolysis of the SMC produced by this process would account for the observed activity in the SDC-liver homogenate system. These observations led to the conclusion that the liver esterase hydrolyses SMC but not SDC. The enzyme is provisionally termed "succinylmonocholine esterase" (SMC-esterase). The SMC-hydrolysing activities of rat, guinea pig, rabbit and human liver are shown in Table II.

Distribution of enzyme activity in rat liver. A 30% homogenate of rat liver in 0.25 *M* sucrose was fractionated by differential centrifugation at 0°C into nuclear, mitochondrial, microsomal and soluble fractions. As shown in Table III, most of the SMC-hydrolysing activity is located in the mitochondrial fraction. The slight activity observed in the nuclear fraction is probably due to the presence of some unbroken cells. In preliminary work on SMC-esterase, suspensions of guinea pig and rat liver mitochondria, prepared by fractional centrifugation in 0.25 *M* sucrose, were used as a source of the esterase. However, owing to the relative instability of these preparations, an attempt was made to prepare a stock of semi-purified enzyme in concentrated solution.

Preparation of a purified SMC-esterase. Acetone-dried rat liver was prepared by homogenizing rat liver with ice-cold acetone in a Waring blender, filtering by suction and washing several times on the filter with cold acetone. The powder was dried and stored in a vacuum desiccator at 0°C. The resulting powder contained an active SMC-esterase, but the enzyme could not be extracted from the powder with water. A suitable extraction medium was found to be 0.25 *M* ammonium

TABLE II. Hydrolysis of SMC by Liver Tissues.

Enzyme preparation	Rate of hydrolysis of 0.01*M* SMC, μl CO_2/hr/100 mg liver
Rat liver homogenate	30, 31, 33, 36
" " slices	34
Guinea pig liver homogenate	26, 26, 27, 28, 34
Rabbit liver homogenate	7, 8
Human liver homogenate (36 hr post-mortem)	13
Human liver homogenate* (fresh)	7

* This sample was removed as a small slice (250 mg) during a cholecystectomy operation and immediately frozen in an acetone-solid CO_2 mixture. Enzyme activity was determined 1 hr later. The low value may be due either to freezing procedure employed or to liver damage in the patient.

TABLE III. Hydrolysis of SMC by Cellular Fractions of Rat Liver.

Fraction	SMC-hydrolysing activity, μl CO_2/hr/mg nitrogen*
Whole homogenate	10
Nuclear fraction	15
Mitochondria	130
Microsomes	5
Soluble	1

* Determined by micro-Kjeldahl method.

hydroxide. A 5% suspension of the rat liver powder in this medium was homogenized for 2 minutes in a Waring blender in the presence of a few drops of octyl alcohol. The suspension was allowed to stand at 5°C for 15 minutes and then centrifuged at 20,000 x gravity for 15 minutes. The viscous supernatant was brought to pH 7.4 with 5% acetic acid and the heavy precipitate formed was removed by centrifugation at 3,300 x gravity for 10 minutes. The resulting supernatant contained 60% of the total activity present in the original acetone powder. The extract was then fractionated with ammonium sulfate. The precipitate formed at 33% saturation with ammonium sulfate had only slight esterase activity with SMC as substrate, but when the concentration of ammonium sulfate was raised to 50% saturation, a precipitate was formed which contained 60% of the activity of the unfractionated extract. The supernatant from this precipitate contained no detectable activity. The activity of all fractions was determined after dialysis against running tap-water overnight.

The fraction with the high SMC-esterase activity (33-50% saturation) had a very low cholinesterase activity with acetyl- or benzoylcholine as substrate. Further, the SMC-esterase was unaffected by 10^{-4} *M* TEPP, whereas the cholinesterase was completely inhibited. Work is now in progress to determine the substrate specificity of the purified preparation of SMC-esterase and to study its resistance to other esterase inhibitors.

Discussion. SMC-esterase might prevent the accumulation of SMC in the body after SDC administration if present in an active form in man. This would suggest that SMC accumulation is not responsible for the prolonged effect of SDC sometimes observed. However, it is possible that in such cases the liver SMC-esterase level and not the serum cholinesterase level is abnormally low and thus allows the accumulation of SMC. It is of interest that a choline ester, namely SMC, should be hydrolyzed in liver by an enzyme which is distinct from the cholinesterases of Whittaker's classification(14).

Summary. 1. The livers of rat and of other animals, including man, contain an esterase, distinct from known cholinesterases, which hydrolyses succinylmonocholine. The enzyme is located in the mitochondria and can be extracted from a liver acetone-powder with dilute ammonium hydroxide. It has been partially purified by ammonium sulfate fractionation of a liver extract, and the final preparation was almost free of cholinesterase activity. The enzyme is TEPP insensitive. 2. The possible role of this enzyme in recovery of muscular control after succinyldicholine administration is discussed.

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