acetigens was pronounced at concentrations of lactone as low as $1 \times 10^{-5} M$. The time curves of oxygen uptake during pyruvate oxidation illustrated the blocking action (which prevents the self-perpetuation of the cycle) of the impurity and the prepared lactone; the oxidation of pure pyruvate proceeded at a fairly steady level until completion, whereas with impure pyruvate or in the presence of the lactone the rate suddenly fell to a low level when the small quantities of malate had been utilized. Neither the oxidation nor the oxalacetogenic ability of succinate was inhibited by the lactone.

These results might be interpreted to mean that this potent inhibitor blocked some reaction between isocitrate and the α-ketoglutarate-succinate step in the cycle. The inhibition did not seem to be on the isocitric dehydrogenase, since there was good oxidation of isocitrate in the presence of impure pyruvate or the lactone, although no oxalacetate was formed and no pyruvate was oxidized. A photometric determination of TPN reduction by isocitrate as catalyzed by isocitric dehydrogenase in an acetone powder of rat heart mitochondria (*5*) showed that the lactone had no inhibitory effect in concentrations up to 5 mM.

Essentially complete conversion of 5 mM pyruvate to α-ketoglutarate was obtained when 1 mM lactone was present, and equally good conversion was seen from isocitrate. Since these results would point to a block on the α-ketoglutarate oxidase, we examined the effect of the lactone on this enzyme isolated from pig heart (*6*) by one of us (C.M.M.) with the help of D. R. Sanadi at the Institute for Enzyme Research, Madison. When α-ketoglutarate and the lactone were present in equimolar concentration, inhibition of 50 percent was observed. This inhibition would seem to be competitive, since increasing the concentration of the α-ketoglutarate reduced the inhibition. The oxalacetigenic ability of α-ketoglutarate in the mitochondria was inhibited even more potently than the oxidase preparation. The block in the cycle would, therefore, seem to be due to this competitive inhibition of the α-ketoglutarate oxidase. Blocking of the initiation of pyruvate oxidation by citrate or isocitrate, or of the normally operating cycle, would be much more complete owing to the low concentrations of α-ketoglutarate present under these circumstances.

Titration of the lactone demonstrated two acidic groups with $pK_1 = 2.35$ and $pK_2 = 6.95$. The first dissociation constant refers to the normal free carboxyl group, but pK_2 is too high for a carboxyl group and would argue strongly against the presence of the straight-chain dimer (where pK_2 would be expected to be between 4.5 and 5.0). Cleavage of the lactone ring near neutrality is unlikely, since rapid back titration showed identical values for the pK's and it is doubtful that ring closure would occur rapidly under these conditions. There remained the possibility that enolization takes place and that form III is prevalent around neutrality, the pK_2 referring to the enolic hydroxyl group whose acidic strength is increased by the surrounding groups and chelation with cations. Titration of diethyl-oxalacetate, which possesses a similar —CO—CO—CH_2— grouping showed that the pK of the enolic hydroxyl here was 7.6. Thus we assume that the inhibitor is present in aqueous solution as the lactone and around neutrality in the enolic form (III). Permanganate reduction indicated an active double bond in the prepared lactone at neutrality, again suggesting the enolic form.

Preliminary work on liver and kidney mitochondria demonstrated that the cycle is not blocked so readily by the lactone as in heart mitochondria. We are at present attempting to purify the lactone and related substances, investigating the more intimate mechanism of the inhibition, as well as studying the action of the inhibitor on the functional activity of rat cardiac muscle.

References and Notes

1. This investigation was supported by grants from the National Heart Institute, National Institutes of Health (H-1391C), and the Life Insurance Medical Research Fund, and aided by facilities supplied by the Allan Hancock Foundation.
2. The term *oxalacetigen* is used to designate a substance that, in the presence of the mitochondrial preparation, will form oxalacetate with which pyruvate may condense to enter the tricarboxylic acid cycle.
3. V. E. Price and L. Levintow, *Biochemical Preparations*, Eric G. Ball, Ed. (Wiley, New York, 1952), p. 22.
4. A. W. K. de Jong, *Recueil trav. chim.* **20**, 81 (1901).
5. G. W. E. Plaut and S.-C. Sung, *J. Biol. Chem.* **207**, 305 (1954).
6. D. R. Sanadi, J. W. Littlefield, and R. M. Bock, *ibid.* **197**, 851 (1952).

2 August 1954.

Carbon Dioxide Utilization by Rabbit Liver

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During the investigation of labeled carbon dioxide fixation into the organic acids of liver homogenates, it was observed that such incorporation of the isotope in the liver of the rabbit differed from that of other species examined (*1–5*). Fixation was evident within the times described into a component of the acetone extracts insoluble in the solvent that readily dissolves the organic acids. Unlike any other species examined, the extent of the incorporation into the insoluble compound was as great and at times greater than that into such acids as succinic, fumaric, and malic.

Liver tissue from three adult rabbits was incubated with $NaHC^{14}O_3$ (*6*) under the conditions previously described (*5*). Incubations were for 10 min except where indicated. Acetone extracts were prepared. The chromatography and radioactive assays have been outlined in other experiments (*3, 5*). The paper chromatographic separations were those of Denison and Phares (*7*) and Benson *et al.* (*8*) and the tests for sugars followed the procedures of Dische (*9*).

The data of Table 1 indicate that the fixation of the

Table 1. Distribution of radioactivity within acetone extracts of rabbit liver homogenates incubated with $NaHC^{14}O_3$, 0.47 gm tissue was incubated in 1 ml modified (*5*) Krebs ringer solution containing 0.005 mc of the isotope; cts means "counts," where the counter efficiency is 2 percent.

Solute	Rf*	Time of incubation (min)									
		3		5		10		20		30	
		(cts/min†	%)	(cts/min	%)	(cts/min	%)	(cts/min	%)	(cts/min	%)
Compound insoluble in amyl alcohol-chloroform	0	1562	39.0	1632	38.1	3264	70.6	4224	81.6	5990	77.9
Malic acid	0.38	1907	47.4	2112	49.2	1088	23.5	819	15.8	1427	18.6
Succinic and lactic acids	0.75	544	13.6	544	12.7	275	5.9	134	2.6	275	3.5
Total activity of labeled compounds		4013		4288		4627		5177		7692	

* Ether-acetic acid-water (13-3-1) was the developing solvent for the paper chromatograms.
† Approximately one-twentieth of the total extract of the liver was applied in each case.

label into the amyl alcohol-soluble compound was not limited to an incubation time of 10 min exclusively. The percentages of the total fixation into malate at 3 and 5 min exceeded those at 10, 20, and 30 min. Since the chemical concentration of the malate did not change, these percentages mirror the changes in specific activity. On the other hand, the abundance of the label in the insoluble residue was lower at 3 and 5 min and greater at 30 min. In butanol-acetic acid-water (4-1-5), the insoluble residue moved on paper as a single spot (Rf 0.2), which gave a positive test for phosphate with acid molybdate, benzidine, and sodium acetate (*8*). When the eluted spot from butanol-acetic acid-water was hydrolyzed in HCl and rechromatographed, it was not found to be a hexose or triose. The rechromatographed spot, tested with diphenylamine, showed that difference (*9*) between the optical density D at 660 and 580 mμ ($D_{660} - D_{580} = D_{400}$) that is characteristic of glycolaldehyde. However, the spectral absorption between 500 and 700 mμ was not identical to that of the product from the known glycolaldehyde, even though the mobility of the compound on paper in the butanol-acetic acid-water system was like that of the authentic glycolaldehyde.

Such relative incorporation into the organic acids and the insoluble compound at the several times of incubation shown here distinguishes carbon dioxide fixation in the liver of the rabbit from similar findings in other species. If the utilization of the label by the organic acids and the compound insoluble in amyl alcohol-chloroform occurs by independent mechanisms and if the insoluble compound is glycolaldehyde phosphate, the data could be explained by current views concerning the path of carbon in animal cells (*10*). By such views it would be presumed that the carbon was first fixed into a hexose, the precursor of sedoheptulose which later cleaved between carbons 2 and 3 to produce the labeled glycolaldehyde.

References and Notes

1. M. M. Khan, K. O. Donaldson, and L. M. Marshall, *Am. J. Physiol.* **176**, 461 (1954).
2. L. H. Newman, K. O. Donaldson, and L. M. Marshall, *ibid.*, in press.
3. L. M. Marshall and F. Friedberg, *J. Biol. Chem.* **199**, 783 (1952).
4. L. C. Leeper, F. Friedberg, and L. M. Marshall, *Nature* **17**, 748 (1953).
5. L. M. Marshall and F. Friedberg, *Biochim. et Biophys. Acta* **13**, 61 (1954).

6. This investigation was supported in part by a research grant from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.

7. F. W. Denison and E. F. Phares, *Anal. Chem.* **24**, 1628 (1952).
8. A. A. Benson *et al.*, *J. Am. Chem. Soc.* **72**, 1710 (1950).
9. Z. Dische and E. Borenfreund, *J. Biol. Chem.* **192**, 583 (1951).
10. B. L. Horecker *et al.*, *ibid.* **207**, 393 (1954).

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Activity in Electrogenic Organs of Knifefishes

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The knifefishes, which comprise the family Gymnotidae, and inhabit fresh tropical waters of Central and South America, are close relatives of the electric eel, *Electrophorus electricus*. They possess structures that resemble the electric organ of the eel, but identification of these with functional electric organs has been doubted (*1*). Only a single report (*2*) mentions electric activity in *Gymnotus carapo*.

Three species of knifefishes examined in a preliminary survey in this laboratory have all proved to be electrogenic. These are *Eigenmannia virescens, Gymnorhamphichthys hypostomus,* and *G. carapo*. Unlike the eel, which emits single or short bursts of pulses of high intensity, up to 600 v and 1 amp, these fish emit low intensity pulses (Fig. 1) continuously and with remarkable regularity (Fig. 2). In this they resemble the Mormyridae of Africa, *Gymnarchus* (*2*), *Mormyrops boulengeri* (*2*), and *Mormyrus kannume* (*3*).

The responses were recorded from intact fishes swimming freely or lying on the inclined floor of a tank with only the head immersed. The electrodes were wires, spaced 5 mm to several centimeters apart, insulated except at their tips, and placed near the swim-