TABLE I. Average Daily Values of Free HCl Secretion from Heidenhain Pouches Expressed as mEq. Before and After Gastrojejunostomy to the Main Stomach and After Gastrojejunostomy Take-Down.

Dog No.	mEq. free HCl/day — Before gastrojejunostomy	After gastrojejunostomy	% rise	mEq. free HCl/day after gastrojejunostomy take-down	% drop
A-67	29.6	95.3	225	—	—
39	31.7	84.3	172	—	—
83	35.7	70.7	98	—	—
40	64.8	131.8	103	64.4	51
C-10	14.2	43.2	204	25.3	41
Avg	35.2	85.1	142	—	46

jejunostomy figure, but much lower than that obtained during the gastrojejunostomy period. These data are summarized in Table I.

Discussion. The mechanisms responsible for this phenomenon are not definitely known to us at this time. We realize that the Heidenhain pouch differs from the normal stomach, but the former is an accurate indicator of the response of the parietal cells to humoral influences.

It is clear from the foregoing that gastrojejunostomy in some way is responsible for an increased output of HCl by the parietal cells. This could represent a compensatory response of these cells to intragastric neutralization of HCl to beyond physiologic limits —the "rebound phenomenon." Storer *et al.* (2) have shown that complete intragastric regurgitation of alkaline duodenal juices will cause a rise in Heidenhain pouch secretion of 100% or more. The possibility of increased antral stimulation due to repetitious passage of food through a normally patent pylorus and back into the stomach via the afferent loop of the gastrojejunostomy also may exist in some instances. Undoubtedly, in the main stomach the various neutralizing factors will tend to keep the concentration of free HCl at lower levels than obtain in the pouch. It seems reasonable to assume that the increased acid production may not always be neutralized and may play a part in the frequent failure of gastrojejunostomy as a treatment for peptic ulcer.

Conclusion. Under the conditions of this experiment, gastrojejunostomy of the main stomach of Heidenhain pouch dogs results in a significant rise in free HCl secretion from the pouch.

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Localization of the Enzymatic Block in Kidneys of Rats Treated with Fluoroacetate. (19814)

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Recent experiments have indicated that a "fluorotricarboxylic acid" formed from fluoroacetate inhibits each of the reactions of the aconitase enzyme *in vitro*(1). This finding would appear to explain the accumulation of citrate in the tissues of animals treated *in vivo* with fluoroacetate(2,3) as well as its accumulation and failure to be oxidized *in vitro* when fluoroacetate is present in reaction mixtures otherwise adequate for citrate oxida-

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TABLE I. Oxidation of Substrates by Homogenates of Kidneys of Rats Treated with Fluoroacetate Compared with Controls.

Substrate	60 min. total: No. of animals	60 min. total: μl O_2 (avg and range) FAc	60 min. total: μl O_2 (avg and range) Control	1st 10 min.: No. of animals	1st 10 min.: μl O_2 (avg, range) FAc	1st 10 min.: μl O_2 (avg, range) Control
Citrate	8	117 (66-144)	300 (254-357)	10	31 (7-45)	55 (34- 79)
Cis-aconitate	7	251 (177-275)	260 (207-323)	6	48 (13-73)	53 (34- 63)
α-ketoglutarate	2	318 (296-340)	358 (324-411)	3	68 (64-71)	78 (64- 93)
Pyruvate and fumarate	5	237 (188-284)	289 (226-325)	6	56 (44-66)	76 (64-100)

tion and synthesis(4,5). Nevertheless, it seemed desirable to compare the oxidative metabolism of citrate and other acids of the Krebs cycle *in vitro* following the administration of fluoroacetate *in vivo*, in an attempt to learn whether all of the reactions that are presumed to require the participation of aconitase were equally affected, since there was already evidence that aconitase was not blocked in some circumstances(2,6).

Materials and method. Dose of fluoroacetate. To establish the fluoroacetate block *in vivo*, male rats (weighing 200-300 g) obtained from the Holtzman-Rolfsmeyer Rat Co., were injected intraperitoneally with 5 mg of fluoroacetate per kg and sacrificed one hour later. Poisoning was evidenced by accumulation of citrate in the tissues(2,3) and/or convulsive states. *Oxidation studies.* The kidneys were excised and placed in ice-cold isotonic KCl solution; after weighing, they were homogenized in sufficient isotonic HCl to produce a 10% final homogenate, weight to volume. In addition to the basic medium reported previously(4), each flask contained substrates added as the potassium salt, brought to pH 7.2-7.4. Center wells contained 0.2 ml of 2.5 N NaOH. Cold homogenates were added to cold reaction mixtures and the vessels were equilibrated 10 min. before manometric readings were begun. The gas phase was air and the temperature was 38°C. Reactions were stopped by addition of 0.3 ml of 50% perchloric acid to the main chamber. Citrate determinations on the protein free filtrates were carried out by the method of Natelson *et al.*(7). *Aconitase studies.* 40 μM of cis-aconitate were added to the solution containing the homogenate preparation, cysteine, and ferrous ammonium sulfate at the concentrations recommended by Dickman and Cloutier(8). After 10 min. of incubation at 30°, sufficient 20% trichloroacetic acid was added to produce a final concentration of 5%. Citrate was determined as noted above. *Aconitase and oxidations.* To test both the aconitase and oxidative functions simultaneously, 20 μM of cis-aconitate were added to flasks containing kidney homogenates, basic medium and sufficient arsenite to depress α-ketoglutarate oxidation, yet not depress oxygen uptake (0.0002M). At various time intervals, oxygen uptake, α-ketoglutarate formation and citrate formation were measured.

Results. The data of Table I indicate that oxidation of all added substrates was significantly less in flasks containing homogenates of poisoned kidneys as compared with the controls, but the greatest effect observed was on oxidation of citrate. Not only was the initial oxidation of citrate depressed to 45% of the control, but the total oxidation was only 32% of the control. By contrast, the oxidation of cis-aconitate and α-ketoglutarate were respectively 96% and 89% of the control values for the total period of incubation, while the initial oxidation was 91% and 87% of the control. In agreement with previous findings of inhibition of oxidation reactions prior to the level of citrate in the Krebs cycle, it was found that oxidation of pyruvate and fumarate was 82% of the control for the total period while oxidation in the first 10 minute interval was 74% of the control.

As in previous investigations(2,6), the aconitase activity of these preparations was not diminished, as measured either by conversion of cis-aconitate to citrate or conversion of isocitrate to citrate.

It seemed possible that the failure of citrate oxidation could be related to defects in hydrogen transport, or a relative excess of

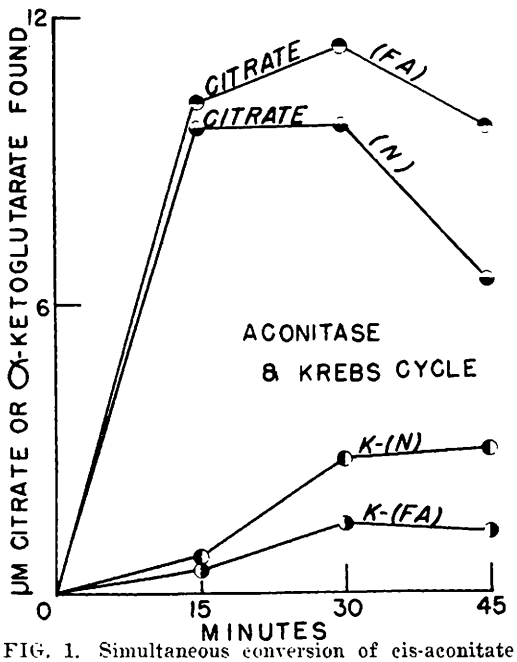

FIG. 1. Simultaneous conversion of cis-aconitate to citrate and oxidation of cis-aconitate. Conditions as in text, but flasks contained 60 mg kidney tissue from normal (N) or fluoroacetate-treated FA) rats and 5 μM ATP in these experiments. Figures presented are avg for two experiments each performed in duplicate.

dephosphorylation. To test this point, experiments were carried out with cis-aconitate as the substrate and citrate formation and disappearance was measured. As Fig. 1 shows, the conversion of cis-aconitate to citrate was the same in both control and fluoroacetate systems; yet the latter failed to reconvert the citrate pool during the course of the incubation period. The result was obtained over a time period where the rate of oxidation was essentially the same in both homogenates. It appears that there is no defect in phosphate transfer or hydrogen transport.

The data from this experiment as well as the data from Table I show that in kidney homogenates from fluoroacetate-treated animals there is a marked decrease in the ability to convert citrate to cis-aconitate with little if any diminution in the ability to carry out the reverse reaction. These findings are in agreement with those of Elliott and Kalnitsky(5) and of Lotspeich, Peters, and Wilson(1) who used different experimental approaches to this problem, and show that the main block occurs at the citrate to aconitate step. These findings might be explained if the dissociation constant for cis-aconitate and aconitase is small enough compared to that of citrate to permit it to compete effectively with the fluoroacetate derivative under conditions in which citrate could not compete(1). Alternatively, one would have to seek additional mechanisms for interconverting the two compounds(5).

Discussion. In consideration of the physiological effects produced by fluoroacetate, it is evident that in the affected cell, a particularly vulnerable site in energy metabolism is attacked. Essentially, progress at the tricarboxylic acid level is effectively blocked, inasmuch as such little cis-aconitate or isocitrate as could be mobilized from sources other than citrate would be converted to citrate. Maintenance of the energy requirements of the blocked cell is then dependent upon conversion of glutamate and aspartate to components of the Krebs cycle and further oxidation of the α-ketoglutarate and oxalacetate formed; since the block varies from tissue to tissue in the extent of its completeness, the citrate accumulated may also serve in part to overcome the effects of the block. It seems possible that the symptomatic response to fluoroacetate poisoning will occur in tissues which have a high energy demand and a low capacity for conversion of amino acids to substrates of the Krebs cycle. The brain, having a great demand for energy from oxidations, and a low capacity for protein breakdown may be such an organ.

Summary. 1. Oxidation of citrate was markedly depressed in homogenates of kidneys from rats treated with fluoroacetate; oxidation of other substrates of the Krebs cycle, including cis-aconitate, was depressed only to a relatively small extent. 2. No inhibition of aconitase was found in these preparations when cis-aconitate was the substrate. 3. The evidence indicated that the conversion of cis-aconitate to citrate was relatively unaffected under conditions in which the reverse reaction was strongly inhibited.

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The Antibacterial Action of Erythromycin.* (19815)

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Erythromycin is a new antibiotic obtained from *Streptomyces erythreus*. Some features of the isolation and production of this antibiotic and of its physical, chemical and biologic properties were described briefly by McGuire *et al.*(1). They found erythromycin to be active *in vitro* against a wide variety of microorganisms, including strains of penicillin-sensitive and penicillin-resistant staphylococci, and of relatively low toxicity both for laboratory animals and in preliminary trials in patients. The prospect of a new agent that is active and nontoxic and which might prove to be effective against staphylococcal infections prompted us to undertake further studies on erythromycin. A preliminary clinical report has been published elsewhere(2) and the results of the laboratory findings are presented in this and the succeeding 2 papers. Some laboratory and clinical observations have also been presented by Heilman *et al.*(3).

Materials and methods. Erythromycin. A supply of "Ilotycin" (Erythromycin, Lilly) for clinical trial and of the purified crystalline material (890 μg/mg) for laboratory studies was furnished by the Lilly Research Laboratories through the courtesy of Dr. J. W. Smith. Stock solutions of the white crystalline powder were made either in broth or distilled water so as to contain 8 mg or 2 mg of the free base per ml; a small amount of ethyl alcohol was used to facilitate solution. Further dilutions of the antibiotic were made in broth unless otherwise indicated. *Bacterial Strains.* Most of the strains of organisms used in this study were recently isolated from infected material obtained from patients at the Boston City Hospital. Some of them were isolated and identified by Marion E. Lamb and A. Kathleen Daily in the Bacteriological Laboratory of the Mallory Institute of Pathology; the strains of gonococci were isolated by Helen W. Trousdale in the Genito-Urinary Clinic and most of the other organisms were isolated by Clare Wilcox and Marilyn K. Broderick to whom we are also indebted for technical assistance throughout this study. Most of the strains of specific groups and types of hemolytic streptococci were obtained from Drs. Rebecca Lancefield, Lowell A. Rantz and Harry A. Feldman. The strains of *Streptococcus mitis, sanguis* and *faecalis* from cases of bacterial endocarditis were obtained from Dr. Leo Loewe. In addition, a few laboratory strains used routinely for assay of other antibiotics were also included. For routine tests of sensitivity, the cultures used were obtained by picking individual colonies from the surface growth of culture plates; however, in the studies on the development of resistance and on the mechanism of action of the antibiotic, individual colonies were picked from agar pour plates of the cultures which had been so diluted as to contain only a few well segregated colonies. *Sensitivity Tests.* These were carried out with serial 2-fold dilutions of the

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