TABLE I. Effect of Feeding Corn Oil on Duration of Pentothal Anesthesia in Rats on 3 Different Days.

Exp.		No. animals	Mean anesthesia time	Stand. dev.	Probability Student t test
A	Oil	9	25	6.2	
	Control	9	76	38.9	
	Mean diff.		51		<.01
B	Oil	7	11	3.5	
	Control	10	22	6.1	
	Mean diff.		11		<.001
C	Oil	17	13	2.85	
	Control	17	30	15.6	
	Mean diff.		17		<.001

Perhaps the fact that the standard deviation of the oil fed group was lower than that for the control group is also of some significance. It was noticed throughout the experiment that the control group was much more erratic than the oil group. The wide variations in the sleeping time of the control group could be attributed to small variations in the percentage of body fat, which Hermann and Wood(2) demonstrated to have a marked effect on sleeping time. The relative uniformity of the sleeping time of the oil fed group could be attributed to the hyperlipemia which may cause a much greater shift in the thiopental equilibrium to the lipid phase so that small variations in body fat do not result in as marked variations in sleeping time.

Summary. 1. It has been shown in rats that ingestion of oil preliminary to thiopental anesthesia will decrease the anesthesia time by about 50%. 2. A suggested explanation for this action is the production of a hyperlipemia by the oil, with the resultant uptake of the circulating thiopental by the chylomicrons.

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Inhibition of Lysozyme by Heparin.* (20281)

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The function of lysozyme in various mammalian fluids and cells has never been known. The presence of the enzyme has been demonstrated by its lytic effect on various microorganisms(1-3), but a natural substrate in the tissues has not been found(4). Meyer(4) has emphasized certain characteristics which are common to lysozyme and hyaluronidase. Hyaluronidase is inhibited by heparin(5,6), and the inhibition has been assumed but not demonstrated to be competitive because of the chemical similarity of the inhibitor and hyaluronic acid. Similarly, inhibition of lysozyme by heparin and related polysulfonates has been shown(7), again without investigation of the nature of the inhibition. The present study establishes the fact that the

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inhibition of lysozyme by heparin does satisfy the usual criteria for competitive inhibition.

Experimental. One ml of a solution of crystalline egg lysozyme† containing 2.5 μg per ml was added to 1.5 ml of a suspension of a dried preparation of *M. lysodeikticus* (Bacto Lysozyme Substrate) contained in a 75 x 12 mm cuvette kept in a water bath at 37°C. The concentrations of substrate used were 180, 132, 84, 72, 60, and 48 mg %; a wider range was not feasible because of the method used. Turbidity (optical density) was read on the Coleman Junior Spectrophotometer(8) at 5-minute intervals for 20 minutes, and the reading was converted into mg % of unlysed bacteria by a calibration chart. A further correction was found to be necessary: completely lysed bacteria gave a reading very definitely greater than zero. To correct for this, suspensions of bacteria of each concentration used were treated with crystalline egg lysozyme in final concentration of 2 μg per ml (*i.e.*, twice the concentration used previously) for 2 hours to ensure complete lysis. A linear relationship with a coefficient p of value 0.097 was found between this reading and the initial concentration of substrate, and since the calibration curve was also linear in this region, a simple correction was possible. If a is the initial substrate concentration and x is the concentration lysed at t minutes, the concentration unlysed is a—(l-p)x. From this (a-x) was calculated. Values of a—(l-p)x below 12 mg % were rejected because in this range, errors in determinations are relatively large. Six experiments were run, and all results for the same interval and the same substrate concentration were averaged, since they did not differ appreciably from each other.

When log (a-x) was plotted against time, it was seen that the points fell very close to a straight line, as in Fig. 1, indicating a first order reaction. Accordingly, data averaged as described were fitted to straight lines by the method of least squares. The slopes of these lines are the velocity constants. When these lines were extrapolated back to t = 0, they

† Obtained through the courtesy of Armour and Co.

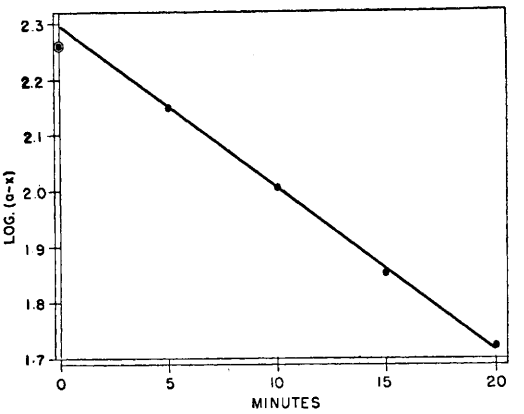

FIG. 1. Velocity of lysis of *M. lysodeikticus* by crystalline egg lysozyme. The logarithms of the mg % of unlysed bacteria are plotted against time. The straight line was drawn through the experimental points by the method of least squares, and the reaction is of the first order. Note that the point for zero time lies some distance from the line, indicating a lag, perhaps as a result of the time necessary for lysis.

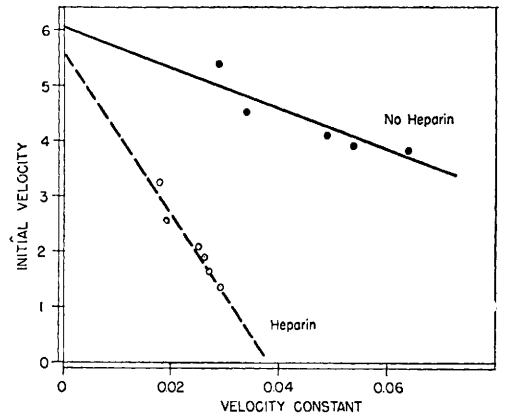

FIG. 2. Effect of heparin. The slope of the lines (drawn through the experimental points by the method of least squares) gives the Michaelis constant; the Y-intercept is V_{max}. Heparin greatly alters the slope without significantly affecting the Y-intercept.

almost invariably indicated a higher initial substrate concentration than was actually used, or rather, a true zero time slightly later than the moment of mixing (see *e.g.*, Fig. 1, point ⊙). This perhaps indicates a slight lag between the combination of lysozyme with its substrate and the actual lysis of the bacterial cell. The velocity constants were plotted against the initial velocities obtained by multiplying the velocity constants by the initial substrate concentrations (Fig. 2).

Again the points fell close to a straight line whose slope, determined by the usual least squares method, is equal to the Michaelis constant(9). Since the molecular weight of the true substrate, *i.e.*, the constituent of the bacterial cell containing the linkage upon which lysozyme acts, is unknown, the constant cannot be given in the usual units.

Simultaneously, a second series of determinations was run which differed from the first only in that the reaction mixture contained in addition heparin in a final concentration of 10 mg per ml. The initial velocity was again plotted against the velocity constant (Fig. 2), and it is obvious to the eye that the slope has changed. The change was found statistically to be highly significant. The intercept on the Y axis is V_{max}, and the difference in the two values for V_{max} is not significant. Inhibition by heparin of the action of lysozyme on *M. lysodeikticus* is therefore competitive(10).‡

Summary. The inhibition of lysozyme by heparin is shown by the usual criteria of the Michaelis-Menton formulation to be competitive in type.

‡ Additional experiments have shown an inhibiting effect of large amounts of heparin on the lysozyme activity of lysates of human leukocytes also. No inhibition is evident at heparin concentrations in the range used in prior experiments for *in vitro* anticoagulation(8).

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Measurement of Complement Fixation in Small Volumes.* (20282)

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The applicability of the complement fixation reaction to the assay and immunological characterization of biological materials is frequently limited or entirely impractical because of the scarcity of the antigen or antiserum available. This is particularly the case in the virus field in which many useful studies have not been possible with the amounts of these materials needed for complement fixation as usually practiced. A specific problem encountered recently in this laboratory has stimulated an investigation of the adaptability of the standard reaction to quantitative work with small volumes. As will be described in this report, the results of the study indicate the promise of considerable success with the reaction in total volumes as small as 12 mm^3.

Materials and methods. The development of the procedure described here has depended principally on modifications of the standard method(1) for complement fixation necessary to overcome the technical difficulties introduced by the minute volumes employed. These have been circumvented in the main by the construction of apparatus eliminating the principal adverse factors. An initial prerequisite to use of the small volumes was

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