	cmm CO_2 liberated in 1 hour
Serum + Ringer solution + Acetylcholine	250
Serum + $NaHCO_3$ + Acetylcholine	325
Serum + Ringer solution + Acetylcholine + $CaCl_2$ (1/500 molar)	146
Serum + $NaHCO_3$ + Acetylcholine + $CaCl_2$ (1/500 molar)	234

It appears that the addition of calcium chloride prevents some of the carbon dioxide from leaving the solution. To prove, that the smaller amount of CO_2 found in the presence of calcium chloride was not due to an inhibitory effect of calcium chloride upon the choline-esterase, but to a methodical error, the acetylcholine was replaced by acetic acid in some determinations:

	cmm CO_2 liberated in 15 minutes
Serum + $NaHCO_3$ + N/100 acetic acid	129
Serum + $NaHCO_3$ + N/100 acetic acid + 0,1 cc $CaCl_2$ molar	120
Serum + $NaHCO_3$ + N/100 acetic acid + 0,2 cc $CaCl_2$ molar	95
Serum + $NaHCO_3$ + N/100 acetic acid + 0,2 cc $CaCl_2$ 1/100 molar	127
Serum + $NaHCO_3$ + N/100 acetic acid + 0,2 cc $CaCl_2$ 1/1000 molar	125

It follows from the above experiments that the titration procedure gives more reliable results, but it has the disadvantage that it can only be used in clear and almost colorless solutions.

Since the manometric method requires a Warburg apparatus we attempted to develop a more rapid procedure for the determination of the activity of cholinesterase in colored solutions. The following was found to be satisfactory: The serum, a $NaHCO_3$ solution and the acetylcholine solution were placed in the outer chamber of a Conway-jar[3] and a N/10 $Ba(OH)_2$-solution in the inner part. The total $Ba(OH)_2$ solution, or 0,5 cc was taken out 40 minutes after the Conway-jar was covered with the glass lid, and the quantity of unreacted $Ba(OH)_2$ was titrated with N/100 acetic acid. The values were satisfactory and in agreement with the results obtained by the titrimetric method mentioned above.

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CHEMICAL STUDIES ON CRYSTALLINE BARIUM ACID HEPARINATE

WE have obtained analytical data for the crystalline barium acid salt of heparin (barium acid heparinate) in comparison with those obtained for the neutral and acid salts of mucoitinsulfuric acid and chondroitinsulfuric acid. The following molar ratio was found for barium acid heparinate. Anhydrohexosamine : anhydrohexuronic acid : SO_3 : Ba = 2.0 : 1.9 : 6.0 : 3.0. Further, N : S : Ba = 2 : 6 : 3. Thus all barium is attached to ester sulfate and the carboxyl group of the uronic acid component is free. Summation (89 per cent.) of the above data, in comparison with the high summation (96 per cent.) obtained for the neutral sodium salt of chondroitinsulfuric acid, does not exclude the possible presence of another constituent. *d*-Glucosamine was identified (as *d*-glucosamine hydrochloride) in the hydrolyzate of the crystalline barium acid heparinate. Sodium heparinate (purified through the crystalline barium acid salt) consumes one mole (per 1,200 equivalent weight) of periodic acid.

The amino group of the *d*-glucosamine component of barium acid heparinate is not acetylated and is not free. Barium acid heparinate loses its anticoagulant potency on repeated crystallization from warm, dilute acetic acid. This change is accompanied by the appearance of a free amino group in the molecule, no sulfate is lost and the material is still stained with toluidine blue. Thus neither sulfate content nor toluidine blue staining power are true criteria of heparin activity.

Crystalline barium acid heparinate is also biologically inactivated by prolonged drying at elevated temperatures and by treatment ("Roche heparin" used in this experiment) with weakly ammoniacal hydrogen peroxide, the latter reaction resulting in appreciable sulfate loss.

Full details will be communicated at a later date.

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[3] E. J. Conway, "Micro-Diffusion Analysis and Volumetric Error."

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