The second of these alternatives was sympathetically considered by Krebs in his early work on the subject of amino acid oxidation, but no decision was reached by him (*5*). On the basis of our present data, we are inclined to favor this second alternative, which is not only consistent with the Bergmann concept of intracellular peptide metabolism, but also supplements earlier work from this laboratory on the enzymatic susceptibility of peptides of *l*-cystine (*3*). In the final analysis, however, the Bergmann concept can only be proved by separation of the enzymes involved, and work on this possibility is in progress.

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Use of Insoluble Penicillin Salts for the Prolongation of Penicillin Blood Levels

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Many methods have been proposed for prolonging blood levels of penicillin. The one usually employed in medical practice is that proposed by Romansky and Rittman (*3*), namely, an intramuscular injection of a suspension of calcium penicillate in beeswax and a vegetable oil. This method has the disadvantage of using a substance (beeswax) of variable composition which may not in all cases be completely absorbable.

In a search for a method of prolonging penicillin blood levels, the writer decided to investigate the insoluble penicillin salts. These compounds have not been used until now because it was believed that the penicillin in such salts was irreversibly inactive. Thus, Abraham and Chain (*1*) found that penicillin was inactivated by a large number of metallic ions—copper, lead, zinc, cadmium, nickel, mercury, and uranium. They also reported that no activity could be recovered by decomposing the inactivated material with acid and extracting with ether. Bacharach and Hems (*2*) state that zinc, copper, mercury, and lead inactivate penicillin rapidly and iron less rapidly. Whether this inactivation is due to the formation of an insoluble penicillin or whether there is a definite chemical change in the penicillin is a subject for future investigation.

It occurred to the writer that there was a possibility that the inactivated insoluble penicillin might be reactivated *in vivo*. If this proved to be true, the insoluble salt would be more slowly absorbed than the soluble sodium, potassium, and calcium salts now in use and would therefore result in a marked prolongation of blood levels. Moreover, all the substances used would be completely absorbable. These suppositions were correct, as shown by the following data.

A control intramuscular injection in a rabbit of 20,000 units/kg. of penicillin suspended in peanut oil gave no readable blood level after 5 hours. On the other hand, a similar injection of silver penicillate produced a blood level of .08 units/cc. at 17 hours and .03 units at 20 hours; one of mercury penicillate, a level of .08 units at 17 hours and one of ferric penicillate, a level of .16 at 17 hours and .02 at 20 hours.

Penicillin produces insoluble salts with iron, copper, tin, vanadium, lead, lanthanum, cesium, zirconium, mercury, bismuth, silver, gold, and probably many other metals. Insoluble salts are also obtained with numerous organic substances, basic or cationic in character, such as the triphenylmethane dyes, namely, gentian violet, brilliant green, crystal violet, methyl violet, and basic fuchsin; with the acridine dyes such as acriflavine and proflavine; with Nile blue, malachite green, toluylene red, safranine, quinine, quinidine, cinchonine, cinchonidine, and hyamine 1622.

The reactivation of penicillin *in vivo* takes place not only with inorganic but also with organic salts. An intramuscular injection in a rabbit of 20,000 units/kg. of brilliant green penicillate produced a blood level of .16 units/cc. at 18 hours, and a similar injection of gentian violet penicillate, a blood level of .04 units at 18 hours.

A more detailed report will appear elsewhere.

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Inhibition of the Enzymatic Hydrolysis of ATP by Certain Cardiac Drugs[1]

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Although many investigations on the action of cardiac drugs have been carried out, only a few studies have dealt with their influence on the enzymatic reactions of heart muscle. Recently, however, Guerra, *et al.* (*2*) have reported that $1:10^6$ ouabain increased the liberation of inorganic phosphorus from adenosine triphosphate (ATP) as catalyzed by a cardiac muscle myosin preparation.

As part of a systematic investigation of the action of certain glycosides on enzyme systems we were interested in the effect of these drugs on the energy-yielding enzymatic reactions in connection with the therapeutic and toxic actions of these substances. The effect of digitoxin and ouabain *in vitro* on the ATP-ase activity of cardiac muscle was, therefore, studied, and the present preliminary report indicates that both of these drugs affect this enzymatic reaction.

ATP-ase activity was measured by the method of DuBois and Potter (*1*) using a Klett-Summerson colorimeter for phosphorus measurements. Normal Sprague-Dawley rats averaging 200 grams were employed. Aqueous solutions of ouabain were added to give a final concentration of 6×10^{-5} M, and 10 per cent alcoholic solutions of digitoxin were added in amounts sufficient to give a final concentration of 4.7×10^{-6} M.

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