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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In order to ascertain whether the action of the drugs was due to an action on the enzyme or on calcium, the activator for this enzyme, the effect of digitoxin was studied in the presence of suboptimal quantities and an excess of calcium. Table 1 shows the effect of varying amounts of calcium on the ATP-ase activity of normal rat's heart. It may be seen that with quantities of calcium below 0.003 M the reaction rate was limited by the calcium concentration. The results presented in Table 2 indicate that a final concentration of 4.7×10^{-6} M digitoxin inhibited the ATP-ase activity of cardiac muscle, the decrease in ATP-ase units being nearly the same, regardless of the calcium concentration. The per cent inhibition decreased with increasing calcium concentrations, since calcium increased both the control and digitoxin-treated samples to the same extent.

TABLE 1

RELATIONSHIP OF CALCIUM CONCENTRATION TO PER CENT STIMULATION OF ATP-ASE ACTIVITY

Molar $CaCl_2$	ATP-ase units	Stimulation (%)
1. 0.0003	14.20	66
2. (None)	8.54	
1. 0.0006	14.43	78
2. (None)	8.09	
1. 0.003	21.32	222
2. (None)	6.62	

TABLE 2

RELATIONSHIP OF DIGITOXIN (4.7×10^{-6} M) TO PER CENT INHIBITION OF ATP-ASE ACTIVITY

Molar $CaCl_2$	ATP-ase units		Decrease in ATP-ase units	Inhibition (%)
	Control	Drug		
1. 0.0003	14.20	11.09	3.11	21.90
2. 0.0006	14.43	11.64	2.79	19.33
3. 0.003	21.32	18.61	2.71	12.71

Ouabain also inhibited the ATP-ase system. In the presence of 0.003 M calcium, ouabain (6×10^{-5} M) produced 13.8 per cent inhibition. A higher concentration of ouabain than digitoxin was, therefore, necessary to produce a similar inhibitory effect.

These experiments indicate that both digitoxin and ouabain inhibit the ATP-ase activity of normal rat cardiac muscle. The amount of inhibition was independent of the calcium concentration, indicating that the drugs did not act through interference with the metallic activator. The difference in the per cent inhibition with various amounts of calcium indicates that the drugs inhibited a dephosphorylation reaction not dependent upon calcium ions for activity. With a limiting amount of calcium, an excess of ATP-ase is present in the test system to react with the drug, and less inhibition would be expected than in the case where the ATP-ase is limiting the reaction rate. The similarity in the decrease of ATP-ase units, regardless of whether calcium or ATP-ase was limiting the reaction rate, indicates that the drugs were inhibiting a dephosphorylation reaction not catalyzed by ATP-ase.

Further studies are necessary on other phosphatases in order to elucidate this inhibitory action of cardiac drugs.

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Creatinuria in Diabetics and an Evaluation of Methods for Determining Total Creatinine

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Diabetics exhibit an above-normal blood sugar, and when the blood sugar rises above the renal threshold there occurs a spilling of the sugar into the urine. In this disease the muscle glucose and glycogen are low and manifest a low tissue carbohydrate metabolism. It is probable that other metabolites present in these tissues in excess of the reduced metabolic requirements will also be found spilled into the urine.

Creatine plays a role in cellular metabolism and proliferation (*2*) as well as in muscular contraction (*4*). A low muscle content of carbohydrate and creatine would account for a reduced metabolism and for the muscular fatigue and degeneration which follows the course of this disease.

Creatine found in the urine of a number of diabetic subjects of both sexes was determined by the method of Folin (*3*). In Table 1 appears a summary of the average creatine spilled during 24 hours. In this series the low excretion of creatine was found to be 176 mg./day, and the high was approximately 1,600 mg./day.

TABLE 1

Age group	No. of subjects	Average creatine (mg./day)
32–37	3	487
43–48	21	670
50–53	18	790
32–53	42	649

Albanese and Wangerin (*1*) reported that in the Folin total creatinine determinations there occurs a decomposition loss of creatinine equivalent to 8 per cent when samples are autoclaved 20 minutes and 9 per cent when they are autoclaved 40 minutes. In order to obtain a more accurate creatine estimation in urine, they proposed a modification which involves autoclaving the standard as well as the preformed creatinine urine.

It seems to us that the loss of creatinine upon autoclaving does not appear to be great in view of the admitted error of ± 10 per cent in the technique involving an optical colorimeter. It is essential to determine in practice whether the Albanese modification will account for the loss of creatinine by the Folin method. By comparing identical samples of the autoclaved urine with the autoclaved as well as nonautoclaved standards of creatinine and creatinine zinc chloride, we will obtain indications of the direction and magnitude of the error