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

in the Folin method. Any autoclaved urine sample should yield a lower creatinine level when compared with the nonautoclaved standard than when compared with the autoclaved standard.

One ml. of a 24-hr. specimen of urine was mixed with 20 ml. of a saturated picric acid and autoclaved for 20 minutes. The solution was then cooled, and 1.5 ml. of a 10 per cent NaOH solution was added. The mixture was allowed to stand 10 minutes, after which it was diluted to volume. The creatinine standards were dissolved in dilute HCl. One creatinine standard was autoclaved with picric acid; the other was not. All solutions were compared and read in a photoelectric colorimeter. A few samples of urine and creatinine standards were autoclaved 40 minutes. The results of the total creatinine found in the various urines are given in Table 2.

TABLE 2

TOTAL CREATININE DETERMINATIONS BY METHOD OF ALBANESE

Urine sample	Calculated from autoclaved (20 min.)		Calculated from nonautoclaved			
	Creatinine standard (mg./ml.)	Creatinine zinc chloride standard (mg./ml.)	Creatinine standard (mg./ml.)	Deviation (%)	Creatinine zinc chloride standard (mg./ml.)	Deviation (%)
(Autoclaved 20 min.)						
H 83	1.36	1.36	1.37	+0.7	1.33	−2.2
C 51	1.00	0.99	1.00	0.0	1.02	+3.0
K 13	0.88	0.88	0.89	+1.1	0.88	0.0
C 64	1.27	1.26	1.28	+0.8	1.33	+5.5
A 2	0.54	0.54	0.55	+1.8	0.55	+1.8
A 7	1.75	1.74	1.76	+0.6	1.77	+1.7
A 1	1.23	1.23	1.20	−2.4	1.20	−2.4
B 2	1.91	1.93	1.88	−1.5	1.87	−3.1
B 3	0.92	0.93	0.91	−1.1	0.91	−2.1
B 9	1.80	1.81	1.76	−2.2	1.76	−2.8
B 10	1.47	1.48	1.52	+3.4	1.52	+2.7
K 88	1.56	1.56	1.60	+2.5	1.60	+2.5
(Autoclaved 40 min.)	Standards autoclaved 40 min.					
A 1	1.17	1.17	1.18	+0.9	1.18	+0.9
B 2	1.83	1.83	1.85	+1.1	1.85	+1.1
B 3	0.89	0.89	0.90	+1.1	0.90	+1.1
8	1.31	1.31	1.30	−0.8	1.30	−0.8

The deviation in results obtained with a nonautoclaved creatinine standard as compared with an autoclaved standard varied from −2.4 to +3.4 per cent, and more than 50 per cent of the determinations were higher when compared with the nonautoclaved standards than when compared with the autoclaved standards.

Summary: Creatinuria was found in all 42 diabetic clinic subjects of both sexes taken at random.

Total creatinine determined in the urines of diabetics by the Albanese modification showed a variable deviation (in magnitude and direction) when compared with total creatinine obtained by the Folin method.

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Interpretation of Lignin: The Synthesis of Gymnosperm Lignin

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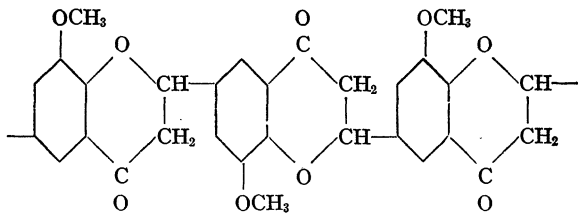
The existence of at least two varieties of lignin appears to be definitely established. One is associated with gymnosperms and gives, as significant fission products, only derivatives of catechol monomethyl ether (guaiacol). The other is associated with angiosperms and gives, as significant cleavage products, the same derivatives of catechol monomethyl ether and also some derivatives of pyrogallol-1,3-dimethyl ether. The possible occurrence of varieties of lignin giving other fission products is not excluded.

Examination of the analytical evidence accumulated during the past 70 years, excludes the improbable elaborate formulas previously proposed and leads to the conclusion that the lignin from gymnosperms is a polymeric 8-methoxy-dihydrobenzopyrone having the structure:



(I)

The lignin from angiosperms is likely constituted in a similar way but having pyrogallol-1,3-dimethyl ether nuclei terminally or otherwise attached or introduced. Such nuclei obviously could not replace directly the catechol monomethyl ether nuclei in (I) unless the migration of a methyl group occurs during degradation.

The material (I) is a cyclicized condensation (aldolization, loss of water—Claisen condensation) polymer of 2-hydroxy-3-methoxy-5-formylacetophenone (III) and is at once available by Fries rearrangement of vanillin monoacetate (II) through the steps (II) (III) (IV) (I).

This synthesis has been accomplished and the amorphous synthetic product, although somewhat darker in color, is qualitatively indistinguishable from a specimen of the lignin from gymnosperms. It has to give the same fission products, and its solubility characteristics and general behavior are the same. Moreover, absorption curves for the synthetic and the natural product are in agreement, and, so far as such comparisons are valid for amorphous materials, quantitative analytical measurements of the synthetic material and its derivatives are in harmony with calculated values and with the reported values for the lignin from gymnosperms.

For comparison purposes an exactly similar synthesis was carried out starting with p-acetoxybenzaldehyde. The product is analogous to that obtained from vanillin monoacetate.

It is to be noted that, in the above synthetic work, use has

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