TABLE II. Influence of Different Ions on the Action of Cobra Venom and Cardiotoxin on Isolated Toad's Heart.

Substances in the perfusion fluid	Condition of heart on perfusion after washing—with the solution	with Ringer
a. Perfused with KCl	Stops in diastole	—
b. The same heart is then perfused with KCl and cobra venom	Heart revives and then stops in systole	Remained so
a. Perfused with NaCl, KCl	Heart stops in diastole	
b. The same heart is then perfused with NaCl, KCl and cobra venom	Heart revives and then stops in systole	,, ,,
a. Perfused with KCl	Stops in diastole	
b. The same heart is then perfused with KCl and cardiotoxin	Heart revives and then stops in systole	,, ,,
Cobra venom (alone)	Stops in systole	,, ,,
Cardiotoxin (alone)	,,	,, ,,

4-5 days at −20°C in the same solution. It seemed of interest to investigate the possible effect of cobra venom on the recombination of actin and myosin in pyrophosphate treated glycerol-fibres(9); such fibres after treatment with cobra venom did not show any contraction on the addition of ATP over 0.26 M KCl. If association has been effected by cobra venom, one should expect contraction up to the concentration of .45 M KCl like glycerinated muscle fibres do.

Threads made of actomyosin prepared according to Weber(10) show no contraction on addition of cobra venom or cardiotoxin. Actin and myosin prepared according to the method of Straub(11) and Szent-Györgyi(12) still show superprecipitation on the addition of ATP and respective co-partner after treatment with cobra venom or cardiotoxin, indicating that these two muscle proteins are not affected directly by these compounds.

These results suggest that the pharmacological action of snake venoms requires the presence of the cell membrane, without which there will be hardly any contraction.

Summary. Cobra venom, cardiotoxin, saponin and drugs of the digitalis series bring about the stoppage of the movements of the excised heart. The cardiac arrest caused by cobra venom, unlike that by saponin and digitalis, is irreversible, *i.e.*, cannot be washed out. Unlike living muscle fibres, glycerinated muscle fibres and actomyosin threads show no contraction under the influence of cobra venom or cardiac drugs. These drugs have no effect on myosin and actin either.

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Specific Determination of Serum Creatinine.* (19107)

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The estimation of serum creatinine by Jaffe's alkaline picrate reaction does not differentiate between creatinine and non-creatinine chromogen. This has led many workers

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to seek improved methods. Miller and Dubos developed an enzymatic method(1), based on the isolation of a soil microorganism capable of decomposing creatinine with a high degree of specificity(2). The difference between the Jaffe-reactive chromogen values before and after treatment with a suspension of the organisms represents true creatinine.

Borsook described a method for the determination of creatinine in tissues which involved separation of creatinine from other chromogenic compounds by adsorption on Lloyd's reagent(3). The results reported for tissues containing very high non-creatinine chromogen compared favorably with those obtained later by Baker and Miller, who employed the specific enzymatic method(4). Recently Hare adapted Borsook's method to the determination of creatinine in serum(5). A high degree of specificity was claimed, but this was not established by comparison with a specific method. To assess the specificity of the adsorption method, we have compared the enzymatic and adsorption methods on normal and uremic human serum.

Methods. We have obtained more consistent results with tungstic acid filtrates than with CCl_3COOH filtrates, such as Hare and Borsook employed. In all our determinations protein was precipitated by adding 3 ml of H_2O, 0.5 ml of 10% sodium tungstate, and 0.5 ml of 2/3 N H_2SO_4 for each ml of serum or plasma, according to Folin and Wu(6). The mixture was centrifuged for 10 minutes after standing at room temperature for 5 minutes with occasional shaking, and the supernatant was filtered through Whatman No. 42 paper.

Determination of creatinine by the adsorption method. Duplicate aliquots of this 1:5 filtrate, diluted, if necessary, to contain 5 to 15 μg of creatinine, are pipetted into conical centrifuge tubes and diluted to 4 ml. For normal sera, 4 ml of filtrate is a satisfactory volume. Glass-stoppered centrifuge tubes are preferable, but ordinary centrifuge tubes, sealed with Parafilm may be used. A 4 ml water blank and a creatinine standard containing 10 μg in 4 ml are set up in duplicate. Four-tenths ml of saturated oxalic acid and 30 to 40 mg Lloyd's reagent are added to each tube. The stoppered tubes are shaken vigorously 4 or 5 times during a period of 10 minutes and then centrifuged for 10 minutes. The clear supernatant fluid is aspirated carefully and discarded. In contrast to the determination in tissue, it is unnecessary to wash the Lloyd's reagent at this stage.

Creatinine is eluted by the addition of exactly 8 ml of alkaline picrate, prepared by mixing 6 parts of H_2O, 2 parts of approximately 0.04 M picric acid,§ and 2 parts of exactly 0.75 N NaOH. The Lloyd's reagent is dislodged from the bottom of the centrifuge tube and suspended in the alkaline picrate by vigorous shaking. The tubes are stoppered and allowed to stand for 10 minutes, during which they are shaken 4 or 5 times. They are again centrifuged for 10 minutes. The color intensity is determined in the Evelyn photoelectric colorimeter on 6 ml aliquots of clear supernatant, employing the 520 filter and the 6 ml aperture. Standards and unknowns are read against a reagent blank tube, set at 100% transmission.

Determination of specific creatinine by the enzymatic method. Total initial chromogen and chromogen remaining after treatment with bacterial enzyme suspension(7) are estimated by the Bonsnes and Taussky method(8) on 3 ml of a 1:5 tungstate filtrate containing 5 to 15 μg of creatinine. Specific creatinine represents the difference between these two

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§ This is prepared from picric acid containing approximately 10% water by dissolving 10.1 g in water and diluting to 1 liter.

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TABLE I. Comparison of Creatinine Values Obtained by the Enzymatic and Adsorption Methods.

Serum creatinine, mg %		
Apparent	Enzymatic	Adsorption
.67	.50	.51
.68	.49	.46
.74	.58	.49
.78	.56	.63
.85	.66	.67
.88	.71	.66
.90	.77	.68
.90	.78	.80
.94	.76	.72
.98	.76	.71
.99	.80	.76
.99	.84	.82
1.01	.75	.82
1.17	.95	.93
1.17	1.04	.95
1.19	.99	.99
1.21	1.05	.94
1.68	1.45	1.49
2.90	2.57	2.78
3.02	2.59	2.80
3.45	3.16	3.22
3.80	3.41	3.78
4.10	3.84	3.86
5.83	5.18	5.45
7.16	6.70	6.40
10	9.48	9.70
11.4	10.8	11
12.6	11.5	11.7
13.6	12.6	13.2
18.7	17.9	18

values.

Results. The results obtained with 30 serum samples are shown in Table I. There is good agreement between the true creatinine values obtained by the enzymatic and the adsorption methods. To demonstrate further that Lloyd's reagent adsorbs creatinine chromogen specifically, a sample of blood fairly high in residual chromogen was incubated with the bacterial enzyme until all the creatinine had been decomposed. The bacteria were centrifuged off and the supernatant fluid was treated with Lloyd's reagent in the usual manner. No non-creatinine chromogen was adsorbed.

A report has appeared from Newburgh's laboratory which indicates that the use of 1:4 tungstate filtrates of plasma or serum give considerably lower creatinine values than 1:10 filtrates(9). Even though our experiments were performed with 1:5 tungstate filtrates, it seemed desirable to check this finding. We determined the creatinine content of 6 normal human sera in 1:5 and 1:10 tungstate filtrates. The values for the 1:10 filtrate averaged 7% higher than the 1:5 filtrate (range, 3 to 13%). This is considerably less than the 21% average difference observed by Camara *et al.*(9), but still represents a significant discrepancy. We, therefore, recommend the use of 8 ml of 1:10 tungstate filtrate instead of 4 ml of 1:5 filtrate in the adsorption procedure. The volume of the blank tubes and standards should be adjusted accordingly. Otherwise, the details of the method are unchanged.

Conclusions. Serum creatinine can be determined specifically on tungstate filtrates by using either the Miller-Dubos creatinine-splitting enzyme, or by adsorption on Lloyd's reagent and subsequent elution by alkaline picrate.

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Comparative Effects of Cortisone and 11-Deoxycorticosterone on Tissue Arginase Levels of Adrenalectomized Lactating Rats. (19108)

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Arginase levels of rat liver(1-3), kidney (2,3) and mammary gland(2) are decreased

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