obviously could yield more important results than could *in vitro* tests. It may be mentioned that diphtheric toxin is devoid of hemotoxin, while tetanal toxin as usually produced (by incubation of the culture for about 2 weeks) is also devoid of hemotoxin but contains considerable hemotoxoid.

It will be noted (Tables I and II) that sulfanilamide-therapy had absolutely no effect on the survival of guinea pigs receiving diphtheric toxin, and very little effect on those receiving tetanal toxin. It seems probable that in the experiments with tetanal toxin the slight differences in favor of the guinea pigs receiving sulfanilamide may be explained by chance variation.

Conclusion. Sulfanilamide in concentrations of 1-1000 or less does not inactivate *in vitro* significant amounts of the hemotoxins of the *beta* hemolytic *streptococcus,* hemolytic *staphylococcus aureus, Cl.oedematis-maligni, Cl. tetani,* or *B. perfringens.* Sulfanilamide-therapy did not significantly affect the course of intoxication in guinea pigs initiated with as little as 1 MLD of diphtheric or tetanal toxin.

10084

Preparation of Extracts of the Renal Pressor Substance.*

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During recent years, evidence has accumulated which suggests that elevation of blood pressure may be produced by some substance or substances which occur in renal tissue. In the course of investigations on the relationship of certain types of experimental hypertension to this renal pressor substance, one difficulty frequently encountered has been that of obtaining uniform and potent renal extracts. An attempt was made to determine the factors which affect the yield of this pressor substance, and to develop a standardized method of preparation.

Fresh kidneys of hogs were obtained from the slaughter house. The kidney cortex was dissected with scissors from the medulla, and the cortical tissue passed through a meat grinder. The minced kidney cortex was ground to a fine paste with carborundum. The possibility that carborundum might adsorb some of the pressor substance was

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excluded by comparing yields from fractions ground with large amounts, small amounts, and none of this substance. Comparisons of fractions ground for different periods of time were also made. The observations showed that a better yield was obtained when the tissue was macerated in a mortar with carborundum, and that the amount of abrasive and the duration of grinding did not influence the yield appreciably, provided that the tissue was ground to a uniform paste.

Ninety-five percent alcohol was added to the paste in an amount equal to 9 cc per gram of original kidney cortex. The time required to obtain maximum precipitation of the pressor substance was investigated. It was found that when the alcohol was separated within a few minutes, the residue was less active than that obtained from a kidney-alcohol mixture which had been allowed to stand in the ice-box for 24 hours. Longer periods of standing—up to 10 days—decreased the activity of the final product. The temperature at which the mixture was kept was found to be of importance; residues from mixtures kept in an incubator at 37°C were less active than

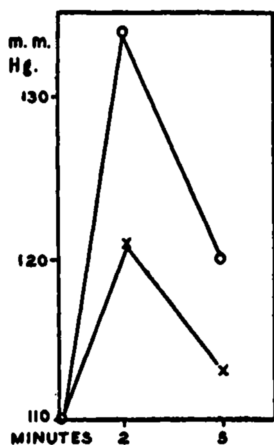

FIG. 1.

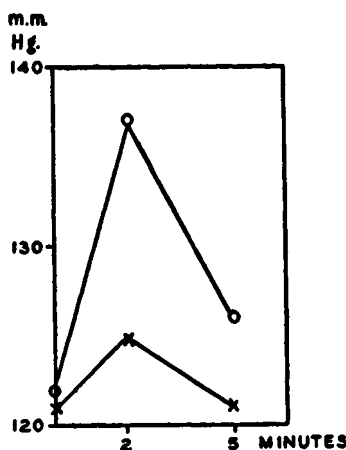

FIG. 2.

Effect of temperature on potency of extract (average of 8 comparisons).
o: Alcohol-kidney mixture separated at 10° C.
x: Mixture warmed before separation.

FIG. 2.

Effect of drying on potency of extract (average of 7 comparisons).
o: Alcohol-insoluble residue dried to powdery state.
x: Residue dried to paste.

those from similar material kept in the refrigerator at approximately 10°C. Centrifuging the mixture as soon as it was taken from the refrigerator, and as rapidly as possible, resulted in preparations which were somewhat more active than those obtained from material which was allowed to stand at room temperature before separation. (Fig. 1.)

After the alcohol-soluble fraction had been decanted and discarded, the alcohol-insoluble residue was washed once with 2 to 4 volumes of ether. The mixture was centrifuged again, the ether discarded, and the sediment spread out on flat plates and dried in a current of air at room temperature. Material desiccated to a powdery state yielded more potent extracts than material which had been dried only to a paste which was free of alcohol and ether. (Fig. 2.) The ether washing resulted in more rapid drying and removal of a lipoidal cloud from the final preparation, without causing any diminution in potency.

The dried powder was triturated with various aqueous solutions. It was found that somewhat better yields of pressor substances were obtained when 0.5% sodium bicarbonate was used than when water or slightly acid sodutions were employed, and that the extract was most potent when the sodium bicarbonate solution was warmed to 40 to 45°C. The amount of solution added to the dry powder was equal to 1 cc per gram of original cortical tissue. After centrifuging, the residue was twice reëxtracted with one-half this volume of warm sodium bicarbonate solution, and the 3 fractions of supernatant fluid obtained by the serial extraction combined.

Attempts were made to further purify the preparations by reprecipitation of the alcohol-insoluble pressor substance. Nine volumes of alcohol were added to one volume of the aqueous extract. The precipitate which formed in this mixture, after it had stood in the refrigerator for 24 hours, was then separated and treated as described above. "Refined" extracts of this type did not differ in their physical and pharmacological characteristics from the original extracts. It was found, however, that quantitative recovery of the pressor substance could not be even approximated (Fig. 3), and this attempt at refinement of the preparation was therefore abandoned. Attempts at isolation of the active pressor principle by salting out with various concentrations of ammonium sulphate, and subsequent dialysis, were also unsuccessful.

The final extract, prepared as described above, is a slightly turbid solution, which has a pronounced and prolonged pressor effect when injected into the experimental animal. The white rat has been found

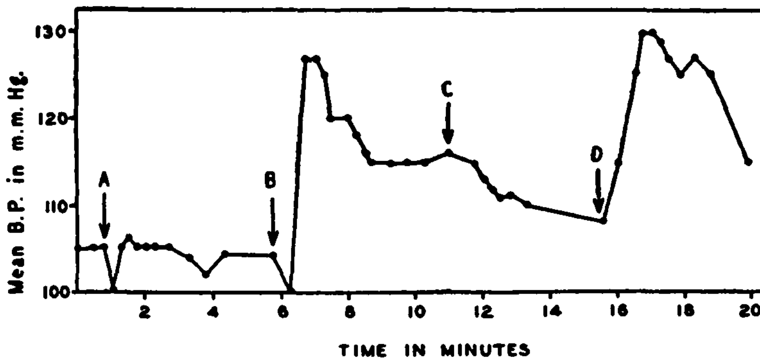

FIG. 3.
Loss of potency of extract on reprecipitation. Heat inactivation of renin.
A: Injection of extract from 0.25 g kidney, "refined" by 2 precipitations from alcohol, then heated at 60°C for 10 minutes.
B: Same as A, but extract not heated.
C: Extract from 0.12 g kidney, not reprecipitated, heated at 60°C for 10 minutes.
D: Same as C, but not heated.

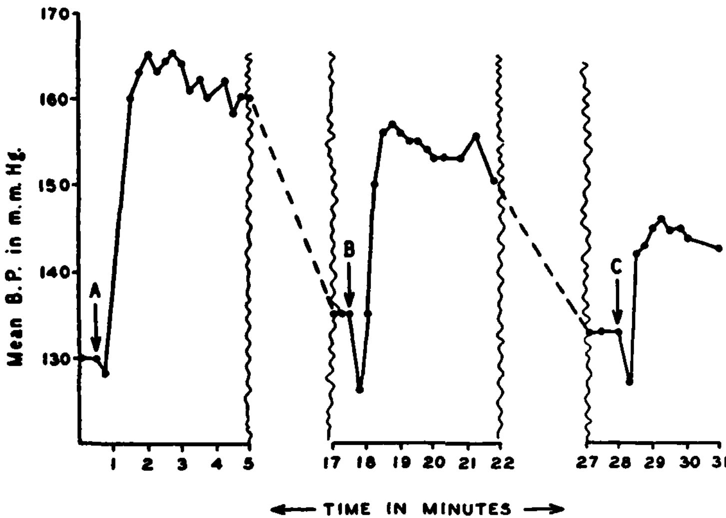

FIG. 4.
Diminishing effect of successive injections of renin. Influence of dialysis on potency of extracts. At points A and C, dialyzed extracts representing 0.25 g kidney injected; at B, equivalent amount of extract which had not been dialyzed.

to be a very satisfactory test animal. Repeated injections into the same animal result in progressively diminishing responses. (Fig. 4.) The pressor substance is heat labile, and is destroyed by exposure to a temperature of 60°C for 10 minutes. (Fig. 3.) It is not dialyzable through Visking membranes. (Fig. 4.) Its effect is not abolished by cocainization of the test animal. Application of the

same extractive technic to other organs (spleen, liver) does not yield a preparation of similar pharmacological properties.

Summary. Some technical factors which are of importance in the preparation of extracts of the renal pressor substance (the "renin" of Tigerstedt and Bergmann) have been investigated. A method which has been found to be satisfactory for the preparation of potent extracts of this substance is described in detail.

10085 P

Metabolism of Nitrogen, Calcium, Magnesium and Phosphorus in Thymectomized Rats.

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In the course of our work on the influence of the glands of internal secretion on mineral metabolism in albino rats, nitrogen, calcium, magnesium and phosphorus metabolism in thymectomized rats was studied.

Twenty male and 22 female albino rats were used, 6 animals of each sex being kept as paired feeding controls. They were thymectomized at 7 weeks of age. Details regarding procedure and meth-

TABLE I.

	Nitrogen Metabolism in Thymectomized Rats				Paired Feeding Controls			
	% of Intake				% of Intake			
Date	Total N Urine	Excretion Feces	Intake mg	% of Intake Retent.	Total N Urine	Excretion Feces	Intake mg	% of Intake Retent.
Control Period:								
Feb. 25-Mar. 2	11	10	294	79	15	10	290	75
Mar. 3-9	15	12	265	73	17	11	268	71
*								
Mar. 14-18	28	21	291	51	23	18	290	59
Mar. 19-23	25	24	287	51	18	15	290	67
Mar. 24-30	29	20	297	51	18	19	302	63
Mar. 31-Apr. 6	27	19	297	54	21	20	300	59
Apr. 7-13	33	24	320	43	20	19	318	61
Apr. 14-20	39	23	313	38	22	20	315	58
Apr. 21-27	37	23	310	40	18	19	308	63
Apr. 28-May 4	38	20	310	42	25	21	311	54
May 5-11	38	21	310	41	24	17	313	59
May 12-18	40	22	342	38	25	16	340	59

*Thymectomized on March 11th.