and 30 minute periods following the injection of 2.26 g of sodium hippurate.* Typical results are recorded in Table I.

It will be noted that the excretion of hippuric acid in normal men is very rapid. The average output of 15 subjects was 0.88 g in 15 minutes which is 44% of the amount injected. On this basis, an adult can excrete at least 3.5 g of hippuric acid in an hour. Since the average hour output of hippuric acid in a similar group of subjects injected with 1.77 g sodium benzoate was found to be 1.25 g of hippuric acid, one can conclude that the excretory capacity of the kidneys is over 2.5 times as great as the speed of synthesis.

In patients with various types of kidney pathology the excretion of hippuric acid is often definitely diminished. Curiously, the output is frequently considerably higher for the second 15-minute period than for the first, which is difficult to explain by any of the current theories of kidney function. Since the excretion of hippuric acid is diminished in certain renal disorders, it is to be recommended that when liver function is determined by the hippuric acid test in patients with suspected concomitant kidney dysfunction, the excretion rate of hippuric acid should also be estimated.

Summary. The excretion of hippuric acid in normal adults following intravenous injection is exceedingly high: about 44% is eliminated in 15 minutes when 2.26 g of sodium hippurate are administered. In various renal disorders the speed of excretion is diminished.

13785

Restoration of Blood Pressure by Renin Activator After Hemorrhage.†

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That a renin-like substance may be found in the blood stream after hemorrhage was first indicated by Sapirstein, Ogden and Southard,[1] who pointed out that a homeostatic mechanism for the regulation of blood pressure might be involved. Independently, similar

* Ampules of sodium hippurate were kindly furnished by George A. Breon, Inc., Kansas City, Mo.

† Aided by a grant from the Board of Research of the University of California.

1 Sapirstein, L. A., Ogden, E., and Southard, F. D., Jr., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 505.

conclusions were drawn by Hamilton and Collins,[2] and more recently by Braun-Menendez,[3] in both cases from experiments which clearly demonstrated the renal origin of the substance in question and showed, moreover, that it appeared also in shock and other hypotensive states.

The fact that the renin-like substance is actually renin is indicated first, by its renal origin, and secondly, by the fact that the assay methods of the different groups of workers cited are based on different pharmacological properties characteristic of renin, Braun-Menendez making use of its pressor properties (with added renin activator) and Sapirstein, Ogden and Southard using the effect on the guinea pig ileum with and without added activator.

It appears then that renin is secreted in hemorrhage and perhaps in any acute hypotensive condition.

The fact that the injection of renin into a normal animal will produce tachyphylaxis or exhaustion of activator raised the question whether in prolonged hypotension an animal might, as a result of continued secretion of renin, eventually become tachyphylactic to its own renin; *i. e.*, the substrate (renin activator) on which the enzyme (renin) acted to produce the actual vasoconstrictor (angiotonin) might become exhausted. An experimental basis for this hypothesis is the observation (Ogden, Hildebrand and Page[4]) that in acute renal ischemia the animal becomes progressively less sensitive to renin. This may be explained by the consumption of renin activator by the animal's own renin.

If the organism, by the continued release of renin, exhausts its supply of renin activator in hypotensive states (hemorrhage or shock) then the addition of renin activator to the blood stream of such an animal should exert a pressor effect similar in character to that produced in the normal animal by the injection of renin. The experiments here reported indicate that this is indeed the case.

Preparation of Renin Activator. The substance hereafter referred to in this discussion as renin activator is the serum globulin fraction of ox blood precipitated by bringing to .4 saturation with ammonium sulfate. This is stated to contain renin activator by Page[5] and also by Braun-Menendez.[6]

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[2] Hamilton, Angie S., and Collins, Dean A., *Am. J. Med. Sci.*, 1941, **202**, 914.

[3] Braun-Menendez, E., personal communication to author.

[4] Ogden, E., Page, E. W., and Hildebrand, G. J., *Proc. Federation Am. Soc. for Exp. Biol.*, 1942, **1**, 63.

[5] Page, I. H., McSwain, B., Knapp, G. M., and Andrus, W. D., *Am. J. Physiol.*, 1941, **135**, 214.

[6] Braun-Menendez, E., Fasciolo, J. C., Leloir, L. F., and Munoz, J. M., *J. Physiol.*, 1940, **98**, 283.

Fresh beef blood was allowed to clot and the serum removed. The serum was brought to .4 saturation with saturated ammonium sulfate and then supercentrifuged. The precipitate was dissolved in a small amount of distilled water, dialyzed against tap water for 12 hours, made up to 1/10 the original volume of serum used and brought into solution by the addition of 1 g of sodium chloride per 100 cc. The protein concentration of the preparations used ranged from 8-11%. The non-protein nitrogen was negligible.

Experimental. Ten dogs were used in these experiments, 7 under nembutal, 2 under ether and one under chloretone. A mercury float manometer recorded the carotid blood pressure.

When the normal blood pressure had been recorded, the dog was bled through the femoral artery, 14-21 cc of blood being removed per kilo body weight. The bleeding was stopped and the recording continued until the blood pressure began to show a definite rise. Another 14 cc per kilo was then removed, the process being repeated until the pressure either failed to show recovery or began to fall after a bleeding.

An injection of 10-25 ml of 10% gelatin was then made to control any osmotic or volume effects which might occur incidentally with the subsequent injection of the renin-activator preparation.

The injections of gelatin occasionally produced a slight rise in blood pressure, but this never exceeded 10 mm Hg and was of very brief duration.

After the effect of the gelatin had disappeared, an equal volume of the renin activator preparation described above was injected, a new preparation being used for each dog.

The injection of this preparation, in sharp contrast to the control gelatin solution, produced a very marked rise in blood pressure ranging from 15-80 mm of Hg, which persisted for 20-120 minutes and was succeeded by a gradual fall in pressure (Fig. 1). Further injection of the renin activator preparation during the period of restored blood pressure produced no pressor effect, but after the pressure had fallen to lower levels, whether spontaneously or as the result of further bleeding, renewed injection again restored the blood pressure toward its normal level.

Since renin activator disappears from the blood with time (Page[7]) one renin activator preparation was allowed to stand two weeks before its effect was determined, and another was prepared from serum a week old. Neither of these preparations had any pressor effect; no recovery of blood pressure occurred, and the animals died soon after

[7] Kohlstaedt, K. G., Page, I. H., and Helmer, O. M., *Am. Heart J.*, 1940, **19**, 42.

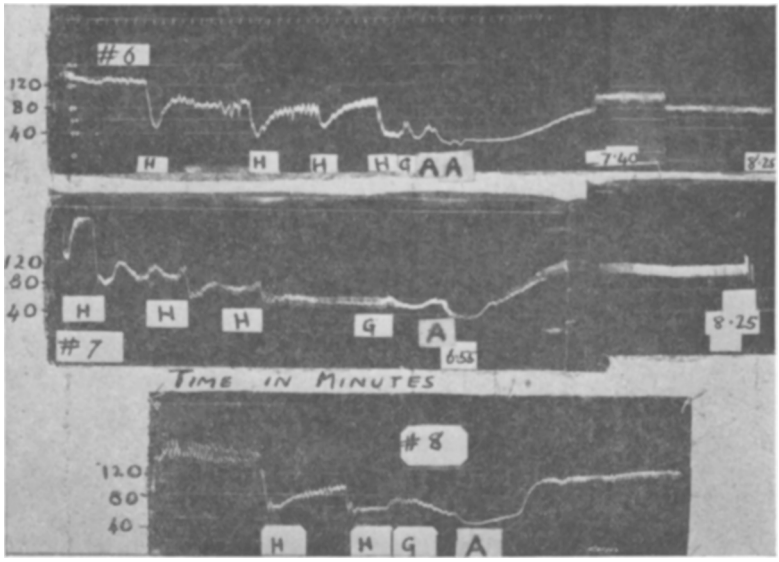

FIG. 1.
The figure shows records of 3 experiments in which successive hemorrhages (H) were followed by injections of gelatin (G), and activator extract (A). For quantities and detailed procedure, see text.

injection. The failure of these two preparations to restore the blood pressure affords another control on the injection of fresh fractions and adds further evidence that our positive findings are, in fact, due to renin activator.

It may be seen in the figure that there was a slight initial drop in blood pressure after the injection of renin activator before the pressor effect appeared. This phenomenon occurred also with the two control preparations just mentioned, and is probably ascribable to foreign-protein effects from ox globulin other than renin activator contained in the preparation.

The pressor effect of the renin activator preparation used in these experiments may be attributed to the fact that the exhausted ingredient of the homeostatic renin-renin activator system had been replenished. In support of this conclusion may be cited the fact that the preparations used had no pressor effect on normal dogs, or dogs whose blood pressure had already been elevated by previous injections of renin activator. In these cases the reno-pressor system was already functioning adequately.

Conclusions. Injection of a renin-activator preparation of ox-plasma restored the blood pressure of dogs after hemorrhage. This restoration was observed with quantities of the preparation containing

only one-tenth or less of the total plasma protein removed.

Corresponding quantities of 10% gelatin or of control plasma concentrations failed to restore the blood pressure.

From these observations it is concluded that the secretion of renin in severe hemorrhage (and perhaps in other forms of shock) is sufficient to produce exhaustion of renin activator. The resulting failure of the reno-pressor system is followed by a fatal collapse of blood pressure. This collapse may be staved off and the blood pressure restored by replacing the exhausted activator by a suitable preparation of ox plasma.

These investigations suggest the possibility of improving the transfusion therapy of hemorrhage and shock by fortifying the plasma with suitable preparations of renin-activator, or possibly by substituting relatively small quantities of activator preparations for plasma in emergency treatment. Work in this direction is in progress.

13786 P

Failure of Heparin to Inhibit Coagulation of Citrated Blood and Plasma in Presence of Staphylococci.*

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Experimental and clinical observations have shown that heparin inhibits the clotting of blood and the formation of thrombi. Recently we have studied the effect of heparin on the coagulation of citrated blood and plasma by staphylococci.[1] Some strains of staphylococci will coagulate human plasma.[2, 3] One, 0.5 and 0.1 cc of heparin† (Liquamine 1.0 cc = 10 mg) was added to a series of tubes containing 3.0 cc of both rabbit and human plasma and sterile saline (3 parts of plasma and one of saline). One-tenth of a cubic centimeter of a broth culture of staphylococci was added to each of these tubes. The medium in each of the tubes was completely coagu-

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† The heparin was supplied by the Roche-Organon, Inc., of Nutley, New Jersey.

1 Rigdon, R. H., and Haynes, Anne, to be published.

2 Loeb, L., *J. Med. Res.*, 1903, **10**, 407.

3 Chapman, George H., Conrad, Burns, and Merrit, H. Stiles, *J. Bact.*, 1941, **41**, 431.