α,β,γ-Triphenylbutyric Acid (III).—During the catalytic hydrogenation of II in dioxane, it was necessary to add fresh catalyst several times.[8] Apparently the hydrogenation product, like other known phenylbutyric acids, was intensly adsorbed at the active surface, thereby hindering the attack on new unsaturated molecules. After evaporation of the dioxane, there was left a crystalline mass. Recrystallization from benzene–ligroin yielded the acid III in beautiful needles, m. p. 158°. The methyl ester, prepared by means of diazomethane, melted at 158° (isopropanol).

Anal. Calcd. for $C_{23}H_{22}O_2$: OCH_3, 9.4. Found: OCH_3, 9.5.

III gave no reaction with concd. sulfuric acid. When III was dissolved in boiling sodium carbonate solution, its sodium salt crystallized on cooling. It is easily soluble in ethanol, and can be recrystallized from isopropanol as long, fine needles, m. p. 278–280°. Twenty-one grams of III and phosphorus pentachloride (15 g.) in benzene (175 cc.) were heated on a steam-bath for four hours. The solvent and phosphorus oxychloride were removed *in vacuo*, whereafter the acid chloride remained as a yellow solid. On trituration with concd. ammonia, the α,β,γ-triphenylbutyramide was formed; from ethanol as fine needles, m. p. 168–169°.

Anal. Calcd. for $C_{22}H_{21}ON$: N, 4.4. Found: N, 4.7.

2,3-Diphenyl-1-keto-1,2,3,4-tetrahydronaphthalene (IV).—The above acid chloride was dissolved in benzene, and after addition of aluminum chloride (11 g.) stirred for four hours at 0°, and eight hours at room temperature. The mixture was then decomposed with ice and hydrochloric acid, and the solvent removed by steam. The crude ketone was dissolved in carbon tetrachloride and washed with sodium carbonate solution. About one-half of the tetralone IV crystallized directly, the second part crystallized only after distillation, b. p. 205–207° (0.02 mm.) and trituration with ligroin; m. p. 146–147°; yield, 13.5 g.

(8) Hydrogenation of 50 g. of II required about nine hours.

1,2,3-Triphenyl-3,4-dihydronaphthalene (V).—To a Grignard solution, prepared from bromobenzene (4 g.) and magnesium (0.6 g.) the above tetralone (4 g.) was added in benzene solution. No reaction occurred. The solvent was then distilled off and the residue heated to 100° for twelve hours. After decomposition with ice and sulfuric acid, carbon tetrachloride was added in order to dissolve the sirupy organic material. The crude carbinol (3.5 g., 70%) could not be induced to crystallize, apparently because it presented a mixture of stereoisomers. It was therefore directly dehydrated with potassium bisulfate (6 g.) at 160° for two hours, and the reaction product again dissolved in carbon tetrachloride. The main bulk of V (2 g.) crystallized immediately after evaporation of the solvent, on trituration with acetone and methanol. An additional crop (0.5 g.) was obtained by distillation of the filtrate, b. p. 215° (0.5 mm.). Recrystallization from high-boiling petroleum ether gave plates of m. p. 176°; yield, 2.5 g., 52%.

Anal. Calcd. for $C_{28}H_{22}$: C, 93.9; H, 6.1. Found: C, 93.6; H, 6.1.

1,2,3-Triphenylnaphthalene (VI).—0.5 gram of the foregoing dihydronaphthalene derivative was heated with an equal weight of selenium to 280–300° for eighteen hours. The hard material was pulverized and extracted several times with boiling acetone. The residue of this solution crystallized directly from ligroin as yellowish needles, m. p. 153–154°; yield, 0.35 g., 70%. No picrate could be obtained in alcohol-benzene solution.

Summary

Reaction of 1,2,3-triphenylallylsodium with carbon dioxide opens a new route to α,β,γ-triphenylbutyric acid, and therefore to 2,3-diphenyl-α-tetralone and its derivatives. The synthesis of 1,2,3-triphenylnaphthalene by this method is described.

REHOVOTH, PALESTINE RECEIVED JULY 21, 1941

[CONTRIBUTION FROM THE MEDICAL CLINIC OF THE PETER BENT BRIGHAM HOSPITAL, AND THE DEPARTMENT OF MEDICINE, HARVARD MEDICAL SCHOOL]

Preparation and Properties of Renin[1]

BY OTTO SCHALES

Tigerstedt and Bergman[2] found that the intravenous injection of saline extracts made from fresh kidneys, causes a prolonged rise in blood pressure. They named the substance responsible for this effect *Renin* and concluded that it is a protein, soluble in water and dilute salt solutions, insoluble in alcohol and inactivated by heat. Bingel and Strauss[3] confirmed these results and purified crude kidney press juice to a certain extent by isolating the fraction soluble in $^1/_3$ saturated and precipitated by $^7/_{12}$ saturated ammonium sulfate solution. Hessel[4] gave a general outline of a further purification of renin, but has not published the detailed procedure.

(1) Presented before the Division of Biological Chemistry at the Atlantic City meeting of the American Chemical Society, September 11, 1941.

(2) R. Tigerstedt and P. G. Bergman, *Skand. Arch. Physiol.*, **8**, 223 (1898).

(3) A. Bingel and E. Strauss, *Deutsch. Arch, klin. Med.*, **96**, 476 (1909).

(4) G. Hessel, *Klin. Wchnschr.*, **17**, 843 (1938).

The work of Goldblatt[5] on the production of experimental hypertension in dogs by means of renal ischemia has stimulated interest in renin as the substance which might possibly cause hypertension of renal origin.

In this paper a new procedure for the preparation of renin is described. Summarized in Table I is a comparison between this method and the two most widely used procedures thus far published.[6,7]

TABLE I

SUMMARY OF RECENT METHODS FOR THE PREPARATION OF RENIN

Authors	No. of steps	Yield in R. U. per kg. cortex	Purity as μg N/R. U. (30 mm.)
Helmer and Page (1939)	8	1000	93 (120)*
	10	?	40
	11	?	27 (25)*
Collings a. o. (1940)	18	1900	8–37
		R. U. per kg. kidney	
Schales (1941)	4	6400–7500	72–93 (34–43 Prot. N.)
	6	4000–5000	24–36
	8	2500–3600	4–5

* In parentheses are results of this Laboratory using the method of Helmer and Page and confirming their results.

The yield is expressed in units which Schales and Haynes[8] define, one rabbit unit (R. U.) being the amount of renin (expressed as micrograms of nitrogen) that is required per kilogram of body weight to cause a rise in blood pressure of 30 mm., when injected intravenously in adult rabbits. It was found that a given amount of pig renin per kilogram of body weight causes the same rise in blood pressure in dogs as in rabbits. Therefore it is permissible to compare on a quantitative basis the results of this Laboratory (using rabbits) with those of Helmer and Page[6] and of Collings and coworkers,[7] who used dogs as test animals.

Experimental

Preparation of Kidney Powder.—Pig kidneys were passed through an electric meat grinder as soon as possible after the slaughtering of the animals and two liters of ice-cold acetone added to each kilogram of finely ground material. The mixture was put in the refrigerator and stirred occasionally. After about two hours, the acetone was removed by suction and the solids were again treated with the same amount of acetone. After a second two hours, the solids were collected on Büchner funnels and the acetone was sucked off as completely as possible. The residue was washed several times with cold acetone and then with cold ether and spread out in a thin layer to dry in air. The drying was completed in vacuum desiccators over calcium chloride. The dry material was ground into a fine powder until it passed a no. 35 sieve, so that the particle size was below 0.5 mm. No change in the activity of the powder was observed after storing it for eight months in vacuum in the icebox. From 1000 g. of pig kidneys an average of about 200 g. of dry powder was obtained, varying in single batches between 180 and 220 g. It was found convenient to work up amounts of 10–15 kilograms of kidney in one day.

Extraction of Renin.—150 grams of dry powder was shaken mechanically for twenty-five minutes with 500 ml. of ice cold 2% sodium chloride solution and then centrifuged for twenty-five minutes at high speed (International centrifuge, size 2, conical head, 4500 r. p. m.). The clear brown supernatant solution was decanted and the residue again treated with 500 ml. of sodium chloride solution. The combined extracts (660–690 ml.) were brought to *p*H 4.2–4.3 (glass electrode) and stored in the refrigerator for ten to fifteen hours. The precipitate, consisting of inactive proteins, was removed by centrifugation and the supernatant solution was filtered through fluted filter paper (Schleicher and Schüll no. 605). The filtrate (630–660 ml.) was dialyzed against running water at 4° for about forty hours, and the precipitated proteins were removed by filtration. About 690–730 ml. of filtrate was obtained. In this fraction, referred to as fraction A, one rabbit unit was contained in 0.12–0.15 ml., a yield of 6400–7500 R. U. per kilogram of fresh kidney. These results were consistent in twenty individual preparations. One R. U. was equivalent to 72–93 μg total nitrogen or 34–43 μg protein nitrogen. Fraction A showed no loss in activity over three months when stored in the refrigerator using toluene to inhibit bacterial growth.

Fraction B.—Some degree of purification of fraction A was achieved by adjusting the *p*H to 3.75, where most of the pigment and about 30–40% of the inert proteins precipitated. However, this procedure resulted in a loss in activity of 20–25% and consequently was abandoned. Further purification of fraction A was facilitated by working with more concentrated solutions. The active material was therefore precipitated by saturating the solution with sodium chloride at *p*H 4.3. After standing in the icebox for eighteen to thirty-six hours, the material was filtered with suction through a thin layer of hyflo supercel and the filtrate discarded. The filter cake was dialyzed until free of sodium chloride and filtered. The filtrate (fraction B) contained 4000–5000 R. U. per kilogram of fresh kidney in ten individual preparations and one R. U. was represented by 24–36 μg nitrogen. The *p*H in the sodium chloride precipitation was found to be important as shown by the following yields from a fraction A which contained 6500 R. U.:

*p*H	4.3	3.6	2.0	6.0
Yield (R. U./kg. kidney)	4700	3100	2600	320

The precipitation was carried out in the cold. When the solution at *p*H 4.3 was maintained at 37° for fifteen hours, only 3200 R. U. could be recovered, even though both the precipitate and the supernatant solution were assayed.

(5) H. Goldblatt, J. Lynch, R. F. Hanzal and W. W. Summerville, *J. Exp. Med.*, **59**, 347 (1934).

(6) O. M. Helmer and I. H. Page, *J. Biol. Chem.*, **127**, 757 (1939).

(7) W. D. Collings, J. W. Remington, H. W. Hays and V. A. Drill, *Proc. Soc. Exptl. Biol. and Med.*, **44**, 87 (1940).

(8) O. Schales and F. W. Haynes, *ibid.*, **47**, 315 (1941).

Fraction C.—Saturated ammonium sulfate solution was added to fraction B until 0.33 saturation was reached. After the solution stood for several hours in the icebox, the precipitate was filtered or centrifuged off and discarded as inactive. The filtrate, which contained about 95% of the renin, was brought to 0.46 saturation by a further addition of saturated ammonium sulfate solution. This precipitated the renin and left most of the pigment in the supernatant solution. The precipitate was centrifuged off and dialyzed against running water in the cold until free from ammonium sulfate. The yield, in form of fraction C, was 2500–3600 R. U. per kilogram of kidney and one unit corresponded to 4–5 μg nitrogen. In some preparations it was necessary to repeat the fractionation with ammonium sulfate to get a product of this purity. This end-product is about twice as pure as the best preparation of Collings and co-workers.[7] Concentrated solutions (100–200 R.U./ml.) have a light yellow color, the dried material is practically colorless and keeps its activity in vacuum in the icebox.

The outlined procedure is simpler than earlier methods and all steps have been checked many times, so that the yields and purities mentioned should be reproducible.

Chemically, renin seems to be a pseudoglobulin. No evidence for a removable prosthetic group has been found so far. Iron-porphyrin-proteids, which accompany renin in the first steps of the procedure, could be removed as inactive and the removal of a green fluorescent compound by prolonged dialysis was without effect on the activity.

Human Renin.—Renin in form of fraction A was also prepared from human kidneys. It was sterilized by filtration through Seitz filters. Adsorption of human or pig renin on Seitz filters was not observed, in contrast to the finding of Collings and co-workers.[7] Table II shows the activity of human renin when tested in human beings, dogs and rabbits. It is interesting to note the difference in response of rabbits and dogs to human renin; dogs were about twenty times as sensitive as rabbits. There was no difference, however, in the sensitivity of these two species to pig renin.

In the experiments of Battro and co-workers[9] on human renin, extracts from 1.20 g. of human kidney were required per kilogram of body weight to produce a rise of 30 mm. in systolic pressure. The outlined procedure yields therefore 15–30 times as much renin from human kidneys as the method used in the South American laboratory. In 5 preparations of each type no essential difference in the renin contents of kidneys from normal and from hypertensive human beings could be detected.

This is in agreement with the findings of Pickering and Prinzmetal[10] on rabbit kidneys and with those of Williams, Grollman and Harrison[11] and Beckwith and Chanutin[12] both studying rat kidneys. Increased amounts of renin in hypertensive kidneys had been found by Williams, Grollman and Harrison[11] in dogs with experimental hypertension, confirming the results of Harrison, Blalock, Mason and Williams[13] and of Prinzmetal, Friedman and Abramson.[14] Slightly larger amounts of renin in hypertensive human kidneys than in normal kidneys have been found by Prinzmetal, *et al.*,[14] but the results with normal and hypertensive cases overlapped extensively. Landis[15] could not find any clear relation between blood pressure during life and the renin content of kidney tissue after death in human beings.

TABLE II

EFFECT OF HUMAN RENIN ON HUMAN BEINGS, DOGS AND RABBITS

Effect on	μg N/unit	Units/kg. kidney	1 Unit from g. of kidney
Human Beings	42– 51[a]	20000–25000	0.04–0.05
	70– 84[b]	12000–14000	0.07–0.08
Dogs	38– 40	25000	0.04
Rabbits	700–775	1300	0.75

[a] 10 experiments on 3 different persons. [b] 3 experiments on one additional person.

Physiological Properties of Renin

The injection of one R. U. of pig renin into rabbits caused a rise in blood pressure which reached its maximum about two to three minutes after the start of the injection. It required ten to fifteen minutes for the blood pressure to return to normal. The effect of human renin on rabbits persisted much longer, up to one hour. Large doses of pig renin (5–7 R. U./kg. body weight) administered to rabbits five to six times daily for thirty days did not produce permanent hypertension. This is in contrast to the claim of Hessel[4] that similar treatment caused permanent high blood pressure.

Pig renin in the form of fraction C was also administered to human beings. Amounts up to 1000 R. U. (13 R. U./kg. body weight) did not cause any rise in blood pressure nor other symptoms. This confirms the report of Turnoff and Rowntree,[16] who observed no effect after the administration of about 200 R. U. The rise in blood pressure caused by human renin in human beings reached its maximum four to seven minutes after the start of the intravenous injection. The only subjective symptom after large doses was a slight headache at the maximum of the pressure rise. It is interesting that renin not only caused

(9) A. Battro, E. Braun-Menendez, A. Lanari and L. F. Leloir, *Rev. Soc. Arg. Biol.*, **16**, 376 (1940).

(10) G. W. Pickering and M. Prinzmetal, *Clin. Sc.*, **3**, 211 (1938).

(11) J. R. Williams, A. Grollman and T. R. Harrison, *Arch. Int. Med.*, **67**, 895 (1941).

(12) J. R. Beckwith and A. Chanutin, *Am. J. Physiol.*, **128**, 562 (1940).

(13) T. R. Harrison, A. Blalock, M. Mason and J. R. Williams, *Arch. Int. Med.*, **60**, 1058 (1937).

(14) M. Prinzmetal, B. Friedman and D. I. Abramson, *Ann. Int. Med.*, **12**, 1604 (1939).

(15) E. M. Landis, *Am. J. Med. Sc.*, **202**, 14 (1941).

(16) D. Turnoff and L. G. Rowntree, *Science*, **93**, 281 (1941).

a rise in arterial but also a rise in venous pressure. In five experiments the venous pressure rise in three normal persons was 8–83 mm. water simultaneously with rises of 13–54 mm. mercury in systolic pressure. There was also a considerable decrease of blood flow in the hand, which fell from an average of 27 cc. to 3 cc. per minute per 100 cc. of tissue during a pressor response of 43–67 mm. systolic in two normal persons. The cardiac output showed in three determinations a very slight decrease of 0.2 liter per minute, while the blood pressure rose 42–56 mm. systolic and 36–42 mm. diastolic.

Summary

A method is described by which renin, a pressor protein of the kidney, can be prepared in good yields and with a high degree of purity. The effect of human renin and hog renin in human beings, rabbits and dogs has been studied. Repeated injections of large amounts of pig renin did not produce permanent hypertension in rabbits.

BOSTON, MASS. RECEIVED OCTOBER 2, 1941

[CONTRIBUTION FROM THE LABORATORIES OF THE NEWARK BETH ISRAEL HOSPITAL]

Specificity Studies on Enzymes Hydrolyzing Esters of Substituted Amino and Nitrogen Heterocyclic Alcohols[1]

BY DAVID GLICK

Earlier work on the specificity of cholinesterase[2] led to certain generalizations, among which was one that the presence of nitrogen groups, such as amino, alkyl-substituted amino, or heterocyclic structures in the acid component of an ester renders the compound refractory to hydrolytic enzyme action. Evidence given in the present communication extends this rule to include esters of mono- and dialkylamino alcohols as well as those of piperidyl alcohols, even though the latter are attacked by an enzyme which, from data to follow, is probably not the same as cholinesterase.

There is no term in the literature for the class of esterases that act on the nitrogen-alcohol esters. Therefore, it is proposed that the term "azolesterase" be applied to this group to distinguish it from other types of esterase. Among the "azolesterases" are cholinesterase, morphinesterase,[3] and certain tropinesterases such as atropinesterase, cocainesterase, and tropacocainesterase. From differences in occurrence, evidence has been presented that the three tropinesterases may be distinct enzymes.[4]

Differentiation of enzyme entities among the azolesterases has been suggested, within certain limitations, by the use of various sera as enzyme sources.[4] Thus, horse serum contains the usual lipase and esterase and, in addition, it is a rich source of cholinesterase; but it is devoid of all of the tropinesterases studied except tropacocainesterase. There are rabbit sera with and without atropinesterase; both types contain cholinesterase, cocainesterase, and tropacocainesterase. Horse serum and both kinds of rabbit serum were employed in the present study.

Most of the substrates used in this investigation are local anesthetics or antispasmotics; their enzymatic hydrolysis might be a significant factor in determining the duration, and possibly the intensity, of the action of these drugs *in vivo*. The following groups of substrates were used: esters of β-diethylaminoethanol, certain alkyl-substituted aminoalkyl benzoates, dialkylaminoalkyl 2-furoates, and piperidylalkanol esters.

Experimental

The manometric method employing the Warburg apparatus was used for the enzyme measurements in the same manner as in preceding studies.[2,4] In the present case, enzyme activity has been expressed in terms of cmm. of carbon dioxide liberated in two hours at 30° with 1% substrate and 2.5% serum in a total volume of 4 ml. One % substrate was chosen since this concentration is sufficiently high to ensure maximum hydrolytic velocities. Manometer readings were taken every fifteen minutes, and activity was determined by the slope of the linear portion of the activity-time curve.

In a number of cases it was necessary to bring the substrate solutions to the desired *p*H of 7.4 by addition of alkali. The weighed substrate was dissolved in a small volume of bicarbonate Ringer solution, either *N* or 0.1 *N* sodium hydroxide (depending on the amount of base required) was added a drop at a time until the solution was neutralized to brom thymol blue used as an outside indi-

(1) Aided by a grant from the Sidney C. Keller Research Fund.

(2) D. Glick, *J. Biol. Chem.*, **125**, 729 (1938); **130**, 527 (1939); **137**, 357 (1941).

(3) C. I. Wright, *J. Pharmacol. Exp. Therap.*, **71**, 164 (1941).

(4) D. Glick and S. Glaubach, *J. Gen. Physiol.* **25**, 197 (1941).