Engel, L. L., Slaunwhite, W. R., Carter, P. & Nathanson, I. T. (1950). *J. biol. Chem.* **185**, 255.
Glueckauf, E. (1949). *Disc. Faraday Soc.* **7**, 239.
Howard, G. A. & Martin, A. J. P. (1950). *Biochem. J.* **46**, 532.
Levi, A. A. (1949). *Symp. biochem. Soc.* **3**, 83.
Martin, A. J. P. (1949). *Symp. biochem. Soc.* **3**, 4–20.
Martin, A. J. P. & Synge, R. L. M. (1941). *Biochem. J.* **35**, 1358.
Mather, A. (1942). *J. biol. Chem.* **144**, 617.
Migeon, C. J. (1953). *J. clin. Endocrin. Metab.* **13**, 674.
Slaunwhite, W. R., Engel, L. L., Scott, J. F. & Ham, C. L. (1953). *J. biol. Chem.* **201**, 615.
Stimmel, B. F. (1946). *J. biol. Chem.* **162**, 99.
Swyer, G. I. M. (1952). *Ciba Foundation Colloquia on Endocrinology*, II, p. 82. London: J. and A. Churchill.
Swyer, G. I. M. & Braunsberg, H. (1951). *J. Endocrin.* **7**, lx.

A New Method of Large-Scale Preparation of Hypertensin, With a Note on its Assay

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The isolation and identification of hypertensin (Braun-Menendez, Fasciolo, Leloir & Munoz, 1939) or angiotonin (Page & Helmer, 1940) has so far been unsuccessful, and this paper describes a new method of preparation designed to produce large quantities of hypertensin for purification.

Hypertensin is formed during the incubation of the enzyme renin, derived from the kidney, with hypertensinogen, a substrate in the globulin fraction of the plasma. Owing to the low yield of hypertensin (probably not more than 1 mg./l.) it must be made on a large scale. Previous workers have reduced the large volumes necessary by salting out the plasma globulins before activation (Braun-Menendez, Fasciolo, Leloir, Munoz & Taquini, 1946). Finally the reaction has been stopped by precipitating the proteins with heat (Edman, 1945) or ethanol (Page & Helmer, 1940), and the filtrate concentrated by vacuum distillation. The renin hypertensinogen reaction is complicated by the concurrent destruction of hypertensin by 'hypertensinase activity' present in both serum and kidney extracts. The serum activity has been destroyed previously by acidification (Braun-Menendez *et al.* 1946), and it is possible to prepare renin with low hypertensinase activity (Pickering & Prinzmetal, 1938).

When starting volumes of 80 l. of blood are used all these described methods are cumbersome, and it has been found that rapid concentration of hypertensin can be achieved by adsorption on to charcoal from the serum and subsequent elution by glacial acetic acid. The hypertensinase activity of the serum does not affect the yield.

MATERIALS AND METHODS

The various types of kieselguhr, Celite 545 and Standard Supercel were supplied by Johns Manville Co. Ltd., Artillery Row, London, S.W. 1.

The charcoal for adsorption was 'animal charcoal technical powder', British Drug Houses Ltd. It was suspended in glacial acetic acid for a few hr., filtered by suction, washed with distilled water and methanol, and dried in an oven at 105°.

For the assay, urethane (British Drug Houses Ltd.), pentapyrollidinium tartrate in polyvidone solution ('Ansolysen Retard' May and Baker Ltd.), (–)-noradrenaline (Bayer Ltd.) and heparin 5000 units/ml. (Evans) were used.

Renin preparation. This was modified from that described by Pickering & Prinzmetal (1938). Rabbit kidney from animals killed 12–24 hr. previously was minced and stirred at 2° with ethanol (2 ml./g. of kidney) for 12 hr. The pulp was gauze-filtered and dried on filter paper at room temperature. This coarse material was powdered and kept its activity indefinitely in the cold (2°). Renin was extracted from the kidney powder by stirring it with 0·9% NaCl (10 ml./g. powder) at 2° for 12 hr. The pulp was filtered through gauze with squeezing and the cloudy filtrate clarified by filtering through a bed of Celite 545 after the addition of Celite 545 to the solution. The renin was precipitated by the addition of 40 g. of $(NH_4)_2SO_4$/100 ml. of filtrate and the precipitate allowed to settle at 2° overnight. The precipitate was filtered through a Whatman no. 50 paper, and the filter paper suspended in distilled water to dissolve the renin. This solution was then ready for use. The optimum amount of renin/l. of serum was discovered by a pilot experiment. (Usually the renin obtained from 150 g. of kidney powder was sufficient to treat 40 l. of serum.) Hypertensinase activity was very low in this renin preparation.

Hypertensin preparation. Stirred ox blood was obtained from the slaughter house in 80 l. batches, and the serum was separated in a no. 5 Alfa Laval milk separator. Frothing was stopped by the addition of a few drops of tributyl citrate or phosphate.

The 40 l. of serum were stirred with an electrically driven stirrer, and powdered animal charcoal was added (7 g./l.). After stirring for 10 min. the charcoal was allowed to settle for 10 min., then the supernatant serum was pumped through the Alfa Laval centrifuge to remove all suspended charcoal. This charcoal was discarded.

The charcoal-treated serum was incubated with renin at room temperature and the temperature of the serum was always close to 20°. More animal charcoal powder was stirred into the serum (7 g./l.), followed by addition of the renin solution. Incubation with stirring was carried on for 30 min., when the charcoal was allowed to settle for 10 min. The serum was pumped through the centrifuge as before, but to facilitate subsequent removal of the charcoal from the centrifuge bowl the centrifuge fins were left out and it was used as an empty bowl. The charcoal in the bowl was then added to that in the bottom of the incubation bin and suspended in 10 l. of distilled water.

Celite 545 filter aid was stirred by hand into the suspended charcoal (300 g. Celite), and it was then filtered through a bed of Standard Supercel filter aid in a large Büchner funnel. Finally it was washed on the filter with 1·5 l. of ethanol. The charcoal was scooped out of the Büchner funnel leaving the filter bed intact. Elution of the hypertensin was carried out by suspending the charcoal cake in 3 l. acetic acid and stirring for 10 min. The acetic acid eluate was then filtered through the same filter bed. After sucking dry, the charcoal was resuspended in 1·5 l. acetic acid and the process was repeated.

The green eluate was then distilled to dryness at about 20 mm. in a 5 l. flask on a boiling-water bath. The residue was taken up in 50, 25, and 25 ml. of distilled water and filtered through a bed of Standard Supercel in a small Büchner funnel. The filtrate was clear yellow and would keep for months at 2° without loss of activity.

Method of assay

The yield was assayed by the pressor response produced in the anaesthetized rat (urethane 100 mg./kg. intraperitoneally). Albino or hooded rats of 250–350 g. were suitable. Occasionally it was necessary to insert a glass tracheal cannula. The blood pressure was lowered by pentapyrollidinium tartrate in polyvidone solution (2·5 mg./100 g. subcutaneously). The blood pressure was measured directly from the carotid artery with a mercury manometer of the type described by Crawford & Outschoorn (1951), and all doses were given into the external jugular vein. A small glass cannula was connected by thin rubber tubing to a 1 ml. tuberculin syringe graduated in 0·01 ml., containing 0·9% saline. This was held by a rubber band on a heavy steel block. Doses were given from a similar tuberculin syringe into the rubber tubing and washed in with 0·15 ml. saline. The dose volume varied from 0·02 to 0·15 ml. Coagulation was prevented by heparin intravenously (200 units/100 g.). Comparisons were made with stock solutions of hypertensin and with (–)-noradrenaline.

RESULTS AND DISCUSSION

It was possible to make large quantities of hypertensin with this method. The yield in terms of (–)-noradrenaline was 0·5–1·0 mg./l. of serum and the dry weight of material in the acetic acid eluate 100–200 mg./l. The basis of this method is a preliminary cleansing treatment of the serum with charcoal, followed by incubation with renin in the presence of more charcoal. Hypertensin is then eluted from the charcoal. While the method was designed for large-scale production, it can be used for volumes of serum as small as 10 ml.

Type of charcoal. The only suitable type was animal charcoal, which was not activated. It was not possible to get full recovery from activated charcoal.

Hypertensinase. Most methods of hypertensin production involve treatment of the serum or purified substrate to destroy 'hypertensinase activity'. This was not found necessary in the present method, the yield was as great with or without such treatment. Hypertensinase activity was usually present in the serum, shown by the greatly decreased amount of hypertensin obtained when the charcoal was added at the end of the period of incubation, instead of at the start. Once adsorbed, hypertensin was immune to destructive factors in the serum, and the charcoal was often left in the serum or in distilled water overnight at room temperature before elution by acetic acid.

Temperature of incubation. The reaction which released hypertensin was shown to be complete at a temperature of 20° within 30 min.

Comparison with other methods. The yield of hypertensin by the present method was always greater than that obtained by three other methods. These methods were: (1) incubation of renin and serum for 10 min. at 37° followed by protein precipitation in an equal volume of boiling distilled water; (2) similar incubation, with protein precipitation in 4 vol. of ethanol (Page & Helmer, 1940); (3) incubation at 2° for 24 hr. within cellophan tubing so that the hypertensin diffused out into surrounding distilled water. In the first two methods previous destruction of the hypertensinase activity in the serum was achieved by acidifying the serum to pH 3·6–4·0 with hydrochloric acid for 20 min. at 25° (Braun-Menendez *et al.* 1946) before return to pH 7·0 with sodium hydroxide. In the last method this was not necessary as hypertensinase is inactive at 2° (Bean, 1942; Sapirstein, Reed & Southard, 1944).

Elution. Glacial acetic acid was always used. Pyridine (20%, v/v, in water) was partially effective.

Assay. Control of all stages of the manufacture of hypertensin and of the subsequent steps in purification was only achieved by having a reliable method of assay. Previous workers have used the blood-pressure responses in the dog (Braun-Menendez *et al.* 1946) or cat (Edman, 1945); Skeggs, Kahn & Shumway (1951) have also used the rat. An improved method has recently been described in the rat by Dekanski (1954). The present method has the advantage that the test animal is easy to obtain; is sensitive to small amounts of hypertensin, and gives reproducible results over long periods (up to 8 hr.). The blood pressure is usually steady at pressures of 50–80 mm. Hg for 4–8 hr., and there is the further

advantage that under these conditions it is difficult to elicit depressor responses, even when crude extracts are given. A typical comparison of noradrenaline with hypertensin is shown in Fig. 1. While comparisons were made with stock hypertensin, it was of interest to compare it with the most potent pressor substance known.

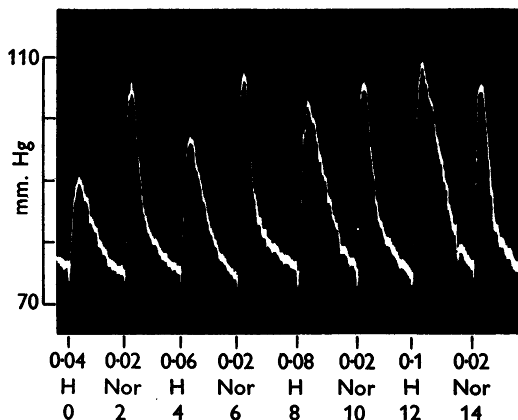

Fig. 1. Comparison of hypertensin with (−)-noradrenaline, showing that 0·02 μg. of noradrenaline is equivalent to between 0·08 and 0·1 ml. of hypertensin. Doses of noradrenaline in μg., of hypertensin in ml. Time in min.

SUMMARY

1. A new method of large scale production of crude hypertensin is described, depending on charcoal adsorption with subsequent elution by glacial acetic acid.

2. A reliable sensitive method of biological assay using the pressor responses in the rat is described.

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REFERENCES

Bean, J. S. (1942). *Amer. J. Physiol.* **136**, 731.
Braun-Menendez, E., Fasciolo, J. C., Leloir, L. F. & Munoz, J. M. (1939). *Rev. Soc. argent. Biol.* **15**, 420.
Braun-Menendez, E., Fasciolo, J. C., Leloir, L. F., Munoz, J. M. & Taquini, A. C. (1946). *Renal Hypertension.* Springfield: C. C. Thomas.
Crawford, T. B. B. & Outschoorn, A. S. (1951). *Brit. J. Pharmacol.* **6**, 8.
Dekanski, J. (1954). *Brit. J. Pharmacol.* **9**, 187.
Edman, P. (1945). *Ark. Kemi Min. Geol.* **22A**, 1.
Page, I. H. & Helmer, O. M. (1940). *J. exp. Med.* **71**, 29.
Pickering, G. W. & Prinzmetal, M. (1938). *Clin. Sci.* **3**, 211.
Sapirstein, L. A., Reed, R. K. & Southard, F. D. jun. (1944). *J. Lab. clin. Med.* **29**, 633.
Skeggs, L. T., Kahn, J. R. & Shumway, N. P. (1951). *Circulation*, **3**, 384.

The Constitution of the Aliphatic Alcohols in Human Sebum

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Human sebum consists mainly of wax esters, free fatty acids, hydrocarbons and a small amount of glycerides. Whilst the free fatty acids have been investigated in detail (Weitkamp, Smiljanic & Rothman, 1947), little is known about the composition of the other fractions. The unsaponifiable matter has long been known to contain cholesterol (for review of earlier literature, see Čmelik, 1952) and squalene has recently been identified (Sobel, 1949; Mackenna, Wheatley & Wormall, 1952). By chromatographic studies these last authors found 14–20 % of aliphatic alcohols in the unsaponifiable matter, but apart from an impure preparation of eicosanol (?) no individual alcohol was isolated.

The present paper describes an investigation into the aliphatic alcohols of human sebum. Sebum (surface skin 'fat') was obtained by extraction of hair from the heads of African (Bantu) males.

METHODS

Reversed-phase partition chromatography of fatty acids. The method of Howard & Martin (1950) (cf. Silk & Hahn, 1954) was extended to effect the resolution of six normal fatty acids ranging in chain length from six to eleven carbon atoms. It was found unnecessary for the present purpose to protect eluate and developing solvent against atmospheric CO_2; also, nitrogen was not purified before entering the titration vessel. The columns were maintained at tap-water temperature (18–22°). For loading of the columns, the acid mixtures were usually dissolved in the developing solvent. It was necessary to prepare a mull only when the mixed acids differed widely in chain length (such as C_5–C_{12}).

The efficiency of separation is illustrated by the elution curve (Fig. 1) for a chromatogram of a synthetic mixture of pentanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic, hendecanoic and dodecanoic acids (about 7 mg. of each). The C_5 and C_6 acids (unresolved) were eluted with water; C_7 with 10 % (v/v) aqueous acetone, C_8 with 25 %,