RESEARCH PAPERS

THE ANALYSIS OF PHARMACOPŒIAL SAMPLES OF ADRENALINE; A LIMIT TEST FOR NORADRENALINE IN ADRENALINE

BY G. B. WEST

From The Department of Pharmacology and Therapeutics, University of St. Andrew's Medical School, Dundee

Received October 17, 1951

IT is now well established that noradrenaline is present with adrenaline in the suprarenal glands of most mammals, although its relative amount varies from species to species. Tullar[1] in 1949 was the first worker to isolate *l*-noradrenaline from suprarenal extracts, and in the same year Goldenberg and his co-workers[2] showed by paper chromatography that preparations of adrenaline complying with the requirements of the United States Pharmacopœia may contain up to 36 per cent. of noradrenaline. The biological assay of adrenaline in the United States Pharmacopœia is based on the pressor effect in dogs, and since noradrenaline is about 1·5 times more pressor than adrenaline, these two amines would not be differentiated by this test. The monograph on adrenaline in the British Pharmacopœia, 1948, states "it may be prepared from an acid extract of the suprarenal glands of certain mammals, or by synthesis." The difficulties of synthesis and the search for cortisone-like activity in the adrenal cortices of mammals make the natural product still a practicable proposition in this country.

Two of the chief therapeutic uses of adrenaline are in circulatory collapse and in asthma. Whereas noradrenaline as well as adrenaline has already proved valuable in combating low blood pressure in operational shock, noradrenaline is considerably less active than adrenaline in producing relaxation of the circular fibres of the bronchioles in the treatment of asthma. Inaccurate dosage may result, therefore, when a natural adrenaline (containing much noradrenaline) is used in the treatment of these two conditions. For these reasons, we have investigated the nature of four pharmacopœial samples of natural adrenaline purchased on the open market in this country and of one similar sample from the United States. The standard material used was a sample of pure synthetic adrenaline. The results suggest that a limit should be made on the amount of noradrenaline present in samples of natural adrenaline so as to achieve accurate dosage.

EXPERIMENTAL

All samples were dissolved in 0·1N hydrochloric acid and diluted with water to give a 1 in 1000 solution in 0·01N hydrochloric acid. Biological assays for adrenaline and noradrenaline contents were completed on the spinal cat by the method of Burn, Hutcheon and Parker.[3] The chemical assays were performed by the method of Euler and Hamberg,[4] which is based on the formation of coloured compounds with iodine. Paper chromatography was carried out using the ascending method with

butanol-acetic acid-water as solvent and potassium iodate or ferricyanide[5] as developer. In each experiment, duplicate strips of paper were used but these were not developed after drying. Instead, the areas on the paper corresponding to the position of the standard adrenaline (R_F value 0·36) and noradrenaline (R_F value 0·28) spots were each cut out, extracted with 0·01N hydrochloric acid, and tested on the isolated rabbit ileum against standard solutions of the two amines. Determination of the specific rotation and melting-points of all specimens gave values lying within the pharmacopœial limits.

TABLE I

BIOLOGICAL AND CHEMICAL DATA ON SAMPLES OF ADRENALINE OF NATURAL ORIGIN, EXPRESSED AS PERCENTAGE ACTIVITY COMPARED WITH STANDARD SYNTHETIC ADRENALINE AND NORADRENALINE

Sample	Colour	Adrenaline per cent.		Noradrenaline per cent.		
		Biological	Chemical	Biological	Chemical	Chromatography
1. British	Light brown	100	89	0	11	3
2. British	Pale buff	92	84	8	16	9
3. British	Light brown	90	88	10	12	10
4. British	Almost white	100	93	0	7	0
5. American ..	Very pale buff	81	73	19	27	20

RESULTS

The results of the assays on the samples of natural adrenaline are shown in Table I. The values for noradrenaline percentages obtained by biological assay and by paper chromatography showed good agreement, but chemical assays gave raised values, suggesting the presence of an interfering substance. The one American sample (U.S.P. Reference Epinephrine Standard—1949) contained much more noradrenaline than that found in any of the four British samples. We suggest that a limit of 10 per cent. of noradrenaline be imposed on samples of adrenaline; by this means, adequate dosage would be achieved in the treatment of both circulatory collapse and asthma. A suitable method of testing samples is described below.

Limit test for noradrenaline in adrenaline. The chromatogram is carried out by the ascending method in a glass tank of suitable size (e.g., 15 × 7 × 22 in.) containing the solvent to a depth of ½ in. The solvent is prepared by shaking *n*-butanol (4 vol.), glacial acetic acid (1 vol.) and water (5 vol.) together and discarding the lower layer. The butanol and acetic acid are of ordinary reagent quality and need not be purified before use. Sheets of Whatman No. 4 filter paper (up to 12 in. in width) are suspended from horizontal glass rods placed 18 in. above the liquid surface, the paper being kept taut by means of a thin glass tube (up to 12 in. in length), closed at both ends and threaded through vertical slits ⅜ in. long and 3 in. apart cut near the bottom of the sheet. The top of the tank is sealed (e.g., by a vaselined glass plate).

Test solutions are applied from a graduated glass syringe as single drops each 0·01 ml., 1 in. apart, along a line 2 in. from the foot of the paper and at least 2 in. from the lateral edges of the paper where the flow tends to be erratic. When the drops have dried, chromatography is carried out at

room temperature for 18 hr. during which time the solvent travels approximately 12 in. from the starting line. After drying for 15 minutes in a cabinet through which a current of warm air circulates, the paper is sprayed with a 1 per cent. w/v aqueous solution of potassium iodate. On development in an air oven at 100° to 110° C. for not more than 2 minutes, adrenaline and noradrenaline are rendered visible as pink and violet spots respectively. Adrenaline (R_F value 0·36) travels slightly faster than noradrenaline (R_F value 0·28) so that separate spots are usually seen. Care must be taken however to avoid overheating which causes both spots to become brown and indistinguishable by colour. At room temperature, the spots assume a uniform brown colour within a few hours. By this technique, spots containing 1 μg. of adrenaline or 2 μg. of noradrenaline can be readily detected.

For the test, 0·01 ml. of a 1 in 1,000 solution of the sample and of synthetic adrenaline in 0·01N hydrochloric acid (i.e., 10 μg. each) are chromatographed. On developing the paper, no violet spot at R_F 0·28 should be visible (indicating under 1 μg. of noradrenaline). In addition, controls of 0·01 and 0·02 ml. of a 1 in 10,000 solution of standard synthetic noradrenaline (i.e., 1 and 2 μg.) may be used to indicate the sensitivity of the method and the correct position and colour of the noradrenaline spot.

Discussion

The results of the assays on samples of adrenaline of natural origin clearly indicate that one-fifth of the activity may be due to noradrenaline. Since this latter amine is much less effective in relaxing bronchial muscle and in causing glycogenolysis than is adrenaline, it is advisable to restrict its concentration. Besides, noradrenaline predominates in the adrenal glands of whales, of young calves and bullocks, and of most other young mammals, and all of these are possible sources of pharmacopœial adrenaline.

Concerning the chromatographic limit test, mention should be made of the fact that separation of adrenaline and noradrenaline by a similar technique was reported[5] in 1948. In the original ferricyanide method, the spots are viewed against a yellow background; in the suggested limit test using potassium iodate, the spots are viewed against a white background and sensitivity is thereby increased.

Summary

1. 5 samples of pharmacopœial adrenaline of natural origin have been subjected to chemical, biological, and chromatographic examination. Noradrenaline was found in 4 of the samples, in amounts representing up to 20 per cent. of the total activity.

2. A limit test for noradrenaline in adrenaline is described using paper chromatography. It is suggested that pharmacopœial adrenaline should contain not more than 10 per cent. of noradrenaline.

References

1. Tullar, *Science*, 1949, **109**, 536.
2. Goldenberg, Faber, Alston and Chargaff, *Science*, 1949, **109**, 534.
3. Burn, Hutcheon and Parker, *Brit. J. Pharmacol.*, 1950, **5**, 142.
4. Euler and Hamberg, *Science*, 1949, **110**, 561.
5. James, *Nature, Lond.* 1948, **161**, 851.