ON THE STABILITY OF ADRENALINE IN INJECTIONS: A COMPARISON OF CHEMICAL AND BIOASSAY METHODS

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The stability of adrenaline in some injections has been investigated by chemical assays based on determinations of adrenochrome and of adrenolutine, while the rat blood pressure method and the rat uterus inhibition method served as biological control procedures. The colorimetric method, in which adrenaline is oxidised to adrenochrome by means of potassium ferricyanide in acid solution, proved unable to detect any deterioration of adrenaline in procaine and adrenaline injections. The corresponding fluorimetric method, in which the adrenochrome formed is rearranged to adrenolutine by addition of strong alkali, gave results which agreed well with the biological results. In injections, adjusted to pH 3 with hydrochloric acid, adrenaline was stable for at least 20 months at 4° in solutions heated at 120° for 20 min., at 100° for 20 min. or unheated. Replacement of the hydrochloric acid by sodium metabisulphite (pH 3·6) prevented the discoloration. In injections of procaine and adrenaline, adjusted to pH 3 with hydrochloric acid, the adrenaline content decreased over the 20 month period, and was most pronounced in the solutions that had been heated before storage. Sodium metabisulphite significantly increased the stability of adrenaline in these injections and also prevented any colour formation.

Few papers have been published on the stability of adrenaline in injections as measured by bioassay procedures, yet the specificity of the chemical methods cannot be generally accepted. A chemical determination of racemisation is only possible in injections with an adrenaline content above 1 mg./ml., and an estimation of a fall in adrenaline content because of deterioration will depend on the method used. Consequently, a bioassay method utilising the effect on the blood pressure of rats, has been adopted for use by the Nordic Pharmacopoeia of 1963.

In the present work we have used some of the experimental conditions adopted by the Nordic Pharmacopoeia. By using chemical and biological methods side by side, we hoped to assess the specificity of the chemical methods used and the role played by the racemisation of adrenaline as a part of the total deterioration processes.

One colorimetric and one fluorimetric method were chosen for the chemical assays, the former based on the oxidation of adrenaline to adrenochrome by potassium ferricyanide (Ehrlén, 1948), the latter being the corresponding adrenolutine method (Hellberg, 1960). The rat blood pressure method of the Nordic Pharmacopoeia, Volume IV, and the modification of Jensen and Vennerød (1961) of the rat uterus method, each based on basically different pharmacological effects of adrenaline, served as reference methods.

EXPERIMENTAL

Adrenaline Injections (1 mg. of adrenaline per ml.)

Series A (pH 3). Adrenaline bitartrate 1·82 g. Sodium chloride 8·00 g. Hydrochloric acid N 3·00 ml. Water for injection to 1000 ml.

Series B (pH 3·6). Adrenaline bitartrate 1·82 g. Sodium chloride 8·00 g. Sodium metabisulphite 0·50 g. Water for injection to 1000 ml.

Procaine and Adrenaline Injections (30 μg. of adrenaline per ml.)

Series C (pH 3·0). Procaine hydrochloride 20·00 g. Adrenaline injection, Series A 30·00 ml. Sodium chloride 4·50 g. Hydrochloric acid N 10·00 ml. Water for injection to 1000 ml.

Series D (pH 3·6). Procaine hydrochloride 20·00 g. Adrenaline injection, Series A 30·00 ml. Sodium chloride 4·50 g. Sodium metabisulphite 0·50 g. Hydrochloric acid N 3·00 ml. Water for injection to 1,000 ml.

The injections were prepared aseptically, dispensed in 10 ml. single-dose containers and immediately sealed by fusion of the glass. No precautions were taken to exclude atmospheric oxygen.

Within each series one batch of sealed containers was heated at 120° for 20 min., another was heated at 98 to 100° for 20 min. and the rest were left unheated. All the ampoules were stored in the dark at 4°.

Chemical Methods

Colorimetric method. The method was that of Ehrlén (1948). To approach the experimental conditions of the corresponding fluorimetric method mentioned below, the pH during oxidation was raised to 6·2 and the concentration of the buffer increased, according to Hellberg (1960). When the oxidation was completed (2 min.), the pH was immediately lowered to about 3·5 with 2 N hydrochloric acid to secure maximal stability of the adrenochrome formed. The extinction was read at 485mμ in a spectrophotometer against the reagent blank.

Each of the results given in Tables I to IV was based on 4 single determinations. The interference of the colour in injections of series A was rendered negligible by appropriate dilution of the solutions; for the procaine and adrenaline injections of series C the interference of colour was eliminated by subtraction of the corresponding blank. The interference observed in the injections of series D is discussed under Results.

Sodium metabisulphite was destroyed before the oxidation of the adrenaline by adding a small excess of iodine 0·22 N to the strongly acid solution; again, the excess of iodine was removed by the addition of arsenite as described by Hellberg (1960).

Fluorimetric method. The method described by Hellberg (1960) was used. Each of the results of Tables I to IV was based on 8 single determinations, the concentrations of the standard solutions being adapted to the level of the test solutions. The average standard deviation, calculated from the autoclaved injections of series D, was 3·6 per cent.

Biological Methods

The rat blood pressure method of the Nordic Pharmacopoeia, Volume IV, based on the modification of Mörch (1960), was used.

The rat uterus inhibition method, as modified by Jensen and Venneröd (1961), was used. In one case (the determination after 20 months of the autoclaved procaine and adrenaline injection, series C, Table III) the

TABLE I

THE STABILITY OF ADRENALINE INJECTIONS, SERIES A (1 MG. OF ADRENALINE PER ML.)
(pH adjusted to 3·0 by addition of hydrochloric acid)

Heat treatment	Storage period (months)	pH	Colour	Adrenaline recovery in per cent of original content			
				Colorimetric method	Fluorimetric method	Rat b.p. method	Rat uterus method
Unheated	0	3·0	colourless	100	—	—	—
	6	3·0	light red	99	102	98 (96–104)*	102 (86–116)
	20	3·0	light red	99	100	94 (95–106)	90 (92–109)
100° 20 min.	0	3·0	colourless	101	106	99 (98–103)	99 (87–115)
	6	3·0	light brown	99	96	99 (97–103)	98 (89–112)
	20	3·0	light brown	98	97	97 (94–106)	104 (92–109)
120° 20 min.	0	3·0	light red	99	100	92 (95–105)	94 (94–106)
	6	3·0	brown, dark ppt.	99	95	93 (97–104)	88 (90–111)
	20	3·0	brown, dark ppt.	96	95	92 (91–110)	83 (88–113)

* Fiducial limits, in parentheses, are expressed as percentages (P = 0·05)

procedure was altered by the addition of procaine to the standard solutions.

RESULTS

The results are given in Tables I to IV.

During the colorimetric analysis of heated adrenaline injections containing procaine hydrochloride and sodium metabisulphite (series D), the

TABLE II

THE STABILITY OF ADRENALINE INJECTIONS, SERIES B (1 MG. OF ADRENALINE PER ML.)
(pH adjusted to 3·6 by adding sodium metabisulphite)

Heat treatment	Storage period (months)	pH	Colour	Adrenaline recovery in per cent of original content			
				Colorimetric method	Fluorimetric method	Rat b.p. method	Rat uterus method
Unheated	0	3·6	colourless	99	102	—	—
	6	3·4	—	100	97	100 (95–105)*	101 (92–109)
	20	3·4	—	100	100	100 (93–108)	87 (89–113)
100° 20 min.	0	3·4	colourless	99	101	96 (94–106)	97 (93 108)
	6	3·3	—	100	96	100 (97–103)	96 (93–108)
	20	3·3	—	100	101	100 (95–105)	98 (90–111)
120° 20 min.	0	3·4	colourless	98	94	97 (93–108)	100 (84–120)
	6	3·3	—	99	95	98 (94–106)	98 (89–113)
	20	3·3	—	99	98	97 (95–106)	94 (83–121)

* Fiducial limits, in parentheses, are expressed as percentages (P = 0·05)

ferricyanide oxidation gave rise to interfering colour reactions, which made the colorimetric method inapplicable. It was shown that a similarly interfering substance (or substances) was formed in solutions of procaine hydrochloride and sodium metabisulphite, upon heating or even when stored in the cold.

Schriever (1956) postulated the formation of a hydroxy derivative of procaine through the action of bisulphite ions and oxygen. The resulting *o*-aminophenol, being a readily oxidizable substance, may be the contaminant involved. Qualitative tests have given some support to this suggestion.

TABLE III

THE STABILITY OF PROCAINE AND ADRENALINE INJECTIONS, SERIES C (30 μG. OF ADRENALINE PER ML.)

(pH adjusted to 3·0 by addition of hydrochloric acid)

Heat treatment	Storage period (months)	pH	Colour	Adrenaline recovery in per cent of original content			
				Colorimetric method	Fluorimetric method	Rat b.p. method	Rat uterus method
Unheated	0	3·0	colourless	101	100	—	—
	6	3·1	light yellow	102	99	96 (96–104)*	93 (88–114)
	20	3·0	yellow	103	77	78 (94–106)	86 (87–114)
100° 20 min.	0	3·0	light yellow	100	94	92 (96–104)	97 (87–115)
	6	3·1	light yellow	102	87	90 (96–104)	91 (87–115)
	20	3·1	yellow	96	72	71 (97–103)	68 (86–117)
120° 20 min.	0	3·0	light yellow	101	86	84 (97–103)	93 (92–108)
	6	3·1	light yellow	100	74	78 (98–103)	77 (90–111)
	20	3·1	yellow	96	65	65 (97–103)	63 (90–112)

* Fiducial limits, in parentheses, are expressed as percentages (P = 0·05)

DISCUSSION

Specificity of the Chemical Methods

Table III shows that the colorimetric method is not a reliable assay method for adrenaline in procaine and adrenaline injections. As the simple adrenaline injections proved to be stable over 20 months (Tables I and II), from the present results it is not possible to claim that the colorimetric method should be abandoned. The results, however, certainly suggest that the specificity of the method should be further controlled.

TABLE IV

THE STABILITY OF PROCAINE AND ADRENALINE INJECTIONS, SERIES D (30 μG. OF ADRENALINE PER ML.)

(pH adjusted to 3·6 by addition of sodium metabisulphite and hydrochloric acid)

Heat treatment	Storage period (months)	pH	Colour	Adrenaline recovery in per cent of original content			
				Colorimetric method	Fluorimetric method	Rat b.p. method	Rat uterus method
Unheated	0	3·6	colourless	102	98	—	—
	6	3·5	—		93	90 (95–105)*	98 (90–111)
	20	3·3	—		77	73 (94–107)	78 (90–111)
100° 20 min.	0	3·5	colourless		90	102 (95–106)	100 (92–108)
	6	3·5	—		88	88 (94–106)	93 (89–112)
	20	3·3	—		88	85 (93–107)	85 (92–109)
120° 20 min.	0	3·5	colourless		88	101 (95–105)	96 (93–108)
	6	3·4	—		90	94 (94–106)	85 (88–114)
	20	3·3	—		82	74 (92–108)	81 (92–109)

* Fiducial limits, in parentheses, are expressed as percentages (P = 0·05)

The agreement between the fluorimetric and the biological results (Tables III and IV) establishes the specificity of the fluorimetric method

and makes clear that no significant racemisation had taken place under the chosen conditions. From an analytical point of view the latter observation is rather interesting. The specificity of the fluorimetric method is limited as it does not differentiate between the optical isomers of adrenaline. If by maintaining a suitable pH the racemisation of adrenaline can be neglected, the fluorimetric method may be regarded as an alternative to the bioassays.

The simultaneous use of the rat blood pressure method and the rat uterus inhibition method assured a safe control of the adrenaline content of the injections examined. While the increase in blood pressure is mainly due to a stimulation of the adrenaline α-receptors in the smooth muscles of the arterioles, the relaxing effect on the rat uterus is caused by a stimulation of the adrenaline β-receptors in the uterus (Ahlquist, 1948). As the range of adrenaline concentrations needed for the rat uterus assay is only 0·1 to 1 ng./ml., compared to 0·5 to 2 μg./ml. for the blood pressure method, the former method must be ascribed a high degree of specificity. The results obtained by the two methods agreed satisfactorily, indicating that any interference from deterioration products was insignificant.

Stability of Adrenaline in Injections

The conditions for optimal stability of adrenaline in injection solutions now seem fairly well established (Heacock, 1959). Of the various factors affecting the stability the pH of the solution is of special interest, as different routes of deterioration of adrenaline have different optimum pH values. It is now generally accepted that, for physiological reasons as well as for pharmaceutical purposes, the pH interval 3 to 3·8, as adopted by the Nordic Pharmacopoeia for adrenaline injections, represents the best possible compromise for the limitation of oxidation and racemisation of adrenaline.

Accordingly, for the series A and C (Tables I and II) the pH was adjusted to 3·0 by hydrochloric acid.

According to Higuchi and Schroeter (1960), the use of sodium metabisulphite as a stabilising agent for adrenaline gave rise to a physiologically inactive sulphonate of adrenaline which may be formed by the action of bisulphite ions. More recently, this substance was claimed to be found in some adrenaline injections (Dibbern and Picher, 1961).

In the metabisulphite-containing injections, series B and D (Tables II and IV), the pH was initially adjusted to 3·6, to ensure that the pH should not fall below 3·0 during the storage period.

No decomposition could be detected in the adrenaline injections of series A and B, not even in those autoclaved. As the colouring in the series A injections, probably attributable to a very slight degree of oxidation of adrenaline, is pharmaceutically inelegant, the results suggest the use of metabisulphite if atmospheric oxygen is not strictly excluded. The good keeping qualities of adrenaline injections reported by West (1950) were confirmed.

In the procaine and adrenaline injections of series C and D, a significant decomposition was observed in both series, and this was most pronounced when metabisulphite was absent. Thus, while the possibility of the formation of adrenaline sulphonate may not be ruled out, in this experiment the metabisulphite increased the stability of adrenaline. The rate of decomposition observed in the series D injections closely corresponds to results recently reported by Vieillefosse, Hanegraaff and Khalili-Varasteh (1961).

The heat treatment of the series C injections increased the rate of decomposition of adrenaline. This fact may be due to some hydrolytic cleavage of procaine, leading to the formation of diethylaminoethanol (Woolfe, 1941). Still, for the aerobic conditions, oxidative routes of deterioration are possible, e.g. the quinone-amine reaction mentioned by Förster (1961).

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