biological activity in partial hydrolysates of the protein hormone which had an average of 7–8 amino-acid residues per peptide molecule. The results of the papain hydrolysis suggest that at least one peptide link is necessary for biological activity. The physiological significance of the dissociation of the adrenocorticotrophic protein, especially as regards its release from the pituitary cells into the circulation and its entry into the target organs, the adrenals, must await further work.

SUMMARY

1. The fractionation of ox anterior pituitary glands with a view to the concentration of the adrenocorticotrophic hormone is described.

2. The conditions for ultrafiltration of the bio-

logical activity have been investigated, and ultrafiltrates of high potency obtained.

3. The total biological activity of the protein hormone prepared by the method of Sayers *et al.* (1943) has been shown to be ultrafilterable under these conditions.

4. The chemical and biological properties of the ultrafiltrates are described.

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Determination of β -Naphthoxyethanol in Blood

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β -Naphthoxyethanol (I) has been shown by Francis & Parry (1949) to be a useful short-acting anaesthetic particularly for the horse. It was desired to correlate the pharmacological action of this compound with

its concentration in the blood, and to study its absorption and excretion. Attempts were therefore made to develop a method of determining it in

biological material. At first potential colorimetric methods were examined. Nitration of β -naphthoxyethanol, followed by reaction of the polynitro compound with butanone in the presence of alkali, gave a red colour; but this was unstable, and its intensity was greatly influenced by small variations in the conditions of nitration. The possibility of hydrolysing the drug to β -naphthol and determining the latter by reaction with diazonium salts did not appear more promising. However, it was found that β -naphthoxyethanol shows selective and characteristic light

absorption in the ultraviolet and the following method of determining it is based on this property. The conditions described have been applied to numerous other aromatic drugs showing selective ultraviolet absorption.

EXPERIMENTAL

Absorption spectrum of β -naphthoxyethanol. β -Naphthoxyethanol was purified by three recrystallizations from light petroleum (b.p. 40–60°) and dried at 60° *in vacuo* for 1 hr. (m.p. 75°). The ultraviolet absorption spectrum of a 0.0005% solution in CHCl_3 (w/v) was examined in a 10 cm. silica cell in the Beckman quartz spectrophotometer, model DU, and is shown in Fig. 1; in Table 1 maxima, minima and inflexions

Fig. 1. Absorption spectrum of β -naphthoxyethanol in chloroform.

are summarized. The maximum at 262 $m\mu$. was thought to be unsuitable for study because of the nearness of this wavelength to the region of critical sensitivity of the Beckman instrument. The maxima at 272 and 325 $m\mu$. were therefore selected. The validity of Beer's law at these wavelengths for solutions of β -naphthoxyethanol ranging in concentration from 0.05 to 0.5 mg./100 ml., and the linearity of instrument response within this range were established (Fig. 2), and showed that solutions of β -naphthoxyethanol in CHCl_3 could be accurately analysed by comparison with a single standard. The effect of varying slit width at both wavelengths was negligible (Fig. 3). It was decided to analyse unknown solutions by determining the optical densities at both 272 and 325 $m\mu$., and to take the average of the two results, since this would enable the identity of β -naphthoxyethanol, isolated from biological material, to be checked, and also increase the accuracy of analysis.

Extraction. Preliminary experiments on the isolation of β -naphthoxyethanol from blood and urine showed that a single extraction with CHCl_3 removed it quantitatively, but also that normal blood and urine did not give transparent

Fig. 2. Optical density of chloroform solutions of β -naphthoxyethanol at 272 (B) and 325 (A) $m\mu$.

Fig. 3. Variation of optical density of a chloroform solution of β -naphthoxyethanol with change in slit width, at 272 (A) and 325 (B) $m\mu$.

extracts. Fig. 4 shows the ultraviolet absorption of chloroformic extracts of 2 ml. of blood or 2 ml. of urine. The absorption of both extracts, although non-specific, was not negligible. Attempts were made to reduce this absorption by varying the pH at which the extraction was performed (Table 2), but the effect was small, some slight advantage being achieved by extracting from a basic medium. The extraction was cleanest using Na_2HPO_4 , which was therefore

Table 1. Maxima, minima and inflexions from the ultraviolet absorption spectrum of β -naphthoxyethanol

Maxima			Minima			Inflexions		
$\lambda m\mu$.	$E_{1\text{ cm.}}^{1\%$	ϵ	$\lambda m\mu$.	$E_{1\text{ cm.}}^{1\%$	ϵ	$\lambda m\mu$.	$E_{1\text{ cm.}}^{1\%$	ϵ
262	243.5	4570	266	229	4310	254	197	3710
272	259	4870	279.5	178	3345	298	47.5	843
281	182	3420	315.5	73.3	1377	—	—	—
311	81.4	1530	319	74.0	1391	—	—	—
317.5	74.4	1397	—	—	—	—	—	—
325	103	1938	—	—	—	—	—	—

selected for use. Table 2 indicates that the blank varies in different samples of blood, and it is therefore necessary to analyse a sample of normal blood with the unknowns, and to subtract the blank in the usual way. It is probable that the

Fig. 4. Absorption spectra of chloroform extracts (40 ml.) of normal rabbit blood (B, 2 ml.) and urine (A, 2 ml.), buffered with Na_2HPO_4 .

blank could be further reduced, for example, by introducing chromatographic techniques; but accurate analyses are readily possible without thus increasing the complexity of the method. Urine must be analysed by constructing full absorption spectra of extracts because only traces of β -naphthoxyethanol are excreted (Spinks, 1950).

in that order, and is dried in the oven. Just before use it is rinsed with CHCl_3 . The most suitable spectrophotometer is a photoelectric type such as the Beckman, but a Hilger Spekker photometer and medium quartz spectrograph have been used successfully.

Reagents. 0.2M- Na_2HPO_4 ; CHCl_3 (B.P.); 1% stock solution of β -naphthoxyethanol in ethanol (w/v); 5 mg./100 ml. standard solution of β -naphthoxyethanol. CHCl_3 (B.P.) contains ethanol as a preservative, but has been found to be satisfactory. The 5 mg./100 ml. standard solution of β -naphthoxyethanol is freshly prepared by diluting 0.5 ml. of the 1% stock solution to 100 ml. with distilled water.

Procedure. Pipette 2 ml. of blood into 4 ml. of 0.2M- Na_2HPO_4 in a 60 ml. glass-stoppered bottle. Add 40 ml. of CHCl_3 and shake vigorously for 5 min. When the lower layer has separated, remove it by means of a pipette provided with a rubber teat and filter it into a 10 cm. silica spectrophotometer cell. Read the optical density (a) at 272 and 325 $m\mu$. against a blank. The blank and a standard are prepared by substituting 2 ml. of water and 2 ml. of a 5 mg./100 ml. solution of β -naphthoxyethanol for blood in the above procedure. Normal blood (2 ml.) from the same animal is also analysed. The optical densities of the extracts of standard (b) and normal blood (n) are also read at 272 and 325 $m\mu$. Then the concentration (c mg./100 ml.) of β -naphthoxyethanol in the unknown is given by the formulae:

$$c_{272} = 5(a_{272} - n_{272})/b_{272} \quad \text{and} \quad c_{325} = 5(a_{325} - n_{325})/b_{325}.$$

The two results should not differ by more than 10%. Their average is taken.

Table 2. Effect on the apparent β -naphthoxyethanol concentration of normal blood and urine of extracting with chloroform at varying pH

Material	Apparent concentrations of β -naphthoxyethanol (mg./100 ml.)									
	0.2M- KH_2PO_4		0.2M- Na_2HPO_4		10% Na_2CO_3		N-NaOH		2.5N-NaOH	
	272 $m\mu$.	325 $m\mu$.	272 $m\mu$.	325 $m\mu$.	272 $m\mu$.	325 $m\mu$.	272 $m\mu$.	325 $m\mu$.	272 $m\mu$.	325 $m\mu$.
Rat blood	0.97	0.93	0.80	0.77	0.67	0.72	0.68	0.74	1.57	1.28
Rat blood	1.57	1.73	0.98	1.00	0.83	0.69	0.38	0.32	—	—
Rat blood	1.44	1.31	1.00	0.93	0.83	0.91	0.72	0.83	1.11	0.89
Rabbit blood	1.17	1.37	0.74	0.63	0.46	0.47	0.40	0.42	0.43	0.42
Rabbit urine	3.66	5.22	2.23	1.87	3.04	3.79	3.61	3.18	3.14	2.71

METHOD

Apparatus. Glass apparatus is washed in soap and alkali to remove adhering biological material, then in tap water, chromic-nitric acid mixture, tap water and distilled water

Table 3. Recovery of β -naphthoxyethanol from blood (2 ml.)

Added ($\mu\text{g.}$)	Found ($\mu\text{g.}$)	Recovery (%)
25	23.7	95
50	52.0	104
50	47.2	94
50	50.6	101
100	93.3	93
100	97	97
100	103	103
100	100	100
150	152	101
200	196	98
		Mean 99

Notes. An alternative method is available, using 10 ml. of benzene as solvent instead of 40 ml. of CHCl_3 . The extracts

Table 4. Comparison of the concentrations of β -naphthoxyethanol found in the blood of dosed rabbits by determination at 272 and 325 $m\mu$.

Concentration found (mg./100 ml.)			
272 $m\mu$.	325 $m\mu$.	272 $m\mu$.	325 $m\mu$.
5.52	5.22	2.28	2.27
3.85	3.77	2.08	2.02
3.47	3.39	2.04	1.94
2.87	2.74	1.54	1.32
2.41	2.41	1.18	1.13
		0.81	0.70

are examined in 2 cm. cells. The blank in normal blood is thereby reduced almost to zero, but the optical density can be read only at 325 $m\mu$., at which sensitivity is low. However, this method is useful when it is not possible to obtain a sample

of normal blood. The validity of the procedure using CHCl_3 has been established in three ways. (1) The recovery of β -naphthoxyethanol from blood (Table 3) and urine has been examined. (2) The specificity of the method has been checked by establishing the identity of the ratio d_{272}/d_{325} for β -naphthoxyethanol extracted from the blood of experimental

Fig. 5. Blood concentrations of β -naphthoxyethanol after intravenous administration of 80 mg./kg. to sheep (A) and rabbit (B).

animals, with that for authentic β -naphthoxyethanol (Table 4). (3) The method has been used for some time to determine β -naphthoxyethanol in the blood of sheep, horse and rabbit. Its accuracy is adequately illustrated by the smoothness of the typical curves shown in Fig. 5. The curves also illustrate the rapid elimination of β -naphthoxyethanol.

DISCUSSION

The development of photoelectric spectrophotometers (Beckman, Hilger, Unicam, etc.) has greatly facilitated the estimation of new drugs and related compounds, most of which contain aromatic or heterocyclic structures, and therefore show selective absorption in the ultraviolet. It is the practice in this laboratory, where ten to twenty new compounds are estimated annually, to examine a compound, first for fluorescence, and secondly for ultraviolet

absorption. Only rarely has recourse to be had to alternative methods of estimation. The disadvantages of ultraviolet analysis are lack of specificity, and, very often, low sensitivity. These disadvantages can be partly neutralized by the proper choice of extraction conditions. The extraction of interfering materials from normal blood, and often of metabolites (Brodie, Udenfriend & Baer, 1947) is reduced by selecting a solvent of low polarity; moreover, the latter is less likely to give emulsions when shaken with large volumes of biological fluids. On the grounds of both specificity and sensitivity, therefore, the order of preference is, for example, light petroleum, benzene, chloroform, butanol. The least polar solvent that extracts the compound quantitatively is chosen. In the present study light petroleum did not extract the compound quantitatively, and chloroform was preferred to benzene because it permitted analysis at two wavelengths, a useful device that increases accuracy and confers an increased degree of specificity.

β -Naphthoxyethanol is eliminated very rapidly from the blood in conformity with its use as a short-acting anaesthetic. In the experiments illustrated here (Fig. 5), and other similar ones, it was possible to correlate the degree of anaesthesia with the blood concentration of β -naphthoxyethanol. For example, the reappearance of the righting reflex in the rabbit was usually associated with a concentration of about 2 mg./100 ml. The rapid elimination is effected by degradative processes, which are described in the following paper (Spinks, 1950).

SUMMARY

1. β -Naphthoxyethanol has been determined in blood by extracting it with chloroform and measuring the optical densities of the extract at the absorption maxima of 272 and 325 m μ .

2. β -Naphthoxyethanol is rapidly eliminated from the blood, in conformity with its use as a short-acting anaesthetic.

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