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Brief Communication

DETERMINATION OF AMPHETAMINE IN URINE AND BLOOD

The determination of amphetamine in body fluids is of interest in veterinary toxicology because of the possible use of amphetamine in the doping of race horses. Many types of methods for its detection and determination have been developed. In the newest methods gas chromatography and mass spectrometry have been applied, making it possible to detect and identify 1 μg amphetamine in blood samples (*Bonnichsen et al.* 1970).

The rôle of amphetamine in the doping of race horses has recently been reviewed by *Schubert* (1967) in a supplement to this journal. In his own experiments with horses *Schubert* used thin layer chromatography and could detect amphetamine in samples of 200 ml urine after 150 mg amphetamine sulfate i.m. The concentration of amphetamine in the urine was estimated to be in the order of 0.1 mg per 100 ml urine (1 μg per ml).

In 1958 the authors developed a quantitative method for the determination of amphetamine in urine and blood based upon the principle of coupling amphetamine with p-nitrobenzenediazonium chloride (*Beyer & Skinner* 1940) at the School of Veterinary Medicine, Davis, California. The method was sensitive and offered reproducible results when conditions such as time, temperature, and especially pH were maintained at optimal values (Fig. 1). The method and the results were not published at that time since the newer chromatographic methods were supposed to be superior to the colorimetric methods. By using steam distillation of 25 ml alkalinized urine, extraction of distillate with hexane and diazotizing at pH 7.25 and 38°C it was possible to detect amphetamine down to 2.5 μg, corresponding to a maximal sensitivity of 0.1 μg per ml urine. The calibration curve was a straight line over a sufficient range of concentrations. The recovery of amphetamine sulfate in this method was about 65 %. In a similar way amphetamine could be determined in blood after precipitation with trichloroacetic acid and centrifugation.

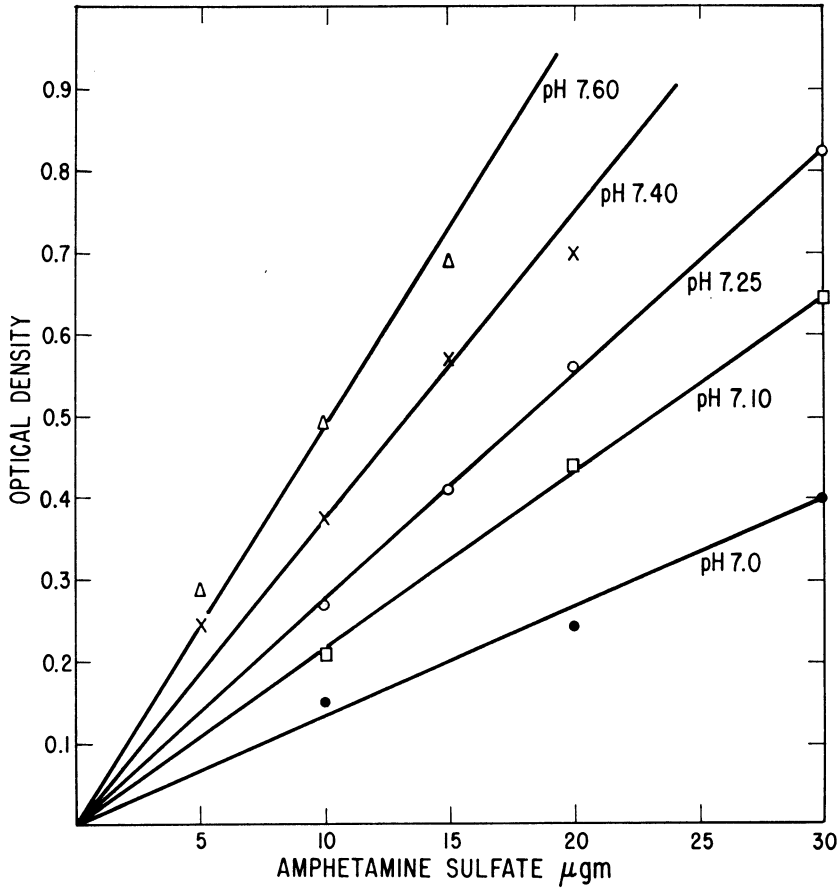

Figure 1. Calibration curves for amphetamine sulfate at different values of pH. (Diazo reaction max. 530 mμ, min. 430 mμ).

Our experiments with amphetamine in man, dog, and horse may be of interest since they give information about the detectable concentrations of amphetamine and the rate of excretion.

The experiments in human subjects (Table 1) indicate that after moderate doses of amphetamine detectable concentrations may persist in the urine for 48 hrs., confirming the results of previous investigations.

The experiments in dogs (Table 2) show that very large doses must be applied to give a detectable concentration in the blood.

Usual doses of amphetamine (Table 3) given intramuscularly to horses did not reach a detectable concentration in the blood

Table 1. Determination of amphetamine in human urine (μg/ml).

Dose orally mg/75 kg	Hours										
	3	4	5½	7	12	20	24	28	33	48	50
5	0.4		1.3		0.8	0.3					
5		2.0		3.5				0.8			neg.
10				1.6	0.6		1.6	2.7	1.3	0.4	neg.

Table 2. Determination of amphetamine in dog plasma (μg/ml).

Dose s.c. mg/kg	Hours							
	½	¾	1	1½	2	3	4	5
1.0					neg.		neg.	neg.
1.0	0.2		0.1		0.1			
3.0		0.5		0.3		0.2		0.16

Table 3. Determination of amphetamine in horse urine (μg/ml).

Dose i.m. mg/500 kg	Hours				
	2	6	9	12	24
100	0.2	0.4			neg.
200	0.1	1.0			neg.
200	4.6	5.2	1.4	1.0	neg.

samples. Amphetamine is more rapidly eliminated in horse, than in man, and could not be detected in the urine longer than 12 hrs. after the administration.

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