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Plasma Levels and Urinary Excretion of Injected Myanesin in Dogs.*

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The recent publications of Berger and Bradley[1,2] describing a new synthetic curarizing agent, α-β-dihydroxy-γ-(2-methylphenoxy)- propane (myanesin), and the initial clinical report by Mallinson[3] of its use as a substitute for curare in 118 cases, are of potential interest in the field of anesthesia.

The brevity of action of myanesin aroused our interest in its physiological disposition, and it was considered desirable to study blood levels and urinary excretion. This report describes a method developed for the estimation of myanesin in plasma and in urine, and experimental data of plasma levels and urinary excretion in dogs.

Principle of the Method. The method described here is dependent upon the nitration of myanesin in aqueous solution, and the development of a strong yellow-green color when made alkaline with sodium hydroxide. The yellow-green color is stable for at least 60 minutes, and its intensity is measured with the photoelectric colorimeter. The myanesin is extracted from blood plasma, or from urine, with chloroform and the chloroform evaporated to dryness. The residue is then dissolved in water and an aliquot analyzed. By acid hydrolysis of the urine before the extraction with chloroform much larger quantities of myanesin can be recovered, indicating the presence of a conjugated derivative of myanesin in urine.

Method of Analysis. Extraction. (1) Plasma.‡ Five ml of plasma obtained from oxalated blood are placed in a small separatory funnel, and 10 ml of chloroform (technical grade) are added. After thorough shaking for 8 minutes the chloroform layer is separated from the plasma, and filtered through Whatman No. 40 filter paper. Occasionally, especially in hot weather, the plasma and the chloroform may emulsify, but rarely is it impossible to obtain 5 ml of clear chloroform filtrate. A 5 ml aliquot of the chloroform filtrate is evaporated just to dryness on a water bath heated to 90°C, being careful not to overheat the residue. Two and five-tenths ml of distilled water are added to the residue, and gently warmed to aid solution of the myanesin. After a few minutes, 2 ml of this aqueous solution are placed in a test tube 16.5 x 150 mm, to be submitted to analysis. This 2 ml aqueous fraction represents the extracted myanesin from 2 ml of plasma.

(2) Urine.‡ (a) Extraction of free myanesin. Four and five-tenths ml of urine are placed in a small separatory funnel and 1.5 ml of buffer having a pH of approximately 7.3 (prepared by adding 0.1 M citric acid to 0.2 M Na_2HPO_4 in the proportion of 2:13) are added. Twelve ml of chloroform are added, and the procedure as outlined for plasma followed, except that an aliquot of 8 ml of chloroform filtrate is evaporated to dryness, and the residue is dissolved in 3 ml of water; the 2 ml of aqueous solution of urine extract placed in the test tube for analysis represent 2 ml of urine.

(b) Extraction of free and conjugated myanesin. In a small flask 2.5 ml of urine are placed, and 2.5 ml of conc. HCl (sp. gr. 1.178-1.188) are added. The contents are then heated on a boiling water bath for 10 minutes.

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2 Berger, F. M., and Bradley, W., *Lancet*, 1947, **1**, 97.

3 Mallinson, F. B., *Lancet*, 1947, **1**, 98.

‡ Reagent blanks of myanesin-free plasma and urine must be run concomitantly, since small amounts of plasma and urine constituents are extracted and nitrated.

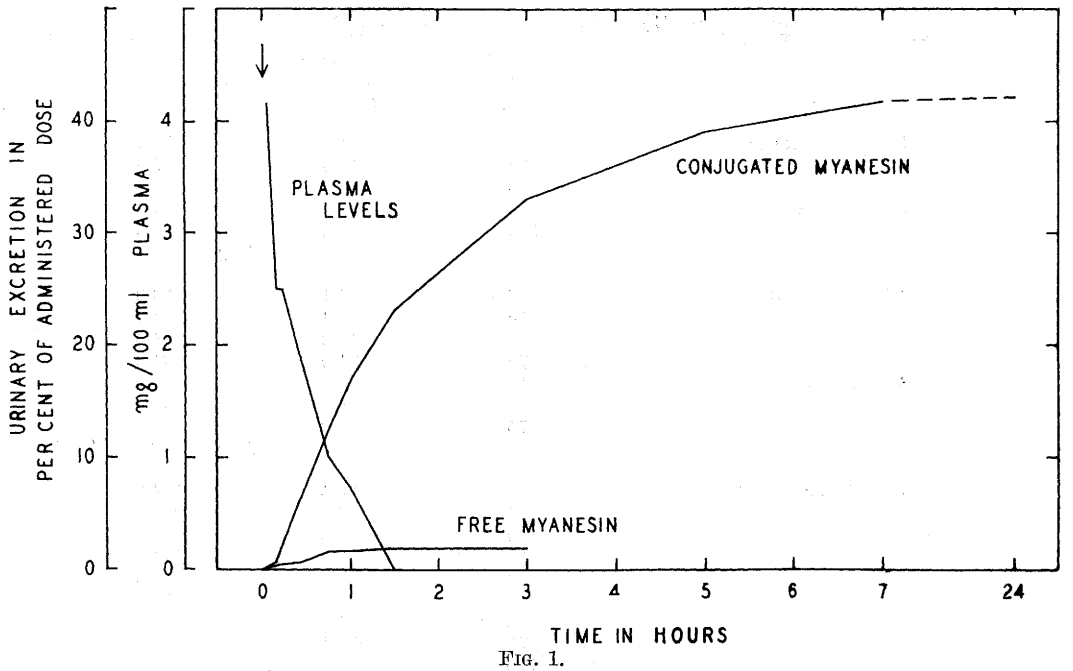

FIG. 1.
Plasma Levels and Urinary Excretion of Myanesin.
The arrow in the upper left hand corner represents the time of the intravenous injection of myanesin, 50 mg per kg. Values plotted correspond to those of Dog No. 4 in Table II.

(Autoclaving similar solutions for 2 and 4 hours at 115-120° C did not increase the recovery of myanesin.) After removal from the bath, 5 ml of water are added to the flask, and well mixed. Five ml are then withdrawn and placed in a small separatory funnel, and the procedure as outlined under plasma followed. The 2 ml of aqueous solution eventually analyzed represent 0.5 ml of urine; consequently the final concentration value must be multiplied by 4 to yield a value reading in mg per 100 ml for the 2 ml analyzed. Note: Analysis of urine for free myanesin must be accomplished within a few hours after the sample is obtained, since spontaneous hydrolysis of the conjugated fraction gradually increases the values of free myanesin.

Analysis. Two ml of the aqueous solution of the plasma or urine extract are placed in a test tube. To each tube is added 1 ml of conc. nitric acid (sp. gr. 1.42). The tubes are then placed in a boiling water bath for exactly 4 minutes. The tubes must not be inserted until the temperature of the water is at least 98°C. Less than 3 minutes in the bath gives inadequate nitration; greater than 5 minutes contributes heavily to the destruction of some of the myanesin present. At the end of 4 minutes the tubes are removed and placed immediately in a beaker of cold water. When the solutions have reached room temperature, 2 ml of 40% sodium hydroxide are slowly added to each tube, with swirling to insure complete mixture.

Ten minutes after the addition of the sodium hydroxide, the contents of each tube are transferred to a clean, dry Klett tube and read in the colorimeter. The plasma or urine reagent blanks are set to zero; readings can then be converted into concentration values directly by comparison with the standard curve.

A Klett-Summerson photoelectric colorimeter was used in our determinations. employing the No. 42 (blue) filter (400-460 millimicrons wave length transmission). Using the method outlined above, the standard curve was prepared with aqueous solutions of myanesin of known concentration, and

TABLE I.
Recovery of Myanesin Added to Plasma and to Urine.

Sample	Myanesin added (γ/ml)	Myanesin recovered (γ/ml)	% recovery
5 ml dog plasma	25	24.0	96.0
	50	48.5	97.0
	75	69.0	92.0
5 ml human plasma	10	10.0	100.0
	100	86.8	86.8
		Avg	94.4 ± 2.3%
4.5 ml dog urine*	7.5	5.1	68.0
	10	7.4	74.0
	50	42.3	84.6
	75	61.0	81.3
4.5 ml human urine	10	9.0	90.0
	25	24.0	96.0
	75	67.0	89.3
		Avg	83.3 ± 4.0%
2.5 ml dog urine†	10	12.4	124.0
	25	27.6	110.4
	50	47.8	95.6
	50	52.0	104.0
	50	38.0	76.0
	50	38.1	76.2
	50	34.5	69.0
	75	70.1	93.5
	100	95.6	95.6
		Avg	93.8 ± 5.9%

* Analyzed directly for free myanesin.
† Submitted to procedure for hydrolysis before analysis.

plotted to read in mg per 100 ml. The reading of the reagent blank was adjusted to zero on the colorimeter. A linear relationship exists between concentrations of myanesin and colorimeter readings for values from 0.5 to 10.0 mg per 100 ml (5 to 100 gamma per ml) of solution analyzed. Periodic checks on aqueous solutions of myanesin, ranging from 0.001 to 1.0% in concentration, showed that such solutions undergo no deterioration for at least 30 days, if stored in a refrigerator.

Experimental. Recovery Data. Table I shows recovery data obtained from analysis of dog and human plasma and urine to which known amounts of free myanesin have been added. Recovery from plasma yields values 94.4 ± 2.3% of the theoretical concentration values; from urine by analysis for free myanesin 83.3 ± 4.0% of the theoretical values; from urine containing free myanesin but submitted to treatment with hot hydrochloric acid 93.8 ± 5.9% of the known values. No conjugated myanesin was available to test hydrolysis and recovery by this method. Probably the increase in % recovery from urine after acid treatment was due to greater extractability. However, the method outlined gave maximal values with urine from dogs previously given myanesin.

Plasma Levels. (Table II). Four dogs after absence of dehydration was assured were given myanesin intravenously in doses of 50 mg per kg and one dog, 75 mg per kg, employing 2-3% supersaturated aqueous solutions.[1] Three were followed as to plasma levels by periodically withdrawing blood samples. Fig. 1 shows a typical plasma level curve (Dog No. 4 of Table II). The sharp decay of the curve of free myanesin correlates well with the response of the animal. Immediately following the injection of 50 mg per kg, the dogs exhibited a flaccid paralysis, affecting predominently the hind legs. No reflexes or responses to painful stimuli could be elicited. Within 2 minutes after the injection all dogs were able to stand, though unsteadiness and muscular weakness persisted for about 20 minutes. Three of the 5 dogs

TABLE II.
Plasma Levels and Urinary Secretion.

	Dog	Solution	Time in min. 5	10	15	25	40	60	90	180
Mg myanesin per 100 ml plasma	1	Plasma	4.90	2.85	2.42	2.35	0.85	0.70	0.0	—
	3	,,	2.68	2.10	—	1.22	1.50	0.88	0.0	0.0
	4	,,	4.16	2.50	2.50	1.90	1.00	0.73	00	—
			Time in min. 10	20	25	40	45	60	90	120	180	300
Free myanesin in % of administered dose	1	Urine	—	0.016	—	0.054	—	0.084	0.107	0.119	0.133	—
	3	,,	0.132	—	0.275	0.433	—	0.527	0.527	—	0.527	—
	4	,,	0.19	—	0.32	—	1.57	1.62	1.93	—	1.93	1.93
			Time in min. 10	25	40	60	90	180	240	300	420	24 hrs
Combined myanesin in % of administered dose	2†	Urine	—	—	—	—	—	—	33.7	—	38.1	40.2
	3	,,	1.51	5.31	10.41	14.92	23.02	27.06	—	30.98	31.18	31.18
	4	,,	0.62	6.2	12.4	16.8	22.4	32.6	—	39.0	41.9	42.0
	5*†	,,	—	—	—	—	—	28.2	—	—	—	34.0

* Given 75 mg per kg myanesin.
† Foley bag catheter not employed here.

given myanesin vomited within 2 minutes after the injection. No free myanesin could be detected in the 90-minute plasma sample of any of the dogs. Conjugated myanesin, though probably present in plasma, could not be detected quantitatively by the above method of acid hydrolysis.

Urinary Output. (Table II). Output of free myanesin was followed in 3 dogs by means of periodic urine samples withdrawn through an indwelling Foley bag catheter. To insure urine blanks that give low readings one must obtain dilute urine for the determinations. In this experimental work on dogs it was found that water diuresis, the water being administered by stomach tube the evening before and every 2 or 3 hours throughout the day of the experiment in quantities of 50 to 75 ml per kg of body weight, greatly enhanced the accuracy of the determinations, since an output of light straw-colored urine could thus be insured.

The rate of excretion of free myanesin was found to parallel the concentration of myanesin in the plasma. However, only from 0.133 to 1.93% of the administered dose was detected as free myanesin in the urine; and the excretion of free myanesin was complete when the plasma level reached zero. (Fig. 1). The concentrations of free myanesin obtained in urine are so low, that the failure of urine collected from rabbits, after large doses of the drug, to have any paralytic effect on mice[1] is readily understood.

Output of conjugated myanesin was followed in 4 dogs; in 2 samples were collected by means of the Foley bag catheter, and in two by recovering the urine from the cage as it was normally passed. From Fig. 1 and Table II it can be seen that the excretion of conjugated myanesin persists for 24 hours or more in some dogs, although the greatest quantity is excreted within 3 or 4 hours. From 32 to 42% of the administered dose is excreted in conjugated form in 24 hours. However, the water diuresis necessary for this procedure may have given results somewhat at variance from normal animals.

Conjugation Product. Myanesin bears a chemical resemblance to propylene glycol

which has been shown by Neubauer[4] and confirmed by Fellows *et al.*,[5] to stimulate an increase in the excretion of glucuronic acid (conjugation?). However, glycerol itself and ethylene glycol did not produce such an increase when administered gastrically or hypodermically in rabbits.[5]

Preliminary observations would seem to indicate that myanesin is conjugated with glucuronic acid, at least in part. Neuberg and Schewket[6] have devised a method for the extraction and identification of conjugated glucuronic acid which they claim to be specific. By employing this method of extraction unequivocal reactions were obtained with naphthoresorcinol,[6,7] although inconsistent reactions were obtained with orcinol,[6,7] on urine excreted after the administration of myanesin, whereas similarly treated control urines gave only the weakly positive reactions expected of normal urine.

Summary. A colorimetric method has been presented for the determination of myanesin in plasma and in urine. The rapid decay curve of myanesin in dog plasma explains the brevity of its pharmacological action. In the dog, from 0.1 to 2.0% of the administered dose is excreted as free myanesin; from 32 to 42% of the administered dose is excreted as conjugated myanesin in 24 hours, possibly as the glucuronide.

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The Null Effect of Hemorrhage on Intestinal Absorption of Chloride in the Presence of Sulfate.*

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Goldschmidt and Dayton[1] showed that sodium sulfate increases the absorption of sodium chloride in the large intestine of dogs. Burns and Visscher[2] later demonstrated that the presence of sodium sulfate and certain other anions of the lyotropic series caused the chloride ion, at a concentration below that in the blood plasma, to leave the small intestine and enter the blood against its diffusion gradient.

Van Liere, Northup, and Sleeth[3] noted that isotonic sodium chloride solution was absorbed significantly faster from the intestine of dogs which had sustained a severe hemorrhage (3.2% of their body weight). Since hemorrhage thus simulated the effect of the sulfate ion, it was of interest to study their combined effect.

Methods. Dogs, selected in pairs as nearly alike in weight and age as possible, were fasted 24 hours previous to the experiment. One served as a control and the other was subjected to hemorrhage. Fifteen experiments were performed on a total of 29 animals (one control died).

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2 Burns, H. S., and Visscher, M. B., *Am. J. Physiol.*, 1934, **110**, 490.

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