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METHODS FOR DETERMINING THE PRESENCE OF TRANQUILIZERS AND OTHER NEW DRUGS IN URINE OF DOGS[1])

By

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The purpose of this work has been to investigate whether the presence of certain tranquilizers and other new drugs can be detected especially in the urine of dogs. One may, as a matter of fact, suspect that dog owners give nervous dogs tranquilizing drugs in connection with dog shows or field trials. In other cases it may happen that stimulating drugs — i. e. "dopes" — are given in order to increase a dog's capability. This may lead to misjudgement of the dog in question.

The possibility of detecting the presence of stimuli has been subject to many investigations in connection with the doping of race horses (4, 9, 12, 13, 14, 16, 19).

For this reason the present investigation has included substances which act tranquilizing and quiet mental reactions. Within this group of substances — psycholeptics — one distinquishes between sedatives, hypnotics and ataraxics or tranquilizers. The first-mentioned substances include barbituric acid derivatives. From an analytical point of view, they have already been thoroughly investigated (1, 2, 3, 6, 10, 18).

Ataraxics show a quieting effect but do not induce sleep. Such substances are especially effective in cases of psychic tension

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Dedicated to Professor *Hans von Euler* on his 90th birthday.

[1]) Investigation carried out by a grant from the Swedish Kennel Club.

and consequently very suitable for giving mentally disturbed dogs in order to withhold possible mental disorders.

On the whole, there are three groups of substances which may be regarded as tranquilizers.

1. Rauwolfia alkaloids
2. Promazines
3. Meprobamate

The following drugs belonging to the above-mentioned groups have been investigated:

Serpasil, Ciba (Reserpin)
Hibernal, Leo (Chlorpromazine)
Centractil, Astra (Promazine)
Lergigan, Recip (Promethazine)
Meprobamat, ACO

Besides, experiments have been made for obtaining methods to prove the presence of the following psycholeptic drugs:

Neurosedyn, Astra (Thalidomide)
Myanesin, Hässle (Mephenesin)
Haldol, Leo (Haloperidol)
Atarax, U.C.B. (Hydroxizine)
Truxal, Lundbeck (Chlorprothixen)
Azacon, Draco (Protipendylchloride)
Metadon, ACO (Methadone)
Diparalene, Abbott (Chlorcycline)
Dramamine, Searle (Dimenhydrinate)
Melufin, Hoechst
Polamivet, Hoechst
Cliradon, Ciba
Palfium, Leo
Preludin, CHB

The first group — Rauwolfia alkaloids — consists of substances derived from the roots of a shrub growing in India — the *Rauwolfia serpentina*. Reserpine, one of these alkaloids, has a decided quieting effect and this effect is utilized. In man, where the drug is perhaps above all used for counteracting high blood pressure, conditions of depression have, however, appeared after prolonged treatment. But this side effect does not have to be taken into consideration as far as the dog is concerned.

The next group — promazine — is a derivative of phenothiazine. Such compounds have proved effective in many connections and have been used in various fields. They are used in treatment for parasitic worms, as histamine antagonists, and as tranquilizers. It was seen at an early stage that antihistamines containing a nucleus of phenothiazine acted as a sedative as well as a hypnotic. At first this was considered an undesired side effect. Nevertheless, it was gradually realized that this effect in reality was a valuable property worth developing. This led to the discovery of chlorpromazine a phenothiazine derivative and a decided tranquilizer. A number of different promazine compounds have since then been produced, all of which showing on the whole, the same mental effect.

A third link in the production of psychopharmaceutics was the introduction of meprobamate. This is also a decided tranquilizer acting on mental as well as muscular tension.

A number of fairly recent drugs not belonging to any of the above-mentioned groups but acting very similarly, are also available.

There is consequently a fairly great variety of drugs that may affect a dog mentally and as a result its behaviour. Unfortunately, such drugs are detrimental to the mental health of dogs, since individuals which should have been culled out as mentally defective might appear as well-balanced and be awarded high prizes, including whatever this might mean to breeding. Therefore, the work of finding methods to prove the presence of such drugs in body fluids or excretions must be considered very urgent.

Kinberger (6, 7) has worked out methods to detect ataraxics in urine of humans. He uses colour reactions with nitric acid and sulfuric acid and diazotization with sulfanilic acid. As far as urine of dogs is concerned, the only reliable of these methods is the colour reaction with concentrated sulfuric acid.

Burger and *Berninger* (4) use paper chromatography and spectrometry in UV. By these methods it is possible to separate and identify different phenothiazine derivatives.

Meprobamate can not be shown by these methods. *Hoffman* and *Ludwig* (5) have, however, worked out a sensitive colorimetric method which has also proved useful for testing the urine of dogs.

METHODS

It was desirable to find a method as general as possible, and one suitable also for the determination of "dopes".

The substances to be tested were first analyzed in a pure form or as a commercial preparation. They were dissolved or dredged in water. The solution was shaken out with chloroform first from acid and then from alkaline solution. Most substances were basic and were extracted in the chloroform phase at alkaline reaction. For extraction of phenothiazine derivatives, *Burger* and *Berninger* (4) recommend a mixture of hexane, benzene and ethyl ether in equal parts. The present authors have, however — found that chloroform is suitable. By it, other drugs are also extracted.

The layer of chloroform was removed and filtered through cotton. It was diluted to a suitable volume. Of this solution amounts were taken corresponding to between 5 and 50 μg. The samples were subjected to the following tests.

Table 1.

Preparation	Conc.	H_2SO_4	Paper Chromatography				Absorption in UV		Reaction at shaking out
	μg	Colour	μg	UV	K_2PtJ_6	Rf	Max.	Min.	
Serpasil	—	—	—	—	—	—	265	245	alkaline
Plegicil	50	rose	5	+++	+	0.74	280	250	„
Hibernal	25	redish violet	10	+	+	0.83	257	226, 280	„
Centractil	10	rose	10	+++	+++	0.80	255	221, 280	„
Lergigan	25	red	10	+	+	0.85	255, 305	223, 277	„
Meprobamat	—	—	—	—	—	—	295	275	„
Neurosedyn	—	—	—	—	—	—	295	255, 260	„
Myanesin	10	rose	10	++	—	0.90	272	250, 252	alk. & acid
Haldol	—	—	5	+	+	0.67	222, 243	232	alkaline
Atarax	25	yellow	5	—	+	0.83	230	—	„
Truxal	25	rose	5	+	+	0.83	227, 270	255	„
Azacon	25	rose	5	—	+	0.77	280	260, 265	„
Metadon	5	yellow	5	+	+	0.84	293	275	acid
Di-Paralene	25	yellow	10	+	++	0.83	231	219	alkaline
Dramamine	10	yellow	10	—	++	0.84	260	250	„
Melufin	—	—	10	+++	+++	0.88	—	—	„
Polamivet	—	—	10	+	+	0.88	—	—	acid
Cliradon	—	—	25	—	+	0.74	—	—	alkaline
Palfium	—	—	25	+	++	0.82	—	—	„
Preludin	—	—	10	—	—	0.74	—	—	„

Table 2.

The following special reactions gave a positive result. For composition of reagent see *Stewart & Stolman* (17).

Serpasil:	Dragendorff's reagent cubes, Marquis' reagent blue-greyish, green-brown colour, Fröhde's reagent blue colour, Mandelin's reagent blue-brownish, purple.
Plegicil:	$AuCl_3$ small needles, Dragendorff's reagent squares, rectangles.
Hibernal:	$AuBr_3$ needles, $AuCl_3$ plates, $HgCl_2$ needles, stars, $PtCl_4$ violet, round, Dragendorff's reagent cubes, needles, Marquis' reagent redish violet-purple colour, Fröhde's reagent redish violet colour.
Centractil:	$AuCl_3$ long rods, KJ_3 needles, $KHgJ_3$ needles, $PtBr_4$ prisms, Marquis' reagent pink-orange colour.
Lergigan:	K_2CrO_4 needles, $PtCl_4$ rhombi, small needles.
Meprobamat:	$HgCl_2$ long needles, stars, Fröhde's reagent blue colour, p-dimethyl-amino-benzaldehyde + $SbCl_3$ redish violet colour, sensitive 1 μg.
Neurosedyn:	K_2CrO_4 needles, Fröhde's reagent blue colour.
Myanesin:	PbJ_2 bundles, stars, $HgCl_2$ bundles, stars, Dragendorff's reagent rods, rosettes.
Haldol:	$HgCl_2$ bundles, stars,
Atarax:	Marquis' reagent blue-greyish μg green-brown colour, Fröhde's reagent blue colour, Mandelin's reagent bluish purple colour.
Truxal:	Fröhde's reagent redish violet colour.

Table 2 (cont.)

Metadon:	$HgCl_2$ needles, rosettes, $PtCl_4$ needles, rosettes, PtJ_4 needles, rosettes, Dragendorff's reagent rods, KJ_3 needles, branches, Marquis' reagent orange red-purple, Fröhde's reagent brown-greyish, green, Waschy's reagent purple (in heat).
Di-Paralene:	$PtCl_4$ small needles, Marquis' reagent yellow colour, Fröhde's reagent blue colour.
Dramamine:	$AuCl_3$ stars, needles, PbJ_2 squares, rectangles, $HgCl_2$ small needles, Picric acid needles, rosettes, $PtCl_4$ leaves, rosettes.
Melufin:	PbJ_2 stars, $HgCl_2$ stars, rods, $PtCl_4$ stars, leaves, Dragendorff's reagent small stars.
Polamivet:	$HgCl_2$ bundles, stars.
Cliradon:	$HgCl_2$ bundles, $PtCl_4$ leaves, Dragendorff's reagent needles, stars.
Preludin:	$AuCl_3$ leaves, Dragendorff's reagent long needles, stars.

1. Concentrated sulfuric acid was added to the chloroform solution and possibly appearing colour was checked.

2. Paper chromatography. a) For substances shaken out from acid solution the following solvents were used: Equal parts of n-butanol and iso-amyl alcohol saturated with concentrated ammonia. Paper: Munktell OB, descending chromatography. b) For substances shaken out from alkaline solution the solvent consisted of 60 parts of n-butanol, 15 parts of concentrated acetic acid and 25 parts of water. The same paper but buffered with M/5 potassium dihydrogen phosphate.

After drying the paper chromatogram, one checked possible fluorescence. Then the paper was sprayed with potassium iodo-

platinate solution (10 ml. of 5 per cent platinum chloride solution mixed with 240 ml. of 2 per cent potassium iodide).

3. Spectrometry. In other experiments the rest of the solution was dissolved after the chloroform had been evaporated in a water bath in ethanol 95 per cent pH 4.5. The absorption spectrum of this solution was determined with Beckman DU between the wavelength of 215 and 315 $m\mu$.

4. Furthermore, attempts were made to prove the substances in question by means of microcrystalline identification or colour reactions with different reagents. Concerning the proof of meprobamate, reference is made to *Hoffman* and *Ludwig* (5).

In experiments with dogs similar methods were used. The dogs were given varying doses of the drugs, on the one hand, perorally and, on the other hand, intramuscularly. After certain intervals, the urine was collected. Between 10 and 20 ml. of urine was shaken out with chloroform partly from acid and partly from alkaline solution. The chloroform was evaporated. The residue was dissolved in 25 ml. of 0.1 n-hydrochloric acid, filtered and perhaps alkalized. After that it was again shaken out with chloroform. The chloroform solution was examined according to methods 1 and 2.

RESULT

Tables 1 and 2 give the results obtained with pure substances and with commercial drugs. The number of plus signs indicates the degree of reaction. Minus shows that the reaction has been negative. In Table 1 the smallest provable amount of the preparations is also given.

The results show that it is possible to detect and distinguish between the substances investigated, even if there is some uncertainty in a few cases, where the Rf values and the absorption curves coincide more or less.

Experiments with Dogs

The following 10 substances were tested on dogs: Serpasil, Plegicil, Hibernal, Lergigan, Haldol, Atarax, Truxal, Azacon, Metadon and Meprobamat.

The positive results are found in Table 3.

Table 3.

Preparation	mg./kg. of body weight	Way of administration	Hours after administration of drugs	Conc. H_2SO_4	Paper chromatography		
					UV	K_2PtJ_6	Rf
Plegicil	0.25	i.m.	2.5	—	++	++	0.73
			24.0	+	+	—	0.45
	1.0	„	3.5	++	+++	+	0.70
			24.0	+	+	—	0.47
	0.25	p.o.	1.25	—	+	—	0.76
			2.25	—	—	—	—
			24.0	—	—	—	—
	0.5	„	1.0	+	—	—	—
			3.0	++	+++	+++	0.75, 0.47
			24.0	—	+	—	0.40
Hibernal	1.0	i.m.	1.5	—	—	—	—
			3.5	+	—	+	0.70
			24.0	++	—	—	—
	2.0	„	2.0	+	—	—	—
			3.5	+	—	—	—
			24.0	+	—	+	0.83
	4.0	„	1.0	+	—	—	—
			2.0	+	—	—	—
			3.0	+++	+	+	0.74
			24.0	+++	+	+++	0.74
	4.0	i.m.	3.0	+	+	+	0.75
			4.0	—	+	+	0.76
			5.0	++	++	++	0.81
			24.0	++	+	+	0.71
	1.0	p.o.	1.0	+	+	+	0.81
			2.5	++	+	++	0.81
			3.5	+++	+++	+++	0.81
			24.0	+++	+	—	0.81
	2.0	„	1.0	+	—	+	0.85
			2.0	++	+	++	0.77
			3.0	+++	+	++	0.85
			24.0	+	—	+	0.85
Lergigan	1.0	i.m.	2.5	—	—	+++	0.65
			4.0	+	—	—	—
			24.0	+	—	—	—
	2.0	„	3.5	+	+	—	0.85
			24.0	+	+	+	0.81
	4.0	„	3.0	+++	+	+	0.74
			24.0	+++	+	+++	0.74
	1.0	p.o.	24.0	++	+	+	0.85
			48.0	++	—	—	—
	2.0	„	24.0	+	+	++	0.85
			48.0	+	—	—	—
	4.0	„	24.0	+	+	++	0.82
			48.0	+	—	—	—

Table 3 (cont.)

Preparation	mg./kg. of body weight	Way of administration	Hours after administration of drugs	Conc. H_2SO_4	Paper chromatography		
					UV	K_2PtJ_6	Rf
Haldol	2.0	p.o.	1.0	—	—	+	0.49
			24.0	—	—	+	0.67
			48.0	—	—	—	—
Atarax	3.0	i.m.	1.0	+	—	—	—
			2.0	+	—	—	—
			24.0	++	—	+++	0.87
	6.0	„	1.0	+	—	—	—
			3.0	++	—	+	0.87
	3.0	p.o.	5.0	++	+++	+++	0.81
	6.0	„	5.0	++	+++	+++	0.82
Truxal	1.0	i.m.	2.0	++	—	—	—
			3.0	+	—	—	—
			5.0	+	—	—	—
			24.0	++	—	—	—
	2.0	„	1.0	—	—	—	—
			3.0	—	—	—	—
			24.0	—	—	++	0.75
	2.0	p.o.	24.0	+	—	—	—
			48.0	—	—	—	—
Azacon	1.0	i.m.	1.0	+	—	—	—
			2.0	+	—	+	0.77
			3.0	+	—	++	0.77, 0.44
			24.0	+	—	—	—
	2.0	„	1.0	—	+	+++	0.78, 0.40
			2.0	+	+	++	0.78, 0.42
			3.0	—	+	+++	0.76, 0.42
			24.0	++	++	++	0.79, 0.42
	1.0	p.o.	3.0	—	—	—	—
			5.0	—	—	++	0.46
			24.0	—	—	+++	0.73, 0.45
	2.0	„	3.0	+	—	+++	0.72, 0.44
			5.0	—	—	+++	0.72, 0.38
			24.0	—	—	—	—
Meprobamat	2.0	p.o.	3.0	+++	(spec. reaction)		
			5.0	+++			
			24.0	+			
	5.0	„	5.0	+++			
			24.0	+			

Plegicil could be detected in the urine between one and three hours after administration. Evidently this substance was transformed to a certain extent in the organism, since chromatography showed spots with an Rf value of between 0.40 and 0.47 in addition to the spot produced by Plegicil showing a Rf value of 0.74. After 24 hours only the first-mentioned spot appeared.

Hibernal, Lergigan and *Atarax* gave positive reactions between 3 and 24 hours after administration.

Serpasil, Haldol and *Metadon* could not for sure be proved by the methods used. Therefore, continued investigations must be considered desirable.

Truxal gave a reaction with conc. H_2SO_4 within between 2 and 24 hours but could not for sure be identified chromatographically.

Azacon is transformed to some extent, since in addition to the real Rf value at about 0.77 a spot was obtained at a Rf of between 0.40 and 0.46. It could, however, in most experiments be proved within between 1 and 24 hours.

Meprobamat was proved by the mentioned special reaction between 3 and 5 hours after administration and gave a weaker but evident reaction after 24 hours.

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SUMMARY

An attempt has been made to work out general methods of determining the presence of recent drugs, among others, tranquilizers. Colour reaction with conc. H_2SO_4, paper chromatography, and absorption in UV and certain special reactions have been tried. Ten of these substances have been given to dogs. After that attempts have been made to determine the presence of them in the urine from the dogs. Seven of these preparations were identified for sure.

ZUSAMMENFASSUNG

Methoden zur Identifiezierung von Transquilizern und einiger anderen neueren Artzneimitteln im Harn von Hunden.

Versuche, um generellen Methoden zum Nachweis von neueren Artzneimitteln, besonders Transquilizern, zu finden, sind ausgeführt worden. Hierbei sind folgende Methoden benutzt worden: Farbereaktion mit konz. Schwefelsäure, Absorption in UV, Papierchromatographie und gewisse Spezialreaktionen. 10 von den untersuchten

Stoffen wurden Hunden gegeben, wonach Versuche die Stoffe im Harn wiederzufinden gemacht wurden. Sieben von den Stoffen könnten sicher wiedergefunden werden.

SAMMANFATTNING

Metoder att påvisa ingivna tranquilizers och en del andra nyare farmaka i urin från hund.

Försök har gjorts att utarbeta generella metoder för att påvisa nyare läkemedel däribland tranquilizers. Härvid har använts färgreaktion med konc. H_2SO_4, papperskromatografi, absorption i UV samt vissa specialreaktioner. 10 av dessa ämnen har givits till hundar, varefter försök gjorts att påvisa dem i urinen. 7 av preparaten kunde säkert påvisas.

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