

*4-n-Propyl-2-phenyl-phenol*

Benzoate	Petroleum ether	96	C	84.2	83.5
			H	6.35	6.33
Cinnamate	Petroleum ether	72.5	C	84.7	84.2
			H	6.39	6.44

*5,7-Dichloro-8-hydroxyquinoline*

Benzoate	Alcohol	129.5-130.5	Cl	22.09	22.33
<i>K salt of 4-n-propyl-2-phenyl-phenyl hydrogen sulphate</i>	Water	Decomposes 180-190	S	9.07	9.69
<i>K salt of 6-chloro-2-phenyl-phenyl hydrogen sulphate</i>	Water	Decomposes 225-230	S Cl	9.80 10.29	9.92 10.98

The biological tests on compounds reported herein were made in the Biological Research Laboratories of E. R. Squibb and Sons and we gratefully acknowledge their assistance.

## SUMMARY.

A number of esters of the three phenols and some of their substitution products were prepared and shown to have no value as urinary antiseptics.

## REFERENCES.

- (1) Leonard, V., *J. A. M. A.*, 83, 2005 (1925).
- (2) Baumann, *Ber.*, 9, 55 (1876).

## SALIVA TESTS. III. DETECTING THE ADMINISTRATION OF SOME OPIUM DERIVATIVES TO HORSES.\*

BY JAMES C. MUNCH.<sup>1</sup>

Previous papers in this series (1, 2) report the development of a method for detecting morphine and heroin in the saliva of horses after the subcutaneous or intramuscular injection of known drugs (that is, the investigators knew that the horses had received morphine or heroin at the time tests upon mice were conducted).

TABLE I.—THRESHOLDS FOR MOUSE TESTS WITH OPIUM ALKALOIDS.

Product.	Mg./Kg.	10. Med. 70% Effective Dose. Gamma/20-Gm. Mouse.
Morphine	4.00	80
Codeine	3.00	60
Dionine	1.00	20
Dilaudid	0.6	12
Heroin	0.05	1

The normal salivas collected from over one hundred untreated horses have been injected into mice; in no instance has an effect been observed resembling that produced by the opium alkaloids. The solution obtained by dissolving morphine or heroin in such a saliva, or in normal horse serum, produced the same effects

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<sup>1</sup> Sharp and Dohme, Philadelphia, Penna.

upon mice as the same concentration in water. The quantities of these drugs producing definite symptoms in seventy per cent of the injected mice (ten or more mice being used on each dose) are given in Table I. In making critical tests, each mouse should be scrutinized before use, as we have found some normal mice tending to show some (but never all) of the symptoms produced by the opium alkaloids. Unless this precaution is taken, misleading results may be obtained.

Since the "doping" of race horses is not permitted by Racing Commissions, it seemed advisable to conduct a series of experiments simulating race-track conditions. Twenty horses were injected subcutaneously with colorless solutions, the composition of which was unknown to the investigators. Gelatin capsules, whose contents were unknown, were administered by mouth to seventeen horses. The general procedure outlined in the first paper of this series was followed:

The saliva was collected from each horse immediately before injection or administration, and at definite intervals afterward (15, 30, 45, 60, 90 and 120 minutes, and sometimes 3 hours and 24 hours). In some instances  $\frac{1}{4}$  grain of arecoline hydrobromide was administered or injected. This facilitated the collection of saliva, but did not influence the results of the test.

The mouth of the horse was not drenched or washed, but every effort was made to obtain undiluted saliva. The opening of a 5-cc. vial was brought in immediate contact with the gingival margins, cheeks and tongue, and the thick slime obtained directly. When the contents were not sufficiently fluid, 1 cc. of distilled water was added to each vial and thoroughly shaken with the slime. A volume of 0.5 to 1 cc. was then injected into one or more mice weighing about 20 Gm.

TABLE II.—SPECIFICITY OF MOUSE TESTS FOR DETECTION OF "DOPED" HORSES.  
DRUG SOLUTIONS INJECTED SUBCUTANEOUSLY.

Horse No.	Weight Kg.	Veterinary Deductions.	Deductions from Mouse Tests.		Product Injected.	Treatment of Horse. Dose Injected.	
			Horse Treated.	Size Dose.		Mg./Horse.	Gamma/Kg.
339	560	Excitable	No	...	Water	...	...
372	455	Normal	No	...	Water	...	...
341	455	Normal	Yes	Small	Morphine	100	220
340	520	Excited	Yes	Large	Morphine	200	380
380	465	Normal	Yes	Small	Heroin	2	4
379	450	Excited	Yes	Large ?	Heroin	4	9
371	445	Normal	Yes	Large ?	Heroin	6	13
342	435	Normal	Yes	Small ?	Heroin	6.5	15
343	430	Excited	Yes	Large	Heroin	6.5	15
347	420	Refractory	Yes	Large	Heroin	15	36
346	475	Very excited	Yes	Large	Heroin	50	105
377	480	Normal	Yes	Small	Codeine	25	50
378	495	Normal	Yes	Large ?	Codeine	100	200
374	410	Normal	Yes	Large	Codeine	200	490
375	445	Normal	Yes	Small	Dilaudid	2	5
370	455	Stimulated	Yes	Large ?	Dilaudid	4	9
382	465	Stimulated	Yes	Large	Dilaudid	6	13
349	445	Stimulated	Yes	Large	Dilaudid	10	22
352	510	Stimulated	Yes	Large	Dilaudid	40	79
345	455	Stimulated	Yes	Large	Dilaudid	100	220

The effects upon the mice were noted over a period of half an hour, although positive reactions usually developed within ten to twenty minutes. In some instances injected mice died over night, but no relation could be established between death and the drug administered. In some tests, in which the saliva was obtained by swabbing the horse's mouth, and diluting to approximately

300 cc. with water, unsatisfactory results were obtained; the amounts of the active substances in the saliva had been too greatly diluted. Such samples must be concentrated by suitable chemical methods before testing.

The solutions and the capsules were prepared by a member of the laboratory (A. Q.) and given code numbers. This worker retained the code and took no part in the administration or mouse testing. Based upon our observations upon mice, an attempt was made to answer two questions: (1) Do the mouse tests on the salivas from a horse suggest that the animal has been "doped?" (2) If "doped," was a large or a small amount of drug administered?

After we recorded our opinions as to "doping" and dose, contact was reestablished with A. Q. and the code consulted for the first time. The detailed data of these tests are given in Tables II and III.

TABLE III.—SPECIFICITY OF MOUSE TESTS FOR DETECTION OF "DOPED" HORSES.  
DRUG ADMINISTERED IN GELATINE CAPSULES.

Horse No.	Weight Kg.	Veterinary Deductions.	Deductions from Mouse Tests.		Product Administered.	Treatment of Horse. Dose Given.	
			Treated.	Size Dose.		Total (Mg.).	Gamma/Kg.
9681	400	Normal	No	....	Water	..	..
342	435	Normal	No	....	Water	..	..
343	425	Normal	No	....	Water	..	..
9960	475	Uneasy	Yes	Large	Morphine	100	210
369	415	Slight stimulation	Yes	Large	Morphine	65	158
381	425	Normal	Yes	Small	Heroin	2	5
373	415	Normal	Yes	Small	Heroin	5	12
141	445	Restless	Yes	Large	Heroin	10	22
376	450	Normal ?	Yes	Large	Codeine	65	145
366	450	Stimulated ?	Yes	Large	Dilaudid	2	4
365	460	Stimulated	Yes	Large	Dilaudid	5	11
9609	470	Restless	Yes	Medium	Dilaudid	10	21
9925	490	Stimulated	Yes	Small	Pantopon	10	20
368	435	Stimulated ?	Yes	Medium	Pantopon	10	23
367	460	Stimulated	Yes	Medium	Opium-gran.	650	1415
9851	370	Normal ?	Yes	Large	Opium-gran.	1000	2720
9988	405	Quiet	Yes	Large	Opium-gum.	1000	2475

The results obtained in making tests on the saliva of twenty horses which were injected with unknown solutions are given in Table II. It will be observed that the opinions of the veterinarians were not always in agreement with the subsequent information in indicating whether a horse had or had not been "doped." The mouse tests were correct in every instance, in showing whether a horse had or had not been "doped," and also in suggesting whether a large or a small dose had been administered. In the case of heroin and Dilaudid, the smallest doses injected were 2 mg. per horse. These doses correspond to the single doses of these drugs administered to humans. It will be noted that the veterinarians were unable to observe any evidence of abnormality in these horses.

Through the courtesy of Jervis Spencer of the Maryland Racing Commission, and of Doctors W. B. D. Penniman and T. A. Ladson, we obtained samples of saliva collected from a Maryland race horse, using the customary race-track pro-

cedure: the horse's mouth was washed with water, the gauze and gloves being added to the washings and the entire collection made up to about 300 cc. with distilled water. Samples of saliva were obtained 30 and 45 minutes after administering 10 grains of morphine sulphate in a gelatine capsule: the horse was galloped for about two miles, and saliva samples collected approximately one hour and one and one-half hours after administration. The injection of 1 cc. of each sample as collected produced a positive reaction when tested on May 14th, or the day after the saliva had been collected. These saliva samples were stored in a refrigerator and retested with the same positive results after one week, and after two months. Since the horse was known to have received morphine sulphate, the results are not included in Table III. The veterinarian was unable to observe any abnormalities in this horse, nor could the jockey detect any differences in his behavior.

Based upon this test, the experiments recorded in Table III were undertaken. Veterinary observations were not always indices of the treatment accorded the horses. The mouse test was again correct in each instance in answering the question whether a horse had or had not been "doped." Morphine appeared to be somewhat more potent than the corresponding dose of opium. Pantopon was moderately effective.

Our studies are being continued in an attempt to detect characteristic symptoms for each of these products, and some success has already been attained. We do not feel justified at this time in asserting, based *solely* upon these mouse tests, which of these opium derivatives was given to a horse. However, we do feel that a positive mouse test would justify the time, labor and expense of a chemical search. Chemical tests of the saliva may be able to identify these products.

#### CONCLUSION.

1. Mouse tests upon the undiluted salivas collected from 37 horses correctly showed, in every instance, whether the horse had or had not received an opium derivative (opium, morphine, heroin, codeine, Dilaudid or Pantopon).

2. Present information does not permit identification of the specific product administered in all cases. Characteristic symptoms for such identification are being sought.

FOOT-NOTE: Through the courtesy of Commissioner of Narcotics H. J. Anslinger, supplies of morphine, heroin, Pantopon and opium were made available for this investigation. Dilaudid was purchased on the open market. The technical assistance of Arnold Quici in the preparation of these solutions and capsules, and Dr. J. C. Horner, Dr. W. A. Paxson, Robert Moore, Harry J. Pratt and Aaron B. Sloane in the administration of these drugs, the collection of saliva and the conduct of the mouse tests is gratefully acknowledged.

#### REFERENCES.

- (1) Munch, J. C., "Saliva Tests I. Morphine," *Jour A. Ph. A.*, 23, 766 (1934).
  - (2) Munch, J. C., "Saliva Tests II. Heroin," *Ibid.*, 23, 1185 (1934).
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