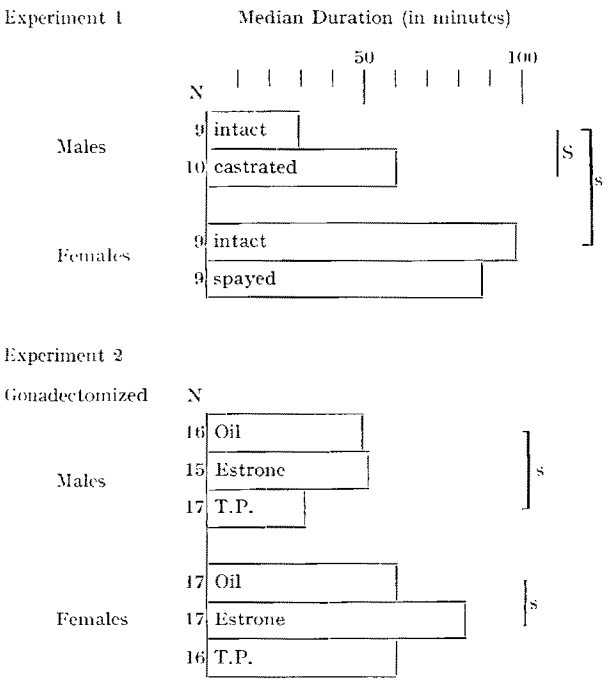
and the experiment was run on three separate occasions (18 and 25 May and 15 June). Treatment was rotated from week to week so that all animals received both compounds and served as controls.



Effects of castration and gonadal hormones on the duration of hexobarbital hypnosis in rats. N = number of rats in group. S = difference between bracketed groups significant at the 1% level; s = difference between bracketed groups significant at the 5% level; by Wilcoson Rank Sums Method[2].

The results of the two experiments are shown in the Figure. These data confirmed the sexual difference reported by BRODIE, females sleeping significantly longer than males. Castration of the males caused an increase in length of sleeping-time, so that the castrated males did not differ significantly from the normal females ($P > 0{\cdot}10$), although the median duration of sleeping time was much shorter for the castrated males. Hypnosis was shorter for spayed than for intact females, but this difference was not significant ($P > 0{\cdot}10$). This experiment was repeated on another group of rats on August 28, 2 weeks after gonadectomy. Results were essentially similar, except that the spayed females slept longer than the normal females. Median sleeping times for the four groups were: normal males 22 min, castrate males 64 min; normal females 105 min, spayed females 120 min.

In the second experiment control males and females showed essentially similar responses.

Of particular interest was the fact that testosterone propionate shortened the sleeping period of the males while estrone had no effect. In the females estrone produced a lengthening of hypnosis whereas testosterone propionate had no effect.

These data suggested that both male and female sex steroids affected hexobarbital hypnosis, but that the responses of the animals to the steroid depended upon the pre-castration state of the animal. BRODIE has shown that hexobarbital metabolism resulted from enzyme concentrations in the liver which markedly differed from males to females, the latter showing a much smaller concentration. The data from the present experiments suggested that removal of the primary sources of sex steroid production resulted in an intermediate rate of hexobarbital destruction, which in normal animals is delayed by female estrogens and increased by male androgens, and that replacement therapy normalized the responses in the appropriate sex. The failure of testosterone propionate to shorten sleeping time of spayed females and of estrone to increase it in castrated males suggested that pre-castration sexual development had affected liver enzyme systems in such a way that they were capable of responding only to the hormone-type which stimulated early development[3]. If these considerations be correct then BRODIE's experiments would appear to have measured pituitary blockade by testosterone in the females and by estradiol in the males or peripheral estrogen-androgen antagonism as suggested by PELLERIN *et al.*[4] for pentobarbital anaesthesia.

CREVIER *et al.*[5], studying pentobarbital anaesthesia, showed that the duration of hypnosis was decreased by testosterone in gonadectomized animals of both sexes whereas estradiol appeared to have little effect in ovariectomized females. PELLERIN *et al.*[4] present data suggesting that progesterone may also lengthen sleeping time.

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Division of Biological Research, G. D. Searle & Co., Chicago 80, Ill., September 4, 1956.

Zusammenfassung

Die Schlafdauer ist nach Hexobarbitalbehandlung bei weiblichen Ratten länger als bei männlichen. Durch Kastrierung wird sie bei männlichen Tieren verlängert, bei weiblichen verkürzt. Bei ovariektomierten Ratten wird sie durch Oestron verlängert. Testosteron-Propionat ist wirkungslos. Bei kastrierten männlichen Ratten wird sie dagegen durch Testosteron-Propionat verkürzt während Oestron keinen Einfluss hat.

[2] F. WILCOXON, Biom. Bull. *1*, 80 (1945).

[3] B. B. BRODIE (Fed. Proc. *11*, 632 [1952]) has shown that hexobarbital localizes in the body fat, from which it is slowly released into the circulation, suggesting that the lipo-tropic effects of the sex steroids may also be involved.

[4] J. PELLERIN, A. D'IORIO, and E. ROBILLARD, Rev. canad. Biol. *13*, 257 (1954).

[5] M. CREVIER, A. D'IORIO, and E. ROBILLARD, Rev. canad. Biol. *9*, 336 (1950).

Action of Reserpine on the 5-Hydroxytryptamine (Enteramine) Biosynthesis and Metabolism in Dogs and Rats

SHORE *et al.*[1] and PLETSCHER *et al.*[2] have shown that reserpine produces a release of 5-hydroxytryptamine (5-HT) from the gastrointestinal mucosa, blood platelets and brain, and the contemporaneous appearance of an increased urinary excretion of 5-hydroxyindoleacetic

[1] P. A. SHORE, S. L. SILVER, and B. B. BRODIE, Science *122*, 284 (1955). – P. A. SHORE, A. PLETSCHER, and B. B. BRODIE, J. Pharmacol. *116*, 51 (1956).

[2] A. PLETSCHER, P. A. SHORE, and B. B. BRODIE, Science *122*, 374 (1955); J. Pharmacol. *116*, 46 (1956).

Table I

Urine collection periods	Daily urinary excretion of 5-HIAA in the rat (in μg per kg of body weight)		
	Group *A* (10 rats) (0·1 mg/kg reserpine)	Group *B* (9 rats) (0·5 mg/kg reserpine)	Group *C* (12 rats) (1 mg/kg reserpine)
1st control period	80 (1·04)	93 (1·43)	67 (1·26)
2nd control period	97 (1·78)	57 (2·16)	86 (1·82)
3rd control period	–	104 (1·10)	–
Reserpine injections started			
1st day	106 (3·10)	158 (2·50)	73 (2·75)
2nd day	109 (2·50)	176 (2·24)	62 (2·64)
3rd day	62 (3·30)	–	–
4th day	63 (1·90)	186 (4·20)	–
5th day	55 (3·40)	211 (3·16)	–
6th day	105 (1·90)	–	–
7th day	–	–	119 (6·07)
10th day	70 (1·21)	–	–
14th day	69 (1·18)	–	–
16th day	–	148 (4·55)	81 (3·34)
21st day	82 (2·54)	–	–
28th day	140 (2·00)	–	–
34th day	74 (3·10)	–	–
41st day	124 (3·55)	–	–

The 10 rats of group *A* were killed 24 h after the 41st reserpine injection. Four rats of group *B* died after 5, 6, 9, 13 days, respectively; the remainder were killed 24 h after the 16th reserpine injection. One rat of group *C* died after 10 days, 6 after 12 and 13 days, 4 after 14 and 16 days, the last was killed after the 15th reserpine injection. The 5-HT content of serum and various tissues of the rats, in terms of μg free base per ml serum or g fresh tissue, was as follows:

Group *A*: Serum 0·07, spleen 0·24, gastrointestinal tract 1·61, lung 0·52, ears 0·64, snout skin 1·10, posterior paws 0·45, peritoneum 0·40;
Group *B*: Serum 0·04, spleen 0·23, ears 0·47, snout skin 0·43;
Controls: Serum 0·59, spleen 2·68, gastrointestinal tract 2·47, lung 0·60, ears 1·16, snout skin 1·14, posterior paws 0·86, peritoneum 0·64.

acid (5-HIAA), which is the major recognizable metabolite of 5-HT.

The observations of the above research workers were confirmed and extended by others[3] who, however, noted conspicuous species differences in the response to reserpine by the gastrointestinal tract and the brain.

For a better understanding of the mechanism of action of reserpine, it appeared interesting to investigate the rate of biosynthesis and metabolism of 5-HT following repeated administration of different doses of reserpine. This was accomplished by the estimation, in dogs and rats, of the daily urinary excretion of 5-HIAA. The method of UDENFRIEND *et al.*[4] was used throughout the experiments. It gave satisfactory results in the case of dog urine, but not so in the case of rat urine. The values given for the rat must, therefore, be considered approximate.

The experiments were carried out on 6 dogs, of which only 4 gave reliable results, and on three groups of 9–12 albino rats weighing 180–230 g. After 2–3 control collections of urine, over 24 h periods, the animals were given daily intraperitoneal doses of 0·1, 0·3, 0·5 and

[3] V. ERSPAMER, Exper. *12*, 63 (1956); Naturwissenschaften *43*, 61 (1956). – P. CORREALE, Boll. Soc. ital. Biol. Sper. *32*, 188 (1956).

[4] S. UDENFRIEND, E. TITUS, and H. WEISSBACH, J. biol. Chem. *216*, 499 (1955).

Table II

Urine collection periods	Daily urinary excretion of 5-HIAA in the dog (in μg per kg of body weight)			
	Dog 1 (0·1 mg/kg reserpine)	Dog 2 (0·3 mg/kg reserpine)	Dog 3 (1 mg/kg reserpine)	Dog 5 (1 mg/kg reserpine)
1st control period	124 (2·04)	75 (0·79)	64 (1·10)	61 (1·00)
2nd control period	138 (1·43)	102 (1·15)	148 (1·21)	110 (1·25)
3rd control period	144 (1·26)	–	95 (3·20)	–
Reserpine injections started				
1st day	221 (3·14)	235 (4·60)	180 (9·30)	217 (11·60)
2nd day	136 (1·75)	56 (3·30)	83 (2·30)	73 (6·60)
3rd day	130 (1·66)	75 (4·90)	61 (1·10)	204 (6·80)
4th day	–	died after 4 days	41 (3·30)	died after 4 days
5th day	–		80 (7·60)	
7th day	79 (6·88)		died after 9 days	
13th day	107 (1·01)			
14th day	124 (3·92)			
21st day	72 (7·66)			
	died after 22 days			

1 mg/kg of reserpine, respectively. All the doses proved to be lethal for the dogs, and the 0·5 and 1 mg/kg doses were lethal for the majority of the rats.

The daily urinary excretion of 5-HIAA in the experimental animals is shown in the Tables I and II. In parentheses is the 5-HIAA content per ml of urine (in μg).

SHORE *et al.*[1], ERSPAMER[5], and FISCHER and LECOMTE[6] observed that the rate of excretion of 5-HIAA markedly increased during the first hours following the administration of high doses of reserpine (2–5 mg/kg, intraperitoneally). This was now confirmed in dogs and in group *B* of rats.

It clearly appears, however, from the tabulated data that, after this initial increase in the 5-HIAA urinary output, the daily excretion of the metabolite of 5-HT returns to normal values, in spite of the continuous administration of reserpine and the persistent very low levels of 5-HT in serum and spleen tissue of reserpine treated rats. An apparent, unexplained exception is group *B* of rats in which the urinary excretion of 5-HIAA remained constantly above the normal levels.

Present results show that reserpine, even in lethal doses, does not appreciably interfere in the biosynthesis of 5-HT in the dog and rat organism. This is in accordance with the observations of HAVERBACK *et al.*[7], who found that reserpine, in a dosage known to lower platelet 5-HT, did not change, in normal human subjects, the excretion of the 5-HT metabolite.

We can conclude that the only hitherto demonstrated action of reserpine on 5-HT is that of causing a more or less conspicuous liberation of the amine from some body depots.

5-HT creatinine sulphate and 5-HIAA were synthetized in the Farmitalia Research Laboratories, Milan.

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Institute of Pharmacology, University of Parma, and Farmitalia S.p.A. Research Laboratories, Milan, November 3, 1956.

Zusammenfassung

Wiederholte intraperitoneale Reserpindosen (0·1–1 mg/kg), die bei vielen Tieren tödlich wirken, sind beim Hund und bei der Ratte kaum imstande, die Biosynthese des 5-Oxytryptamins (Enteramin) zu beeinflussen, obwohl sie eine erhebliche Ausschüttung der Substanz aus einigen Körperdepots verursachen können.

[5] V. ERSPAMER, Exper. *12*, 63 (1956); Naturwissenschaften *43*, 61 (1956).

[6] P. FISCHER and J. LECOMTE, C. r. Soc. Biol. Paris *150*, 1026 (1956).

[7] B. J. HAVERBACK, A. SJOERDSMA, and L. L. TERRY, New England J. Med. *255*, 270 (1956).

Absence of Uricolytic Activity in Human Parotid Glands*

For the past fifty years investigators have unsuccessfully sought for an uricase in human tissues[1]. The search has been given new impetus by the observation that the amount of urate lost from the body pool is 100–250 mg greater than that excreted in the urine[2]. This finding is suggestive evidence for the presence of uricolytic activity in the body. GEREN *et al.*[3] have shown that N-labelled uric acid, administered by mouth to humans, was extensively degraded, a result quite different from that obtained when the same preparation was administered parenterally. Thus, the localization of a uricase in humans may reside in either (or both) the intestinal flora or in the host tissues.

Recently STERN and IGLESIAS claim to have demonstrated the presence of a uricolytic ferment in human saliva and particularly in human parotid gland[4]. Their method of uricase detection was not described, nor did the authors present their scheduled paper orally at the International Physiological Congress, Montreal, 1953.

Inasmuch as the occurrence of a uricase in human tissues would be of prime importance in considerations of purine metabolism and gout we endeavored to confirm the presence of this enzyme in human parotids. Two fresh human parotid glands were minced, passed through a tissue press, homogenized and diluted with phosphate buffer. An aliquot of each suspension (equivalent to 130 mg of parotid) was incubated with urate under conditions of pH, temperature and time which permit the direct relation of uricase concentration to disappearance of urate in mammalian (other than primate) liver and kidney homogenates[5]. Urate concentration was measured by standard photometric procedures.

There was absolutely *no* disappearance of urate from the parotid tissue, urate-containing medium. Thus uricase, as determined by conventional methods is absent in human parotid. This conclusion is in keeping with the very many past failures to demonstrate uricase in any or all human tissues.

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Zusammenfassung

In homogenisierter menschlicher Speicheldrüse liess sich entgegen den Angaben von STERN und IGLESIAS[4] keine Urikase nachweisen.

* This work was performed during the tenure of Grants A 139 and A 139-C from the National Institute of Arthritis and Metabolic Disease, Department of Health, Education and Welfare, Public Health Service.

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[3] W. GEREN, A. BENDICH, O. BODANSKY, and G. B. BROWN, J. biol. Chem. *183*, 21 (1950).

[4] W. STERN and O. IGLESIAS, Abstr. XIX. Int. Physiol. Congress, Montreal, September 1953, p. 803.

[5] A. H. SCHEIN, E. PODBER, and A. B. NOVIKOFF, J. biol. Chem. *190*, 331 (1951).

Electrophysiological Investigation on the Antennal Receptors of the Silk Moth During Chemical and Mechanical Stimulation

Progress in the biochemical isolation of the sexual attracting-substance of the Silkmoth (*Bombyx mori*) was recently achieved by the use of new methods (BUTENANDT[1] and HECKER[2]). The extract of the attracting

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[2] E. HECKER, Chem. Ber. *88*, 1666 (1955); *Verteilungsverfahren im Laboratorium* (Verlag Chemie, Weinheim 1955).