

since this compound is considered to be a normal urinary constituent.<sup>6</sup> The ketonic fraction which was not precipitated by digitonin yielded the 2 main 17-ketosteroids which are found in the urine of normal women,<sup>6</sup> androsterone and etiocholanol-3( $\alpha$ )-one-17.

*Summary.* Isoandrosterone, androsterone, and etiocholanol-3( $\alpha$ )-one-17 have been isolated from the urine of a woman suffering from virilism. No dehydroisoandrosterone has been found.

While this paper was in press Pearlman<sup>7</sup> reported the isolation of isoandrosterone from the urine of normal women and of patients with cancer. The yield which is given only for normal female urine is appreciably lower than that obtained from this case of virilism.

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### Enhancement of Certain Toxic Effects of Codeine and Morphine by Sulfapyridine.\*

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In a previous paper it has been demonstrated that sulfapyridine enhances the narcotic and toxic effects of papaverine.<sup>1</sup> The increase of the anaesthetic and toxic effects of barbituric compounds on rats when treated with sulfanilamide has been shown by Adriani.<sup>2</sup> It was considered of interest to investigate whether the action of other drugs affecting the central nervous system is also increased by sulfapyridine. The present paper deals with the influence of sulfapyridine on the effects of codeine and morphine.

The codeine experiments were performed on rats, mice, and rabbits, those of morphine only on mice. The weights of the animals ranged from 190 to 240 g for the rats, 20 to 23 g for the mice, and

<sup>6</sup> Callow, N. H., and Callow, R. K., *Biochem. J.*, 1939, **33**, 931.

<sup>7</sup> Pearlman, W. H., *J. Biol. Chem.*, 1940, **136**, 807.

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<sup>1</sup> Glaubach, S., *Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 325.

<sup>2</sup> Adriani, J., *J. Lab. and Clin. Med.*, 1939, **24**, 1066.

2000 to 2500 g for the rabbits. Sulfapyridine was administered orally to rats and mice in 5 g/kg, and to rabbits in 4 g/kg doses 15 hours prior to the subcutaneous injection of codeine or morphine. None of the animals receiving sulfapyridine alone showed central nervous system symptoms such as analgesia, catalepsy or muscle twitching.

*Codeine Investigations.* Rat Experiments: The type of symptoms produced by codeine is dependent upon the dosage used. Smaller doses give only sedation and analgesia which is evident by the lack of response to ear pinching. With increasing doses after the initial depression a peculiar cataleptic stage appears in which the animal can be placed first in the side, then in the sitting and back position; in this latter rigidity often occurs; the animal placed on its back remains stiff and the spine resists flexion. Competitive with these symptoms is the increase in reflex excitability due to the stimulation of the spinal cord. Large doses give rise to muscular twitching and finally convulsions. With fatal doses death occurs from respiratory failure.

Table I shows the effects of 150 mg/kg of codeine phosphate on 4 rats with and without sulfapyridine treatment. In the first ex-

TABLE I.  
Effect of Subcutaneous Injection of 150 mg/kg of Codeine Phosphate into Rats  
With and Without Sulfapyridine.

Exp. No.	Rat No.	Date	Sulfa-pyridine	Time in minutes											
				Analgesia		Back position		Muscle twitching		Con-vulsions		Death			
				O.	D.	O.	D.	O.	D.	O.	D.				
1	132	12/6/39	without	20	150	39	81	0	0	0	0				
2	"	12	with	20	330	34*	296	80	10	0	0				
3	"	22	"	24		32		32	13	45	30	75			
1	134	6	without	21	120	31	59	0	0	0	0				
2	"	12	with	38	250	53*	267	0	0	0	0				
3	"	22	"	25		35*		47	13	60	50	110			
1	133	6	without	20	150	39	81	0	0	0	0				
2	"	12	"	48	122	48	82	0	0	0	0				
3	"	22	with	28	296	40*	270	40	70	105	7				
4	"	1/12/40	without	45	95	33	70	0	0	0	0				
1	135	12/6/39	without	21	154	38	68	0	0	0	0				
2	"	12	"	23	139	38	79	0	0	0	0				
3	"	22	with	31	314	31*	260	50	145	0	0				
4	"	1/5/40	without	31	†	30	88	0	0	0	0				

O. Onset.

D. Duration.

\*Animal becomes rigid when placed upon its back.

†Not checked.

periment the 4 rats were tested for their reaction to codeine alone. Five days later rats No. 132 and No. 134 of this group were fed with sulfapyridine, the other 2 animals remained without treatment. On the next day codeine was again administered to all 4 rats. The effects on the rats treated with sulfapyridine were enhanced in every respect, in contrast with the effects on the untreated animals. The analgesia was more persistent, the back position was maintained longer, rigidity occurred, and in No. 132 muscle twitching was observed. In the third experiment all 4 rats were treated with sulfapyridine before the codeine injection. The codeine effect was now markedly increased in rats No. 133 and No. 135 and rats No. 132 and No. 134 were thrown into convulsions and died from respiratory failure. In the fourth experiment the 2 surviving rats were tested again for their reaction to codeine alone; the effects were the same as observed in experiment 2 with codeine alone.

The experiments with 150 mg/kg codeine phosphate on another group of rats consisting of 8 animals, with 250 mg/kg on 4 rats showed results similar to those presented in Table I. After sulfapyridine the codeine effects were enhanced and delayed return to normal occurred. Of 4 animals receiving 400 mg/kg of codeine phosphate, one died. After a rest period of 6 days the remaining 3 rats received a preliminary dose of sulfapyridine before the codeine injection and all of these died.

*Mice Experiments.* The codeine symptoms in mice consist of an initial depression with muscular weakness and dragging of the hind legs. Elevation and bending of the tail (Straub reaction) usually occurs in this state. After toxic doses the depression is followed by restlessness, increased reflexes and convulsions; death occurs from respiratory failure. One group of 6 mice received 100 mg/kg of codeine phosphate. Straub reaction, muscular weakness and sagging

TABLE II.  
Increase of Toxicity of Codeine and Morphine in Mice Treated with Sulfapyridine.

Drug	Group	No. animals	Sulfapyridine	No. of animals	
				Convulsions	Died
Codeine phosphate 200 mg/kg	A	10	with	10	10
	B	15	without	4	1
	C	12*	with	12	11
Morphine sulfate 300 mg/kg	D	15	with	15	13
	E	21	without	9	3
	F	7†	with	7	5

\*The rats in this group had been used in group "B" 8 days earlier.

†The rats in this group had been used in group "E" 10 days earlier.

of the hind quarters were observed, but no convulsions occurred. The same animals treated with sulfapyridine, and then tested for their codeine reaction showed marked prolongation of the codeine effects and 2 of the animals had convulsions.

Table II shows the fatal effect of 200 mg/kg of codeine phosphate administered to 10 mice (group A) which have been fed with sulfapyridine. All animals had convulsions and died; the same doses given to 15 mice (group B) which had not received sulfapyridine produced convulsions only in 4 animals; one of which died. When 12 of these animals (group C) were fed with sulfapyridine, and then injected with codeine, all had convulsions and 11 out of 12 mice died. From this it is apparent that the toxicity of codeine is greatly increased.

*Rabbit Experiments.* The symptoms produced by codeine are initial depression, muscular weakness, increase in reflexes, catatonia and convulsions.

The administered doses were 20, 25 and 30 mg/kg of codeine phosphate.

The results of these experiments are as follows: 30 mg/kg of codeine phosphate administered to 3 normal animals produced toxic symptoms but no death; the same rabbits treated with sulfapyridine and then injected with codeine reacted much more strongly to the same amount of the drug, and 2 died. One rabbit which had not been tested for its reaction to codeine alone, but received sulfapyridine in the very beginning died after the injection of 30 mg/kg of codeine phosphate. Four rabbits received doses of 25, and 2 others 20 mg/kg, before and after treatment with sulfapyridine. All animals survived but their reaction to codeine was enhanced in the latter experiment. In 6 of these rabbits sulfapyridine estimations were performed immediately prior to the codeine injection. Two of the sulfapyridine rabbits which died after injection of 30 mg/kg of codeine phosphate showed blood levels of 27 and 31 mg% respectively. The values in 2 rabbits injected with 25 and 2 animals with 20 mg/kg of codeine phosphate ranged from 19-35 mg%. Six hours later when the animals appeared to have recovered completely the blood levels were still about the same.

*Morphine Investigations.* The effects of morphine in mice are essentially the same as those of codeine. The morphine doses used in these investigations ranged from 100 to 300 mg/kg.

For the experiments with 100 and 200 mg/kg of morphine sulfate, 12 mice were used. The animals were first tested for their reaction to morphine alone; and then after a rest period of 7 to 9 days, sulfa-

pyridine was given preliminary to the morphine treatment. The symptoms then were more pronounced and lasted for a longer time.

The increased toxicity of morphine when preceded by sulfapyridine medication may be seen from Table II (Morphine Exp.). Fifteen mice (group D) which received 300 mg/kg of morphine sulfate after sulfapyridine, without having been tested for their reaction to morphine alone, had convulsions and 13 out of 15 died. The same amount given to 21 mice receiving no sulfapyridine resulted in only 3 fatalities. After a rest period 7 of these mice (group F) received sulfapyridine prior to the morphine injection; all had convulsions and 5 of them died.

*Summary.* The results reported are similar to those demonstrated for papaverine; the effect of both codeine and morphine being enhanced by sulfapyridine. The increased activity of the drugs after sulfapyridine becomes evident not only in the prolongation of their action but also in the intensity of their effects and in the increase of their toxicity.

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### Attempt to Recover Sodium Pregnanediol Glucuronidate from Chimpanzee Pregnancy Urine.\*

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Using the technic developed by Browne and Venning<sup>1, 2, 3, 4</sup> for

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<sup>1</sup> Venning, E. M. and Browne, J. S. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 792.

<sup>2</sup> Venning, E. M., Henry, J. S., and Browne, J. S. L., *Can. Med. Assn. J.*, 1937, **36**, 83, 297.

<sup>3</sup> Venning, E. H., *J. Biol. Chem.*, 1937, **119**, 473.

<sup>4</sup> Venning, E. H., *J. Biol. Chem.*, 1938, **126**, 595.