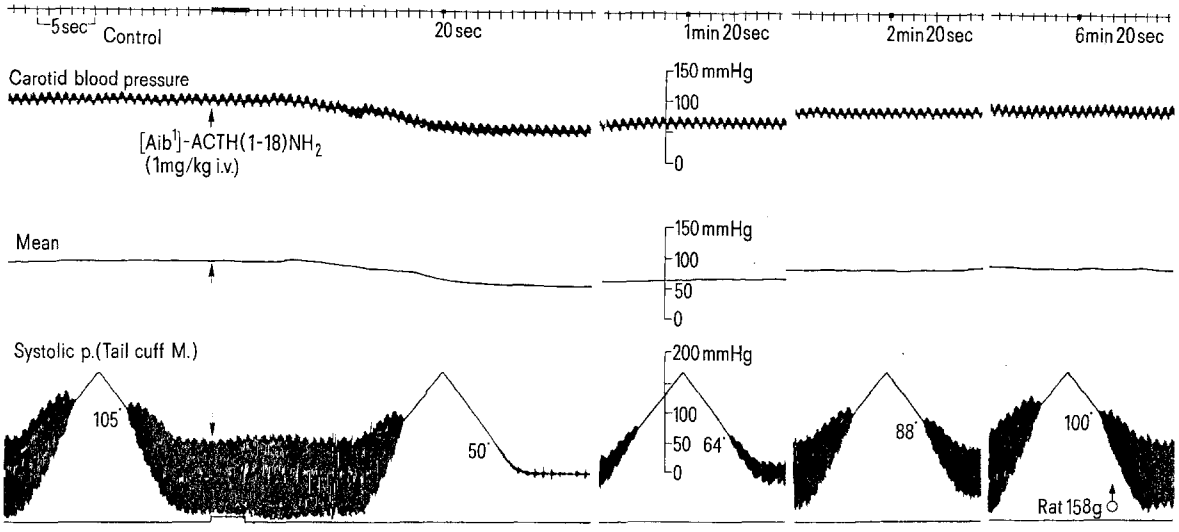

Effect of [Aib^1]-ACTH(1-18)NH_2[3] on arterial pressure in rat.

rats. [β-Ala^1, D-Phe^7, Orn^{15}]-ACTH(1-18)NH_2(II) at a dose of 0.25 mg/kg decreased the tail blood pressure by 40–50 mm Hg in intact rats and its potency was almost equal to that of I. On the whole, I, II, and synthetic porcine ACTH seemed to have a similar depressor potency.

Previous experiment in this laboratory have shown that the relative MSH potencies against human β-MSH in vivo of I, II and synthetic porcine ACTH were 1, 100 and 0.2 on weight basis respectively[4,5] and their ACTH potencies were 480, 6 and 180 units/mg, respectively[6,7]. These results suggest that the depressor activity of ACTH peptides may not be correlated with their melanocyte-stimulating and adrenal-stimulating activity.

Since the depressor response to ACTH seemed to vary from species to species[1], further studies regarding this are now in progress.

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The Accumulation Pattern of Ingested Gossypol in Selected Organs of the Rat

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Summary. Bound gossypol levels in the spleen and kidney of rats ingesting dietary gossypol (0.98%) varied directly with the feeding intervals of 6, 14, 28 and 35 days. Free gossypol level in the kidney, spleen and lungs increased for 14 days and then tended to decrease as the feeding period was extended.

Dietary gossypol accumulates in various organ tissues of swine[1]. Studies have shown that the gossypol concentration in swine tissue is directly related to the level of free gossypol consumed and to the duration of the feeding period, up to 28 days[2]. The administration of a single dose of ^{14}C-labeled gossypol to rats resulted in an initial high concentration of ^{14}C in those organs most likely involved in the elimination of gossypol[3]. However, ^{14}C-radioactivity in the spleen, lungs, and kidneys was undetected by 13 days post-injection. The liver retained a high radioactivity. The relationship of the duration of the gossypol feeding period to gossypol accumulation in organ tissues of the rat have not been reported. This investigation was designed to determine the distribution of gossypol and its pattern of accumulation in selected organs of rats ingesting gossypol for feeding periods of varying duration.

Methods. Male rats (56 days of age) of the Holtzman strain were housed individually in metabolism cages under uniform conditions of light (10 h light, 14 h dark) and temperature (68–72 F). Animals were randomly divided into 2 diet groups. The compositions of the control and experimental diets are shown in Table I. The animals in each diet group were also randomly subdivided into 4 groups of 9 animals, each subgroup corresponding to feeding intervals of 6, 14, 28, and 35 days. The liver, kidneys, spleen, and lungs were collected at the end of

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each feeding period. The organs were stored at −20 °C until analyses were made for free and bound gossypol[1].

Results and discussion. The results from organ analyses for free and bound gossypol are presented in Table II. The concentration of bound gossypol in the liver and spleen increased accumulatively during the 35-day feeding period. It has been reported that there is no accumulative increase in pig liver gossypol concentration beyond a feeding period of 28 days[2]. The lack of further accumulation was attributed to a reduced feed intake as the duration of the feeding period was increased. The experimental rats in this study did not exhibit a depressed food intake throughout the 35-day feeding period, however, there was a depression in growth exhibited by the experimental animals during each feeding interval (Table III). The report that gossypol concentrations in organ tissues are directly related to the level of free gossypol consumed[2] may account for increased bound gossypol concentrations in the liver and spleen. The accumulation of high concentrations of bound gossypol in the liver and spleen may be due to the involvement of these organs in the elimination of gossypol. It has been postulated that gossypol located in other tissues is released and subsequently deposited in the liver and spleen[5]. The chelation of gossypol with the large quantities of iron in the liver and spleen may also contribute to the high concentration of bound gossypol found in these organs. The level of bound gossypol in the kidney was highest at the end of the 14-day feeding interval and then underwent a marked reduction through the 28-day and 35-day

Table I. Composition of rations

	Percentage
Cottonseed meal[a]	20.00
Purified soybean protein	10.00
Corn oil	10.00
Salts USP XIV	4.00
Cornstarch	30.20
Purified cellulose	2.00
Corn dextrose	23.00
B vitamins[b]	0.05
Choline chloride	0.20
Vitamins A and D mixture[c]	0.05
NaCl	0.50

[a] Degossypolized cottonseed meal was used in the control diet. The cottonseed in the experimental diet contained 0.98% free gossypol. [b] Described by OLTJEN et al.[4]. [c] Contained 20,000 IU and 2,500 USP units/g of vitamin A and D, respectively.

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Table II. Gossypol content of organs of rats fed a gossypol-containing diet[a]

Organ	Gossypol Form	Days on diet			
		6	14	28	35
Liver					
	F	82.1 ± 7.3	35.2 ± 4.9	68.8 ± 3.8	92.0 ± 16.7
	B	46.9 ± 15.7	131.9 ± 17.7	233.7 ± 17.9	273.4 ± 7.0
Kidney					
	F	101.2 ± 9.4	125.4 ± 20.4	34.8 ± 13.2	30.9 ± 11.7
	B	36.4 ± 16.8	106.2 ± 38.5	21.5 ± 5.8	8.0 ± 3.5
Spleen					
	F	87.2 ± 47.3	142.2 ± 23.4	45.1 ± 17.0	36.8 ± 13.9
	B	0.0 ± 0.0	275.5 ± 66.0	906.7 ± 299.7	1,407.0 ± 266.5
Lungs					
	F	39.6 ± 12.2	93.2 ± 7.9	9.9 ± 3.7	19.4 ± 7.3
	B	25.6 ± 8.1	90.6 ± 56.8	183.2 ± 22.6	140.0 ± 19.0

[a] Mean values (μg/g wet tissue) ± SE.

Table III. Effect of gossypol-containing diet on weight response of rats[a]

Body weight (g)	Days on diet			
	6	14	28	35
Initial				
Control	198.2 ± 4.4	198.8 ± 5.2	199.8 ± 4.2	200.9 ± 4.3
Experimental	199.0 ± 4.8	197.8 ± 4.9	200.2 ± 4.6	200.6 ± 4.6
Final				
Control	213.0 ± 8.2	240.2 ± 9.8	328.0 ± 12.4	352.8 ± 10.4
Experimental	166.0 ± 10.4	117.0 ± 7.2	174.3 ± 9.7	167.6 ± 8.3

[a] Mean values ± SE.

feeding intervals. Bound gossypol concentration in the lungs increased accumulatively through the 28-day feeding interval but demonstrated a decline at the end of the 35-day feeding interval.

Free gossypol levels in the kidney, spleen and lungs were greatest during the shorter feeding periods and generally displayed decreased levels at the end of the 28- and 35-day feeding periods. With the exception of the 14-day feeding period, the free gossypol concentration in the liver remained nearly constant.

Although this study was of relatively short duration, it clearly indicates the wide distribution of gossypol in the organs of the rat. It also adequately establishes the relationship between the duration of gossypol ingestion and the accumulation of gossypol in the organs of the rat. If gossypol is an accumulative poison, its concentration in each organ should vary with the duration of the ingestion period. This was found to be the case for bound and total (bound plus free) gossypol concentrations in the liver and spleen, but not for the kidneys and lungs. It is apparent that total gossypol levels in the kidneys and lungs reach a maximum and then decrease as the length of the feeding period is increased. A possible explanation may be that there is a shift of accumulated gossypol in the kidneys and lungs to other organs. The presence of two reactive carbonyl groups on the gossypol molecule and the multiplicity of chemicals available in each organ for interaction with gossypol allows for a variety of bound forms of gossypol to exist. It is possible that these compounds vary widely in their motility within the organism and account for an extremely complex accumulation pattern.

Interaction of Atropine or Methylatropinium with Four Effects of Two Cholinergic Drugs

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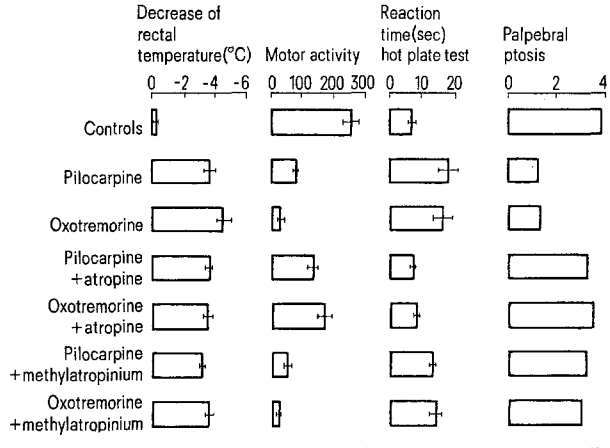
Summary. In mice, pilocarpine – or oxotremorine – induced decrease in locomotor activity and increase of the reaction time to pain were antagonized by atropine and not by methylatropinium. Identical doses of atropine and methylatropinium suppressed the antagonism of the cholinergics towards reserpine-induced palpebral ptosis. Cholinergics-induced hypothermia was not clearly antagonized by atropine or methylatropinium.

The sedative effects of the cholinergic drugs are antagonized by atropine but not by its quaternary derivative. So is the decrease in locomotor activity[1], the catalepsy[2], the suppression of an avoidance-conditioning[3], the potentiation of the barbiturates effects[4] and the antagonism of the amphetamine-induced hyperactivity[5].

However, Spencer[6] and Janssen and Niemegeers[7] have shown that higher doses of the quaternary derivatives of atropine-like drugs could exert the same antagonism as atropine towards the effects of the cholinergic drugs.



Influence of atropine or methylatropinium on four effects of pilocarpine or of oxotremorine. The doses of pilocarpine and of oxotremorine were different according to the test (see materials and methods). The total length of the horizontal bars represents 2 SEM. Locomotor activity results are expressed by the mean of beams crossed by one mouse during 30 min. Palpebral ptosis was appreciated from 0 to 4 according to Rubin et al.[12].

Among the various common effects in mice of two cholinergic drugs, oxotremorine and pilocarpine[8], we chose four of them; in order to dissociate the central component from the peripheral component of these effects we tested whether atropine or methylatropinium could antagonize the effects of oxotremorine and pilocarpine in these four experimental situations at doses of the anticholinergic agent which do not modify these tests when injected alone[9].

Materials and methods. For all experiments, groups of 10 male mice (19–23 g) were used (six for the palpebral ptosis). Atropine or methylatropinium (1 mg · kg^{-1}) were always given i.p. 15 min before pilocarpine or oxotremorine. Rectal temperature was measured 30 min after pilocarpine (64 mg · kg^{-1}) or oxotremorine (0.25 mg · kg^{-1}) administration. Locomotor activity was measured between the 30th and the 60th min after pilocarpine (4 mg, kg^{-1}) or oxotremorine (0.06 mg · kg^{-1}) administration. During this period, each mouse was placed in an actograph box[10]. The reaction time to a nociceptive stimulus

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