cold trapping without reduction were unsuccessful. Since sensitivity to oxidation of this compound is a considerable problem, on-site environmental analysis may be required if dimethylarsine itself is to be detected.

Dimethylarsinic acid is a major and ubiquitous form of arsenic in the environment. It is particularly involved in biological systems. Methylarsonic acid, although found, was generally present in smaller concentrations than dimethylarsinic acid, a probable consequence of its being only an intermediate in the arsenic methylation sequence. Dimethylarsinic acid is very resistant to oxidation. It is not oxidized by bromine water, or, quantitatively, even by aqua regia. It could then have a considerable residence time in natural waters, unless subject to bacterial oxidation.

Both dimethylarsinic acid and methylarsonic acid are pesticides. Since they are identical with the biologically produced methylarsenic acids the detection of the effect of added methylarsenic pesticides will be difficult.

Finally, the introduction of arsenic compounds into the environment may eventually result in a general increase in their concentrations in water and air because of the bacterial mobilization of all the forms of arsenic. Information is needed on the effect of all the forms of arsenic on ecological systems.

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Hypertensive Action of 18-Hydroxydeoxycorticosterone

Abstract. *18-Hydroxydeoxycorticosterone is an adrenal steroid hormone causing salt and water retention and is secreted in greatly increased amounts in response to the pituitary hormone adrenocorticotropic hormone. Its production is abnormally high in some forms of hypertension in man and rat. Direct proof that 18-hydroxydeoxycorticosterone is capable of causing hypertension is present. Daily subcutaneous injections of 200 micrograms, a low physiological dose, significantly increase the blood pressure of unilaterally nephrectomized saline-treated rats after 2 weeks. This strengthens the hypothesis that 18-hydroxydeoxycorticosterone contributes to the etiology of hypertension, possibly by a mechanism involving stress-induced release of adrenocorticotropic hormone.*

18-Hydroxydeoxycorticosterone (18-OH-DOC) is an adrenal steroid native to several species including rat (*1*) and man (*2*). In both rat and man its secretion is increased greatly by adrenocorticotropic hormone (ACTH) (*2, 3*), a pituitary hormone liberated in response to stress. 18-OH-DOC is produced by the rat in quantities that may exceed those of corticosterone, the principal steroid affecting carbohydrate metabolism in this species (*4, 5*). It affects electrolyte and water excretion (*3, 6*) and has been implicated as a possible hypertensive agent in rats as well as man (*7*). However, the crucial experiment, namely to establish whether 18-OH-DOC can elicit hypertension, had to await its availability in sufficient quantity for biological assay. This experiment has now been performed with 18-OH-DOC prepared by a new organic synthesis (*8*). Weanling male Wistar rats were unilaterally nephrectomized and given 1 percent saline, a common procedure employed for the assay of hypertensive steroids (*9, 10*). One week after the operation the animals were separated into four groups of ten rats each. Three groups received subcutaneous injections, at 9 a.m., of steroid suspended in cottonseed oil, the fourth group received only cottonseed oil. Corticosterone (B) and deoxycorticosterone acetate (DOCA) were administered daily at a dosage of 200 μg for 21 days, 18-OH-DOC was given at a dosage of 200 μg for 20 days and of 140 μg on day 21, because of insufficient material. Blood pressure was determined in the afternoons by the tail-cuff method under light ether anesthesia (*11*), at the onset of the experiment and on days 2, 3, 9, 10, 16, 17, and 21. Body weights were checked on days 2, 9, 16, and 21. On day 21 the rats were decapitated, and the organs were removed and weighed.

The changes in blood pressure with time are depicted in Fig. 1. The blood pressures of the 18-OH-DOC–treated animals were elevated compared to those of the vehicle-treated animals on day 16 (155 ± 3 as compared to 137 ± 5 mm-Hg, $P < .01$), day 17 (160 ± 2 as compared to 143 ± 4 mm-Hg, $P < .01$), and day 21 (158 ± 3 as compared to 142 ± 5 mm-Hg, $P < .02$). On days 9 and 10 the increase was of marginal significance (140 ± 5 as compared to 128 ± 4 mm-Hg and 151 ± 3 as compared to 142 ± 4 mm-Hg, respectively, $.05 < P < .1$ by two-tailed t-test, $P < .05$ by one-tailed t-

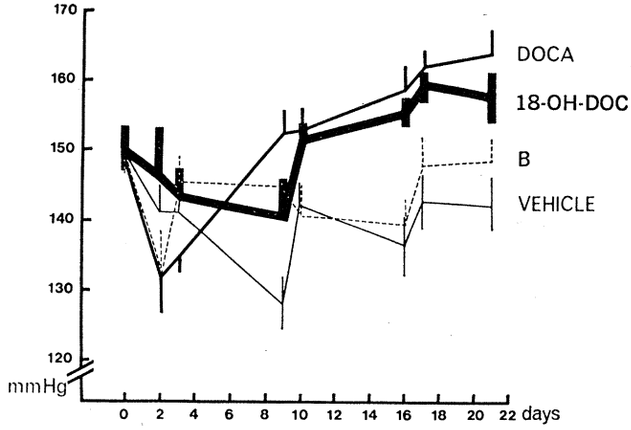

Fig. 1. Hypertensive action of 18-OH-DOC. Ten rats per group were injected subcutaneously with 200 μg of steroid daily for 21 days, except for the 18-OH-DOC – treated group, which received 200 μg for 20 days and 140 μg on day 21. One group was injected with vehicle only (0.1 ml of cottonseed oil). Blood pressures were measured by the tail-cuff method (*9*). Significant differences from the vehicle-injected rats occurred on days 16, 17, and 21 in the 18-OH-DOC–injected rats, on days 9, 16, 17, and 21 in the DOCA-injected rats, and on day 9 only in the corticosterone-injected rats. *B*, corticosterone.

Table 1. Effects of corticosterone, 18-OH-DOC, and DOC on organ weights. The dose was 200 μg per day, given subcutaneously. Body weights remained the same for the 21-day period.

Organ	Organ weights			
	Vehicle	Corticosterone	18-OH-DOC	DOC
Pituitary (mg)	7.3 ± 0.5	7.0 ± 0.5	7.0 ± 0.5	6.9 ± 0.3
Adrenals (mg)	40.5 ± 1.2	35.8 ± 0.9*	37.3 ± 1.6	39.1 ± 1.8
Testes (g)	2.87 ± 0.05	2.77 ± 0.06	2.91 ± 0.05	2.89 ± 0.05
Heart (g)	0.96 ± 0.03	0.91 ± 0.03	0.93 ± 0.03	1.05 ± 0.03*
Kidney (g)	1.83 ± 0.05	1.76 ± 0.04	1.72 ± 0.05	1.96 ± 0.05†
Thymus (mg)	632 ± 29	545 ± 32*	544 ± 28‡	591 ± 24
Spleen (mg)	722 ± 27	675 ± 35	684 ± 34	768 ± 21
Liver (g)	11.2 ± 0.3	10.4 ± 0.3†	10.4 ± 0.3†	11.1 ± 0.2

* $P < .05$. † $.05 < P < .1$. ‡ $P < .02$, compared to vehicle-injected animals.

test). The blood pressures of the DOCA-injected animals were elevated compared to those of the vehicle-injected animals on days 9, 16, 17, and 21, in confirmation with the findings of Hall and Ayachi (*10*), who noted that hypertension may be evoked in rats at much lower doses than those commonly used (1 to 2 mg per rat per day). The blood pressures of the DOCA-injected animals did not differ significantly from those of the 18-OH-DOC–injected animals. Significant increases over values before injection occurred in the DOCA-treated rats on days 17 and 21. In the 18-OH-DOC–treated rats the increases over values before injection were significant on day 17, but not on day 21, possibly because the full dose had not been administered on that day. No increases over values before injection were obtained in the corticosterone-treated rats. Decreases in average blood pressure values from averages before injection occurred in all groups in the early stages, probably reflecting accommodation to the experimental procedure. In the 18-OH-DOC–treated rats these did not attain statistical significance, but they were significant in the vehicle-treated rats on days 3 and 9, in the DOCA-treated rats on days 2 and 3, and in the corticosterone-treated rats on day 2. The blood pressures of the corticosterone-treated rats differed from those of the vehicle-treated rats only on day 9, the day on which the vehicle-treated rats had attained their lowest values.

The steroid injections had no effect on body weights which increased linearly throughout the experiment (Table 1). 18-OH-DOC elicited a significant decrease in thymus weight, equaling that evoked by corticosterone. Both these steroids also caused a marginal decrease in liver weight. In contrast to corticosterone, 18-OH-DOC did not reduce adrenal weight significantly. DOCA caused a significant increase in heart weight and a marginally significant increase in kidney weight.

Our observations indicate that 18-OH-DOC is capable of evoking hypertension in the unilaterally nephrectomized rat exposed to saline when administered in amounts that are well within the physiological range for this animal. At a daily dosage of 200 μg administered for 3 weeks, 18-OH-DOC was equipotent with DOCA and elicited increases in blood pressure comparable to those reported for a daily dosage of 250 μg of *dl*-aldosterone (*9*). The hypertensive potency of these steroids therefore appears to be of the same order and not proportional to their effects on electrolyte and water excretion in the adrenalectomized rat, which may be summarized as follows on the basis of equal mass (*6, 12*). For K^+ excretion: aldosterone $\gg$ DOC $\gg$ 18-OH-DOC; for Na^+ retention: aldosterone $\gg$ DOC $\geq$ 18-OH-DOC; for urinary Na^+/K^+ ratios: aldosterone $\gg$ DOC $>$ 18-OH-DOC; for the ratio of H_2O retained to Na^+ retained: 18-OH-DOC $>$ DOC $>$ aldosterone.

The thymolytic action of 18-OH-DOC is also observed in rats after a 3-day exposure to a higher dosage, and such rats usually respond to a moderate stress applied 4 hours after the last injection with abnormally high increases in circulating corticosterone concentrations (*13*). This suggests that 18-OH-DOC does not inhibit but may even enhance ACTH secretion, or that it interferes with the disposal of corticosterone. The biological effects of 18-OH-DOC may therefore involve the action of other steroids as well. The inhibition of carrageenan-induced rat paw edema by 18-OH-DOC is more readily observed in normal (*14*) than in adrenalectomized animals. Finally, it should be noted that the blood pressure of rats developing adrenal regeneration hypertension, a syndrome to which 18-OH-DOC may contribute, correlates positively with the ratios of corticosterone to 18-OH-DOC in adrenal vein blood and negatively with thymus weights (*5, 15*).

Note added in proof: Rapp *et al.* have obtained a significant rise in the blood pressure of unilaterally nephrectomized saline-fed rats 2 weeks after the administration of 18-OH-DOC at a dose of 1 mg per 100 g of body weight per day (*16*).

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15. We, furthermore, found that heart and kidney weights correlated negatively with corticosterone and 18-OH-DOC secretion rates in such rats (*5*); this would be in accord with the fact that the average weight of these organs was reduced by corticosterone and 18-OH-DOC in the present work, although the difference was not significant. This work has appeared in abstract form in *Fed. Proc.* **32**, 352 abstr. (1973).
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Spiroplasmas and Acholeplasmas: Multiplication in Insects

Abstract. *The helical wall-free microorganism,* Spiroplasma citri, *which is associated with citrus stubborn, a disease with no known vector, multiplied in the leafhopper vector of corn stunt but multiplied to higher titer in the vector of aster yellows and decreased the longevity of that insect.* Acholeplasma laidlawii *and* A. granularum *also multiplied in both leafhoppers.*

The existence of prokaryotic plant pathogens other than eubacteria was unsuspected until 1967, when Doi *et al.* (*1*) suggested that the dwarf disease of mulberry might be caused by a mycoplasma-like or chlamydia-like agent. Electron microscopy has since revealed that plants with symptoms of many other diseases harbor prokaryotes in their vascular tissues. These microorganisms are extremely diverse (*2*). For example, it is now believed that the bodies associated with mulberry dwarf disease and a major cluster of "yellows" diseases (*3*), such as aster yellows (*4*), most closely resemble mycoplasmas (class Mollicutes, order Mycoplasmatales). In other instances, affinity of presumed pathogens to mycoplasmas is dubious. For this reason the organisms associated with, but not demonstrated to cause, the citrus stubborn disease are of interest. These phloem-localized organisms, which in ultrathin cross sections closely resemble mycoplasmas (*5*), have been shown to be cultivable (*6*), motile (*7*), and capable of assuming helical configurations (*7*) despite the apparent simplicity of their membrane system (*8*). The name *Spiroplasma citri* was proposed for this organism, which is serologically distinct from all known members of the Mollicutes and several spirochetes (*9*). However, its gram-positive reaction, its method of motility, and the ordered structures on the outer surface of its limiting membrane (*7*) raised doubts about its placement in higher taxa (*9*). Helical filaments of the organism resemble motile bodies (*10*) associated with, and suspected to cause (*11*), corn stunt, a disease from which no cultivable prokaryote has been obtained consistently (*11, 12*). The presumed agents of corn stunt and citrus stubborn diseases are serologically related (*13*). The value of attempts to fulfill Koch's postulates for proof of pathogenicity with *S. citri* is thus evident. One of the best means of introducing infectious organisms into the phloem of a healthy plant would be through the use of an insect vector. Unfortunately, although most of the phloem-localized plant disease agents multiply in insects, no vector has been discovered for *S. citri.* Since leafhoppers (Homoptera: Cicadellidae) transmit many plant disease agents, we tested these insects for their ability to support multiplication of *S. citri.*

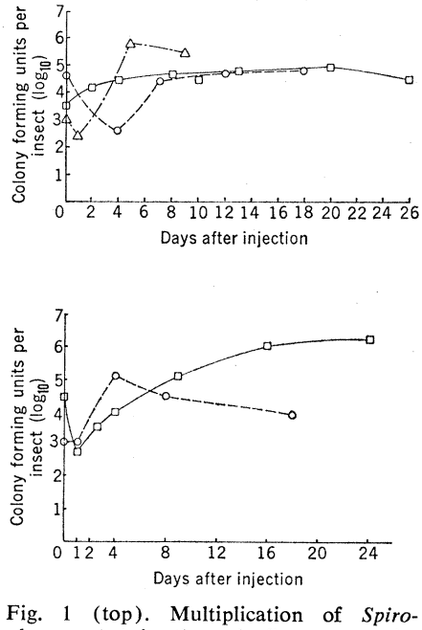

Fig. 1 (top). Multiplication of *Spiroplasma citri* in three leafhopper species (△ *Macrosteles fascifrons*; ○ *Draeculacephala* spp.; and □ *Dalbulus elimatus*). Fig. 2 (bottom). Multiplication of ○ *Acholeplasma granularum* and □ *A. laidlawii* in *Dalbulus elimatus.*

The Moroccan isolate of *S. citri* was cultivated as described (*6*). For comparison, cultures of two acholeplasmas (*Acholeplasma laidlawii*, strain PG-8 and *A. granularum*, strain BTS-39) were produced in conventional mycoplasma broth media containing 1 percent bovine serum fraction. Early experiments established that *S. citri* was recoverable from extracts of the leafhopper *Dalbulus elimatus* (Ball) with little or no loss of titer by plating portions of the extract on conventional mycoplasma agar plates containing 20 percent horse serum and bacterial and fungal inhibitors (*14*). In our studies, the presence of penicillin, thallium acetate, and amphotericin was sufficient to exclude all fungi and eubacteria from the plates while permitting recovery of all three prokaryotes we introduced. No other wall-free prokaryotes were recovered from the leafhopper extracts. The identity of colonies was confirmed periodically with specific fluorescein-conjugated antiserums by the epifluorescence technique (*15*). Experimental infections were initiated by injecting (*16*) 0.1 to 0.2 μl of liquid cultures into the abdomens of groups of 300 to 1000 leafhoppers. Immediately after injection, and on various later days, 30 insects were ground with 1 ml of mycoplasma medium, centrifuged for 10 minutes at 1800*g* to remove coarse debris from the extract, and plated in 0.1-ml portions on conventional mycoplasma plates.

Spiroplasma citri multiplied in all leafhoppers tested. In *Draeculacephala* spp. (*17*), an initial decrease in titer observed 4 days after injection was followed by a gradual increase until 18 days after injection when the experiment was terminated (Fig. 1). In all experiments a relative loss of 0.5 to 1.0 $\log_{10}$ units of organisms occurred between the time of extraction and the first plating ("zero time"). These losses presumably occurred during centrifugation. Thus, in *Draeculacephala*, the relative increase of more than 2 $\log_{10}$ units of organisms between days 4 and 12 after injection resulted in a titer of 4.4×10^4 colony-forming units (CFU) per insect. This restored the titer to the initial amount recoverable after injection level, but not to the initial input amount of 2.6×10^5 CFU per insect, calculated on the assumption of an injection volume of 0.2 μl.

Multiplication of *S. citri* in *D. elima-*