every 2 months to a maximum of 100 mg/day. He noted clinical improvement with 15 mg, but said it was less rapid than that with 50 and 100 mg/day. However, the clinical picture is not generally considered to be consistent enough to allow such fine discrimination in drug response, and in most clinical trials it has been found necessary to assess the drug by the bacteriologic response in groups of lepromatous cases, each group on a particular dosage for a year or more.

Thus it seems indicated to test the therapeutic effect in human leprosy of lower dosages of DDS and less frequent administration. The margin between toxic level and therapeutic level of the drug appears to be so large as to allow considerable innovation in the regimen. DDS is excreted only slowly and Ross(15) has reported detectable concentrations in blood 2 to 4 weeks after the last administration of sulfone. The results suggest that DDS might have an especially useful role in chemoprophylaxis, a contral measure potentially adaptable to many leprosy endemic areas.

Summary. 1. The activity against *Mycobacterium leprae* of 4,4'-diaminodiphenyl sulfone (DDS) was tested in mice by feeding a series of diets containing the drug in concentrations ranging from 0.03 to 0.00001%. Multiplication of *M. leprae* was completely suppressed at all levels. 2. The lowest DDS intake was a hundred times less that the least amount required to produce chemically detectable amounts in the blood (0.2 to 0.3 μg/ml).

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An Anomalous Effect of Theophylline on ACTH and Adenosine 3',5'-Monophosphate Stimulation.* (31283)

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The mechanism of action of a set of peptide and amine hormones in their target tissues has been shown to involve a coupling of the primary hormone reaction with effector systems by adenosine-3',5'-monophosphate (3',5'-AMP)(1-4).

In general 3 criteria have been used to establish an intermediary role for 3',5'-AMP in hormone action. First the hormone is shown to have a specific effect to increase the concentration of 3',5'-AMP in the target tissue; second, 3',5'-AMP is shown to simulate the effect of the hormone on its target tissue; third, inhibition of the breakdown of 3',5'-AMP in target tissues by methyl xanthines is shown to either stimulate or potentiate the hormonal action(5).

The participation of 3',5'-AMP as an inter-

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mediary in the action of adrenocorticotrophic hormone (ACTH) on adrenal steroidogenesis has been well established by the first two criteria(6,7). We were therefore interested to test the effect of methyl xanthines in adrenals to determine whether inhibition of 3′,5′-AMP breakdown would lead to steroidogenesis, or potentiate the action of ACTH upon this process. Relatively high concentrations of 3′,5′-AMP are required to stimulate steroidogenesis in surviving adrenocortical preparations due, it is generally believed, to a poor penetration of the cyclic nucleotide into cells, but possibly also due in part to a rapid destruction of the added 3′,5′-AMP. This latter possibility added further interest to an examination of the effect of methyl xanthines upon ACTH and 3′,5′-AMP stimulation of adrenal steroidogenesis. The results of this study are reported here.

In these studies addition of the methyl xanthine theophylline to the incubation medium has not produced the expected potentiation of the action of either ACTH or 3′,5′-AMP on steroid production by rat adrenals *in vitro*. However, we have found that concentrations of theophylline normally used in this type of study markedly inhibit protein synthesis in this tissue, as evaluated by the incorporation of amino acid into adrenal protein. In view of the reported blockade of the steroidogenic action of both ACTH and 3′,5′-AMP in adrenals by inhibitors of protein synthesis(8), it is suggested that the anomalous behavior of theophylline in this system is due to its effect upon protein synthesis.

Experimental. Adrenal glands were obtained from male Sprague-Dawley rats (160-180 g) following cervical fracture and decapitation. The glands were trimmed free of fat, bisected, weighed and preincubated at 37°C for 1 hour in 2 ml (2 glands) of Krebs bicarbonate Ringer-0.2% glucose with and without addition of theophylline at various concentrations. The gas phase was 95% O_2-5% CO_2. At the end of the preincubation period the tissue was transferred to fresh medium, from which glucose was omitted, but which contained glycine-1-C^{14} (0.3 μC/ml; 1.6 μmoles/ml). Where required ACTH (a^s-ACTH, by courtesy of Dr. C. H. Li, California), 3′,5′-AMP (Calbiochem), glucose-6-phosphate and triphosphopyridine nucleotide (NADP) were added in 0.2 ml of Ringer and the incubation continued for a further hour. At the end of the second incubation the tissue was transferred to 2 ml of 10% trichloracetic acid and the incubation medium extracted twice with 2 volumes of methylene chloride. The incorporation of ^{14}C-label from glycine-1-^{14}C into the protein of the adrenal tissue was determined in the trichloracetic acid insoluble fraction of the tissue by procedures previously described(9). The corticosteroids liberated into the incubation medium were estimated using a blue tetrazolium method(10). As theophylline is extracted from the medium by methylene chloride, and has a small inhibitory effect on the blue tetrazolium reaction, the extracts from theophylline containing media were compared with standards (corticosterone) containing an equivalent amount of theophylline. The results are expressed as μg corticosteroids (blue tetrazolium chromagens) per 100 mg wet tissue weight, and as ^{14}C counts per minute (liquid scintillator) per mg protein as determined by the method of Lowry *et al*(11).

Results. The effects of different concentrations of theophylline on the stimulation of corticosteroid output in rat adrenal bisects by ACTH, 3′,5′-AMP and glucose-6-phosphate plus NADP, and the incorporation of ^{14}C-label from glycine-1-^{14}C into adrenal protein were studied concurrently in a series of experiments in which control and theophylline-treated tissues were directly compared. Table I summarizes the results of corticosteroid determinations on the media from second incubations of control adrenal bisects and those incubated with theophylline at concentrations of 10^{-2}, 10^{-3} and 10^{-4} M. Steroidogenesis was stimulated by ACTH (10, 50 and 250 mU/ml), 3′,5′-AMP (1 and 5 mM) or glucose-6-phosphate plus NADP (3 plus 2 mg/ml) with some of the tissues serving as unstimulated controls. The mean value for corticosteroids (μg/100 mg) is given together with the percent increase over the level found in the unstimulated tissues. It will be seen from Table I that the highest concentration of theophylline tested (10^{-2} M) did not po-

TABLE I. Effect of Theophylline on Steroidogenic Activity of ACTH, 3′, 5′-AMP and Glucose-6-Phosphate plus NADP in Rat Adrenal Bisects.

Theophylline concentration (molar)	Corticosteroids (μg/100 mg wet wt)						
	ACTH (mU/ml)				3′, 5′-AMP (mM)		Glucose-6-P + NADP
	0	10	50	250	1	5	3+2 mg/ml
0	12.8 ± .5* (31) —	21.9 ± .6 (19) +71%†	28.5 ± 1.6 (15) +123%	35.5 ± 1.3 (16) +177%	15.0 ± 1.1 (16) +17%	29.4 ± 1.7 (12) +130%	23.3 ± .7 (15) +82%
10^{-2}	15.8 ± 1.02 (12) —	20.5 ± 2.1 (7) +30%	25.0 ± 2.5 (8) +58%	27.5 ± 2.1 (8) +74%	20.5 ± 1.5 (4) +30%	31.2 ± 2.6 (4) +97%	25.1 ± 3.6 (4) +59%
10^{-3}	12.4 ± .9 (7) —	16.2 ± .4 (4) +31%	33.1 ± 1.0 (4) +167%	40.6 ± .8 (4) +228%	17.3 ± 1.2 (4) +40%	36.2 ± 2.3 (4) +192%	24.2 ± 2.5 (4) +95%
10^{-4}	14.7 ± 1.1 (7) —	22.8 ± 2.4 (4) +55%	28.9 ± 3.0 (4) +97%	35.2 ± 2.8 (4) +140%	16.3 ± 1.7 (4) +10%	30.4 ± 2.5 (3) +107%	23.3 ± 1.3 (4) +59%

* Mean ± standard error. Number of observations in parentheses.
† Percent increase of mean over value for unstimulated tissue at each level of theophylline.

tentiate the steroidogenic action of any of the ACTH concentrations used. In fact there was a small but significant inhibition of the hormonal effect. This concentration of theophylline had some potentiating action on the effect of 3′,5′-AMP added at 1 mM concentration but had only a negligible action on the response to 5 mM 3′,5′-AMP. At 10^{-3} M theophylline showed a small but significant potentiation of the steroidogenic action of ACTH added at 50 and 250 mU/ml, but not at the 10 mU/ml level, and enhanced the effect of the higher concentration (5 mM) of 3′,5′-AMP. Theophylline at 10^{-4} M did not significantly influence the steroid output stimulated by either ACTH or 3′,5′-AMP. At all concentrations tested theophylline had no significant action on steroidogenesis induced by glucose-6-phosphate plus NADP.

Table II summarizes the results of determinations of the specific activity (counts per minute per mg protein) of adrenal protein obtained from the same incubations as those used for compiling the results in Table I. It will be seen that theophylline strongly inhibits amino acid incorporation into protein, the reduction of the values for unstimulated tissues being 60% at the 10^{-2} M level, 23% at 10^{-3} M and 10% at 10^{-4} M. It will also be seen that the previously described(9) dose dependent inhibition of amino acid incorporation into protein, obtained with steroidogenic concentrations of ACTH or 3′,5′-AMP, occurs in addition to the reduction due to the-

TABLE II. Effect of Theophylline on Glycine-1-^{14}C Incorporation into Adrenal Protein in Rat Adrenal Bisects.

Theophylline concentration (molar)	Glycine-^{14}C incorporation into protein (counts/min/mg protein)						
	ACTH (mU/ml)				3′, 5′-AMP (mM)		Glucose-6-P + NADP
	0	10	50	250	1	5	3+2 mg/ml
0	234 ± 7* (30)	207 ± 7 (20)	165 ± 6 (16)	117 ± 5 (16)	234 ± 8 (15)	148 ± 10 (12)	229 ± 12 (16)
10^{-2}	94 ± 9 (12)	67 ± 7 (7)	53 ± 7 (8)	40 ± 5 (8)	77 ± 5 (4)	58 ± 5 (4)	91 ± 7 (4)
10^{-3}	180 ± 11 (8)	139 ± 4 (4)	124 ± 13 (4)	91 ± 10 (4)	210 ± 12 (4)	118 ± 8 (4)	178 ± 19 (4)
10^{-4}	211 ± 12 (8)	205 ± 30 (4)	163 ± 17 (4)	106 ± 13 (4)	235 ± 25 (4)	177 ± 30 (4)	222 ± 20 (4)

* Mean ± standard error. Number of determinations in parentheses.

ophylline at all concentrations of the methyl xanthine used.

Discussion. High concentrations of theophylline, or other methyl xanthines, are required to inhibit the enzymatic transformation of 3′,5′-AMP to adenosine-5′-monophosphate. Thus, a concentration of 8×10^{-3} M theophylline is required to inhibit by 50% the transformation of 3′,5′-AMP to adenosine-5′-monophosphate by the isolated enzymes of beef heart muscle(12). In their studies of 3′,5′-AMP action on sodium transport in toad bladder, Orloff and Handler(13) used theophylline at 10^{-2} M to mimic vasopressin effects. In most studies of the effect of 3′,5′-AMP on free fatty acid release in adipose tissue preparations, a concentration of 10^{-3} M theophylline simulates epinephrine action on this parameter(14).

The present results show that theophylline at 10^{-3} M does not influence adrenal steroidogenesis. A small potentiation of ACTH or 3′5′-AMP action on corticosteroidogenesis is obtained, but the effect is not pronounced and is observed only at certain concentration of ACTH and 3′,5′-AMP. When the concentration of theophylline is raised to 10^{-2} M the steroidogenic action of ACTH and 3′,5′-AMP is reduced rather than potentiated. This inhibitory effect of theophylline upon ACTH and 3′5′-AMP action on steroidogenesis may be the consequence of the concurrent reduction of amino acid incorporation into protein to 40% of control levels by 10^{-2} M theophylline. This possibility arises because of the requirement for protein synthesis for ACTH and 3′,5′-AMP stimulation of adrenal steroidogenesis apparent from the work of Ferguson(8) who showed that inhibitors of protein synthesis, such as puromycin, inhibit the steroidogenic action of both ACTH and 3′,5′-AMP in the *in vitro* rat adrenal system. These studies have recently been confirmed and extended(15,16,17,18). In Ferguson's studies a rough relationship between the degree of inhibition of protein synthesis was obtained; when ACTH action was fully inhibited, amino acid incorporation into adrenal protein was virtually abolished(8). Farese (16), however, found that another inhibitor of protein synthesis, chloramphenicol, completely blocked ACTH action on steroidogenesis in rat adrenals when protein synthesis was inhibited by only 50%. As it is not necessary to block protein synthesis completely before ACTH or 3′,5′-AMP stimulation of steroidogenesis is affected, the partial inhibition of protein synthesis in rat adrenals obtained with 10^{-2} M theophylline may result in a reduced steroidogenic response.

The foregoing considerations raise the possibility that in rat adrenal tissue theophylline has two antagonistic actions, (1) an enhancement of ACTH or 3′,5′-AMP stimulation of steroidogenesis by protection of the cyclic nucleotide formed intracellularly, or derived from the medium; and (2) a reduction of the stimulatory action of ACTH or 3′,5′-AMP on steroidogenesis by a partial inhibition of the synthesis of protein(s) essential for the steroidogenic action. Whether potentiation or inhibition of the steroidogenic action of ACTH or 3′,5′-AMP is observed depends upon the relative concentrations of theophylline and the steroidogenic stimulator.

Summary. The action of several peptide and amine hormones on their target tissues involves the generation of adenosine-3′-5′-monophosphate (3′5′-AMP). Inhibition of the enzymatic breakdown of 3′5′-AMP by high concentrations of theophylline results, in most of these hormone-tissue systems, in a simulation of the hormone effect or in a potentiation of its action. Adrenocorticotrophin (ACTH) action on steroidogenesis in adrenals, which involves 3′,5′-AMP formation, is not affected by theophylline to the same degree as the actions of other hormones on their target tissues. A possible explanation for this discrepancy may be found in the fact that high concentrations of theophylline inhibit protein synthesis in surviving rat adrenal tissue, a process which is known to be involved in the steroidogenic action of both ACTH and 3′,5′-AMP. It is suggested that theophylline action in rat adrenals has two antagonistic components, (1) potentiation of the steroidogenic action by protection of 3′,5′-AMP, and (2) reduction of the steroidogenic action by inhibition of protein synthesis.

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Muscle Cell pH in Relation to Chronicity of Potassium Depletion. (31284)

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Rats fed a K-deficient diet and provided dietary sodium chloride undergo a loss of skeletal muscle K which is incompletely replaced by a gain of muscle Na(1,2). Simultaneously, the plasma HCO_3 concentration becomes elevated and the plasma Cl concentration is reduced. Cooke *et al*(3) proposed that an intracellular acidosis in association with an extracellular alkalosis occurs in K deficiency due to transfer of H^+ from the extracellular compartment. This entrance of H^+ into muscle cells was predicted, among other things, on the fact that the sum of Na and K in K-deficient rat muscle is less than in control muscle and the hypothesis that H^+ enters to compensate for the cation deficit. The results of subsequent *in vivo* studies with K-deficient rats(4,5,6,7) have shown an increase, to varying degrees, in the intracellular H^+ concentration of skeletal muscle.

In previous work from this laboratory(8,9) rats placed on low-K diets for periods up to 35 days exhibited an initial rapid loss of muscle K which was quantitatively greater than the gain in Na. Subsequently, during the dietary period 14 through 35 days, K losses were balanced by essentially equivalent gains of Na. Hence, the Na plus K deficit in muscle was constant during the final 21 days of the depletion period. Meanwhile, the plasma HCO_3 content continually increased throughout the entire 35-day period of K deprivation; this was indicative of a continuing decrease in plasma and extracellular H^+ concentration. If extracellular H^+ is transferred to the intracellular compartment of skeletal muscle in compensation for the Na plus K deficit, there should have been no further rise in plasma HCO_3 content during the time the muscle cation deficit was constant. It was therefore of interest to evaluate any changes in intracellular pH (pHi) during the course of K depletion by utilizing the DMO (5,5-dimethyl-2,4-oxazolidinedione) distribution method.

Methods. Young adult male rats of the Wistar strain weighing approximately 270 g were placed either on a control or a low-K, normal Na and Cl diet representing a modification of the diets formulated by Orent-Keiles and McCollum(10). The animals were housed in individual cages and given food