infection with this PPLO strain. Whether the effects here described are due to an intracellular action of the mycoplasma(1) or to mycoplasma-associated products, is yet to be determined. Other effects on FL cells after infection with this PPLO include not only early cytolytic effects, but also the subsequent appearance of a "PPLO-resistant" cell population showing changes in morphology and division rate of the cells(3). It seems reasonable to assume that these changes, and at least some of the chromosome aberrations, are related. The heteroploid FL cells were particularly susceptible to this mycoplasma and were therefore chosen for these initial studies. Further investigations now in progress will include other cell types, also diploid cells, in order to evaluate how generally the effects here described occur as a consequence of infection with this and other strains of mycoplasma.

Summary. Characteristic chromosome changes were observed in PPLO-infected FL human amnion cells. These changes included a gradual reduction in chromosome numbers, increase in chromosome aberrations, and the appearance of 3 new varieties. Although some of the aberrations appeared early after infection, most changes developed slowly, over a period of several months.

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Specificity of Enzyme Inhibition by DDD.* (30146)

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The chlorophenyl position isomers of 2,2-bis (chlorophenyl)-1,1-dichloroethane (DDD) produce adrenal cortical atrophy in the dog (1) and inhibition of steroidogenesis in the human and dog. The o,p′ isomer is more active in causing atrophy than the p,p′ and the m,p′ is the most active(2,3). In other species tested (rat, rabbit, guinea pig, cat, hamster) the atrophy does not occur under conditions of dosage and time similar to those producing it in the dog(1). In a non-responsive species Frenkel *et al*(4) have found lack

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of inhibition of steroid secretion in the DDD-fed hamster. Although intravenous administration of DDD inhibits steroidogenesis and glucose-6-phosphate dehydrogenase (G-6-PDH) activity in dog adrenals within 2 hours, Cazorla and Moncloa(5) have found that addition of DDD to normal dog adrenal slices produces no inhibition of G-6-PDH activity. These investigations suggest that the decreased production of reduced triphosphopyridine nucleotide (TPNH) resulting from the lowered G-6-PDH activity might be one factor in the decreased steroid synthesis which requires this reduced nucleotide. It has been reported that, in the human, decreased conversion of corticoids to reduced metabolites occurs as a result of DDD administration(6,7,8), indicating an extra-adrenal (liver) site of action as well. Recent work(9) indicates that DDD administration increases the conversion of cortisol to metabolites not measured in the usual Porter-Silber procedure.

The work reported here was undertaken to (1) determine whether or not the decreased corticoid reductase activity could also be explained by the relative unavailability of reduced pyridine nucleotides, (2) compare the G-6-PDH inhibition in a responsive (dog) and non-responsive (rat) species, (3) compare the effect of DDD on the activity of another TPNH-producing enzyme, 6-phosphogluconate dehydrogenase (6-P-GDH), and (4) obtain direct evidence concerning the DDD effect on steroidogenesis in another non-responsive species (rat).

Materials and methods. Mongrel dogs were fed 100 mg/kg o,p' DDD by capsule daily for 2 weeks. The dogs were anesthetized with sodium pentobarbital and a 10% homogenate of the adrenals and the liver was prepared in water. Similar homogenates were prepared from rats killed by decapitation after a stunning blow. Rats were fed Purina rat chow containing 0.25% o,p' DDD for 10 days and controls and pair-fed controls received chow only.

The G-6-PDH and 6-GDH activities were assayed under the conditions indicated in Table I. Diluted homogenates containing 2.5-3.5 μg of adrenal tissue or 5-7 μg liver tissue were added to 100 μl of the appropriate reagent and incubated for 30 minutes at 37°C. The necessary blanks and standards were carried through the procedure along with the samples. Following incubation, the TPNH formed was measured fluorometrically (10). During this period the TPNH formed was directly proportional to time and tissue concentration within the limits cited. Proteins were determined by the method of Lowry *et al*(11). The *in vitro* effect of DDD on G-6-PDH was studied in control homogenates with 1 mM DDD added in propylene glycol; an equivalent volume of propylene glycol was added to control incubations.

Liver homogenates from DDD-fed and control dogs were assayed for cortisol reductase activity by incubation with cortisol-1,2-H^3 according to the method of DeMoor *et al* (12). Final concentrations in a 3.0 ml final volume containing 1.0 ml of a 10% liver homogenate were: Cortisol, 0.009 mM (0.5 μc/m mole cortisol-1,2-H^3); TPN, 0.02 mM; and glucose-6-phosphate, 0.1 mM. After a 30-minute incubation in an air atmosphere at 37°C the contents of each flask were extracted with ethyl acetate, evaporated to dryness under N_2 and chromatographed on paper (13). The areas on the chromatograms corresponding to cortisol (F), cortisone (E), tetrahydrocortisol (THF), and tetrahydrocortisone (THE) were located by scanning with a Vanguard Chromatogram Scanner and then cut out and placed in a counting vial containing 15 ml scintillation solution (2,5 di-

TABLE I. Conditions for Determination of Glucose-6-Phosphate Dehydrogenase and 6-Phosphogluconate Dehydrogenase.

Enzyme	Buffer	Substrate	Other additions
G-6-PDH	Tris (hydroxy methyl) amino methane, 100 mM, pH 8.9	glucose-6-phosphate, 1 mM	TPN, 0.3 mM; bovine serum albumin, 0.05%
6-PGDH	2-amino, 2-methyl propanediol, 100 mM, pH 9.0	6-phosphogluconate, 0.5 mM	TPN, 0.5 mM; bovine serum albumin, 0.05%

phenyl oxazole, 6 g/l; 2,2-p-phenylene bis (5-phenyloxazole), 0.15 g/l toluene). Radioactivity was assayed in a Packard Tri Carb Liquid Scintillation Spectrometer.

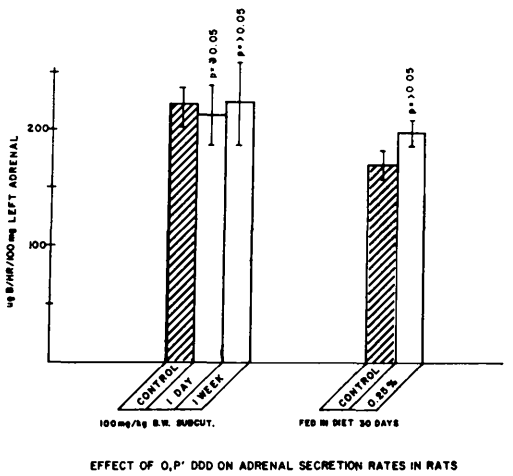
Rat adrenal steroid secretion rates were determined as described by Huffman and Azarnoff(14) on DDD-fed and normal animals.

Results. Significant inhibition of G-6-PDH was seen in adrenals of dogs, but not rats fed DDD (Table II). No significant effect upon liver G-6-PDH of either species was demonstrated. Studies of *in vitro* addition of DDD at the 1 mM level showed no inhibition of G-6-PDH activity of adrenal or liver in either species which is consistent with the observation of Cazorla and Moncloa(5) with dog adrenal slices. We also found no inhibition of G-6-PDH activity when purified yeast enzyme was assayed in the presence of 1 mM DDD.

No significant inhibitory effect of DDD feeding on the 6-P-GDH of liver or adrenal in either the rat or dog was found (Table III). Similarly, there was no decrease in ability of liver homogenates from DDD-fed dogs to convert cortisol to its tetrahydro derivatives (Table IV).

Fig. 1 illustrates the results of feeding and of subcutaneous injection of DDD upon adrenal secretion rates in rats. These data indicate no significant alteration in the adrenal corticosterone secretion rate of rats as a result of DDD administration and are consistent with the lack of histological changes observed in the rat adrenal even at high dosage schedules.

Discussion. It is possible that differences



EFFECT OF O,P' DDD ON ADRENAL SECRETION RATES IN RATS

FIG. 1. Adrenal steroid secretion rates of DDD-treated and control rats.

in inhibitory effect of DDD feeding on G-6-PDH activity in sensitive and non-sensitive species and tissues may be related to concentration of the drug in the tissues. Shortly after a single intravenous dose of DDD to dogs and rats the adrenal shows a higher concentration of the drug than does any other tissue(15). However, after a week or more the levels in liver and adrenal approach the same concentration and those tissues with high fat content (nerve, depot fat) may show a higher concentration than the adrenal. Therefore, in the experiments reported here it is unlikely that there were great total differences in liver and adrenal drug concentration although there is the possibility that differences in concentration in various cellular components may exist. Lack of *in vitro* inhibition of G-6-PDH suggests the possibility of the need for conversion of DDD to

TABLE II. Glucose-6-Phosphate Dehydrogenase Activities in Control and DDD-Fed Dog and Rat Tissues.

Tissue	No. of animals	Control	Pair-fed control	DDD-fed	Groups compared	P
		moles/kg protein/hr				
Dog liver	5	.88 ± .11*		.86 ± .20	Control *vs* DDD-fed	>.05
" adrenal	5	3.27 ± .69		2.22 ± .40	Control *vs* DDD-fed	<.001
Rat liver	12	.80 ± .46	.99 ± .60	.71 ± .26	Control *vs* DDD-fed	>.05
					Pair-fed control *vs* DDD-fed	>.05
" adrenal	6	4.56 ± .42	4.44 ± .68	5.72 ± .82	Control *vs* DDD-fed	<.02
					Pair-fed control *vs* DDD-fed	<.02

* Mean ± standard deviation.

TABLE III. 6-Phosphogluconate Dehydrogenase Activities in Control and DDD-Fed Dog and Rat Tissues.

Tissue	No. of animals	Control	Pair-fed control	DDD-fed	Groups compared	P
		moles/kg protein/hr				
Dog liver	2	.24 ± .01*		.67 ± .10	Control *vs* DDD-fed	<.025
" adrenal	2	.15 ± .07		.31 ± .06	Control *vs* DDD-fed	>.05
Rat liver	6	1.00 ± .36		1.16 ± .32	Control *vs* DDD-fed	<.025
" adrenal	6	2.16 ± .92	2.16 ± .20	2.41 ± .44	Control *vs* DDD-fed	>.05
					Pair-fed control *vs* DDD-fed	>.05

* Mean ± standard deviation.

an "active" form before inhibition can occur or that due to its water insolubility, it is not available to the enzyme in the form in which it was added. The enzyme 6-P-GDH is not affected by DDD nor did our experients demonstrate a change in cortisol reduction by liver homogenates from DDD-fed animals.

The lack of effect of DDD on rat adrenal steroid secretion rates corroborates the histological evidence and fits the concept of DDD responsive (dog) and DDD resistant (rat) species.

Summary. Dog adrenal glucose-6-phosphate dehydrogenase is inhibited by DDD feeding while the same enzyme is not changed in the adrenals of similarly treated rats. The activity of the liver enzyme is not affected in either species. Inhibition of neither purified yeast G-6-PDH, normal liver, nor adrenal enzyme could be demonstrated after addition of DDD to incubation mixtures. DDD feeding does not decrease the activity of 6-phosphogluconate dehydrogenase in the liver or adrenal of either species. No effect of DDD on dog liver cortisol reductase could be demonstrated. Direct evidence for lack of effect of this drug on rat adrenal steroid secretion rate is presented.

TABLE IV. Effect of DDD Feeding on Conversion of Cortisol to Reduced Metabolites by Dog Liver Homogenates.

No. of animals	Radioactivity ratio	Control	DDD-fed	P
9	THF:F	.05 ± .02	.04 ± .01	>.05
10	THE:E	.54 ± .30	.69 ± .35	>.05

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