(1,2). It was shown that when 25 γ or more testosterone propionate was injected, the sexual behavior was comparable with that of the intact controls; while the group receiving 12.5 γ always had a lower drive. As in the part of the study summarized earlier it was not possible to differentiate between those animals receiving daily injections of 25, 50, or 100 γ testosterone propionate/100 g body weight.

Summary. A non-directed hyperexcitability, dependent on the level of androgen, has been described in the male guinea pig. An increase in the frequency of its occurrence took place after castration; and with the daily administration of testosterone propionate in amounts of 25 γ or more per 100 g body weight there was an immediate return to the level of the intact control. Injection of 12.5 γ of the hormone did not eliminate the increase which followed castration. The similarity of these results to other data bearing on the effect of testosterone on sexual behavior is noted.

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Effect of Quinones on Adaptive Enzyme Formation in a Strain of *Pseudomonas aeruginosa.* (20945)

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A washed suspension of a strain of *Pseudomonas aeruginosa* oxidizes benzoic acid after a latent period of 100-120 minutes(1). The latent period can be decreased and the rate of benzoate oxidation increased by the previous addition of succinate and ammonium sulfate. These effects are proportional, within limits, to the amount of succinate added(2). As a result of the oxidation of succinate, ammonia is assimilated, precursors, possibly peptides or proteins(3) are stored in the cell, and these can subsequently be utilized to form the enzyme when benzoic acid is added. It was thus possible to study the effect of a compound on enzyme formation in 2 ways provided the rate and extent of succinate oxidation is not affected. If the compound is added at the beginning with succinate and ammonia, it could increase or decrease the formation of the precursors and, if it is not metabolized, also affect the utilization of the precursors for enzyme formation. If the compound is added with the benzoate after the succinate oxidation is completed, it could only affect the utilization of precursors. The following is a study of the effect of catechol, protocatechuic acid and related compounds on the formation of the enzyme for benzoic acid.

Methods. A strain of *Pseudomonas aeruginosa* originally obtained from Dr. Koppers was grown in 100 ml of nutrient broth (Difco) for 48 hours at 37°C. The cells were centrifuged down, suspended in 50 ml distilled water, centrifuged again, and finally suspended in 7.0 ml of 0.05 M Na-K-phosphate buffer pH 7.8. 0.5 ml of this suspension was used in each Warburg vessel which had a final fluid volume of 2.0 ml. All the compounds added were dissolved in buffer and when necessary adjusted to pH 7.8. 0.2 mg of succinic acid and 0.2 mg ammonium sulfate were added to the bacteria at zero time with or without the compound to be tested and the rate and extent of the oxygen uptake measured. The succinate oxidation was usually completed in one hour. At the end of 2 hours, 1.0 mg of sodium benzoate was added from the side arm with or without the

TABLE I. Effect of Various Compounds on Oxidation of 1 mg of Sodium Benzoate. Cells were incubated 2 hr at 37°, pH 7.8 with 0.2 mg succinic acid and 0.2 mg $(NH_4)_2SO_4$. Compounds were added (a) at zero time with succinic acid and ammonia, or (b) at the end of 2 hr with the benzoate. In the concentrations used none of the compounds affected the rate or extent of succinate oxidation. Figures, in mm³ oxygen uptake, represent oxidation of benzoate from which the appropriate control uptakes have been subtracted.

γ/ml	Compound added	mm³ O_2 uptake				
		60 min.	75 min.	90 min.	105 min.	120 min.
	0	11	49	113	201	304
50	Catechol with benzoate	6	8	9	13	14
25	" " "	10	27	44	72	113
5	" " succinate	26	78	116	282	401
10	" " "	57	132	238	359	481
15	" " "	66	142	246	365	487
50	Protocatechuate with benzoate	29	87	147	244	350
5	" " succinate	22	81	150	253	371
10	Dopa with benzoate	1	5	15	48	96
10	" " succinate	13	52	118	210	314
50	Epinephrine with benzoate	6	27	70	142	191
50	" " succinate	4	15	45	112	153
	0	18	62	128	214	316
10	Hydroquinone with benzoate	6	10	21	42	75
10	" " succinate	17	61	111	189	285
10	p-aminophenol with benzoate	10	37	78	138	216
10	" " succinate	8	12	17	32	41

compound. The rate of benzoate oxidation was an index of the amount of enzyme formation.

Results. Both catechol and protocatechuic acid have been identified as intermediates in the oxidation of benzoate by *Pseudomonas fluorescens* (4) and by certain mycobacteria (5). Both were oxidized slowly by our strain of *Pseudomonas*, and one or both were formed during benzoate oxidation as shown by the addition of 5-amino uracil which condenses with them to form a yellow compound. It was therefore rather surprising to find that catechol added with benzoate markedly inhibited the formation of the benzoic acid enzyme, and this effect was unchanged after 2 recrystallizations of the catechol. On the other hand, small amounts of catechol added at zero time with succinate stimulated enzyme formation as shown by decreased latent period and increased rate of benzoate oxidation. This effect might be described as a type of back adaptation. Protocatechuate, whether added at zero time or with benzoate, only stimulated enzyme formation. In order to test the possible effect of the carboxyl group, 3,4 dihydroxyphenalanine (dopa) was compared to epinephrine. When added with benzoate the former was 4-5 times as effective as an inhibitor. Thus a carboxyl group on the side chain does not neutralize the effect of the rest of the molecule. When dopa was added at zero time it had little effect, whereas epinephrine was almost equally active when added at zero time and with benzoate. These results are shown in Table I.

The percentage inhibition by catechol and dopa was independent of the amount of succinate. The oxidation rate of benzoate when it was added after 0.3 mg of succinate had been oxidized was for a time 3 times that after 0.1 mg of succinate, yet during this period the percentage inhibition varied only between 84 and 90 for both compounds at both concentrations of succinate. It is thus improbable that they are reacting directly with the precursors to make them unavailable for enzyme formation. The percentage inhibition by both compounds was also independent of the amount of benzoate when it was varied from 0.1 to 1.0 mg. Therefore mass action effects are not directly involved.

Other quinones were also active. The results with hydroquinone and p-aminophenol are shown in Table I. It is obvious that the latter was much more effective when added

with succinate which indicates that it inhibited the formation of precursors more than their utilization. But hydroquinone like dopa had little effect when added with succinate which suggests that both compounds were rapidly inactivated in the presence of the bacteria oxidizing succinate and much less rapidly inactivated after the succinate oxidation was completed. It is obvious that all these compounds would readily autoxidize at pH 7.8 to quinones, and quinone added as such has exactly the same effect as hydroquinone. Comparison of naphthoquinones with anthraquinones showed that the former were more effective inhibitors in concentrations which had no effect on succinate oxidation and that they behaved like hydroquinone.

Discussion. Quinones readily inhibit enzyme formation. Catechol even though an intermediate in benzoate oxidation still behaves like other quinones until the enzyme for its oxidation is formed. If 10 γ/ml of catechol is added with succinate, then not only is some benzoate enzyme formed but also some catechol enzyme, and under these conditions the subsequent addition of 50 γ/ml of catechol with the benzoate causes little or no inhibition. At present there is no obvious reason why in the orthoquinone series dopa is the most active inhibitor or why p-aminophenol behaves differently from hydroquinone.

Summary. Catechol added with benzoate inhibits the formation of the enzyme which oxidizes benzoate in a strain of *Pseudomonas aeruginosa.* Added with succinate and ammonium sulfate 2 hours before benzoate it facilitates the formation of the benzoate enzyme. Protocatechuic acid only facilitates the formation. Dopa and epinephrine also inhibit enzyme formation, the former only when added with benzoate, the latter when added before or with benzoate. Hydroquinone, quinone, and p-aminophenol inhibit formation, the first 2 only when added with benzoate. The last is more effective when added before benzoate.

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Effects of a Lysine Deficiency Upon Enzyme Activity in Rat Liver.* (20946)

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Several laboratories(1-4) have reported that liver xanthine oxidase activity is reduced in rats fed low protein diets. It has been noted by Richert and Westerfeld(5) that livers of weanling rats on a low protein ration (8% casein) lose about 50% of their liver choline oxidase activity. Williams and Elvehjem(6) have shown that with a tryptophan deficiency in the adult rat liver xanthine oxidase activity and endogenous respiration are reduced markedly, and the activity of succinic oxidase is decreased to some extent. Williams, Denton, and Elvehjem(7) have reported that in weanling rats the activity of rat liver xanthine oxidase can be completely removed by a methionine deficiency while succinic oxidase activity can be reduced somewhat. Unexpected results were obtained in some recent studies with a histidine deficiency (8) in which it was found that the absence of dietary histidine had no demonstrable effect upon xanthine oxidase, choline oxidase, or succinic oxidase, although weanling animals forcibly fed the deficient ration began to die

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