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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after about 10 days.

In the present paper we wish to present results of the effects of a lysine deficiency upon liver enzymes. It was felt that deficiency of lysine, an amino acid which once deaminated cannot be reaminated, might show drastic effects on liver enzymes, effects which would be possibly comparable to those obtained with a non-protein ration. The present studies were conducted in a manner similar to those reported earlier(7,8) in which results of *ad libitum* feeding were compared with those of forcible feeding by stomach tube. These feeding procedures were compared since it is known that animals receiving an amino acid-deficient ration *ad libitum* show a marked loss in appetite(9). Thus it was considered imperative to keep food intake at a constant level to rule out any complications due to inanition. Also this makes it possible to compare the results directly with those obtained with a methionine or histidine deficiency, which have been previously reported(7,8). The enzyme systems chosen for study were liver xanthine oxidase, succinic oxidase, choline oxidase, and endogenous respiration. These systems are representative in that xanthine oxidase is a soluble cytoplasmic enzyme while succinic and choline oxidase are mitochondrial systems. Endogenous respiration may be considered a measure of general oxidative processes in the liver homogenate.

Procedure. Male weanling rats of the Holtzman strain were divided into 4 groups and placed on the purified ration of Ramasarma *et al.*(10) for one week or until an average weight of 55-60 g was reached. This ration consisted, in brief, of purified amino acid mix (16%), Salts IV(11), corn oil, the known vitamins including B_{12} (20 γ/kilo of ration) and sucrose. Two of the groups were fed *ad libitum* and 2 groups were fed forcibly. The latter groups were fed by stomach tube to insure a constant food intake. In previous similar experiments it was shown that weanling rats consume, on the average, 6 g of the synthetic ration per day. Therefore, this level was chosen to be fed. Feedings were made 3 times daily at 6-hour intervals. All groups were fed the complete purified ration for one week to accustom the animals to feeding procedures and to make sure that the animals were growing well at the beginning of the experiment. All animals were given water *ad libitum*. The results reported in this paper are the combined results of 3 replicate experiments. The animals were sacrificed for the enzyme determinations between 10 and 14 days when animals from the group forcibly fed the lysine deficient ration showed marked deficiency symptoms and appeared near death. Control animals were also removed for analysis when deficient animals were analyzed. The animals were killed by decapitation and the livers removed and placed immediately in cracked ice. A portion of each liver was quickly weighed, homogenized in 5 volumes of 0.039 M sodium potassium phosphate buffer, pH 7.3, and strained through gauze. This homogenate was used for xanthine oxidase determination according to the method of Axelrod and Elvehjem(12) and choline oxidase by the method of Williams *et al.*(13). A portion of the homogenate was diluted with buffer to give a 5% homogenate which was employed for measuring succinic oxidase according to the method of Schneider and Potter(14). Endogenous respiration was calculated from the oxygen uptake of the control flasks in the xanthine oxidase assay during the first 10-minute interval following the temperature equilibration period. Succinic oxidase, choline oxidase and endogenous respiration were assayed at 30°C while xanthine oxidase was determined at 37°C. This higher temperature was found necessary since erratic results have occasionally been obtained at 30°C(15). Nitrogen of the homogenates was determined by a semi-micro Kjeldahl procedure in duplicate.

Results. The enzyme activities in Table I are based on liver nitrogen. Xanthine oxidase activity is expressed in microliters of oxygen uptake per hour per g of liver nitrogen. Choline oxidase, succinic oxidase, and endogenous respiration activities are reported as microliters of oxygen uptake per hour per mg of liver nitrogen.

As seen in Table I, a lysine deficiency exerts a marked effect upon the activities of xanthine oxidase, succinic oxidase, and choline oxidase. A reduction to 58-76% of basal

TABLE I. Effects of Lysine Deficiency upon Liver Enzyme Activities and Liver Nitrogen of Rats Fed Forcibly and *Ad libitum*.

		Forcibly fed rats			*Ad libitum* fed rats		
Enzyme determined	Ration	No. of rats	Enzyme activity based on liver N.*	Ratio of deficient to control	No. of rats	Enzyme activity based on liver N.	Ratio of deficient to control
Xanthine oxidase	Basal	7	5030 ± 113†	.62	6	4800 ± 430	.57
	" -lysine	9	3120 ± 357		6	2780 ± 260	
Succinic oxidase	Basal	14	717 ± 59	.71	12	790 ± 51	.71
	" -lysine	15	510 ± 47		9	560 ± 49	
Choline oxidase	Basal	10	59 ± 6	.76	9	64 ± 8	.67
	" -lysine	13	45 ± 4		10	43 ± 4	
Endogenous respiration	Basal	13	94 ± 8	(.86)	10	69 ± 9	(1.11)
	" -lysine	15	81 ± 8		10	77 ± 5	
			mg N./g fresh liver			mg N./g fresh liver	
Liver nitrogen	Basal		25.8 ± .9	.87		25.8 ± .7	.88
	" -lysine		22.4 ± .3			22.6 ± .7	

* See text for method of expressing enzyme activities. Activity in terms of fresh liver wt can be calculated from enzyme results and figures for N./g fresh liver.

† Stand. error of mean.

activity was noted for these enzymes in terms of liver nitrogen. The cytoplasmic enzyme, xanthine oxidase, was somewhat more greatly affected than the mitochondrial enzymes, succinic oxidase, and choline oxidase. Endogenous respiration was not significantly altered by the lysine deficiency.

It can be noted that the reduction of activity of the enzymes studied was essentially the same whether the lysine deficient ration was fed forcibly or fed *ad libitum*. This was true even though the animals fed *ad libitum* showed a significantly lower food intake.

Nitrogen analyses of the livers of the rats indicate that a lysine deficient ration either fed *ad libtum* or fed forcibly causes a small but significant reduction in the nitrogen content of the liver. It should be noted, however, that the loss of enzyme activity is much greater than the loss of general liver nitrogen. Since a non-protein ration has been shown to reduce drastically the activity of xanthine oxidase in as short a period as 5 days(3), it is conceivable that a deficiency of any of the essential amino acids might have a similar effect. In the absence of one critical amino acid other amino acids would not be expected to be utilized for protein synthesis. Earlier results from this laboratory(7,8) have indicated that xanthine oxidase activity is reduced almost completely by a methionine deficiency while a histidine deficiency exerts no demonstrable effect upon this enzyme. As now observed, the lack of lysine exerts an intermediate effect in that the activity of xanthine oxidase is reduced to somewhat over half that of the normal.

The observed decrease in activity of succinic oxidase is greater than that produced by either of the other 2 amino acids (methionine and histidine) and is comparable to the decrease noted for both xanthine oxidase and choline oxidase. It can be seen that the lysine deficiency produces similar depression of activity in each system while histidine and methionine each produce a completely different pattern. Endogenous respiration has not been shown to be significantly changed by a deficiency of any of the 3 amino acids.

These results suggest that after 10 days to 2 weeks of forcibly feeding a ration devoid of lysine, although the animals are beginning to die, there appear to be sufficient body stores of lysine to maintain these enzymes to a moderate extent when a normal level of other essential and non-essential amino acids are fed. This is in contrast to the drastic reduction in activity obtained with a non-protein ration.

With the 3 amino acids studied thus far, no great differences have been found between the groups receiving the ration *ad libitum* or by stomach tube although in all cases animals receiving the deficient ration by stomach

tube have begun to die before the end of the 2-week period. Therefore, it would appear that when fed a ration deficient in an essential amino acid the young animals used in these experiments are unable to cope with normal quantities of food intake. Thus they begin to die after about 10 days when fed a constant amount of food by stomach tube. Animals of similar weight and age fed *ad libitum*, however, appear to adjust to some extent to the deficient ration by reducing their food intake and consequently survive for longer periods of time.

Summary. 1. A lysine-free ration fed to weanling rats either *ad libitum* or by a forcible feeding procedure reduces the activity of liver xanthine oxidase, succinic oxidase, and choline oxidase to somewhat over half that of normal. 2. A lysine deficiency has little effect on liver endogenous respiration. 3. The deficiency produces an entirely different pattern of liver enzyme activity from that produced by a methionine or histidine deficiency.

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Nutritional Studies with the Guinea Pig. B-Vitamins other than Pantothenic Acid.* (20947)

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The only B-vitamins for which there is unequivocal proof of dietary essentiality for life in the guinea pig are thiamine(1-3), folic acid(4-7), and pantothenic acid(8). There is considerable indication that a dietary supply of niacin also is essential(9,10).

A semi-synthetic diet recently developed for guinea pigs(11) has proven useful in evaluating the essentiality of several of the B-vitamins for these animals. In the present investigations deficiencies of thiamine, choline, folic acid, pyridoxine, niacin, and riboflavin have been demonstrated in the guinea pig, some for the first time. This report is concerned chiefly with a brief description of the gross deficiency symptoms and the effects of the deficiency on the growth rate and survival time. The effect of lack of a dietary supply of para-aminobenzoic acid, inositol, biotin, and vit. B_{12} has also been studied. Supplements of these substances were found

* Part of the data in this paper was presented at the American Institute of Nutrition in Chicago, April 1953 (*Fed. Proc.* 1953, v12, 472.)