Respiratory Activities of Hypo- and Hypervitaminotic-A Rat Liver Homogenates. (29612)

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Although the role of vitamin A in the visual process is now reasonably well understood, its mechanism of action in intermediary metabolism and particularly in structural integrity of various tissues is not clear. Many investigators have sought to learn more about the function of vit. A through studies on the metabolic lesions elicited by a deficiency or an excess of the vitamin. Recently DeLuca *et al*(1) showed an increase in oxygen uptake (measured for 30 min) by liver homogenates of vit. A-deficient rats with a number of Krebs cycle acids. Sampson and co-workers(2) demonstrated that most female rats receiving from 300-525 units of vit. A/g of body weight daily had higher respiratory quotients than litter mate controls. Redfern(3) recorded an increase in endogenous oxidation by liver homogenates from vit. A-deficient rats. On the other hand, Ray and Sadhu(4) reported that liver slices and homogenates of rats fed excess vit. A, showed a significantly decreased oxidation of succinate. Berdjis(5) reported an increase in number of mast cells in almost all organs of rats given toxic doses of vit. A. They further stated that many pathological conditions and metabolic disturbances are directly related to the variations in mast cell population in tissues. Squibb(6) reported that chickens receiving 100,000 units of vit. A/day for 13 days showed a decrease in liver lipids. Brown *et al*(7) suggested that permeability changes associated with hypervitaminosis-A may be due to a change in sulfation of membrane-associated mucopolysaccharides, which appear to be necessary for the integrity of membrane structure.

In this paper results are reported which show a marked initial elevation in oxygen uptake by liver homogenates from either hypovitaminotic or hypervitaminotic-A rats. The initial increase in oxygen consumption is followed by a rapid decline during the following hour. An effect of tri-o-cresyl phosphate (TCP) on respiratory control and liver lipids has also been demonstrated. This substance was studied because previous work(10) had shown it to have a pro-oxidant action in unsaturated lipid systems *in vitro,* to decrease vit. A, and to produce changes in body fat composition *in vivo.*

Materials and methods. Male weanling rats of an Osborne-Mendel derived strain (FDA, Division of Nutrition) from dams maintained on a low vit. A stock diet were used in our deficiency studies. The basal diet contained 18% casein (vitamin free), 64% sucrose, 10% lard, 4% salt mixture (Jones-Foster), and 4% vitamin mixture (vit. A and E free). To this was added vit. E as a mixture of dl-alpha tocopherol and dl-alpha tocopherol acetate at 200 mg/kilo. Six weanling rats were used in each of 4 groups: They were fed 1) the basal diet, 2) basal diet supplemented with 5 mg of vit. A acetate per kilo, 3) basal diet with 0.1% TCP, and 4) basal diet containing both vit. A and TCP. Vit. A deficiency was determined by the onset of loss in body weight, which occurred in 28-40 days. Food and water were available *ad libitum.* In our excess vit. A experiments, young adult rats weighing about 100 g were divided into 2 groups of 5 rats each and maintained on a stock diet (Division of Nutrition). The rats of one of these groups received daily supplements of 50,000 units of vit. A, *per os,* for one week. The rats were sacrificed, their livers quickly excised and placed in cold isotonic saline solution. The tissues were blotted dry, weighed and homogenized in 0.25 M de-ionized sucrose to yield a 10% w/v homogenate, by means of a Potter-Elvehjem homogenizer fitted with a Teflon pestle. The homogenates (0.5 ml) were added to Warburg vessels containing a solution of 40 μmoles sodium phosphate buffer, pH 7.4, 300 μmoles NaCl, 12 μmoles

TABLE I. Effect of Vitamin A Deficiency and Tri-o-cresyl Phosphate (TCP) on Oxidative Decline in Liver Homogenates.*

Dietary supplement	α-Ketoglutarate Time interval 0–30	60–90	% Change	Succinate Time interval 0–30	60–90	% Change
None	8.0 ± .6	4.7 ± .6	−41 ± 5.0	14.0 ± .8	8.0 ± .7	−47 ± 7.0
Vit. A	5.6 ± .3‡	7.0 ± .3	+25 ± 4.0	10.3 ± 1.0	7.8 ± .6	−24 ± 4.0
TCP	7.7 ± .7	9.2 ± .7	+20 ± 3.0	15.4 ± 1.0	11.3 ± .6	−26 ± 9.0
Vit. A + TCP†	6.1 ± .4	8.1 ± .3	+33 ± 6.0	6.8 ± .8	5.8 ± .2	−15 ± 7.0

* Values are expressed as μl O_2/mg homogenate protein/½ hr.
† When fed, vit. A acetate was added at 5 mg/kg of diet, and TCP at 0.1% of diet.
‡ Values are average of 6 rats per group with standard error of mean.

KCl, 4 μmoles $MgSO_4$ and 60 μmoles of substrate; 3 μmoles of nicotinamide-adenine-dinucleotide (NAD) was added when required. The final incubation volume was 3 ml. Oxygen uptake was measured for 1½ hours at 37°C by standard Warburg techniques. The center well contained 0.2 ml of 30% KOH absorbed on filter paper and the gas phase was air. Corrections were made for endogenous oxidation. The results of the oxygen uptake are expressed as μl of oxygen consumed/30 minute/mg homogenate protein (QO_2 protein). An aliquot of each homogenate was analyzed for protein by the Biuret technique, total nitrogen by Kjeldahl method, vit. A by the method of Ames *et al*(8). Total lipid was determined gravimetrically.

Results: Vitamin A deficiency. Oxidation of α-ketoglutarate and succinate by homogenates from the deficient rats exhibited an initial increase of about 40% in the first 30 minutes. This was followed by a rapid decline (Table I and Fig. 1). Data in Table I and Fig. 1 also show that dietary supplementation of vit. A prevented the initial increase, as well as the subsequent decline in oxygen uptake. Addition of TCP to the basal diet also prevented the initial increase and the subsequent decline of α-ketoglutarate oxidation by the vit. A deficient homogenates. Vit. A and TCP did not have the same protective effect with all substrates (Table I). In the oxidation of succinate by the homogenates an appreciable decline was observed (approx. 24%). The same magnitude of respiratory decline occurred in all groups including those supplemented with vit. A. Vit. A deficiency has no effect on total liver fats or liver protein (Table II). On the other hand, when TCP was added to the vit. A deficient diet, a 36% increase in total liver fat and a slight but insignificant decrease in liver proteins was noted. When vit. A and

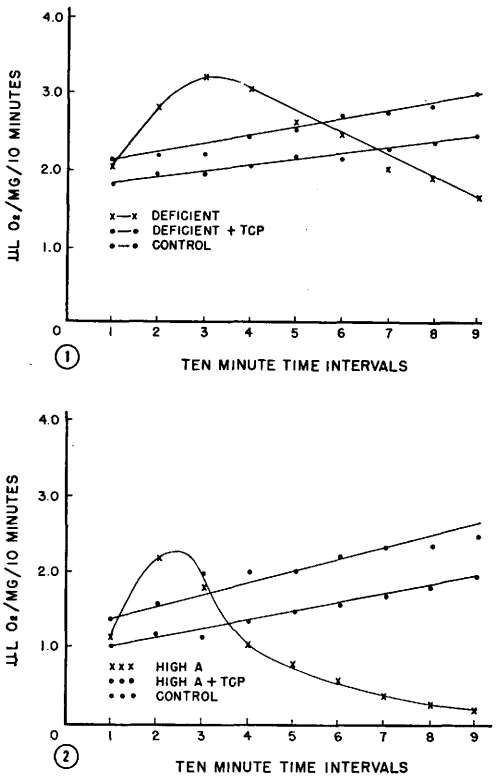

FIG. 1. Prevention of decline of α-ketoglutarate oxidation in liver homogenates of vit. A deficient rats by dietary addition of vit. A and/or TCP. Each point on the curve is average of 5 experiments.

FIG. 2. Prevention of decline of α-ketoglutarate oxidation by *in vitro* addition of TCP in liver homogenates from rats receiving excess vitamin A. Each point on the curve is the average of five rats. The rats received 50,000 units of vitamin A acetate/day for five days. The TCP was rehomogenized with homogenates at a concentration of 20 μg/50 mg of liver.

TABLE II. Effects of Vitamin A and TCP on Liver Constituents.

Diet supplements	Fat (%)	Vitamin A (μg/g)	Protein (%)
None	6.0 ± .2	0	18.3 ± 2.0
Vit. A	5.9 ± .4†	92 ± 11.0	18.0 ± 1.0‡
TCP	9.3 ± .3	0	17.6 ± 2.1
Vit. A + TCP	10.4 ± .8	50 ± 3.0	17.8 ± 1.5
High vit. A*	6.0 ± .4	1517 ± 11.0	15.9 ± .3‡

* Rats received 50,000 units of vit. A/day for 5 days.
† Values are average of 6 rats/group (± S.E. of mean).
‡ $P < .02$.

TCP were both added to the diet a greater increase in total fat content of the liver, no change in liver protein and an approximately 45% decrease in the liver storage of vit. A, were observed.

Hypervitaminosis-A. When excess vit. A was given to rats for 5 days there was an initial increase in oxygen consumption by liver homogenates when pyruvate, citrate, α-ketoglutarate, β-hydroxybutyrate and succinate were used as substrates (Table III). When compared to the control values for the first 30 minutes the increases in oxidation by the homogenates from hypervitaminotic-A rats were 160, 38, 76, 11, and 54% for pyruvate, citrate, α-ketoglutarate, β-hydroxybutyrate and succinate, respectively. Subsequently there was a marked decline in respiratory activity. The addition of TCP *in vitro* to liver homogenates from the hypervitaminotic-A rats at 20 μg/50 mg of tissue, restored oxidation almost to normal, and prevented oxidative decline (Fig. 2). Data in Table II show that excess vit. A did not affect total liver fat, but caused an increase in liver storage of the vitamin and a significant decrease in liver proteins. These results are similar to those found in chickens by Squibb(6).

Discussion. The present observations have shown that in a vit. A deficiency, the metabolic lesion is manifested through an initial increase in liver respiration followed by a rapid decline. This metabolic disturbance can be prevented by dietary supplementation of vit. A or TCP. The increase in oxygen consumption and subsequent decline occurred in both a vit. A deficiency and with an excess of the vitamin, suggesting involvement of a common mechanism. Brown *et al*(7) have postulated that toxic dosages of vit. A cause a change in membrane permeability due to a decrease in sulfation of mucopolysaccharides associated with cellular lipoproteins. A permeability change could cause the initial increase in oxidation by increasing the accessibility between enzyme and substrate. Schwarz *et al*(13), in a study of respiratory decline associated with vit. E deficiency, found that vit. E exerted its protective effect on the enzyme at trace element sensitive sites. Any of several trace metals induced respiratory failure. The similarity of the respiratory response found in both a vit. E and vit. A deficiency, would suggest that the metabolic lesions occurring in both deficiencies might be of similar origin. Permeability changes associated with a vit. A deficiency become more important in light of the recent work of Wolf and Varandiani(11) and Moretti and Wolf(12), who demonstrated that mucopolysaccharide biosynthesis is impaired in this deficiency. The present observations provide further support for the hypothesis that vit. A and other fat soluble vitamins(7) act through at least one common function in biological systems.

TABLE III. Effects of Excess Vitamin A on Liver Respiration.*

Substrates	Normal 0–30 min	Normal 60–90 min	Normal % Change	High vitamin A† 0–30 min	High vitamin A† 60–90 min	High vitamin A† % Change
Pyruvate	3.2 ± .2	7.1 ± .7	+119 ± 5.0	8.3 ± .7	.3 ± .3	—97 ± 4.0
Citrate	6.4 ± .5	7.2 ± .5	+ 13 ± 1.0	8.8 ± .5	3.4 ± .5	—61 ± 4.1
α-Ketoglutarate	5.5 ± .2	9.5 ± .7	+ 73 ± 3.1	9.6 ± .5	.7 ± .5	—93 ± 5.0
β-OH-butyrate	4.5 ± .3	8.8 ± .5	+ 96 ± 3.5	5.0 ± .3	8.8 ± .7	+76 ± 4.2
Succinate	8.9 ± .7	6.9 ± .3	— 22 ± 5.0	13.7 ± .8	12.4 ± .8	—11 ± 2.0

* Values are average of 5 rats/group, expressed as μl of O_2/mg of homogenate protein/½ hr.
† Rats received 50,000 units of vit. A/day for 5 days.

The fact that TCP added *in vivo* as well as *in vitro* prevented the oxidative decline of α-ketoglutarate is, for the moment, unexplained. The data obtained support, in part, the view of Squibb(6) that animals receiving high dosages of vit. A showed a significant decrease in liver proteins.

Summary. The rates of oxidation of several Krebs cycle acids by rat liver homogenates were similarly affected by either a vit. A deficiency or a vit. A excess. In both conditions the initial increases of about 40% were followed by marked respiratory declines. Tri-o-cresyl phosphate *in vivo* or *in vitro* prevented the initial increase with subsequent decline in respiration of livers from abnormal vit. A-nurtured rats. However, this chemical in the diet (0.1%) reduced the liver vit. A level by 50%, decreased liver protein and markedly increased liver fat.

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Sodium Mobilization in the Perfused Rat Tail Artery.* (29613)

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Closed circuit perfusion of the rat tail with a medium in which sodium is reduced and replaced by sucrose produces a decline in the resistance of the vascular bed(1). In such a system, continuous monitoring with Na^+ and K^+ glass electrodes indicates that sodium is added to the medium from regions of the tail tissue not accessible to inulin. In contrast, when potassium or choline are the replacements for sodium, resistance of the vascular bed does not decline, and little, if any, sodium is added to the perfusing medium. This relationship between mobilization of sodium and peripheral vascular resistance suggests that vascular smooth muscle cells themselves are directly involved in the exchange. If so, this supports the view that the transcellular distribution of sodium is a determinant of vascular smooth muscle tension. Accordingly, in the present experiment, sodium and potassium distribution in the rat tail artery was measured by conventional chemical techniques following perfusion with low sodium solutions in which lactose, potassium chloride, or choline chloride served as substitutes. As anticipated, a differential mobilization of sodium was observed.

Methods. Male albino rats of an inbred Wistar strain weighing 300 g or more, in 4 groups of at least 8 animals each, and anesthetized with a pentobarbitone-phenobarbitone combination(2) were used. The ventral tail artery and a companion vein were cannulated and the tail then perfused at 0.65 ml/min and 37°C. The venous effluent was discarded. The system was first allowed to run freely for 3 minutes using a basal solution of the following composition in mM/

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