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Vitamin E Deficiency and Ion Transport in Rat-Liver Slices

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Rat-liver slices lose potassium when placed in cold saline solution and reaccumulate potassium when placed in warm oxygenated Ringer solution (McLean, 1963). Weanling rats fed on a yeast diet which is deficient in vitamin E and selenium suffer acute liver necrosis at 17 ± 4 days after starting to eat the diet. Liver slices taken from rats given this diet for 7 days or more cannot reaccumulate potassium after cooling (McLean, 1960*c*). It was postulated that failure of ion transport might be the basic lesion in dietary liver necrosis. Such a failure of ion and water transport would fit in well with Himsworth's (1947) theory that swelling of the liver cells and occlusion of the sinusoids was the final step leading to dietary liver necrosis.

Schwarz (1958) found that either vitamin E or selenium added to the diet would prevent liver necrosis in rats fed on a yeast diet. Chernick, Moe, Rodnan & Schwarz (1955) found that liver slices from rats given the yeast diet show a progressive decrease in oxygen uptake after incubation for 30 min. *in vitro*. This failure was called 'respiratory decline' and it was postulated that a failure in oxidation, probably at a mitochondrial level, was the basic defect in dietary liver necrosis. Neither α-tocopherol nor substrates such as isocitrate prevented respiratory decline when added to the incubation medium.

α-Tocopherol fed to the animals rapidly cured the defect, and feeding with selenium decreased the amount of respiratory decline observed. A number of antioxidants such as *NN'*-diphenyl-*p*-phenylenediamine prevented respiratory decline when added to the incubation medium (Corwin & Schwarz, 1959, 1960; Schwarz, 1961).

The present investigation was designed to find out which of the factors lacking in the yeast diet was responsible for the failure of ion transport and respiratory decline, and to try to decide between the two theories of pathogenesis of dietary liver necrosis. Rats deficient only in vitamin E were investigated, as well as rats fed with the necrogenic diet, with and without addition of vitamin E and selenium.

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MATERIALS AND METHODS

Animals and diets. Ten-day-old rats and their mothers were removed from all contact with stock diet and given a vitamin E-deficient casein diet. The young rats were weaned at 21 days after birth and fed on one of two diets (Table 1). The amounts of water-soluble vitamins recommended by Ramalingaswami & Sinclair (1954) were added to the diet. Where selenium was added it was given as sodium selenate enough to give 50 μg. of selenium/100 g. of diet. To reverse vitamin E deficiency 20 mg. of α-tocopherol acetate mixed in olive oil was given orally.

Liver-slice techniques. Ion transport in liver slices was measured by the technique described by McLean (1963). Briefly, potassium was leached out of the liver slices by keeping them in cold saline [containing NaCl (150 mM) and KCl (5 mM)] for 35 min. The slices were then put into warm oxygenated Ringer solution (McLean, 1963), and potassium reaccumulation was measured by taking samples at intervals. The sodium and water contents of slices were also measured and found to alter in the opposite way to potassium.

Oxygen uptake by liver slices was measured by the Warburg technique.

Experiments were carried out on the ability of liver slices to maintain their original potassium content *in vitro*, as distinct from the ability to recover potassium lost during cooling. In these experiments liver slices were cut as rapidly as possible after the rat was killed, and the slices were put into warm Ringer solution with oxygen bubbling through the solution. Care was taken that the liver and slices did not cool down at any stage.

Table 1. *Composition of diets given to the rats*

	Composition (%)	
	Casein diet	Yeast diet
Corn starch (Thistle brand; Brown and Polson Ltd.)	51	63
Casein (Low vitamin content; Genatosan Ltd.)	25	—
Dried yeast (DCL baker's yeast; The Distillers Co. Ltd.)	—	18
Salt mixture (Glaxo Laboratories Ltd.)	3	3
Cod-liver oil (B.P.)	1	1
Sucrose (A.R.)	10	10
Lard (Zwan brand)	10	—
Olive oil (B.P.)	—	5

RESULTS

The casein diet is deficient only in vitamin E. Weanling rats fed on the diet grow well. After a few weeks they develop the testicular atrophy and loss of dental pigment characteristic of vitamin E deficiency.

When liver slices from animals given the deficient diet for several months are investigated, two sets of abnormalities are found. First, there is a disturbance of ion transport, the slices are unable to reaccumulate the potassium lost during cooling, and, after an initial few minutes of extrusion, sodium and water gradually accumulate in the slice (Fig. 1). Secondly, the oxygen uptake by cooled slices is defective. The initial Q_{O_2} is normal but declines to a low level during incubation (Table 2).

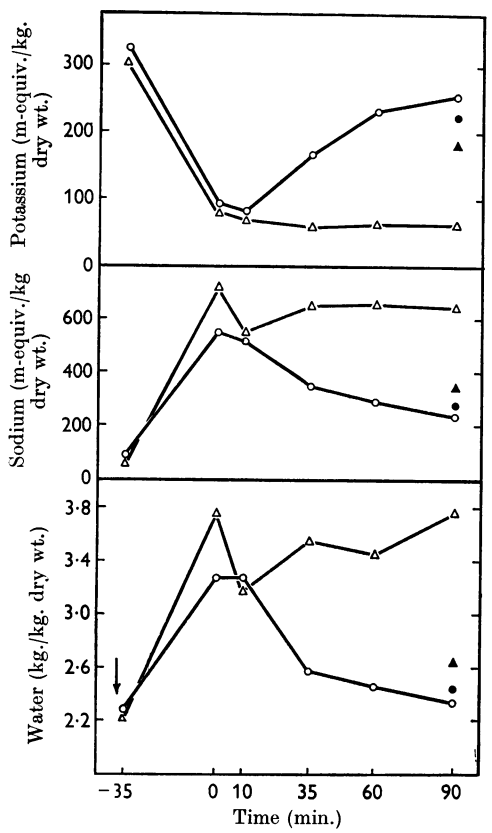

Fig. 1. Potassium, sodium and water contents of liver and liver slices. The arrow indicates the time at which the original liver was sliced. The period of cooling was from −35 to 0 min., and the period of incubation from 0 to 90 min. The cooling and the subsequent incubation in either Ringer solution (△, ○) or in Ringer solution containing Phenergan (0·1 mM) (▲, ●) were as described in the text. △ and ▲, Liver from a rat fed on the casein diet for 3 months from weaning. ○ and ●, Liver from a litter-mate rat fed on the same diet but given α-tocopherol acetate (20 mg.) 4 hr. before death.

Fig. 1 shows that the defect of ion transport is promptly reversed by feeding with α-tocopherol, and that the addition of Phenergan to the incubation medium restores ion transport almost to the normal level.

Table 2 illustrates that the cooled liver slices from a vitamin E-deficient rat fed with selenium show a defect in ion transport at a time when oxygen uptake is normal, that the oxygen uptake later falls to a low level, and that in the presence of the calcium salt of EDTA ion transport is restored to a large extent and oxygen uptake entirely maintained. It also shows that, if the slices are not cooled, but incubated as soon as they are cut, then the ionic composition is maintained and the oxygen uptake does not fall.

Table 3 illustrates that slices from animals given a yeast diet for 28 days maintain oxygen uptake when incubated immediately, but show the phenomenon of respiratory decline in a marked fashion after brief cooling. It shows that the slices can maintain their potassium content if incubated immediately, but cannot recover potassium lost in cooling (McLean, 1960*c*).

Vitamin E deficiency does not affect the water, sodium or potassium contents of the original liver. The effect of leaching in cold saline is similar in slices from vitamin E-deficient animals, fed on yeast or casein diets, in slices from animals fed on stock diet (MRC 41B cubes), and in slices from animals fed on a deficient diet and then given α-tocopherol.

DISCUSSION

The two defects found in liver slices from rats fed on casein or yeast diets are a failure to reaccumulate potassium and a falling oxygen uptake after cooling. Both defects are due to dietary deficiency of vitamin E. The addition of selenium to the diets does not abolish the defects, whereas α-tocopherol promptly reverses them both. The low protein content of the yeast diet does not lead to a declining oxygen uptake as long as adequate amounts of α-tocopherol are given (McLean, 1960*c*). The defects are of more rapid onset in rats given the yeast diet presumably because this causes a more severe vitamin E deficiency (Corwin & Schwarz, 1959).

In all liver slices cooling leads to movement of sodium and water into, and of potassium out of, liver cells. But in the vitamin E-deficient slice this disturbance seems to set in motion some process that has two consequences: initially, active movement of ions is no longer carried out by the cells; later on oxygen uptake fails, perhaps as a result of disturbances of intramitochondrial ion and water contents.

Table 2. *Composition and oxygen uptake of liver slices*

A male rat was given the casein diet for 5 months from weaning and then weighed (330 g.). Selenium (50 μg./100 g. of diet) was added for 10 days. Incubation and cooling of the liver slices were carried out as described in the text.

	Duration of incubation (min.)	Potassium content (m-equiv./kg. dry wt.)	Sodium content (m-equiv./kg. dry wt.)	Water content (kg./kg. dry wt.)	Initial Q_{O_2} (20–30 min. from placing in Warburg bath)	Rate of O_2 uptake at 140 min. (% of initial rate)
Original liver	0	343	89	2·30	—	—
Slices cooled in saline for 35 min.	0	111	665	3·78	—	—
Slices incubated in Ringer soln. at 38° with O_2, after cooling	—	—	—	—	5·6	40
	10	89	570	3·43	—	—
	35	63	650	3·57	—	—
	60	82	580	3·37	—	—
	170	56	760	4·21	—	—
Slices incubated in Ringer soln. containing EDTA (calcium salt) (1 mM) at 38° with O_2, after cooling	—	—	—	—	5·2	117
	170	145	415	2·60	—	—
Slices incubated in Ringer soln. at 38° with O_2 immediately after slicing, i.e. without cooling	—	—	—	—	5·8	99
	170	269	225	2·50	—	—

Table 3. *Effect of cooling on the respiratory decline and on the final potassium content*

The rats were given the yeast diet for 28 days. All slices were weighed and put straight into Warburg flasks. These were kept cold or else put into the Warburg bath. The flasks were gassed with O_2 for 7 min. after being placed in the bath. At the end of the incubation the potassium content of the slices incubated immediately was 380 m-equiv./kg. dry wt., and that of the slices previously cooled for 10 min. was 175 m-equiv./kg. dry wt.

Time from death of animals (min.)	Q_{O_2}	
	Incubated immediately	Cooled for 10 min.
30–40	6·9	—
40–50	8·5	5·3
50–60	8·8	4·5
80–100	8·3	3·7
150–170	7·3	2·5

It is a commonplace to suggest that the effects of vitamin E deficiency are due to autoxidation. The interesting point that arises here is that the effects of vitamin E deficiency seem to be specifically connected to ion transport. Liver slices from vitamin E-deficient animals are able to maintain their ionic composition and oxygen uptake so long as there is no period of cooling with its attendant sodium entry and potassium loss.

The first defect that is noted in cooled slices is the inability to reaccumulate potassium. This may indicate that ion transport involves a step that is unusually sensitive to autoxidation, and that, in the search for enzyme systems involved in ion transport, vitamin E-deficient tissues might be useful.

Both Phenergan and EDTA have antioxidant activity (Ottolenghi, 1959; Bernheim, 1959; McLean, 1960*a*). Many antioxidants protect vitamin E-deficient slices from respiratory decline (Mertz & Schwarz, 1959), and it seems most likely that Phenergan and EDTA act as antioxidants in protecting slices.

Relation of the defects in vitro *to dietary liver necrosis*

Schwarz (1961) has postulated that the basic defect in dietary liver necrosis is a failure of dehydrogenation in the mitochondria. He bases this theory on the decline of oxygen uptake of liver slices from rats given a yeast diet, and on the decline of oxygen uptake by liver homogenates and mitochondria when these are incubated in the various unusual media, containing excess of sodium or of NAD (Corwin & Schwarz, 1959, 1960). In all these circumstances an alteration in mitochondrial permeability due to autoxidation is an equally likely explanation for the effects found. It is likely that Schwarz (1961) allowed his liver slices to cool before incubation and that the respiratory decline that he describes would also be abolished by immediate incubation.

Since rats given 25% casein diets never show dietary liver necrosis, but show the same defects as rats given the yeast diet, it is no longer possible to call these defects the basic lesion of dietary liver necrosis. The failure of selenium, which prevents liver necrosis, to prevent the decline in oxygen uptake of liver slices or to restore potassium reaccumulation points to the same conclusion and is

evidence against the suggestion that selenium is involved in electron transport.

The associated defects of failure to reaccumulate potassium and of decline in oxygen uptake, after cooling, are part of the inability of vitamin E-deficient tissues to stand up to certain conditions *in vitro*. However, it is possible that the defects shown up in vitamin E-deficient tissues by the stress of cooling *in vitro* can also be brought about *in vivo* by some other stress. Analyses of the dystrophic muscles of vitamin E-deficient animals have shown high sodium and low potassium contents. These were attributed to expansion of the extracellular space. But when the values given are recalculated it becomes clear that these changes are at least in part due to loss of potassium and gain of sodium in the intracellular phase (Blaxter & Wood, 1952; Morgulis & Osheroff, 1938). We can postulate that vitamin E-deficient tissues are unable to recover from the situations that stress the mechanisms responsible for ion movements.

The way in which selenium prevents dietary liver necrosis is as yet unknown, but perhaps it acts by preventing the situation which stresses the ion-transport mechanism. If this were found to be so, then we would have an explanation for the need for simultaneous deficiency of vitamin E and selenium for dietary liver necrosis to occur.

SUMMARY

1. Rats were made deficient in vitamin E by feeding them on casein or yeast diets.

2. Liver slices from these animals were unable to reaccumulate potassium removed by leaching in the cold.

3. Cooled slices show a falling off in oxygen uptake.

4. These defects were not prevented by feeding with selenium but were promptly reversed by giving α-tocopherol or by the addition of anti-oxidants to the incubation solution.

5. It is concluded that the defect *in vitro* in oxygen uptake by vitamin E-deficient tissues follows disturbances of ionic composition due to cooling.

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Biochem. J. (1963) **87**, 167

Condensed Tannins

15. INTERRELATIONSHIPS OF FLAVONOID COMPONENTS IN WATTLE-BARK EXTRACT*

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Wattle-bark extracts (*Acacia mearnsii*, formerly *A. mollissima*), known commercially as 'mimosa' extract, is mainly composed of polymeric leuco-robinetinidin and leuco-fisetinidin tannins (Roux, 1957*a*, *b*, 1958; Roux & Evelyn, 1958*a*) accompanied by closely related flavonoids, (−)-robinetinidol (Roux & Maihs, 1960), robinetin (Kirby & White, 1955) and fisetin (Roux, 1952). Isolation of (+)-catechin and (+)-gallocatechin (Roux &

* Part 14: Roux & Paulus (1962*b*).

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