adenosine triphosphate(6) reserves.

Summary. Vanadium administration results in decreases in coenzyme A, coenzyme Q and succinoxidase activity in rat livers. A marked protection against these changes is afforded by simultaneous administration of calcium pantothenate, l-cysteine hydrochloride and disodium adenosine triphosphate. The results are discussed in relation to other reported observations on the effects of vanadium administration.

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Cytochrome Oxidase in Radiosensitive and Radioresistant Amoebae.* (26794)

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Two species of the giant amoeba *Pelomyxa* are known to differ by a factor of approximately 10 in their sensitivity to x-radiation. The x-ray LD_{50} for *P. carolinensis* is about 100 kr(1-3), whereas that for *P. illinoisensis* is about 10 kr(2,4,5). It seemed possible that there might be some fundamental metabolic difference responsible for this wide dissimilarity in radiosensitivity. Very little is known about the metabolic characteristics of *P. illinoisensis*. However, *P. carolinensis* is known to contain cytochrome pigments(6), and 70% of its respiration is cyanide-sensitive(7). Møller and Prescott(6) were unable to demonstrate absorption peaks characteristic for cytochrome *c* by direct spectrophotometric examination of ruptured cells, and several investigators have failed to detect cytochrome *c* oxidase activity (see 6). Møller and Prescott suggested that a cytochrome *e*-cytochrome *c* oxidase system was operating as the terminal oxidase in *P. carolinensis*.

It occurred to us that *P. illinoisensis* might contain cytochrome *c* oxidase, in contrast to the more radioresistant species. Thus we assayed the 2 species for this enzyme. We have found, however, that both species contained approximately the same amount of cytochrome *c* oxidase activity, although the sys-

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tem is rather labile, particularly in *P. carolinensis*. For comparative purposes we looked for this enzyme in *Amoeba proteus*. This large uninucleate amoeba is radioresistant, with an LD_{50} of at least 120 kr(2,3,8).

Methods. The *Pelomyxa* species (Illinois strains, isolated by Kudo(9,10)) were grown on *Paramecium multimicronucleatum* and *Chilomonas paramecium*. *Amoeba proteus*, originally obtained from General Biological Supply House, Inc., Chicago (1957), was grown predominantly on *Tetrahymena pyriformis* (strain W). *Paramecium* and *Chilomonas* organisms similar to those which were fed to the pelomyxae were used for supplemental food. All amoebae used in these experiments were starved for 4 days prior to use; they were thoroughly washed before and at daily intervals during starvation to eliminate food remnants.

The amoebae were disrupted by homogenization in ice-cold water. Homogenizers were made from barrels and matched plungers of 0.5 ml syringes, with all surfaces ground to fit closely. The packed volume of cells (one *g* of force for 15 minutes) used in each homogenate was about 0.15 ml for the pelomyxae and 0.06 ml for *Amoeba proteus*, representing approximately 1400 organisms of *P. carolinensis*, 3300 of *P. illinoisensis*, and 16,000 of *A. proteus*. Total volume of each homogenate was about 0.3 ml. Microscopic examinations of homogenates revealed that cellular disruption was complete after 20 or 30 seconds of rotation and compression of the plunger.

Cytochrome oxidase was measured by the spectrophotometric method of Hogeboom and Schneider(11). Aliquots of the homogenate were added to the reaction mixture (reduced cytochrome *c* (Sigma), Al^{+++}, and phosphate buffer, pH 7.4) within one minute after homogenization. Readings were made at intervals of 30 or 60 seconds at 550 mμ to measure the rate of oxidation of reduced cytochrome *c* at 25.0°C. Initial rates were calculated by extrapolation to zero time. Total nitrogen was measured by nesslerization of an H_2SO_4-H_2O_2 digest of a portion of the homogenate.

In some experiments, the homogenates were incubated for varying lengths of time, either at 0°C or 25°C, prior to assay, so that information could be obtained about the stability of the system.

TABLE I. Cytochrome Oxidase Activities of Protozoa and Mammalian Tissues.

Source	No. exp.	μmoles cytochrome *c* oxidized/hr/mg N*
Pelomyxa illinoisensis	3	7.4 ± .1
Pelomyxa carolinensis	3	8.3 ± 1.5
Amoeba proteus	2	.6 ± .3
Paramecium aurelia	3	1.5 ± .7
Rat thymus	9	24.6 ± 1.8
Mouse liver	17	322 ±16

* Averages with avg deviation.

Results. Table I shows the cytochrome *c* oxidase activities of 3 species of amoebae assayed immediately after disruption of the cells. For purposes of comparison, the activities of *Paramecium aurelia* and of 2 mammalian tissues, assayed by the same procedure, are also included. It is clear that the activities of the 2 *Pelomyxa* species are nearly the same, and considerably lower than those of mammalian tissues: ⅓ that of thymus and 1/40 that of liver. Mammalian tissues, especially the thymus, are considerably more radiosensitive than the most radiosensitive of the amoebae that have been studied. *Amoeba proteus* contains an almost negligible amount of cytochrome oxidase; *Paramecium aurelia* has a somewhat higher concentration, but levels of activity of both are extremely low and variable, and at least in the case of *Amoeba proteus* of questionable significance.

The necessity of carrying out the assay as soon as possible after disruption of the cells is revealed by Fig. 1. The activity of the homogenized cells is apparently stable in 0°C, at least for 20 to 40 minutes, but disappears rapidly at 25°C. The activity of *P. illinoisensis* decreased to 50% after 15 minutes, whereas that of *P. carolinensis* dropped to 50% in about 3 minutes. After 45 minutes at room temperature, 80 to 90% of the activity of both species had disappeared. In one experiment in which *P. illinoisensis* was homogenized at room temperature, then assayed about 5 minutes later, the initial rate was only 25% of that observed when homogenization was carried out at 0°C and the sam-

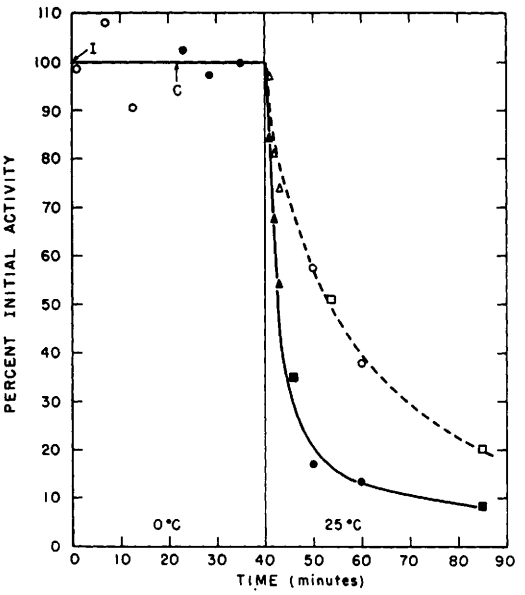

FIG. 1. Lability of cytochrome *c* oxidase at 25°C. Solid line and symbols, *P. carolinensis*; dotted line and open symbols, *P. illinoisensis*. Samples of *P. illinoisensis* were homogenized at 0°C at zero time (arrow at I), and 3 assays were made beginning 1, 7, and 12.5 min. later. *P. carolinensis* was homogenized at 23 min. (arrow at C) and 3 assays similarly made. At 40 min., both homogenates were transferred to a 25°C bath. Readings were made 10 and 20 min. later (circles). Data from other experiments are incorporated in this graph (squares). The points at 41, 42, and 43 min. (triangles) were calculated from rate of decrease of activity during the assays, corrected for anticipated first-order decrease due to substrate utilization.

ple assayed immediately afterward.

To rule out the possibility that the changes observed in optical density represented merely a swelling of subcellular particulates and not a true oxidation of cytochrome *c*, assays were carried out in the presence of 0.006 M NaCN. Virtually complete inhibition occurred; there was negligible change in optical density.

Discussion. It should be emphasized that these experiments indicate only that there is present within the cells of the giant amoebae an enzyme that can catalyze the oxidation of reduced cytochrome *c* by molecular oxygen. The activity of this enzyme is relatively low, and there is no reason to presume that a cytochrome *c*-cytochrome *c* oxidase system represents a significant metabolic pathway *in vivo*, particularly in view of the inability of Møller and Prescott(6) to demonstrate absorption bands of cytochrome *c* in *P. carolinensis*. It is very possible that the protozoan enzyme that oxidizes cytochrome *c in vitro* is not identical with the mammalian enzyme, and that mammalian cytochrome *c* is not its physiological substrate.

According to a recent report(12), cytochrome oxidase (prepared from beef heart) is a copper-hemoprotein complex consisting of equimolar quantities of copper and cytochrome *a*. Møller and Prescott(6) detected an absorption band at 605 mμ, which they attributed to either cytochrome *a*, cytochrome *e* oxidase, or both. They made no mention, however, of the other absorption bands characteristic of cytochrome *a*. Furthermore, the band at 605 mμ was weaker in *P. carolinensis* than in *Amoeba proteus*, whereas according to our data, the latter has much less cytochrome *c* oxidase activity.

Nevertheless, there is now definite evidence that these cells possess some sort of cytochrome *c* oxidase. The extreme lability of the system, particularly in *P. carolinensis*, is probably the reason why this enzyme has not been hitherto detected in these protozoa. Møller and Prescott(6) indicated the necessity of maintaining their homogenates at 0° to −10°C in order to preserve the absorption bands.

Summary. Cytochrome *c* oxidase activity has been demonstrated in 2 species of the giant amoeba *Pelomyxa*, one of which is radioresistant and the other radiosensitive. The differences observed in this enzyme do not appear to be sufficient to account for the 10-fold difference in radiosensitivity of the living organisms. The level of cytochrome *c* oxidase activity is about one-fortieth that of mammalian liver, and is highly labile at room temperature. The radioresistant *Amoeba proteus* was also assayed for cytochrome *c* oxidase activity but almost negligible amounts were found.

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Hypergammaglobulinemia in Mink.* (26795)

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Lesions found in mink affected with Aleutian disease resemble many of those described for such diseases as periarteritis nodosa, lupus erythematosus, and other associated diseases of man. Certain of the gross and microscopic lesions of Aleutian disease have been described by Hartsough and Gorham (1), and Helmboldt and Jungherr(2). Obel (3), in describing a similar condition of mink in Sweden, has pointed out that this may be a form of plasma cell myeloma, since one of the prominent changes is plasma cell proliferation. Recently Page(4) found that affected mink have an accompanying hypergammaglobulinemia. This report is concerned with the determination and comparison of serum protein values of normal and Aleutian disease affected mink using zone electrophoresis.

Materials and methods. Blood samples were taken from normal and affected mink on 2 ranches which had a history of the disease. In addition, blood samples were collected from a ranch where the disease had never occurred and the animals were in good health. The blood was collected from 18 normal and 18 affected mink by heart puncture while the animals were under ether anesthesia. Immediately after collection of the blood samples, the mink were sacrificed and complete necropsies performed. Animals were designated normal or affected on the basis of history, clinical signs, and post-morten lesions. Serum was removed from blood samples and stored at –26°C until electrophoretic determinations were made.

All the mink in the affected group were homozygous for the Aleutian gene as defined by Shackelford(5). The control mink, which had no evidence of disease, included 11 animals which were homozygous for the Aleutian gene and 7 which were not homozygous for this gene.

Samples of serum from normal and affected mink were subjected to electrophoresis at room temperature on filter paper (S and S 2043-A mgl) in veronal buffer (ionic strength 0.075 and pH 8.6) for 16 hours at a constant current of 0.104 milliamp/cm width. Spinco Model R paper electrophoresis apparatus was used.† The sample size was 0.006 ml of serum.

At the end of 16 hours, the paper strips were heated at 125°C for 30 minutes, fixed and stained in a 1% methanolic bromphenol blue solution(6). Prior to scanning in a Spinco Model RB Analytrol integrating densitometer, the dried strips were exposed to ammonium hydroxide vapor. A B-5 cam and 500 mμ interference filters with a 1.5 mm slit width were used. Total serum proteins were determined by the biuret method(7).

Results. Results of the electrophoretic de-

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