destroyed without any surviving cells under our conditions.

Schimke and Barile reported(8) the presence of a 3-enzyme system that degrades arginine to ornithine in PPLO-contaminated but not PPLO-free cell cultures. No association was made between the presence of this enzyme system and destruction of the cells. These workers have not reported differences between PPLO strains in this regard and have even suggested that assays of one of these enzymes, arginine deaminase, might be a suitable general screening method for PPLO detection in cell cultures. Experiments are now in progress to ascertain whether or not our lytic factor is identical to these enzymes. It nevertheless appears certain that the peculiar response of murine lymphoma cells to these phenomena is both distinctive and intriguing, and one is reminded of the antilymphoma properties that have recently been associated with guinea pig serum asparaginase(9). Our preliminary results indicate that these cells have an *in vitro* arginine requirement of about 0.15 mM, and can utilize citrulline but not ornithine in place of arginine. In this respect they are not unusual(10-12). Further work is now being done to clarify the nature of the macromolecular principle and its effect on L5178Y cells.

Summary. Evidence is presented which indicates that some mycoplasma strains which are cytocidal for murine lymphoma cells *in vitro* liberate into the medium non-dialyzable substances that are sufficient for production of the cytocidal effect. This effect is prevented if enough L-arginine is added to the medium.

Appreciation is extended to Dr. Vittorio Defendi for his active interest and advice throughout these studies.

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Enzyme Histochemistry of Rabbit Aorta, in Spontaneous Lesions and After Acute Exercise.* (28872)

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In studying the effect of exercise on enzyme content of experimental cholesterol atherosclerosis(5) and spontaneous medial degeneration of the rabbit aorta(6) it was found that there were no changes in aortic content of succinic dehydrogenase, cholinesterase, nonspecific esterase, glucose-6-phosphatase, alkaline phosphatase, cytochrome oxidase, and diphosphonucleotide diaphorase attributable to the exercise. The experiments cited above were carried out over 60-day periods. It was then postulated that observable but evanescent changes in enzyme content of the aortic wall might occur after a single intense period of exercise which were not evident in the chronic experiment. It

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was also postulated that there might be enzymes other than those studied in the previous experiment which might be influenced by exercise. Thus, the present report concerns the study of the content of triphosphopyridine nucleotide diaphorase (TPN diaphorase), phosphorylase and leucine aminopeptidase (LAP), as well as the enzymes listed above, in the aorta of normal rabbits sacrificed after a single period of intense exercise.

Materials and methods. New Zealand white rabbits weighing about 4000 g were used. One hundred grams of Purina rabbit chow were fed daily. Water was provided *ad libitum.* Since most of the rabbits were at first unable to run well in the treadmill previously described(5), they were subjected to 2 days of training before they were used for the experiment. Ten rabbits were exercised in the treadmill until they became completely exhausted. The time required for complete exhaustion was generally from 20 to 45 minutes. As soon as they were exhausted, the rabbits were sacrificed by air injection through the auricular vein and a complete autopsy was performed. Five normal rabbits were sacrificed as concurrent controls. Specimens of tissues were taken for cryostat and paraffin sectioning. Sections were frozen immediately after removal at −70°C, and then cut in a cryostat at −28°C.

Succinic dehydrogenase, nonspecific esterase, cholinesterase, glucose-6-phosphatase, alkaline phosphatase, cytochrome oxidase, and DPN diaphorase were demonstrated by the same methods as in the previous experiments (5,6): TPN diaphorase by the method of Nachlas, *et al*(11), and phosphorylase by the method of Grillo(3). Since this reaction is based on detection of the glycogen produced from glucose-1-phosphate by the phosphorylase, control slides were incubated simultaneously in the same medium without glucose-1-phosphate to detect pre-existing glycogen. The difference of glycogen content between control and substrate-incubated slides indicates phosphorylase activity. Leucine aminopeptidase was demonstrated by the method of Nachlas *et al,* as described by Pearse(13).

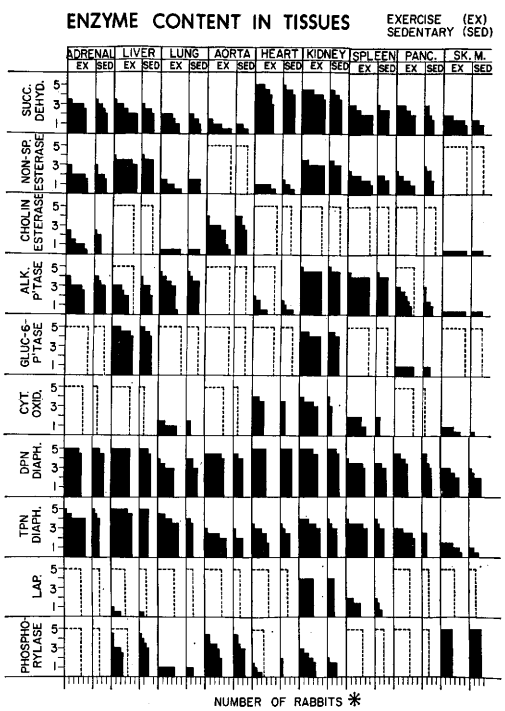

FIG. 1. Histogram of enzyme content of tissues from sedentary and exercised rabbits. Ordinate represents intensity of the reaction; abscissa, number of animals examined, each division representing 2 animals. Dotted enclosures represent negative reactions in the designated number of animals. Reactions of spontaneous lesions are not indicated.

The quantitative evaluation of the histochemical reaction was made by visual estimation on a scale of 1 to 5.

Results. The distribution of enzymes in the tissues of the rabbits is indicated in Fig. 1. No difference in enzymatic activity was observed between the exercised and sedentary group, or between the animals of this experiment and those of the chronic experiment previously reported(5).

Spontaneous lesions were found in 2 of the 10 acutely exercised, and one of the 5 sedentary animals.

In this experiment the enzymes studied, in addition to those previously reported, were distributed as follows:

TPN diaphorase reaction was widely distributed throughout all of the tissues in the same locations as DPN diaphorase(5), but present in lesser intensity. In the normal aorta, it was present in the cytoplasm of the

medial muscle fibers, endothelial cells, and adventitia, and absent in elastic tissue (Fig. 2). It was also present in the medial muscle cells of small arteries of solid tissues. In the spontaneous lesions it was decreased, but not absent, in areas of muscle degeneration (Fig. 3).

Phosphorylase was present in liver, renal collecting tubules, skeletal muscle, heart muscle cells, and smooth muscle cells of the aorta (Fig. 4), and the bronchus. In the spontaneous lesions, it was remarkably decreased in the areas of degeneration (Fig. 5).

Leucine aminopeptidase was present principally in the proximal convoluted tubules of the kidney and in the spleen, and absent from the aorta and small arteries. It was also absent from the spontaneous lesions.

Discussion. Although the inhibitory effect of exercise on development of atherosclerosis has been recognized experimentally(5,7,8,10, 12,14), and clinically(1,2,4,9,15), no changes in the enzymatic activity were observed in the aorta of chronically exercised rabbits. In this experiment, no changes were observed in the aorta of acutely exercised rabbits in activity of enzymes previously studied(6), or of TPN diaphorase, phosphorylase, and leucine aminopeptidase also studied in the present experiments. However, it is still possible that other enzymes may vary in exercised animals, and further studies are in order. The decrease of TPN diaphorase and phophorylase in the spontaneous lesion seems to be related to the degeneration of medial muscle fibers, and is similar to that observed with DPN diaphorase and succinic dehydrogenase (6).

Summary. 1. The activities of succinic dehydrogenase, nonspecific esterase, cholinesterase, glucose-6-phosphatase, alkaline phosphatase, cytochrome oxidase, DPN diaphorase, TPN diaphorase, phosphorylase and leucine aminopeptidase in the frozen sections of adrenal, liver, kidney, lung, spleen, pancreas, heart, striated muscle, small arteries and aorta of acutely exercised and sedentary rabbits were studied histochemically. 2. There were no enzymatic changes attributable to the acute exercise in any organs. 3. TPN diaphorase is present in the intima, medial muscle fibers and adventitia of the aorta. 4. Phophorylase is present in the medial muscle fibers of the aorta. 5. Leucine aminopeptidase is absent in all parts of the aorta. 6. In the spontaneous lesions, TPN diaphorase and phosphorylase are decreased as the medial muscle fibers are degenerated.

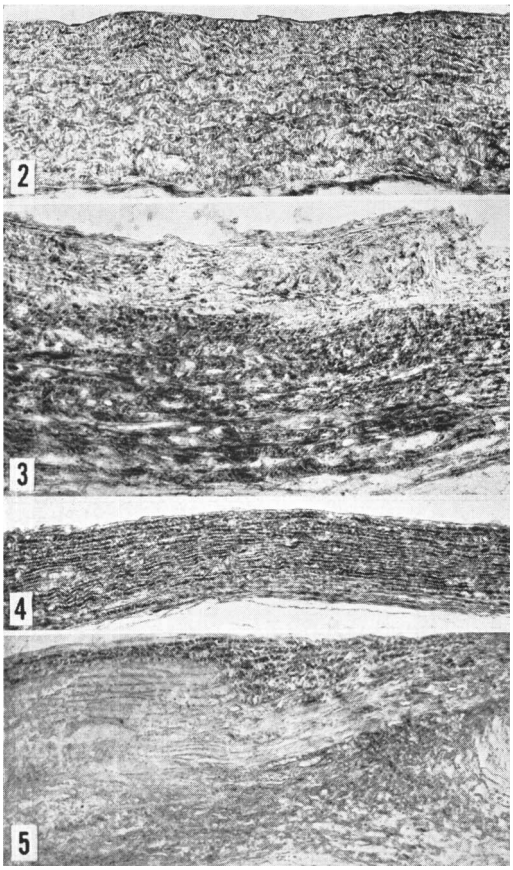

FIG. 2. TPN diaphorase in normal aorta showing generalized distribution of enzyme in medial muscle cells, intima and adventitia. Elastic tissue is unstained. (Rabbit RD-4 × 80)

FIG. 3. TPN diaphorase in spontaneous lesion of aorta showing decreased activity of enzyme in area of muscle degeneration. (Rabbit RA-4 × 80)

FIG. 4. Phosphorylase in normal aorta. Activity of enzyme is seen in medial muscle cells. Elastic and fibrous tissue is unstained. (Rabbit RC-2 × 80)

FIG. 5. Phosphorylase in spontaneous lesion showing disappearance of enzyme activity in center of lesion and normal intensity of that in unaffected area (upper portion). (Rabbit RA-7 ×80)

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Fatty Acid Composition of the Phosphatide and Triglyceride Fractions of Human Epidermis.* (28873)

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Although considerable work has been done on the determination of the lipid composition of sebum(1-5), and on the lipid fractions obtained from sebum, particularly by Haahti and co-workers(5), the lipid composition of epidermis has interested few investigators recently. This may be due in part to the relative ease in obtaining sebum as compared with adequate amounts of epidermis. Reinertson and Wheatley(6) have carried out lipid analysis not only on total human epidermis, but also on the "Malpighian" layer and stratum corneum. In this paper the fatty acid composition of the triglycerides and phosphatides of human epidermis is reported.

Methods. Human skin† was obtained from extremities and radical breast operations as soon as feasible or within a few hours after surgery. Aside from the pooled breast skin samples, 3 were obtained from females of ages 18, 61, and 83, and 6 from males of ages 41, 56, 58, 59(2) and 71. The extremities had been removed from patients having such afflictions as arteriosclerosis, diabetes, frostbite, and tumors. After removal of the skin from the extremities, it was cut into about 4 × 6 inch square pieces, washed free of blood, etc., and frozen immediately at –25°C if not used at once. Prior to removal of the epidermis the adipose layer was scraped off thoroughly, and the skin surface was cleaned in one of two ways: (A) The epidermal surface was washed with cotton soaked in distilled water, dried with clean cotton, then the epidermis was removed by the procedure of Baumberger *et al*(7). The epidermis was dipped into anhydrous ether quickly several times, set at room temperature to remove the excess ether, then weighed for immediate extraction or for storage at –25°C. (B) The epidermal surface was washed with cotton wet with distilled water, dried with cotton, and rubbed gently with cotton soaked with anhydrous ether. The surface was then washed again with distilled water, dried with cotton, and the epidermis was removed as described under (A). Both procedures gave the same results, but the second was preferred. Care is needed in cleaning the epidermis for the following reasons: (A) Ex-

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