rich in Na and although much of this is in cells only a portion is ionized and, to an unknown but important degree, active.

*Summary.* The rat tail artery contains more than 5 times as much Na and about half as much K as gastrocnemius muscle. Inulin distribution indicates that cellular and extracellular water phases are about equal in magnitude. Calculation using the inulin space or any other reasonable estimate of water partition shows that much of the Na burden is necessarily cellular and non-ionized.

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## Some Observations on Lipid Metabolism and Adipose Tissue in the Vitamin E-Deficient Rat.* (28510)

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It is well known that vitamin E-deficiency coupled with a large intake of long chain poly-unsaturated fatty acids results in the development of yellow pigmentation of the body fat. This gross change is accompanied by striking histological changes consisting of the deposition of acid-fast and PAS-positive material within fat cells(1-3). Biochemical studies of this altered fat have revealed high levels of lipo-peroxides and increased levels of nitrogen, leading to the proposal that the lipids have undergone auto-oxidation, polymerization and coupling with proteins(4). The present studies were undertaken to define further some of the biochemical and metabolic changes associated with the development of this abnormality in the adipose tissue.

*Materials and methods.* A group of 18 rats weaned at 3 weeks of age were transferred to a Vit.E-deficient diet in which 30% of the calories were supplied as the free fatty acids of linseed oil(5). Controls consisted of weanlings and of adults receiving the E-deficient diet supplemented with 25 mg of α-tocopheryl acetate twice weekly. All animals were given oral supplements of Vit. A and D. Animals on the deficient diet were sacrificed in pairs at intervals up to 15 weeks. Samples of pararenal adipose tissue were removed and evaluated grossly. Lipids were extracted from the adipose tissue with chloroform:methanol and chloroform, and the solvents then evaporated with nitrogen. After further drying in a vacuum desiccator, the lipids were weighed on an analytical balance. Chromatograms of both the phosphatides and non-phosphatides were made on paper impregnated with silicic acid. After staining with Rhodamine 6G the lipid spots were detected under ultraviolet light(6).

Silicic acid column chromatography was used for quantitative separation of the lipid fractions into 4 groups(7). An additional group of non-phosphatides which has not been completely characterized was eluted from the column with 50 ml of ethyl ether immediately after the normal non-phosphatides.

To obtain further information concerning phospholipid metabolism in adipose tissue, orthophosphate labeled with P-32 was injected subcutaneously into 2 Vit. E-deficient and 2 control animals using a dose of one microcurie per gram of body weight. Animals were killed 24 hours later. Total lipids were extracted as above from liver and adipose

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† The data in this report form part of a thesis submitted in partial fulfillment of the requirements for the degree, M.D. with Honor.

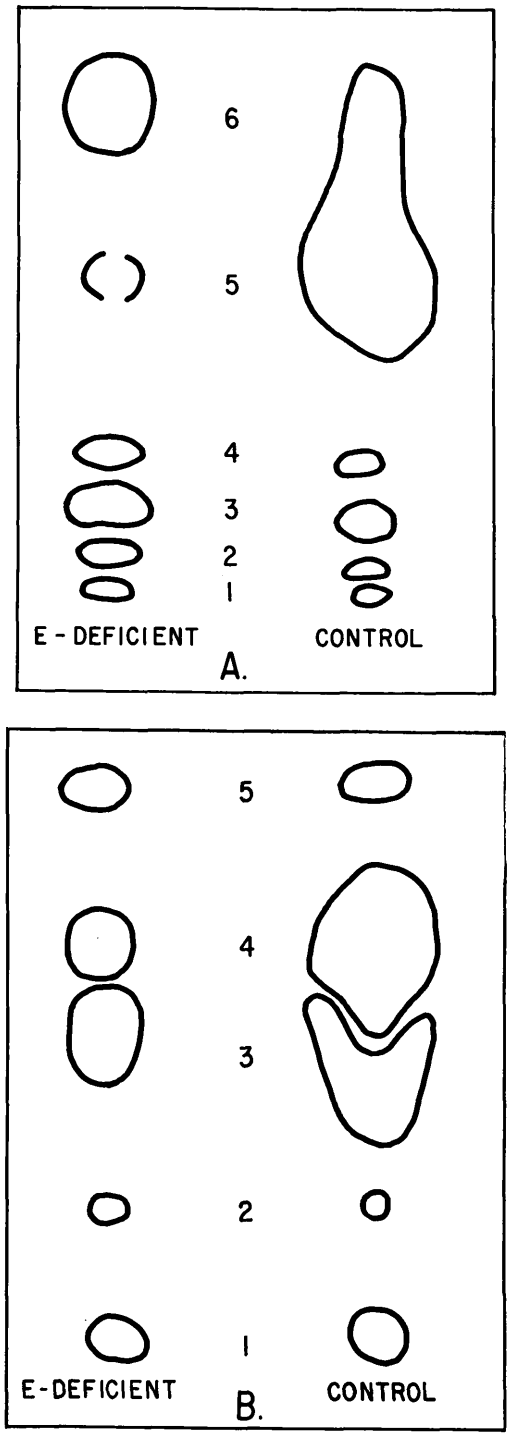


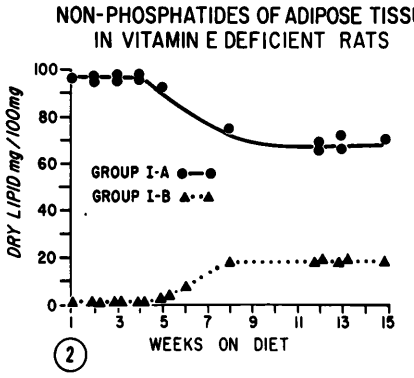

FIG. 1. Tracings of typical chromatograms of lipids extracted from adipose tissue. A. Phosphatides: 1, lysolecithin; 2, sphingomyelin; 3, lecithin; 4, phosphatidyl ethanolamine; 5, polyglycerol phosphatides; 6, non-phosphatides. B. Non-phosphatides: 1, monoglycerides; 2, free cholesterol; 3, free fatty acids; 4, triglycerides; 5, cholesterol esters.

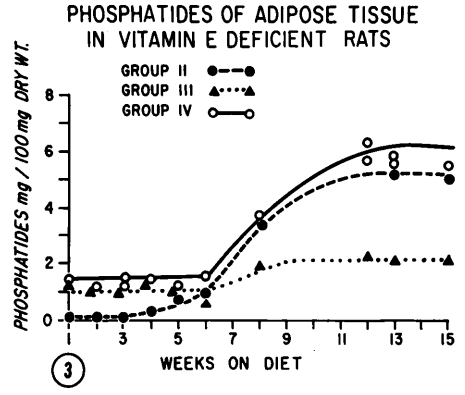
tissue, and aliquots were analyzed for radioactivity and for phosphorous by the method of Bartlett(8). The specific activities of the extracts were calculated on the basis of counts per minute per microgram of lipid phosphorus. To form some estimate of the rate of phospholipid degradation, specific activities were also determined on the extracts of adipose tissue which had been allowed to autolyze for 6 hours at 37°C.

*Results.* Gross changes in adipose tissue began at about 5 weeks and progressed from the appearance of scattered small white flecks through a lemon yellow to a medium brown color and firm consistency by 12 to 15 weeks.

On paper chromatograms the adipose tissue of deficient and control animals showed qualitatively the same lipid components, although there appeared to be a reduction in the free fatty acid and trigylceride fractions in the E-deficient animals and an increase in phosphatides in these same animals. This is illustrated in tracings of typical chromatograms (Fig. 1). The quantitative column chromatography showed that after the animals had been on the deficient diet for 5 weeks the normal non-phosphatides (Group I-A) began to decrease progressively. Concomitantly there appeared an altered non-phospholipid (Group I-B) which has not yet been completely characterized (Fig. 2). These alterations in the non-phosphatides were accompanied by an increase in all of the phospholipid fractions (Groups II, III, and IV), as shown in Fig. 3. Observations on the adipose tissue of a stock animal whose depot fat had been severely reduced by fasting showed no change from control values in the ratio of phosphatides to non-phosphatides.

In the incorporation studies with P-32 the specific activity of the adipose tissue in relation to that of the liver from deficient animals was slightly greater than the relative specific activity of adipose tissue from the controls (Fig. 4, fresh samples). There was, however, a much greater amount of labeled phospholipid per unit weight of adipose tissue



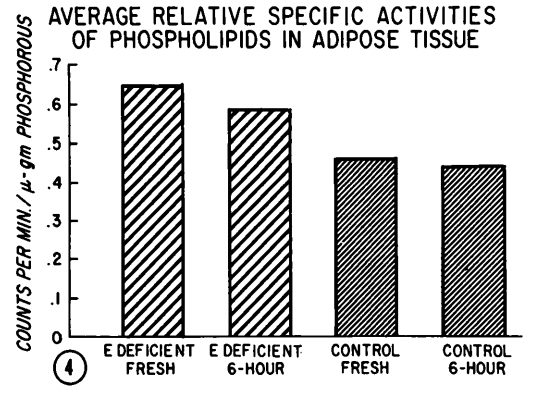




FIG. 2. Quantitative changes in non-phosphatides extracted from adipose tissue. Group I-A: mono- and tri-glycerides, free fatty acids, free cholesterol and cholesterol esters. Group I-B: an abnormal non-phosphatide, not fully characterized.

FIG. 3. Quantitative changes in phospholipids extracted from adipose tissue and fractionated by silicic acid column chromatography. Group II: poly-glycerol phosphatides; Group III: phosphatidyl ethanolamine; Group IV: lysolecithin, sphingomyelin and lecithin.

FIG. 4. Relative specific activities of adipose tissue after administration of P-32. Fresh samples: lipids were extracted immediately after sacrifice. Six-hour samples: extracts were made after tissue had been allowed to autolyze at 37°C for 6 hours. Relative specific activity was calculated as the ratio of specific activity of the adipose tissue to that of the liver.

in the deficient animals, since relatively more total phospholipid was present. Autolytic reduction in specific activity was slight, and about the same in deficient and control animals.

*Comment.* It should be noted that these findings are based on extractable lipid, and that there is an increasing amount of non-extractable pigmented material present in the adipose tissue after 6 weeks on the diet.

The occurrence of the unusual non-phosphatides (Group I-B) in animals on the deficient diet for longer than 5 weeks is of interest. Although it has not been completely characterized, its behavior in silicic acid column and paper chromatography resembles that of di-glycerides. It is also possible that this material represents polymerization products of auto-oxidized lipids.

It is interesting and unexpected that the gross and histological changes were accompanied by a marked increase in the phospholipid fractions of the extractable lipid. That the phospholipid in the deficient animals has a specific activity equal to or slightly greater than that of the controls indicates that this is metabolically active phospholipid. Indeed, on the basis of inorganic P-32 uptake no significant distinction can be made between the phospholipid extracted from the adipose tissue of deficient and control animals, despite the increased amount of phospholipid in the former.

If the group I-B non-phospholipid should prove to be di-glyceride, it is of interest to note that di-glyceride is a precursor of lecithin. Thus, an increase in di-glyceride at a time when lecithin is on the increase would not seem surprising.

*Summary.* 1. Gross, microscopic and biochemical observations were made on adipose tissue of rats receiving a diet deficient in Vit. E and rich in long-chain unsaturated fatty acids. 2. A progressive increase was found in the ratio of extractable phospholipids to non-phospholipids. 3. Studies of the uptake

of P-32 indicate that the metabolic activity of phospholipids in abnormal adipose tissue is comparable to that in normal adipose tissue.

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## Preservation of Rabbit Spermatozoa: Ethylene Glycol *vs* Glycerol for Frozen Semen.* (28511)

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Live young have been produced by female rabbits inseminated with previously frozen semen(1). The semen diluent contained 8% glycerol (G). For practical use, however, a higher fertility rate than first reported was needed. It has been demonstrated(2-4) that for the rabbit, ethylene glycol (EG) may be more satisfactory than glycerol for sperm preservation.

Two alternative procedures for incorporating G into a semen diluter prior to freezing have been: (a) initial semen dilution with a nonglycerolated diluter at room temperature, subsequent cooling to 5°C, and then stepwise addition of G at 5°C; or (b) initial (all-at-once) incorporation of G in the diluter with semen dilution performed at 37°C and subsequent cooling to 5°C. There are contradictory reports(5-9) based almost exclusively on the bovine as to the relative effectiveness of these two methods.

In seeking to improve the initial diluter, we set up 4 experiments to evaluate the effects of substituting EG for G and to obtain some evidence on the influence of the method of glycerol addition on survival of frozen rabbit spermatozoa.

*Materials and methods.* Semen was collected in an artificial vagina from primarily strain III (New Zealand White) bucks of The Jackson Laboratory rabbit colony. Semen was collected 5:30-7:30 AM, when distracting noises and influences were minimal. It was then evaluated for motility (rated 0-5), concentration (haemocytometer count), and live sperm (number of live sperm in 100 counted, using the differential stain fast green FCF and Eosin B as reported by Mayer *et al.*[10]). Samples having less than either 50% live sperm or 100 million/cc were eliminated from treatment comparisons on the grounds that they were of such inferior quality that results might be biased. The split sample technique was used with 0.15 cc undiluted semen/treatment in all comparisons. Immediately after collection and initial dilution all semen was cooled slowly to 5°C during the course of 1 hour. It was frozen at 3:30 PM the same day by the procedure of Fox(1) and stored at –90°C in a 2-phase mechanical freezer. The basic diluters used in all the experiments are listed in Table I.

Exp. 1. Semen was initially diluted 1:5 at 20°C with a nonglycerolated diluter and 3 hours later 1:2 in a stepwise manner at 5°C with the same diluter, having either 16% EG

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