and ammonium sulfate led to the prompt discovery that the accompanying phospholipid varied from none to half of the total plasma phospholipid depending upon the salting agent employed. The results demonstrate the necessity of separating the individual protein fractions with minimal chemical alteration, before information can be secured concerning the protein-phospholipid complexes of blood plasma.

Summary. The phospholipid partition (lecithin, cephalin and sphingomyelin) of hemophilic plasma revealed no lack of the clot-aiding phospholipid cephalin.

A tested technic showed only minute traces of "free" phospholipid in normal and hemophilic plasma. Measurable amounts of uncombined cephalin and other phospholipids were found, however, in lipemic plasmas. Attempts to liberate cephalin by tryptic digestion of plasma were unsuccessful. Added cephalin combined with the plasma proteins to a large extent, except in lipemias.

The lysis of platelets in the presence of calcium (yielding an active thromboplastic solution) gave lysates containing little or no phospholipid, free or combined.

11757

Effect of Alpha-Tocopherol on Lesions of Skeletal Muscles in Rats on Vitamin A-Deficient Diets.

CECIL KRAKOWER AND JOSEPH H. AXTMAYER. (Introduced by D. H. Cook.)

From the School of Tropical Medicine, San Juan, Puerto Rico.

Degenerative lesions of skeletal muscles have been described in rats on vitamin A-deficient diets.[1] We too have constantly observed such lesions in our experimental rats on a standard vitamin A-free diet.

In recent years muscular dystrophy in rats has been shown to be related to a deficiency in vitamin E. It was first produced in the suckling young of mother rats placed on a diet deficient in vitamin E by Evans and Burr[2] and the nature of the lesions in the muscles of the affected young were first described by Olcott[3] and recently by

1 Bessey, O. A., and Wolbach, S. B., "The Vitamins"—a Symposium, American Medical Association, 1939.

2 Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 273.

3 Olcott, H. S., *J. Nutrition*, 1938, **15**, 221.

Pappenheimer[4] and by Telford, *et al.*[5] Muscular dystrophy has also been described in older rats on vitamin E-deficient diets by Blumberg,[6] Ringsted,[7] Burr, Brown and Moseley,[8] Einarson and Ringsted,[9] Evans, Emerson and Telford[10] and Knowlton and Hines.[11] Some of these authors have given histological descriptions of the lesions.

While it has been demonstrated that the administration of vitamin E concentrates or wheat germ oil will prevent muscular dystrophy, Barrie,[12] Demole and Pfaltz,[13] and Goettsch and Ritzmann[14] have shown that alpha-tocopherol is similarly effective in preventing the disease in the suckling young and Knowlton, Hines and Brinkhous[15] in adult rats.

Inasmuch as the muscular lesions in rats on vitamin A-free diets resemble those of muscular dystrophy due to lack of vitamin E and as these vitamin A-free diets do not contain vitamin E, it was considered desirable to determine whether these muscular lesions were related to the deficiency of vitamin A or were due to lack of the vitamin E factor.

To avoid the use of an additional source of vitamin A in either wheat germ oil or its concentrates synthetic alpha-tocopherol* was employed. The standard vitamin A-free diet we have used consists of: Purified casein 18%, corn starch 68%, baker's yeast 10%, Osborne & Mendel's salt mixture 4% and viosterol 1 cc per kilo of diet.

In the first series there were 3 litters, the mothers of which had been previously fed Purina dog chow but were placed on the vitamin A-free diet 17, 14 and 14 days respectively postpartum. The young

4 Pappenheimer, A. M., *Am. J. Path.*, 1939, **15**, 179.

5 Telford, I. R., Emerson, G. A., and Evans, H. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 291.

6 Blumberg, H., *J. Biol. Chem.*, 1935, **108**, 227.

7 Ringsted, A., *Biochem. J.*, 1935, **29**, 788.

8 Burr, G. O., Brown, W. R., and Moseley, R. L., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 780.

9 Einarson, L., and Ringsted, A., *Nutritional Abstracts*, 1938, **8**, 52.

10 Evans, H. M., Emerson, G. A., and Telford, I. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 625.

11 Knowlton, G. C., and Hines, H. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 665.

12 Barrie, M. M. O., *Nature*, 1938, **142**, 799.

13 Demole, V., and Pfaltz, H., *Schweiz. Med. Wchnschr.*, 1939, **69**, 123.

14 Goettsch, M., and Ritzmann, J., *J. Nutrition*, 1939, **17**, 371.

15 Knowlton, G. C., Hines, H. M., and Brinkhous, V. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 804.

* Kindly contributed by Merck & Co. and Hoffman-La Roche, Inc.

were weaned at 28 days and were henceforth continued on the vitamin A-free diet. There were 17 young in this series, 8 of these were fed 1.5 mg of alpha-tocopherol (Merck's) in olive oil weekly; the remaining 9 controls were fed olive oil alone. No cod liver oil supplement was given. Sixteen of the animals were killed when they were between 60 and 75 days of age. One rat died (63 days old) and was immediately autopsied. The initial weights at the time of weaning ranged from 33 to 57 g and the final weights between 55 to 89 g.

In the second series 2 litters with a total of 17 rats were employed. The mothers were maintained on Purina dog chow during gestation and lactation. The young were weaned at 28 days and placed on the vitamin A-free diet. Eight of these were given weekly injections subcutaneously of 5 mg d l alpha-tocopherol acetate (Hoffman-La Roche) in olive oil. The remaining 9 were left as controls. The diet of all animals was supplemented once or twice and in one instance 3 times with one or 2 drops of cod liver oil. All animals died at or were allowed to survive to ages ranging from 113 to 183 days. The animals that died were autopsied as soon as possible. The initial weights at weaning varied between 44 and 73 g and at death between 104 and 172 g.

Experimental and control animals were killed simultaneously when possible.

Results. In the first series (acute vitamin A deficiency) there were microscopic (but no macroscopic) muscular lesions in all control animals except the youngest one (60 days). These were of the character of hyaline necroses with or without reactive cellular changes and with little evident regeneration. The lesions were generally few but in some groups of muscle they were either absent or quite frequent. The most pronounced lesions in this series occurred in the oldest control rat (75 days).

In the experimental group that was fed tocopherol no muscular lesions were observed except a very rare hyaline necrosis in the muscles of the hind legs of one animal and of the forelegs of a second.

In the second series (chronic vitamin A deficiency) there were extensive muscular lesions in the control group, particularly the older ones (macroscopically clearly apparent only in one, due to calcification of the hyaline necroses). The lesions were as above with more active repair and regeneration.

In the experimental groups that received injections of tocopherol no lesions were observed except a single hyaline necrosis in the mus-

culature of the forelegs of one animal and a rare lesion in the abdominal musculature of the second.

Many of the animals, particularly the older ones, whether control or experimental, exhibited varied degrees of weakness and some incoördination towards the latter part of the experimental period. The central and peripheral nervous systems were not examined. As might be expected the alpha-tocopherol had no effect upon either the extent or character of the characteristic lesions of vitamin A deficiency present in all animals, and testicular atrophy occurred as readily in the experimental as in the control animals. Focal areas of interstitial myocardial infiltration occurred as frequently in the one as in the other.

Conclusions. Muscular lesions hitherto described in vitamin A deficiency in rats can be prevented by the administration of alpha-tocopherol and hence are not related to the vitamin A-deficient state. Vitamin E or alpha-tocopherol should form a part of the basal diet used to produce vitamin A deficiency in rats.

11758

Histamine in the Rabbit Skin.

M. ROCHA E SILVA.* (Introduced by A. C. Ivy.)
From the Instituto Biologico, São Paulo, Brazil.

Using the trypan blue test we have shown[1] that histamine injected intradermally in the very young rabbit does not cause a seepage into the injected area of the dye injected intravenously. This fact is consistent with an observation described by Feldberg and Kellaway[2] on the pharmacological effect of histamine on the cat. Very young cats are very resistant to the injection of histamine. In particular, the pressure in the pulmonary artery is only slightly altered by the intravenous injection of histamine in young cats, while the same dose injected into old cats may produce marked changes. By determining the histamine content of the lungs in cats varying in weights from 0.5 to 6 kg, they have shown a parallelism between the increasing amount of histamine in the lung tissue and the pharma-

* Fellow of the John Simon Guggenheim Memorial Foundation.

1 Rocha e Silva, M., *Nature*, 1940, **145**, 591.

2 Feldberg, W., and Kellaway, C. H., *Aust. J. Exp. Biol. and Med. Sc.*, 1937, **15**, 81.