mal rabbit serum and anti-sera prepared against a strain of *Willia* and against Fleischmann's yeast were negative at 1:100 dilution of the polysaccharide. The activity of a 1:100,000 solution was apparently undiminished after heating 30 minutes at 100° C.

In view of the specificity of most of the other polysaccharides isolated by various workers it is of interest to note that precipitin rings were obtained with *Monilia psilosis* polysaccharide using antisera† prepared against similar yeast-like organisms, but either at a lower dilution or less rapidly, in the majority of cases. (These included *Monilia albicans, Monilia parapsilosis,* Ashford, *M. krusei,* Castellani, a non-pathogenic monilia, and two strains of monilia isolated from erosio interdigitalis.)‡ This raises a possible question as to the individuality of the species so far tested and is further confirmation of the findings of Hopkins and Benham,[3] who observed cross-agglutinations among these same organisms, although certain distinctions could be obtained by agglutinin absorption. Work is in progress to obtain the carbohydrates from related species with the object of studying cross-precipitin reactions and precipitin absorption.

With the isolation of additional active material it is hoped to amplify both the chemical and immunological studies including skin and serum reactions of individuals harboring monilia.

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The Dietary Production of Dystrophy of the Voluntary Muscles.*

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Vitamin E has been shown by Evans, Bishop, and their coworkers[1, 2, 3] to be necessary for normal reproduction in the rat, and by

† Kindly furnished by Miss R. W. Benham, Department of Dermatology, College of Physicians and Surgeons, Columbia University.

‡ It is interesting that the monilia polysaccharide precipitated with a sample of Type II pneumococcus antiserum furnished by Dr. M. Heidelberger.

[3] Hopkins, J. G., and Benham, Rhoda W., *N. Y. State J. Med.*, 1929, xxix, 793.

* This work has been conducted with the aid of the departmental Research Fund of the Chemical Foundation.

[1] Evans, H. M., and Bishop, K. S., *J. Met. Research*, 1923, iii, 201, 223.

[2] Evans, H. M., and Burr, G. O., *Memoirs of U. of Calif.*, Berkeley, 1927, viii.

[3] Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, lxxvi, 273.

Beard,[4] in the mouse. This investigation was begun in order to study the effect of an E-free diet on the guinea pig; and as the problem of feeding these animals simplified diets, such as are ordinarily used in vitamin E experiments, was rather a difficult one, it was decided to use instead, a natural food diet, in which the vitamin E had been destroyed by treatment with ethereal ferric chloride, a method discovered by Waddell and Steenbock.[5] When guinea pigs were reared on this diet, they stopped growing after 1 to 2 months, maintained a constant weight for another month, rapidly declined for 2 or 3 days, and suddenly died. The oestrus rhythm was found to be normal but further studies upon reproduction were impossible. Upon autopsy, these animals showed marked macroscopic changes in the skeletal muscles throughout the body.

The diet was prepared as follows:

Rolled Oats (Quaker)	355 parts
Wheat Bran (Pillsbury)	180 "
Casein (Merck Technical)	75 "
Lard	80 "
Cod Liver Oil (Meads)	10 "
NaCl	10 "
$CaCO_3$	15 "

The ingredients were shaken in a closed container with 10 gm. of ferric chloride, U.S.P. lump, that had been taken up in about 125 cc. of ether and a little water, and the mixture set aside. After half an hour, the contents were transferred to a tray and the ether allowed to evaporate. Then there was added:

Skimmed Milk Powder (Merrell Soule) 275 parts.

Each guinea pig was given daily by pipette 3 cc. of orange juice, to protect it from scurvy.

Rats, both male and female, reared upon this diet, gave symptoms that were typical of vitamin E deprivation; and the females were cured after true resorption of the embryos by transferring them to a diet, similar in all respects except that the ethereal ferric chloride treatment had been eliminated.

Guinea pigs that had been born in the laboratory were used for these experiments, and upon weaning at 21 days, they were given the experimental ration with greens and orange juice. After about 10 days, when they had become more or less accustomed to the new diet, the greens were discontinued. Control guinea pigs were given

[4] Beard, H. H., *Am. J. Physiol.*, 1926, lxxv, 682.

[5] Waddell, J., and Steenbock, H., *J. Biol. Chem.*, 1928, lxxx, 431.

either a similar diet in which the ethereal ferric chloride treatment had been omitted or else the stock diet of oats, bran, hay and greens.

Thus far 20 animals on the experimental diet have been studied and 10 controls. The experimental group had rather similar histories, which differed markedly from litter-mate controls. The growth rate for the first month or two was normal. After the animals had attained a weight of about 400 gm., there was a sudden cessation in the growth of the experimental group accompanied by general flabbiness; and it was observed that the animals had difficulty in righting themselves if they were placed on their backs, a test used by the California workers[3] to determine the degree of paralysis in baby rats. During the following month, a constant weight was maintained after which the animals went into rapid decline for 2 to 3 days and suddenly died on about the hundredth day of the experiment, although one animal is still living after 7 months on the diet. The controls showed no break in the growth curve and continued to grow steadily. A few of the experimental group recovered spontaneously from the flabbiness, made a moderate gain in weight, remained stationary at a higher level, and died.

While most of the animals were killed and autopsied when apparently on the point of death, a few were sacrificed before the cessation of growth. Of the 19 experimental guinea pigs that have come to autopsy, all but one showed striking macroscopic changes in the voluntary muscles. The muscles of the thigh and abdomen were particularly involved. They appeared atrophied and pale, and had a yellowish color, quite different from those of the normal controls. Sometimes they were gritty looking, and streaked as though calcified or infiltrated with fat. In 2 cases, the thigh muscles were markedly hemorrhagic. The muscles seemed to have lost their irritability.

The other organs appeared normal. In no case were there any scorbutic lesions. The animals were thin and flabby, but not particularly emaciated, as indicated by normal deposits of fat in the subcutaneous tissue. Inanition was not the cause of the muscle dystrophy because some of the most pronounced lesions were found in actively growing animals that showed no decline in weight.

Rats reared on this diet seemed normal in all respects except for the sterility caused by the absence of vitamin E from the diet.

Two rabbits born in the laboratory were given the same diet with 6 cc. of orange juice when they were 4½ weeks of age. They maintained their weight and even grew to some extent for 12 days, then lost the use of their muscles completely, and were killed and autop-

sied 3 and 4 days later. The muscles were small, extremely pale, and without irritability. Other organs seemed normal.

Experiments are in progress in which guinea pigs are to be killed at different stages in order to determine the time of onset of the disease. Curative, as well as prophylactic doses of wheat germ oil are being administered to guinea pigs and rabbits in order to ascertain whether vitamin E deficiency is the cause, or whether the toxicity of the ferric chloride itself, or its effect on the natural food diet in some way other than vitamin E destruction may be responsible. The muscles of paralyzed baby rats as produced by Evans and Burr and their coworkers[3] upon vitamin E-free diets are being investigated.

The only conclusion which may be drawn at present is that the diet described in this paper produces in the guinea pig and rabbit a general dystrophy of the voluntary muscles unaccompanied by obvious lesions in the other organs.

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Pathological Changes in the Skeletal Muscles Produced by Dietary Means.

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Guinea pigs maintained on the diet described in the previous paper[1] for periods ranging from 35 to 133 days, develop extreme degeneration of the skeletal muscles of the trunk and extremities.

The primary alteration is a waxy or hyaline necrosis of the fibers. This is followed by great proliferation of the muscle nuclei, leading to the formation of so-called "Muskelzellenschlauche" within the intact sarcolemma. There is also active regeneration of muscle cells in the later stages. The disappearance of the degenerated fibers is accompanied by a variable amount of interstitial fibrosis and lipomatosis.

The affected muscles are characterized grossly by a striking pallor. The prevailing color is yellowish grey, with fine stippling. The muscular tone and elasticity are lost, and the muscle bulk is much reduced in comparison with that of control litter mates.

Careful histological study of the principal organs and tissues other

[1] Goettsch, M., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 564.