arteries exposed to an injurious agent showed destruction of the endothelium lining the vessel. In the instances where the weaker dilutions of turpentine were employed, poorly stained remnants of this layer could be seen, but with the other injurious agents it was completely absent. When 50% and 75% croton oil and higher dilutions of turpentine were used the nuclei of the muscle cells in the inner third of the media were pyknotic and a slight fraying of the elastic fibers in the media, including the internal elastic lamella, was present. This latter change appeared to be of a corrosive type. There was no exudate in the intima or the tissues immediately beneath the internal elastic lamella, but as the adventitia was approached a sparse scattering of small round cells and occasional polymorphonuclear leucocytes was observed in 2 instances. Small clumps of polymorphonuclear leucocytes were seen surrounding the vessels in the periadventitial connective tissue in all the sections, but the number of cells did not exceed that observed in the controls. From these observations it must be deduced that the tissues of blood vessel walls differ strikingly from other tissues in the body with respect to their response to the injurious agents employed.

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Possible Relationship Between Cod Liver Oil and Muscular Degeneration of Herbivora Fed Synthetic Diets.*

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We have made numerous attempts to formulate a satisfactory synthetic diet for herbivorous animals. In a previous communication,[1] in which partial success with an adult goat was recorded, it was reported that the synthetic diet caused paralysis in rabbits with a histological picture of muscle degeneration. These studies have been continued with goats, rabbits and guinea pigs, using diets as follows:

* Supported by a grant from the Heckscher Research Fund.

[1] Woodward, J. C., and McCay, C. M., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 241.

Goats		Rabbits and Guinea Pigs
20	Regenerated Cellulose	20
26	Corn Starch	40
20	Casein	15
15	Sucrose	10
7	Yeast	5
6	Lard	4
6	Salt Mixture	4
12 cc. per week	Cod Liver Oil	2
	Tomato or Orange Juice	Daily

Six kids were transferred to the diet directly after weaning. They consumed it readily and grew at a normal rate for 2½ to 3 months, when the growth rate declined but all animals continued to eat well. The first death occurred after 133 days on the diet and 4 others died during the following 2 months. The remaining animal lived until the 315th day. All animals died suddenly without obvious symptoms.

The characteristic finding on autopsy was the evidence of failing circulation, consisting of intermuscular edema of the legs, increased fluid in body cavities, and congestion and edema of the lungs. There was frequently a severe hemorrhagic enteritis, often associated with large amounts of blood stained fluid in the lumen of the small intestine. Fatty degeneration of the liver and kidneys was also a frequent finding. Although no case of stiffness, such as is found regularly in the rabbits, was observed, gross muscle lesions were found in several of the goats. On histological examination muscle lesions were found in all cases. The skeletal muscle lesions were similar to hyaline, waxy or Zenker's degenerative changes. There was also a proliferation of fibroplastic tissue which tended to replace the degenerating muscle.

The heart muscle was also involved in all animals. Gross alterations were often evident and consisted of dilations especially of the right ventricle. In some cases the heart was entirely normal in shape but the myocardium was usually paler than normal suggesting degeneration and fibrosis. On section, several local well defined grayish areas were found. On histological examination hyaline and granular degeneration changes of the heart muscle fibers were seen. In some areas the fibers were necrotic and in process of absorption. These changes were accompanied by an increase in connective tissue which often completely replaced portions of the degenerated muscle.

In continuing the studies with rabbits, the degenerative changes in the leg muscles were again found. They were similar to those

seen in the goats but more extensive. The replacement of muscle by connective tissue is no doubt one of the chief causes of muscle paralysis or stiffness. Many of the rabbit hearts have shown marked gross alterations, often dilated, the muscle very flabby, the ventricles stretched and reduced in thickness. The changes usually appeared first and were more marked in the right ventricle. On histological examination hyaline changes and vacuolar degeneration of the muscle fibers were present. Hemorrhage in the heart muscle was found in a few cases. Fibroplastic changes were not as marked as in the goat hearts. However, the goat lesions were probably of longer standing.

When guinea pigs were fed the synthetic diet the results were similar to those obtained with the rabbits. Similar lesions were found in both the skeletal and heart muscles. In all the species the skeletal muscle changes resemble very closely those reported by Goettsch and Pappenheimer.[2] However, these investigators have not found any changes in the heart.

Dr. Olafson, of the Department of Pathology and Bacteriology, who first found the histological changes in the goat hearts, called our attention to the similarity of lesions to those reported by Agduhr[3] in rabbits and other species receiving large doses of cod liver oil, and suggested that this might be the causative agent in our results. Agduhr stressed the responsibility of cod liver oil in causing heart lesions but little attention has been given to his work. We, therefore studied the cod liver factor in relation to our results.

A group of 10 rabbits were placed on the synthetic diet, omitting the cod liver oil and without any added source of vitamins A or D. The fat previously supplied by the oil was furnished as lard. These rabbits made larger gains and lived longer than rabbits receiving 2 to 3% of cod liver oil in their synthetic ration, which usually die between the 30th and 40th day. By the 43rd day 2 of the rabbits on the synthetic ration without cod liver oil had died showing symptoms of vitamin A deficiency. The remaining 8 rabbits, which were starting to lose weight, were divided into 2 groups of 4 each. Group 1 received a supplement of 3% of cod liver oil and Group 2 was given a vitamin AD concentrate plus cottonseed oil to make up the fat content. All of the animals receiving the cod liver oil became stiff and died in from 11 to 19 days after the oil was introduced. There were no symptoms of A deficiency. Two of the animals

[2] Goettsch, M., and Pappenheimer, A. M., *J. Exp. Med.*, 1931, **54**, 145.

[3] Agduhr, E., *Acta Paediatrica*, 1926, **5**, 319.

placed on the concentrate refused to eat and died of A deficiency. The other 2, after a period of further weight loss, recovered and started to gain. One died after 80 days. It was not typically stiff but the heart was found flabby and dilated. The second animal is still alive and apparently normal after 270 days.

Another group of 4 rabbits was given the concentrate from the start. Two died at 36 days. They were not stiff but in both cases autopsy showed the lungs congested and the right ventricle slightly dilated. The other 2 survived for 155 days and 197 days respectively. The one surviving the longest was stiff for 147 days. Both showed the skeletal and heart muscle lesions on autopsy.

To study the problem further, 40 guinea pigs were divided into 4 groups as follows: (I) Basal synthetic diet with medicinal cod liver oil; (II) Basal synthetic diet with animal cod liver oil; (III) Basal synthetic diet with AD concentrate; (IV) Stock diet of alfalfa, grain and cabbage with cod liver oil.

In the previous experiment an animal grade of cod liver oil was used. Thus groups were included to ascertain whether a more refined product would give different results. The oils were fed separately as 0.5 cc. per animal per day. Group III received an equal amount of cottonseed oil containing the concentrate. The vitamin B and C sources were also fed separately. To date, after 125 days, the results of this experiment are as follows: After 70 days 7 of the animals in Group I had died, 4 of which were stiff and the survivors were stiff. Two of the latter died 10 days later. The remaining animal was changed to the stock diet and is showing some recovery. In Group II, 6 animals were dead within 70 days, all stiff. The survivors were stiff. One of these died 20 days later. The remaining animals are still alive, 2 of which are showing some recovery as a result of a change to the stock diet. In Group III, 8 animals still survive with no signs of stiffness. Several have more than doubled in weight. The other 2 were killed for autopsy at a time when they were apparently normal. No gross lesions were found. Two of the animals in Group IV died early in the experiment without stiffness. The remainder have continued to grow rapidly and are apparently normal. It is clear that the replacement of the cod liver oil by an AD concentrate has greatly prolonged the survival period on the synthetic diet. In general, it has at least greatly delayed the onset of the stiffness and presumably the onset of the muscle lesions which apparently precede the stiffness, though not preventing the eventual development of the lesions in the few cases (rabbits) examined.

It is tentatively suggested that some factor in the cod liver oil, primarily in the saponifiable fraction, is concerned in the production of the muscle lesions and the other pathological symptoms observed in herbivorous animals fed a synthetic diet. The fact that no deleterious effects have been observed from the same level of cod liver oil when added to a stock diet, may mean that the effects are the combined result of the oil and some other factor peculiar to the synthetic diet, or it may be that a toxic effect of the oil is partially counteracted by some ingredient of the stock diet of natural foods. Our earlier results showed that rabbits which had become stiff frequently recovered when changed to a diet of alfalfa and that the inclusion of alfalfa in the synthetic diet had some protective influence.[4]

The literature contains several reports of deleterious effects of cod liver oil on herbivora. Slagsvold[5] reports a poisoning of cattle by cod liver oil, with stiffness and muscle lesions as a characteristic symptom. Wahlin[6] found degenerative changes in the cardiac and skeletal muscles of guinea pigs which he attributed to cod liver oil. Golding[7] showed that cod liver oil has a specific effect not shown by other oils in lowering the percentage of fat in cow's milk. This finding has been confirmed by several others. Of special interest in connection with our work, is Golding's findings that the unsaponifiable fraction of cod liver oil was without influence.

The significance of our results will become much clearer when the experiments now in progress are finished and when the histological examination of the some 90 animals which we have had on these various experiments are completed. We are aware that muscle lesions have been reported as an accompaniment of various diseases and dietary deficiencies, and that much further study is needed to work out the specific relationships involved in our findings.

[4] McCay, C. M., Madsen, L. L., and Maynard, L. A., *J. Biol. Chem., Proc.*, 1933, **100**, lxviii.

[5] Slagsvold, L., *Norsk. Vet. Tids.*, 1925, **6**, 161. *Abs. J. A. V. M. A.*, 1925, **68**, 236.

[6] Wahlin, B., *Acta. Med. Scand.*, 1931, **74**, 430.

[7] Golding, J., *Proc. World's Dairy Congress*, 1928.