found in our normal animals). Although not abundant, sperm were found in many tubules of the testes of all 5 rabbits. They were more plentiful in the cured animals than in those dying with dystrophy. Aside from a slight sloughing of germ cells in the testes of rabbit No. 218, which died with dystrophy, and in the testes of rabbit No. 227, which was cured of the disease, the germinal epithelium of all animals was normal. This confirms and extends our recent finding[7] that widespread muscle lesions occur in vitamin E-deficient rabbits in the absence of testicular degeneration.

In this experiment the dystrophic rabbits developed no symptoms other than those referable to changes in the skeletal muscles. Furthermore, the remarkable stimulus given to muscle repair by α-tocopherol therapy was not lost even after 6 attacks of the disease.

Conclusions. As many as 6 successive attacks of nutritional muscular dystrophy have been produced and cured in rabbits. Continued α-tocopherol therapy following the last attack resulted in the complete repair of hyalinization and necrosis of the thigh muscles. Testicular degeneration was not observed.

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Production of Subcutaneous Sarcoma by Azo Dye and the Influence Thereon of Liver Feeding.

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Interest in the liver tumors produced by azo dyes has recently been directed chiefly along two lines, to wit: (a) studies of the inhibition of carcinogenesis by accessory food substances, particularly the demonstration that extracts of liver or yeast will protect rats against the butter-yellow liver cancer,[1] and (b) the apparent difference in biological activity between the azo compounds and the polynuclear hydrocarbons which might be dependent upon the presence

[7] Mackenzie, C. G., and McCollum, E. V., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 148.

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[1] Sugiura, K., and Rhoads, C. P., *Cancer Research*, 1941, **1**, 3.

or absence of nitrogen in the molecule.[2] " . . . . the hydrocarbons usually produce tumors at the site of application whereas all of the above nitrogen-compounds except styryl 430 tend to exert an effect at a remote site." " . . . . it might be helpful even at this early stage to learn if the presence of nitrogen in the majority of compounds which act at a distant site is significant or merely a coincidence."[2] Observations made in this laboratory during the past few months would appear to bear both upon the problem of the inhibition of carcinogenesis by food supplements and upon the relation of nitrogen content to carcinogenic activity. We have found that the nitrogenous compound *o*-aminoazotoluene may in fact produce tumors at the site of injection as well as in the liver, and moreover that the development of such local tumors, in contrast to hepatomas, appears not to be inhibited by the addition of liver to the diet.

Liver cancer may be induced in rats or mice by the incorporation of *p*-dimethylaminoazobenzene (butter yellow) or *o*-aminoazotoluene in the food, and by subcutaneous injection of these dyes dissolved in olive oil or suspended in glycerin.[3] The development of local tumors at the site of application has not been reported.

Our studies were begun in December, 1940, for the purpose of procuring a liver cell carcinoma with which to carry out certain immunological investigations. Sixty young adult mice of the highly inbred C strain were given *o*-aminoazotoluene in a course of injections extending now more than a year. The mice belonged to a family bred in this laboratory from pedigreed C strain parents obtained from the Roscoe B. Jackson Memorial Laboratory in Bar Harbor, Me. Males and females were employed in equal number. Every mouse received 2.5 mg of dye weekly until the termination of the experiment. The dye was dissolved in corn-oil ("Mazola") and injected subcutaneously into the back through a No. 24 needle, 0.1 cc of oil being used for each dose. Forty of the animals were maintained on a stock diet of Purina chow pellets, while the remaining 20 were given a supplement of dried fresh beef liver, 20% by weight added to powdered Purina pellets and finally made into small cakes by moistening with water and drying.

Almost a year after the beginning of the experiment, tumors began to appear in some of the mice. Meanwhile about half of the animals, both of liver-fed and control groups, had died, presumably from the toxicity of the carcinogen. This report is based upon

[2] Fieser, L. F., *Cause and Growth of Cancer*, University of Pennsylvania Press, 1941.

[3] Shear, M. J., *Am. J. Cancer*, 1937, **29**, 269.

TABLE I.
Tumors in Treated C Strain Mice.

	Controls Total No., 7*		Liver fed Total No., 3
Interscapular tumor	5 (71%)		3 (100%)
Normal liver	1		2
Cirrhotic "	3		0
Cirrhosis and adenoma	1	71%	1
Carcinoma	1		0

*One liver specimen lost.

the findings in 10 mice brought to autopsy during November and December of 1941, some 11 months after the beginning of the experiment. At the present writing, 22 additional mice are still under treatment; these will be reported upon at a later date.

Results are shown in Table I. Subcutaneous sarcomata† developed in 8 of the 10 mice, and these were found invariably in the interscapular region, a site at which blebs of injected oil may often be seen. Spontaneous subcutaneous sarcomas are found very rarely in the C strain mouse.[4]

While the feeding of liver protected against liver cirrhosis and cancer, it does not appear to influence the development of local sarcomata since these tumors developed in all of the liver-fed animals

It would appear from the data that the presence of basic nitrogen in a compound does not signify that its carcinogenic activity is limited to an organ or tissue remote from the site of application. It may act locally if given in sufficient concentration, over a long period of time, and in an oily medium.

Summary. 1. Sarcomata develop near the site of injection of *o*-aminoazotoluene given subcutaneously to mice. 2. Liver feeding does not appear to protect against the development of such sarcomata.

† We are indebted to Dr. E. E. Sproul of the Department of Pathology for interpretation of the histological sections.

[4] Personal communication from Roscoe B. Jackson Laboratory.