

the fact that the annual budget of the Botanic Garden has fallen from nearly \$229,000 in 1930 to \$195,000 in 1940 (\$168,000 in 1934). This falling off of income has, of course, resulted in curtailing many of the activities, in the temporary suspension of some of them, and in some loss of personnel.

The total general attendance at the garden for the first thirty years of its existence was nearly 22,500,000, or almost three times the population of Greater New York, as shown by the 1940 census, and more than eight times the population of Brooklyn by the same census. During this period nearly 18,000 teachers have brought to the garden more than 1,000,000 pupils for instruction in all aspects of plant life and horticulture by members of the garden staff. In addition to this, there has been an attendance of adults and children of more than 1,000,000 at classes organized independently of the schools. The garden has also during its first three decades given talks, lectures and addresses at schools to audiences totaling nearly 2,550,000.

The director points out that in order to carry on this educational program and to maintain a large collection of plants indoors and under glass, in proper condition of health and culture and properly labelled, requires the presence of a scientific, as well as an educational staff, and the inclusion of a certain amount of scientific research in such subjects as plant disease, plant breeding and other aspects of plant life. During these thirty years the city has made a total appropriation in its tax budget of a little more than \$2,200,000, which is about twenty-eight cents per inhabitant, or a trifle less than one cent per inhabitant per year. Contributions of private funds by the Board of Trustees of the Brooklyn Institute of Arts and Sciences, of which the garden is a department, have been nearly \$1,715,000.

When the development of the plantations of the garden began, in 1911, part of the area was a dumping ground where refuse was daily burned. The remainder of the fifty acres comprising the grounds was almost wholly undeveloped. By a happy combination of botanists, horticulturists and a landscape

architect, the garden has now become one of the most beautiful spots in Greater New York and is generally recognized as one of the most beautiful and educationally effective botanic gardens in the world.

The annual reports that have been issued since 1911 have shown that the garden is by no means a parochial institution serving merely Brooklyn and New York City, but that its scientific and educational publications and its work of public education are known throughout the world. The report records the steady growth of the library, open free daily to the public, and of the herbarium, open daily for consultation for those competent to use such collections. Special attention is called to the cooperation of the Botanic Garden in scientific and educational work with such organizations and institutions as the New York World's Fair; Works Progress Administration; Department of Parks; Board of Higher Education; Board of Education; the American Association of Botanical Gardens, which the Brooklyn Botanic Garden helped to found; the Brooklyn Civic Council; the Garden Club of America; New York Bird and Tree Club; five hospitals of Brooklyn, and numerous other organizations.

The increasingly serious financial situation throughout the world has made it conspicuously difficult to finance the scientific and educational activities of the garden and to maintain the plantations and grounds in a proper state of upkeep. The director calls special attention to the need of additional endowment. By a special system of economy during the past twenty years, involving the expenditure of only a portion of the income from its permanent funds, the Botanic Garden has added more than \$150,000 to its endowment. In order to maintain its present activities and to meet the constantly increasing demand of the public and of various departments of the City of New York for service, an addition of \$1,000,000 to the endowment fund is urgently needed.

The report of the director is followed by reports of the heads of the various departments of the garden and a detailed report of the Botanic Garden Budget for the year 1940.

SPECIAL ARTICLES

THE PREVENTION BY CHOLINE OF LIVER CIRRHOSIS IN RATS ON HIGH FAT, LOW PROTEIN DIETS¹

IN connection with experiments on the feeding of wheat germ oil, it was reported² that diffuse nodular cirrhosis of the liver was produced in 7 rats which received large amounts of the oil (3 to 5 cc per day) for 243 days or longer. This regimen was notably high in

¹ This investigation was aided by a grant from William R. Warner and Co., Inc., New York City.

² H. Blumberg, *Pub. Health Rep.*, 55: 534, 1940.

fat and low in protein. In subsequent investigations by Blumberg and Grady,³ this observation on the dietary production of cirrhosis was repeated and extended with wheat germ oil and also with corn oil; a description of the pathology of the cirrhosis and of the extensive fatty changes was presented.

The production of cirrhosis of the liver in 3 dogs on high fat diets has been reported by Chaikoff and

³ H. Blumberg and H. G. Grady, *Proc. Am. Soc. Biol. Chem.*, April, 1941; also, in press.

Connor.⁴ By means of a deficient diet Rich and Hamilton⁵ have produced cirrhosis in rabbits, and have reported its prevention by yeast. Spellberg and Keeton⁶ observed cirrhosis apparently of dietary origin in 1 guinea pig and 1 rabbit in their experiments. According to a recent publication by Gyorgy and Goldblatt,⁷ liver injury (necrosis, cirrhosis) was produced in rats on a diet containing 10 per cent. casein and 22 per cent. fat, with thiamin, riboflavin, pyridoxin and pantothenic acid supplied as members of the vitamin B complex.

In view of the fact that cirrhosis had been produced in our earlier experiments^{2, 3} by diets high in fat and low in protein, we prepared more purified diets that were specifically low in choline, shown by Best, Hershey and Huntsman⁸ to be a lipotropic factor. Diet A (55 per cent. fat) had the following composition: casein 10 per cent., sucrose 29, lard 51, corn oil 2, cod liver oil 2, and Salt Mixture No. 51 (McCollum) 6. This basal ration, deficient in the vitamin B complex, was supplemented with 10 mg of thiamin hydrochloride, 20 mg of riboflavin, 10 mg of pyridoxin hydrochloride and 20 mg of calcium pantothenate⁹ per kilogram of diet. Diet C (70 per cent. fat) was exactly the same as Diet A, except that the lard content was increased 15 per cent. at the expense of the sucrose, so that the composition was as follows: casein 10 per cent., sucrose 14, lard 66, corn oil 2, cod liver oil 2, and Salt Mixture No. 51 (McCollum) 6. The 4 members of the vitamin B complex were added as in Diet A.

When rats of the McCollum piebald strain, half-grown to almost full-grown animals weighing 140 to 250 gm, were placed on these diets, cirrhosis of the liver developed on Diet A in approximately 125 to 150 days. On Diet C, cirrhosis was observed in animals which died or were sacrificed after approximately 100 to 140 days. The cirrhosis was frequently accompanied by severe ascites and hydrothorax, with occasional hydropericardium.

Microscopically, the cirrhotic livers showed scarring with alteration of the architectural pattern. In some cases there was considerable accompanying necrosis, with little in others. Variable amounts of fat were observed. Usually there were appreciable quantities of a yellow pigment, apparently similar to that previously observed to a slighter degree in the experiments with wheat germ oil.³

⁴ I. L. Chaikoff and C. L. Connor, *Proc. Soc. Exp. Biol. and Med.*, 43: 638, 1940.

⁵ A. R. Rich and J. D. Hamilton, *Bull. Johns Hopkins Hosp.*, 66: 185, 1940.

⁶ M. A. Spellberg and R. W. Keeton, *Am. Jour. Med. Sci.*, 200: 688, 1940.

⁷ P. Gyorgy and H. Goldblatt, *Proc. Soc. Exp. Biol. and Med.*, 46: 492, 1941.

⁸ C. H. Best, J. M. Hershey and M. E. Huntsman, *Am. Jour. Physiol.*, 101: 7, 1932.

⁹ Kindly supplied by Merck and Co., Inc., Rahway, N. J.

The addition of l-cystine, at a level of 25 mg per rat per day, did not retard the development of the cirrhosis. Preliminary results with d, l-methionine indicated a slowing of the cirrhotic process but not protection at a level of 25 mg per rat per day, although higher levels have not yet been tried. However, the cirrhosis was prevented by the addition of 1 per cent. choline hydrochloride (10 mg per gm of diet, or approximately 40 to 60 mg per rat per day). Normal livers were secured by the addition of the choline alone, in combination with methionine, or even with cystine, a substance which itself causes hepatic damage at toxic concentrations¹⁰ and has been reported by Earle and Victor¹¹ to produce cirrhotic changes in rats when fed at the excessive levels of 5 per cent. or 10 per cent. of the diet.

It is interesting that Gyorgy and Goldblatt⁷ in their experiments did not prevent liver injury by choline, although they found that "10 to 20 mg of choline daily reduced the incidence and severity of the liver injury but not to a great extent." They also reported an accentuation of cirrhosis of the liver by addition of cystine, and found that "the daily addition of from 10 to 20 mg of choline or of 1 gram of yeast, or better, of both, neutralized more or less completely the effect of cystine on the liver." Under our considerably different experimental conditions, the addition of choline was attended by protection against the hepatic cirrhosis itself. The possible relation of choline to other types of experimental cirrhosis and to the protective action of yeast, as reported by other workers, is under investigation.

Presumably, at least a large part of the beneficial effect of choline is brought about through its lipotropic activity, whereby it prevents the process of long-continued fatty infiltration which, according to Connor,¹² may lead to cirrhosis of the liver in diabetes and in chronic alcoholism in man. However, it may be that choline also exerts its protective action through some additional mechanism.

Cirrhosis of the liver has been produced in more than 30 rats fed the described high fat, low protein diets, whereas the development of cirrhosis has been prevented in a like number of animals by the addition of choline. Details of these experiments, together with observations on the effect of protein level and of yeast, will be reported elsewhere.

HAROLD BLUMBERG
E. V. MCCOLLUM

SCHOOL OF HYGIENE AND PUBLIC HEALTH,
THE JOHNS HOPKINS UNIVERSITY

¹⁰ A. C. Curtis and L. H. Newburgh, *Arch. Int. Med.*, 39: 828, 1927.

¹¹ D. P. Earle, Jr., and J. Victor, *Jour. Exp. Med.*, 73: 161, 1941.

¹² C. L. Connor, *Am. Jour. Path.*, 14: 347, 1938.