DIETETIC HEPATIC INJURIES AND THE MODE OF ACTION OF TOCOPHEROL

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In this paper, three different types of fatal liver injuries are to be described which can be produced by dietary means; *i.e.*, by varying a purified regime in several directions, so that conditions of special metabolic stress may arise. These three kinds of dietary liver injury are not only fundamentally different in their causal conditions but they can also be distinguished histologically. Furthermore, they show differences in the way they can be influenced by some liver protective agents, for instance, by sulphur-containing amino acids. The three kinds of liver damage are: (1) the disturbances developing after feeding diets containing a highly purified alkali-treated casein, the so-called casein VI[1, 2]; (2) the damage produced by feeding diets containing 10 to 20 per cent cod-liver oil[3]; and (3) the damage which can be produced by using yeast as a main protein source.[4]

It was found that growing rats, under these different conditions, die after some weeks. The disturbance of liver function was the main reason for their death. Each of these three types of liver injuries can be inhibited by vitamin E.

It is now known why, in these experiments, the liver damage was so severe and sometimes appeared before any other sign of vitamin E deficiency could be seen. It is remarkable that calcifications were sometimes found in these livers, especially in smaller lobes. Calcifications also have been described by Fenn and Goetsch[5] in degenerated muscles. This may indicate that the deviation of biochemical functions in damaged livers is of the same kind as in degenerated muscles. Our diets have been varied in several ways without remarkable effects upon the capacity to induce liver injuries when no protecting agents were given. The only ingredient of the diets which nevre has been changed is the salt mixture, and experiments in this direction may be of some interest.

In 1940, we intended to find out whether p-amino benzoic acid is a vitamin for rats. Casein has a very high content of this substance,[1] the amount of p-amino benzoic acid found in crude casein being as high as in yeast or liver. It is difficult to prepare casein which is free of this growth-promoting factor. Therefore, as starting material, a highly purified casein from Merck was used. It was found that treatment at pH 8.5 in the heat followed by precipitation with acid results in a product which is practically free of amino-benzoic acid. This product was named casein VI. Young rats on diets containing 15 per cent casein VI died after several weeks. No outstanding external symptoms could be seen. In every case, death occurred following a coma of a few hours duration. Several times it has been possible to induce coma and death in small mice by transferring a filtrate of the blood of dying rats. The dead rats show a striking degeneration of the liver, localized in the central parts of the lobules. The cells in the degenerated

centers are partly, but not too much, infiltrated with fat. Hemorrhages occur. The endothelium of capillaries is free of fat, but shows ceriod pigment.

It may be mentioned that, in about 1,300 rats with liver damages, a typical cirrhosis has never been seen. Possibly this is because the animals did not live long enough.

It ought to be mentioned also that it is important not to give too much vitamin E before beginning the dietary experiment. As a rule, the young rats were put on experimental diets when at 28–30 grams in weight. The mothers and their litters were fed rice and skimmed milk and nothing else, starting from the day of birth.

Casein VI has nearly the same elementary composition and quite the same optical rotation as the starting material. P-amino benzoic acid was completely ineffective regarding the liver damage. Many other substances were tested and also found to be negative. Twenty milligrams of choline chloride were ineffective. Usually, every rat in these experiments received one milligram of choline chloride daily. This was necessary and sufficient to avoid hemorrhagic kidney degenerations.

Sulphur-containing amino acids were without any effect upon the casein VI damage, though it is quite certain that in casein VI cystine is transformed into lanthionine by the alkaline treatment. To make certain that this change is not the reason for the liver injury, purified diets containing a certain amount of lanthionine were fed. In these experiments, no effect of lanthionine upon the livers of growing rats could be seen. Therefore, it seems rather unlikely that the content of lanthionine is the cause of the casein VI damage. It may be mentioned that ethionine also did not induce liver damages.

The fact that sulphur-containing amino acids are not concerned in this liver injury is noteworthy. This is one of the main differences between the casein VI damage and the third type of liver injury to be discussed here, the so-called "rat eclampsia." It is reasonable to conclude that, by the alkaline treatment, another protecting substance in the casein must be split off or destroyed. It has not been possible to demonstrate this factor, although several attempts have been made.

We have found a few substances which are able to inhibit the development of the casein VI injury. Twenty milligrams of xanthine have a protective influence. Xanthine was formerly found by Forbes and McConnell,[6] Neale and Winter,[7] and other groups to protect against the liver damage induced by chloroform and similar agents.[8-10]

In the search for factors in natural materials active against casein VI damage, it was found in 1941 that wheat germs had a high capacity for preventing the liver injury. In 1942 to 1943, the active compound of wheat germs was concentrated and nearly isolated, and it was found to be identical with vitamin E. This result was surprising, since all of the animals received 50 micrograms of synthetic dl,α-tocopheryl acetate every week. This seemed to be sufficient to protect young rats against the development of disturbances in the sexual sphere. As a matter of fact, an approximately 17-fold increase in the amount of vitamin E was necessary to protect the

animals against liver damages. They needed 120 to 130 micrograms of synthetic dl,α-tocopheryl acetate daily for full protection. In subsequent experiments, the vitamin E was added to the fat before mixing the diets. Five milligram per cent was sufficient in every case to inhibit liver injuries, though it is quite evident that a part of this amount is destroyed when the components of the diet have been heated during its preparation.

Thus, it has been established that vitamin E has an influence on the functions of the liver. When this effect was found, experiments were started to ascertain whether other liver damages also could be inhibited by vitamin E. In the veterinary literature, a special toxic liver dystrophy had been described as arising in pigs when large doses of cod-liver oil are administered.[11-13] This induced a similar experiment with diets containing normally purified casein combined with relatively large amounts of cod-liver oil. When fed with these diets, young rats showed a severe failure of growth. They were seriously damaged—the muscles getting dystrophic, the backs hunching extremely, and the fur being rough and unkempt. When the amount of cod-liver oil was 20 per cent, all animals died after several weeks. They showed severe alterations of the liver, and, in some cases, but not in all, an evenly distributed degeneration of liver cells was found. The breakdown of liver functions seemed to be the immediate cause of death. The animals showed no body fat at all, while their liver cells were well-filled with big lipoid granules. This may indicate, perhaps, a derangement of fat metabolism.

With 5 milligram per cent of synthetic dl,α-tocopheryl acetate, no animal died. The growth of this group was much improved but not quite comparable with the rate of growth in control groups. The animals were sacrificed after 140 days of experiment. No pathological findings except a calcification of the kidneys could be found. The calcification can be traced back to the hypervitaminosis D. It can be reproduced by administration of vitamin D alone without cod-liver oil.

The protective effect of vitamin E upon the cod-liver oil injury in rats is in accordance with the well-known findings of other investigators, especially of Mackenzie,[14] Dam,[15-17] and Mason.[18] The literature about toxic effects of large doses of cod-liver oil is rather complicated and difficult to survey. In part, injuries are described which are typical symptoms of vitamin E deficiency.[3]

The third dietary liver injury found to be influenced by tocopherol is the damage developed if yeast protein is fed as the main source of protein in synthetic diets. Yeast is very poor in cystine and low in methionine, so that this damage surely is comparable with the well-known findings of Weichselbaum,[19] György,[20-22] and other groups[23-32] working on yeast diets or on low protein diets. The liver injury in these animals begins in the lobular periphery. The cells are filled with fat, as are star-cells. It is remarkable that the kidneys of these animals show a severe glomerulo-nephrosis,[33] a condition very seldom found in rats. A certain percentage of the animals developed convulsions shortly before death occurred.[34] It is difficult to observe these convulsions, since most of the animals lapse into coma during the night and are found dead in the morning. The whole syndrome with liver damage, glomerulo-nephrosis, and convulsions was named "rat-eclampsia."

Further investigations are necessary to learn whether the rat-eclampsia may be compared with human eclampsia, and if it can be regarded as a model for studies on problems connected with this group of diseases.

In earlier publications, it was thought that this liver damage was caused only by the lack of sulphur-containing amino acids.[28-32] This is not the only reason, as has been proved by these experiments and the independent experiments of György.[22] The absence of tocopherol is necessary at the same time. Vitamin E alone, or sulphur-containing amino acids alone are able to inhibit the developments of rat-eclampsia. Therefore, it is clear that this disease is not identical with the isolated lack of sulphur-containing amino acids or with the lack of Vitamin E alone. It is no simple combination of two diseases but is a special case involving two quite different dietary components. The term "ambogen" has been introduced for this type of deficiency disease. The existence of an ambogen disease seems to prove a special metabolic correlation between the compounds affected.

Several groups of investigators have not been able to find liver damage on diets low in sulphur-containing amino acids.[35-37] The explanation for their failure can be found in the amount of tocopherol administered in the diets. The relation between vitamin E supply and prevention of liver injuries has been stressed recently by Himsworth and Lindan.[38]

The connection between sulphur-containing amino acids and vitamin E seems to be the reason for the protein-sparing effect of tocopherol found by several groups of investigators.[39-41] Cystine should be regarded as the limiting factor in diets low in casein.

Recently, experiments were started which combined yeast protein with 20 per cent cod-liver oil. In the first group of animals on this diet, death occurred before liver lesions could develop. The rats were in a severely damaged condition, and, when they died, a new syndrome was seen. The lungs were infiltrated and the hearts were pale and seemed, to the naked eye, to be degenerated. There were impressive hydrothoraces and hydropericardia. The histological examination, however, which has not yet been completed, demonstrated that only small parts of the heart muscle really were degenerated. The fluid in the lungs is not an acute edema but seems to be infiltrated through the pleura. A pleuritis and pericarditis with fibrinosis are found. Further investigations must be made before it can be established whether or not an infection is affiliated with these changes. At any rate, it is remarkable that 5 milligram per cent of dl,α-tocopheryl acetate was able to protect all the control animals against this disease.

In summary, it should be stated that these results demonstrate that tocopherol is important for the liver and that, in its functions, it is closely correlated to several other agents, especially to sulphur-containing amino acids, to xanthin, and to unsaturated compounds in fat. These relations are complicated and must be elucidated by subsequent experiments of a quantitative nature. This may, perhaps, help in understanding the versatility of the symptoms of vitamin E deficiency under different conditions.

It is impressive that 1 mol of tocopherol has the same effect in preventing "rat eclampsia" as 200–400 mols of cystine or methionine. This may, *perhaps*, indicate that vitamin E has a catalytic function, where cystine, or

compound derivatives are used as substrates. It is quite certain, for chemical reasons, that tocopherol itself cannot participate directly in transmethylation, but it may be possible that the function of tocopherol is, in some way, affiliated with transmethylation steps. Thus, vitamin E would be important for fat metabolism and for sulphur-containing amino acids at the same time.

Attempts have been made to discover the effects of tocopherol-therapy in cases of human liver diseases. The influence upon epidemic hepatitis is difficult to demonstrate because this disease has a ready tendency for recovery. A certain percentage of the cases do not respond immediately to vitamin E therapy, while others seem to be influenced. Though the results look favorable generally, further careful experiments are necessary. The application of tocopherol in cases of liver damage is complicated if the bile-flow is reduced or stopped. A vicious cycle exists. Insufficient absorption of vitamin E permits further injury to the liver, and this reversibly reduces the bile-flow and tocopherol-absorption, *et cetera*.

In order to break this cycle, it is necessary to give water-soluble vitamin E preparations or to inject vitamin E in oil solution intramuscularly. These injections are not well absorbed, and it is not possible to estimate the amount of tocopherol which actually reaches the liver. It will, perhaps, be possible to find special water-soluble vitamin E compounds which can be hydrolyzed specificially in the liver. It was found that rat liver is rather active in hydrolyzing tocopherol-phosphate.

When large doses of dl,α-tocopheryl acetate were administered to cases of jaundice due to occlusion of the bile ducts, and impressive reduction of bilirubin in blood occurred. This treatment may obtain a certain value in preparing patients for operations. In about 30 per cent of these cases, bilirubin did not react. In the urine of others, but not in every case, a colorless substance was detected which can be oxidized to a dark green pigment, thus disturbing the Gmelin reaction for bilirubin.

Many more experiences must be collected before final judgment of the value of vitamin E therapy in human liver injuries can be given. In order to avoid deprecation of the real value of vitamin E therapy, one should be careful and not be too optimistic. Perhaps, it will be favorable and necessary to combine vitamin E with other liver-protecting agents.

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Discussion of the Paper

DR. N. S. SCRIMSHAW (*Department of Obstetrics and Gynecology, University of Rochester, School of Medicine and Dentistry, Rochester, New York*): In Dr. Schwarz's generally excellent presentation, I must object to his use of the term "rat eclampsia." He feels justified because he has used it to describe a condition of sudden onset, characterized by kidney and liver pathology and convulsions. However: (1) The renal and hepatic lesions of human eclampsia are not generally regarded as specific, and they do differ from those described by Dr. Schwarz in his rats. (2) Convulsions occur in both man and experimental animals for a very wide variety of causes and are not in themselves suggestive of eclampsia. (3) It seems most unwise to confuse the literature by applying the term "eclampsia" to a condition which not only is not comparable to human eclampsia in any specific fashion, but which also is not associated with pregnancy. None of the animals in which Dr. Schwarz described "rat eclampsia" was pregnant. (4) The term "eclampsia-like syndrome" has already been guardedly applied to several experimentally induced conditions in experimental animals, including the rat. In none of these was there definite assurance that the syndrome was really comparable to human eclampsia.

I do not intend this specific criticism to detract from the very challenging suggestions which Dr. Schwarz has advanced, particularly in regard to the efficacy of alpha-tocopherol therapy in human infectious hepatitis and obstructive jaundice. He has been modest in his presentation, for, in private conversations, he revealed that he has had the opportunity to make very extensive clinical trials and objective evaluations of the drop in serum bilirubin in a large number of cases.

This is certainly work which deserves to be seriously considered and carefully repeated in this country.