HEPATIC TUMORS INDUCED BY SELENIUM

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The possibility of inducing a tumor by an antimetabolite has attracted particular attention because in this way the critical link in the tissue metabolism whose disturbance may cause the cells to become malignant may be revealed. An important step in the study of the origin of tumors is therefore to determine the ability of ethionine to induce benign or malignant hepatic tumors [6].

The ethyl analog of methionine, and its biochemical antagonist ethionine block many of the biochemical functions of methionine, and in particular they suppress the action of the methyl groups of methionine in the biosynthesis of choline, although these groups may still be used to form creatine [25]. Naturally, such a block may lead to an endogenous choline insufficiency. Because chronic choline insufficiency [5] and protein-choline insufficiency [2, 3] lead to the formation of hepatic tumors, it appears possible that the mechanism of the blastomogenic influence of ethionine amounts to a secondary choline insufficiency. It appeared however that whereas methionine protects completely against ethionine carcinogens, choline exerts very little protective action [8]. There is therefore reason to suppose that the blastomogenic influence of ethionine is associated with biochemical functions of methionine other than its participation in methylation.

If there were some other method of inducing in the organism abnormal chemical processes resembling those brought about by ethionine, and if under these circumstances cells became malignant, then there would be evidence in favor of the hypothesis of a direct or an indirect connection between some forms of functional methionine insufficiency and the blastomogenic process. It occurred to us that an approximation to such a situation could be realized by the injection of selenium.

Ethionine is taken up by protein molecules with the result that abnormal cell proteins are formed, and the incorporation of methionine is prevented [9]; this process accounts for the metabolic disturbances which result in ethionine being an antagonist of methionine. Selenium is also taken up by a certain fraction of the proteins to form analogous of the sulfur-containing amino acids seleniummethionine and seleniumcysteine; the function of the cell proteins is also disturbed by this process. These circumstances apply both to the animal [15, 16, 17] and to plant [26] organisms. It is highly probable that disturbances induced by selenium in animals are to a large extent due to its antimetabolic effect on the metabolism of the sulfur-containing amino acids, just as in plants [22].

It is important that selenium is taken up most intensely in the liver proteins, as has been shown by experiments carried out in our laboratory [1], and by McConnell [14]. Just like ethionine, the selenium analogs of the sulfur-containing aminoacids retain many of the biochemical functions of the latter. Selenoglutathione, for example, is active in bringing about oxidative processes [27], while the selenium analog of S-adenosylmethionine – Se-adenosylmethionine – may replace the latter in enzymatic methylation systems [19]. If selenium, like ethionine, can induce the formation of tumors, there is then hope that by eliciting those biochemical functions of methionine which are blocked by both selenium and ethionine we may get nearer to understanding the metabolic disturbances associated directly or indirectly with the development of malignancy.

Nelson and his co-workers [20] studied the relationship between cirrhosis of the liver and the development in it of tumors, and showed that when selenium was included in the diet, rats might develop an adenoma or a low-grade carcinoma; no metastases were found. No one who has studied the subsequent chronic poisoning due to selenium observed any tumor formation [4, 10]. However, because ethionine leads to the development of a marked blastomatous growth,

it seems to us that selenium must be able to induce a more clearcut condition than was observed by Nelson and his co-workers. The experiments that were carried out fulfilled our expectations.

METHODS

Our experimental conditions differed from those of Nelson [20]. To increase the period of action of selenium, we tried to extend survival time. To do so, firstly we used male rats; they were less sensitive to selenium than females, as used by Nelson. Secondly we gave a rather smaller dose of selenium (0.43 mg per 100 g food), than the smallest dose used by Nelson (0.5, 0.7, and 1 mg per 100 g food).

The selenium was added as sodium selenate (Na_2SeO_4 10 mg/kg food) to food of the following composition: casein 12%, maize starch 79%, sunflower oil 5%, and salt mixture 4% [13], and crystalline vitamins of group B were added. In addition, they received daily 20 IU of vitamin A, 8 IU of vitamin D, and 1 mg once per week of vitamin E. (In Nelson's experiments the food consisted of seeds; the vitamins of group B were supplied in the form of yeast and dried liver; selenium was given either as organic compounds of selenium in the grain itself, from plants grown in soil containing the element, or as a solution consisting of a mixture of the potassium ammonium salt of selenic acid and ammonium potassium sulfate.)

The animals received unlimited amounts of food; they were kept in individual cages with a double wire bottom to prevent their consuming faeces.

Altogether 40 heterozygote rats were used having an initial weight of 40-50 g. Because large amounts of riboflavine may prevent the development of tumors induced by dimethylaminoazobenzene [18], or by chlorine insufficiency [21], from the seventh month onwards we included in the diet 10 mg per day of riboflavine; at this stage, a biopsy showed certain changes in the liver, which will be described later. By the seventh month, 34 rats remained alive, and of them 18 received additional riboflavine.

RESULTS

The rats lived to 32 months, whereas Nelson's died at 24 months.

Of the 23 rats which survived for 18 months, tumors developed in 10, and the additional ration of riboflavine had no influence on survival. In three rats, cancer of the liver was found, and in two of them there were also metastases in the lungs; both of these animals received riboflavine. In four rats a sarcoma developed (two of them received riboflavine), and in all cases the tumors were found to have spread along the peritoneum. In three of the rats, a hepatic adenoma was found. The first tumors were found at the 19th month. In Nelson's experiments [20], of the 53 rats which survived 18-24 months, tumors developed in 11; they were identified as hepatic adenomas, and in five cases as "low-grade hepatic carcinoma"; in four rats there was an "adenometoid hepatic cell hyperplasia" which was thought to represent a state of transition towards tumor formation. In no case were any metastases found. Three rats with "low-grade carcinoma" received 10 parts, and one 7 parts of selenium per 1,000,000 of food, which was more than any of our animals received.

Microscopical investigations of the liver of our animals dying at between 7 and 18 months, and liver biopsies made during this period (some of them repeated) showed that the normal distribution of trabeculae were preserved. There was however an uneven basophilia, and a variation in size of the cells and nuclei of the hepatic epithelium; there was also a diffuse fatty degeneration of the hepatic cells, which contained small fat droplets.

In three of the animals in which a tumor developed, the microscopical study revealed nodules in the parenchyma and a diffuse fibrosis. In two of these rats, metastases were found in the lungs. Figure 1 shows a micrograph of a nodule from the liver of one of them, and Fig. 2 shows a long of the same rat with metastases of the hepatic cell carcinoma.

Of the four connective tissue tumors, three were polymorphocellular sarcomas (19th, 19th, and 29th months), and one was a spindle-cell sarcoma (25th month). In one case the polymorphocellular sarcoma took the form of a large nodule in the right lobe (Fig. 3); in the three remaining cases, the sarcomatous tissue had spread into the subcapsular regions; in four of the rats the sarcomas were diffusely spread along the peritoneum.

Adenomas were found in three rats, and consisted of extensive hepatic nodules of the parenchyma.

Of the 13 rats in which no tumors developed, in four the epithelium of the small bile ducts had proliferated to form groups of the so-called oval cells near the portal fields; in two of these rats, the initial stages were found of the so-called cysts. The general appearance was very reminiscent of what is found in the early stages of the reaction of

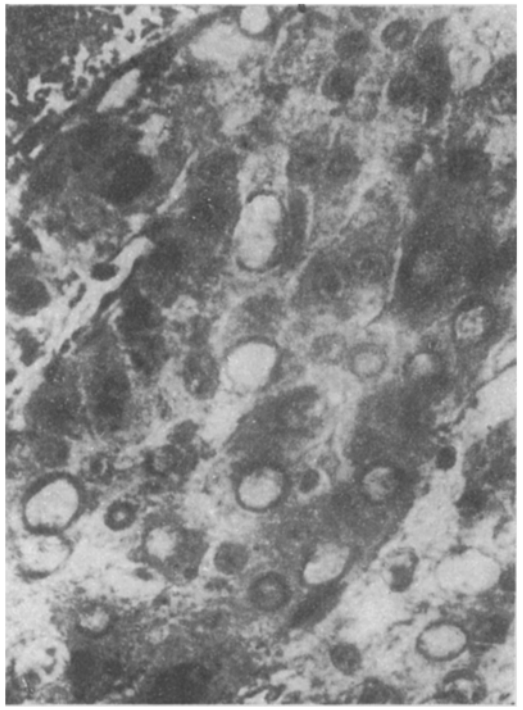

Fig. 1. Nodule from a hepatic carcinoma in rat No. 56. Experiment lasting 24 months. Micrograph. Stain hematoxilin-eosin. Magnification 400 ×.

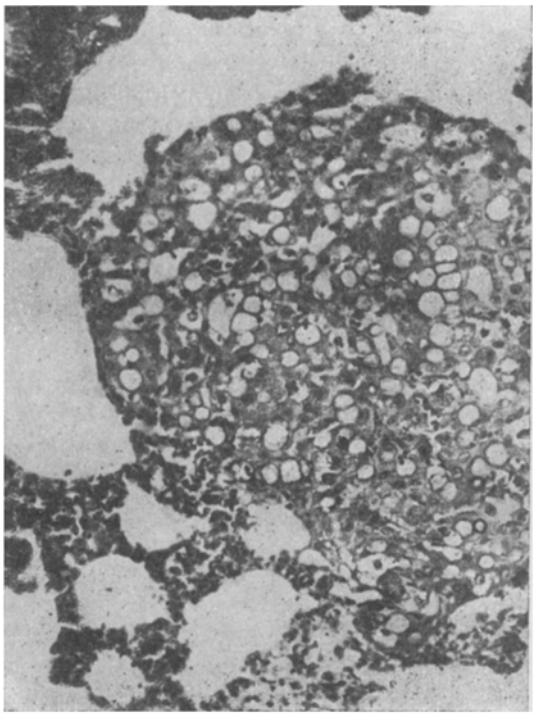

Fig. 2. Lung of rat No. 56 with metastases from hepatic carcinoma. Micrograph. Stain hematoxilin-eosin. Magnification 120 ×.

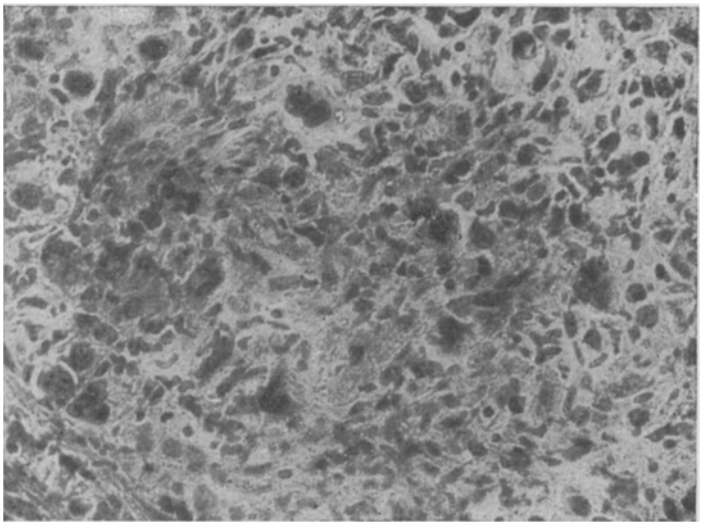

Fig. 3. Polymorphocellular sarcoma in rat No. 58. Micrograph. Stain hematoxilin-eosin. Magnification 240 ×.

hepatic tissue to various carcinogens, or to the inclusion of ethionine in the food [7]. They are usually described as precarcinogenic. In nine rats the changes observed were typical of the usual chronic selenium poisoning, and there were dystrophic changes (up to complete necrosis) with indications of regeneration and sclerosis.

Therefore the inclusion of 10 mg Na_2SeO_4 per kg of the diet of heterozygous rats led to the formation of a blastomatous growth which was much more clearly defined than was the "low-grade carcinoma" described by Nelson and others [20]. At the present time so many carcinogens are known that the discovery of one more should not attract particular attention. However, in the case of selenium, what is important is that the biochemical action is known to take place at a particular site. In neither case are we concerned merely with the uptake of "traumatic" substances into the protein molecules of the liver, but rather with the elimination of a particular metabolic stage. The similarity of the results of our experiments with selenium to the reverse effects with ethionine [6] can scarcely be missed.

A comparison of the results obtained with selenium and ethionine stimulates investigation into a possible direct or indirect pathogenic disburbance of some of the stages of the metabolism of methionine leading to hepatic tumors. As far as ethionine is concerned, such a hypothesis is favored by the fact that the induction of a tumor by this compound is blocked by methionine [8].

The problem regarding selenium is more complex. Although in the organism selenium is methylated, and a considerable proportion excreted in this form [11], its inclusion in the diet does not reduce the amount of chlorine in the tissues [12]. Therefore, just as for ethionine [8] there is no reason to suppose that the cause of the development of selenium tumors is an endogenous choline deficiency. We must now renounce this hypothesis, which we advanced previously [3], especially because further unpublished observations we have made have shown that choline cannot prevent selenium poisoning. However, the addition of methionine alone to the diet is not sufficient to prevent selenium toxicosis; only when methionine is given together with α-tocopherol is there any preventive effect on selenium poisoning [21].

This last circumstance is important in indicating ways in which the facts we have considered are to be interpreted, because in the metabolic processes there are definite physiological relationships between selenium and tocopherol [23].

SUMMARY

Fourty heterozygotic rats received Na_2SeO_4 (10 mg per kg of food). Out of 23 rats, which survived for over 18 months, the tumors developed in 10: in 3 cases there was cancer of the liver (in 2 rats – metastases into the lungs), in 4 – sarcoma, and in 3 – hepatic-cellular adenoma. Addition of riboflavin to the diet (10 mg daily), beginning from the 7th month of the experiment, did not change the pathological process. A suggestion is made that the blastomogenic effect of selenium is connected with the fact that it is methionine antimetabolite and the mechanism of its action is possibly very similar to that of ethionine.

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