THE ROLE OF THE MICROBIAL FLORA IN THE INDUCTION OF INTESTINAL TUMORS IN RATS

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The frequency of appearance of tumors and the extent of the neoplastic process in the intestine was studied after exposure of rats of varied microbiological status to the action of 1,2-dimethylhydrazine. Gnotobiotophore rats developed intestinal tumors much less frequently than ordinary animals, and when they did develop administration of Bacillus acidophilus of rats by mouth led to the more intensive development of tumors in the proximal portion of the small intestine than in animals receiving an ordinary diet.

KEY WORDS: 1,2-dimethylhydrazine; gnotobiotophore rats; intestinal tumors.

Numerous epidemiological investigations have revealed distinct differences in the geographic spread of carcinoma of the large intestine in man [8, 10, 14, 16]. These differences in the frequency of appearance of carcinoma of the large intestine in different countries can be partly explained by variations in the intestinal microflora caused by the character of the diet [5-7, 9, 11]. Experimental investigations have also revealed the important role of the intestinal microflora for the induction of tumors in this region. It has been shown, for instance, that cycasin does not induce tumors of the intestinal tract in bacteria-free rats because of the absence of β-glucosidase of bacterial origin, an enzyme that converts this substance into its active carcinogenic metabolite, methylazoxymethanol [12, 13].

In experiments on the induction of intestinal tumors by 1,2-dimethylhydrazine, the role of the microbial flora in the production of the carcinogenic effect has also been hinted at [3].

The object of this investigation was to study the special features distinguishing carcinogenesis in gnotobiotic animals and in ordinary animals that constantly received a culture of Bacillus acidophilus.

EXPERIMENTAL METHOD

Experiments were carried out on 5 male Wistar rats of line AF-49, with a body weight of 150 g at the beginning of the experiment, and reared under bacteria-free conditions. The animals contained only 3 species of microorganism – Bacillus subtilis, Clostridium putrificus, and Saccharomyces – and they therefore belonged to the category of gnotobiotophores [1]. Throughout the experiment the animals were kept in isolators under microbiologically controlled conditions. The control series consisted of 15 male rats of the same line and the same weight as the gnotobiotophore rats, but kept under ordinary animal house conditions. Both groups of rats received an injection of sterile 1,2-dimethylhydrazine dihydrochloride (DMH) once a week in a dose of 30 mg/kg (calculated as the base). The compound was dissolved in distilled water, the pH of which was adjusted to 6.5 with sodium bicarbonate. The DMH solution was packed in ampules, sterilized, and tested for sterility.

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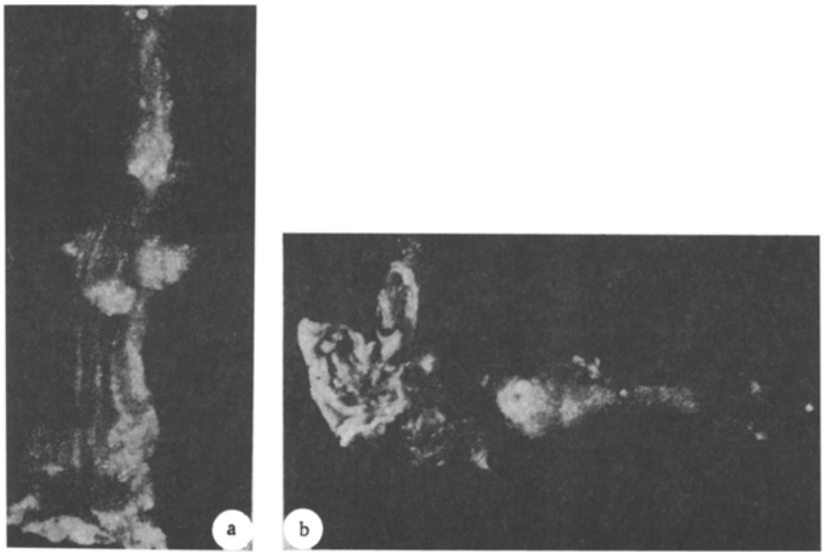

Fig. 1. Tumors developing in Wistar rats of line AF-49: a) large exophytic tumors of the rectum (2×); b) saucer-shaped tumor of the duodenal bulb (natural size).

Fig. 2. Tubular adenocarcinoma with growth of exophytic character in a gnotobiotophore rat. Van Gieson, 50×.

In addition, 25 noninbred male rats weighing initially 140-160 g, bred at the "Rappolovo" Nursery, Academy of Medical Sciences of the USSR, were given instead of drinking water acidophilic milk prepared on the basis of Bacillus acidophilus of rats. These rats 3 weeks after the beginning of receiving the acidophilic milk began to received weekly subcutaneous injections of DMH in a dose of 21 mg/kg, prepared in the same way as in the previous experiments but without subsequent sterilization. The results obtained in this series of experiments were compared with those of experiments on rats receiving an ordinary diet performed by one of us (K.M.P.) previously [2, 4]. At the end of the experiments the cadavers of the animals were autopsied and a histological investigation of the tumors found in the various parts of the intestinal tract without any macroscopic evidence of neoplasms was carried out.

EXPERIMENTAL RESULTS

From 4 to 4.5 months after the beginning of the experiments the Wistar rats kept under ordinary animal house conditions began to develop signs of a disease, with the appearance of liquid stools mixed with blood and loss of weight. In most of the animals abdominal tumors were palpated. In the interval between the 146th and 164th days after the beginning of DMH administration all the rats of this series were killed. At autopsy multiple tumors were found in their intestine, mainly in the large intestine and, in particular, in its descending part. In addition, 8 of 15 rats had tumors of the duodenum and the proximal portion of the jejunum. The intestinal tumors were of the nature of endophytic and exophytic neoplasms (Fig. 1) and in their microscopic structure they were chiefly adenocarcinomas and mucous and prickle-cell carcinomas. In one case metastasis of a prickle-cell carcinoma was observed in the lymph glands of the ileocecal angle. Both in their macroscopic appearance and in their microscopic structure the tumors found were indistinguishable from neoplasms of the small and large intestine usually arising in rats under the influence of DMH [2, 4].

The gnotobiotophore rats were killed on the 178th day after the beginning of the experiment when they had no evidence of a disturbance of their general condition. Only in 1 of the 5 rats were intestinal tumors found at autopsy; 2 of them, exophytic tumors 2-3 mm in diameter on a broad base, were localized in the

descending colon and 1 small saucer-shaped tumor was located in the duodenal bulb. Microscopic examination of the various segments of the intestinal tract revealed no additional neoplastic changes in any of the rats studied, and macroscopically the tumors discovered were tubular adenocarcinomas (Fig. 2).

Of the 25 rats receiving acidophilic milk, 22 survived until the discovery of the first intestinal tumor, and these were killed on the 178th-185th day of the experiment. All these rats had multiple intestinal neoplasms. However, by contrast with the similar experiments performed previously, but in which no acidophilic milk was given, and which acted as the control for the present investigation, in these experiments more tumors were found in the duodenum and in the proximal portion of the jejunum. For example, whereas usually in experiments with the induction of intestinal tumors by DMH on the average 0.9-1.2 tumors (in the various series of experiments) were counted per rat in the proximal portion of the small intestine; in the present investigation 1.8 tumor per rat was found in the same situation ($P < 0.05$). It was impossible to estimate the effect of continuous administration of acidophilic milk on the development of tumors in the large intestine, for multiple neoplasms of different sizes, often confluent and forming extensive neoplastic masses, were discovered in the large intestine (both in the experiment and in the control).

Intestinal tumors thus appeared much less frequently in the gnotobiotic rats than in the ordinary animals, and when they did arise the tumors were smaller. These results indicate that the intestinal microbial flora participates in the metabolism of the carcinogen. It will be noted that gnotobiotophore rats with a limited intestinal flora were used in these experiments. The possibility cannot be ruled out that the microorganisms present possess some type of enzyme activity that is essential for the metabolic conversions of DMH, and that this could be responsible for the appearance of tumors in one of the experimental gnotobiotic rats.

This investigation also revealed that the administration of acidophilic milk leads to the appearance of more tumors in the initial part of the small intestine. Because the metabolism of DMH has received very little study it is difficult to give a reliable explanation of this newly observed fact. However, if Weisburger's hypothesis [15] is true that compounds conjugated with glucuronic acid are formed during DMH metabolism and enter the intestine, it can be postulated that Bacillus acidophilus possess β-glucuronidase activity and, as a result of this, it liberates the active carcinogenic metabolite into a part of the intestine where the usual microbial flora is poor. Further investigations into the question of carcinogenesis in animals with a deliberately modified bacterial flora will help to elucidate some important aspects of the pathogenesis of tumors of the gastro-intestinal tract.

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