toxicity studies at an experimental level would not have been likely to reveal potential intrauterine developmental defects in embryos to whose mothers the drug may have been given"(14), a recourse to protozoa for studying toxicity was in order. Protozoa have been used for studying antimetabolic actions of drugs; with some drugs they mimic man and other animals in their response(15,16, 17), despite their lack of metazoan metabolic detoxification mechanisms.

Summary. The toxicity of thalidomide and some of its breakdown products were tested on protozoa, mainly the flagellate *Ochromonas danica*. Toxicity attributed to the phthalimide ring or phthalic acid was reversed by nicotinic acid. Glutamine contributed to this reversal, especially with compounds having a phthalimide group or a partly hydrogenated phthalic acid; glutamine was especially effective with Doriden which has a glutarimide group, but lacks a phthalimide group. These results with protozoa are in accord with the reported teratogenicity of nicotinamide antagonists.

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Distribution of an Antihistamine Substance(s) in Extracts of Human Tumor and "Normal" Tissues.* (28666)

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The existence of a naturally occurring antihistamine substance(s) in extracts of human and animal tissues has been amply confirmed (1,9). Recently, we reported the presence and distribution of this active principle(s) in different kinds of human tissues and described a standard method for its extraction and

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testing(10). The present paper provides evidence that in extracts of tumor tissues the amount of the antihistamine substance(s) is significantly higher than in extracts prepared from the non-tumorous part of the same tissue. Data are also presented on the histamine content of the tumor and "normal" tissues.

Method. Collection of tissue specimens: Immediately after surgical removal of a tumorous tissue, appropriate pieces (2-20 g) were selected for extraction. One piece was obtained from the obviously pathological region and another piece was taken from the non-tumorous part, as normal control tissue. Tumor tissues were carefully examined to exclude the use of necrotic tissue for extraction. The selected tissues were weighed and kept frozen at −10°C until used for extraction. The active principle was extracted by the method described previously(10).

Extraction and assay of histamine was performed according to the method of Barsoum and Gaddum(11) as modified by Code(12).

Testing of Antihistamine Activity: Antihistamine activity was tested on the isolated guinea pig ileum preparation. Terminal segments of the ileum (6-7 cm long) were suspended in Tyrode solution in a 16 ml bath at 34°C. Histamine dihydrochloride (calculated as base) was always added to the bath at 3 minute intervals, left in contact for 20 seconds, then washed out. Tissue extracts were dissolved in Tyrode solution immediately before use (adding 1 ml of Tyrode solution per 0.2 g of original tissue, the pH adjusted to 7.2 if necessary) and kept in contact with the preparation for 2 minutes. Extracts prepared from the tumor and "normal" part of an organ were always tested on ileum strips obtained from the same animal and possibly on the same strip in order to avoid any discrepancy in evaluation of activity of the extracts.

The antihistamine activity of extracts is expressed in arbitrary units as described previously(10). One unit represents the amount of tissue, in terms of its wet weight, which is capable of suppressing completely the contractions brought about by the standard dose of histamine.

Results. Antihistamine content of tissues: Forty-two extracts were tested and antihistamine activity could be demonstrated in variable amounts in each. When the values of activity in the different tissue extracts were examined, the amount of active principle found in the tumor extracts was much greater than that of the "normal" tissue in the majority of instances.

Results of 19 experiments are shown in Table I. Column 6 gives the amount of tumor tissue and column 7, the amount of "normal" tissue taken from the same specimen, in grams, which contains one unit of activity. In 4 experiments, 2 extracts were prepared and tested from the "normal" tissue, one from the adjacent tissue of the tumor and another taken from a distance of 8-16 cm from the tumor. The results of the latter are given in brackets.

Comparing the mean values of the 19 experiments, it can be seen that from the tumor tissue 1.66 g and from the "normal" tissue 5.20 g contained one unit of activity. That is, the antihistamine activity of tumor extracts was significantly higher than that of the "normal" tissue extracts ($P - < 0.05$). Elevated antihistamine activity in the tumor tissues (up to 40 times) was found in 12 of the 19 organs. In 3 experiments there was no difference between tumor and "normal" tissue and in 4 out of the 19 organs the activity of the "normal" tissue was higher (up to 5 times) than that of the tumor tissue.

Histamine content of tissues: Only a few tissue samples were assayed for histamine because of the small quantities available. The experiments performed indicate that in case of large bowel, the histamine content of the tumor tissues is higher than that of "normal" tissues. The results of 4 experiments are shown in Table II. It can be seen that the average histamine content of the tumor tissue was 6.6 μg/g tissue, while the "normal" tissue contained 2.5 μg/g histamine only.

Discussion. These results confirm the existence of a natural antihistamine substance(s) in human tissue extracts. The findings that the active principle(s) is present in a significantly higher amount in tumor tissues shows striking similarities to the results described with plant tumor extracts. It has been re-

TABLE I. Amount of Tumor and "Normal" Human Tissues, Expressed in Grams, Containing One Unit of Activity (Complete Inhibition Against the Standard Dose of Histamine) from the Antihistaminic Substance.

Sample No.	Type of tissue	Patient Age	Patient Sex	Pathological diagnosis*	Tumor tissue	Normal tissue
82	Sigmoid	64	♂	Adenocarcinoma chronic ulcer	.5	1.3
85	Rectum	45	♀	Adenocarcinoma (A)	.9	6.0
88	Stomach	77	♀	Carcinoma (B) acute ulcer	1.9	1.8
95	Colon	62	♀	Adenocarcinoma (B)	4.2	16.8 (12.0)
98	Rectum	62	♂	Carcinoma (B)	.3	12.0
100	Colon	54	♂	Carcinoma (B) Polyposis	1.6	.5
106	Sigmoid	63	♀	Carcinoma (A), transitional cell type basophil	1.5	16.0 (18.0)
114	Colon	68	♂	Carcinoma (A)	.5	1.0 (4.0)
117	"	59	♂	Polyposis no malignancy	.5	1.5
120	Stomach	58	♀	Carcinoma, ulceration at eosophago-gastric junction	.6	.6
121	"	62	♂	Carcinoma mucin production	1.0	12.0
126	Colon	69	♀	Adenocarcinoma, Polyposis	2.6	3.2
130	"	53	♀	Adenocarcinoma small leyomyoma in walls	5.0	2.3 (1.0)
138	Lung	42	♂	Carcinoma (A)	1.0	2.0
140	Colon	58	♀	Carcinoma extention serosa	1.2	2.0
141	"	63	♀	Carcinoma (B)	3.1	.8
142	Caecum	74	♀	Carcinoma (B), Polyposis	1.3	1.3
144	Lung	40	♀	Carcinoma, moderate anaplasia Sec. carcinoma	1.5	1.2
155	"	71	♂	Carcinoma (A) papillary pattern Node invasion	2.4	3.3
				Average	1.66	5.20

* (A) Poorly differentiated; (B) well differentiated.

ported that extracts of plant tumors (crown gall, oak gall) when injected into guinea pigs, protected the animals against a lethal histamine aerosol(13,14,15). The active principle was found to be present in large amounts in the tumor, but not in the normal part of the plants. At present, we do not know whether the same or a similar substance is responsible in both instances for the antihistamine activity; nor do we know whether the accumulation of a biologically active substance(s) in the human and the plant tumors has any direct connection with the abnormal tissue growth. The accumulation, however, of an antihistamine-like substance(s) in such different types of tumor tissues may lead to new possibilities in the research of tumor etiology.

TABLE II. Histamine Content (μg/g) of Tumor and "Normal" Tissues.

No. of sample	Histamine content (μg/g) Tumor tissue	Normal tissue
126	12.0	3.1
140	3.0	1.6
141	4.4	1.2
142	7.3	4.1
Avg	6.68	2.5

There is no evident explanation for those results in which antihistamine activity was found to be higher in the "normal" than in tumor tissue extracts. We have some indication, however, that the activity of "normal" tissues is frequently unusually high when the histological examination shows the presence of polyposis. Thus, it seems possible that in case of reverse results there may be chemical changes in the tissue which are not apparent histologically, but which could relate to the increased antihistamine activity.

We do not know whether the clinical history will show any difference in patients where the activity of tumor extracts was particularly high compared with those where the opposite was obtained.

Summary. An evaluation of the naturally occurring antihistamine property of human tissues was made. It was found that in general the activity was considerably higher in tumors as compared to non-tumorous "normal" tissue.

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Effect of Dihydrotachysterol (AT10) and Epinephrine on Serum and Tissue Cholesterol and Calcium Levels. (28667)

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Numerous reports(1-5) have appeared implicating calcium deposition with atheroma formation. Since dihydrotachysterol (AT10) has been shown(6,7) to increase serum calcium levels and produce calcification, it appeared of interest to see if an elevated calcium level in the blood together with hypercholesteremia would induce lesion formation.

Intravenous administration of epinephrine, 0.025 mg/kg once daily for 5 days and 0.05 mg/kg twice daily for the next 10 days, has been reported to produce alterations in the aorta of the rabbit(8). Epinephrine administration therefore was included in this study.

Methods and materials. Female Holtzman rats were housed 3 rats per cage, with food and water supplied *ad libitum.* The hypercholesteremic diet (base diet) employed was that previously described(9). It is essentially a high sucrose diet containing 2% cholesterol. To this base diet 400 units per g of Vit. D was added, since it had been found that Vit. D intensified the degree and rate of development of hypercholesteremia in the rabbit(10) and in the rat(9) on a cholesterol diet.

Epinephrine was administered daily 5 days a week by the subcutaneous route to one group of rats at a dose of 100 μg per rat (approximately 563 μg/kg based on the median weight of the rats in the group). In the first experiment AT10, dissolved in corn oil, was injected subcutaneously once a week at a dose of 100 μg per rat (approximately 584 μg/kg) for the first 12 weeks. The dose was increased to 150 μg (approximately 876 μg/kg) for the next 8 weeks. This dose was too high since the rats started to lose weight and 4 deaths resulted.

The dose was reduced to 100 μg for the remainder of the experiment. In the second experiment AT10 was injected as before but at a dose of 125 μg per rat (approximately 600 μg/kg) throughout the experimental period.

The animals were sacrificed by decapitation and a blood sample was taken. A sample of the liver, 100-200 mg from the left lobe, was quickly removed, weighed, and placed into 2N K OH for digestion. The entire aorta was removed, stripped of connective tissue, and weighed. Before digestion it was cut open along its entire length and was examined grossly for lesions. Cholesterol was determined by the method previously described (11). Calcium was determined by a modifi-