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Carcinogenic Activity of a Chlorinated Polyether Polyurethan[1]

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SUMMARY

On the basis of the results of an earlier study, a particular polyurethan sample (Y-238) was selected for further evaluation of its carcinogenic potential. This sample was subjected to physical and chemical tests for elucidation of its chemical structure, molecular weight, and molecular weight distribution. Additional biological tests were conducted on male NBR rats by implanting various quantities of the sample i.p., while others received an intrabronchus implant. Tumors, assessed histologically as malignant, were observed following both routes of implantation. The most common neoplasms of the pulmonary site was epidermoid carcinoma, while fibrosarcoma was the most common neoplasm in the peritoneal cavity. Data from the i.p. implantation suggested a dose-related incidence of cancers.

INTRODUCTION

The carcinogenicity from the i.p. implantation of 17 polyurethans and a polyethylene in black Bethesda NBR rats was previously reported (2). The assumption that the area under the corrected cumulative tumor mortality *versus* time curve is directly related to the carcinogenic potential of the particular polymer afforded a quantitative assessment of relative tumorigenicity. Sample Y-238, a chlorinated polyether polyurethan, demonstrated the highest relative tumorigenicity of the polymeric materials studied. This polymer has been characterized in more detail by physical and chemical analysis, and its tumorigenic properties have been examined more thoroughly. These data serve as the basis of this report.

The tumors following i.p. implantation in a previous study (2) were predominantly fibrosarcomas. Lung implantation studies were undertaken to determine whether the nature of the tumor was dependent upon the polymer or the site of implantation. Various quantities of the polymer were implanted i.p. to explore the possibility of a dose-response relationship for tumor formation.

MATERIALS AND METHODS

Polymer Characterization. Although a general structure of the polymeric sample was proposed (2), it was desired to better characterize the polymer. Elemental analyses[2] were performed by means of a CHN analyzer; chloride was determined by amperometric titration, and oxygen was determined by difference.

The molecular weight and molecular weight distribution[2] were determined by gel permeation chromatography. The sample was eluted with 0.01 M LiBr in dimethyl formamide. Calibration was based on polystyrene standards. The molecular weight was obtained by multiplying angstrom size by molecular weight per angstrom (estimated to be 40 to 50). The number average and weight average molecular sizes were obtained by graphical integration of the chromatogram with appropriate calculations.

The polymer was soluble in hexafluoroisopropanol. Films were cast from this solution for infrared analysis.[2]

In calculating the number of repeating units per molecule, the assumption was made that all chlorine was from MOCA.[3] The average values of the repeating units could then be calculated on the basis of the experimental C/H ratio and the theoretical molecular weight on the basis of the chlorine analysis.

Thermogravimetric analysis was performed in our laboratory under a purified nitrogen atmosphere at a flow rate of 200 ml/min and a temperature program rate of 10°/min. The thermal stability of the polymer provides another means of characterizing it by quantitating weight loss versus temperature.

Lung Implantation. The polyether polyurethan sheet was cut into strips approximately 1 × 1 × 15 mm. The strips were washed with ethanol and distilled water and dried in an oven at 70° for 24 hr. Strips were then cut into samples of 2 different sizes, approximately 6 mm long (5 to 8 mg) and approximately 1 cm long (9 to 13 mg).

Stainless steel 28-gauge surgical wire was passed through each sample and bent with the aid of instruments to form 2 hooks. The finished samples were small enough to pass through an 18-gauge hypodermic needle. Controls consisted of inserting the stainless steel hooks without the attached plastic. A diagram of the hooks and test samples are shown in Chart 1.

Male black Bethesda rats about 3 months old were anesthetized with anesthetic ether. Tracheotomy was performed asceptically, and the polymer samples or control hooks were impacted into the left inferior bronchus by means of an 18-gauge trochar. The trochar was removed, the tracheal rings were closed with silk sutures, and the skin was closed with stainless steel autoclips. (Clay-Adams, Inc., Parsippany, N. J.) One to 2 weeks later, the stainless steel clips were removed.

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[2] Experimental data determined by the DeBell and Richardson Testing Institute, Enfield, Conn.

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[3] The abbreviation used is: MOCA, 4,4'-methylene-bis(2-chloroaniline) (also called methylene-bis-o-chloroaniline).

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Thirty-five rats were implanted with polymer samples of each weight range (5 to 8 mg and 9 to 13 mg), as described above, and the same number of rats received stainless steel hooks for controls.

The rats were kept for a period of 2 years and examined frequently. If the animal was sufficiently moribund that immediate survival was not anticipated, it was sacrificed and autopsied. Tissues and organs were carefully examined grossly, and all suspicious lesions, as well as specimen samples of brain, heart, aorta, lungs, liver, gall bladder, spleen, kidney, adrenals, bladder, gonads, pancreas, stomach, small intestine, large intestine, tongue, esophagus, trachea, thyroid, thymus, salivary gland, and lymph nodes, were preserved in 10% buffered formalin for processing and histological examination. It was not possible to obtain all tissues in many of the rats that died during the night because of autolysis and/or cannibalism.

Implantation i.p. Small disks (0.31 cm in diameter) were punched from the sheet of polyurethan, Sample Y-238. These were washed and dried, as previously described, prior to implantation. Groups of 35 NBR males received i.p. implants of a sufficient number of disks to provide 750, 375, 187.5, and 93.8 mg/rat. The implantation technique was the same as previously described (2). The tumorigenic data for the group implanted with 1500 mg/rat was reported previously (2) in which the sample consisted of rectangularly shaped pieces of the polyurethan the longest dimension of which did not exceed 0.3 cm. Data from the control group of rats have also been reported (2). The control group received the same surgical treatment, but nothing was placed in the abdominal cavity.

All animals were observed frequently, and a record was maintained of their general health and individual weights. The protocol included a 2-year observation period; however, when examination revealed a rat with a significant tumor, or if the rat was sufficiently moribund that it was not expected to survive much longer, it was sacrificed and autopsied. A careful gross examination was conducted at autopsy for suspicious lesions and tumors. Tumors, if present, as well as organs and tissues (as listed above), were excised and preserved in 10% buffered formalin for histological examination. All suspected tumors were confirmed by histological examination.

RESULTS

Polymer Characterization. The results of elemental analysis, gel permeation chromatography, and infrared analysis (not shown) suggest that the polymeric material has the structure presented in Chart 2. On the average, the oxybutylene group is repeated about 16.5 times, ($\bar{Y} \sim 16.5$). There is probably a distribution of oxybutylene chain lengths but this distribution is not known. X and n have average values of 0.86 and 11 respectively.

The number average molecular weight, $\bar{M}_n$, is estimated to be in the vicinity of 16,000 to 20,000, and the weight average molecular weight, $\bar{M}_w$, is estimated to be in the range 58,000 to 72,500. This represents a $\bar{M}_w/\bar{M}_n$ ratio of 3.6.

The polymer is soluble in hexafluoroisopropanol; thus suggesting it is not highly cross-linked.

Infrared evidence (not shown) suggests that the polymer is a polyether polyurethan containing aromatic groups. It appears likely that the polymer was formed from the reaction of toluene diisocyanate with the polyether and cured with MOCA. The structure written in Chart 2 indicates the toluene diisocyanate was the 2,4 isomer; however, it is probable that a mixture of isomers was present. If all the chlorine came from MOCA, as indicated in Chart 2, then this moiety represents 16.5% by weight of the material.

The elemental analysis is presented in Table 1.

Another parameter characteristic of the polymer sample is its relative stability to heat. Decomposition of this polymer in a nitrogen atmosphere occurs at 197°–500° and appears to proceed by a 1-step mechanism.

Lung Implantation. The rats tolerated the surgical procedure quite well; there were no deaths among the 105 rats during the 1st 7 months postimplantation, and the growth rate of all rats was satisfactory throughout the 24-month period.

Moribund rats, according to the protocol, were to be sacrificed and autopsied. However, several rats died during the night and, by morning, due to autolysis and/or cannibalism, autopsy and histological examination were frequently incomplete. The results, then, may represent an underestimation (but not an overestimation) of tumorigenicity from the implants, since histological confirmation

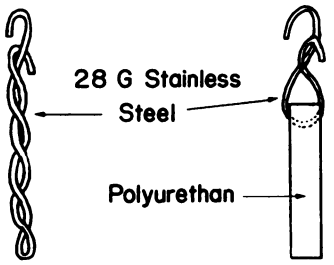

Chart 1. Lung implants: *left*, stainless steel hook and body of controls; *right*, polyurethan sample with stainless steel hook.

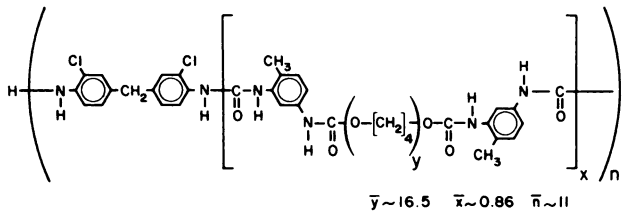

Chart 2. Proposed structure of polyurethan Y-238 from analyses reported and information provided by the supplier.

Table 1
Elemental analysis

Element	Wt (%)
C	62.4
H	8.4
N	4.9
Cl	4.4
O (by difference)	19.9
Total	100.0

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was required before cancers were reported.

Of the 35 rats in each group entering the study, 34 of the controls, 31 of the small-sample (5 to 8 mg) implant group, and 32 of the large-sample (9 to 12 mg) implant group survived for 1 year or more. Malignant tumors of the lungs, at or near the implant site, were epidermoid (squamous cell) carcinomas; 1 in the 5- to 8-mg group was detected at 21.5 months, 3 in the 9- to 12-mg group were found 17 to 22 months after implantation, and none were found in the stainless steel hook control group through 24 months. A low-power (×100) photomicrograph of one of the lung epidermoid carcinomas is shown in Fig. 1, while a higher magnification (×400) is presented in Fig. 2.

Another rat in the 9- to 12-mg group revealed a tumor of the lung. This, however, was an adenocarcinoma, believed to have metastasized from the pancreas to the lung, liver, and bladder, rather than a tumor of pulmonary origin.

Acute and chronic bronchopneumonia, pneumonitis, bronchitis, and multiple abscesses were noted in the implanted lungs of both experimental groups, as well as controls (stainless steel hooks). This response was seen in animals that had pulmonary tumors, as well as those that did not exhibit such tumors. A few of the animals, in both the treated and control groups, revealed occasional instances of fibrosarcoma, adenocarcinoma, and myeloma in areas remote to the implant site.

The finding of squamous cell carcinomas in the rats' lungs in this study is in agreement with the study of Laskin *et al*. (4), who found a similar type of tumor in rats repeatedly exposed to polyurethan dust by inhalation.

Implantation i.p. The rats tolerated the surgical procedures quite well. There were no deaths in any of the groups during the 1st 4 months following implantation, and no more than 1 death in any group during the 1st year except for the high-dose group (1500 mg/rat). Weight gains of rats in all groups were essentially comparable throughout the experimental period.

Malignant tumorigenic incidence for each sample weight is indicated in Chart 3 in which the data are corrected for nontumor deaths, according to the method of Pilgrim and Dowd (6), and plotted as percentage of survivors *versus* duration of implants in months. Data on the controls and the high dose group are the same as reported previously (2). Chart 4 shows the number of rats in each group that developed fibrosarcomas during the study. The dose-response relationship of the data in these charts indicates there is a greater risk of tumorigenesis in the rat as the weight of sample implanted is increased. The trend is similar whether malignant tumors (Chart 3) or those that are specifically fibrosarcomas (Chart 4) are considered.

The time required for detection of malignant tumors in 2 or more implanted rats as a function of sample weight is presented in Chart 5. There appears to be a linear relationship between the time required for 2 or more rats to develop malignant tumors and weight of sample implanted within the range of 187.5 to 1500 mg/rat. Rats implanted with the smallest sample weight (93.8 mg) did not reveal malignant tumors during the 24-month observation period, and no tumors were detected in the controls prior to the 24th month.

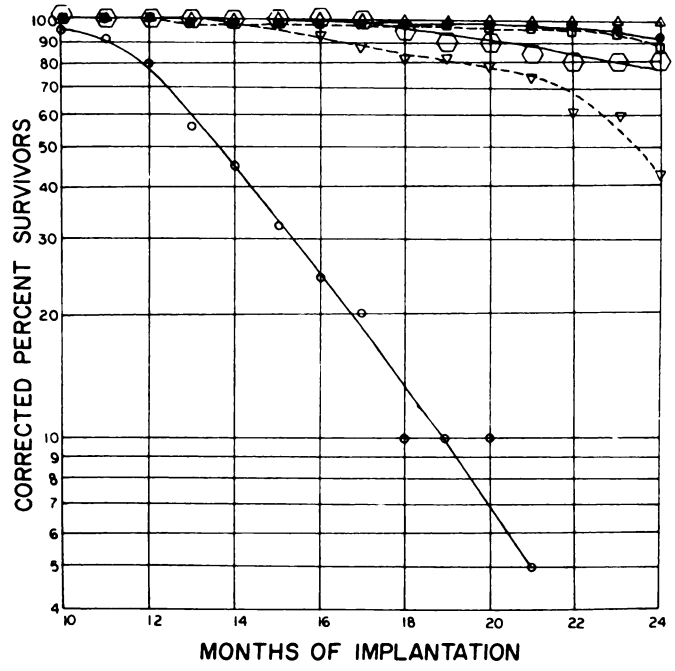

Chart 3. The influence of quantity of polyurethan implanted (i.p.) on duration of animal survival, corrected for nontumor-related deaths. ●, controls; △, 93.8 mg/rat; □, 187.5 mg/rat; ⬡, 375 mg/rat; ▽, 750 mg/rat; ○, 1500 mg/rat.

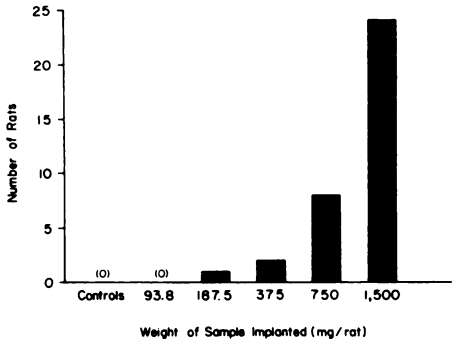

Chart 4. Number of rats developing fibrosarcomas within 24 months following polyurethan implantation i.p., as a function of the quantity implanted.

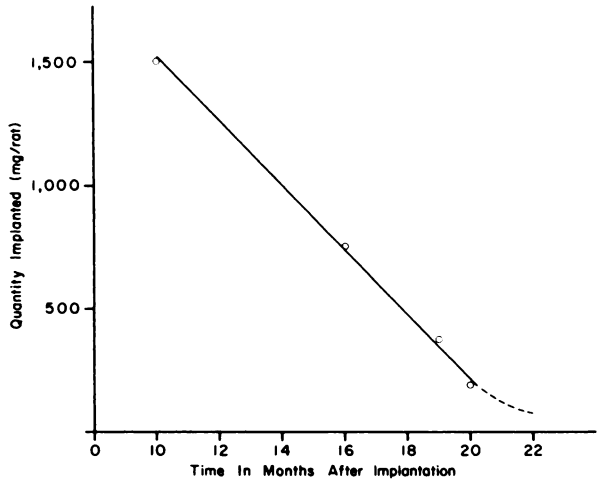

Chart 5. The relationship between time for detecting malignant tumors in 2 or more rats and the quantity of i.p.-implanted polyurethan.

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DISCUSSION

Based upon the accumulated information about the polymer investigated in this report (Y-238), a proposed structural formula for the polyurethan is presented in Chart 2.

As reported previously (2), biodegradation of this polyurethan had been suspected because of an apparent loss of a portion of the material after many months of i.p. implantation. This subjective impression is in agreement with the observations of Hueper (3), who also reported disappearance of the polyurethan sample he implanted, and with Sherman and Lyons (7), who investigated the *in vivo* degradation of a ^{14}C-labeled polyurethan.

Laskin *et al.* performed a number of experiments with different carcinogens and radioactive compounds using the intrabronchial implantation technique (5) and inhalation of polyurethan dust (4) in rats. The lung tumors produced in our study were very similar to those observed by these investigators (4, 5).

Fibrosarcomas have predominated as the major type of tumor found by the authors in rats implanted i.p. with polyurethans (2). If the number of rats with fibrosarcomas at the end of the 24-month study is compared with the quantity of polyurethan implanted (Chart 4), a dose-response relationship is apparent.

Hueper (3) has proposed that carcinogenicity may result from biodegradation products of polyurethans. Since the size of the polyurethan disk and rectangular samples in this study were both below the critical size for solid-state carcinogenesis, as reported by Alexander and Horning (1), our data are consistent with a mechanism of biological degradation of the polymer to yield an active carcinogenic compound.

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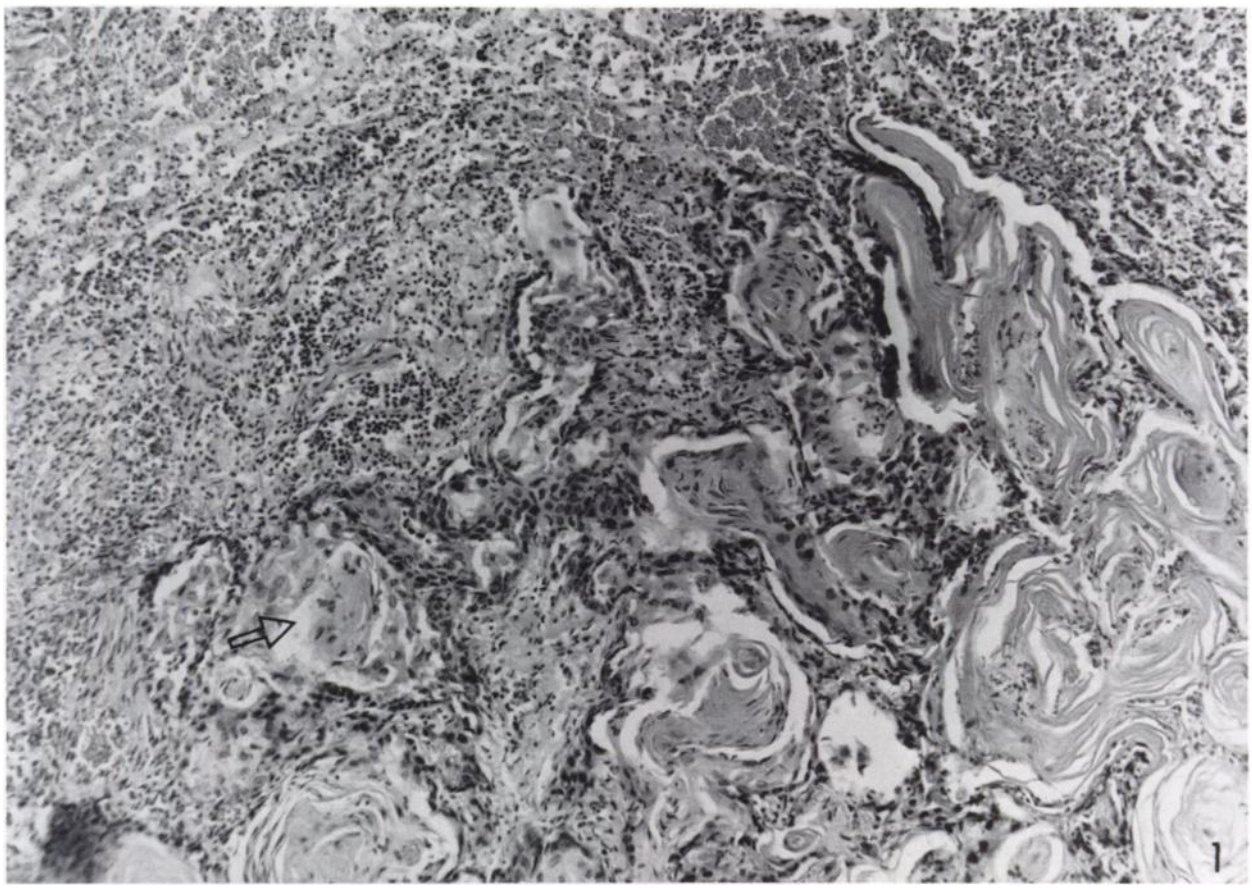

Fig. 1. A section of the lung revealing chronic inflammation and well-differentiated epidermoid carcinoma. The nest of tumor cells (*arrow*) are shown at higher magnification in Fig. 2. × 100.

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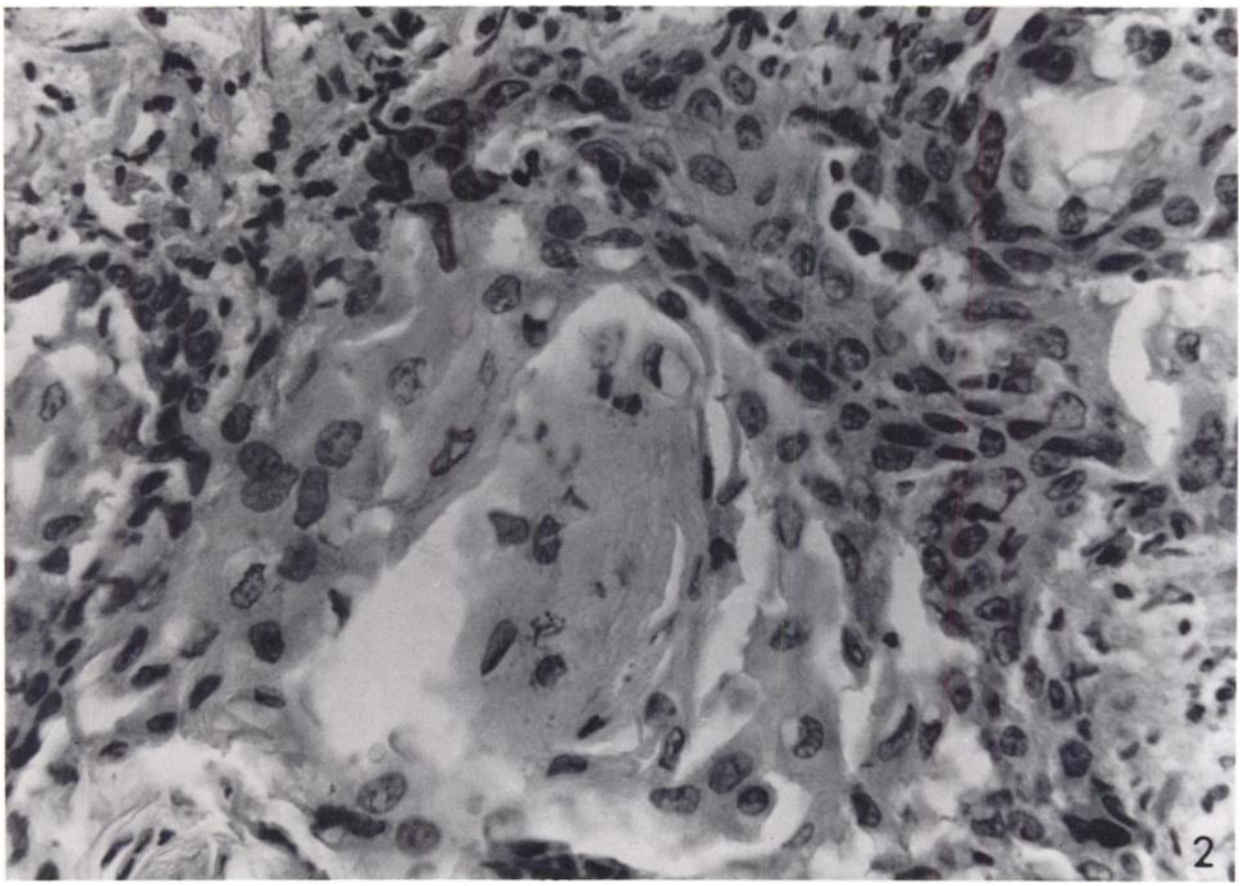

Fig. 2. Epidermoid carcinoma of the lung revealing well-differentiated tumor cells and keratin formation. × 400.

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