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Chronic Biological Effects of Methyl Methacrylate Vapor

II. Body and Tissue Weights, Blood Chemistries, and Gross Metabolic Performance in the Rat[1]

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Chronic exposures of mature male Sprague–Dawley rats to near threshold-limit value (TLV) concentrations of methyl methacrylate (MMA) monomer vapor were conducted in order to assess the effects of such exposures on gross metabolic performance. There was no significant effect on food or water intake or water excretion. Average fecal excretion during the exposure portion of the week tended to be higher in the exposed than in sham-exposed rats. These differences were absent for average values obtained on weekends. Final mean body, adrenal, epididymal fat pad, and popliteal fat pad weights were not significantly different.

INTRODUCTION

In a companion article (Tansy *et al.*, 1976) to the present one we reported that chronic 3- and 6-month exposures of rats to near TLV concentrations of MMA monomer vapor in air are associated with changes in body weights, blood chemistries, intestinal motor activities, and total body fat.

Rats which had been chronically exposed to daily concentrations of 116 ppm MMA vapor in air showed significantly decreased average small intestinal transit performance. These effects were considered to be highly significant because the observations were made 48 hr following the last exposure on a near starvation background.

The same *in vivo* observations were made in anesthetized dogs acutely exposed to high concentrations of MMA vapor (Tansy *et al.*, 1977). Surgical interruption of the long arc reflexes of the autonomic nervous system, appropriate pharmacological blockades, and cross-circulation experiments led us to conclude that the inhibitory effect of MMA was due to the direct action of the vapor on the canine gastrointestinal motor effector system and that the vapor was delivered to the sites of action via the cardiopulmonary systems (Tansy *et al.*, 1977). The inhibition of spontaneous motor activities in the small bowel and uterine segments of the rat *in vitro* confirms the presence of a direct inhibitory effect (Tansy *et al.*, 1975).

Other than intestinal motility effects of MMA exposure, the most striking change was the deficiency of abdominal fat in the 3-month MMA-exposed group (Tansy *et al.*, 1976). The observations of lower body weights and reduced body fat

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suggest three possible mechanisms that may be associated with chronic exposure to MMA vapor:

(1) changes in feeding behavior,
(2) absorptive-motor dysfunctions of the gastrointestinal tract, and
(3) alterations in fat metabolism.

No case excludes the additional possibility that whatever mechanisms are involved may be to some extent age and time dependent.

Inasmuch as nothing is known of the time requirement for the appearance of the fat deficiency, we decided to monitor more closely the development of the rats through the exposure period. During the current year we first examined the rate of growth of all MMA-exposed and sham-exposed animals, as well as a daily sampling of food and water intake, and rates of excretion. Thus, the purpose of these studies was to determine what gross metabolic performance changes could be inferred to exist in rats which received chronic exposure to this vapor.

MATERIALS AND METHODS

Forty-six male Charles River Sprague–Dawley rats weighing between 174 and 216 g were used in this study. All animals were kept in closed colony cages under controlled conditions of temperature and illumination for one week prior to being committed to any experiment in order to screen sick, suspicious, or overly aggressive types. During the first week, Purina Laboratory Chow and tap water were provided *ad libitum*.

Statistical populations involving sham and experimental groups were chosen from the same shipment in order to insure that the individuals in each group were approximately the same weights and ages. Groups were selected according to a procedure which uses a table of random numbers, as previously described (Tansy *et al.*, 1976). Animals were separated into sham and experimental groups consisting of 23 each by means of the true random process prior to commencing exposures. A subset of nine animals from each group was designated for special metabolic performance studies by an independent true random process. The remaining sham and experimental animals were housed singly in galvanized cages in an air conditioned room under constant temperature (22°C) and light cycles and fed pelleted forms of the Purina Laboratory Chow and tap water *ad libitum*.

The metabolism cages which were used were the Econo-Cage No. 110 with chrome extenders. These metabolism units were obtained from Maryland Plastics, Inc., New York, New York, and were equipped with conventional urinary–fecal separators. All animals in this subset were identified by coded ear markings and were weighed daily. The refused water and food, urine, and feces were collected daily. Tap water and Purina Laboratory Chow pellets were supplied *ad libitum*. The usual supply of food was approximately 40 g of pelletized chow and 100 ml of tap water. Both quantities were sufficient for more than 2 consecutive days of consumption. Daily food and water intake were determined by differences in the weights and volumes that were supplied the previous day. The collected feces from each animal were counted and weighed and then dried in an oven at 55°C over a 24-hr period. The dry fecal weight was defined as net solid output. The loss in weight of the dry feces represented the weight of the fecal moisture. Net fluid output was obtained by adding the difference between wet and dry fecal weight in grams to the urine volume excreted in milliliters.

Metabolic performance data were obtained under two different conditions. During the weekly MMA exposure cycle the rats were unavailable for observation for 7 hr during exposure. On weekends the rats were housed in the metabolism cages for the entire time between cessation of exposure on Friday afternoon until commencement of exposure on Monday morning; consequently mean performance values were analyzed separately according to whether the data were collected during the exposure period or on weekends.

The exposure of colonies of rats to MMA monomer vapor in air was conducted in the manner described previously by us (Tansy *et al.*, 1976). Essentially, short-term chronic and sham exposures were conducted using two 11.4 ft^3 stainless-steel commercial chambers manufactured by Young and Bertke Company, Cincinnati, Ohio. Exposure doses were set by the constant rate infusion of liquid MMA monomer into a heated reaction vessel through which room air was passed at a known constant rate. This procedure produced virtually instantaneous vaporization of the monomer. Flow rates were calculated to yield the desired gas concentrations and verified by an analysis of gas samples by gas chromatography. Routine gas samples were obtained twice daily and analyzed chromatographically and rotameters were checked periodically to insure constancy of the exposure parameters. The average concentration was 116 ± 6 ppm.

Following the first week, MMA vapor was introduced into the experimental chamber for an average of 7 hr per day, 5 days per week (excluding Saturdays and Sundays), until 542 hr of exposure were recorded. To minimize possible differences in feeding behavior during exposure periods, both the sham and experimental groups were deprived of food during the exposure periods. Tap water was provided *ad libitum* during the exposure period to prevent dehydration. The water supplies for both groups were equipped with standard glass tubes which provided suitably small meniscae which minimized the solution of MMA vapor in the water.

At the end of the exposure day the metabolic subsets were placed overnight in the metabolism cages and the appropriate measurements were made (body weight, food intake, water intake, urine output, wet feces weight, and fecal pellet number). At the end of the 3-month experimental period the following additional studies were performed:

(1) SMA 12/60 blood analyses,
(2) terminal body weight, organ weights (adrenals, epididymal and popliteal fat pads), and
(3) routine histological examination of heart, lungs, kidneys, small bowel, and liver.

Data for each sham control and experimental group were tabulated. Means and standard deviations were computed and comparisons were made by the Student's *t* test under the null hypothesis that the means were equal. The alternate hypothesis was two tailed: The means were different. The 5% confidence level was set in advance.

RESULTS

Upon necropsy, mature rats which were chronically exposed for 3 months (542 hr) to daily concentrations of 116 ppm of MMA vapor in air showed no visual evidence of reduced visceral or subcutaneous fat when compared to rats sham-exposed for the same period. As can be seen in Table 1, the mean weights of the

TABLE 1
BODY AND TISSUE WET WEIGHTS RESULTING FROM EXPOSURE TO 542 hr OF METHYL METHACRYLATE VAPOR

Tissue	Sham control group (23)[a] (g)	Experimental group (23)[a] (g)
Whole body	493.59 ± 48.43	487.20 ± 41.74
Adrenals	0.02 ± 0.01	0.02 ± 0.01
Left epididymal fat pad	0.26 ± 0.08	0.27 ± 0.11
Left popliteal fat pad	0.38 ± 0.07	0.41 ± 0.14

[a] No of animals.

whole body, adrenals, and left epididymal and popliteal fat pads were not significantly different between the groups.

Blood serum analyses from all MMA-exposed animals at necropsy demonstrated a significant decrease in total bilirubin and an increase in total cholesterol (Table 2). These differences were hinted at in our previously published 3-month study (Tansy *et al.*, 1976), but the small population of that study (eight animals per group) may have prevented their determination as significant.

Metabolic performance data were computed according to two classifications: 5-day mean values (because only 17 hr per day of performance are observable during the course of weekly exposure) and 48-hr weekend values when all hours of

TABLE 2
SMA 12/60 BLOOD SERUM ANALYSES AFTER 542 hr EXPOSURE TO METHYL METHACRYLATE VAPOR[a]

Test	Sham control group	Experimental group
T.P. (g%)	6.98 ± 0.21 (23)	7.03 ± 0.25 (23)
Alb. (g%)	4.13 ± 0.21 (23)	4.17 ± 0.25 (23)
A/G ratio	1.45 ± 0.09 (23)	1.46 ± 0.12 (23)
Ca^{2+} (meq/liter)	5.04 ± 0.23 (23)	5.06 ± 0.17 (22)
Inor. Phos. (mg%)	6.20 ± 0.52 (23)	6.22 ± 0.43 (23)
Chol. (mg%)	91.78 ± 16.81 (23)	108.22 ± 27.49 (23)*
Glucose (mg%)	110.04 ± 11.78 (23)	108.74 ± 14.36 (23)
BUN (mg%)	15.09 ± 2.66 (23)	15.61 ± 2.10 (23)
Uric acid (mg%)	1.47 ± 0.33 (23)	1.71 ± 0.62 (23)
T. Bili. (mg%)	0.45 ± 0.10 (23)	0.38 ± 0.10 (23)*
Alk. Phos. (mU/ml)	105.22 ± 23.42 (23)	111.87 ± 26.99 (23)
LDH (mU/ml)	669.36 ± 226.67 (22)	734.70 ± 240.80 (23)
SGPT (mU/ml)	53.57 ± 8.27 (23)	55.00 ± 9.96 (23)
SGOT (mU/ml)	255.74 ± 53.70 (23)	269.00 ± 51.11 (23)

[a] T.P., total protein; Alb., albumin; A/G, albumin/globulin; Ca^{2+}, calcium; Inor. Phos., inorganic phosphate; Chol., cholesterol; BUN, blood urea nitrogen; T. Bili, total bilirubin; Alk. Phos., alkaline phosphatase; LDH, lactic dehydrogenase; SGPT, serum glutamate-pyruvate transaminase; SGOT, serum glutamate-oxaloacetate transaminase. No. of animals given in parentheses.

* Statistically significant difference when compared with the mean values of sham control rats ($p < .05$).

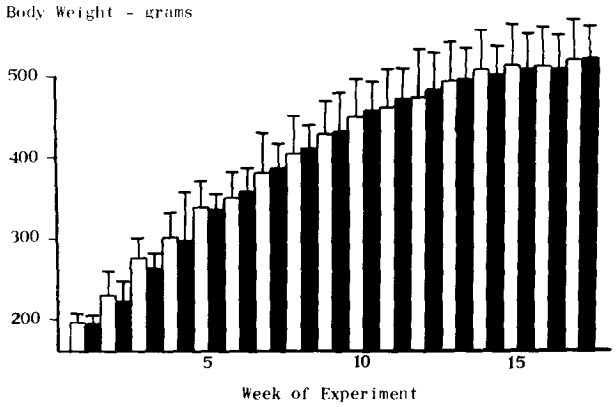

FIG. 1. Growth rate. Each bar represents the mean value of 23 animals plus standard deviation. Black bar: sham rats; white bar: MMA-exposed rats (same for Figs. 4 and 5).

performance are observable. Figure 1 illustrates that the growth rates for each subset of metabolism animals were similar. There were no significant differences in mean weekly values of food intake (Fig. 2) nor were there any differences in mean water intakes regardless of whether the means represent 5-day exposure periods (Fig. 3) or 2-day nonexposure periods when all excreta could be collected (Fig. 4). Average weekend fecal excretion (Fig. 5) was similar in both groups during the course of the experiment. However, average weekday values (Fig. 6)

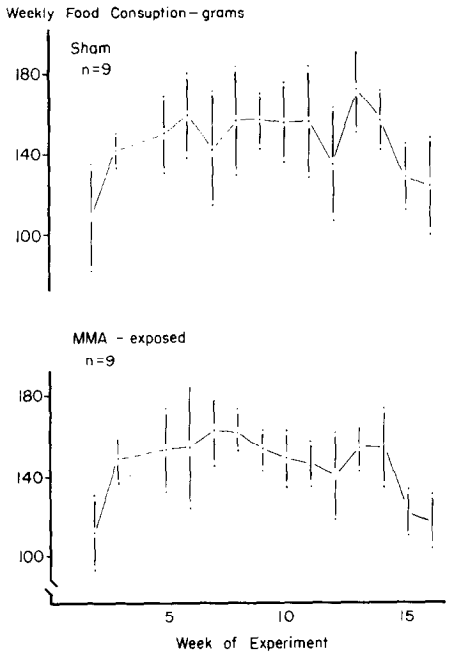

FIG. 2. Food consumption. Each sham or MMA-exposed point represents the mean value of nine animals plus standard deviation.

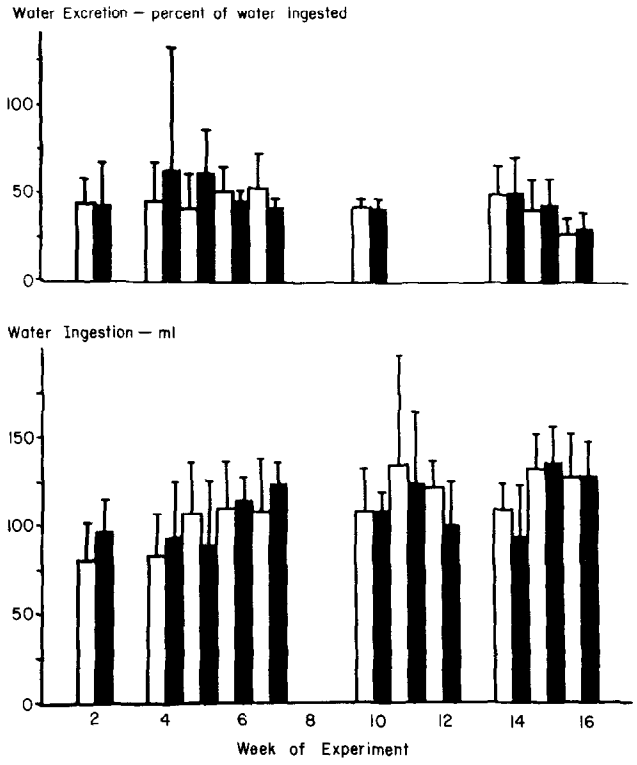

FIG. 3. Fluid balance. Each bar represents the mean value of nine animals plus standard deviation of either water ingested or percentage of water excreted as urine daily from Monday to Friday.

exhibited more heterogenicity with the result that the mean values of the exposed rats for Weeks 7, 10, and 11 were significantly increased.

Preliminary histological observations of sections from the livers of rats chronically exposed to daily concentrations of 116 ppm vapor in air for 3 months only indicated the possible presence of liver damage. These observations will be repeated in animals which are currently being chronically exposed to higher gas concentrations. Interestingly enough, preliminary unpublished observations from our original studies (Tansy *et al.*, 1976) also suggested that chronic exposure of rats to 116 ppm of MMA vapor in air might be associated with frank liver damage.

DISCUSSION

We reported previously that average body mass was significantly less in rats which had received the same MMA exposure as in this study. We believe that the lack of corresponding findings during this study is attributable to the age of the population of animals and dietary restriction. In the original study (Tansy *et al.*, 1976) starting body weights were in the 90- to 110-g range; in this study, the starting body weights were in the 174- to 216-g range. In the former study (Tansy *et al.*, 1976) all of the food was consumed; in this study, excess food was always present. At the end of the 3-month experiment in the previous study (Tansy *et al.*, 1976), half of the rats in both groups were removed permanently for various

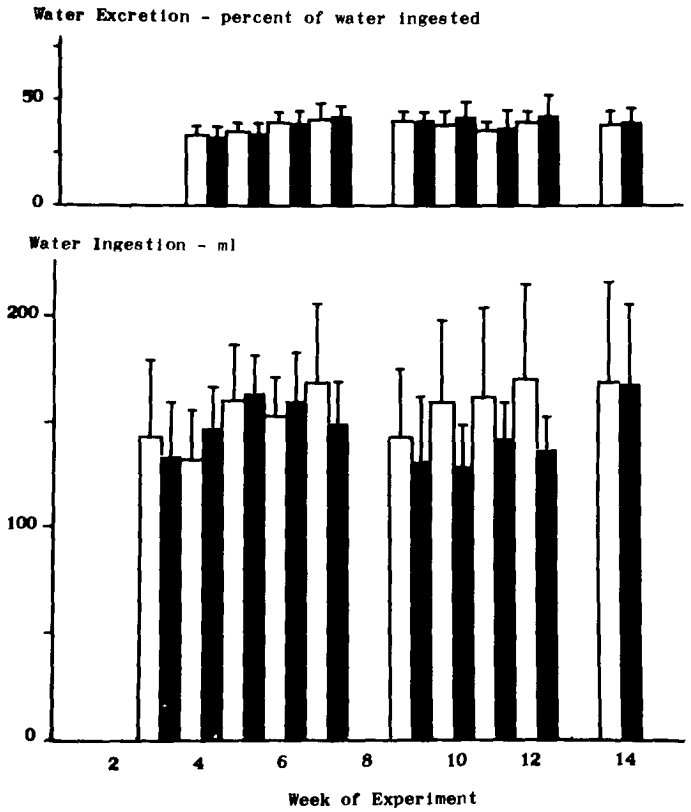

FIG. 4. Fluid balance. Each bar represents the mean value of nine animals plus standard deviation of either water ingested or percentage of water excreted as urine daily from Friday to Monday.

studies. Thus for the balance of 6 months the remaining rats had twice as much food available. It is therefore understandable why in the former study that although the 6-month sham-exposed animals were significantly heavier than the MMA-exposed rats, the shams themselves were still much less massive than would be expected.

We can thus ascribe the leaner appearance of the 3-month-exposed rats in that study to the combined effects of dietary restriction (not present in this study) and MMA vapor exposure. Our observations that, on the average, MMA-exposed rats tend to have higher oxygen consumption values, support this possibility. Studies are currently being conducted by one of us (Hohenleitner) to determine whether MMA exposure can be inferred to affect fat metabolism under conditions of restricted diet.

Figures 5 and 6 suggest a relationship between systematic vapor exposure and gastrointestinal motor performance. The divergence in mean weekday values of fecal excretion in the exposed group is absent when mean weekend values are computed for the same groups. If the direction of changes for the weekday values can be ascribed to an inherent tendency of the exposed group that was manifest at the beginning of the experiment, this contention is not supported by a similar

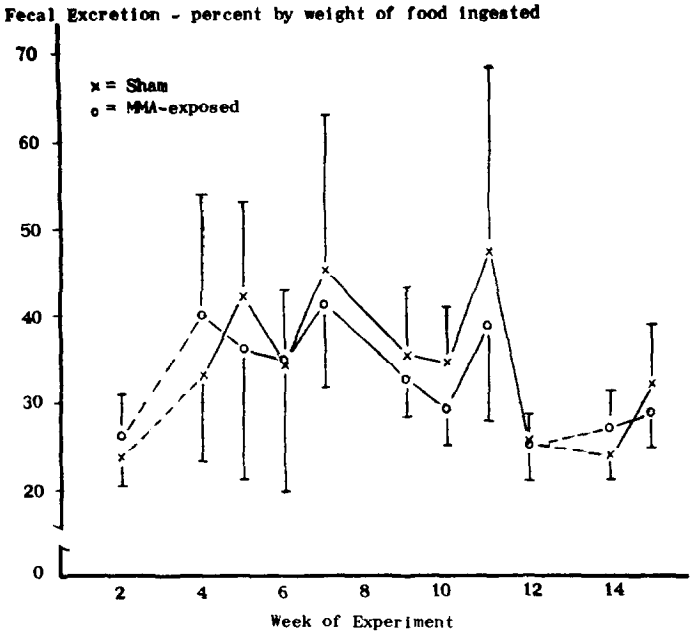

FIG. 5. Fecal excretion. Each point represents the mean value of nine animals plus standard deviation of measurements of fecal excretion from Friday to Monday.

systematic difference in weekend values at the beginning of the experiment. Thus the increases in percentage of fecal excretion seem more plausibly related to the actual conduct of gas exposure.

This interpretation is not unreasonable inasmuch as we have already demonstrated reductions in spontaneous activity of the small bowel segments exposed to

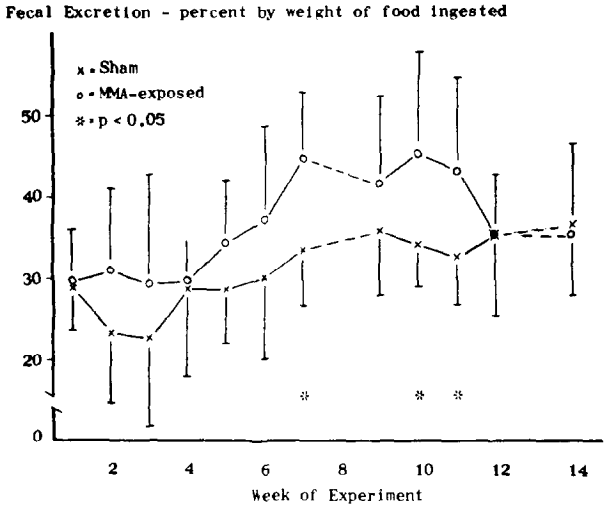

FIG. 6. Fecal excretion. Each point represents the mean value of nine animals plus standard deviation of daily measurements of fecal excretion from Monday to Friday.

MMA vapor *in vitro* (Tansy *et al.*, 1975) and reduced intestinal transit rate *in vivo* (Tansy *et al.*, 1976). The weekend fecal excretion data suggest that the effects observed during the exposure week are not apparent on the weekend and thus may be of a transitory nature. Nevertheless a systematic change of gastrointestinal motor performance for 5 of 7 days for many weeks may have significance when its effects are superimposed on those provided by dietary restriction, growth, and/or chronic stress.

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