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Some effects of experimentally-produced cigarette smoke on the growth, vitamin C metabolism and organ weights of guinea-pigs

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Guinea-pigs receiving a controlled dietary intake of L-xyloascorbic acid (ascorbic acid, vitamin C) inhaled experimentally produced cigarette smoke for periods of up to 20 min each day. Growth rate was significantly depressed by the smoke treatment, an effect at least in part attributable to a reduction in food intake. Growth of individual organs was not depressed to the same extent as that of the body as a whole. The lungs of the animals receiving smoke were significantly heavier than those of control animals ($P < 0.05$). The concentration of ascorbic acid in the adrenal glands was significantly lower in the animals receiving smoke than in the controls ($P < 0.01$). The smoke-induced depression of the adrenal gland ascorbic acid was apparent after 4 days; after 18 days marked adrenal hypertrophy accompanied the lowered ascorbic acid levels.

Lupu, Velican & Mihaescu (1954) found that exposure of guinea-pigs and rabbits to cigarette smoke resulted in a fall in the ascorbic acid concentration in the adrenal glands of both species. We have not found any reports of smoke-induced changes in the ascorbic acid content of any other organs. There is evidence that in man smokers have lowered blood levels of vitamin C (Venelut, 1954; Calder, Curtis & Fore, 1963).

The experiments now described were designed to cast further light on this relation. Guinea-pigs were used since their complete dependence upon dietary vitamin C enables tissue levels to be established at pre-determined values. The animals were exposed to controlled amounts of experimentally produced cigarette smoke and its effects on the growth rate and on the ascorbic acid content and weight of certain organs were recorded.

EXPERIMENTAL

Animals and diet

Male albino guinea-pigs, initial weight 250–300 g, were used. The diet had the following composition (g); ground oats 37, wheat bran 35, dried skim milk (1% fat) 10, dried full-cream milk (26·5% fat) 10, dried yeast 6·5, salt mixture 1·0, magnesium oxide 0·5. The dried milks were from Unigate (Milk Products) Ltd., the dried yeast from Distillers Co. (Yeast) Ltd. and the salt mixture from Glaxo Laboratories Ltd. Before incorporating the milk powders into the diet they were heated for 24 h at 110° to destroy any residual ascorbic acid. Each animal received a weekly supplement of 0·05 ml of menaphthone in arachis oil (5% w/v), 0·05 ml of DL-α-tocopherol acetate in arachis oil (5% w/v) and 0·05 ml of cod liver oil. Previous work had already indicated that this diet, when supplemented with ascorbic acid, supported normal

growth of guinea-pigs; without the ascorbic acid the guinea-pigs developed scurvy (Hughes & Hurley, 1969). A daily dose of ascorbic acid was given orally on a body-weight basis to all animals.

The guinea-pigs were housed in individual galvanized zinc cages with removable 7/10-inch mesh bottoms. They were allowed to eat the diet freely. When measurements of food intake were required, 50 g of diet was given daily to each animal and the amount left uneaten 24 h later measured.

Organ weights and ascorbic acid analysis

At the end of each experiment the animals were killed by stunning and decapitation. The appropriate organs were rapidly dissected, dried once between filter paper and weighed. An extract for ascorbic acid determination was prepared by grinding the weighed organ with sand and 6% metaphosphoric acid; the reduction of a standard acid solution of phenol-indo-2,6-dichlorophenol by a portion of the extract was measured photometrically (Hughes, 1956).

Production of the cigarette smoke and its presentation to the guinea-pigs

The cigarettes were smoked in a standard smoking machine (R. W. Mason, Clevedon, Somerset). Each cigarette was drawn on once a minute by the machine for 2 s at a suction pressure of 350 mm of water. These values are based on measurements quoted for human smokers (Fabricant, 1946; Shepherd, 1951; Greenberg, Lester & Haggard, 1925; Hilding, 1956; van Proosdy, 1960). The cigarettes were burnt to a stub length of 20 mm (Doll, Hill & others, 1959; Korteweg, 1959).

The smoke entered an adaptor (manufactured and fitted by East & Co. Ltd., Cowley, Oxford) where it was mixed with air to give a composition similar to that known to be present in the lungs of smokers whilst smoking (about 500 ml of air containing 34 ml of smoke). The mixture was propelled along a Perspex tube containing a row of ports along each side fitted with rubber adaptors to accommodate heads of different sizes. The guinea-pigs were placed in wood–metal restrainers fitted with adjustable metal positioning rods and moved into position so that the head, up to, but excluding, the eyes, projected through the port into the tube. They appeared normal when replaced in their cages after being subjected to the smoke. The control animals were similarly treated but were not exposed to smoke. This procedure produces a situation where, instead of receiving a smoke–air mixture once a minute, the lungs are continuously filled with it. Methodological limitations make it difficult to simulate more closely the human situation.

Plan of experiments

Expt 1. Two groups of 12 animals were used. One was a control group and the other was exposed to the smoke–air mixture for a single period of 10 min daily, for 51 days, the animals being weighed daily. The animals were then killed, the adrenals, heart, lungs, kidney, brain, liver and testes removed and weighed and the ascorbic acid content of the adrenals, testes and brain determined.

Expt 2. Two groups of 15 animals were used. The experiment continued for 29 days and the animals were exposed to the smoke–air mixture for two periods of 10 min daily (at 09.30 and at 21.30 h). Food intake was measured and the ascorbic acid content of the liver, testes and adrenal glands determined.

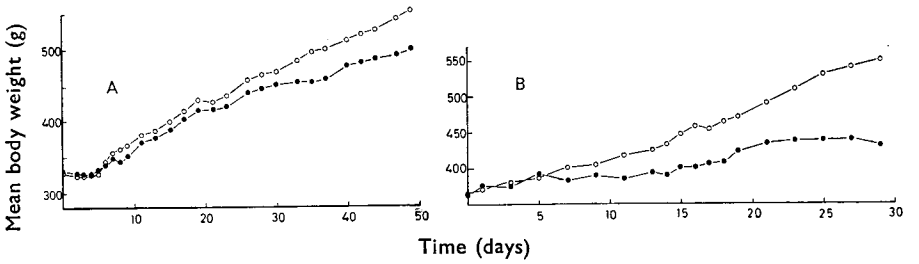

FIG. 1. Effect of smoke-air mixture [either one daily exposure (A, 12 animals/group) or two (B, 15 animals/group), each of 10 min] on growth of guinea-pigs. ○—○, control group; ●—●, smokers. Exposure to the smoke commenced on the fifth day.

Expt 3. Two groups of eight animals were used. The experimental conditions were as in Expt 2 but the experiment was terminated after 18 days. The weights and ascorbic acid contents of the adrenal glands and spleen were determined.

Expt 4. This was designed to determine how rapidly the smoking effects appeared. Two groups of animals were treated as in Expt 3 for 4 days.

RESULTS

These are given in Fig. 1 and in Table 1. Analysis of variance tests for significance were applied and a probability of $\leqslant 0{\cdot}05$ accepted as revealing a significant difference. Guinea-pigs exposed to the cigarette smoke for a single period of 10 min daily (Expt 1) had a lower growth rate than the corresponding control group (Fig. 1) the mean body-weights of the two groups being significantly different ($P \leqslant 0{\cdot}05$) from the 35th day of treatment. The ascorbic acid levels in the organs

Table 1. *Effect of smoke treatment (two 10-min exposures daily) on weight and ascorbic acid content of guinea-pig organs.* The daily intake of ascorbic acid was 0·5 mg/100 g. (Mean values with their standard errors)

Period of smoke treatment (days)	Guinea-pigs per group	Weight (g)	Weight as % of body-weight	Ascorbic acid (mg/100 g)
Adrenal glands				
C 4 (Expt 4)	8	0·127 ± 0·007	0·039 ± 0·002	30·5 ± 0·76†
S 4 (Expt 4)	8	0·129 ± 0·016	0·039 ± 0·005	21·9 ± 0·58
C 18 (Expt 3)	8	0·204 ± 0·004*	0·047 ± 0·009*	31·7 ± 0·76†
S 18 (Expt 3)	8	0·271 ± 0·007	0·067 ± 0·003	19·8 ± 0·88
C 29 (Expt 2)	15	0·236 ± 0·010	0·040 ± 0·002*	31·1 ± 1·5†
S 29 (Expt 2)	15	0·270 ± 0·011	0·062 ± 0·006	22·0 ± 2·2
Spleen				
C 4 (Expt 4)	8	0·409 ± 0·004	0·128 ± 0·001	8·6 ± 0·34
S 4 (Expt 4)	8	0·391 ± 0·005	0·169 ± 0·013	7·9 ± 0·24
C 18 (Expt 3)	8	0·300 ± 0·020	0·084 ± 0·004	8·1 ± 0·24
S 18 (Expt 3)	8	0·350 ± 0·015	0·085 ± 0·001	6·5 ± 0·24
Testes				
C 29 (Expt 2)	15	2·70 ± 0·08	0·46 ± 0·02	8·88 ± 0·16
S 29 (Expt 2)	15	1·96 ± 0·18	0·45 ± 0·034	8·09 ± 0·05

* Difference of means for controls (C) and smoke-treated animals (S) significant at 5% level.
† Difference of means for controls and smoke-treated animals significant at 1% level.

examined (adrenals, testes, brain) were not significantly different. Of the organs examined only with the lung was there a significant difference between the absolute weights. The mean absolute weight for the lungs of the controls was 3·16 g $\pm$ 0·11 (0·55 $\pm$0·01 as % of body-weight) and for the smoke-treated animals 3·64 $\pm$ 0·19 (0·71 $\pm$ 0·02 as % of body-weight) ($P < 0·05$).

In Expts 2–4 the animals were subjected to two 10 min periods of smoke treatment daily. This intensification of the treatment produced a still greater divergence in the growth curves of the two groups, the difference in mean body-weight being significant ($P < 0·05$) from the 11th day of treatment (Fig. 1B). The adrenal ascorbic acid was in this experiment significantly lower in the animals receiving smoke than in the controls ($P < 0·01$; Table 1). During the period when food intake measurements were made (days 12–28) the controls ate significantly more food on a body-weight basis than the smoke-treated animals ($P < 0·01$).

Experiments 3 and 4 were short-term (18 and 4 days respectively). In the 18-day experiment exposure to the smoke produced both adrenal hypertrophy and a lowered adrenal ascorbic acid level (Table 1). In the 4-day experiment there was no hypertrophy of the adrenal glands although their ascorbic acid level was depressed ($P < 0·01$; Table 1). There was no significant change in the ascorbic acid content of the spleen (Expts 3 and 4) or of the testes (Expt 2) of the smoke-treated animals (Table 1).

DISCUSSION

Evidence suggestive of a possible relation in man between smoking and ascorbic acid derives primarily from measurements of blood levels of the vitamin (Venelut, 1954; Calder & others, 1963; Brook & Grimshaw, 1968). It is generally accepted that in man the level of ascorbic acid in the leucocytes (and to a less extent, in the plasma) provides a useful index to the ascorbic acid status of the body as a whole (Vilter, 1967). In guinea-pigs, however, this is not so, particularly at the sub-optimal dietary levels we used (Hughes & Jones, 1970). On the other hand, the ascorbic acid content of organs such as the adrenal glands and the spleen appears to reflect dietary intake over a very wide range and its determination in such organs presents none of the difficulties associated with its determination in leucocytes. In most experiments, the ascorbic acid content of the adrenals and spleen was, therefore, assumed to reflect the ascorbic acid status of the animal.

The guinea-pigs received a controlled daily intake of ascorbic acid of 0·3 mg/100 g body-weight (Expt 1) or 0·5 mg/100 g body-weight (Expts 2–4). This is sufficient to maintain growth and development but is well below the intake necessary to produce tissue saturation (Evans & Hughes, 1963). Restriction of the ascorbic acid intake to these pre-determined levels meant that any smoke-induced changes in the vitamin C economy of the body as a whole would be reflected as changes in the tissue levels.

The most striking result was a fall in the ascorbic acid concentration of the adrenal glands of the animals receiving smoke. Stressing agents such as low temperature, are known to produce a fall in adrenal ascorbic acid and Larson, Haag & Silvette (1961) suggested that smoking should be considered a stressing agent. This is now reinforced by two further findings; (*a*) the animals receiving smoke over the prolonged period had hypertrophied adrenal glands—this too being a condition characteristic of stressed animals, and (*b*) none of the other organs examined displayed

a fall in ascorbic acid. When the vitamin C status of the animal as a whole changes any change in the adrenal glands is paralleled by changes in other organs such as the spleen (Hughes & Jones, 1970).

It is unlikely that the lowered growth rate of the smoke-treated guinea-pigs is a consequence of changed tissue ascorbic acid levels. A large reduction of tissue ascorbic acid is necessary to produce the depressed growth rate characteristic of hypovitaminosis C (Evans & Hughes, 1963). Furthermore, the growth depression observed with the animals receiving smoke is attributable (at least in part) to a reduced food intake. Thus in Expt 2 from day 12 to day 28 of the experimental period, on a body-weight basis the food intakes of the controls were significantly higher ($P < 0{\cdot}01$) than those of the smoke-treated animals. This inanition presumably resulted from a direct effect of the smoke on the food intake control mechanism(s) although during the initial week of the experiment a depression of growth was recorded without any reduction of food intake (Fig. 1B).

Although the animals receiving smoke appeared to have larger organs on a body-weight basis, only the lungs (in absolute terms as well as on a body-weight basis) were significantly greater than in the controls. This would appear to indicate a true smoke-induced hypertrophy of the lungs in guinea-pigs, a phenomenon that does not appear to have been previously reported.

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