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**EFFECT OF AIR POLLUTING CHEMICAL GASES
UPON IMMUNOLOGIC PROCESSES IN ANIMALS**

by

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EFFECT OF AIR POLLUTING CHEMICAL GASES
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In our preliminary studies, it appeared that antibody production was enhanced when antigen was aerosolized to the experimental animals following the certain period of SO₂ gas exposure. It was also found that skin sensitizing antibody against house dust antigen appeared to be higher in titer among the human subjects with chronic respiratory diseases or with smoking habit.¹⁾ Present studies were carried out to extend the preliminary studies to confirm it and to investigate the influence of air polluting chemical gases upon immunological processes in animals.

MATERIALS

Animals :

Male guinea pigs weighing 400 to 500 gm, male albino rabbits weighing 3,500 to 4,000 gm, and adult white Leghorn hen were used in this studies.

Antigen :

Bovine serum albumine (BSA) (Armour Co.) and five times crystallized egg albumine (EA) (Nutritional Biochemical Co.) were used for immunization.

Exposure boxes :

As shown in Fig.1, two fans were installed in exposure box securing uniform distribution of chemical gases and aerosolized antigens. Absorbing tube contained glass fiber which had been immersed to saturated sodium hydroxide solution and dried. This tube was connected between box and outlet in an attempt to absorb SO₂ as much as possible. Sulfur dioxide bomb was purchased and concentration of SO₂ gas in exposure box was adjusted, controlling SO₂ and outlet flow. Concentration of SO₂ gas was measured by sensitive detecting tube available at Takachiho Co. (Tokyo).

METHOD

Immunization procedure :

Guinea pigs and rabbits were exposed to 250 to 400 p.p.m. of SO₂ in exposure box for 30 minutes. Following this, 1 % of ESA or EA was aerosolized for 30 minutes from both side of inlet into exposure box, where SO₂ preexposed experimental animals and control animals were placed together. This was repeated for seven times every other day. Two weeks after last exposure, blood was drawn from each animals and serum was frozen until use.

Two groups of rabbits, consisting of four animals in each were exposed to SO₂ in an identical manner as stated previously. One group of animals received 5 mg of BSA intramuscularly with Freund's adjuvant and another 5 mg of BSA subcutaneously immediately after 1st, 4th and 7th exposure. Blood was drawn two weeks after the last injection. Control two groups were immunized with BSA in an identical manner but without preexposure to SO₂. This study was done to see whether SO₂ exposure itself could promote systemic or local antibody production showing adjuvant effect.

Antibody production :

Titer of antibody produced was measured by tannic acid treated red cell hemagglutination test²⁾ and passive cutaneous anaphylaxis³⁾ (PCA). One guinea pig was tested with two sera in PCA : one is from the experimental animal and the other from control for comparison.

Serial two fold dilution from 1 : 10 was employed in hemagglutination test and serial three fold dilution in passive cutaneous anaphylaxis.

Histological studies :

These animals were sacrificed after SO₂ exposure and sent for histological studies.

Activity of cilia :

Leghorn hen was placed on board and one drop radioactive macroagglutinated human serum albumine was placed into lower part of trachea and movement of radioactive material was checked by scintigram. These studies were done with and without SO₂ exposure in the same hen. Hen was particularly chosen because of length of neck to make comparison easier.

Inhalation of radioactive material :

¹³¹I labelled human serum albumine was aerosolized to rabbits preexposed to high concentration of SO₂ and rabbits without preexposure. Blood was drawn periodically to follow fate of inhaled material.

RESULTS

Antibody production :

As shown in Table 1 and 2, titer of antibody was higher in SO₂ preexposed animals measured by hemagglutination test and PCA, when immunized with aerosolized antigen.

When antigen was given intramuscularly with Freund's adjuvant or subcutaneously, there appeared no significant difference of antibody titer in comparison between SO₂ preexposed group and control, although statistic analysis appeared not to be feasible, because of variation of titer and small number of animals (Table 3).

Histological studies :

Infiltration of leucocytes, hemorrhage, edema, atelectatic changes, increased excrete in bronchi etc. were major changes in acute exposure of SO_2 .

Activity of cilia :

Movement of radioactive material due to ciliary activity was measured by scintillation counter using hen. Because of coughing, it was sometimes difficult to ascertain the speed of movement. In addition, because of insignificant number of subjects and preliminary stage of the study at the present time, definite statement could not be made at this time, but some delay of movement was seen in SO_2 preexposed hen.

Inhalation of radioactive material :

At the present stage of studies, no definite statement cannot be made as only preliminary studies were done so far.

DISCUSSION

SO_2 is thought to be one of the most important air polluting gases at the present time. From the investigation presented here, it became clear that antibody production is accelerated when animals are immunized through air way following exposure to SO_2 . SO_2 inhalation itself does not appear to stimulate immunologically competent cells as shown in Table 3, but SO_2 inhalation produce damage of air way tract. This probably cause permeability of air way since any kind of inflammatory changes increases permeability. Activity of cilia decreases when SO_2 is inhaled. According to Kensler and Battista⁴), ciliary activity is markedly reduced when exposed to SO_2 . This confirmed our preliminary findings. Consequently, inhaled

antigen would stay longer period in air way tract since ciliary activity is reduced. In addition, permeability of air way tract membrane is increased. Therefore, inhaled antigen has more chance to touch with immunologically competent cells in body through the circulation. This is thought to be the main cause of acceleration of antibody production when SO₂ was preexposed, although this was not proved definitely at this stage.

Currently it was found that the incidence of positive skin test to house dust was higher among the smokers and among the subjects with chronic respiratory diseases¹⁾. This fact can be explained with the identical mechanism.

In human daily life, high concentration of SO₂ such as used in this study is seldom encountered, but chronic exposure of low concentration of SO₂ could produce an identical changes in respiratory tract. Anyhow, the findings in this study is certainly important, taking present air polluting problem into consideration.

To know the mechanism, more studies are needed. For instance, activity of alveolar macrophage, fate of inhaled antigen, etc. should be investigated further.

CONCLUSION AND SUMMARY

Antibody production was accelerated when animals were preexposed to SO₂ and aerosolized with antigen. Because of premature investigation at the present stage, the mechanism could not be clarified definitely but from the available investigative results, it was most likely due to : 1) increased permeability of air way tract membrane, and 2) decreased activity of cilia which cause accumulation of antigen in longer period when SO₂ was preexposed.

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Table 1. Antibody titer in rabbits

Pretreatment	No. of animals	Antigen	Hemagglutination titer (reciprocal)	PCA (reciprocal)
SO ₂	8	EA	1280-5120 (3360)	90-2780 (900)
None	6	EA	320-2560 (907)	10-90 (50)
SO ₂	10	BSA	640-5120 (2112)	not done
None	9	BSA	10-5120 (780)	not done

Note: Number in () shows mean value.

Table 2. Antibody titer in guinea pig

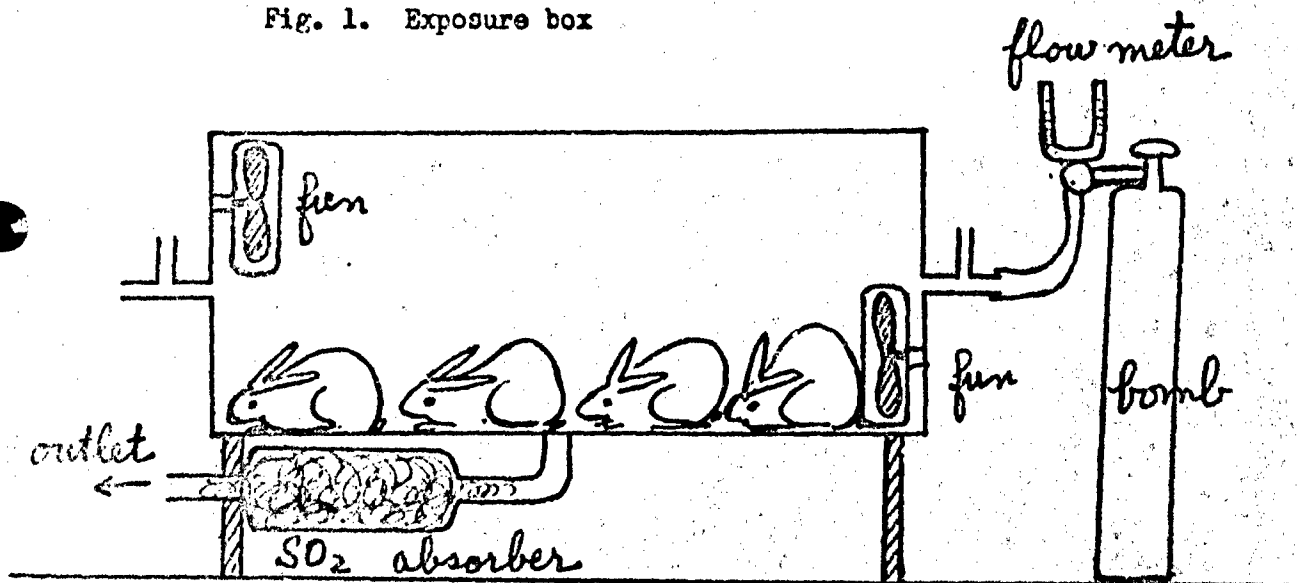
Pretreatment	No. of animals	Antigen	Hemagglutination titer (reciprocal)	PCA (reciprocal)
SO ₂	9	EA	160-10240 (1866)	30-270 (174)
None	11	EA	20-10240 (1285)	10-90 (38)
SO ₂	9	BSA	0-320 (57)	not done
None	11	BSA	0-80 (18)	not done

Note: Number in () shows mean value.

Table 3. Antibody titer in rabbit

Pretreatment	No. of animals	Immunization method	Hemagglutination titer
SO ₂	4	BSA with Freund's adjuvant	640-10240
None	4	BSA with Freund's adjuvant	640-20480
SO ₂	4	BSA S.C	160-640
None	4	BSA S.C	160-320

Fig. 1. Exposure box



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13. ABSTRACT Antibody production was accelerated when animals were preexposed to SO ₂ and aerosolized with antigen. Because of premature investigation at the present stage, the mechanism could not be clarified definitely, but from the available investigative results, it was most likely due to: (1) increased permeability of air way tract membrane, and 2) decreased activity of cilia which cause accumulation of antigen in longer period when SO ₂ was preexposed. (Author)		

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