

Experimental Model of Asthma by Toluene Diisocyanate (TDI)

Ken-ichi TANAKA<sup>1)</sup>, Mitsuru KAWAI<sup>2)</sup> and Nobuo MAEKAWA<sup>2)</sup>

<sup>1)</sup> *Toxicology Laboratory, Research and Development Planning Department, Toray Industries, Inc., 2-7-35 Sonoyama, Otsu, 520 Japan*

<sup>2)</sup> *The First Department of Medicine, Chest Disease Research Institute, Kyoto University, Sakyo-ku, Kyoto, 606 Japan*

(Received, January 14, 1983)

INTRODUCTION

Toluene diisocyanate (TDI) is a chemical substance having a broad industrial use. Respiratory disorders caused by this substance have been frequently published since Fuchs and Valade<sup>1)</sup> reported the first case. In particular, attention has been paid to an asthma-like symptom of the workers who are exposed to this substance in their workshops. Although hypersensitivity to TDI has been suspected in connection with the symptom by many authors, the underlying etiology has not been entirely clarified<sup>2-5)</sup>. For the better understanding of the problem, we have developed a new experimental model of asthma in guinea pigs.

MATERIALS AND METHODS

**Chemicals.** TDI used in the present study was purchased from Katayama Chemical Industries, Ltd., Osaka, Japan. It was a mixture of two isomers, 2,4- and 2,6-TDI, 80/20. This was used after it had been dissolved in ethyl acetate, an organic solvent not reacting with TDI.

**Animals.** Male and female Hartley strain guinea pigs weighing 300-600 g were employed as experimental animals. A group of 10 guinea pigs was used with or without a control group of the same number. The experiment was repeated several times under the same condition.

**Sensitization.** A 10% TDI solution was painted on the mucous membrane of the bilateral nasal cavities of guinea pigs with a thin cotton applicator (46514 #100, Kawamoto Hotai Zairyo Company, Osaka, Japan) once daily for five consecutive days.

The amount of the TDI solution which was painted on the both sites each time was about 10 mg in all. The application of TDI in this concentration did not bring about any serious effects on the general condition of the test animals, although some of them happened to cough to a slight degree after the painting of TDI. It was found that the concentration of TDI less than 5 %

did not cause any noticeable effect except for scratching of the nose with fore-extremities. The scratching behavior was found also in the control animals to which only ethyl acetate was applied. Challenge. When three weeks had passed after the sensitization procedures mentioned above, the animals were challenged with a 5% TDI solution in the same way.

Passive transfer of asthma. Sera obtained from guinea pigs which were considered sensitive to TDI were injected into the peritoneal cavities of non-treated animals. The recipients were challenged with a 5% TDI two days later.

Histopathology. The experimental animals were dissected at various timepoints throughout the experiments. Microscopic studies of the removed lungs were carried out by using  $3\mu$  sections which were completed with hematoxylin-eosin or Giemsa staining.

Hematology. White blood cell differentials were examined by using blood which was obtained from the ears of the animals.

## RESULTS

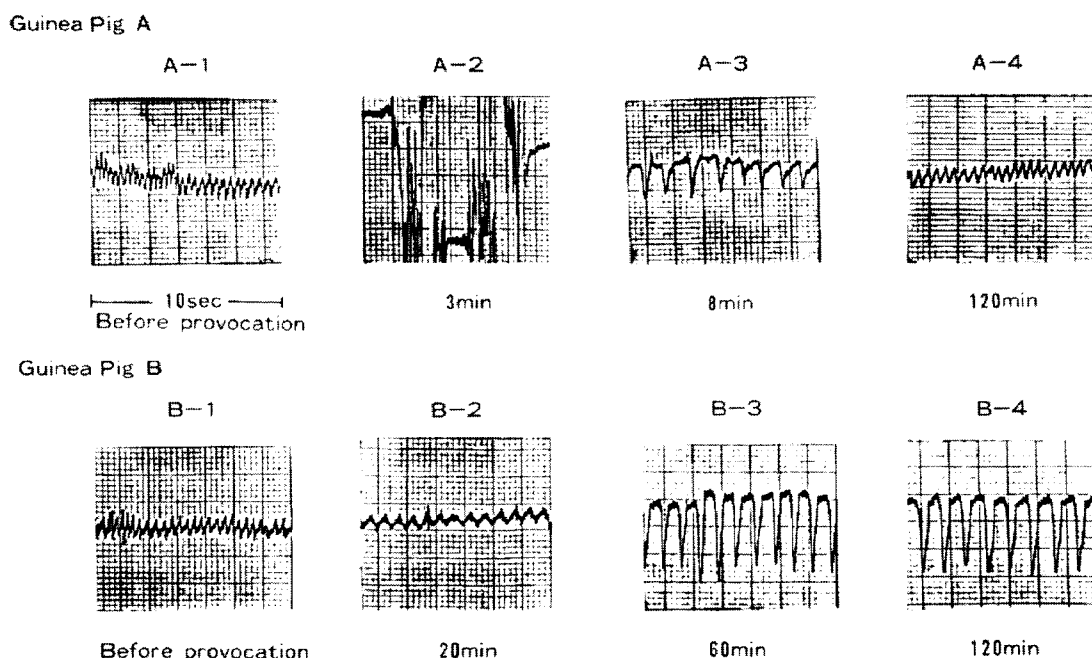
Abnormal phenomena which had not been found until then were observed among the test animals challenged. Within 1 to 10 minutes after the painting of TDI, they coughed strongly and demonstrated exertional breathing resembling asthma. They elevated their heads, opened the mouths, and gasped. Wide abdominal fluctuation with the prolongation of expiratory phase was observed. These symptoms were very often accompanied by hyperrhinorrhea, sneeze, and tears in eyes.

Some of the test animals not only suffered from the exertional breathing but also became anaphylactic. Cyanosis in eyes appeared suddenly together with other signs such as piloerection, urination, evacuation of feces, and so on. Then they fell down with convulsion of extremities. They, however, stood up within several minutes and recovered after a while.

The exertional breathing was usually observed to disappear within half an hour. Sometimes, however, it was observed for two hours or more. The exertional breathing could be found with or without these immediate reactions when an hour or more had elapsed after the application of TDI. Fig. 1 shows the respiration curves of two test animals before and after the provocation. One of them, Guinea Pig A, suffered from the exertional breathing within several minutes after the application of TDI but recovered from the state within half an hour. On the other hand, another animal, Guinea Pig B, demonstrated the wide abdominal fluctuation when 60 minutes had passed after TDI was applied, not showing the immediate asthma-like symptom.

The attacks mentioned above could be induced recurrently by the same provocation with an interval, a day or more. It seemed that sensitivity to TDI differed a great deal among each individual guinea pig. Even in the same animal, the intensities of the attacks varied on the days when the attacks were provoked.

The number of the animals which suffered from the attacks increased with the repetitive provocations. Table 1 shows the number of the animals which suffered from the attacks at least once during the three months after they were initially challenged. The booster effect by the repetitive provocations was marked as shown in the table. During the test period, even the



**Fig. 1.** Respiration curves of two guinea pigs before and after provocation.

A rubber tube sensor filled with water was attached to the abdomen of each guinea pig. Pressure fluctuation by respiration was conducted to a transducer (Nihon Kohden, MPU 0.5) connected with the sensor. Guinea Pig A coughed when 3 minutes had passed after the application of TDI (A-2) and demonstrated exertional breathing afterwards (A-3). It disappeared within half an hour. The respiration curve when 120 minutes had passed after the provocation was nearly as same as that before it was provoked (A-4). Without the immediate reaction as observed in Guinea Pig A, Guinea Pig B showed a slow gasping pattern with the prolongation of expiratory phase when 60 minutes had passed after the treatment with TDI (B-3). The abnormal breathing continued more than 120 minutes (B-4).

**Table 1.** Number of of animals which exhibited asthma-like symptoms during the three months after initial challenge

	Total number of animals	Number of animals which exhibited asthma-like symptoms	
		by initial challenge	by repetitive challenge
Control animals <sup>1)</sup>	20(100%)	0(0%)	0(0%)
Test animals	60(100%)	10(16.7%)	39(65.0%)

1) Guinea pigs treated with only ethyl acetate

drastic reactions such as anaphylactic shocks hardly brought about deaths in the test animals. Only one fatal case was found among all the eighteen anaphylactic cases of the test animals throughout the experiments.

The asthma-like symptoms could be provoked with TDI in a very low concentration, e.g., 0.1%, when the test animals had become sufficiently sensitive to TDI.

Further, it was found that passive transfer of asthma was possible in our experimental system.

Sera obtained from the guinea pigs which were considered sensitive to TDI were transferred to non-treated guinea pigs intraperitoneally. In the eight cases among the twelve, the asthma-like symptoms were not induced in the recipients. In the remaining four cases, however,

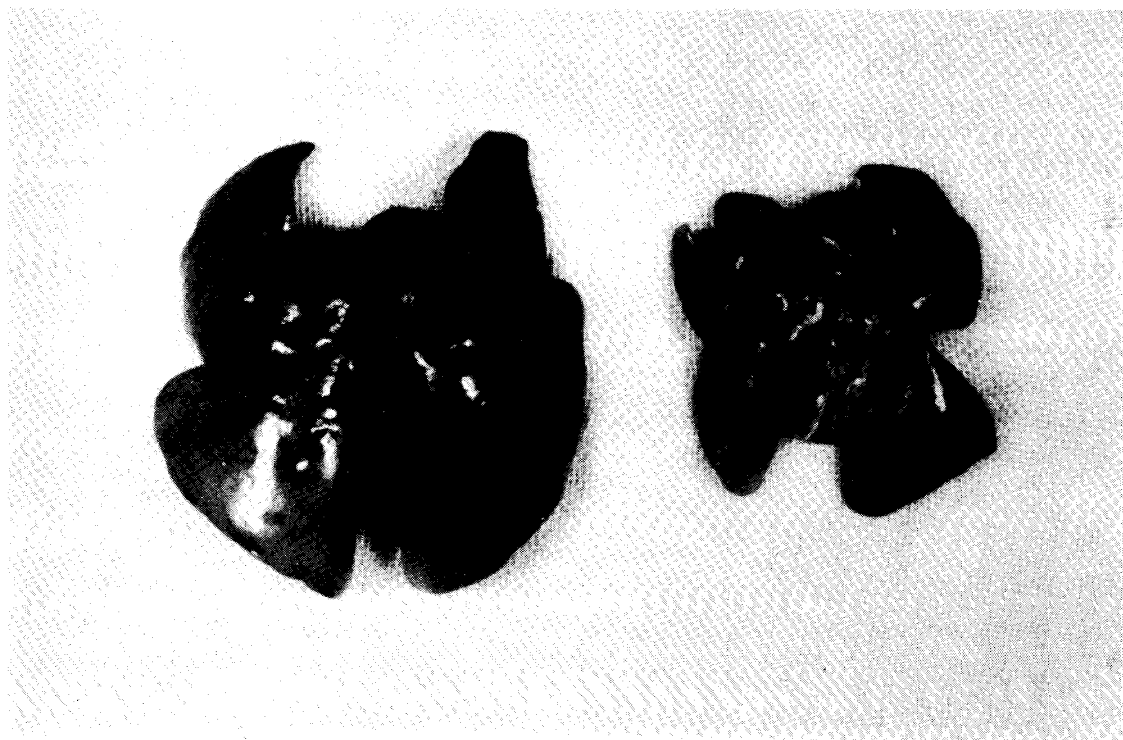
the passive transfer was successful.

Two ml of the serum itself, and the twofold diluted serum with physiological saline solution, and the fourfold diluted one, were respectively injected into the abdominal cavities of three different recipients. When each recipient was challenged with a 5% TDI, the asthma-like symptoms mentioned above were induced with dose-dependency in the recipients. The exertional breathing was more intense in the first recipient than in the second recipient and was not found in the third recipient. The application of TDI did not induce any symptom in control animals which had received sera from non-treated guinea pigs.

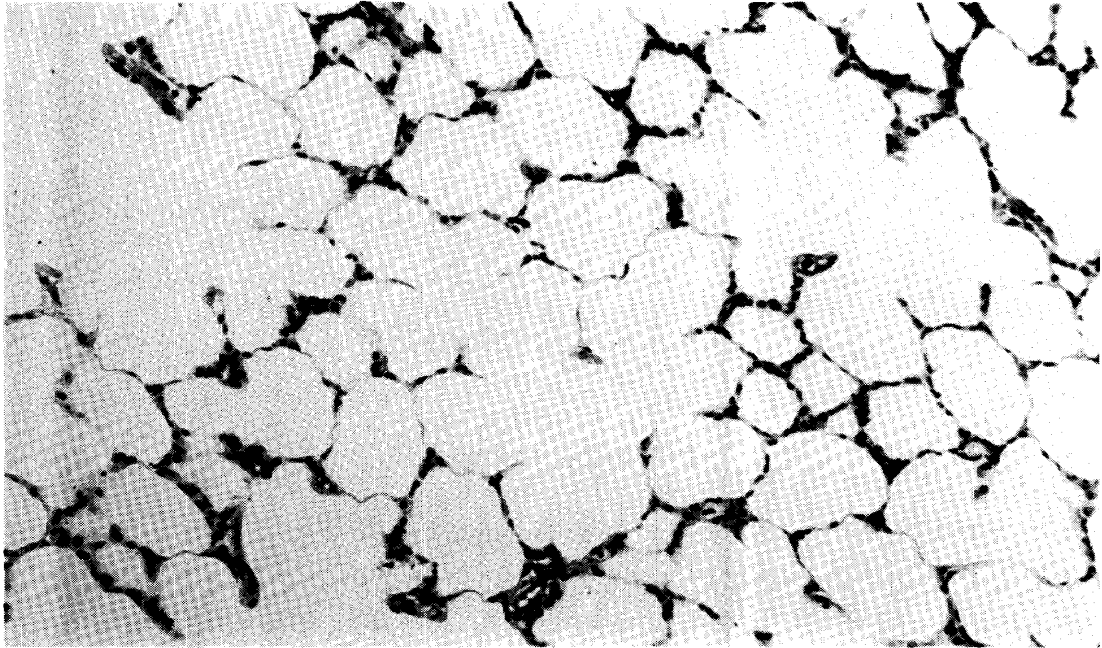
The lungs which were removed immediately after the attacks, were gray-white colored and elastic in consistency. Sometimes their size was enormously large compared with that of the lungs from the control animals (Fig. 2). Corresponding with the macroscopic findings, the emphysematous change was observed microscopically (Fig. 3).

Some other changes were observed in the lung sections from the test animals which had frequently suffered from the attacks. Bronchial epithelials were hypertrophied with the enlargement of goblet cells. Thickening of the smooth muscle layer of the bronchus was observed at the same time. One of the characteristic figures of the lung was infiltration of eosinophils. It was found not only in the subepithelial layer of the bronchus but also in the alveolar walls and the interstitial tissues fibrously thickened. Moreover, the eosinophils were found in the mucous plug together with the desquamated epithelial cells (Fig. 4).

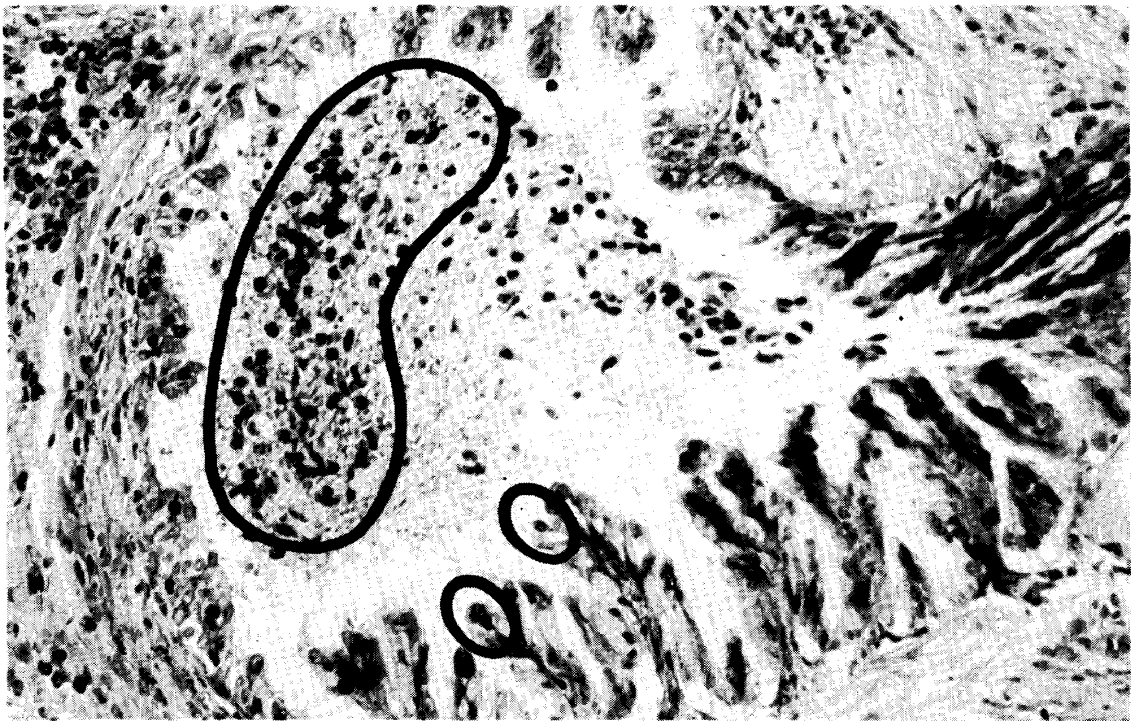
Eosinophilia was found in the peripheral blood of the test animals. It was not outstanding at that time when the animals were initially challenged but was found remarkably after the attacks had been frequently repeated (Table 2).



**Fig. 2.** Lungs removed from a test animal immediately after provocation (left) and those from a control animal (right)



**Fig. 3.** Microscopic view of the lung demonstrated in Fig. 2.  $\times 100$  H.E. staining



**Fig. 4.** Eosinophils in mucous plug

Eosinophils (enclosed with black lines) are observed not only in subepithelial layer of the bronchus but also in mucous plug. Bronchial epithelials and muscular layer are hypertrophied.  $\times 100$  Giemsa staining.

**Table 2.** Frequency distribution of eosinophils in peripheral blood

	Number of samples tested	Percentage of eosinophils among white blood cells						
		0-5	6-10	11-15	16-20	21-25	26-30	>31
Control animals <sup>1)</sup>	20	19	1	0	0	0	0	0
Test animals								
Before sensitization	20	16	4	0	0	0	0	0
Early stage <sup>2)</sup>	20	16	2	2	0	0	0	0
Later stag <sup>3)</sup>	20	7	3	6	2	1	1	0

- 1) Guinea pigs treated with only ethyl acetate
- 2) When test animals were initially challenged
- 3) When three months passed after initial challenge

### DISCUSSION

Many efforts have been made to clarify the mechanism of suspected hypersensitivity in the TDI disorders through the *in vitro* antigen-antibody reactions. Nevertheless, no clear-cut conclusion has been drawn because of conflicting results presented by different authors.

Tse *et al*<sup>6)</sup>. supposed that the controversial aspects of immune response ascribed to TDI might be responsible for the variability of hapten-protein conjugates.

TDI is an extraordinarily reactive substance. Its reaction with proteins easily leads to their polymerization through cross-linking by the diisocyanate. Karol *et al*<sup>7)8)</sup>. synthesized an antigen through the reaction of proteins with *p*-tolylmonoisocyanate (TMI) instead of TDI in order to avoid the autopolymerization by the action of the diisocyanate. Both experimental and clinical studies which were carried out by Karol *et al*<sup>7)~12)</sup>. using the monoisocyanate-derived antigen demonstrated that an immunological mechanism was involved in the TDI disorders.

On the other hand, there is another view that the essence of the TDI disorders is rather pharmacological than immunological<sup>13)~17)</sup>. While admitting the immunological involvement in the physical disturbances caused by TDI, Sangha and Alarie<sup>18)</sup> reported that TDI was the most sensory irritant and that repeated exposures to TDI at or above 0.023 ppm resulted in cumulative effects.

These arguments have encouraged us as an initial step of our studies to develop a new model of asthma apart from testing the *in vitro* reactions by synthesized antigens.

Animal model of the asthmatic state is well reviewed by Patterson and Kelly<sup>19)</sup>. In the paper, the authors mentioned that guinea pigs have been traditionally used as a model of antigen-induced airway obstruction and that the main symptom observed in the challenged animals is anaphylaxis which leads them to death due to the airway obstruction.

Karol *et al*<sup>8)</sup>. evaluated the respiratory hypersensitivity of guinea pigs using their formula called "respiratory index". The index was calculated on a basis that an increase in respiratory rate and a decrease in tidal volume appeared in the sensitized animals by the challenge prior to a slow gasping type respiratory pattern and collapse<sup>8)</sup>. The authors<sup>8)</sup> had guinea pigs inhale a conjugate of TMI with ovalbumin in a plexiglass inhalation chamber for ten minutes each day on five consecutive days. When the animals were challenged after a certain period, the hypersensitive state defined by the authors was found in the test animals.

Later, Karol *et al.*<sup>11)</sup> had the animals inhale TDI vapor itself in the same inhalation chamber. Although the hypersensitive responses of the animals were not found by the authors, tolyl-specific antibodies were proved by way of both passive cutaneous anaphylaxis and Ounchterlony test. More recently, Karol *et al.*<sup>20)</sup> exhibited that also the hypersensitive responses by them could be induced by the inhalation challenge with TDI vapor in the guinea pigs which had been exposed to this substance percutaneously.

The above information gave us a suggestion that the topical application of TDI dissolved in a volatile substance to the upper respiratory tract of experimental animals might bring about asthma-like symptoms which were considered allergic in nature.

The model was produced by painting TDI itself dissolved in ethyl acetate onto the nasal membrane of guinea pigs. Because the solvent is highly volatile, the technique can provide the animals an effective inhalation of TDI vapor of a high concentration even if the duration is temporary. It was found that neither nose nor general condition of the test animals was seriously affected by the procedures. Neither use of any adjuvants nor conjugation of hapten with heteroproteins was necessary in our experimental system.

In other words, the present model was developed through the application of a simple chemical alone to the respiratory tract of the animals.

A lot of information interesting from the standpoint of both clinical immunology and occupational health has been obtained. From the former standpoint, it seems noteworthy that even a drastic attack like anaphylaxis hardly causes death in the challenged animals. Since human asthmatic attacks are rarely mortal, this phenomenon attracts our attention a great deal.

The immortal and reproducible attacks have brought about morphological changes characteristic of asthma. It is reviewed that a thickened basement membrane in the bronchus and eosinophilia can be found in human asthma of long duration, while these findings are hardly observed in minimal asthma<sup>19)</sup>. This information well corresponds with our experimental results. It is interesting that the remarkable eosinophilia in our model appeared after the attacks had been frequently repeated. It seems that this experimental system can be used as an appropriate model of eosinophilia in asthma.

The present model is also interesting from the standpoint of occupational health. In connection with the clinical signs of the TDI asthma, some authors<sup>3,16,21,22)</sup> have indicated that the patterns of the asthma can be immediate, late, and/or dual. As we already mentioned, the exertional breathing could be found in our model without the immediate reaction after the provocation. This phenomenon is interesting in connection with the clinical observation as described above.

Our experimental data correspond also with the well-known fact that the workers who have already become sensitive to TDI react with the substance in a very low concentration<sup>21,23)</sup>.

The main purpose of our work is to propose an experimental model applicable to further studies of asthma induced by TDI and not to decide here whether the very essence of the TDI is hypersensitivity or not. Detailed studies on TDI-derived antibodies have not yet been carried out. It seems, however, the experimental results obtained so far suggest that an immunological mechanism is involved in the pathogenesis of the TDI disorders, as shown in Karol's papers<sup>7~12,20)</sup>.

One of the characteristic features of our model is that the experimental technique is quite easy. We believe that this animal model is useful not only for studies of asthma induced by TDI but also in the various research fields of asthma because of the simplicity of the methodology.

### SUMMARY

Attention has been paid to respiratory hypersensitivity in workers who are exposed to toluene diisocyanate (TDI) in their workshops. An experimental model of asthma in guinea pigs by TDI has been developed. A 10% TDI solution dissolved in ethyl acetate was painted on the nasal cavities of guinea pigs with a thin cotton applicator once daily for five consecutive days. Three weeks later, the animals were challenged with a 5% TDI solution.

Exertional breathing accompanied by the prolongation of expiratory phase was observed among the test animals. The number of the animals suffering from the attacks increased by the repetitive provocations. Although some of the guinea pigs suffered from anaphylaxis by the provocation procedures, the attacks including the anaphylactic shocks hardly brought about deaths in the animals.

It was found that passive transfer of the symptom was possible in our experimental system. Both eosinophilic infiltration in the lung and eosinophilia in the peripheral blood were found among the test animals. This experimental model was developed by the application of a simple chemical alone to the respiratory tract of guinea pigs. A significance of the model is discussed with the review of the literatures concerned.

### ACKNOWLEDGEMENT

We thank Professor Katsuya Ohata and Doctor Shigekatsu Kohno, Kyoto College of Pharmacy, for their helpful advice.

### REFERENCES

- 1) Fuchs, S. and Valade, P.: Etude clinique et expérimentale sur quelques cas d'intoxication par le Desmodur T (diisocyanate de toluène 1-2-4 et 1-2-6). *Arch. Mal. Profess.* 12: 191-196, 1951.
- 2) O'Brien, I. M. *et al.*: Toluene di-isocyanate-induced asthma. I. Reactions to TDI, MDI, HDI and histamine. *Clinical Allergy*. 9: 1-6, 1979.
- 3) O'Brien, I. M. *et al.*: Toluene di-isocyanate-induced asthma. II. Inhalation challenge tests and bronchial reactivity studies. *Clinical Allergy*. 9: 7-15, 1979.
- 4) Danks, J. M. *et al.*: Toluene-diisocyanate induced asthma: evaluation of antibodies in the serum of affected workers against a tolyl mono-isocyanate protein conjugate. *Clinical Allergy*. 11: 161-168, 1980.
- 5) Gallagher, J. S. *et al.*: Diverse profiles of immunoreactivity in toluene diisocyanate (TDI) asthma. *J. Occup. Med.* 23: 610-616, 1981.
- 6) Tse, C. S. T. *et al.*: Induction of murine reaginic antibodies by toluene diisocyanate. (An animal model of immediate hypersensitivity reactions to isocyanates). *Am. Rev. Respir. Dis.* 120: 829-835, 1979.
- 7) Karol, M. H. *et al.*: Tolyl-specific IgE antibodies in workers with hypersensitivity to toluene diisocyanate. *Am. Ind. Hyg. Assoc. J.* 39: 454-458, 1978.
- 8) Karol, M. H. *et al.*: Hapten-specific respiratory hypersensitivity in guinea pigs. *Am. Ind. Hyg. Assoc. J.* 39: 546-556, 1978.
- 9) Karol, M. H. *et al.*: Presence of tolyl-specific IgE and absence of IgG antibodies in workers exposed to toluene diisocyanate. *J. Environ. Sci. Health. C13(3)*: 221-232, 1979.



- 10) Karol, M. H. *et al.*: Longitudinal study of tolyl-reactive IgE antibodies in workers hypersensitive to TDI. *J. Occup. Med.* 21: 354-358, 1979.
- 11) Karol, M. H. *et al.*: Immunologic sensitization and pulmonary hypersensitivity by repeated inhalation of aromatic isocyanates. *Toxicol. Appl. Pharmacol.* 53: 260-270, 1980.
- 12) Karol, M. H. and Alarie, Y.: Antigens which detect IgE antibodies in workers sensitive to toluene diisocyanate. *Clinical Allergy.* 10: 101-109, 1980.
- 13) VanErt, M. and Battigelli, M. C.: Mechanism of respiratory injury by TDI (toluene diisocyanate). *Ann. Allergy.* 35: 142-147, 1975.
- 14) Butcher, B. T. *et al.*: Toluene diisocyanate (TDI) pulmonary disease: Immunologic and inhalation challenge studies. *J. Allergy Clin. Immunol.* 58: 89-100, 1976.
- 14) Butcher, B. T. *et al.*: Longitudinal study of workers employed in the manufacture of toluene-diisocyanate. *Am. Rev. Respir. Dis.* 116: 411-421, 1977.
- 15) Davies, R. J. *et al.*: The in vitro effect of toluene diisocyanate on lymphocyte cyclic adenosine monophosphate production by isoproterenol, prostaglandin, and histamine. *J. Allergy Clin. Immunol.* 60: 223-229, 1977.
- 16) Butcher, B. T. *et al.*: Inhalation challenge and pharmacologic studies of toluene diisocyanate (TDI) sensitive workers. *J. Allergy Clin. Immunol.* 64: 146-152, 1979.
- 17) Butcher, B. T.: Approaches to the understanding of mechanisms in occupational asthma. *Europ. J. Respir. Dis.* 61: 183-191, 1980.
- 18) Sangha, G. K. and Alarie, Y.: Sensory irritation by toluene diisocyanate in single and repeated exposures. *Toxicol. Appl. Pharmacol.* 50: 533-547, 1979.
- 19) Patterson, R. and Kelly, J. F.: Animal models of the asthmatic state. *Annu. Rev. Med.* 25: 53-68, 1974.
- 20) Karol, M. H. *et al.*: Dermal contact with toluene diisocyanate produces respiratory tract hypersensitivity in guinea pigs. *Toxicol. Appl. Pharmacol.* 58: 221-230, 1981.
- 21) Pepys, J. *et al.*: Asthma due to inhaled chemical agents—toluene di-isocyanate. *Clinical Allergy.* 2: 225-236, 1972.
- 22) Weill, H. *et al.*: Respiratory effects in toluene diisocyanate manufacture: A multidisciplinary approach. *Environ. Health Perspect.* 11: 101-108, 1975.
- 23) Butcher, B. T. *et al.*: Toluene diisocyanate (TDI) pulmonary disease: Immunologic and inhalation challenge studies. *J. Allergy Clin. Immunol.* 58: 89-100, 1976.