Conclusions

This interim report shows that in men engaged in the manufacture of TDI, under good conditions, the annual average rate of deterioration of both FEV_1 and FVC appears to be significantly greater than predicted and suggests a cumulative effect.

The validity of these conclusions is, however, dependent on the validity of the predicted values. As stated, these predicted values were obtained from a North American survey and it is doubtful if they would apply to the north-west of England.

The dangers of using predicted values from a different area are demonstrated by Lowe *et al.* (1968), who showed that the values at Ebbw Vale were 5% lower than those taken at Port Talbot, only thirty miles away.

The next step in this investigation is to obtain a direct comparison of lung function in men working on a nearby non-TDI plant.

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Immune Responses to Tolylene Diisocyanate (TDI) Exposure in Man

The major problem in the investigation of the effects of human exposure to TDI is to separate its irritant properties from its potentially sensitizing properties. Evidence for the immunogenicity of TDI in man is almost entirely derived from clinical observation that sensitized individuals may respond in an asthmatic manner to concentrations of TDI which do not produce symptoms in nonsensitized individuals (Swenson *et al.* 1955, Woodbury 1956). In a situation in which a suspect sensitizer may also produce symptoms of primary irritation it is important to demonstrate immunogenicity in as objective a manner as possible. Bronchial challenge with TDI in man has only rarely been carried out and is potentially dangerous and difficult to control because of the very ready breakdown of TDI which makes dosage uncontrollable. Laboratory evidence of sensitization in man is limited to the demonstration of lymphocyte transformation in sensitized individuals induced by TDI-conjugated proteins (Bruckner *et al.* 1968).

In animals there is good evidence that the administration by injection of TDI-protein conjugates results in a circulating antibody response directed against the hydrolysed TDI determinant (Scheel *et al.* 1964). In addition these workers showed a circulating antibody response in rabbits to prolonged inhalation of TDI vapour. The present work was undertaken in an attempt to detect circulating antibodies to TDI in individuals apparently sensitized to the compound.

Methods

Specimens of serum were obtained from 55 subjects all with symptoms suggestive of TDI sensitivity. They had suffered asthma-like symptoms after periods of exposure to TDI varying from hours to 16 years. There was considerable variation in the time interval between last exposure to TDI and the time at which the serum sample was obtained. This time interval was between zero (i.e. still exposed) and 5½ years. As a control, serum was taken from 40 cotton textile workers who almost certainly had not been exposed to TDI.

Three methods were used to detect antibody:

(1) A complement-fixation method (Fulton & Dumbell 1949) (CFT): As antigen bovine serum albumin lightly conjugated with tolylene 2:4 diisocyanate (molar ratio 1:2) was used. The method of conjugation was modified from that of Scheel *et al.* (1964). As control antigen unconjugated bovine serum albumin was used. Serum samples were tested at a dilution of 1 in 5 and three antigen concentrations were used.

(2) A red cell linked antiglobulin technique (RCLAT): A subagglutinating dose of a mixture of the 2:4 and 2:6 isomers of TDI was added with rapid mixing to a large volume of 2% sheep erythrocytes suspended in isotonic phosphate buffered saline (pH 7·4). After incubation at 4°C for 30 minutes the cells were washed three times. 'Sensitized cells' were now incubated with serum samples for 30 minutes at 37°C, washed four times in phosphate-buffered saline and then tested for the attachment of immunoglobulin to the cell surface by the addition of a rabbit anti-human globulin reagent. All sera were absorbed at 37°C and 4°C with large volumes of sheep cells before use and controls using unsensitized cells were set up with each serum.

(3) A modified passive cutaneous anaphylaxis test in cynomologous monkey skin (PCA): Intradermal injections of serum (0·1 ml) were made into the abdominal skin of cynomologous monkeys. After 48 hours a monkey was given an intravenous dose of 2% Evans blue sufficient to

cause 'bluing' of the gums. It was then exposed to a saturated atmosphere of TDI vapour for 3 minutes using a simple anæsthetic machine. The monkey was very deeply tranquillized and the operators used protective masks. Areas of bluing were read after 30 minutes. The monkey was not allowed to survive.

Results

Twenty-three subjects gave positive results in one or more tests. Three gave positive CFT, 12 positive RCLAT and 14 positive PCA. Five individuals were positive in more than one test: one subject in all three tests; 3 positive with RCLAT and PCA; and 1 positive with CFT and RCLAT. None of the 40 control sera gave positive results. The lack of correlation between the three techniques suggests that they detect either antibodies of slightly different specificity or of different immunoglobulin class. The suggested difference in specificity may relate to the area of the carrier molecule on which the TDI attaches.

Sera giving positive CFT were all taken within a relatively short time of acute reaction to exposure. On the other hand PCA positive tests occurred as long as five years after last known exposure to TDI. The positive RCLAT sera occupied an intermediate position. There appeared to be no correlation between positivity with respect to any of the three tests and either age or delay in the onset of symptoms after first exposure.

Of particular interest are 6 sera taken seven months after a single exposure to an unusually high concentration of TDI which produced severe symptoms. All 6 had positive serology: 3 positive RCLAT, 2 positive PCA, and one positive by both techniques.

Peripheral blood eosinophil counts taken at the same time as the serum sample were available in 23 subjects. No correlation existed between eosinophilia and positive serology. Sputum samples from 19 subjects were examined for eosinophilia and again no clear correlation existed with seropositivity.

Conclusions

It has been demonstrated that exposure to TDI vapour may give rise to circulating antibodies in man. It is suggested that the three techniques used detect antibodies differing either in specificity or immunoglobulin class. The etiological relationship between circulating antibody and symptoms of clinical sensitivity remains to be investigated. Although approximately half the subjects tested gave positive results, as yet the techniques available do not provide a suitable laboratory diagnostic test for TDI sensitivity.

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The Effect of Tolylene Diisocyanate on Certain Laboratory Animals

Despite the now considerable amount of work carried out on the effect of tolylene diisocyanate (TDI) on experimental animals (Zapp 1957, Duncan *et al.* 1962, Niewenhuis *et al.* 1965), little information has emerged from animal work to throw any light on the allergic sensitization effects of this chemical reportedly observed in man at levels in the atmosphere as low as the threshold limit value (TLV 0·02 ppm).

The effects of TDI atmospheres on the respiration of guinea-pigs and of monkeys have been examined to see if any support could be obtained for the view that the effects of TDI on respiration in man can involve an immune mechanism. Guinea-pigs were chosen for this work because of their ability to show delayed hypersensitivity effects, and rhesus monkeys because of their immunological similarity as primates to man. It was felt that, if an allergic sensitization effect were to be produced in the lungs of experimental animals, it would appear as a grossly altered breathing pattern.

Three-month-old albino guinea-pigs were exposed to atmospheres of TDI of concentrations varying between 0·01 and 5 ppm for three periods of about six hours, and their respiratory patterns were recorded by plethysmography. Three weeks later these and other, previously unexposed, guinea-pigs were exposed again to levels of TDI of around the TLV of 0·02 ppm. Similar experiments were performed with mature monkeys (*Macaca mulatta*) and the breathing pattern was recorded in this case using a telemetric strain-gauge device fixed to a strap running around the animal over the lower portion of the rib cage.

Table 1 shows that TDI levels of 0·02–0·05 ppm TDI do not significantly affect the breathing rate of guinea-pigs not previously exposed to

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