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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Table 1
Guinea-pigs: effect of TDI atmospheres on breathing rate: Exposure (single animals)

Level of exposure (ppm)	*Breaths/min at times after first exposure* 0 min	40 min	160 min	5 h
0·02–0·05	100	100	100	100
0·18	100	60	50	50
0·50	100	50	36	40

Table 2
Guinea-pigs: effect of TDI atmospheres on breathing rate: Exposure then re-exposure three weeks later (groups of animals); standard deviation stated

Level of exposure for 3×6 h (ppm)	*Level of re-exposure (ppm)*	*Breaths/min at times after first re-exposure* 0 min	40 min	160 min	5 h
0 (Controls)	0·02	116±7	113±13	97±4	106±8
2–5	0·02	102±2	81±9	59±9	65±1

TDI. Falls in breathing rate of about 50% occur with 0·18 ppm TDI in previously unexposed animals (Table 1) and even greater falls occur at higher concentrations. Occurring along with this reduced breathing rate is an increased depth of breathing and an altered pattern of breathing. This effect on breathing is probably of the non-specific type as described by Davis *et al.* (1967) involving responses of receptors in the upper respiratory tract.

In batches of animals (Table 2) previously exposed to high levels of TDI (2–5 ppm) significant reductions in breathing rate were noted on re-exposure to TDI at levels as low as 0·02 ppm. These previously exposed animals, showing increased reactivity to TDI exposure, were shown by skin patch tests to be skin-sensitized to TDI. Other tests for sensitization – for instance, tests for precipitating and hæmagglutinating antibodies – were, however, negative. The lymphocyte transformation test was also negative, but it is doubtful if this test has value in guinea-pigs since their lymphocytes, unlike human lymphocytes, did not transform with standard mitogenic agents such as PHA. Guinea-pigs pre-exposed to rather lower levels of TDI (e.g. 0·5 ppm) showed no greater sensitivity on re-exposure to low (0·02 ppm) levels of TDI three weeks later.

In the experiments with batches of animals, the atmospheres were analysed specifically for isocyanate content only (Reilly 1968) as well as for TDI plus hydrolysis products (Marcali 1957). The analyses show that the 0·02 ppm atmospheres giving profound changes in breathing pattern contained about 0·01 ppm of the isocyanate.

Monkeys showed great acute sensitivity to TDI atmospheres (Table 3). The results indicated no allergic sensitization effect detectable in the breathing rate either during a 3-day exposure followed by re-exposure three weeks later or during more chronic exposure. The extreme sensitivity of monkeys to the acute effects of TDI was noted, even at comparatively low levels (0·4 ppm), and this may be due to the damaged state of the lungs of these wild animals resulting from previous exposure to respiratory disease. Tests of the blood of monkeys exposed to the higher levels of TDI (Table 3) for precipitating or hæmagglutinating antibodies were negative. Monkeys exposed to the TLV of TDI for extended periods of time (Table 4) were apparently unaffected.

Our work has led us to conclude: (1) That gross exposure of the respiratory system to TDI may render that system more sensitive to contact with low levels of TDI, but that this greater sensitivity may not involve an allergic mechanism. (2) That the value of investigative work in this field is greatly limited by the essentially complex analytical problems encountered with TDI atmospheres. (3) That presently available immunological techniques do not, because of the difficulty of preparing suitable antigenic systems, allow us to determine whether animals exposed to TDI undergo any process with a significant immunological component.

Table 3
Rhesus monkeys: effect of TDI atmospheres on breathing rate: Exposure and re-exposure

Animal	*Time and level of exposure*	*Time and level of re-exposure*	*Result*
1	3×6 h, 0·4 ppm●	—	Animal died following exposure; death due to pulmonary œdema
2	2×6 h, 0·7 ppm●	1×6 h, 0·02 ppm	No effect of re-exposure
3	1×6 h, 0·13 ppm	1×6 h, 0·02 ppm	No effect of re-exposure

●These levels induced lacrimation during exposure

Table 4
Rhesus monkeys: effect of TDI atmospheres on breathing rate: Chronic exposure

Animal	*Time and level of exposure*	*Result*
4	5×6 h, 0·02 ppm	No effect
5	23×6 h, 0·02 ppm	No effect
6	23×6 h, 0·02 rising to 0·18 ppm	No effect

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DISCUSSION

Dr Pamela M Fullerton (*The Middlesex Hospital, London W1*) said that 14 of the firemen discussed by Dr McKerrow had developed a neurological syndrome after exposure to high concentrations of TDI. Proof that this substance itself had been responsible was lacking, but no other substance in the factory was known to produce such a syndrome and none had been present in high concentration. Anoxia had not been responsible.

Five of the men had developed an acute effect at the time of the fire, becoming euphoric and ataxic; 2 had lost consciousness. These 5 men and 9 others had appeared to have a mild encephalopathy during the subsequent few weeks. The symptoms had been of difficulty in concentration, poor memory and odd behaviour, and some had suffered from headaches. Abnormal physical signs had been scanty, but 5 men had had some degree of ataxia when seen three weeks after the episode. The symptoms had suggested an organic disturbance, but in addition, the syndrome was thought not to be hysterical because in several instances abnormalities had first been noticed by someone other than the man himself; most had complained of symptoms before knowing that others had been similarly affected, and they had improved rapidly at a time when the authorities had become increasingly concerned.

In a few men some residual difficulties were thought to be largely psychogenic. Three had been seen over a year later and assessed psychiatrically by Dr Raymond Levy of the Middlesex Hospital. Two of them had had definite evidence of mild organic impairment, the history clearly suggesting that this dated from the time of the fire. The residual symptoms in the third patient were thought to be entirely psychogenic.

Dr D L Caldwell (*Wallasey*) asked what was the effect of acute exposures to high concentrations of TDI.

Dr Adams said that fortunately there had been no cases of men exposed to high concentrations of TDI. Even small concentrations of 1–2 ppm, however, could cause irritation of the eyes, throat and lungs. Experimental work, carried out on animals, had shown that exposure to high concentrations of over 50 ppm resulted in destruction of lung tissue.

Dr M L Newhouse (*London School of Hygiene and Tropical Medicine*) asked what the speakers considered the best method of surveillance of (*a*) sensitized workers continuing in exposure, and (*b*) workers continuing to work where they were exposed and not known to be sensitized.

Dr Peters replied that a sensitized worker should not continue to be exposed. He believed that the worker exposed to TDI but not known to be sensitized should be followed with periodic questionnaires and pulmonary function (FEV_1) on an annual basis.

Dr McKerrow said it was suspected that in both groups mentioned by Dr Newhouse there might be an unduly high attack rate of bronchitis and also an annual decrement in the ventilatory capacity larger than expected. Their surveillance should include questions on attacks of bronchitis and preferably serial measurements of the forced expiratory volume and vital capacity.

Dr K S Williamson (*Birmingham*) asked whether the findings of short-term falls in FEV_1 in TDI workers observed by Dr Peters could be considered entirely due to that material. Normal values quoted in the literature related to shift workers while Dr Peters' population were day-workers, and hence it seemed that diurnal rhythmic changes might account for a part of the changes he had observed.

Dr Peters replied that three pieces of evidence suggested that these acute falls in FEV_1 were attributable to TDI: (1) Identical measurements had been made on a group of welders in which no significant change in FEV_1 occurred. (2) The 5 members of the survey team had measured themselves in their laboratory and in the factory in the morning and afternoon. No change took place in the laboratory and a change similar to that seen in the polyurethane workers occurred in the factory. (3) They had just finished surveying another factory in which exposure was conveniently divided into very low ($<0{\cdot}001$ ppm) and quite high (up to 0·03 ppm). Those in the low exposure area had had no change in FEV_1 while those in the high exposure area showed a fall of 0·2 litre.

Dr R N Hill asked whether measurements of isocyanate in air were available for the studies described by Dr McKerrow and Dr Adams.

Dr McKerrow replied that no air measurements were available for the firemen; in the case of the group of factory workers, air samples had been taken from time to time by the managements concerned. These were unlikely to detect high concentrations of TDI lasting only brief periods following spillages.

Dr Adams said that air measurements were done on the TDI plants throughout the day, at all points where there was liable to be TDI. Although there obviously must be occasions when a higher concentration was missed, it was very unusual for the readings to rise above the TLV of 0·02 ppm.

Dr Munn commented that Dr Peters' studies had included men whose exposure to TDI varied from two to nine years. He asked whether there was any evidence that those with long exposure had poorer lung function than those with relatively short exposure.

Dr Peters replied that there was no evidence that long exposure was worse than short exposure, but because of small numbers it was not possible to examine this relationship adequately. The questionable reliability of predicted normal values complicated the relationship. Pre-exposure data on pulmonary function to establish the individual's normal value would be very helpful; they were attempting to do that.

Wing Commander C C G Rawll (*Ministry of Defence, London*) asked if any investigation had revealed the existence of antibodies to HDI. The RAF used large quantities of a paint based on HDI, and he had clinical records that strongly suggested that 4 paint sprayers had become sensitized by the small amount of unreacted HDI in the pot-mix.

Dr Taylor answered that he was aware of clinical evidence very suggestive of sensitivity to HDI, but did not know of anyone who had succeeded in demonstrating any immune response to this material.