Clin. exp. Immunol. (1969) **4,** 585–596.

IMMUNOLOGICAL INVESTIGATION OF INDIVIDUALS WITH TOLUENE DIISOCYANATE ASTHMA

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(*Received* 3 *December* 1968)

SUMMARY

Thirteen subjects who experienced only the expected irritant respiratory and ocular effects from occupational exposure to toluene diisocyanate (TDI) were compared with eight workers who appeared clinically to be sensitized to this substance, as manifested by the prompt development of asthmatic symptoms on exposure to minute concentrations of TDI. In an attempt to document an immunological response to TDI in the latter group, several conjugates of TDI with human serum albumin were prepared. Gel diffusion, leucocyte histamine release, PCA in guinea-pigs, passive haemagglutination, passive transfer (P-K) and a few direct skin tests all failed to show antibodies to TDI. In lymphocyte culture, however, TDI–human serum albumin complexes produced stimulation of lymphocytes from seven of the eight subjects suspected of being sensitized and none of the controls.

INTRODUCTION

Toluene diisocyanate (TDI) is a volatile, highly reactive substance which combines notably with hydroxyl or amino groups. It is widely used as a polymerizing agent in the production of polyurethane foams, lacquers, adhesives, insulation, fibres and other items. Exposure to concentrations of TDI in excess of 0·05 ppm causes many workers to develop irritative symptoms including conjunctival burning, itching, lacrimation, dry cough and nasal congestion (Fuchs & Valade, 1951; Reinl, 1953; Swensson, Holmquist & Ludgren, 1955; Gandevia, 1964). Brugsch & Elkins (1963) reviewed more than 300 cases reported in the world literature between 1951 and 1960, including two fatalities (Reinl, 1953; Schurmann, 1955) where lower respiratory tract involvement was severe. Isocyanates also are primary

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skin irritants. It has been observed that some workers, after repeated exposures and a latent period of a few weeks to months, develop asthmatic symptoms upon re-exposure to minute concentrations of TDI (Fuchs & Valade, 1951; Swensson *et al.*, 1955; Woodburry, 1956; Brugsch & Elkins, 1963). Such individuals become unable to work where TDI is used, and this has created a very significant industrial medicine problem, particularly in plastics manufacturing, in many areas of the world (note references). The type of onset and exquisite sensitivity is suggestive of typical extrinsic asthma, but an immunological mechanism for this disorder has not been adequately documented. We, therefore, undertook to determine whether an immunological response to TDI could be demonstrated in these individuals.

MATERIALS AND METHODS

Preparation of antigen

Conjugates of TDI (Eastman) with human and rabbit serum albumin were prepared by a modification of the procedure of Scheel, Killens & Josephson (1964), which involved very slowly adding a solution of TDI in dioxane dropwise to a 1% solution of albumin at a temperature of 4–11°C with constant stirring. The concentration of TDI in dioxane was varied so that approximately equal volumes of TDI–dioxane solution and albumin solution were mixed. Initially the pH was maintained approximately at 8·5 with NaOH (Scheel *et al.*, 1964). Because of the frequent occurrence of precipitates, however, subsequently the pH of the human serum albumin (HSA) solution was adjusted to 4·5 with HCl and maintained at this pH throughout the addition of TDI–dioxane solution. Extensive dialysis against pH 7·4, 0·15 M-phosphate buffered saline was used to remove unreacted TDI and dioxane and to adjust the pH to 7·4. Several conjugates were prepared varying the amount of TDI added from 10 to 160 mg/g HSA, hoping in this manner to make preparations with differing numbers of haptens per molecule of albumin. These TDI–HSA conjugates are referred to as TDI–HSA 10, 20, 40, 80 and 160. TDI–RSA denotes conjugates of TDI and rabbit serum albumin.

The development of protein conjugates was confirmed by ultraviolet spectrophotometry. Comparison of the optical densities of unreacted albumin and the conjugates showed an increase in absorbance maximal at 255 mμ (Fig. 1) and proportional to the amounts of TDI used in the preparation of these particular conjugates (Fig. 2).

Immunological activity of conjugates

In an attempt to demonstrate that the TDI–HSA and TDI–RSA conjugates could elicit TDI specific antibodies, quantities of TDI–HSA 20 or 40 or TDI–RSA 20 or 40 (3·3 mg protein/ml) were emulsified with 2 volumes of complete Fruend's adjuvant (Difco). Individual rabbits were injected with 1·2 ml of the emulsion divided equally into four sites weekly for 1 month. The injections were repeated three additional times over a 2–3-month period.

Subjects

Twenty-one adult subjects who had been exposed to TDI in the course of their employment were studied. Eight of these had typical symptoms of severe asthma on exposure to small amounts of TDI. Thirteen workers had experienced only irritative symptoms on exposure to moderate or high concentrations of TDI. Detailed clinical observations and

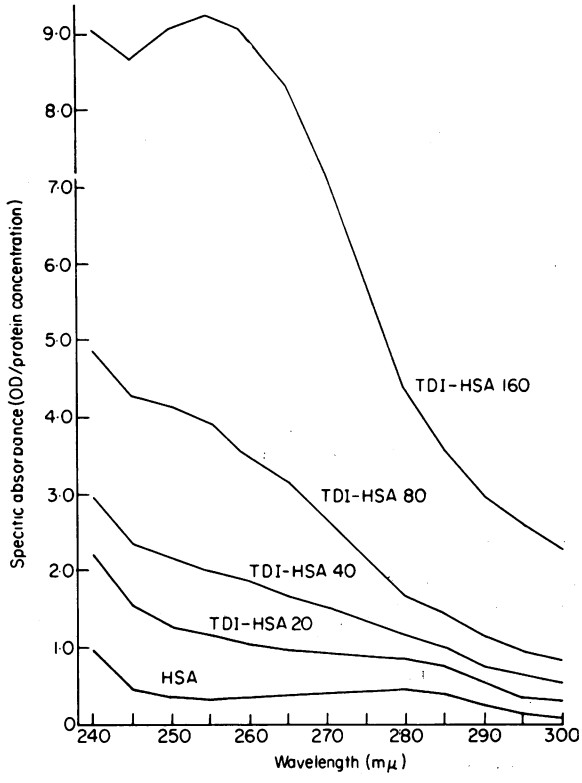

FIG. 1. Ultraviolet absorbance of various conjugates of TDI with human serum albumin (HSA).

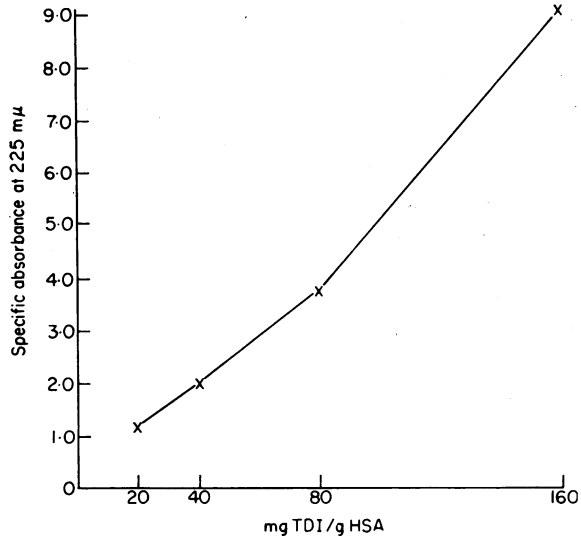

FIG. 2. Ultraviolet absorbance of conjugates prepared by the addition of increasing amounts of TDI.

industrial hygiene data concerning the work environment of most of these subjects have been reported upon previously (Bruckner *et al.*, 1968).

Immunological studies

In vitro histamine release from human leucocytes was determined by a procedure similar to that described by Lichtenstein & Osler (1964), using a modified spectrofluorometric method (Shore, Burkhalter & Cohn, 1958) for measuring histamine. Passive cutaneous anaphylaxis (PCA) (Ovary, 1958), tanned cell haemagglutination tests (Boyden, 1951), gel diffusion, skin tests and passive transfer (Prausnitz–Küstner) tests were performed by standard methods.

Lymphocytes were cultured by a modification of the method of Bach & Hirshhorn (1964) and Girard *et al.* (1967). Heparinized blood (1 mg heparin/10 ml blood) was allowed to sediment for 1 hr at 37°C. The leucocyte-rich supernatant was mixed with an equal volume of Eagle's minimal essential medium (MEM, Grand Island Biological Co.) and placed in 2-oz glass prescription bottles for 1 hr to effect a partial separation of lymphocytes from the other leucocytes which tend to adhere to glass surfaces. The decanted cell suspension contained 60–90% mononuclear cells. After centrifugation at 160 ***g*** for 10 min, the cells were washed with 10 ml of MEM and again centrifuged for 5 min at 160 ***g***. The cells were then resuspended in 1 ml of residual supernatant and counted. Aliquots of $2{\cdot}5 \times 10^6$ cells were placed in conical glass tubes in 4 ml of culture medium consisting of MEM to which 15% foetal calf serum (Difco), 200 mM-l-glutamine, 300 units of penicillin and 300 μg of streptomycin had been added. In patients with a history of allergy to penicillin or streptomycin, these were omitted. Then 0·1 ml of TDI–HSA conjugate solution containing 1 mg protein/ml was added to appropriate tubes in all patients studied. In addition, one-tenth of this quantity of conjugate was utilized in many cultures. As positive controls to determine the ability of the lymphocytes to respond to stimulation, cultures containing 2 μl phytohaemagglutin P (PHA, Difco) were incubated in tightly stoppered tubes in the vertical position for 3 days at 37°C along with 3-day control cultures to which no PHA was added. Tubes to which TDI–HSA conjugates were added were harvested along with antigen-free control tubes at 6 days. All cultures were done in duplicate.

Following culture the tubes were centrifuged at 40 ***g*** for 5 min and the supernatant discarded. Four drops of a fresh 3:1 solution of absolute methanol and glacial acetic acid and 3 ml of 1% sodium citrate were added to swell the cells, and the tubes were centrifuged at 40 ***g*** for 5 min. As much of the supernatant as possible was removed, and 3 ml of the methanol–acetic acid fixative were added. After 10 min the tubes were centrifuged at 40 ***g*** for 10 min and all but 0·5 ml of supernatant was removed. The cells were resuspended, and a drop of the suspension was placed on a coverslip and allowed to air dry. The coverslips were inverted into a drop of 0·5% aceto orcein on a slide and examined microscopically. To determine the percentage of blast cell transformation, 2000 cells from each duplicate set of tubes was counted by an observer who did not know the source of the slides.

Subsequently, additional studies were performed to confirm the microscopic observation of lymphocyte stimulation by tritiated thymidine uptake employing a modification of the method of Moorhead & McFarland (1966). Heparinized blood (1 mg heparin/10 ml) was drawn into a plastic syringe in which the blood was allowed to sediment for 1 hr at 37°C. The supernatant plasma was expressed through a bent 18 guage needle into a plastic tube, and the white blood cells (WBC) were counted. The leucocyte suspension was added in

quantities sufficient to give a final concentration of $0{\cdot}625 \times 10^6$ WBC/ml to the same culture medium (MEM, 1-glutamine, penicillin and streptomycin) used previously in a plastic Erlenmeyer flask. Additional autologous plasma, obtained by centrifuging the residual red cell layer remaining in the syringe, was added to bring its final concentration up to 20%. After mixing, 4-ml aliquots were distributed by plastic pipettes into 15-ml plastic culture tubes (Falcon). Antigen was added in the manner described previously to triplicate tubes.

After 6 days in culture, 1·5 μC of tritiated thymidine (specific activity approximately 16·3 C/mM, New England Nuclear Coporation) was added to each tube 2 hr prior to harvest. Then the tubes were centrifuged at 180 ***g*** at 4°C for 5 min and all except 1 ml of the supernatants was discarded. The cells were resuspended in the residual supernatant and transferred to glass tubes (because the plastic tubes were dissolved by subsequently used reagents). The plastic tubes were washed twice with cold saline to assure adequate removal and transfer of the cells. The glass tubes were centrifuged at 275 ***g*** at 4°C for 10 min, and the cells were washed once more with 5 ml cold saline. Then 5 ml of cold 10% trichloroacetic acid (TCA) were added. The precipitates were resuspended vigorously, and the tubes allowed to stand at room temperature for at least 20 min. After centrifugation at 4°C at 1700 ***g*** for 20 min, the precipitates were washed with 5-ml quantities of 5% TCA. Care was taken to remove all of the TCA solution possible following the last centrifugation. Then 0·5 ml of NCS solubilizer (Nuclear Chicago Corporation) was added and the tubes vigorously agitated on a Vortex mixer. When the precipitate had completely dissolved, the solutions were transferred to scintillation vials. The tubes were washed twice with 5·5 ml of scintillation fluid (5·0 g PPO and 0·3 g POPOP/l toluene), and the washings were added to the scintillation vials. The radioactivity was measured on a Packard Tri-Carb (Series 3000) refrigerated liquid scintillation spectrometer.

RESULTS

PCA tests

Six hours after intracutaneous injections with 0·1 ml of serum from rabbits immunized with TDI conjugates, 1 ml of undiluted TDI–HSA 40 or TDI–RSA 20 and 0·5 ml Evans blue dye were injected intravenously into albino guinea-pigs. When killed after 30 min, extravasation of dye was found in both rabbit anti-TDI–HSA and rabbit anti-TDI–RSA sites when challenged with TDI–HSA 40 or TDI–RSA 20, respectively. Positive PCA reactions also were obtained with TDI–HSA 40 challenge of an anti-TDI–HSA serum neutralized with HSA, while HSA failed to elicit a reaction with this neutralized serum. Likewise, TDI–RSA elicited reactions with rabbit anti-TDI–RSA serum 'neutralized' with RSA, while RSA failed to produce PCA with the anti-TDI–RSA serum with or without 'neutralization' with RSA. These results suggest that some of these rabbit antibodies were TDI-specific, though reactivity with denatured albumin cannot be excluded.

PCA tests were carried out as described above using sera from six sensitive and three non-sensitive human subjects challenged with undiluted TDI–HSA 40 antigen. Rabbit anti-TDI–HSA 40 was used to provide a positive control. PCA reactions were not elicited with any of the human sera.

Gel diffusion tests

The undiluted sera from seven sensitive and six non-sensitive subjects were diffused on

micro-Ouchterlony slides against undiluted antigen and a 1:10 dilution of TDI–HSA 20, 40, 80 and 160. No lines of precipitation were formed.

In vitro histamine release from subjects' leucocytes

No histamine release was observed when the washed leucocytes from six sensitive and three non-sensitive individuals were incubated with TDI–HSA conjugates. As expected, histamine release did occur when pollen extracts were added to the leucocytes of allergic subjects.

Passive haemagglutination

The sera of six sensitive and three non-sensitive subjects were studied. Tanned red cells were sensitized with TDI–HSA 40 and TDI–HSA 160 in varying dilutions. Rabbit anti-TDI–HSA serum again served as a positive control and produced agglutination in dilutions greater than 1:10,000, largely due to anti-HSA antibodies. None of the human sera caused agglutination.

Passive transfer (P-K) tests

These were performed on several occasions employing sera from six sensitive and three non-sensitive persons. Twenty-four hours after injecting 0·1-ml aliquots of serum into volunteers known to serve as satisfactory recipients, the sensitized sites were challenged with undiluted TDI–HSA 40, 80 and 160, which had been sterilized by Millipore filtration. The tests were repeated with TDI–HSA 40 and 640 concentrated by negative pressure dialysis to a protein content of 15 mg/ml. The P-K tests were all negative except for one control serum which repeatedly reacted to the concentrated TDI–HSA 40. This patient did not have a history of asthma on exposure to TDI but had had asthma when working with other amines.

Direct skin tests

These were restricted in number in order to facilitate access to subjects at a large chemical manufacturing corporation where most of the blood specimens were obtained. Both scratch and intracutaneous tests using undiluted TDI–HSA 40 (3 mg protein/ml) and 160 (2·55 mg protein/ml) were negative on three sensitive subjects.

Lymphocyte cultures

As shown on Table 1, lymphocytes from none of eleven exposed, non-sensitive subjects in the first group studied showed significant blast cell transformation when cultured with TDI–HSA 40 (0·1 mg protein). Cultures with one-tenth of this quantity of antigen also were negative. All subjects responded to PHA. In addition, the cells of two subjects never exposed to TDI were similarly cultured with no response to TDI.

Table 2 demonstrates that lymphocytes from six of seven sensitive subjects showed significant blast cell transformation when cultured with TDI–HSA 40 (0·1 mg protein in 4 ml culture). Cultures with one-tenth of this antigen concentration showed similar results. Patient L.S. did not exhibit a definite response, but his last exposure to TDI was over $3\frac{1}{2}$ years previous to his culture. Patient K.B. did not show unequivocal stimulation with TDI–HSA 40 but did when TDI–HSA 160 (0·1 mg protein) was used. Positive tests were obtained on three occasions with both K.A.'s and F.F.'s cells using TDI–HSA 40. Both of these subjects also showed positive responses to TDI–HSA 80 and 160. Subjects C.P. and E.P. (No.

1 in Tables 1 and 2, respectively) were of special interest in that these brothers had similar exposure to TDI in their joint work making polyurethane foams for insulation. Only E.P. clinically appeared to be sensitized, and on simultaneous lymphocyte cultures only his cells were stimulated by TDI–HSA 40.

TABLE 1. Lymphocyte cultures of clinically non-sensitive subjects

Subject	Exposed	Asthma	% blasts/2000 cells counted		
			6-day control	TDI–HSA 40 (0·1 mg protein)	PHA
1. C.P.	+	−	9	8	32*
2. W.D.	+	−	5·5	4·5	53
3. R.T.	+	−	7·3	7·4	57
4. G.K.	+	−	6·2	6·7	69
5. R.S.	+	−	6	7·5	51·7
6. V.M.	+	−	1·4	1·8	65
7. M.M.	+	−	1·7	2·3	50
8. W.A.	+	−	0·7	0·9	73
9. M.S.	+	−	2·8	4·1	66
10. N.V.	+	−	1·7	3·9	51·5
11. T.R.	+	−	3·8	6·6	71

* A different lot of PHA was used in this experiment than in subsequent ones.

TABLE 2. Lymphocyte cultures of clinically sensitive subjects

Subject	Exposed	Asthma	% blasts/2000 cells counted		
			6-day control	TDI–HSA (0·1 mg protein)	PHA
1. E.P.	+	+	8	20	30‡
2. K.A.†	+	+	1	37·5	69
3. L.S.	+	+	4	7·7	50
4. J.H.	+	+	6·5	19	59
5. F.F.†	+	+	7·5	33	63·5
6. E.D.	+	+	4·9	23·8	62
7. K.B.	+	+	1	14·5*	62

* TDI–HSA 160 (0·1 mg protein).
† Also cultured with TDI–HSA 80 and 160 (0·1 mg protein).
‡ A different lot of PHA was used in this experiment than in subsequent ones.

The second group studied is shown on Table 3. F.F. was also included in the previous group. A.G. and F.F. showed significant ($P<0·01$) increase in tritiated thymidine uptake as well as blast cell transformation when cultured with antigen. Culture of A.G.'s cells with

TDI–HSA 80 (0·1 mg protein) showed 13·8% blast cell transformation, but the tritiated thymidine cultures with this antigen were technically unsatisfactory. One subject with a history of irritative symptoms had negative cultures.

TABLE 3. Lymphocyte cultures evaluated by tritiated thymidine (^{3}H) uptake

Subject	Clinically sensitive	Control		TDI–HSA 40*		TDI–HSA 160†	
		^{3}H uptake (CPM)	% blasts‡	^{3}H uptake (CPM)	% blasts‡	^{3}H uptake (CPM)	% blasts‡
A.G.	+	77	4·0	342	10·2		
		77		494			
		121		445			
F.F.	+	209	4·6	ND	ND	848	13·2
		289				715	
		354				794	
O.H.	−	82	5·5	51	6·4	ND	ND
		86		75			
		64		94			

CPM, Counts/min of triplicate cultures; ND, not done.

* 0·1 mg protein. † 0·01 mg protein. ‡ Based on counting 2000 cells.

DISCUSSION

Since TDI has a marked irritant action on mucous membranes, respiratory symptoms and injury would be expected following inhalation of high concentrations of this substance. This has been well documented both in humans and experimental animals (Woodbury, 1956; Zapp, 1957; Duncan *et al.*, 1962; Silver, 1963; Brugsch & Elkins, 1963; Niewenhuis *et al.*, 1965). The occurrence of symptoms in a few individuals at much lower concentrations of TDI poses the problem of whether these persons are simply unusually reactive to its irritant effects or whether they have acquired immunologically mediated hypersensitivity. Difficulty in making this distinction is compounded by the fact that highly reactive substances, such as TDI, are not only likely to be irritants but also are apt to sensitize, presumably by readily conjugating with suitable carriers to form antigens. Several facts indirectly support the possibility that sensitization occurs in some exposed individuals: the usual latent period of weeks to years between the beginning of exposure and the development of extreme reactivity to TDI (Bruckner *et al.*, 1968), a progressively shorter exposure–response interval, the specificity of this marked intolerance to TDI-type compounds alone, the relatively common past history of personal allergy in this group (Bruckner *et al.*, 1968), the fact that the symptoms are quite typical of bronchial asthma, and the not infrequent occurrence of eosinophilia (Woodbury, 1956; Silver, 1963; Brugsch & Elkins, 1963). Sweet (1968) provoked asthmatic symptoms and a significant decrease in vital capacity and FEV_1 in an apparently sensitized subject by inhalational challenge with a concentration of TDI which produced no effect on normals or other asthmatic individuals. However, no direct evidence for an immunological

response to TDI in these individuals appears to have been recorded. Attempts to sensitize guinea-pigs by inhalation of TDI have generally been unsuccessful (Zapp, 1957). Scheel *et al.* (1964) reported TDI specific antibodies to be present in the sera of rabbits immunized by intravenous injection of TDI–egg albumin conjugates. PCA tests in guinea-pigs and gel diffusion were reported positive, but their data do not exclude reactivity with the carrier protein used in these tests. More recently Thompson & Scheel (1968) reported inability to sensitize Pertussis immunized rats by one or two prolonged inhalational exposures to TDI. Konzen *et al.* (1966), working with *p, p*-diphenylmethane diisocyanate (MDI), reported that humans subjected to MDI inhalations produced a transient antibody response as demonstrated by PCA in guinea-pigs. However, there was no indication that the exposed subjects became sensitized, and, conversely, sera of asthmatics usually fail to produce PCA in guinea-pigs with ordinary allergens, such as pollen extracts (Fisher, Middleton & Menzel, 1965), even when P-K tests are strongly positive. Direct skin tests of apparently sensitized humans with TDI itself have failed to show positive results (Swennson *et al.*, 1955; Sweet, 1968).

Failure, in our studies, of gel diffusion, PCA and passive haemagglutination to demonstrate the presence of circulating antibodies to TDI in our apparently sensitized patients was not unexpected. Precipitin reactions with unconcentrated serum and PCA in guinea-pigs, as just noted, are almost invariably negative when studying other allergens causing extrinsic asthma. Although Ishizaka & Ishizaka (1968) have recently shown that IgE antibodies are capable of producing haemagglutination, there is much evidence that antibodies other than atopic reagins are responsible for most of the haemagglutinating activity of allergic serum (Mathews & Speer, 1961; Perelmutter, Freedman, & Sehon, 1962).

There were greater expectations that skin tests, P-K tests and *in vitro* histamine release from leucocytes might provide relevant evidence of sensitization. Among the several factors which might account for failure to have obtained positive results in these tests, possible unsuitability of the TDI–HSA test antigens merits major consideration. Because TDI will react even with water and with itself, it is impossible accurately to estimate from our data the number of TDI haptens per molecule of HSA in the various conjugates which were prepared. It is possible, therefore, that the increasing ultraviolet absorbance noted when larger amounts of TDI were added to a fixed amount of HSA (Fig. 2) may largely have been due to conjugation with increasing numbers of HSA molecules rather than the expected increasing degree of conjugation of each HSA molecule. Thus, the immunological valence of TDI–HSA conjugates may not have been suitable for eliciting biological effects such as skin test reactivity.

Clinical assessment of which TDI-exposed subjects appeared to exhibit sensitization to this compound was made by one individual independently of knowledge of the results of the lymphocyte cultures. The finding of lymphocyte stimulation in seven of eight apparently sensitized subjects and none of thirteen non-sensitized individuals indicates a significant association ($P<0{\cdot}01$), and the results were reproducible. The possibility of error in the microscopic identification of blast cells is well recognized but was minimized in the present work by having one experienced individual examine all the slides. More importantly, all preparations were read 'blind'. In a limited number of experiments the results were confirmed by finding significant, albeit slight, lymphocyte stimulation by the tritiated thymidine uptake technique. Work in this laboratory using the described methods has shown the tritiated thymidine uptake to be less sensitive than the microscopic technique with some other antigens, in agreement with some observations elsewhere (Mills, 1966). Also, the experiments

employing tritiated thymidine were done at a time when the subjects' TDI sensitivity seemed to be waning, as judged by microscopic criteria.

In a limited number of observations comparing results after 4, 5, 6 and 7 days of culture, 6 days appeared to be optimal. In a few experiments with serial dilutions of TDI–HSA 40 and 80, the largest amount of antigen used (final concentration of 25 μg protein/ml culture) gave the greatest degree of lymphocyte stimulation. With TDI–HSA 160, one-tenth of this antigen concentration sometimes was optimal, larger amounts appearing to have a toxic effect on the cells. With the tritiated thymidine method, the use of autologous plasma, rather than foetal calf serum, employing plastic culture tubes, and omitting adsorption of the leucocytes on to glass yielded less variable results. Larger amounts of suitable antigens might have been more stimulating, but problems in preparing highly substituted conjugates and their toxicity for cells set limitations on this approach. Only HSA was tried as a carrier.

While lymphocyte transformation initially was found to occur in association with delayed hypersensitivity (Pearmain, Lycette & Fitzgerald, 1963), it also occurs in apparent association with immediate hypersensitivity, including atopy (Lycette & Pearmain, 1963; Wiener & Brasch, 1965; Zeitz, Van Arsdel & McClure, 1966; Girard *et al.*, 1967). Of particular relevance to this study is the fact that several investigators (Hirschhorn *et al.*, 1963; Holland & Mauer, 1964; Ripps, Fellner & Hirschhorn, 1965; Girard *et al.*, 1967; Halpern, Ky & Camache, 1967) have found stimulation of lymphocytes by drug allergens. Possibly the TDI conjugates used in this work have an analogous effect, the mechanisms of which are not very well understood.

Finally, it should be pointed out that this work does not prove that the immunological mechanisms responsible for the susceptibility of the lymphocytes from TDI-sensitive asthmatics to stimulation by TDI conjugates are the same as those responsible for their asthma. As shown so clearly by the ubiquity of immunological response to penicillin antigens (Levine *et al.*, 1966), for example, the occurrence of some forms of immunological response to drugs and other low molecular weight compounds is not necessarily associated with clinical manifestations of hypersensitivity. However, the fact that in the present work evidence of an immunological response was found only in individuals suspected of being sensitized supports, but does not prove, the hypothesis that TDI asthma is allergic.

ACKNOWLEDGMENTS

This investigation was supported in part by United States Public Health Service Training Grant 5 TI AI 44-09 from the National Institute of Allergy and Infectious Diseases. We are indebted to Dr J. J. Ronayne and Dr V. N. Dodson for making available most of the individuals studied.

REFERENCES

Bach, F. & Hirschhorn, K. (1964) Lymphocyte interaction: a potential histocompatibility test *in vitro*. *Science*, **143**, 813.

Boyden, S.V. (1951) The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera. *J. exp. Med.* **93**, 107.

Bruckner, H.C., Avery, S.B., Stetson, D.M., Dodson, V.N. & Rohayne, J.J. (1968) Clinical and immunologic appraisal of workers exposed to diisocyanates. *Arch. environm. Hlth*, **16**, 619.

BRUGSCH, H.G. & ELKINS, H.B. (1963) Toluene diisocyanate (TDI) toxicity. *New Engl. J. Med.* **268,** 353.

DUNCAN, B., SCHEEL, L.D., FAIRCHILD, E.J., KILLENS, R. & GRAHAM, S. (1962) Toluene diisocyanate inhalation toxicity: pathology and mortality. *Amer. indust. Hyg. Ass. J.* **23,** 447.

FISHER, J.P., MIDDLETON, E., Jr & MENZEL, A.E.O. (1956) Study on passive cutaneous anaphylaxis with serum of allergic patients. *J. Allergy*, **27,** 256.

FUCHS, S. & VALADE, P. (1951) Etude clinque et experimentale sur quelques cas d'intoxication par le desmodur T (diisocyanate de toluène 1-2-4 et 1-2-6). *Arch. Mal. prof.* **12,** 191.

GANDEVIA, B. (1964) Respiratory symptoms and ventilation capacity in man exposed to isocyanate vapor. *Aust. Ann. Med.* **13,** 157.

GIRARD, J.P., ROSE, N.R., KUNZ, M.L., KOBAYASHI, S. & ARBESMAN, C.E. (1967) *In vitro* lymphocyte transformation in atopic patients: induced by antigens. *J. Allergy*, **39,** 65.

HALPERN, B., KY, N.I. & AMACHE, N. (1967) Diagnosis of drug allergy *in vitro* with lymphocyte transformation test. *J. Allergy*, **40,** 168.

HIRSCHHORN, K., BACH, F., KOLODNY, R.L., FIRSCHEIN, I. L.& HASHEM, N. (1963) Immune response and mitosis in human peripheral blood lymphocyte *in vitro*. *Science*, **142,** 1185.

HOLLAND, P. & MAUER, A.L. (1964) Drug-induced *in vitro* stimulation of peripheral lymphocytes. *Lancet*, **i,** 1368.

ISHIZAKA, K. & ISHIZAKA, T. (1968) Skin reactive properties of soluble antigen- γE antibody complexes. *Fed. Proc.* **27,** 316.

KONZEN, R.B., CRAFT, B.F., SCHEEL, L.D. & GORSKI, C.H. (1966) Human response to low concentrations of p, p-diphenylmethane diisocyanate (MDI). *Amer. indust. Hyg. Ass. J.* **27,** 121.

LEVINE, B.B., FELLNER, M.J., LEVYTSKA, V., FRANKLIN, E.C. & ALISBERG, N. (1966) Benzylpenicilloyo-specific serum antibodies to penicillin in man. II. Sensitivity of the hemagglutination assay method, molecular classes of the antibodies detected, and antibody titres of randomly selected patients. *J. Immunol.* **96,** 719.

LICHTENSTEIN, L.M. & OSLER, A.G. (1964) Histamine release from human leukocytes by ragweed pollen antigen. *J. exp. Med.* **120,** 507.

LYCETTE, R.R. & PEARMAIN, G.E. (1963) Further observations on antigen-induced mitosis. *Lancet*, **ii,** 386.

MATHEWS, K.P. & SPEAR, H.J. (1961) A comparative study of the hemagglutinating and skin sensitizing activities of ragweed sensitive human sera. *J. Immunol.* **87,** 274.

MILLS, J.A. (1966) The immunologic significance of antigen induced lymphocyte transformation *in vitro*. *J. Immunol.* **97,** 1185.

MOORHEAD, J.F. & MCFARLAND, W. (1966) Efficiency of scintillation counting of tritium labelled DNA-protein in the mixed leucocyte reaction *in vitro*. *Nature* (*Lond.*), **211,** 1157.

NIEWENHUIS, R., SCHEEL, L., STEMMER, K. & KILLENS, R. (1965) Toxicity of chronic low level exposures to toluene diisocyanate in animals. *Amer. indust. Hyg. Ass. J.* **26,** 143.

OVARY, Z. (1958) Immediate reactions in the skin of experimental animals provoked by antibody–antigen interaction. *Progr. Allergy*, V, 459.

PEARMAIN, G., LYCETTE, R.R. & FITZGERALD, P.H. (1963) Tuberculin-induced mitosis in peripheral blood leukocytes. *Lancet*, **i,** 637.

PERELMUTTER, L., FREEDMAN, S.O. & SEHON, A.H. (1962) Demonstration of multiple hemagglutinating factors in sera of ragweed-allergic individuals with the help of immunosorbents. *J. Immunol.* **89,** 623.

REINL, W. (1953) Über Erkrankungen bei der Verarbeitung von Kunststoffen auf der Basis der Polyurethan. *Zbl. Arbeitsmed.* **3,** 103.

RIPPS, C., FELLNER, M.J. & HIRSCHHORN, K. (1965) Response of lymphocyte to tetracycline. *Lancet*, **ii,** 1965.

SCHEEL, L.D., KILLENS, R. & JOSEPHSON, H. (1964) Immunochemical aspects of toluene diioscyanate (TDI) toxicity. *Amer. indust. Hyg. Ass. J.* **25,** 179.

SCHURMANN, D. (1955) Gesundheitsschaden durch neuartige Lacke und Schaumstoffe. *Dtsch. med. Wschr.* **80,** 1661.

SHORE, P.A., BURKHALTER, A. & COHN, V.H., Jr (1959) A method for the fluorometric assay of histamine in tissues. *J. Pharmacol. exp. Ther.* **127,** 182.

SILVER, H.M. (1963) Toluene diisocyanate asthma. *Arch. intern. Med.* **112,** 401.

SWEET, L.C. (1968) Toluene-diisocyanate asthma. *Univ. Mich. med. Center J.* **34,** 27.

SWENSSON, A., HOLMQUIST, C. & LUNDGREN, K. (1955) Injury to the respiratory tract by isocyanates used in

making lacquers. *Brit. J. industr. Med.* **12,** 50.

THOMPSON, G.E. & SCHEEL, L.D. (1968) Alteration of lung pathology from diisocyanate by glycemic or sensitizing agents. *Arch. environm. Hlth,* **16,** 363.

WIENER, S. & BRASCH, J. (1965) The mitogenic effect of grass-pollen extract. *Med. J. Aust.* **1,** 148.

WOODBURY, J.W. (1956) Asthmatic syndrome following exposure to tolylene diisocyanate. *Industr. med. Surg.* **25,** 540.

ZAPP, J.A., JR (1957) Hazards of isocyanates in polyurethane foam plastic production. *Arch. indust. Hlth,* **15,** 324.

ZEITZ, S.J., VAN ARSDEL, P.P., JR & MCCLURE, K.K. (1966) Specific response of human lymphocytes to pollen antigen in tissue culture. *J. Allergy,* **38,** 321.