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Use of Hexyl Isocyanate Antigen to Detect Antibodies to Hexamethylene Diisocyanate (HDI) in Sensitized Guinea Pigs and in a Sensitized Worker

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ABSTRACT

Use of Hexyl Isocyanate Antigen to Detect Antibodies to Hexamethylene Diisocyanate (HDI) in Sensitized Guinea Pigs and in a Sensitized Worker. Karol, M.H. and Hauth, B.A. (1982). *Fundam. Appl. Toxicol.* 2:108-113. Hypersensitivity to hexamethylene diisocyanate (HDI) has been reported following occupational exposure. Diagnosis of sensitivity is usually made from clinical evaluation of symptomatology. An *in vitro* serologic assay for HDI sensitivity was developed by immunizing guinea pigs with HDI and with hexyl isocyanate (HMI). Animals injected intradermally with HMI produced hapten-specific antibodies whereas guinea pigs injected with HDI produced antibodies specific for larger determinants which included the HDI hapten. The larger determinants were assumed to be composed of portions of "self" molecules which reacted *in vivo* with HDI. Serum albumin appeared to be one such molecule. No cross reactions were noted between antibodies to HDI and another widely used industrial isocyanate, toluene diisocyanate (TDI). Antigens effective in detecting antibodies to HDI or HMI were tested for ability to detect reaginic antibodies in a worker with clinical "HDI" asthma. Using a radioimmunoassay (RAST), antibodies reacted with conjugates containing either HDI or HMI as haptens. In addition, the prevalance of HDI polyisocyanates (Desmodur N) in spray paints prompted its use as a hapten. Antibodies reacted with Desmodur N antigen conjugates in RAST. RAST inhibition further indicated that Desmodur N antigen reacted more readily with the patient's antibodies than did HDI or HMI antigens. These results suggest that the patient may have been exposed to HDI polyisocyanates in spray paint application. Use of Rast inhibition for diagnosis of sensitivity may indicate the precise sensitizing agent within a mixture.

INTRODUCTION

Isocyanates have found widespread use in the production of polyurethane for manufacture of cars, paints, furniture, bedding and many other goods. Toluene diisocyanate (TDI), the most widely used of the isocyanates, has frequently been associated with clinical sensitization of workers (Pepys *et al.*, 1972; Bruckner *et al.*, 1968). Use of other diisocyanates has also resulted in cases of isocyanate sensitivity (Charles *et al.*, 1976; Konzen *et al.*, 1966; Zeiss *et al.*, 1980).

Diagnosis of isocyanate sensitivity using *in vitro* serologic techniques has been notoriously difficult (Porter *et al.*, 1975; Butcher *et al.*, 1976). Recently, however, several groups of investigators have reported successful identification of IgE antibodies to TDI as a result of employing a p-tolyl isocyanate protein conjugate as the test antigen (Karol *et al.*, 1978a; Baur *et al.*, 1980; Butcher *et al.*, 1980). Use of the monofunctional isocyanate for antigen preparation prevented cross-linking polymerization of protein and resulted in homogeneous antigen preparations which were readily characterized (Karol *et al.*, 1978b). Confirmation of the ability of p-tolyl isocyanate antigen to detect antibodies to TDI was obtained from an animal model for isocyanate sensitivity (Karol, 1980; Karol *et al.*, 1980). Guinea pigs sensitized to TDI produced antibodies which were detected using protein conjugates composed of p-tolyl isocyanate or o-tolyl isocyanate as haptens.

Recently, other diisocyanates have gained use in manufacture of polyurethane based products. Hexamethylene diisocyanate (HDI) and HDI polyisocyanates, for example the biuret structure of HDI trimer (DES-N, Mobay Chemical Corporation) which contains between 0.6 - 2.0% free HDI, are major ingredients in two component polyurethane spray paints. These paints are used in automotive and airplane coatings. Cases of clinical sensitivity to HDI (Charles *et al.*, 1976) and trimethyl HDI (Hjorth, 1975) have been reported.

Development of a serologic diagnostic assay for antibodies to HDI presents a problem analogous to that encountered with TDI, *i.e.* preparation of a suitable test antigen. Previously, we reported that guinea pigs sensitized by repeated inhalation of hexyl isocyanate-ovalbumin conjugate responded with production of antibodies directed exclusively to the hexyl isocyanate (HMI) hapten (Karol *et al.*, 1979a). It remained to be investigated whether animals exposed to HDI would similarly produce hapten (HDI)-specific antibodies and whether such antibodies were best detected using antigens prepared with hexyl (*mono*) isocyanate as hapten. This study reports the production and specificity of antibodies produced in guinea pigs following intradermal injection with HDI and with HMI. Antigens were also tested for ability to detect antibodies in a worker with clinical HDI sensitivity.

MATERIALS AND METHODS

Sensitization

Female, English smooth haired guinea pigs (Hilltop Lab Animals, Scottsdale, PA) weighing 250-300 g were used throughout the study. Groups of 4 animals were sensitized by intradermal injection with 50 μL HDI (Mobay Chemical Corpo-

ration 98% purity) or 50 μL HMI (Eastman Organic Chemicals) into each of two shaved dorsal sites. Blood was drawn from guinea pigs 14 days following immunization.

Isocyanate conjugate antigens

Isocyanate conjugates were prepared as previously described (Karol, 1980). Briefly, 100 μL isocyanate were added to 100 mL of 0.5% solution of guinea pig serum albumin (GSA, Fraction V, Sigma Chemicals) in borate buffer, pH 9.4. Dependent upon the reactivity of the isocyanate hapten, the reaction was performed at a temperature ranging from 0 °C to 60 °C for 0.5 to 3.0 hr. Reactions were terminated by addition of excess monoethanolamine. Reaction mixtures were filtered, the supernatant dialyzed against distilled water and conjugates were isolated by lyophilization. The extent of haptenic coupling to protein was estimated using trinitrobenzene sulfonic acid (Synder and Sobocinski, 1975) to detect unreacted amino groups. The following isocyanate-protein conjugates were prepared: HDI-GSA (22% average hapten substitition of amino groups), HDI-human serum albumin (HDI-HSA 37% substitution), HDI-ovalbumin (HDI-OA, 79% substitution), HMI-GSA (45% substitution), HMI-HSA (62% substitution), HMI-OA (83% substitution), DES N-HSA (80% substitution), n-butyl isocyanate-GSA (85% substitution), cyclohexyl isocyanate-GSA (78% substitition), phenyl isocyanate-GSA (96% substitution), p-tolyl isocyanate-GSA (90% substitution), methyl isocyanate-GSA (5% substitution), methyl isocyanate-OA (79% substitution), TDI-GSA (90% substitution), diphenylmethane diisocyanate-GSA (MDI-GSA, 62% substitution) and dicyclohexylmethane diisocyanate-GSA (Hylene-GSA, 95% substitution). Monoisocyanates were purchased from Eastman or Aldrich Chemicals. Diisocyanates were a gift from Mobay Chemical Corporation and had the following purities: HDI (Mondur HX, 98% pure), TDI (80:20 mixture 2,4/2,6 TDI, Mondur TD_{80}, 99.7% pure), MDI (Mondur MR, 50% monomer, remainder higher molecular weight polymers, 99.5% pure) and dicyclohexylmethane diisocyanate (Hylene W, 99.3% pure). The HDI trimer (DES-N) was also a gift from Mobay and contained 1% free HDI.

Serological techniques

Blood was drawn from guinea pigs by clipping the nailbed. Animals were bled prior to sensitization and 14 days following sensitization. Terminal bleedings were obtained by cardiac puncture. All sera were stored at −20 °C.

Immunodiffusion

Immunodiffusion was performed using slides coated with 1% agarose (Biorad). Where indicated, sera were concentrated five fold using Minicon macrosolute concentrators (Amicon, A125). Antigens were used as 0.1% solutions in 0.05M borate buffer, pH 8.3.

Passive cutaneous anaphylaxis (PCA)

Cytophilic antibodies were detected by injecting 0.1 mL serum (diluted with saline) intradermally into the shaved backs of normal guinea pigs. Following a 6 hr latent period, animals were challenged by intravenous injection with 0.5 mL solution containing 2.5 mg antigen and 5 mg Evans blue dye. Reactions were read after 30 min.

Antibody binding to HDI [^{125}I] GSA

Anti-HDI antibodies were additionally detected using the ammonium sulfate precipitation technique (Minden and Farr, 1978). HDI-GSA was radiolabelled with ^{125}I using the lactoperoxidase method (Radioiodination System, New England Nuclear). The radiolabelled antigen had a specific activity of 4400 cpm/ng (Packard Gamma Scintillation Counter, 70% efficiency). For the assay, 100 μL serum (diluted with borate buffer, pH 8.3 containing 0.1% GSA) were incubated with 25 μL HDI-[^{125}I] GSA (1.38ng, 10 000 cpm) at 37 °C for 2 hr. The reaction mixture was chilled in ice and globulin-bound HDI-[^{125}I] GSA was separated from free HDI-[^{125}I] GSA by addition of 100 μL saturated ammonium sulfate solution (SAS). After 1 hr at 0 °C, tubes were centrifuged at 9080g. The amount of free radiolabelled antigen was determined by counting 150 μL supernatant.

Inhibition of antibody binding

The percentage of HDI-[^{125}I] GSA precipitated by each serum dilution was plotted versus the serum dilution. From this plot, the dilution of antiserum which precipitated approximately 30% of the antigen was determined and used for evaluation of antibody specificity. For inhibition, 100 μL serum dilution were incubated with 50 μL unlabelled hapten-protein conjugate (1 μg to 50 μg) in borate buffer, pH 8.3. Incubation was for 10 min at 37 °C. HDI-[^{125}I] GSA (25 μL, 10 000 cpm) was then added and incubation continued at 37 °C for 2 hr. Reaction tubes were placed in an ice bath and 150 μL chilled SAS were added to precipitate serum globulin. The amount of HDI-[^{125}] GSA remaining in the supernatant in tubes containing isocyanate hapten-protein inhibitors was compared with amounts remaining in the supernatant when protein alone was added as inhibitor.

Human studies

Serum was obtained from a car painter with an impressive history of occupational asthma. Sensitivity was assumed to have been induced by HDI exposure. The serum was a kind gift from Dr. Lars Belin, Goteburg, Sweden. It was found to have a total IgE content of 58 Units/mL (PRIST, Pharmacia Diagnostics).

Radioallergosorbent tests (RAST)

RAST assays were performed to detect specific IgE antibodies in human sera. For the assays, 50 μL serum were incubated with an antigen coated disc (Karol, 1981) for 16 hr at ambient temperature. Discs were washed, then 50 μL [^{125}I] rabbit anti-human IgE (RAST Reagent, Pharmacia Diagnostics) were added and discs incubated an additional 16 hr at room temperature. Following thorough washing, discs were counted using a gamma scintillation counter. Titers were expressed as the percent of added radioactivity bound to discs. Control sera were from 5 non-sensitized persons involved in isocyanate production. Each of the control sera had a total IgE content comparable to that of the sensitized individual (40 - 80 Units/mL).

RAST inhibition

The ability of various isocyanate conjugates to inhibit RAST reactions was determined using a modification (Karol, 1981) of a standard procedure (Gleich *et al.*, 1974). Sera (25 μL) were first incubated with isocyanate conjugate in 25 μL buffer (PRIST diluent, Pharmacia Diagnostics). Control tubes contained serum and 25 μL buffer. After 2 hr at 37 °C, an antigen-coated disc was added to each tube and samples were incubated for 16 hr at ambient temperatures. Discs were washed and rabbit anti-IgE were added as described above for RAST.

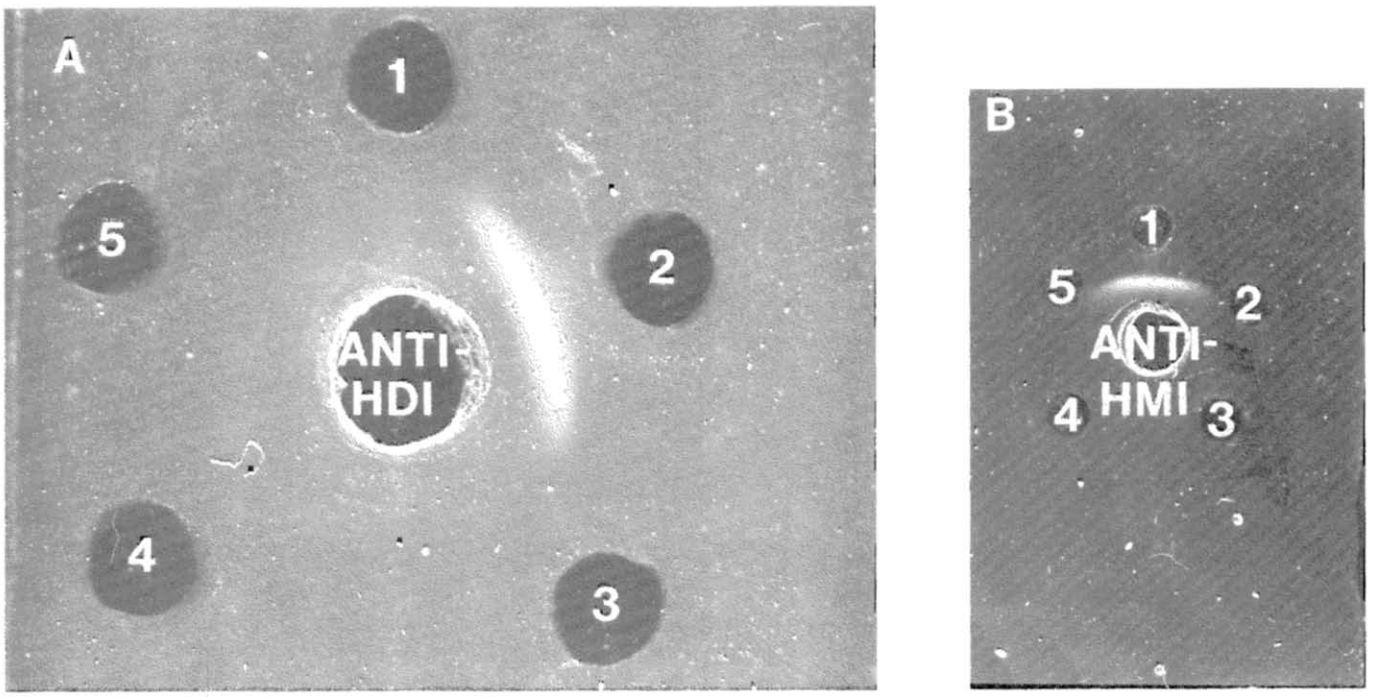

FIG. 1. Double diffusion in agarose. A. Serum from a guinea pig injected with HDI was placed in the central well. Antigens (all 1 mg per mL borate buffer pH 8.3) were placed in peripheral wells as follows: Well 1, TDI-GSA; Well 2, HDI-GSA; Well 3, hexyl isocyanate-GSA; Well 4, GSA; Well 5, p-tolyl isocyanate-GSA. A precipitin band formed between the antiserum and HDI-GSA. B. Antiserum from a guinea pig injected with HMI was concentrated 5 fold and placed in the central well. A precipitation band formed between this antiserum and HMI-GSA (well 1). No bands formed between antiserum and: HDI-GSA (well 2), GSA (well 3), TDI-GSA (well 4) and p-tolyl isocyanate-GSA (well 5).

RESULTS

Gel diffusion

Sera from animals injected with HDI and HMI were evaluated for antibody using double diffusion in gel. Animals within each group displayed similar reactions. Typical results are shown in Fig. 1. Guinea pigs injected with HDI produced antibodies which precipitated with HDI-GSA (Fig. 1A). No reactions were observed when these sera were diffused against TDI-GSA, HMI-GSA, MDI-GSA or GSA. Guinea pigs injected with HMI produced antibodies which precipitated only with HMI-GSA (Fig. 1B) and showed no reaction with HDI-GSA. Using this assay, no cross-reactivity was apparent between antibodies to HDI and HMI. In all instances, sera taken before immunization were negative when diffused against all antigens.

Passive cutaneous anaphylaxis (PCA): anti-HDI antibodies

A more sensitive assay was employed to evaluate specificities of antibodies induced by HDI and HMI. Results of PCA assays for each of the experimental animals injected with HDI are listed in Table 1. Highest titers were demonstrated with HDI-GSA. Prominent reactions were also noted with antigens containing aliphatic monoisocyanates as haptens, for example, HMI-GSA, n-butyl isocyanate-GSA, cyclohexyl isocyanate-GSA and methyl isocyanate-GSA. By comparison, antibodies reacted weakly, if at all, with aromatic isocyanate-GSA conjugates such as phenyl isocyanate-GSA and p-tolyl isocyanate-GSA.

No reactions were detected when GSA was used for antigen challenge in PCA. However, GSA determinants appeared to be involved in reaction of anti-HDI antibody with isocyanate-GSA conjugates since hapten conjugates formed using ovalbumin as carrier protein (HDI-ovalbumin and HMI-ovalbumin) gave only minimal reactions with anti-HDI antibodies.

Anti-HMI antibodies

The PCA technique was used to evaluate specificity of anti-hexyl isocyanate antibodies (Table 2). Greatest reactions were observed with HMI-GSA and n-butyl isocyanate-GSA. Similar to findings observed with anti-HDI antibodies, little reactivity was detected with GSA conjugates containing aromatic isocyanates as haptens. However, antibodies to HMI differed from anti-HDI antibodies in their lack of requirement for GSA determinants. Anti-HMI antibodies reacted with HMI hapten when the latter was presented on ovalbumin as carrier. The strict anti-hapten specificity of HMI-induced antibodies was further demonstrated by the failure of these antibodies to react with methyl isocyanate-GSA. Anti-HMI antibodies also failed to react with HDI-GSA. This result may reflect a lack of externally exposed haptenic groups on HDI-GSA.

Specificity of HDI-[^{125}I] GSA-antibody binding

The specificity of antibodies induced by HDI, and reactive with HDI-GSA, was evaluated by inhibition studies using radiolabelled HDI-GSA and serum dilutions which bound approximately 30% of the antigen. Results of inhibition studies are presented in Table 3. With each of the two sera studied, HDI-GSA was the most potent inhibitor of reaction. HMI-GSA was a less effective inhibitor since approximately 3 fold more HMI-GSA, compared with HDI-GSA, was required to achieve 50% inhibition. Cyclohexyl isocyanate-GSA showed some inhibitory activity, whereas methyl isocyanate-GSA and several aromatic isocyanate-GSA conjugates displayed little activity as inhibitors.

RAST

Serum was obtained from a patient with acute "HDI" pulmonary hypersensitivity. The serum was evaluated for specific IgE antibodies using the RAST technique. For these studies, three different "hexyl isocyanate" containing antigens were used for coating assay discs. Results of all RAST assays are presented in Table 4. Serum from the sensitized patient bound 7.2% of the added radioactivity in the HDI-HSA RAST. This binding is highly significant ($P < .01$) when compared with binding demonstrated by the control sera. The latter sera were from non-sensitized isocyanate workers and were selected because their total IgE content matched that of the patient's. The specificity of binding by the patient's serum was confirmed using RAST inhibition. When 100 μg HDI-HSA was incorporated into the assay buffer, binding by the experimental serum was reduced to 1.4%. The HDI-HSA inhibitor had no effect on binding by control sera to HDI-HSA coated discs.

The patient's serum was also positive in RAST assay using HMI-HSA coated discs as well as DES N-HSA coated discs. Specificity of binding was tested in HMI-HSA RAST. In the presence of HMI-HSA, binding was reduced to background levels (see Legend, Table 4).

To further evaluate the specificity of the patient's antibodies, the various "hexyl-isocyanate" containing antigens were compared with each other as inhibitors in HDI-HSA RAST. Results are shown in Table 5. DES N-HSA was found to be the most effective inhibitor of the reaction. It was more potent than HDI-HSA in inhibiting HDI-HSA RAST.

DISCUSSION

Isocyanates are recognized as etiologic agents of industrial sensitization. Sensitization is believed to occur as the result of interaction of isocyanates with "self" components resulting in formation of complete antigens (Claman *et al.*, 1980). The problem of early identification of persons who have developed sensitivity remains one of the prime objectives of preventive medicine.

Recently a major advance toward early detection of sensitivity to TDI was made by development of a RAST assay employing p-tolyl isocyanate-serum albumin as antigen (Karol, *et al.*, 1978a; Karol *et al.*, 1979c). The assay gained acceptance (Baur *et al.*, 1980; White *et al.*, 1980; Gallagher *et al.*, 1981) when it was demonstrated that animals sensitized to TDI produced antibodies which were detected using the tolyl isocyanate-serum albumin antigen (Karol, 1980; Karol *et al.*, 1980).

Diagnosis of HDI sensitivity is currently based on clinical assessment of symptomatology. Development of a diagnostic serologic assay for this isocyanate may allow early detection of isocyanate sensitivity thus precluding onset of clinical reactions. In the current study, animals were immunized with HDI

TABLE 1
PCA Titers of Sera from Guinea Pigs Injected with HDI

Challenge Antigen	Antibody Titer[A] Animal No. 7	8	9	10
Hexamethylene diisocyanate-GSA[B]	8	8	6	9
Hexamethylene diisocyanate-OA	4	<2	<2	<2
Methyl isocyanate-GSA	6	5	5	6
Methyl isocyanate-OA	<2	<2	-	<2
Hexyl isocyanate-GSA	6	5	5	-
Hexyl isocyanate-OA	<2	<2	<2	<2
n-Butyl isocyanate-GSA	7	7	6	8
Cyclohexyl isocyanate-GSA	5	5	5	-
Phenyl isocyanate-GSA	<3	<3	<3	-
p-Tolyl isocyanate-GSA	<3	<3	-	5
Toluene diisocyanate-GSA	4	3	4	4
Diphenylmethane diisocyanate-GSA	4	<2	<2	-
Dicyclohexylmethane diisocyanate-GSA	<2	-	<2	<2
GSA	<1	<1	-	<1

[A]Titer is the reciprocal of the highest serum dilution yielding a positive reaction site of at least 5 mm diameter, expressed as $\log_2$.

[B]GSA, guinea pig serum albumin; OA, ovalbumin.

TABLE 2
PCA Titers of Sera from Guinea Pigs Injected with Hexyl Isocyanate

Challenge Antigen	Antibody Titer[A] Animal No. 1	2	3	4
Hexyl isocyanate-GSA	8	7	8	8
Hexyl isocyanate-OA	6	5	6	8
Methyl isocyanate-GSA	<2	<2	<2	<2
Methyl isocyanate-OA	<2	-	<2	-
n-Butyl isocyanate-GSA	7	7	7	8
Cyclohexyl isocyanate-GSA	7	6	6	7
Phenyl isocyanate-GSA	<2	-	<2	3
p-Tolyl isocyanate-GSA	<3	<3	3	3
Hexamethylene diisocyanate-GSA	<1	<1	<1	<1
Dicyclohexyl methane diisocyanate-GSA	<4	<4	<4	<4

[A]Titer is the reciprocal of the highest serum dilution yielding a positive reaction site of at least 5 mm diameter, expressed as $\log_2$.

Abbreviations as in Table 1.

TABLE 3
Inhibition of HDI-[^{125}I] GSA Binding to Anti-HDI Sera by Isocyanate Hapten-Conjugates

Inhibitor	Amount Isocyanate Inhibitor Added[A] Serum #7[B]	Serum #9
HDI-GSA	0.8 μg	3.3 μg
HMI-GSA	3.0	8.3
Cyclohexyl isocyanate-GSA	7.0	----
Methyl isocyanate-GSA	$\geqslant$ 25 (8%)	25 (4%)
TDI-GSA	25	35
MDI-GSA	22	35 (40%)
Dicyclohexyl methane-GSA	22	----

[A]From dose-response determinations, the amount of inhibitor required for 50% reduction of antigen binding was obtained and is presented here. In cases where 50% inhibition was not achieved, the maximum degree of inhibition is indicated in parentheses.

[B]Serum #7 was diluted 1:10 for assay, serum #9 was diluted 1:3.

TABLE 4
RAST Evaluation of "HDI" Sensitized Worker

Serum	% ^{125}I Bound		
	HDI-HSA RAST[A]	HMI-HSA RAST	DES N-HSA RAST
"HDI" asthmatic	7.2%[B]	15.3[C]	15.3
Control A	1.5	1.5	1.9
Control B	1.4	1.2	1.6
Control C	1.4	1.2	1.6
Control D	1.4	1.2	1.6
Control E	1.4	1.2	1.6

[A]Abbreviations as in Table 3.

[B]RAST inhibition. Incorporation of 100 μg HDI-GSA into the assay buffer reduced binding to 1.4% and had no effect on binding by control sera.

[C]RAST inhibition. Binding was reduced to 1.3% when 100 μg HMI-HSA was incorporated into the assay buffer.

and responded with production of antibodies. Several "hexyl isocyanate" haptenated antigens were prepared and tested for ability to detect the antibodies produced.

Animals injected with HDI produced a small population of antibodies with specificity toward the "hexyl isocyanate" moiety as indicated in Table 3. The majority of antibodies reacted with HDI only when the hapten was bound to GSA (see Table 1). This reactivity probably reflects contribution of GSA determinants to the antigen formed *in vivo* following HDI administration. The extensive reaction of antibodies with methyl isocyanate-GSA, but not with methyl isocyanate-OA (Table 1) lends support to this assumption. Of significance was the finding that sera from HDI-injected animals showed no reactivity, in PCA assay, with GSA. Antibodies appeared to recognize an "isocyanate-modified" carrier rather than GSA. Failure of anti-HDI antibodies to react with phenyl isocyanate-GSA or other aromatic isocyanate-GSA conjugates indicated that, for recognition by these antibodies, GSA must be "modified" by an aliphatic isocyanate. Recently, Zeiss *et al.* (1980) presented evidence for antibodies to a "modified" HSA in workers exposed to trimellitic anhydride. The latter is a highly reactive chemical used in the manufacture of plastics.

In contrast to the above results, guinea pigs injected with HMI produced almost exclusively hapten-specific antibodies (Table 2). The exclusive anti-hapten specificity of antibodies raised to HMI parallels results obtained previously when guinea pigs were sensitized to an HMI-OA conjugate (Karol *et al.*, 1979a). In the latter study, antibodies were produced to hexyl isocyanate hapten whereas antibodies were not reactive with the ovalbumin carrier.

The hapten specificity shown by anti-HMI antibodies enabled use of the antibodies as a probe to detect HMI determinants on antigens. As seen in Table 2, anti-HMI antibodies showed no reaction with HDI-GSA. This result implies the absence or sparsity of exposed "hexyl isocyanate" hapten on HDI-GSA. It also suggests a cross-linked, polymerized structure for HDI-GSA. TDI has previously been identified as a bifunctional protein cross-linking reagent (Schick and Singer, 1961) and the polymeric nature of TDI-GSA has been demonstrated (Karol *et al.*, 1979b). The bifunctional reactivity of diisocyanates with proteins may explain the limited hapten-specific immune response to HDI observed in the current study.

The availability of serum from a patient with clinically diagnosed HDI asthma enabled evaluation of HDI-HSA and HMI-HSA as antigens to detect antibodies in man as a result of occupational exposure, presumably to HDI. Results of RAST assays (Table 4) indicated that both HDI-HSA and HMI-HSA effectively identified reaginic antibodies in this serum. RAST binding was not the result of total IgE in the serum since control sera contained the same amount of total IgE yet showed no reactivity in RAST. In addition, RAST inhibition studies confirmed the specificity of reactions. Comparison of the two RAST assays indicated that although both systems detected antibody in the patient's serum, HMI-HSA in RAST better distinguished the sensitized serum from control sera.

In the spray painting operation, HDI polyisocyanate (for example, Desmodur N) is frequently used rather than HDI itself. The polyisocyanate always contains from 0.6 - 2.0% free HDI. This occurrence prompted examination of the patient's serum for antibodies to DES-N. RAST, using DES-N-HSA coated discs, effectively detected reaginic antibodies in the serum. RAST inhibition indicated that DES-N antigen had greater reactivity with antibodies than did HDI- or HMI conjugate (Table 5). This finding implies that the patient may have been exposed and sensitized to Desmodur N in the spray paint product.

In summary, HDI and HMI were each found to be immunogenic in guinea pigs. HMI induced predominantly hapten-specific antibodies whereas HDI induced antibodies specific for aliphatic isocyanate-GSA determinants. Using RAST, reaginic antibodies were detected in serum from a patient diagnosed with HDI asthma. The exquisite specificity of the

TABLE 5
Inhibition of HDI-HSA RAST
("HDI" Asthmatic Serum)

Inhibitor	% Inhibition[A]
None	0
20 μg HMI-HSA	75 ± 1
20 μg HDI-HSA	82 ± 0.5
20 μg DES N-HSA	89 ± 1

Assay tubes contained 25 μL serum and 20 μg inhibitor in 25 μL buffer. After 2 hr at 37 °C, an HDI-HSA coated disc was added to each tube and incubation continued for 16 hr at ambient temperature.

[A]Average of duplicate determinations.

immune system indicated that the patient may have been exposed to DES-N, or to HDI and DES-N simultaneously, in the spray paint operation.

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