衛生化学

別冊

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human renal MT-1 and rabbit hepatic MT-2; the primary structures of mammalian metallothionein are similar. Thus, the fuse formation may be due to the same amino acid sequence in the antigenic determinant against antibody in mammalian metallothioneins. On the other hand, Chen and Whanger²²⁾ reported that the two species of MT isolated from the same organ of the rat possess different antigenic properties. Our results of double diffusion test between carp hepatopancreatic and kidney metallothionein showed a clear difference of structures and sequence of antigenic determinant against antibody.

We found that the amino acid composition of carp metallothionein is different from that of mammalian metallothionein, and the antigenic determinant in carp metallothionein is different from that in mammalian metallothioneins.

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家庭用品による接触皮膚炎の原因化学物質の追求（第２報）
市販ゴム手袋中の老化防止剤の分析およびパッチテスト陽性率

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Chemical Approach to Contact Dermatitis Caused by Household Products. II. Analysis of Antioxidants in Commercial Rubber Gloves and Incidence of Positive Reactions to Antioxidants in Patch Testing

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Antioxidants in 37 different brands of commercially available rubber gloves mainly for domestic use were analysed by the method previously reported (M. Kaniwa, S. Kojima, A. Nakamura, M. Ishihara, Eisei Kagaku, **28**, 137 (1982)).

It was clarified that almost every brand of the domestic gloves contained one or two of the following phenolic antioxidants: 2,2′-methylene-bis (4-methyl-6-*tert*-butylphenol) (MBMBP), styrenated phenol (SP), 2,2′-isobutylidene-bis (4,6-dimethylphenol) (IBBDMP), 4,4′-butylidene-bis (3-methyl-6-*tert*-butylphenol) (BBMBP). A nitrogen-containing antioxidant, *N*-isopropyl-*N*′-phenyl-*p*-phenylenediamine (IPPD), known as a strong sensitizer, was detected in one brand of gloves for industrial use. The frequencies of detection of the antioxidants were as follows: MBMBP, 19/37; SP, 8/37; IBBDMP, 6/37; BBMBP, 1/37; IPPD, 1/37.

Patch testing to various antioxidants was also studied in 40 patients with allergic contact dermatitis from rubber or poly (vinyl chloride) materials. No one reacted to the phenolic antioxidants tested, including MBMBP and SP. On the other hand, about one third of the patients reacted to IPPD.

From the results, it is preferred to use the phenolic antioxidants for not only domestic but also industrial rubber gloves.

Keywords —— antioxidant determination; commercial rubber gloves; 2,2′-methylene-bis (4-methyl-6-*tert*-butylphenol); styrenated phenol; 2,2′-isobutylidene-bis (4,6-dimethylphenol); 4,4′-butylidene-bis (3-methyl-6-*tert*-butylphenol); *N*-isopropyl-*N*′-phenyl-*p*-phenylenediamine; patch testing; positive reaction incidence

緒　　言

厚生省家庭用品安全対策室の主宰による"家庭用品による接触皮膚炎に関するモニター病院制度"を通じて，ゴム手袋によると考えられる接触皮膚炎の事例がいくつか挙がってきている.1)

我々はそのうちの１つ，作業用ゴム手袋によるアレルギー性接触皮膚炎の事例について原因化学物質の検索を行い，その結果を前報2)で報告した．そして強い感作性

が知られている老化防止剤の *N*-isopropyl-*N′*-phenyl-*p*-phenylenediamine (IPPD) が主な原因化学物質であると結論づけることができた．

しかし，このように原因化学物質を特定できるアレルギー性接触皮膚炎の事例は少ない．その理由は前報にも述べたように，製品中に含まれる化学物質が不明の場合が多く，パッチテストの対象化学物質の選定が難しいからである．こうした状況を改善するためには市販製品中の化学物質の使用実態を把握しておくことがまず必要である．

そこで，市販ゴム手袋中のゴム添加剤のうち，老化防止剤の使用実態を把握するために小売店で購入したもの，モニター病院を通じて入手したものなど，37点のゴム手袋について，どのような種類の老化防止剤がどのくらいの量でゴム手袋中に存在するかを調査した．その結果，市販ゴム手袋中に使用されている老化防止剤はほとんどフェノール系化合物であることが判った．

ところが，接触皮膚炎の原因物質を詳細に記載した Cronin や Fisher の成書にもフェノール系老化防止剤の感作性に関するまとまった記述は見当らない.[3,4]

そこで，ゴム手袋から高頻度で検出された2種のフェノール系老化防止剤を含めた13種の老化防止剤について，皮膚科外来を受診した接触皮膚炎患者を対象としてパッチテストを行い，各々の老化防止剤の陽性率を求めた．その結果，フェノール系老化防止剤はいずれもほとんど陽性反応が認められなかった．その他では IPPD がとびぬけて高い陽性率を示した．

以上の結果について報告する．

実験方法

A．市販ゴム手袋中の老化防止剤の分析　**1．試料**——分析したゴム手袋37点についてブランド名，メーカー名，用途，色調を Table Ia, Ib に示した．37点のうち30点は1982年2月—4月にかけて東京都，千葉市，大阪市，高松市の小売店より購入したもの，2点は医療用手袋を譲渡されたもの，5点はモニター病院を通じて入手したものである．用途別では，家庭用30点，医療用2点，軽作業用（薄手）4点，前報で分析試料とした重作業用（厚手）1点であった．メーカー，ブランドについては，国内メーカー11社による26ブランド，外国メーカー4社，輸入発売元7社による11ブランドであった．

なお，プラスチックスに繁用され，ゴム製品にも使用されている 2,6-di-*tert*-butyl-4-methylphenol (BHT) も分析対象としたが，BHT を含むゴム手袋が入手できなかったので，その代りに BHT を含むことを確認した医療用のイソプレンゴムシート（昭和ゴム製）を抽出方法の検討の際の試料として用いた．我々の分析法で定量したところ，そのシートの BHT の定量値は 963 µg/g であった．

2．試薬——使用した老化防止剤，可塑剤の化学名，本文中の略称，商品名，メーカー名を Table II に示した．これらは精製せずに使用した．その他の試薬は原則として特級を用いた．なお，2,2′-isobutylidene-bis(4,6-dimethylphenol) (IBBDMP) は以下のように合成し，標品とした．

すなわち，2,4-dimethylphenol 2.44 g (2×10^{-2} mol) を 50 ml のナス形フラスコにとり，エタノール 10 ml を加えて溶かし，さらに濃硫酸約 1 ml を加えた後，isobutyraldehyde 約 1 ml を滴加し，80°C の水浴上で10時間還流した．冷後，水約 40 ml を加えて希釈し，エーテル 50 ml で4回振とうして抽出した．抽出液は合わせ，無水硫酸ナトリウムで乾燥後，エバポレーターでエーテルを留去し，3.52 g の残留物を得た．

内径 20 mm のガラス製クロマト管に Merck 社製のカラムクロマトグラフィー用 Kieselgel 60(70—230 mesh) 30 g をヘキサンで湿式法により充塡したカラムに残留物を吸着させ，ヘキサン，5%，10%，50%-エーテル含有ヘキサン，エーテル各々 100 ml で溶離させた．主生成物は10%エーテル含有ヘキサン分画にほぼ溶出した．その分画について溶媒を留去した後，ヘキサンから再結晶して無色針状晶 (mp 155—156°C) 2.01 g を得た．その IR, NMR スペクトルを Fig. 1 に，MS データを Table III に示した．これらのスペクトルデータは本化合物の構造をよく裏付けている．

3．装置——ガスクロマトグラフ　島津 GC-4CM（水素炎イオン化検出器付き）; ガスクロマトグラフ-質量分析計　島津 LKB-9000型; 質量分析計　日本電子 JMS-01SG-2型; 赤外分光光度計　日本分光 JASCO A-102型; 核磁気共鳴分析計　日本電子 FX-200型．

4．老化防止剤の分析法——分析法は前報の方法を用いた．ただし，試料量を 1g，アセトン：クロロホルム (1:1) 混液の液量を 10 ml に変更した．また，定性，定量分析の条件は以下の通りとした．

GC 条件: 2% OV-1 on Gas Chrom Q (80—100 mesh), 1.5 m×3 mm I.D., ガラスカラム; 温度，カラム 150—275°C (10°C/min), 注入口および検出器 280°C; キャリアガス，窒素 60 ml/min; 検出器，水素炎イオン化法 (FID), 水素 0.4 kg/cm^2, 空気 0.5 kg/cm^2. GC-MS 条件：GC 条件は上記と同じ．ただし，キャリアガスはヘリウム (30 ml/min) を用いた．MS 条件はイオン化電圧 70V，イオン化電流 60 µA，イオン加速電圧

TABLE Ia. Analytical Data of Antioxidants and Plasticizers in Commercial Rubber Gloves by Japanese Manufacturers

No.	Brand	Manufacturer	Use[c]	Color	Content, μg/g							
					MBMBP	IBBDMP	SP	BBMBP	IPPD	DBP	BBP	DEHP[d]
1	New Family	S.T. Chemical	D	Pink	1630	–[g]	–	–	–	–	–	–
2	Family Soft	S.T. Chemical	D	Pink	1490	–	–	–	–	–	–	–
3	Jab	S.T. Chemical	D	Blue-green	3490	–	–	–	–	–	–	–
4	Family	S.T. Chemical	D	Blue-green	–	3710	–	–	–	–	–	–
5	Jab	S.T. Chemical	D	Pink	–	6740	–	–	–	–	–	–
6	Jab	S.T. Chemical	D	Pink	–	–	–	3480	–	–	–	–
7	1327 Gloves	Dunlop Home Products	D	Pink	2630	–	–	–	–	–	–	–
8	Dunlop Gloves	Dunlop Home Products	D	Pink	2250	–	–	–	–	–	–	–
9	Lotus Hand	Dunlop Home Products	D	Orange	2090	–	–	–	–	–	–	–
10	Sumai Robe	Dunlop Home Products	D	Pink	4860	–	–	–	–	–	–	–
11	Sweet Home	Sanko Kagaku Kogyo	D	Orange	–	–	5310	–	–	–	–	–
12	Minori Tebukuro	Sanko Kagaku Kogyo	D	Blue	–	–	5620	–	–	–	–	–
13	Minori Tebukuro	Sanko Kagaku Kogyo	D	Green	–	–	6080	–	–	–	–	–
14	Minori Tebukuro	Sanko Kagaku Kogyo	ND	Black	–	–	2730	–	–	–	–	–
15	***[a]	Sanko Kagaku Kogyo	M	Pale yellow	–	–	3230	–	–	–	–	–
16	Doctor Hand-2[b]	Fuji Latex	D	Pale yellow	–	2150	–	–	–	–	845	4220
17	Doctor Hand	Fuji Latex	M	Pale yellow	–	1590	–	–	–	–	–	–
18	Bilpearl[b]	Okamoto Riken Gomu	D	Brown	–	1970	–	–	–	225	–	–
19	Bilpearl[b]	Okamoto Riken Gomu	D	Green-blue	–	–	–	–	–	100	–	937
20	***[b]	Okamoto Riken Gomu	D	Pink	–	–	–	–	–	274	–	2100
21	New Rubber Hope	Atom	ND	Red-brown	399	–	–	–	–	–	–	–
22	Long Rubber Gloves	Taiyo Kogyo	D	Orange	2190	–	–	–	–	–	–	–
23	Tokyo Tebukuro	Tokyo Latex Co-op.	D	Pale blue-green	2040	2160	–	–	–	–	19000	–
24	Hanaki Tebukuro	Nippon Hanaki Gomu	ND	Black	–	–	5650	–	–	–	–	–
25	Koshin[b]	Koshin Gomu	ND	Dark brown	–	–	1600	–	117	–	–	2450
26	Kogane Stout[e]	Oji Gomu	ND	Black (ontside) Brown(inside)	–	–	–	–	–	–	–	–

TABLE Ib. Analytical Data of Antioxidants and Plasticizers in Commercial Rubber Gloves by Foreign Manufacturers

No.	Brand	Manufacturer[f]	Distributor	Use	Color	Content, μg/g MBMBP[d]
27	Handy	Ansell	Dodwell & Co	D	Yellow	1820
28	Beauty	Ansell	Dodwell & Co	D	Orange	1940
29	New Austral	Ansell	Dodwell & Co	D	Blue	1490
30	Ansell	Ansell	Dodwell & Co	D	Red (outside) Moss-green (inside)	1700
31	Household Gloves	Ansell	Nippon N. R. Drug	D	Blue-green	6810
32	Marigold	London Rubber	Jardin Matheson & Co.	D	Red	244
33	***	Hutchinson Mapa	Showa Kako	D	Pink	645
34	Paris Gloves	Hutchinson Mapa	Showa Kako	D	Red	5170
35	France Baltex	Hutchinson Mapa	Miyai Sansho	D	Pale blue-green	1040
36	Kursaal Lux[e]	S. A. E. Kursaal	Taki Gomu Kagaku	D	Pink	-
37	Mercy Robe[e]	S. A. E. Kursaal	Fumakilla	D	Yellow	-

a) *** : unknown brand-name.
b) Obtained as gloves caused contact dermatitts.
c) D, for domestic use ; ND, for non-domestic use ; M, for medical use.
d) The full names are shown in Table II. Other antioxidants and plasticizers shown in Table II were not detected.
e) Phenolic antioxidants of unknown structures were detected.
f) Ansell, in Malaysia ; London Rubber, in Malaysia ; Hutchinson Mapa, in France, S. A. E. Kursaal, in Spain.
g) — : not detected.

TABLE II. Antioxidants and Plasticizers Used in the Experiment

No.	Compound	Abbreviation	Trade name[a]
Antioxidant			
1	6-Ethoxy-2, 2, 4-trimethyl-1, 2-dihydroquinoline	ETMDQ	Antigen AW
2	Poly(2, 2, 4-trimethyl-1, 2-dihydroquinoline)	TMDQ	Nocrac 224
3	Phenyl-α-naphthylamine	PAN	Nocrac PA
4	*N*-(3′-Hydroxybutylidene)-α-naphthylamine	HBNA	Nocrac C
5	Phenyl-β-naphthylamine	PBN	Nocrac D
6	*p*-Phenylenediamine[b]	PPD	
7	*N*-Isopropyl-*N*′-phenyl-*p*-phenylenediamine	IPPD	Nocrac 810-NA
8	*N*, *N*′-Di-β-naphthyl-*p*-phenylenediamine	DNPD	Nocrac White
9	*N*, *N*′-Diphenyl-*p*-phenylenediamine	DPPD	Nocrac DP-1
10	*N*-(3-Methacryloyloxy-hydroxypropyl)-*N*′-phenyl-*p*-phenylenediamine	MPPD	Nocrac G-1
11	*N*-(*p*-Toluenesulfonyl)-*N*′-phenyl-*p*-phenylenediamine	TPPD	Nocrac TD
12	2, 6-Di-*tert*-butyl-4-methylphenol	BHT	Nocrac 220
13	Styrenated phenol	SP	Nocrac SP-N
14	2, 5-Di-*tert*-butyl-hydroquinone[b]	DBHQ	
15	2, 2′-Methylene-bis(4-methyl-6-*tert*-butylphenol)	MBMBP	Nocrac NS-6
16	2, 2′-Methylene-bis(4-ethyl-6-*tert*-butylphenol)	MBEBP	Nocrac NS-5
17	2, 2′-Isobutylidene-bis(4, 6-dimethylphenol)[c]	IBBDMP	
18	1, 1′-Bis(4-hydroxyphenyl)cyclohexane	BHPC	Antigen W
19	4, 4′-Butylidene-bis(3-methyl-6-*tert*-butylphenol)	BBMBP	Antigen BBMS
20	4, 4′-Thio-bis(3-methyl-6-*tert*-butylphenol)	TBMBP	Nocrac 300
21	Dilauryl 3, 3′-thiodipropionate[b]	DLTDP	
22	Triphenyl phosphite[b]	TPPI	
23	Triphenyl phosphate[b]	TPPA	
Plasticizer			
24	Dibutyl phthalate[b]	DBP	
25	Butylbenzyl phthalate[b]	BBP	
26	Di (2-ethylhexyl) phthalate[b]	DEHP	

a) "Nocrac," by Ouchi Shinko Chemical Ind. Co.
"Antigen," by Sumitomo Chemical Co., Ltd.
b) Purchased from Tokyo Kasei Kogyo Co., Ltd.
c) Synthetized in our laboratory.

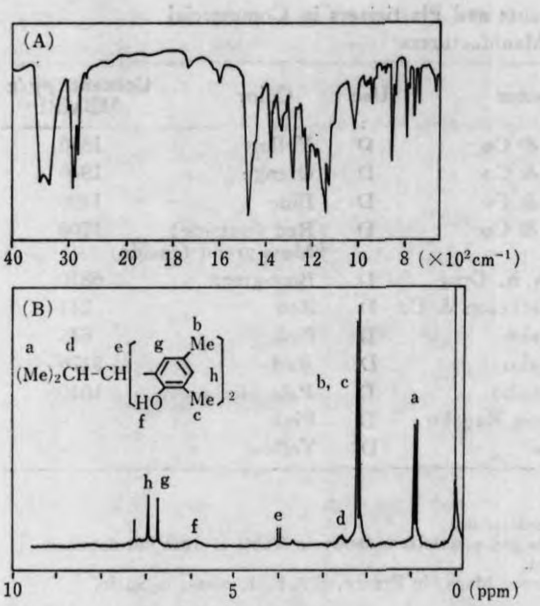

Fig. 1. Infrared Spectrum (A) and Proton Nuclear Magnetic Resonance Spectrum (B), of Authentic IBBDMP

IBBDMP: Colorless needle (recrystallized from hexane), mp 155—156°C.
(A): Measured by KBr method.
(B): ppm, in $CDCl_3$; 0.88, 0.91 (aliphatic methyl, 6H), 2.17, 2.22 (aromatic methyl, 12H), 2.56 (methine, 1H), 3.95, 4.00 (doublet, J=10.5 Hz, benzylic H, 1H), 5.87 (phenolic OH, 2H), 6.72, 6.95 (aromatic H, 4H).

3 KV，イオン源温度330℃とした．MS条件：試料直接導入法による．イオン化電圧70V，イオン化電流200 μA，イオン加速電圧10KV．

B．パッチテスト Table II に示した標品のうち，No. 1—3，6—9，11の含窒素系8種，No. 13，15，16，18，20のフェノール系5種，計13種の老化防止剤を10%，1%，0.1%の割合で白色ワセリンに混和し，鳥居薬品製のパッチテスト用バンソウコウ「ミニプラスター」を用いて被験者に貼付した．貼付後，48時間，72時間，1週間における皮膚反応を観察した．

被験者は1980年から1982年の間に東邦大学病院皮膚科外来を受診した患者のうち，ゴム製品による接触皮膚炎と診断されたもの30名（ゴム群：男9名，女21名，年齢15—58歳），および塩化ビニル製品による接触皮膚炎と診断されたもの14名（塩ビ群：男1名，女13名，年齢18—57歳）で，うち4名が重複していた．

実験結果

1. シリカゲルカラムクロマトグラフィー

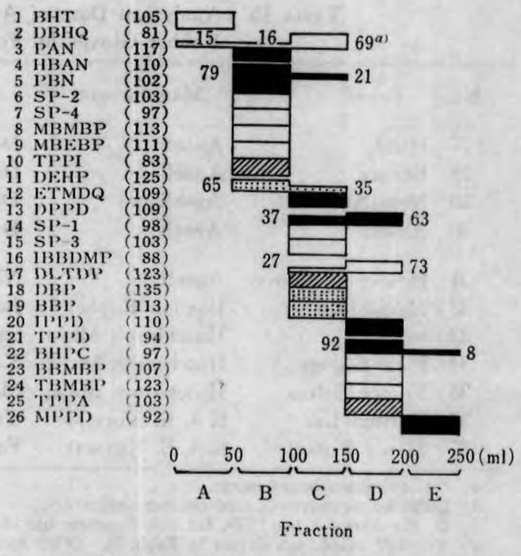

Fig. 2. Elution Behavior of Antioxidants and Plasticizers on Silica Gel Column Chromatography

☐ : phenolic antioxidant,
■ : nitrogen-containing antioxidant,
▨ : other type antioxidant,
░ : plasticizer.

Sample: 500 μg of each compound was loaded.
Column: 5 g of silica gel (Kieselgel 60, 70—230 mesh) dispersed in hexane was packed into a glass column of 10 mm I.D.
Fractionation: Fr. A (with 50 ml of hexane), Fr. B (with 50 ml of 5% ether in hexane), Fr. C (with 50 ml of 10% ether in hexane), Fr. D (with 50 ml of 50% ether in hexane), Fr. E (with 50 ml of methanol).
The values in parentheses show total recoveries (%) from the column.
a) Compounds were found over several fractions in the percentage ratios described along the boxes.

標品500 μgをカラムに吸着させ，シリカゲルカラムクロマトグラフィーの操作を行なったときに，どの分画に溶離されてくるかをFig. 2に示した．複数の分画にわたって溶出したものについては，各分画への溶出量の割合をパーセントで示した．また，カッコ内の数字は各々のカラムからの回収率（%）を示す．

2. ガスクロマトグラフィー

標品についてシリカゲルカラムクロマトグラフィーにより分画A—Eを得た後，各分画について実験方法4のGC条件で分析したときのガスクロマトグラムをFig. 3に示した．各化合物の出現位置はIPPDの保持時間を1.00としたときの相対保持時間で示した．

いずれも対称性のよいピークを示した．ピークの分離の点では，分画Bにおいてstyrenated phenol (SP)の成分の1つであるSP-2（相対保持時間1.34）と2,2′-methylene-bis(4-methyl-6-*tert*-butylphenol) (MBMBP)

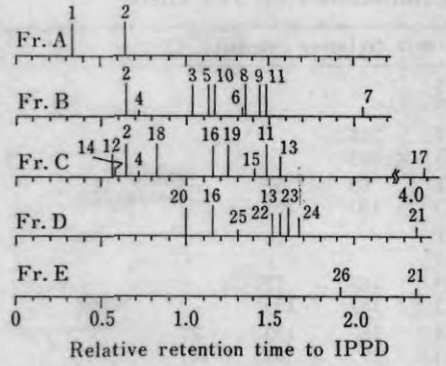

Fig. 3. Gas Chromatograms of Authentic Antioxidants and Plasticizers

Condition : 2% OV-1 on Gas Chrom Q (80-100 mesh), glass column 150 cm × 3 mm I.D. ; temperature, column 150 — 275°C (10°C/min), injection port and detector 280°C ; carrier gas (nitrogen), 60 ml/min ; detection (flame ionization detector), hydrogen 0.4 kg/cm², air 0.5 kg/cm². The retention time of IPPD was 8.05 min. Numbers in Fig. are the same with those in Fig. 2 : 1 BHT, 2 DBHQ, 3 PAN, 4 HBAN, 5 PBN, 6 SP-2, 7 SP-4, 8 MBMBP, 9 MBEBP, 10 TPPI, 11 DEHP, 12 ETMDQ, 13 DPPD, 14 SP-1, 15 SP-3, 16 IBBDMP, 17 DLTDP, 18 DBP, 19 BBP, 20 IPPD, 21 TPPD, 22 BHPC, 23 BBMBP, 24 TBMBP, 25 TPPA, 26 MPPD.

(1.35)，分画Cにおいて 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (ETMDQ) (0.57) と SP の成分である SP-1 (0.58) のピークがほぼ重なった．しかし，SP は SP-1—4 の4つの成分ピークが同時に認められるので，単一のピークしか示さない MBMBP, ETMDQ とは SP のその他のピークの有無を確かめることで区別できる．

定量はピーク高さによる絶対検量線法によった．実験方法4に従って操作して得た検液の量を 0.5 ml とし，その 5 μl を GC に注入し，FID の感度を 10^3 MΩ，レンジを 0.16 V とし，ピーク高さが 0.5 cm 以上の場合をピークとして定量した場合，定量限界は triphenyl phosphate (TPPA)，*N*-(3′-hydroxybutylidene)-α-naphthylamine (HBAN) では約 10 μg/g, SP, *N*-(methacryloyloxy-hydroxypropyl)-*N*′-phenyl-*p*-phenylenediamine (MPPD)，*N*-(*p*-toluenesulfonyl)-*N*′-phenylenediamine (TPPD)，dilauryl 3,3′-thiodipropionate (DLTDP) では約 5 μg/g，その他では約 1 μg/g であった．

3. マススペクトロメトリー

標品について実験方法4の MS 条件（試料直接導入法）でマススペクトルを測定した．ただし，SP は GC-MS 条件で測定し，SP-1—4 の各成分のマススペクトルを得た．その結果を Table III に示した．TPPD, di (2-ethylhexyl) phthalate (DEHP) 以外はすべて分子イオンを示した．TPPD (分子量 338) の場合，トシル基の脱離したイオン (*m/z* 184) が最大質量のフラグメントイオンであった．なお，*N*-phenyl-α-naphthylamine (PAN) と *N*-phenyl-β-naphthylamine (PBN) はほぼ同じマススペクトルを示した．しかし，両者は GC での保持時間が異なっており，実際試料の分析では GC-MS が用いられることから，両者は区別できる．

4. 抽出法の検討

ゴムのように製造時に加熱などの苛酷な処理が行なわれる場合，添加された化学物質の真の存在量を知ることは不可能である．このような場合，至適抽出条件を設定するには分析対象の化合物を含むことを確認した製品を用いて各種の条件で抽出を行い，最大の抽出量を示す条件を至適抽出条件と考えるしかない．

そこで，後述のように市販のゴム手袋から検出された MBMBP, SP, IBBDMP, IPPD, および BHT の5種の老化防止剤を対象にして抽出条件を検討した．抽出溶媒としては，前報で用いたアセトン：クロロホルム (1：1) 混液に加えてアセトン：ベンゼン (1：1) 混液，アセトン，メタノールの4種の溶媒系を用いた．検討の結果を Table IV に示した．

まず，標品 200 μg について溶媒 50 ml 中で 80℃ の水浴上で2時間還流させた．MBMBP, IBBDMP, SP, IPPD は定量的に回収されたが，BHT だけがどの溶媒系においても回収率が低かった．そこで，標品 200 μg について溶媒 50 ml 中で2時間振とうさせたところ，メタノール以外の溶媒系ではほぼ定量的に回収されたことから，BHT は加熱によって損失すると考えられた．

次に，試料 No. 1 (MBMBP を含む)，No. 5 (IBBDMP を含む)，No. 25 (SP および IPPD を含む) のゴム手袋およびイソプレンゴムシート (BHT を含む) 各々約1 g を用いて抽出率を検討した．抽出率はアセトン：クロロホルム (1：1) 混液で30分間，4回繰り返して振とうしたときの抽出量を100(%)として示した．まず，80℃ で還流させたところ，BHT の抽出率がメタノール以外では60% 以下と低かった．また，室温で振とうしたところ，メタノールではいずれの老化防止剤も抽出率が50% 以下と低く，アセトンでは BHT と SP がやや低い回収率を示した．

以上の結果から，抽出法としては還流するよりも室温で振とうする方が良いこと，抽出溶媒としてはアセトン：クロロホルム (1：1) 混液，アセトン：ベンゼン (1：1) 混液がほぼ同程度の抽出率を示し，この両者が

TABLE III. Mass Spectral Data of the Authentic Antioxidants and Plasticizers[a)]

		Molecular weight	Fragment ion, m/z (relative intensity, %)								
ETMDQ		217	217 (14, M^+)	202 (100)	174 (42)	145 (17)					
PAN		219	220 (20)	219 (100, M^+)	218 (70)	217 (55)	216 (20)				
HBAN		193	194 (12)	193 (54, M^+)	192 (18)	71 (26)	69 (18)	57 (25)	55 (25)	44 (100)	
PBN		219	220 (21)	219 (100, M^+)	218 (38)	217 (25)					
IPPD		226	226 (74, M^+)	211 (100)	183 (35)	169 (13)	167 (13)	77 (13)			
DPPD		260	260 (100, M^+)	183 (15)	169 (25)	168 (13)	167 (19)	130 (10)	77 (12)		
MPPD		326	327 (28)	326 (100, M^+)	197 (82)	184 (10)	167 (12)	92 (14)	69 (10)		
TPPD		338	184 (26)	91 (62)	90 (33)	45 (67)	44 (100)	43 (52)	39 (20)		
BHT		220	220 (25, M^+)	205 (100)	177 (7)	145 (12)	57 (16)				
SP	SP-1	198	198 (46, M^+)	183 (100)	165 (25)						
	SP-2	302	302 (67, M^+)	287 (100)	210 (46)	198 (12)	105 (17)				
	SP-3	302	302 (50, M^+)	287 (100)	210 (26)	198 (19)	105 (31)				
	SP-4	406	406 (47, M^+)	391 (70)	314 (20)	302 (20)	235 (15)	105 (100)			
DBHQ		222	222 (75, M^+)	220 (23)	207 (100)	205 (22)	57 (75)	44 (35)	43 (37)	41 (57)	
MBMBP		340	340 (80, M^+)	283 (20)	177 (100)	164 (60)	155 (18)	149 (40)	127 (20)	121 (20)	57 (20)
MBEBP		368	368 (82, M^+)	311 (24)	191 (100)	178 (70)	175 (48)	163 (35)	57 (23)		
IBBDMP		298	298 (30, M^+)	256 (20)	255 (100)	91 (10)	43 (8)				
BHPC		268	269 (30)	268 (90, M^+)	226 (22)	225 (100)	199 (34)	197 (15)	149 (27)	131 (33)	107 (35)
BBMBP		382	382 (18, M^+)	340 (30)	339 (100)	177 (12)	176 (9)	162 (10)	161 (8)	57 (15)	
TBMBP		358	358 (100, M^+)	343 (20)	196 (38)	181 (72)	164 (27)	149 (73)	121 (20)		
DLTDP		514	514 (5, M^+)	346 (10)	329 (10)	178 (40)	161 (35)	143 (60)	105 (22)	89 (36)	85 (25)
			71 (45)	69 (45)	57 (95)	55 (85)	43 (100)				
TPPI		310	310 (58, M^+)	217 (100)	199 (8)	170 (8)	152 (22)	151 (14)	93 (8)	77 (50)	
TPPA		326	326 (18, M^+)	325 (13)	233 (19)	232 (9)	170 (7)	94 (100)	66 (36)	65 (28)	
DBP		278	278 (4, M^+)	223 (6)	205 (4)	149 (100)	104 (5)	76 (4)	57 (6)	41 (8)	
BBP		312	312 (15, M^+)	255 (10)	223 (13)	206 (28)	149 (100)	91 (32)	57 (10)	41 (10)	
DEHP		390	279 (5)	167 (30)	149 (100)	71 (40)	70 (35)	57 (50)	55 (20)	43 (30)	41 (25)

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a) MS condition (direct inlet technique): ionization voltage 70 V, ionization current 200 μA, ion acceleration voltage 10 KV. GC-MS condition (for analysis of SP): GC condition is shown in Fig. 2, except that helium (30 ml/min) was used as carrier gas. MS condition; ionization voltage 70 V, ionization current 60 μA, ion acceleration voltage 3 KV, ion source temp. 330°C.

TABLE IV. Recoveries of Antioxidants

	Recovery (%)							
	Acetone : Chloroform (1 : 1)		Acetone : Benzene (1 : 1)		Acetone		Methanol	
	S	R	S	R	S	R	S	R
Standard solution[b]								
MBMBP	96	118	90	98	96	93	84	108
IBBDMP	94	102	91	110	90	103	77	110
SP	96	97	96	101	86	104	77	103
BHT	98	12	98	30	92	27	59	34
IPPD	92	97	99	97	97	103	85	92
Commercial gloves[c]								
MBMBP (No. 1)[d]	100	93	98	100	97	98	43	93
IBBDMP (No. 5)	100	89	104	92	99	91	40	95
SP (No. 25)	100	102	95	109	85	110	21	85
BHT[e]	100	61	102	60	87	45	30	102
IPPD (No. 25)	100	97	101	128	99	125	35	111

a) S : shaking with 10 ml of solvent, for 30 min, four times, at room temp.
R : reflux with 50 ml of solvent, for 2 h, at 80°C.
b) Added amount is about 200 μg.
c) Sampling amount is about 1 g.
d) The sample numbers in parentheses are the same with those in Table I.
e) Isoprene rubber sheet containing BHT was used in place of commercial rubber gloves.

適当であることが判った．そこで，以後の実験は前報と同じくアセトン：クロロホルム（1：1）混液を用い，室温で振とうして抽出することとした．

5. 定性，定量分析の実際例，および市販ゴム手袋中の老化防止剤の調査

以上の検討により，ゴム手袋中の老化防止剤の分析法を確立したので，市販製品の調査を行なった．試料 No. 23 を例に分析の実際を説明する．

試料 No. 23 はやや厚手で丈の長い家庭用ゴム手袋「東京手袋」で，メーカー名の記載はなく，「東京ラテックス協同組合」の表示があった．抽出物のガスクロマトグラムにはピーク1（相対保持時間 1.16），ピーク2（1.25），ピーク3（1.35）の3つのピークが認められた．ピーク1，ピーク3の保持時間は IBBDMP および MBMBP のそれと一致した．そこで，抽出物をシリカゲルカラムクロマトグラフィーにかけたところ，ピーク1は分画C，Dに，ピーク3は分画Bに溶出した．各分画について GC-MS を測定したところ，ピーク1は *m/z* 298 (M^+)，255 (M^+-43) に，ピーク3は *m/z* 340 (M^+)，283 (M^+-57)，177，164 に主要なフラグメントイオンを示し，各々 IBBDMP，MBMBP の標品のマススペクトルとほぼ一致した．ピーク1，ピーク3の GC-MS によるマススペクトルを Fig. 4 に示した．

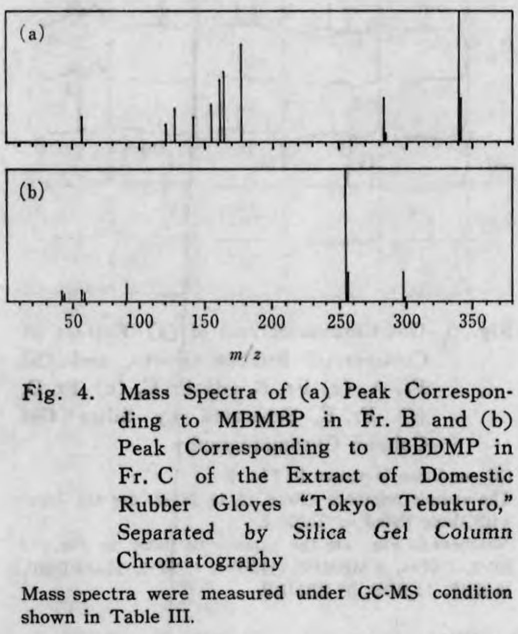

Fig. 4. Mass Spectra of (a) Peak Corresponding to MBMBP in Fr. B and (b) Peak Corresponding to IBBDMP in Fr. C of the Extract of Domestic Rubber Gloves "Tokyo Tebukuro," Separated by Silica Gel Column Chromatography

Mass spectra were measured under GC-MS condition shown in Table III.

以上より，試料 No. 23 には老化防止剤として MBMBP，IBBDMP が含まれることが判った．GC による定量分析の結果，MBMBP 2040 μg/g，IBBDMP 2160 μg/g であった．

なお，ピーク2についてはシリカゲルカラムクロマトグラフィーにおける溶出挙動，GC における保持時間，GC-MS によるマススペクトルデータから可塑剤の butylbenzyl phthalate (BBP) と同定された．その定量

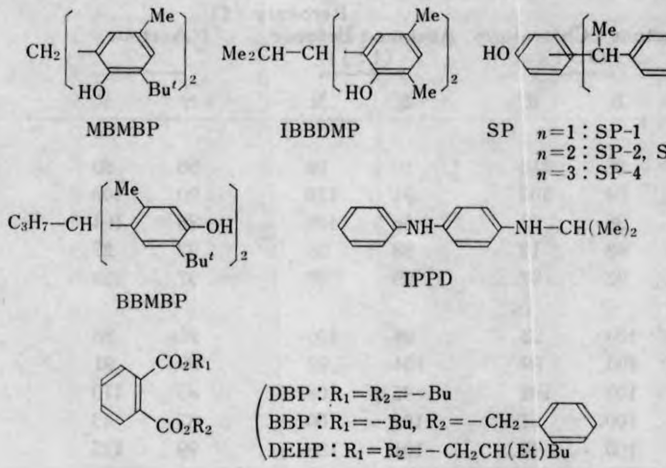

Chart 1. The Structures of the Antioxidants and the Plasticizers Found in Commercial Rubber Gloves

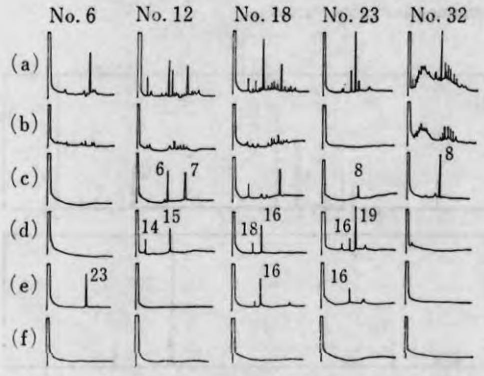

Fig. 5. Gas Chromatograms of (a) Extract of Commercial Rubber Gloves, and (b) Fr. A, (c) Fr. B, (d) Fr. C, (e) Fr. D, (f) Fr. E, Separated by Silica Gel Column Chromatography

GC condition is shown in Fig. 3.
The sample numbers (No. 6, 12, 18, 23, 32) are the same with those listed in Table I.
Numbers in Fig. are the same with those in Fig. 2 : 6 SP-2, 7 SP-4, 8 MBMBP, 14 SP-1, 15 SP-3, 16 IBBDMP, 18 DBP, 19 BBP, 23 BBMBP.

値は 19000 μg/g と高く，ゴム手袋にもフタル酸エステルが何らかの目的で使用されていることが判った．

ゴム手袋から老化防止剤が検出された典型例を Fig. 5 に示した．低分子量のゴム成分やワックス様物質などは分画Aにほとんど溶出してしまい，その他の分画には分析上妨害となるピークはほとんど見られなかった．ただし，BHT は分画Aに溶出してくるため，BHT の抽出量が少なかったり，夾雑物の量が多くなったときには定量が難しくなる．その場合にはゲルクロマトグラフィーなど，吸着クロマトグラフィーとは異なるモードでのクリーンアップを行う必要があるが未検討である．

本分析法を用いて 37 点のゴム手袋を分析した結果を Table Ia, Ib に示した．37 点のうち32点から老化防止剤が検出された．ほとんどが1種で，併用されていたのは試料 No. 23 (MBMBP と IBBDMP), No. 25 (SP と IPPD) の2点だけであった．なお，試料 No. 19, 20, 26, 36, 37からは Table II に挙げた老化防止剤はいずれも検出されなかった．また，No. 26, 36, 37 にはマススペクトルデータからフェノール系化合物と考えられる成分が認められたが，その構造については検討中である．

使用されていた老化防止剤としては外国メーカー品，国内メーカー品ともフェノール系化合物が主であった．すなわち，外国メーカー品では MBMBP が検出されたものが11点中9点 (244—6810 μg/g) と大半を占めていた．一方，国内メーカー品では含窒素系の IPPD が1点 (177 μg/g) 検出された以外はフェノール系化合物で，MBMBP が10点 (399—4860 μg/g), SP が8点 (1600—6080 μg/g), IBBDMP が6点 (1590—6740 μg/g), 4,4′-butylidene-bis (3-methyl-6-*tert*-butylphenol) (BBMBP) が1点 (3480 μg/g) 検出された．

また，フタル酸エステルは外国メーカー品からは全く検出されなかったが，国内メーカー品では4メーカー，6ブランドから，dibutyl phthalate (DBP) が3点 (100

Table Va. Incidence of Positive Reactions to Antioxidants in Thirty Patients with Contact Dermatitis from Rubber Materials

	Incidence of positive reactions (%)											
	48 h						72 h					
	10%		1 %		0.1%		10%		1 %		0.1%	
	++	+	++	+	++	+	++	+	++	+	++	+
IPPD	9	9	18	10	0		45	28	21	11	0	
TMDQ	0		0		0		0		0		0	
ETMDQ	0		0		0		0		0		0	
PAN	0		0		0		0		0		0	
DNPD	0		0		0		0		0		0	
DPPD	0	9	0		0	9	0	9	0		0	
TPPD	0		0		0		0	9	0	9	0	9
PPD	0		9	0	0	9	0		9	0	9	0
SP	0		0		0		0		0		0	
BHPC	0		0		0		0		0		0	
MBMBP	0	9	0		0		0		0		0	
MBEBP	0		0		0		0		0		0	
TBMBP	0		0		0		0		0		0	

Table Vb. Incidence of Positive Reactions to Antioxidants in Fourteen Patients with Contact Dermatitis from Poly (vinyl chloride) Materials

	Incidence of positive reactions (%)											
	48 h						72 h					
	10%		1 %		0.1%		10%		1 %		0.1%	
	++	+	++	+	++	+	++	+	++	+	++	+
IPPD	0		10	20	0		25	50	40	30	0	
TMDQ	0		0		0		0		0		0	
ETMDQ	0		0		0		0		0		0	
PAN	0		0		0		0		0		0	
DNPD	0		0		0		0		0		0	
DPPD	0		0		0		0		0		0	
TPPD	0		0		0		0		0		0	
PPD	0		0		0		0		0		0	
SP	0		0		0		0		0		0	
BHPC	0		0		0		0		0		0	
MBMBP	0		0		0		0		0		0	
MBEBP	0		0		0		0		0		0	
TBMBP	0		0		0		0		0		0	

—274 μg/g), BBP が2点 (845, 19000 μg/g), DEHP が4点 (937—4220 μg/g) 検出された. DBP, BBP, DEHP についてシリカゲルカラムクロマトグラフィーでの溶出位置, GC での保持時間, マススペクトルを Fig. 2, 3, Table III に示した.

なお, 市販ゴム手袋から検出された老化防止剤, フタル酸エステルの構造式を Chart 1 に示した.

6. 老化防止剤のパッチテスト陽性率

パッチテストの結果を Table Va, Vb に示した. 各化合物ごとに (+) 以上の陽性反応を示した患者の割合をパーセントで表示した.

パッチテスト後, 72時間での陽性率を各化合物間, およびゴム群と塩ビ群の間で比較した. MBMBP, SP を含めた5種のフェノール系老化防止剤はいずれも10%濃度においてもゴム群, 塩ビ群両群ともにほとんど陽性反応を示さなかった. 一方, 含窒素系老化防止剤では IPPD が1%濃度以上でゴム群, 塩ビ群のいずれに対しても数10%と, 他の化合物に比べてとびぬけて高い陽性

率を示した．

考　　察

市販の家庭用ゴム手袋37点を分析した結果，使用されている老化防止剤は MBMBP, SP, IBBDMP などのフェノール系化合物が主であることが明らかとなった．すなわち，MBMBP が37点中19点，SP が8点，IBBDMP が6点，BBMBP が1点検出された．なお，含窒素系化合物では IPPD が1点だけ検出された．IPPD が検出された試料 No. 25 は暗色で，厚手の重作業用手袋で，普通家庭で使用されるものではない．Cronin の成書には，「*p*-フェニレンジアミン系老化防止剤は主に工業用ゴム製品に使用され，家庭用ゴム製品には用いられない．」とあり,3a) 我々の分析結果はその記述と矛盾しない．

一方，病院皮膚科外来患者のうち，ゴム製品で接触皮膚炎を起こした患者（ゴム群），および塩化ビニル製品で接触皮膚炎を起こした患者（塩ビ群）を対象にパッチテストを行なった．その結果，現在ゴム手袋に主に用いられている MBMBP, SP などのフェノール系老化防止剤はゴム群，塩ビ群いずれに対してもほとんど陽性反応を示さず，これらの感作性は弱いと考えられた．

前報において作業用手袋（試料 No. 25）によるアレルギー性接触皮膚炎の原因化学物質について，「IPPD は原因化学物質の1つであるが，SP はパッチテストを行なっていないので，原因化学物質か否かは判定できない．」と結論したが，今回のパッチテストの結果から，SP が原因化学物質である可能性は少ないと考えられる．

我々の文献調査ではフェノール系老化防止剤による接触皮膚炎の報告は少なく，市販ゴム手袋から検出されたようなビスフェノール系化合物による事例は見当らなかった．フェノール系老化防止剤による接触皮膚炎の報告例としては，Cronin が感作性物質の1つに挙げている dioxydiphenyl3a) を使用した外科用手袋3b,5) およびゴムシート3c) による事例，2-*p*-methylcyclohexyl-4,6-dimethylphenol を使用した手袋3d) の事例，BHT, butylated hydroxyanisole (BHA)3e,7) による事例が Cronin の成書に挙げられている．また Fisher は monobenzyl hydroquinone が脱色能とともに感作能を持つことを指摘している.4)

一方，IPPD はパッチテストを行なった老化防止剤の中でとびぬけて高い陽性率を示した．関東らは Hartley 系白色モルモットを用いた感作実験により IPPD が強い感作能を有することを認めている.8) また，Cronin, Fisher の成書にも IPPD が強い感作源であることが記述されており,3f,4b) IPPD を使用したタイヤ,9) ゴムチューブ,10) 潜水用ゴムマスク11) などによる接触皮膚炎の事例が報告されている．

なお，フタル酸エステルについては外国メーカー品からは全く検出されなかったのに対して，国内のいくつかのメーカーの製品に偏って DBP, BBP, DEHP が検出された．楠本らは飲食物用ゴム製品から DEHP を検出している.12) 成書によれば，ゴムを軟化させ，配合剤の分散を良くし，押し出し，圧延成型などをやりやすくするために DBP, DEHP, tricresyl phosphate などの可塑剤が添加されるという.13)

以上を総合すると，現在市販されているゴム手袋には老化防止剤として MBMBP や SP などの感作性の低いフェノール系化合物が主に使用されており，老化防止剤に関しては接触皮膚炎患者にとって好ましい状況にあると考えられる．他の老化防止剤に比べてとびぬけて高い陽性率を示した IPPD は家庭用だけでなく，工業用のものも含めてゴム手袋全般に使用しないことが望ましいと考える．

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食品中に残存する抗菌性物質に関する研究（第1報）
高速液体クロマトグラフィーによるテトラサイクリン系抗生物質の定量[1]

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Studies on Residual Antibacterials in Foods (I)
Determination of Tetracyclines by High-Performance Liquid Chromatography[1]

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A simple and reproducible method for the determination of residual tetracyclines (TCs) in livestock products by high-performance liquid chromatography was established.

Tetracycline (TC), oxytetracycline (OTC) and chlortetracycline (CTC) in a sample were extracted with 5% aqueous perchloric acid. The extract was passed through SEP-PAK C_{18} cartridge. After washing the cartridge with water, TCs was eluted with a mixture of *N, N*-dimethylformamide (DMF)-water (4 : 6) containing 0.02 M phosphoric acid. The eluate was injected on a Li Chrosorb RP-8 column by using a mixture of DMF-water (1 : 4) containing 0.02 M phosphoric acid as a mobile phase. The effluent was monitored with a UV detector at a wavelength of 340 nm.

The recoveries of TCs from cattle muscle, liver, kidney and milk fortified with 1 μg/g of TC and OTC and 2 μg/g of CTC were 34.7—52.9% for muscle, 37.7—51.6% for liver, 53.1—68.3% for kidney and 74.8—88.7% for milk, respectively.

The quantitative analysis was carried out by the method of standard addition and the minimum detectable amount of TC and OTC was 0.1 ppm and CTC was 0.2 ppm.

Keywords——livestock product ; HPLC ; tetracycline ; oxytetracycline ; chlortetracycline ; SEP-PAK C_{18} cartridge ; UV detector

緒　　言

テトラサイクリン系の抗生物質（TCs）は，飼料添加物あるいは動物用医薬品として広く使用されており，畜産食品中への残留が懸念される物質である．食品中に残留する TCs の分析法としては，一般に微生物を利用した生物検定法により行なわれており，厚生省乳肉衛生課の「畜水産食品中の残留物質検査法」第1集の3においても，生物検定法を採用している．しかし，この方法は，検定用微生物の保存や検定など煩雑な操作が必要であり，定量値も環境等の他の要因により影響を受けやすく，常に安定した結果を得ることは困難である．一方，TCs の理化学的な分析法としては，ケイ光光度測定法,[2] ガスクロマトグラフィー法,[3] 薄層クロマトグラフィー法,[4] および高速液体クロマトグラフィー（HPLC）法[5] が報告されているが，いずれも前処理が煩雑であったり，分離条件が不充分であったりして，実際に日常分析に使用し得る方法としては認め難い．しかしながら，HPLC 法は，緩和な条件下で分離分析が可能であること，ガスクロマトグラフィー法のような揮発性の誘導体にする操作が不要であることから，TCs のような不安定な物質には適した分析手段であると考えられる．そこで，HPLC の分離条件および試料の前処理法について検討を加え，簡単な操作で畜産食品中に残留するテトラサイクリン（TC），オキシテトラサイクリン（OTC），およびクロルテトラサイクリン（CTC）を精度よく定量する方法を開発した．さらに，この方法を用いて，市販

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A SEARCH FOR CHEMICALS RESPONSIBLE FOR CONTACT DERMATITIS CAUSED BY HOUSEHOLD PRODUCTS II

Analysis of Anti-oxidants in Commercial Rubber Gloves and Positive Reaction to Anti-oxidants in Patch Tests

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and Masaru ISHIHARA[2]

INTRODUCTION

....***### has been surfacing through the "Hospital Monitoring Scheme for Contact Dermatitis Caused by Household Products" organized by the Household Products Safety Unit of the Ministry of Health and Welfare.[1)]

~~Welooked for~~ We looked for the causative agents of allergic contact dermatitis from heavy duty rubber gloves, the incidence of which had been detected by the above scheme ....***### and could conclude that N-isopropyl-N'-phenyl-p-phenylenediamine (IPPD), an anti-oxidant which is known to ...***###, is mainly responsible.

However, cases in which the chemicals responsible can be identifie
are very few. In many cases, it is not known as to what chemicals are prese
in the household product. So, scientists have difficulty in selecting chemicals for patch testing. To improve this situation, first of all it is important to understand the general pattern of the use of chemicals in commercially marketted products.

We, therefore, investigated the types and amounts of anti-oxidants
37 rubber glove samples purchased from retail shops, obtained from monitor

::...***### 1-2 lines of print are missing from near the bottom of almost every page of the original. These are marked in the translated text.

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hospitals, etc to understand the pattern of usage of anti-oxidants (which are common additives in rubber) in commercial rubber gloves. As a result, we found that ~~in~~ almost every make of rubber glove had phenolic anti-oxida compounds. Reports of thorough investigations on the sensitizing property of such phenolic compounds are not available. Even in the books by ~~Cor~~ Cronin,3) and Fischer,4) which give detailed information about causative agent of contact dermatitis, there is no detailed discussion about this subject.

We then carried out patch tests ~~on~~ using 13 different anti-oxidant including two phenolic anti-oxidants found commonly in rubber gloves, on contact dermatitis patients who had come to our dermatology OPD. In the patch test, none of the phenolic anti-oxidants showed a positive reaction. Among the non-phenolic anti-oxidants, IPPD produced a very high~~ly~~ incidence of positive reaction.

TEST METHODS

A. Analysis of Anti-Oxidants in Commercial Rubber Gloves

1. Samples used. The 37 gloves used for analysis are classified according to Brand name, manufacturer, application, and shades in Tables Ia and Ib. Out of these 37 samples, 30 were purchased from retail shops in Tokyo, Chiba, Osaka, and Takamatsu cities during February – April 1982. ~~Tp~~ Two of th samples were gifts supplied for hospital use, and five were obtained from the monitorring hospitals. When we classif~~y~~ied the gloves according to application, 30 were for domestic use, 2 for hospital use, 4 for light work (relatively thin gloves), and one was a heavy duty rubber glove of th type a~~p~~nalysed in our previous study. 26 of the brands were from 11 Japane companies, and 11 brands were from four foreing manufacturers and seven import firms.

In addition to these, we included 2,6-di-tert-butyl-4-methylpheno a widely used additive in plastics which is often used in rubber also...Si we could not obtain rubber gloves containing BHT, we utilized an isoprene rubber sheet meant for h~~a~~ospital use, manufactured by ...###*** Rubber which was confirmed to contain BHT. We used this as a sample for studying the method of extraction. In the method of quantitative analysis used by us, the sheet was determined to have a BHT contetn of 963 micro g/g.

2. Reagents . Table II gives the chemical names of the anti-oxidants and plasticizers tested, their abbreviations used in this paper, their commerci names, and the names of their maunfacturers. These chemicals were used without any purification. For other reagents used, in principle the 'guarenteed reagent grade' chemicals were used. 2,2'-siobutylidene-bis(4,6 dimethylphenol) (IBBDMP) was synthesized by the method described below and used as the authenti~~a~~c sample.

2.44 g (2 x10^{-2}mol) of 2,4-dimethylphenol was taken in a 50 ml round bottomed flask and dissolved by the addition of 10 ml of methanol. 1 ml of conc. sulphuric acid was then added, followed by the drop-wise addition of about 1 ml of isobutyraldehyde. The mixture was then refluxed for 10 h over an 80°C water bath. After cooling, about 40 ml water was added to dilute the product. It was then extracted 4 times by shaking using 50 ml of ether. The extracts were combined, dried with anhydrous sodium suplhate, the ~~ehee~~-- ether evaporated off over an evaporator, and 3.52 g of residue was obtained.

The residue was then allowed to be adsorbed on a 20 mm inner diama glass column containing 30 g Kie~~l~~selgel 60 (70-230 mesh), for column chromatography and manufactured by Merck Ltd. The column had been packed by the wet method using hexane. The column was eluted with 100ml each of hexane and ether-hexane mixtures containing 5, 10, or 50% ether, as well as with 100ml of ether. Almost all of the main reaction product was eluted out in the 10% ether-hexane fraction. By evaporating off the solvent from this fraction and recrystallizing the residue from hexane, 2.01 g of colourless needle shaped crystals with m.p. 155-156°C were obtained. The IR and NMR spectra are shown in Fig. 1 and its MS data are given in Table III. These spectral data well illustrate the structural characteristics of the compou

3. Equipment used. The following equipments were used. Gas chromatograph Shimadzu GC-4CM (with a hydrogen flame ionization detector); Gas chromatog -Mass Sepectrometer: Shimadzu LKB-9000; Mass Spectrometer: Nihon Denshi JMS-01SG-2; IR Spectrophotometer: Nihonbunko JASCO A-102; and NMR Spectrom Nihon Denshi FX-200.

4. Method of analysing anti-oxidants . The methods used in our previous paper were used here also. However, the amount of sample used was 1 g and ~~and~~ of 1:1 acetone-chloroform mixture was used. Qualitative and quantitative analyses were carried out under the following conditions:

GC conditions: 2% OV-1 on Gas Chrom Q(80-100 mesh), 1.5 m x 3 m l glass column; Temperature: Column 150-275 °C (10°C/min), Injection port and detector 280°C; Carrier gas: Nitorgen 60 ml/min; Detector: Hydrogen fl ionization detector (FID), hydrogen 0.4 kg/cm², air 0.5 kg/cm².

GC-MS conditions: GC conditions were the same as given above, exc that helium _ _**/~~ml~~ min was used as the carrier gas. MS conditions were, ionization voltage 70 V ....###***, ionization current ...###*** ~~1~~3 KV, ion source temperature 330°C.

MS conditions: The sample was directly introduced, ionization vol 70 V, ionization current 200 micro A, ion acceleration voltage 10 KV,

B. Patch Test

Patch test was conducted with 13 anti-oxidants selected from those shown in Table II. These were the nitrogen containing compounds 1 - 3, 6

11; and the phenolic compounds 13, 15, 16, 18, and 20. These were mixed wi white vaseline to the extent of 10%, 1%, and 0.1% and applied on the test subjects with the help of "Miniplaster" (adhesive plaster meant for patch testing, manufactured by Tori-i Yakuhin Co.). The skin reaction was observed ~~after~~ at 48 h, 72 h and one week.after application.

The test subjects were patients who visited the Dermatology OPD of the Toho Univeristy Hospital during 1980-82. 30 of the subjects had been diagnosed as having contact dermatitis caused by rubber products. (We shall designate this group as the 'rubber products-sensitive g~~ur~~roup'. This group had 9 males, and 21 females (age 15-58)). 14 of the subjects had been diagnosed to have contact dermatitis from vinyl chloride products ('vinyl chloride sensitive group', 1 male and 13 females, age 18-57). Four of the subjects belonged to both the groups.

RESULTS

....#### **** 500 micro g of authentic sample , when adsorbed in a column and subjected to silica gel column chromatography, got eluted as illustrated in Fig. 2. In the case of samples that got eluted in more than one fraction, the percentages eluted in each fraction are given by the side, in Fig. 2. The numerals given ~~on the left side~~ in parentheses represe per cent recovery of each authentic substance.

2. Gas Chromatography

After obtaining fractions A - E of authentic samples/ through silic gel column chromatography, each fraction was subjected to GC under the conditions specified in Section 4. of Methods. The chromatograms shown in Fig. 3 were thus obtained. The retention times of the compounds given in t Fig. are relative retention times obtained when the retention time of IPPD ~~is~~ was taken as 1.00.

All the fractions gave peaks with good symmetry. In Fraction B, the separation of peaks ...###**** methylene-bis(4-methyl-6-tert-butyl phenol) (MBMBP) (1.35), and in Fraction C, the peaks of 6-ethoxy-2,2,4-trime -1,2-dihydroquinoline (ETMDQ) (0.57) and SP-1 (0.58), a component of SP, ha overlapped. However, since SP shows four peaks corresponding to its four components, it can be differentiated from MBMBP and ETMDQ which show only single peaks.

Quantitative analysis was carried out using the peak heights by the absolute calibration method. The sample obtained by the procedure of 4. (Methods) was made up to 0.5 ml. 5 microlitre of this was injected into the GC column and analysed under FDI sensitivity 10^3 M Ohms and range 0.1 Peaks higher than or equal to 0.5 cm alone were considered. The lower limi of quantitative analysis were about 10 micro g /g for triphenyl phosphate (and N-(3'-hydroxybutylidene)-alpha-naphthylamine (HBAN); about 5 micro g/g for SP, N-(methacryloyloxy-hydroxypropyl)-N'-phenyl-p-phenylenediamine (MP N-(p-toluenesulphonyl)-N'-phenylenediamine (TPPD), and dilauryl-3,3'-thiodiprop (DLTDP); and about 1 microg/ g in the case of the others.

3. Mass spectrometry

The mass sectra of authentic samples were measured under the conditions of sectio 4. (Methods) (the samples were introduced directly). However, for SP, GC-MS ...###*** and the mass spectra of each of the components (SP-1 to were obtained. The results are given in Table III.

All the compounds, except for TPPD and di(2-ethylhexyl)phthalate (D showed molecular ions. TPPD (molecular weight 338) had ion fragment with the maximum mass at m/z 184, i.e. the ion fragment produced by dissociation of the tosyl group. N-phenyl-alpha-naphthylamine (PAN) and N-phenyl-beta-n thylamine (PBN) had almost identical spectra. However, since they had diff retention times in GC, and since GC-MS was used for the actual samples, these two could be differentiated.

4. Method of Extraction

In the case of materials like rubber that undergo harsh treatments like heating during their processing, it is impossible to determine the true amounts of additives used. Thus, in setting the optimum extraction conditio one has to first ~~to~~ carry out extractions under a variety of conditions on the product which has been confirmed to contain the target chemical, and the take the conditions that gave maximum extraction as the optimum conditions.

We examined the conditions of extraction for the five anti-oxidants, MBMBP, SP, IBBDMP, IPPD, and BHT which had been detected in commercial rubbe gloves. Four types of solvents were tried. These are a 1:1 mixture of ace chloroform, which had been used in the earlier study also, a 1:1 mixture of acetone-benzene, acetone, and methanol. The results are summarized in Table

Firstly, 200 micro g of authentic sample was refluxed for 2 h in 50 of the solvent over an 80°C water bath. The MBMBP, IBBDMP, SP, and IPPD could be quantitatively recovered. Only BHT had a poor recovery with all the solvent systems. We therefore gave a 2 hour shaking extraction of 200 ~~microg~~ micro g authentic ~~solvent~~ sample in 50 ml solvent. Under these conditions almost quantitative recovery could be made of all the ~~solvents~~ chemicals, except when methanol was used as the solvent. This indicates that BHT is destroyed by h

Next, we attempted extraction of these compounds from rubber product using Samples, No. 1 (containing MBMBP), No. 5 (containing IBBDMP), and No. (containing SP and IPPD) all taken from rubber gloves; and an isoprene rubber sample (containing BHT). The samples weighed about 1 g each. The amount of compou extracted by shaking for 30 min repeatedly four times in a 1:1 mixture of acetone and chloroform was taken as 100% extraction. Except in the methanol solvent, extraction of BHT ~~we~~ by refluxing at 80°C was very low (60% or less). Extraction of all the anti-oxidants was only about 50% in methanol by shaking at room temperature. When acetone was used as the solvent, the recoveries of BHT and SP were rath low. The above experiments revealed that shaking at room temperature was better than refluxing. Acetone, ...#####***** and 1 : 1 mixture gave almost the same level of extraction showing that they are the best suited solvent systems. Therefore, for the subsequent experiments, as in our previous work, we decided to use a 1:1 mixture of acetone and chloroform and room temperature shaking for extraction.

5. Examples of Qualitative and Quantitative Analysis and investigation Anti-oxidants in Commercial Rubber Gloves

After establishing the methods of analysing anti-oxidants in rubber gloves as described above, we analysed commercial products. We shall now explain the procedure of this analysis taking sample No. 23 as an example.

Sample No. 23 was a rather thick and long rubber glove meant for domestic use. Its commercial name was 'Tokyo Tebukuro'. It carried a disp "Tokyo Latex Co-operative Society", but the manufacturer's name was not giv The gas chromatogram of the extract from this glove showed three peaks, i.e. Peak 1 (relative retention time 1.16), Peak 2 (1.25), and Peak 3 (1.35). The retent times of Peaks 1 and 3 coincided respectively with those of IBBDMP and MBMBP. We then subjected the extract to silica gel column chromatography. Peak 1 was eluted in Fractions C and D, and Peak 3 in Fraction B. When GC-M was done with each fraction, Peak 1 showed the major ion fragments at m/z 29 (M^+) and 255 (M^+-43), while in Peak 3 these were at m/z 340 (M^+), 283 (M^+- 177, and 164. These more or less coincided with the mass spectra of authen IBBDMP and MBMBP. Fig. 4 gives the GC-MS mass spectra of peaks 1 and 3.

...###**** sample No. 23 was found to contain MBMBP and IBBDMP as anti-oxidants. Quantitative analysis by GC revealed the presence of

2040 micro g / g of MBMBP and 2160 micro g/g of IBBDMP.

Regarding Peak 2, from the elution behaviour in silica gel column chromatography, retention time in GC, and ... ####**** was identifie to be butylbenzyl phthalate (BBP). Its concentration was found to be as hig as 19000 micro g /g. Thus, we discovered that phthalic acid esters are also used in rubber gloves for some purpose.

Fig. 5 shows a typical case where an anti-oxidant is present in rubber glove. Rubber component, low molecular weight waxy substances, etc get almost all eluted in Fraction A. There were almost no peaks in the other fractions that would interfere with the analysis. However, ...###*** the amount of ....####*** extracted ~~was~~ is small, or when the amount of miscella neous materials is large, the quantitative analysis becomes difficult. In such cases, clean up by a procedure other than adsorption chromatography, i.e. gel chromatography, etc, would be required. We have not investigated this aspect.

The results of analysis of 37 rubber glove samples using our method of analysis are given in Tables Ia and Ib. 32 of the 37 samples had anti-oxidants in them. Only in two samples, were more than one anti-oxidant pre MBMBP and IBBDMP were present in sample No. 23, and SP and IPPD were present in sample No. 25. Sample Nos. 19, 20, 26, 36, and 37 showed no trace of an of the anti-oxidants listed in Table II. Sample Nos. 26, 36, and 37 showed in their mass spectra the suggestion of the presence of some phenoloc compo We are presently investigating this.

Both in the Japanese and in the foreign-made products, mainly phenolic compounds have been used as anti-oxidants. 9 out of 11 foreign-made sample contained MBMBP in the ~~rage~~ range, 244-6810 micro g/g. Among the Japanese products, one sample contained IPPD, a nitrogen containing compound (177 micro g/g). The others contained phenolic anti-oxidants. 10 of them had M

(399 - 4860 micro g/g), 8 samples had SP (1600 - 6080 micro g / g), 6 sampl had IBBDMP (1590 - 6740 micro g /g), and one sample had 4,4'-butylidene-bis (3-methyl-6-tert-butyl phenol) (BBMBP) (3480 micro g /g).

Phthalic acid esters were not found in the foreign-made products. But among the Japanese products, ... ####**** (100- 274 micro g/ g), BBP ~~(895~~ (845, and 19000micro g / g) was found in two samples; and DEHP (937 - 4220 micro g / g) was found in 4 samples. The elution behaviour in silica gel column chromatography, retention times in GC, and mass spectra of DBP, BBP, and DEHP are given in Figs. 2 and 3 and Table II. ~~Cart~~ Chart 1 shows the structures of the anti-oxidants and phthalic acid esters found in commercial rubber gloves.

......#######*****

Results of patch tests are given ~~Fig~~ in Tables Va and V b. ~~Subjects-~~ The proportion of subjects showing a reaction of (+) or higher is given as percentages in the Tables.

After the patch test, the compounds were compared for the productio of symptoms at 72 h after administering the test. Comparisons were also made between the 'rubber product sensitive' and the 'vinyl chloride sensiti groups'. Five phenolic anti-oxidants including MBMBP and SP caused almost no positive reaction at a concentration of 10% in both the groups. On the other hand, the nitrogen containing anti-oxidants, IPPD ...###*** at ~~10%~~ concentration or higher both in the 'rubber products sensitive' and the 'vinyl chlori sensitive' groups.

DISCUSSION

Analysis of 37 commercially obtained rubber gloves for domestic use revealed that mainly phenolic compounds such as MBMBP, SP, IBBDMP, etc are used as anti-oxidants in these products. In fact, in 19 out of these 3 samples, MBMBP was detected. SP was found in 8 samples, IBBDMP in 6 sampl and BBMBP in one sample. Among nitrogen containing compounds, IPPD was fou in one sample(Sample No. 25). Sample No. 25 was a thick heavy duty rubbe glove that is not normally used in homes. Cronin's book mentions$^{3\ a)}$ that p-phenylenediamine type anti-oxidants are used mainly in industrial rubber products and not in household rubber products. This agrees with what we found.

In patch tests on patients with a history of contact dermatitis caused by rubber products and ~~and~~ subjects who had had contact dermatitis from vinyl chloride products, phenolic axidants like MBMBP and SP, which are i use presently in rubber gloves, caused almost no positive reaction in eith group of subjects. They are, therefore, not cond~~s~~idered to be strong sensitizers.

In our previous report, we had concluded that "IPPD was one of substances responsible for the contact ~~der~~ allergic dermatitis observed to be caused by the heavy duty rubber glove" (Sample No. 25 in the present study). But nothing could be said about SP since no patch test was carrie out with it. The results obtained in the present study suggest that SP is not likely to be a causative agent.

In our study of the relavant literature, we found a few cases of contact dermatitis caused by phenolic anti-oxidants. There were no cases such dermatitis due to <u>bis</u>-phenol type compounds such as the one detected i commercial rubber gloves. As an instance of contact dermatitis by a phenol anti-oxidant, Cronin's book~~s~~ cites a case of dermatitis casued by a surgic rubber glove$^{3\ b),\ 5)}$ and a rubber sheet$^{3\ c)}$ that contained dioxydiphenyl $^{3\ a}$ Cronin identified this compound to be a sensitizer. He also cites the exa of cases caused by a rubber glove$^{3\ d)}$ containing 2-p-methylcyclohexyl-4,6 thyl phenol and also by BHT and butylated hydroxyanisol (BHA)$^{3\ e),\ 7)}$. Fish had pointed out 4) that mono-benzylhydroquinone has a colour removing action as well as a sensitizing action.

IPPD showed a very high incidence of positive reaction, among all t anti-oxidants tried. Kanto et al. 9) had observed the strong sensitizing action of IPPD when applied on Hartly strain of white guinea pigs. The books of Cronin and Fisher mention3 f), 4 b) that IPPD is a strong sensitizer. Cases of contact dermatitis due to IPPD-containing tyres9), rubber tub rubber diving masks11), etc have been reported.

While no phthalic esters were found in the foreign-made products, the products of some Japanese manufacturers showed the presence of DBP, BBP DEHP in rubber ~~predu~~ articles meant for use with eatables.12) According to a certain source13), DBP, DEHP, tricresyl phosphate, etc are used in rubber products to soften the rubber, to disperse the ingredients well, and to make the extrusion-forming, rolling, etc ~~more~~ easier.

To sum up the above results, phenolic compounds like MBMBP, SP, et with low sensitizing action are mainly used as anti-oxidants in rubber glov being marketed at present. Thus, as far as the anti-oxidants are concerned the present trend~~s~~ is desirable from the viewpoint of contact dermatitis patients. IPPD, which showed a far higher incidence of positive reaction compared to other anti-oxidants tested, should not in our opinion be used in rubber gloves for domestic use or in any rubber gloves including those meant for industrial use.

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(Tables and Figs. are given in English in the original)
