REFERENCES

1 Randall MJ, Parry MJ, Hawkeswood E, Cross PE, Dickinson RP. UK-37248, a novel, selective thromboxane synthetase inhibitor with platelet anti-aggregatory and anti-thrombotic activity. *Thromb Res* 1981; 23 : 145

2 Chen XS, Long K, Wan WQ. Effects of CI-914 on platelet aggregation, thrombosis and cAMP content in platelets. *Chin J Pharmacol Toxicol* 1988; 2 : 252

3 Chen XS, Long K, Yue TL, Wan WQ. Selective effects of CI-914 on the metabolites of arachidonic acid via cyclooxygenase pathway. *Ibid* 1989; 3 : 85

4 Sills T, Heptinstall S. Effects of a thromboxane synthetase inhibitor and a cAMP phosphodiesterase inhibitor, singly and in combination, on platelet behaviour. *Thromb Haemosts* 1986; 55 : 305

5 Sheng ML, Chen SB, Jin WF, *et al*. Endothelial cells culture and distinguish. *Acta Acad Med Shanghai* 1987; 14 : 71

6 Si YQ, Li ZJ, Ma KR, *et al*. Radioimmunoassay for 6-keto-$PGF_{1\alpha}$. *Acta Acad Med Sin* 1986; 8 : 310

7 Zhang XY, Ren SQ, Xong J, Di H, Yang BH. A sensitive method to determine cyclic adenosine monophosphate in platelets. *Chin J Cardiol* 1980; 8 : 142

8 Adler B, Gimbrone MA, Schafer AI, Handin RI. Prostacyclin and β-adrenergic catecholamines inhibit arachidonate release and PGI_2 synthesis by vascular endothelium. *Blood* 1981; 58 : 514

9 Hopkins NK, Gorman RR. Regulation of endothelial cell cyclic nucleotide metabolism by prostacyclin. *J Clin Invest* 1981; 67 : 540

10 Brotherton AFA, Macfarlane DE, Heak JC. Prostacyclin biosynthesis in vascular endothelium is not inhibited by cyclic AMP. Studies with 3-isobutyl-1-methylxanthine and forskolin. *Thromb Res* 1982; 28 : 637

11 Whorton AR, Collawn JB, Montgomery ME, Young SL, Kent RS. Arachidonic acid metabolism in cultured aortic endothelial cells. Effect of cAMP and 3-isobutyl-1-methylxanthine. *Biochem Pharmacol* 1985; 34 : 119

12 Martin TJ, Smith IL, Nolan RD, Dusting GJ. Prostanoids in platelet-vascular interactions. *Am J Cardiol* 1983; 52 : 22A

13 Sills T, Cowley AJ, Heptinstall S. Aspirin and dazoxiben as inhibitors of platelet behaviour; modification of their effects by agents that alter cAMP production. *Throm Res* 1986; 42 : 91

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链霉素聚合物引发链霉素速发型过敏反应[1]

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Immediate type anaphylaxis of streptomycin allergy elicited by streptomycin polymers[1]

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ABSTRACT It has been known that streptomycin (SM) can cause some immediate type anaphylaxis even anaphylactic shock in clinical therapy. The characteristic of the substance that elicits the allergic reaction has not been reported. Using gel fil-

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tration and high performance gel permeation chromatography (HPGPC), we have found that some impurities of high molecular weights (HMW) were formed by heating acidic SM solution. The HMW impurities had a colour reaction with citric acid-acetic anhydride reagent and elicited passive cutaneous anaphylaxis (PCA) on guinea pigs sensitized with rabbit anti-SM-BSA serum and general anaphylaxis in guinea pigs immunized by SM-BSA. It is suggested that the impurities, SM polymers (poly-SM) related to some reactions on amino groups of SM, are the allergens of SM allergy.

KEY WORDS streptomycin; allergens; anaphylaxis; polymers; gel chromatography

提要 利用凝胶过滤及HPGPC等技术，证明链霉素(SM)溶液在酸性条件下保温，可形成链霉素聚合物(poly-SM)。Poly-SM可以引发SM抗血清致敏的豚鼠的被动皮肤过敏(PCA)反应和由SM-BSA免疫的豚鼠的全身过敏反应，说明poly-SM是引发SM速发型过敏反应的过敏原。

关键词 链霉素；变应原；过敏症；聚合物；凝胶色谱法

临床中使用链霉素(streptomycin, SM)常引起速发型过敏反应，但引发反应的过敏原仍为未知。最近的研究表明，SM药品中存在的致敏性杂质是引发SM速发型过敏反应的过敏原[1,2]，并发现其主要致敏性杂质是一组色谱行为相似且在320 nm有紫外吸收峰的未知杂质(待发表资料)。抗生素中已知致敏性杂质可分为抗生素—大分子载体(主要是蛋白类载体)的结合物和自身聚合物两类。但商品SM中的致敏性杂质不是蛋白类杂质(待发表资料)。虽然目前还没见SM聚合的报道，为探讨SM过敏反应中过敏原的化学本质，本文对SM能否聚合，SM聚合物(poly-SM)能否引发速发型过敏反应问题进行探讨。

MATERIALS AND METHODS

链霉素样品的处理 SM硫酸盐，效价789 U/mg，本实验室精制。

酸保温处理，将SM硫酸盐水溶液调pH为2左右，55℃水浴保温不同时间，调pH为中性后，冷冻干燥。

SM硫酸盐溶液经葡聚糖凝胶Sephadex G-15 (Pharmacia产品)柱(1.5×40 cm)，蒸馏水洗脱，收集K_{av}=0.3～0.6处(蓝色葡聚糖-2000测定)的洗脱液，冷冻干燥得纯SM。

链霉素中高分子杂质含量的比较

1 凝胶过滤法 200 mg样品溶于2 ml蒸馏水中，通过葡聚糖凝胶Sephadex G-15柱(1.5×40 cm)，蒸馏水洗脱，收集K_{av}=－0.1～0.1处的洗脱液比较其紫外吸收光谱。

2 高效凝胶渗透色谱(HPGPC)法 采用Waters 510型高压泵，SIL-1 A进样阀，岛津SPD-1型uv检测器，CTO-2 A恒温箱，R-772型记录仪，色谱柱(4 mm×30 cm)填充NWG多孔硅胶(天津化学试剂二厂)。

流动相pH 6.4磷酸缓冲液0.02 mol/L。

流速0.8 ml/min，检测波长220 nm，灵敏度0.08 AuFS，柱温25℃，记录纸速5 mm/min，样品浓度100 mg/ml，进样量10 μl。

柠檬酸-乙酸酐反应[3] 在小试管中放入少许固体待测样品，加入一滴柠檬酸-乙酸酐试剂，混合，80℃加热。若有红紫色显现为阳性结果。比较SM和酸保温中形成的高分子杂质冻干品的柠檬酸-乙酸酐反应。

兔抗链霉素血清的制备

1 免疫抗原SM-BSA的合成 SM和牛血清白蛋白(BSA)按10:1(wt:wt)溶于pH 9.4碳酸缓冲液0.5 mol/L中，37℃放置12 h后，于4℃透析除去游离的SM，冷冻干燥。

2 免疫 新西兰兔5只(本所实验动物繁育场提供)，2.5±SD 0.2 kg，每只肌注卡介苗10 mg，1 wk后免疫。基础免疫两次，每周一次，每次在腘窝淋巴结中注射0.2 ml含500 μg抗原的福氏完全佐剂。基础免疫2 wk后加强免疫。每次注射1 ml含500 μg抗原的福氏不完全佐剂于兔的肌肉、脚掌及背部皮下多点，

隔周一次，加强 2-3 次。免疫结束后由颈动脉放血，分离血清，血清于－20℃冻存。间接血凝法[4]测 SM 抗体效价。

I 型过敏反应的动物模型 DHP 纯系白色豚鼠由我所实验动物繁育场提供。

1 豚鼠被动皮肤过敏(PCA)反应[5] 16 只豚鼠 248±18 g，转移 0.1 ml SM 抗血清，16-20 h 后 iv 0.5 ml 1% Evan blue 液，15 min 后用葡聚糖凝胶分级得到的不同 Kav 组分做为攻击抗原，用经亲和层析除去 SM 抗体（未发表资料）后的兔血清做对照。

2 豚鼠全身过敏反应 15 只豚鼠 220±31 g，用 SM-BSA 免疫豚鼠。第 1 次免疫 ip 含 1 mg SM-BSA、37×10^8 百日咳菌苗的 Al(OH)$_3$(2 mg/ml)混悬液 1 ml，第 2、3 次免疫注射液中不含百日咳菌苗。每周免疫 1 次，第 3 次免疫后的 d 21，先 iv 1% Evans blue 溶液 0.5 ml，15 min 后再由静脉攻击抗原。另设对照组。若 10 min 内出现抓鼻、发抖、呼吸急促等过敏症状且眼窝、嘴、鼻、耳根等处出现蓝色染料，为阳性反应。

RESULTS

酸保温前后链霉素中高分子杂质的比较

1 凝胶过滤法 酸保温前、后的 SM，经葡聚糖凝胶分级，$K_{av}=-0.1\sim0.1$ 处组份的 uv 吸收光谱的比较结果见 Fig 1。可见酸保温后，SM 中高分子杂质的含量增加，且增加的高分子杂质在 320 nm 附近有紫外吸收峰。

2 HPGPC 法 HPGPC 分析 SM 样品，发现在 K_{av} 较小处有杂质峰出现，该样品经葡聚糖凝胶除去其中的高分子杂质后制备的 SM 纯品，再经 HPGPC 分析时，K_{av} 较小处的杂质峰消失，说明消失的杂质峰为高分子杂质所致。经酸保温后的 SM 样品，该吸收峰增高，说明其中高分子杂质的含量增加(Fig 2)。

酸保温增加的高分子杂质致敏性的测定

1 豚鼠 PCA 反应 SM 样品或 SM 纯品，经酸保温后，在葡聚糖凝胶 Sephadex G-15 上

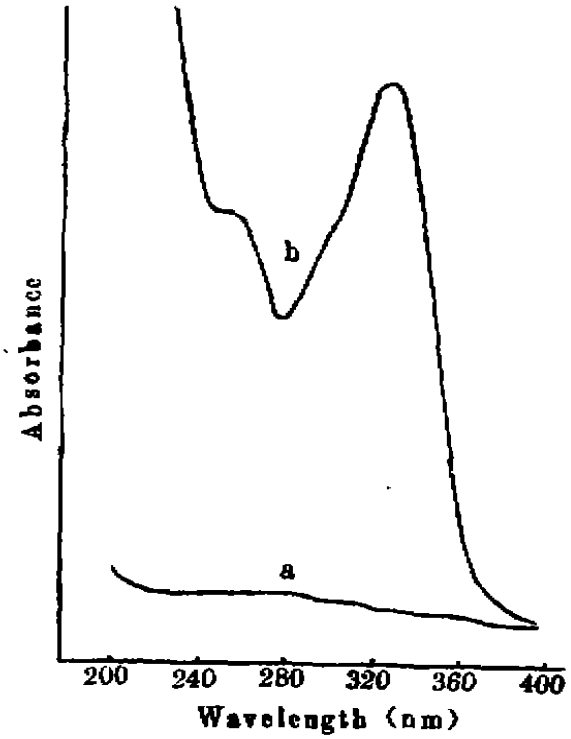

Fig 1. UV spectra of V_0 fraction of streptomycin separated by gel filtration on Sephadex G-15. a) Streptomycin, b) Streptomycin treated at pH 2 and 55℃ for 4 h.

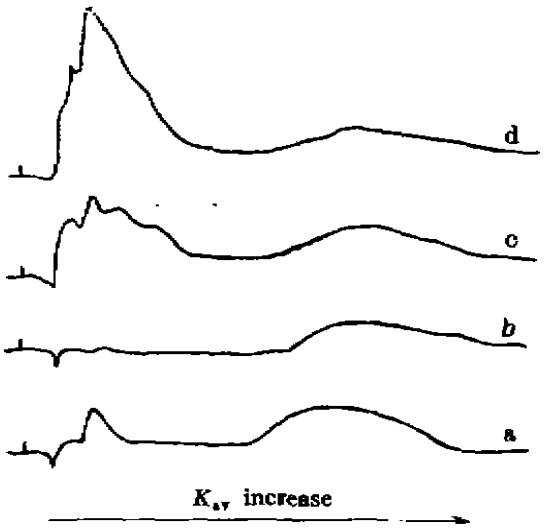

Fig 2. HPGPC chromatograms of the streptomycin by different treatments. a) Non-treated, b) Free from impurities of high molecular weights by gel filtration, c) Treated at pH 2 and 55℃ for 1 h, d) Treated at pH 2 and 55℃ for 2 h.

Tab 1. Passive cutaneous anaphylaxis reaction on guinea pigs elicited by some streptomycin (SM) fractions on gel filtration.

Sample	SM fraction on Sephadex G-15 (K_{av})	Volume challenged (ml)	Hemagg titer of anti-SM serum	Dilution	Diameter of blue spot in PCA reaction (mm) ($n=4$)	
SM*	−0.1−0.1	0.2	1:512	no	Vast stertch	
				1/2	Vast stretch	
				1/4	Vast stretch	
			1:16	no	Negative	
			67 μg anti-SM Ab purified by affinity chromatagraphy		15×16	18×17
					15×18	16×18
SM†	0.1−0.3	1	1:512	no	Negative	
Pure SM††	−0.1−0.3	1	1:512	no	Negative	
	−0.1−0.1	0.5	1:512	no	40×60	40×50
					35×50	40×40
			1:16	no	Negative	

* SM treated at pH 2 and 55℃ for 4 h, † Non-treated SM, †† Pure SM treated at pH 2 and 55℃ for 3 h.

Tab 2. General anaphylaxis elicited by impurities of high molecular weights from SM in guinea pigs immunized by SM-BSA (bovine serum albumin) ($n=3$).

Challenger*	*Reaction*
Immunized guinea pigs	
No impurities of high molecular weights	−
0.5ml V_0 fraction from nontreated SM	−
0.1ml V_0 fraction from SM treated at pH2 and 55℃ for 3 h	+
0.5 ml V_0 fraction from pure SM treated at pH 2 and 55℃ for 3 h	+
Normal guinea pigs	
0.5 ml V_0 fraction from SM treated at pH 2 and 55℃ for 4 h	−

* Each challenger contained Evans blue and the V_0 fraction was on sephadex G-15.

$K_{av}=0$ 附近的组分具有较强的引发 PCA 反应的能力，而 K_{av} 在 0.1～0.3 处的组分，PCA 反应阴性；未经酸保温的 SM 样品，在葡聚糖凝胶 Sephadex G-15 上 $K_{av}=0$ 附近的组分也不能引发反应 (Tab 1)。说明酸保温中增加的高分子杂质可以引发 PCA 反应。

2 豚鼠全身过敏反应 SM 样品或 SM 纯品经酸保温后，用葡聚糖凝胶 Sephadex G-15 上 $K_{av}=0$ 附近增加的高分子杂质攻击由 SM-BSA 免疫的豚鼠，约 2 min 出现抓鼻、发抖、呼吸急促等症状，预先注入血管中的 Evans blue 出现在眼窝、耳根、嘴鼻等部位，而对照组豚鼠不出现上述反应 (Tab 2)。表明酸保温中增加的高分子杂质可以引发全身过敏反应。

酸保温增加的高分子杂质和柠檬酸-乙酸酐试剂的反应 柠檬酸-乙酸酐试剂对叔胺结构有特异反应[3]。酸保温中增加的高分子杂质和柠檬酸-乙酸酐试剂呈阳性反应，而 SM 却呈阴性反应。说明高分子杂质的产生和 SM 的氨基有关。

DISCUSSION

在酸保温条件下，SM 分子形成的高分子杂质是引发 SM 速发型过敏反应的过敏原。引发 SM 速发型过敏反应的过敏原，必然含有两个以上 SM 抗原决定簇；SM 抗原决定簇是以整个 SM 分子为结构基础的[4]；提示形成的高分子杂质是 poly-SM。

用 HPLC 分析 SM 在酸保温中形成的高分子杂质，发现紧随 SM 峰后色谱行为极相似的 3 个杂质峰能引发 SM 速发型过敏反应，经适当处理，该致敏性杂质能分解出 SM(未发表资料)。进一步提示酸保温中形成的高分子杂质是 poly-SM。由于酸保温中形成的高分子杂质仅有 3 个组分能引发 SM 速发型过敏反应，且用葡聚糖凝胶 Sephadex G-25 分级时，分子量最大的组分仅分布在 $K_{av}=0.3$ 附近(未发表资料)。表示 SM 在所用反应条件下形成的 poly-SM 的聚合度并不高。氨苄青霉素的 2-5 聚体均能引发氨苄青霉素的速发型过敏反应[6]。酸

保温中形成的3个poly-SM组分究竟是SM的几聚体，有待研究。

虽然目前还没见SM能形成聚合物的报道，但SM分子既有氨基，又有醛基，在酸催化条件下，醛基和氨基易发生亲核加成反应。Aronson等也曾认为SM在溶液中可以以其醛基和*N*-甲基葡萄糖胺上的仲氨基分子内缩合[7]。提示SM分子间通过醛基和氨基缩合是可能的。实验中我们通过柠檬酸-乙酸酐反应证明聚合反应和SM分子上的氨基有关。Poly-SM的确切聚合机理及聚合后的结构，有待研究

从商品SM中分离到的主要致敏性杂质"高杂1"在320 nm附近有紫外吸收峰[2]，poly-SM在320 nm附近也有紫外吸收峰。通过对SM生产工艺的了解得知，生产中为除去发酵液中的蛋白质需将发酵液用草酸酸化至pH 3左右，并通蒸气至70℃。在此条件下，SM的聚合反应是可能发生的。Poly-SM一旦形成，现有的主要精制手段都不能将其全部除净(未发表资料)，因此生产过程中产生poly-SM并带到商品SM中是完全可能的。

REFERENCES

1 Hu CQ, Zhao JX, Jin SH. Studies on streptomycin allergy. I. Idenfication of the high molecular weight allergenic impurities of streptomycin allergy from commercial streptomycin. *Chin J Pharm Anal* 1989; 9 : 264

2 Hu CQ, Zhao JX, Jin SH. Studies on streptomycin allergy. II. Separating the allergenic impurities of streptomycin allergy from commercial streptomycin. *Ibid* 1989; 9 : (in press)

3 Ohkuma S. *Detection of some nerve stimulants*. II. Color reaction of 1-phenyl-2-dimethylaminopropane and tertiary amines. J *Pharm Soc Jpn* 1955; 75 : 1124

4 Zhao JX, Wu Q, Sun XL. Study on the antigenic determinant of streptomycin allergy. *Acta Pharm Sin* 1981; 16 : 31

5 Ovary Z. Immediate reactions in the skin of experimental animals provoked by antibody-antigen interaction. In: Kallós P, Waksman BH, eds. *Progress in allergy*; Vol 5, Basel: Karger, 1958 : 459-508

6 Jin SH, Jing J. Studies on ampicillin polymers I, Isolation and characterization of ampicillin polymers. *Chin J Antibiot* 1987; 12 : 241

7 Aronson J, Meyer WL, Brock TD. A molecular model for chemical and biological differences between streptomycin and dihydrostreptomycin. *Nature* 1964; 202 : 555

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全胃肠外营养对丁胺卡那霉素药物动力学的影响

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Influence of total parenteral nutrition on amikacin pharmacokinetics

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ABSTRACT Six patients under total parenteral nutrition (TPN) and eight control patients received 200 mg of amikacin by iv infusion in 0.5 h. Amikacin concentrations of serum and urine were determined by fluorescence polarization immunoassay. At 6 h after the beginning of administration.

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