刺激性气体对大鼠鼻腔粘液纤毛作用的研究方法

K T Morgan，蒋学之*，E A Gross，D L Patterson
(Chemical Industry Institute of Toxicology, Research Triangle Park NC 27709, USA)

提要　介绍正常离体大鼠鼻腔粘液纤毛活动功能及大鼠吸入甲醛(15 ppm)，二甲胺(175 ppm)，和氯气(2.5 ppm)，6 h/d，9 d后鼻粘液流动率和流动方式的变化。本方法为研究刺激性气体对鼻腔的作用提供了有用的手段，并可能有助于阐明其毒性机理。

关键词　鼻粘膜；纤毛；大鼠；二甲胺；氯；甲醛

呼吸道表面的许多部分具有粘液纤毛结构[1-3]，它由纤毛上皮细胞的纤毛及覆盖其上的分泌粘液构成(图1)。粘液呈两相：粘胶相(上层)和水相(下层)。Proctor[1]对各种研究人类或动物的鼻腔粘液纤毛功能方法的优缺点作了评述，大部分方法不适用于常用的实验动物——鼠类。Lucus和Douglas[4]曾使用低倍双目显微镜研究了各种动物(包括大鼠)鼻腔粘液纤毛结构和功能，我们改良了他们的方法并研究了正常大鼠鼻腔粘液纤毛功能特性和吸入各种刺激性气体后鼻腔粘液纤毛的变化。

材料与方法

大鼠　F-344大鼠购自Charles River Breeding Lab, Kingston NY。每只不锈钢笼中饲养3鼠，任意进食和饮水，检疫期2周，健康的和鼠病毒检测呈阴性者才用于本实验。

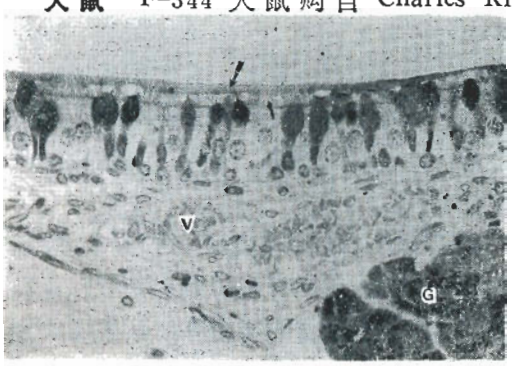

Fig 1.　A glycol methacrylate section of mucosa of rat ventral nasal septum showing the mucus blanket or epiphase (large arrow). The cilia were bathed by perciliary fluid(small arrow). Within the epithelium were mumerous goblet cells (darkly stained). G = glands, V = blood vessels × 840

染毒　每组包括染毒大鼠6只，对照12只，体重250-300 g，染毒组鼠分别在8 m^3不锈钢玻璃染毒柜中吸入被测试的气体6 h/d，每周5 d，共计2周。各种被测毒物浓度分析方法见文献[5-7]。本实验大鼠吸入浓度甲醛为15 ppm，二甲胺为175 ppm，氯气为2.5 ppm。

解剖　大鼠于末次吸入染毒后立即断头，切除下腭和周围软组织，沿冠状缝纵行剖开鼻腔，移除鼻中隔以暴露鼻甲部(图2)。鼻中隔及右侧鼻腔用于检测。在离体情况下，鼻腔粘液纤毛活动功能可保持20-40 min，解剖过程是在3.5-6 min内完成。

记录和分析　粘液纤毛功能测试装置和方法[8]及所用仪器详见图3。鼻腔标本与水平面呈10-15°斜角放置在观察仓内，切除部分凸出的额骨可以得到较好的观察角度。用配有长焦距物镜(×10，8 mm)的双目显微镜观察鼻腔粘液纤毛活动。显微镜配有三通管，使目测与

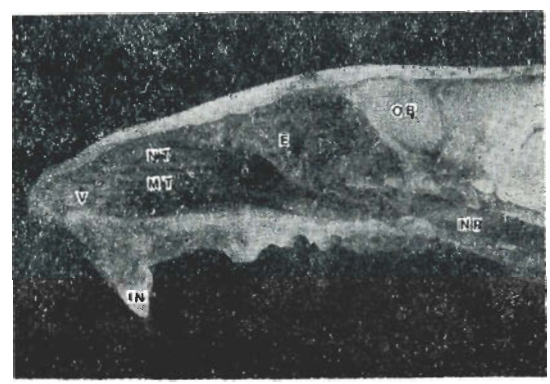

Fig 2.　A rat head opened adjacent to the midline, with the septum removed to display the turbinates. E = ethmoid turbinates; IN = incisor tooth; MT = maxilloturbinate; NP = nasopharynx; Nt = naso-turbinates; OB = olfactory bulb; V = vestibule.

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* Visiting scientist from Shanghai First Medical College, Shanghai 200032, China

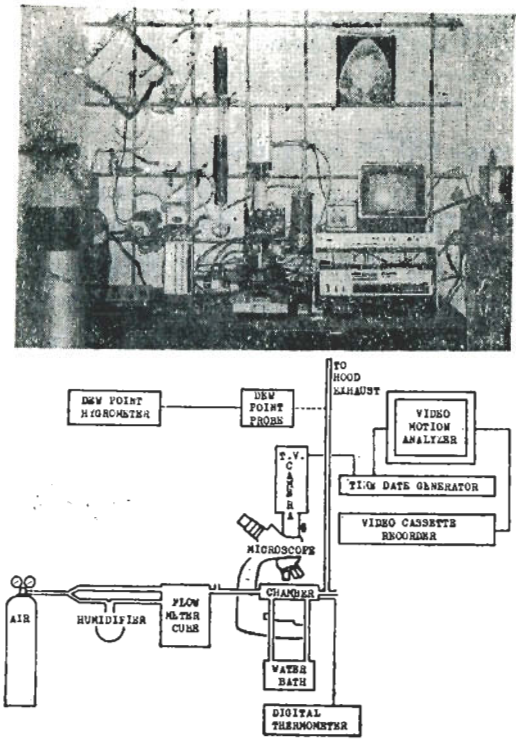

Fig 3. Equipment used for studies of *in vitro* mucociliary activity. Mucus flow was counted by a stop watch and analysed using the video motion analyser.

录像可以同时进行，纤维光束源自上斜方照射于贴在物镜头侧的小片铝箔上，再折射到检测标本表面，即可在屏幕上得到清晰的图像。

录像分析仪可调节速度以测绘器官表面结构图形和流动速度。观察指标计有粘液流动方向和速度(mm/min) 粘液流停滞时间和纤毛活动停滞时间。异常粘液流以及其它观察结果均以图形记录。

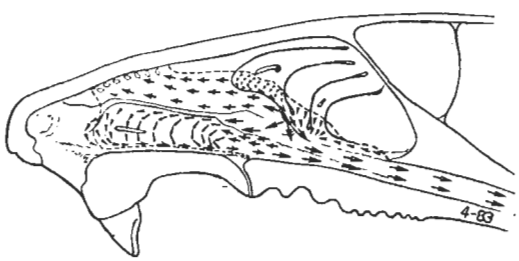

Fig 4. Mucus flow pattern and flow rate in nasal passage of normal rats. The arrow indicated the direction and rate of mucus flow. The open arrow indicated the direction of clearance of this area onto the nasal septum. The broken lines indicated the junction of the respiratory epithelium with the squamous epithelium (anterior line), and the olfactory epithelium(posterior line). Large arrow = 7-12 mm/min; intermediate arrow = 6 mm/min; small arrow = 1-3 mm/min; large arrow head = 0-2 mm/min; small arrow head = 0-5 mm/min

结　　果

正常大鼠鼻腔粘液流见图 4 。鼻腔内各部分粘液流动方向不同，在鼻腔顶部前 1/3，即上鼻甲和下鼻甲的顶端，粘液自后向前方或向前腹侧方向流动，当流达腹侧部时，即转折流向鼻中隔或鼻腔侧壁，然后向后流入咽喉部。筛甲的结构颇为复杂，其表面有粘厚的粘液层，仅在其前缘端才见到粘液流(注：筛甲大部分由嗅上皮构成，在前缘端由呼吸纤毛上皮细胞构成)，流动方向是从后背部向前腹侧流动，最后也是汇入咽喉部。下鼻甲的粘液流动则限于在该鼻甲范围内，有规则地从后向前腹侧方向流动。

观察和测定结果表明鼻腔不同区域的粘液流动速度差别较大，但各鼠间的个体差异不大。大致上，下鼻甲的前部和中部，筛甲的前缘粘液流＜1 mm/min；上鼻甲中部 5 mm/min 左右；咽喉部 5-10 mm/min；鼻腔后端之侧壁部粘液流速可高达 20-30 mm/min；而筛甲前缘粘液流速却十分缓慢，甚至无法测得。非纤毛上皮(nonciliated epithelium)分布区有时也能见到粘液流动现象，嗅觉上皮区则无明显粘液流存在(图 4)。

染毒后的大鼠，无论是吸入二甲胺，氯气或甲醛均出现鼻腔粘液纤毛功能受抑制。这三种刺激性气体对粘液流的抑制作用明显地大于对纤毛活动功能的抑制，它们对鼻腔粘液纤毛功能的损伤表现有相同之处，但又各有其特点。相同之处是最早和最容易受损伤的部位均是在下鼻甲的前部和中部以及上鼻甲的前部和腹侧缘，上鼻甲除中部可以见到少数灶性损伤外，一般顶部不受损害。不同的是，吸入甲醛后，损伤部位纤毛活动停滞，粘液流完全消失(图 5)，而吸入氯气则在下鼻甲后部见到粘液

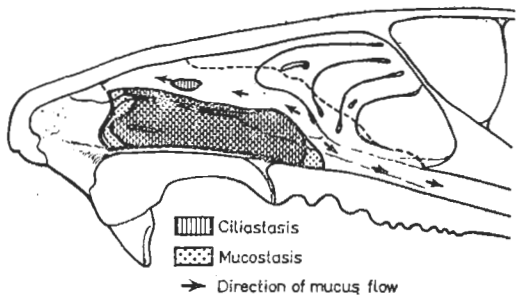

Fig 5. After 9 d exposure (6 h/d) to 15 ppm formaldehyde gas. There were extensive mucostasis and ciliastasis. The dorsal portion of nose in this field of view was unaffected. Mucus flowed around large area of ciliastasis in ventral nose, but flowed over small patches of ciliastasis in nasoturbinate.

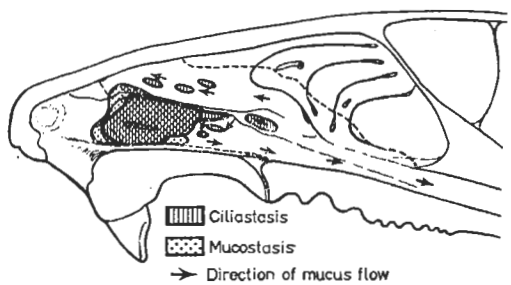

Fig 6. Exposure to 2.5 ppm chlorine for 9 d (6 h/d) induced a distinct area of ciliastasis on posterior lateral wall, but otherwise the distribution of lesions differed only slightly from that seen with dimethylamine and formaldehyde.

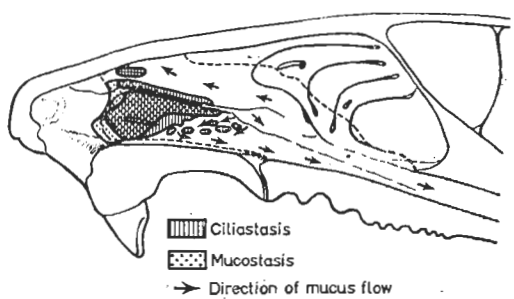

Fig 7. After 9 d exposure (6 h/d) to 175 ppm dimethylamine. The direction of mucus flow was reversed on the posterior lateral wall, and mucus was seen to flow over small areas of ciliastasis in most rats. The ventral margin of nasoturbinate was affected, except in area of fenestration seen in some rats.

流动方向混乱现象(图 6). 吸入二甲胺则在侧壁后部见到极其明显的粘液发生逆向流动现象(图 7). 此外，吸入二甲胺或氯气的大鼠，观察到在已发生纤毛活动停滞的区域仍见到缓慢的粘液流. 这个现象提示影响粘液流动的原因除纤毛外尚有其他目前我们尚不知道的因素.

讨　　论

活体情况下研究呼吸道粘液纤毛功能，很难观察其内部的变化. 离体情况下又难保持温度和湿度[9]. 虽有摄制电影的方法[9]，但难用于研究鼠类鼻腔. 近年报道使用录像方法研究鸡的气管[10]. 我们创制了一具有夹套的观察仓，以流经夹套的温水保持温度，气流通过加湿装置保持湿度，研究了离体蛙腭[8]和大鼠鼻腔的粘液流方法，提供正常和异常情况下鼻腔粘液纤毛功能的变化.

鼻腔粘液流动方式已有少量报道[4,11]，本文描述了鼻腔各个不同部位粘液流动的方式和速度. 大部分区域的粘液流与吸入空气流呈逆向流动，似乎起着清洁吸入气体的作用.

我们看到，鼻腔的粘液层是一连续的覆盖于鼻腔表面的物质，它随纤毛摆动而流动. 因此，虽在呼吸上皮细胞分布区和嗅上皮细胞分布区均存在着粘液层，但粘液流动现象仅见于呼吸上皮细胞区. 此外，在非纤毛上皮细胞区也见到有缓慢的粘液流动现象. 这可能是由于受到邻近粘液流的牵引作用，或其他原因所致，这些表明粘液层具有粘稠弹性的特征[12]. 已证实覆盖在呼吸上皮区的粘液流动可以清除吸入的尘粒.

甲醛对动物[11]及人[13]的鼻腔粘液纤毛功能的影响已有报道，但没有二甲胺和氯气对鼻腔粘液纤毛功能毒性作用的资料. 我们看到这三种气体对鼻腔粘液纤毛功能均有明显的毒性作用，表现为局灶型损伤. 这可能与气体流经鼻腔时的动力学分布有关.

用 γ-示踪方法研究指出，豚鼠吸入 9 ppm 甲醛 4 周后，鼻腔粘液清除功能有明显改

变[14]，但该方法不能提供损害分布及部位的资料。故综合使用不同方法，对了解毒性损伤的分布、性质和严重程度可提供更全面的资料。

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A PROCEDURE FOR STUDY OF EFFECTS OF IRRITANT GASES ON NASAL MUCOCILIARY APPARATUS OF RATS

K T Morgan, X Z Jiang, E A Gross, D L Patterson

(Chemical Industry Institute of Toxicology, Research Triangle Park NC 27709, USA)

ABSTRACT In normal rats the mucus flow rates varied considerally from one area to another, but were fairly consistent between animals for each area. The flow rate ranged from 1 to 30 mm/min in different areas. Rats were exposed to formaldehyde (15 ppm), dimethylamine (175 ppm) and chlorine (2.5 ppm) for 6 h/d × 9 d. The gases inhibited mucociliary function with mucostasis being more variable in extent than ciliastasis. The distributions of areas of ciliastasis and mucostasis were generally similar but not identical for each gas. With all 3 gases, the dorsal surface of the nose were unaffected. Conclusion: This procedure provides useful informations on the effects of irritant gases on rat's nasal mucociliary apparatus and helps to elucidate the mechanisms responsible for nasal toxicity.

KEY WORDS nasal mucosa; cilia; rats; dimethylamine; chlorine; formaldehyde