# Dynamic Rabbit Model of Ear Barotrauma

Binru Wang; Xianrong Xu; Jianhong Lin; Zhanguo Jin

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**BACKGROUND:** Establishing animal models of ear barotrauma (EB) to provide evaluation criteria for Eustachian tube dysfunction.

**METHODS:** Using expansive sponges, 70 rabbits' right pharyngeal openings of the auditory tubes were blocked to cause dysfunction in the right Eustachian tubes. The right tympanic cavities of 65 rabbits were the Model Group (Subgroups 1–13) and these rabbits' left tympanic cavities were the Nonblockage Group. Hypobaric chamber tests (HCTs) at various vertical speeds ($100\ m \cdot s^{-1}$, $75\ m \cdot s^{-1}$, $50\ m \cdot s^{-1}$, and $15\ m \cdot s^{-1}$) and altitudes (13,123 ft and 6562 ft) were conducted. The remaining five rabbits' right tympanic cavities were the Control Group and no HCTs were conducted. After HCTs, observations were made on rabbits' behavioral changes, oto-endoscope and tympanometry results, and pathological changes of the tympanic mucosae.

**RESULTS:** 1) Rabbits in Subgroups 1–12 demonstrated EB, while Subgroup 13 and the Control Group did not. 2) Histopathology showed EB caused by rapid ascent/descent at $100\ m \cdot s^{-1}$ was more severe than that of $75\ m \cdot s^{-1}$ and $50\ m \cdot s^{-1}$ ($P < 0.01$), and that there were no significant differences in EB caused by rapid ascent/descent at $75\ m \cdot s^{-1}$ and $50\ m \cdot s^{-1}$ ($P > 0.05$). There were no significant differences in pathological injuries at the altitudes of 6562 ft and 13,123 ft ($P > 0.05$). 3) Based on tympanic membrane structures, tympanometry, and histopathological results, rabbits' EB can be classified into mild, moderate, and severe.

**DISCUSSION:** EB's dynamic models could be established through HCTs on rabbits with Eustachian tube dysfunction.

**KEYWORDS:** ear barotrauma, Eustachian tube dysfunction, hypobaric chamber tests, disease models, animals.

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In 1937, Armstrong and his colleagues first named a series of symptoms of ear fullness, earache, and hearing loss induced by changes of air pressure as "ear barotrauma (EB)". EB is defined as acute traumatic inflammation caused by a pressure imbalance between the middle ear cavity and the surrounding environment.[1] The Eustachian tube is the tube that connects the nasopharynx to the middle ear. The middle ear cavity of a mammal is filled with air. However, the air in the middle ear does not directly contact the outside atmosphere and the pressure balance in the middle ear cavity is adjusted by the opening of the Eustachian tube. Since the opening of the Eustachian tube cannot be adapted to the sudden increase or decrease of external air pressure, the sudden change of air pressure in the middle ear cavity occurring in a special environment becomes a common cause of pressure injury to the middle ear.[19] EB in the aviation environment is called aero-otitis. Severe aero-otitis could cause pilots' incorrect operations in the air and endanger flight safety.[3,19] EB is not only present in air passengers and flying and diving enthusiasts, but also for people undergoing hypobaric chamber tests (HCTs) or hyperbaric oxygen therapy,[2,5,9,10] although professional flight crews, skydivers, and divers have a much greater chance of EB than the average person.[4,11,13] EB is a relatively common disease in clinical medicine. According to the literature, most patients cannot tolerate rapid changes in air pressure in hyperbaric oxygen therapy, with up to 69% of patients suffering from EB due to exposure to the changing pressure in the chamber, while the proportion of EB in unconscious patients exposed to rapid pressure changes in the cabin is as high as 94%.[5] In addition, approximately 4.2% of patients who experienced general anesthesia during surgeries are also at risk for EB.[12]

The pathogenesis, diagnosis, and treatment of EB have been developed according to the theories of ventilation resistance in the Eustachian tube, Henry's law, Boyle's law, and the unidirectional valve in the Eustachian tube.[2,6] In the past 10 yr, the

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incidence and basal rate of EB have been declining in China, but it is still the main reason for the grounding of flight personnel. Through large epidemiological data surveys in the Chinese Air Force, Xu et al.[18] have evaluated Eustachian tube (ET) dysfunction from 12,812 flight cadets and 120 fighter pilots. The detection rate and elimination rate (unfitness rate for recruitment) were 0.12% and 0.84%, respectively, for cadets and 63.33% and 20%, respectively, for pilots.[18] Xu et al. also reported that among the 230 fighter pilots hospitalized in the Otolaryngology department, EB ranked first in the hospitalized disease spectrum, and ranked 8$^{th}$ in 372 flight personnel's spectrum of grounding diseases in all departments.[14,15] These results indicate that both elimination rate and detection rate caused by ET dysfunction are relatively low in physical examinations for pilot recruitment; however, the hospitalization rate and grounding rate caused by ET dysfunction are high. This may be due to a lack of understanding of the EB characteristics and imperfections in EB testing and evaluation criteria.

The studies found that the functional state of the Eustachian tube and the lesions in its surrounding area are the main factors affecting efficacy and grounding rate.[9,16] Based on the clinical practice of aeromedicine, Xu first proposed the concept of primary EB and secondary EB.[16,19] According to our previous animal experiments in HCTs, guinea pigs with Eustachian tube dysfunction were bound to develop EB symptoms when pressure rapidly decreased with a speed of $100\ m \cdot s^{-1}$.[19] The aim of the current experiment was to establish an EB experimental model in rabbits at different altitudes and vertical velocities in a hypobaric chamber. The animal behavior, the structure of the tympanic membrane, the function of the Eustachian tube, and the pathological changes of the tympanic mucosa cells were observed to further explore the mechanism of EB pathological damage.

## METHODS

### Animals and Groupings

Used for this research project were 70 normal male rabbits, with body weight between 2500 and 3000 g, purchased from Shunli Farm of Beijing. The bilateral external auditory canal and nasal cavity of the tested rabbits were unobstructed without abnormal secretions, and the tympanic membrane of the tested animals was intact without congestion. The rabbits were randomly divided into three groups: the Eustachian tube obstruction group, the Eustachian tube Nonblocking group, and the Control group. The experimental conditions of each group are described as follows: 1) In the Eustachian tube obstruction group, 65 rabbits were randomly divided into 13 groups of 5 rabbits each. The Eustachian tube pharynx of the right ear of these 65 rabbits was blocked with an expansion sponge and the HCT was tested at different decompression speeds. 2) Since the Eustachian tube pharynx of the left ear in the above 65 rabbits was not blocked, the testing results and materials from the left ear from those 65 rabbits were used as the data of the Nonblocking group. 3) In the Control group, the right Eustachian tube pharynx of five rabbits was blocked by an expansion sponge like the Eustachian tube obstruction group, and the left Eustachian tube was not blocked as in the Nonblocking group. The tympanic mucosa cells from the right ear were harvested 30 min after surgery as control, but these five rabbits in the Control group did not undergo the HCT. All procedures regarding the use and care of animals in this study were approved by the Institutional Animal Care and Use Committee in the Air Force General Hospital, People's Liberation Army of China.

### Materials and Equipment

Used were 5% ketamine ($0.5\ ml \cdot kg^{-1}$) combined with Sumianxin II ($0.5\ ml \cdot kg^{-1}$), Merocel high expansive hemostatic sponges (expansive sponges for short; Medtronic–Schneider, Minneapolis, MN, USA), an oto-endoscope (3 mm in diameter, 0° visual angle, Karl Storz, Tuttlingen, Germany), CGK-48M hypobaric chamber (Air Force General Hospital, PLA, Beijing, China), and a tympanometry detector (ZO 901, Madsen, Farum, Denmark).

### Procedure

Five rabbits were euthanized by overdose injection of ketamine and sumianxin. The tympanic cavity was opened and the Eustachian tube was microdissected out for study of the relationship of the adjacent tissue. During the operation, an expanded sponge with the size of $2.5 \times 6.0 \times 10.0$ mm was inserted into the right Eustachian tube and tympanic mucosa specimens were removed for pathological observations.

The external auditory canal of the rabbits in all groups was cleaned under the oto-endoscope and tympanometry detector. The external nostrils of the rabbits were disinfected with iodophor (disinfection radius: 2.5 cm). Under aseptic operation, an expansive sponge was placed into the right nasopharynx with toothed forceps. HCT was performed once the rabbit awakened from anesthesia.

The procedures for performing HCT on rabbits have been previously described in detail.[17] In the experimental condition of the Eustachian tube obstruction group, caged rabbits in the hypobaric chamber were tested at different vertical velocities and heights (**Table I**). The left tympanic cavity of all animals in the Eustachian tube obstruction group was used as the Eustachian tube Non-occlusion group. In the Control group, the right Eustachian tube was blocked and the rabbits were placed in the hypobaric chamber for the same duration as the Eustachian tube obstruction group during the tests, but without HCT. Rabbits' behavioral changes at different vertical speeds and altitudes were observed during HCT.

The functional evaluation of the Eustachian tube is mainly through observation of the oto-endoscope and tympanometry. Wallace et al. classified EB into six grades. Grade 0: symptomatic but without otological signs; Grade 1: diffuse redness and otopiesis; Grade 2: mild hemorrhage in the tympanic membrane (TM); Grade 3: severe hemorrhage in the TM; Grade 4: dark and mild bulging of the TM caused by hematocele in the middle ear and visible fluid level; and Grade 5: hemorrhage in the middle ear, TM perforation, and visible blood in the external acoustic meatus.[7] Xu et al. have classified EB into four

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**Table I.** Rabbit Subgrouping for Different HCT Testing Conditions.

| EFFECT OF ASCENDING/15 $m \cdot s^{-1}$ DESCENDING | ALTITUDE 13,123 ft | ALTITUDE 6562 ft | EFFECT OF DESCENDING/15m $\cdot s^{-1}$ ASCENDING | ALTITUDE 13,123 ft | ALTITUDE 6562 ft |
|---|---|---|---|---|---|
| 100 $m \cdot s^{-1}$, 15 $m \cdot s^{-1}$ | Subgroup 1 | Subgroup 2 | 15 $m \cdot s^{-1}$, 100 $m \cdot s^{-1}$ | Subgroup 3 | Subgroup 4 |
| 75 $m \cdot s^{-1}$, 15 $m \cdot s^{-1}$ | Subgroup 5 | Subgroup 6 | 15 $m \cdot s^{-1}$, 75 $m \cdot s^{-1}$ | Subgroup 7 | Subgroup 8 |
| 50 $m \cdot s^{-1}$, 15 $m \cdot s^{-1}$ | Subgroup 9 | Subgroup 10 | 15 $m \cdot s^{-1}$, 50 $m \cdot s^{-1}$ | Subgroup 11 | Subgroup 12 |
| -- | -- | -- | 15 $m \cdot s^{-1}$, 15 $m \cdot s^{-1}$ | Subgroup 13 | -- |

The rabbit subgroups for different HCT conditions are as follows. The pressure in the hypobaric chamber ascended to a simulated altitude at the set speed to stay for 5 min, and then descended to the simulated horizontal plane to stay for 15 min. The same ascent/descent stimulus was repeated once. For example, five rabbits in Subgroup 1 ascended to 13,123 ft at 100 $m \cdot s^{-1}$ to stay for 5 min, and descended to the horizontal plane at 15 $m \cdot s^{-1}$ to stay for 15 min. Then this same stimulus was repeated. The remaining subgroups of rabbits were completed in the same manner according to different setting conditions.

degrees. Class I: no significant changes in the TM; Class II: mild hyperemia in the TM's malleus handle and the pars flaccida; Class III: hyperemia in the TM's malleus handle and pars flaccida and surrounding areas; and Class IV: diffuse hyperemia in the TM and even exudates or hematocele in the tympanic cavity or TM perforation. Class II and above are considered to be Eustachian tube dysfunction.[16,19] Assignments were given to the above evaluation of Eustachian tube function. The scores of Wallace's Grade 0–5 were 0, 1, 2, 3, 4, and 5, while Xu's Class I–IV were 0, 1, 2, and 3. After HCT, all rabbits were subject to tympanometry. Type A indicates the tympanic function is normal. Type C indicates tympanic negative pressure, which is a dysfunction of the Eustachian tube. Type B indicates there were exudates, hemorrhage, and even fluid level or TM perforation in the tympanic cavities. None indicates there was a relatively high fluid level or errhysis on the external auditory canals after the tympanic membrane, so tympanometry could not be performed. The scores for tympanometry were: Type A = 0, Type C = 1, Type B = 2, and none = 3.

### Histopathological Evaluation

At the end of the experiment, the anesthetized rabbits were decapitated and the bulla was opened. The temporal bone was fixed with 10% formalin in PBS for 7 d. The tympanic mucosa tissues were microdissected out and dehydrated through a graded series of ethanol solution ending with 100%, and embedded in paraffin. The paraffin sections were cut at a thickness of 4 $\mu$m and collected on glass slides. The sections were routinely stained with hematoxylin and eosin. Based on pathological signs observed under the optical microscope, cilia loss and the glandular cavity's inflammatory cell exudation were classified as "mild" for less than 5%, "moderate" for 5–20%, and "severe" for greater than 20%. Inflammatory cell infiltration confined to the subepithelium was classified as mild, exceeding and involving most of the epithelial layer as severe, and in between as moderate. Cilia loss, inflammatory cell infiltration, and ulcers in the epithelial layer were recorded as three pathological parameters, and semiquantitative pathological results were: (−) normal, (+) mild, (++) moderate, and (+++) severe.[8,17] The scores for −, +, ++, and +++ were 0, 1, 2, and 3 and a mean score was calculated.

### Statistical Analysis

The paired Chi-squared test and Spearman correlation analysis were used to analyze statistical significances among different groups (SPSS 22.0). *P*-values less than 0.05 were defined as significant statistical differences.

## RESULTS

All rabbits with the expansive sponge blocking the right Eustachian tube demonstrated some behavioral changes. These behavioral changes include swallowing, runny nose, sneezing, blinking, tearing, scratching the nose, breathing with mouth open, shaking head, etc. After exposure to the HCT, most of the rabbits in the 1–12 subgroups that experienced a 75 $m \cdot s^{-1}$ rise (246 $ft \cdot s^{-1}$) and a 15 $m \cdot s^{-1}$ (49 $ft \cdot s^{-1}$) drop or a 15 $m \cdot s^{-1}$ rise and a 75 $m \cdot s^{-1}$ drop showed transient behavioral changes such as rotation, standing, grabbing the cage, hitting the cage, scratching the ears, and raising their heads while breathing. Rabbits in subgroups 1–4 that experienced a 100 $m \cdot s^{-1}$ (328 $ft \cdot s^{-1}$) rise and a 15 $m \cdot s^{-1}$ drop or a 15 $m \cdot s^{-1}$ rise and a 100 $m \cdot s^{-1}$ drop showed the most significant behavioral changes with 4–6 typical symptoms, while rabbits in subgroups 5–12 showed 3–4 of the above typical symptoms. The behavioral changes were most noticeable when rabbits rapidly ascended to the highest cabin altitude [13,123 ft or 6562 ft (4000 or 2000 m)] or rapidly descended to near the ground level. In contrast, the rabbits in the Control group did not have above typical EB symptoms.

The tympanic membrane of all rabbits in subgroup 13 and the control group remained intact without visible hyperemia (**Fig. 1A**). The rabbits in subgroups 5–12 that experienced a rise or fall of 75 m or 15 $m \cdot s^{-1}$ had intact tympanic membranes in both ears, but the tympanic membrane in the right ear exhibited various degrees of congestion due to occlusion of the Eustachian tube (**Fig. 1B**). In contrast, the right tympanic membrane in all rabbits in groups 1–4 that experienced a rise or fall of 100 m or 15 $m \cdot s^{-1}$ developed tympanic membrane perforation or obvious hematocele (**Fig. 1C**).

The results of the tympanometry showed that the tympanic pressure profiles in the nonobstructed group (left ear) and the control group were all type A curves (**Fig. 2A**). The most common tympanic pressure curve in subgroups 5–12, that experienced a rise or fall of 75 m or 15 $m \cdot s^{-1}$, were C, except for five cases with curve B (**Fig. 2B**). The most common right tympanic pressure in subgroups 1–4 that experienced a rise or fall of 100 m or 15 $m \cdot s^{-1}$ were curve B (**Fig. 2C**). However, in all 65 tested

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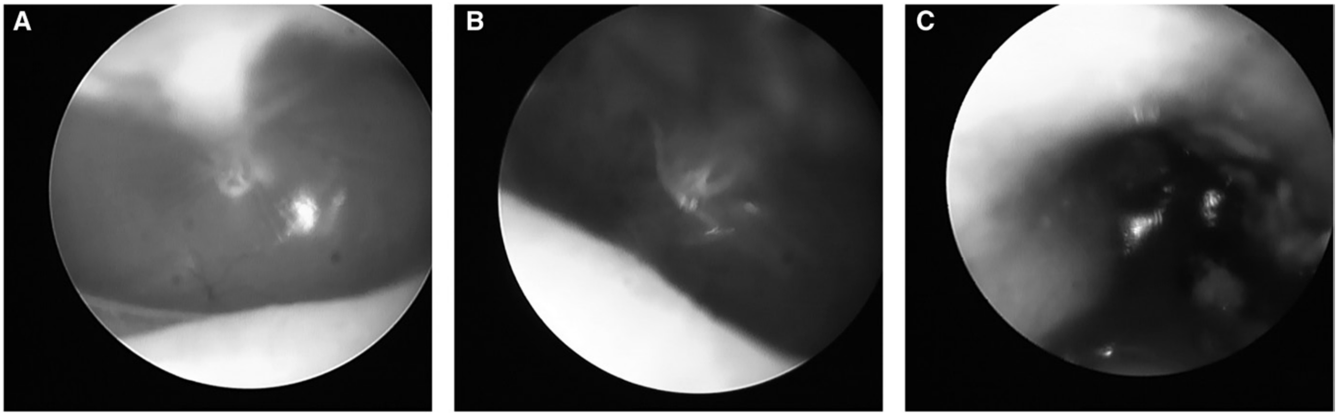


**Fig. 1.** Situations of the right tympanic membrane after HCTs. A) No hyperemia in the tympanic membrane, with cone of light reflection. B) Intact tympanic membrane, obvious hyperemia in the tympanic membrane's malleolar stria, the pars flaccida, and surrounding areas. C) Severe tympanic membrane perforation, unclear tympanic cavity structure, relatively plentiful hematocele in the external auditory canal.

ears, 3 ears were curve C, and 1 ear was curve A, but the pressure value of curve A was −140 mmHg.

The histological examinations of the tympanic mucosa showed no significant pathological damage in the ears in the nonobstructed groups, the control group, and subgroup 13 (**Fig. 3A**). In contrast, significant inflammatory changes, such as cilia loss, inflammatory cell infiltration, and inflammatory cell exfiltration from glandular cavities were detected in the tympanic membrane mucosa for subgroups 5–12. The damage level of subgroups 1–4 **(Fig. 3C)** were more severe than subgroups 5–12 **(Fig. 3B).**

According to the semiquantitative analysis of cilia loss, inflammatory cell infiltration, and inflammatory cell exudation from the glandular cavity, the EB severity in subgroups 1–4 was moderate to severe, and subgroups 5–12 was mainly mild to moderate. However, EB did not occur in the nonobstructed group (left ear) and the control group (**Table II**).

Based on evaluation criteria of Wallace and Xu on ET function and the Chi-squared test, there was high consistency in both classification methods (Kappa = 0.97, $P < 0.001$, **Table III**). Based on oto-endoscope, tympanometry, and histopathological injuries, rabbits' EB was classified into the three degrees (**Table IV**).

## DISCUSSION

HCTs can simulate pressure changes during flight and actually tests the ET function. Therefore, HCT is the main basis and effective method for judging EB as a medical evaluation of ET function of military flight personnel.[9,16] In aeromedical practice, when a pilot flies the aircraft down at high speed and approaches the ground, the pressure difference generated in a short time will be very large, making EB likely to occur. According to reports in the literature, EB in flight mainly occurs at an altitude of 13,123 ft (4000 m), especially around the altitude of 6562 ft (2000 m) above sea level. For fighter pilots, they must withstand a descent rate of more than 20–30 $m \cdot s^{-1}$ (66–98 $ft \cdot s^{-1}$), so they must have good Eustachian tube function. Studies have shown that guinea pigs do not develop EB at a descent rate of 15 $m \cdot s^{-1}$ (49 $ft \cdot s^{-1}$), but EB will be produced after three consecutive drops at a rate of 100 $m \cdot s^{-1}$ (328 $ft \cdot s^{-1}$), yet vestibular pathological damage will not occur.[19,20] The

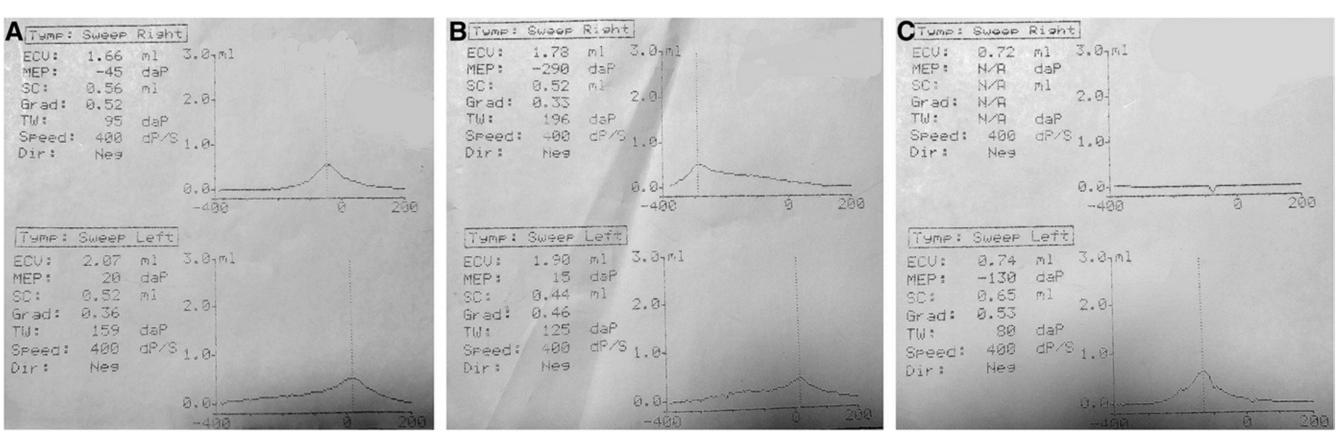


**Fig. 2.** Binaural tympanometry chart. A) Both ears: Type A curve. B) The right ear: Type C curve; the left ear: Type A curve. C) The right ear: Type B curve; the left ear Type A curve.



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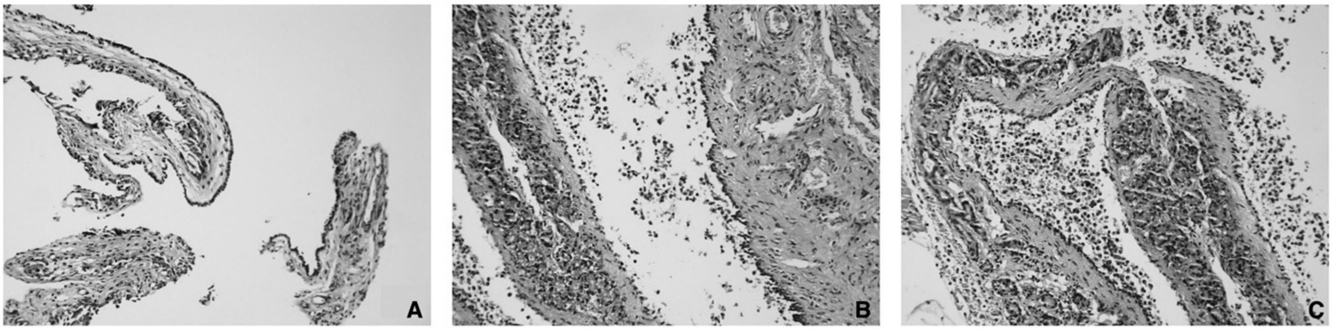

**Fig. 3.** Histopathological evaluation of the right middle ear tympanic cavity mucosa. A) No hyperemia or inflammatory exfiltration in the normal middle ear mucosa. B) Relatively plentiful inflammatory cell infiltration and mild cilia deletion in the middle ear mucosa, visible inflammatory cell exfiltration in the glandular cavities. C) Diffuse inflammatory cell infiltration in the mucosa, severe cilia loss, massive inflammatory cell exfiltration from the glandular cavities, and relatively plentiful RBC exfiltration.

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locations of ear damage induced by rapid changes in air pressure include the external auditory canal, the middle ear cavity, and the inner ear. Barotrauma of the external ear canal is mostly caused by earplugs, while barotrauma of the inner ear usually does not cause permanent pathological changes to vestibular end-organs. Therefore, ear barotrauma caused by HCT in this paper is mainly discussed in terms of the secondary barotrauma in the middle ear.[19] The rabbit experimental model designed in this experiment is based on the theoretical and experimental basis of EB, as well as the characteristics of EB in pilots observed in clinical aeromedical practice.

In subgroups 1–12, the typical changes in EB behavior were found in all the experimental animals, while significant tympanic mucosal hyperemia or tympanic membrane perforation was also detected in the right ears where the right Eustachian tube was successfully blocked. The tympanometry test also confirmed that the C curve or B curve appeared in the right ear in subgroups 1–12 due to the occlusion of the Eustachian tube. In contrast, the animals in the nonblocking group and the control group as well as subgroup 13 did not have any EB-related behavioral changes, nor any histopathological damage in the middle ear cavity or tympanic membrane. According to the results of this experiment, the preliminary conclusions are listed as follows: 1) In the absence of pressure changes, although obstructing the Eustachian tube can cause dysfunction of the Eustachian tube, it does not lead to obvious EB; 2) the rabbits in subgroup 13 did not develop EB under conditions of rise/fall of 15 $m \cdot s^{-1}$ although the right Eustachian tube was blocked with a dilatation sponge; 3) the left ears of rabbits in nonblocking subgroups 1–12 did not develop EB after repeated altitude increase at a speed of 100 $m \cdot s^{-1}$, 75 $m \cdot s^{-1}$ (246 $ft \cdot s^{-1}$), or 50 $m \cdot s^{-1}$ (164 $ft \cdot s^{-1}$); and 4) EB occurred in rabbits during HCT in subgroups 1–12 with occlusion of the right Eustachian tube, indicating that EB will

**Table II.** The Histopathological Injuries of the Eustachian Tube in Rabbits.

| | CILIA LOSS* | | | | IINFLAMMATORY CELL INFILTRATION$^{\dagger}$ | | | | INFLAMMATORY CELL EXUDATION FROM GLANDULAR CAVITIES$^{\ddagger}$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GROUP | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 | SCORE | MEAN |
| 1 | 0 | 0 | 2 | 3 | 0 | 0 | 1 | 4 | 0 | 0 | 2 | 3 | 43 | 2.87 |
| 2 | 0 | 0 | 1 | 4 | 0 | 0 | 1 | 4 | 0 | 0 | 3 | 2 | 40 | 2.67 |
| 3 | 0 | 0 | 1 | 4 | 0 | 0 | 1 | 4 | 0 | 0 | 2 | 3 | 40 | 2.67 |
| 4 | 0 | 0 | 2 | 3 | 0 | 0 | 1 | 4 | 0 | 0 | 2 | 3 | 40 | 2.67 |
| 5 | 0 | 2 | 3 | 0 | 0 | 1 | 4 | 0 | 0 | 4 | 1 | 0 | 23 | 1.53 |
| 6 | 0 | 2 | 3 | 0 | 0 | 2 | 3 | 0 | 0 | 2 | 3 | 0 | 24 | 1.60 |
| 7 | 0 | 0 | 5 | 0 | 0 | 3 | 1 | 1 | 0 | 3 | 2 | 0 | 25 | 1.67 |
| 8 | 0 | 1 | 4 | 0 | 0 | 0 | 5 | 0 | 1 | 2 | 2 | 0 | 25 | 1.67 |
| 9 | 0 | 3 | 2 | 0 | 0 | 2 | 3 | 0 | 1 | 3 | 1 | 0 | 20 | 1.33 |
| 10 | 1 | 3 | 1 | 0 | 0 | 3 | 2 | 0 | 0 | 3 | 2 | 0 | 19 | 1.27 |
| 11 | 0 | 1 | 4 | 0 | 1 | 3 | 1 | 0 | 0 | 4 | 1 | 0 | 20 | 1.33 |
| 12 | 1 | 3 | 1 | 0 | 0 | 2 | 3 | 0 | 0 | 4 | 1 | 0 | 20 | 1.33 |
| 13 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 19 | 1.27 |
| Non- blockage group | 65 | 0 | 0 | 0 | 65 | 0 | 0 | 0 | 65 | 0 | 0 | 0 | 0 | 0.00 |
| Control group | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0.00 |

Based on Kruskal-Wallis rank sum test, *, $^{\dagger}$, and $^{\ddagger}$ indicate $P < 0.05$ for Subgroup 1 vs. Subgroup 5 and Subgroup 9, respectively; $P < 0.05$ for Subgroup 2 vs. Subgroup 6, respectively; $P < 0.01$, 0.05, 0.05 for Subgroup 2 vs. Subgroup 10, respectively; $P < 0.01$, 0.05, 0.05 for Subgroup 3 vs. Subgroup 7, respectively; $P < 0.01$, 0.01, 0.05 for Subgroup 3 vs. Subgroup 11, respectively; $P < 0.05$ for Subgroup 4 vs. Subgroup 8 and Subgroup 12, respectively; $P < 0.05$ for Subgroup 9 vs. Subgroup 12, Nonblockage group and Control group, respectively; $P < 0.05$, 0.01 and 0.01 for Subgroup 10 vs. Subgroup 13, Nonblockage group and Control group, respectively; $P < 0.05$, 0.05 and 0.01 for Subgroup 11 vs. Subgroup 13, Nonblockage group and Control group, respectively; $P < 0.05$, 0.01 and 0.01 for Subgroup 12 vs. Subgroup 13, Nonblockage group and Control group, respectively.

**Table III.** Evaluation of Eustachian Tube Function in Rabbits.

| | | WALLACE (GLADE) | | | | | | | XU (CLASS) | | | | | TYMPANOGRAM | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GROUP | CASE | 0 | 1 | 2 | 3 | 4 | 5 | MEAN | 0 | 1 | 2 | 3 | MEAN | 0 | 1 | 2 | 3 | MEAN |
| 1 | 5 | 0 | 0 | 0 | 1 | 3 | 1 | 4 | 0 | 0 | 1 | 4 | 2.8 | 0 | 0 | 4 | 1 | 2.2 |
| 2 | 5 | 0 | 0 | 0 | 1 | 2 | 2 | 4.2 | 0 | 0 | 0 | 5 | 3 | 0 | 0 | 3 | 2 | 2.4 |
| 3 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 4 | 0 | 0 | 0 | 5 | 3 | 0 | 0 | 5 | 0 | 2 |
| 4 | 5 | 0 | 0 | 1 | 1 | 2 | 1 | 3.6 | 0 | 0 | 1 | 4 | 2.8 | 0 | 0 | 4 | 1 | 2.2 |
| 5 | 5 | 0 | 1 | 3 | 0 | 1 | 0 | 2.2 | 0 | 0 | 4 | 1 | 2.2 | 0 | 4 | 1 | 0 | 1.2 |
| 6 | 5 | 0 | 0 | 3 | 1 | 1 | 0 | 2.6 | 0 | 0 | 4 | 1 | 2.2 | 0 | 4 | 1 | 0 | 1.2 |
| 7 | 5 | 0 | 1 | 3 | 1 | 0 | 0 | 2.0 | 0 | 0 | 3 | 2 | 2.4 | 0 | 3 | 2 | 0 | 1.4 |
| 8 | 5 | 0 | 0 | 3 | 2 | 0 | 0 | 2.4 | 0 | 0 | 4 | 1 | 2.2 | 0 | 4 | 1 | 0 | 1.2 |
| 9 | 5 | 0 | 0 | 4 | 1 | 0 | 0 | 2.2 | 0 | 0 | 4 | 1 | 2.2 | 0 | 4 | 1 | 0 | 1.2 |
| 10 | 5 | 0 | 2 | 2 | 1 | 0 | 0 | 2.2 | 0 | 0 | 5 | 0 | 2 | 0 | 5 | 0 | 0 | 1 |
| 11 | 5 | 0 | 1 | 3 | 1 | 0 | 0 | 2.0 | 0 | 0 | 4 | 1 | 2.2 | 0 | 4 | 1 | 0 | 1.2 |
| 12 | 5 | 0 | 1 | 3 | 1 | 0 | 0 | 2.0 | 0 | 0 | 5 | 0 | 2 | 0 | 4 | 1 | 0 | 1.2 |
| 13 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| Nonblockage group | 65 | 65 | 0 | 0 | 0 | 0 | 0 | 0 | 58 | 7 | 0 | 0 | 0 | 65 | 0 | 0 | 0 | 0 |
| control group | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |

The scores for Wallace's EB Grade 0–5 were 0, 1, 2, 3, 4, 5, while Xu's EB Class I–IV were 0, 1, 2, 3. The scores for tympanometry were: Type A = 0, Type C = 1, Type B = 2, and none = 3. Based on Spearman correlation analysis, there were statistical correlations between Wallace's assessment and Xu's assessment (r = 0.953, $P < 0.001$), between Wallace's assessment and the tympanogram (r = 0.983, $P < 0.001$), and between Xu's assessment and the tympanogram (r = 0.0.955, $P < 0.001$).

only occur if the Eustachian tube is blocked and under a repeated altitude increase in HCT.

According to the evaluation of middle ear histopathology, when rabbits ascended to the same altitude (13,123 ft or 6562 ft) at $100\ m \cdot s^{-1}$, $75\ m \cdot s^{-1}$, and $50\ m \cdot s^{-1}$, respectively, pathological injuries in the middle ear in rabbits ascending at $100\ m \cdot s^{-1}$ were more severe, while there was no significant difference in pathological injuries between ascending speed at $75\ m \cdot s^{-1}$ and at $50\ m \cdot s^{-1}$. It means that when the rabbits ascend to a certain altitude in HCTs (13,123 ft or 6562 ft), the EB caused by the vertical speed of $100\ m \cdot s^{-1}$ is more severe than the EB caused by $75\ m \cdot s^{-1}$ or $50\ m \cdot s^{-1}$. However, the severity of EB caused by $75\ m \cdot s^{-1}$ or $50\ m \cdot s^{-1}$ did not differ much. Likewise, when rabbits descended to the ground from the same altitude (13,123 ft or 6562 ft) at $100\ m \cdot s^{-1}$, $75\ m \cdot s^{-1}$, or $50\ m \cdot s^{-1}$, respectively, pathological injuries in the rabbits descending at $100\ m \cdot s^{-1}$ were more severe, but there was no significant difference in pathological injuries between the rabbits descending at $75\ m \cdot s^{-1}$ and the rabbits descending at $50\ m \cdot s^{-1}$. It means that during a rapid fall from a certain altitude (13,123 ft or 6562 ft) in HCTs, EB caused by the vertical speed of $100\ m \cdot s^{-1}$ is more severe than EB caused by $75\ m \cdot s^{-1}$ and $50\ m \cdot s^{-1}$, but there was no difference between exposure to rapid descent at the speed of $75\ m \cdot s^{-1}$ and $50\ m \cdot s^{-1}$. This indicated that when ascending to or descending from a certain altitude (0 ft–13,123 ft), the fastest vertical speed and obstruction of the Eustachian tube can lead to more severe EB.

Histopathological evaluation showed no significant difference in rabbits ascended at $100\ m \cdot s^{-1}$ to the altitudes of 13,123 ft or 6562 ft. When the rabbits ascended at $75\ m \cdot s^{-1}$ to the altitudes of 13,123 ft or 6562 ft, there was no significant difference in pathological damage. When the rabbits ascended at $50\ m \cdot s^{-1}$ to the altitudes of 13,123 ft or 6562 ft, there were also no significant differences in pathological injuries. In other words, the severity of EB was similar in ascent to 13,123 ft or 6562 ft at the vertical speed of $50\ m \cdot s^{-1}$, $75\ m \cdot s^{-1}$, or $100\ m \cdot s^{-1}$. Likewise, the severity of EB was similar in descent from 13,123 ft or 6562 ft at the vertical speed of $50\ m \cdot s^{-1}$, $75\ m \cdot s^{-1}$, or $100\ m \cdot s^{-1}$. It means when the vertical speed was constant ($50–100\ m \cdot s^{-1}$), there were no significant differences between EB caused by the altitude variation between 13,123 ft and 6562 ft. In other words, EB mainly occurs at altitudes below 6562 ft, which is closely related to atmospheric pressure distribution and similar to the main occurrence of barotrauma at the altitude of 6562 ft in aviation medicine.

Wallace classified EB into six grades during the Second World War. However, in aviation medicine, since diagnosis and classification of EB cannot be achieved through invasive

**Table IV.** Criteria for EB Grading in Rabbits.

| | OTO-ENDOSCOPE EXAMINATION | TYMPANOMETRY | HISTOPATHOLOGICAL LESIONS | SCORE (%) |
|---|---|---|---|---|
| Mild | Tympanic membranes intact, no or mild hyperemia (Class I) | Type A or Type C | At least 2 lesions, up to 1 moderate | ≥4, <6 (27–40%) |
| Moderate | Tympanic membranes intact, moderate or severe hyperemia (Class II–III) | Type C or B | 3 lesions, at least 2 moderate | ≥6, <12 (40–80%) |
| Severe | Obvious tympanic membrane perforation and hematocele (Class IV) | Type B or none | 3 lesions, at least 2 severe | ≥12, ≤15 (80–100%) |

Oto-endoscope examination: the scores for Xu's EB Class I–IV were 0, 1, 2, 3. The scores for tympanometry were: Type A = 0, Type C = 1, Type B = 2, and none = 3. Pathological variables included three items: cilia lose, inflammatory cell infiltration, and inflammatory cell exfiltration from the glandular cavities. Each item included Grades 0–3, so pathological performance scores were 0–9.



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operations like TM incision, it is hard to distinguish between "dark and mild bulging of the TM caused by hematocele in the middle ear and visible fluid level" (Grade 4) and "severe hemorrhage in the TM" (Grade 3), not to mention the identification of "mild hemorrhage in the TM" (Grade 2).[15] The evaluation of ET function indicated there is high relativity between Wallace's and Xu's evaluation methods on EB. Therefore, Xu's preliminary classification based on degrees of hyperemia and integrity of the TM from a clinical aviation medicine practice viewpoint may be more practical.

In summary, when the HCT is performed at a vertical speed ($15\ m \cdot s^{-1}$, $50\ m \cdot s^{-1}$, $75\ m \cdot s^{-1}$, or $100\ m \cdot s^{-1}$) at different altitudes (6562 ft or 13,123 ft), rabbits will exhibit different pathological changes in oto-endoscopy and tympanometry. From this, it can be judged whether a rabbit has EB and the severity of EB. In this experiment, we divided the semiquantitative lesions of EB into the following degrees by oto-endoscopy, tympanometry, and pathological findings. Scores 0–4 indicate normal, 4–6 indicate mild EB, 7–11 indicate moderate EB, and 12–15 indicate severe EB.

The use of rabbits as subjects for the experiment of barotrauma of the middle ear has the following advantages. 1) Rabbits are highly compliant and well tolerated. No rabbits experienced accidental death during anesthesia and Eustachian tube blockage and HCTs.[17] 2) Rabbits can recover quickly after anesthesia and the behavioral changes of rabbits in response to HCTs are obvious and easy to observe. 3) The anatomy of the middle ear of the rabbit is similar to that of humans. The diameter of the external auditory canal in rabbit is relatively large, which is beneficial for observing the congestive changes in the tympanic membrane and the tympanic cavity through the oto-endoscope and the tympanometry detector. The establishment of this experimental model provides helpful reference experience for the classification of EB lesions and the discussion of EB damage mechanisms.

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