INTRODUCTION:
Changes in the mechanical conditions affecting lumbar intervertebral discs (IVD) are thought to be an important factor in lumbar disturbances, and, thus, the influences of overload on the IVD have been studied extensively [1]. However, few studies have investigated the effects of unloading on the IVD. Although it has been reported previously that proteoglycan (PG) levels of the lumbar whole IVD were decreased by unloading in rats during spaceflight [2] and in rats suspended by the tail [3], it has not been determined whether the reduced PG of the IVD caused by unloading could return to the previous state by reloading or whether there are differences in these effects on the anulus fibrosus (AF) and nucleus pulposus (NP).

The purpose of this study was to investigate the effect of reloading after unloading on the lumbar IVD using the rat tail-suspension model by analyzing histological changes and alterations of PG content of the AF and the NP respectively.

MATERIALS AND METHODS:
Tail Suspension Model: Tail suspension was performed using the Morey-Holton method [4] with some modifications. This model is widely accepted as an animal model to simulate weightlessness, and as a model to examine the lumbar spine [3], in which this method removes the compressive load on the lumbar IVD. The tail suspension model has an advantage of no surgical invasion (no inflammatory effect). In addition, the rats are able to move freely on the floor by use of the forelimbs, and, therefore, this is not an immobilization model.

Animal Protocol: Thirty-six 9-week-old male F344/N rats were randomly divided into three groups: caged control (C), tail-suspended (TS), and reloaded (TS+RL). To unload the IVDs, tail suspension was performed for 3 or 6 weeks (TS-3w, TS-6w). Similarly, C rats were subdivided into two groups (C-3w, C-6w). TS+RL rats were allowed to reload for 3 weeks after 3 weeks of unloading. At the end of 3 or 6 weeks, the rats were sacrificed and their lumbar spines were removed.

Histological Examinations: The removed lumbar spines were fixed in 4% paraformaldehyde, and decalcified in 10% EDTA. Discs (bone-disc-bone) were embedded in paraffin, cut into 10 µm central sagittal sections, and stained with hematoxylin-eosin (HE) and safranin-O.

Assay of GAG content/ DNA content: Isolated discs were separated into the AF and NP under a stereoscopic microscope. Six AFs and six NPs were pooled to give one sample, respectively. Each sample was digested in papain overnight. Glycosaminoglycan (GAG) accumulations for PG content were determined using the dimethylmethylen blue (DMMB) method, and the cell number by DNA content was determined by the Hoechst dye assay to normalized GAG data.

Statistical Analysis: Differences of GAG content normalized by DNA content among C, TS, and TS+RL groups were statistically evaluated by ANOVA. P<0.01 was considered significant.

The study protocol was approved by the Guiding Committee of the Center for Laboratory Animal Science, National Defense Medical College.

RESULTS:
Histological Findings (Fig.1): The Safranin-O staining level of the IVD in the TS groups tended to be lower than that in the C and TS+RL groups. In the AF of the TS group, the stained area was more narrow than that in the C and TS+RL group. Histological findings using HE stain indicated that PG metabolism in the IVD is regulated during adaptation to various mechanical states.

Time Course of GAG/DNA Changes in the Anulus Fibrosus (Fig.2):
The GAG content of the AF in the TS groups (TS-3w, TS-6w) were significantly decreased to 29% and 42%, respectively, compared with that of the C groups (C-3w, C-6w). The GAG content of the TS+RL group increased to some extent, but remained significantly lower than that in the C-6w group.

Time Course of GAG/DNA Changes in Nucleus Pulposus (Fig.3):
The GAG content of the NP in TS groups (TS-3w, TS-6w) were also significantly decreased 35% and 27%, respectively, compared with that of the C groups (C-3w, C-6w). In the TS+RL group, the GAG content recovered to the same level as that in the C-6w group.

DISCUSSIONS:
Our results suggest that the reduced PG in the IVD caused by unloading can recover by reloading. Nevertheless, the loss of PG in the IVD is commonly regarded as a "degenerative" and irreversible change, such as that occurring with aging or during a pathologic state. In this study, the PG contents increased from the decreased state without obvious degenerative histological changes. Therefore, these results indicate that PG metabolism in the IVD is regulated during adaptation to various mechanical states.

In addition, our results indicate that the recovery rates of PG contents in the AF are insufficient compared to those in the NP, and that this difference in the response between AF and NP during the reloading phase may be due to different cell types (originally derived from either mesenchymal cells or notochord-derived cells) and/or to structural disparities [5]. These changes in the lumbar IVD may be associated with the low back pain that astronauts experience during and after spaceflight. Although we suggest that a longer follow-up and a detailed study of reloading is required, our results provide a better understanding of the functional properties of the IVD during mechanical stress.

REFERENCES: