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العد النسبى والنوعى للخلايا الرئوية لحيوانات الجولدن هامستر نتيجة التعريض لغبار صناعى

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أجريت هذه الدراسة للوقوف على التغيرات التى تحدث للعد النسبى والنوعى للخلايا الرئوية لحيوانات الجولدن هامستر نتيجة التعريض لغبار صناعى يشبه الذى يسود فى العواصف الرملية بجمهورية مصر العربية . أظهرت النتائج ارتباطا ايجابيا بين وزن الجسم والعد الكلى للخلايا الرئوية . ولقد تبين من معاملات التعريض للغبار الصناعى ان العد النسبى للخلايا الرئوية (عدد الخلايا الرئوية الكلية منسوبا الى وزن الحيوان) يزيد مع زيادة فترة التعريض ، يستمر الزيادة حتى مرحلة ٤٨ ساعة من التعريض حيث كان العد النسبى ضعف مثيله مع حيوانات المقارنة . بعد فترات راحة لعدد شهر وشهرين بعد توقف معاملات التعريض للغبار الصناعى ، حدث انخفاضا فى العد النسبى للخلايا الرئويه حيث قارب المعدل الطبيعى تبين أنه فى المرحلة بين ٤٨ الى ٧٢ ساعة من التعريض للغبار الصناعى بدأ العد النسبى للخلايا الرئويه فى الانخفاض مما يدل على أن قمة المنحنى تقع فى نقطة بين ٤٨ الى ٧٢ ساعة .

تبين أن النسبة المئوية للخلايا الليمفاوية كانت ٦٦ وفى حيوانات المقارنة وصلت بعد ذلك الى ١ر٨ ، ٥ر١٦ ، ٤٨ر١٨ ، ١١ر٣ بعد التعريض للغبار الصناعى لفترات ١٢ ، ٢٤ ، ٤٨ ، ٧٢ ساعة كل على حده كانت النسبه المئويه للخلايا الملتهمة ٣٤ر٩٩ فى حيوانات المقارنة وصلت الى ٢ر٩٨ ، ٥ر٨٣ ، ٥٢ر٨١ ، ٧ر٨٨ بعد التعريض للغبار الصناعى للفترات الأربعة المذكورة كل على حدة . وجد أن فترات الراحة لعدد شهر أو شهرين بعد معاملات التعريض للغبار الصناعى سببت تخفيضا فى النسبة المئوية للخلايا الليمفاوية ، ولقد كان التخفيض أكثر مع زيادة فترة الراحه . ومن الجانب الآخر تسببت فترات الراحة بعد التعريض للغبار الصناعى فى زيادة النسبة المئوية للخلايا الملتهمة ، ولقد كانت الزيادة أكثر مع طول فترة الراحه .

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RELATIVE AND DIFFERENTIAL COUNT OF PULMONARY CELLS OF GOLDEN HAMSTERS AFTER EXPOSURE TO SYNTHETIC DUST

(With 2 Tables & 2 Figures)

By

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SUMMARY

The present work is focused on the evaluation on changes occuring in the relative and differential count of pulmonary cells of golden hamsters animals following acute inhalation exposure to synthetic dust which was prepared so as to simulate that prevailing in sandy storms. The results revealed a positive correlation between the total count of pulmonary cells and the body weight of the animals. The exposure treatments showed that the relative, (in relation to 100 gm. body weight) and total pulmonary cell count in hamsters increases steadily as the period of exposure is prolonged. This increase continues up to 48 hours exposure when the count was more than two folds than that of the control. After one and two months following the cessation of the inhalation experiments, clearance took place and the animals showed a drop in the total count down to a point close to the base line of the control.

The differential count of pulmonary cells showed that the percentage of lymphocyte cells was 0.66 for the control hamsters then reached 1.8, 16.5, 18.48 and 11.3 after exposure for 12, 24, 48 and 72 hours respectively.Pulmonary alveolar macrophage cells percentage was 99.34 for the controls, reached 98.2, 83.5, 81.52 and 88.7 after the mentioned exposure periods respectively. Clearance for one and two months after the latter three exposure treatments respectively decreased the lymphocyte cell percentage than the none-cleared animals, the prolonged clearance gave the lower lymphocyte percentages. The clearance periods previously mentioned increased the pulmonary alveolar macrophage percentages than the non-cleared animals. Values of pulmonary alveolar macrophage increased with increasing clearance periods.

INTRODUCTION

Acute exposure to fine dust particles commonly inhaled by man and animals in Egypt results in clinical manifestations varying from mild non specific irritation of the respiratory tract to increasing susceptibility to acute bacterial and viral infections and allergic reactions. The evaluation of the underlying mechanisms of such manifestations necessitates a through analysis of the concentration, and physical composition of the airborne particles prevailing in such sandy storms, as well as a study of the physiological changes in the respiratory system following exposure to these air pollutants.

The phagocytic alveolar macrophage is thought to play a central role in the lungs defense against environmental contaminants (GREEN, 1968 and GREEN and CAROLIN, 1969). The alveolar macrophage is important not only in the lungs defense against inhaled particulates, but also in interferon production (ACTAN and MEYRVICK, 1966) and in the initiation of antibody synthesis (NOSSAL and AUSTIN, 1966, FISHMAN et al., 1963 and UHR and WEISSMANN, 1965), any alteration of its function by a noxious agent (pollutant) could prove perilous for the ultimate health of the animal. (GROSS et al. 1969) concluded that they have long been considered an integral and vital component of the pulmonary clearance mechanism. It has been established that inhalation of various air pollutants influence the number and function of pulmonary alveolar macrophages (PAM) (GARDNER et al., 1972, COFFIN and GARDNER, 1972 and WATERS et al., 1974).

MATERIAL AND METHODS

Thirty golden hamsters animals, body weight ranged from 60 to 190 gm., were caged individually under standard conditions of room temperature (22 ± 1 C) and relative humidity (50 ± 5%). A mixture of fresh and commercially processed feed containing approximately 20% protein and water were available to the animals.

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Synthetic dust was prepared by mixing six chemical compounds with finely ground dust particles,diameter less than 300 um,so as to adjust their levels to values normally existing in dust samples (El-Sheikh et al.1974) i.e., ferric oxide 0.2, calcium chloride 0.02, zinc oxide 0.05, cobaltous sulfate 0.02,copper sulfate 0.005 and magnesium sulfate 0.006 gm./100 gm. dust.

Nine of the hamsters were used as controls. The remaining were exposed to the synthetic dust using Animal Inhalation Chamber, the average concentration of the dust in the air inhaled by the experimented animals was 0.4917 mg./ liter of air. Periods of exposure to dust were 12, 24, 48 and 72 hours respectively. Animals of the latter three periods were sacrificed after 30 and 60 days respectively from the end of exposure. The latter two categories were used to study lung dust clearance.

Pulmonary cell suspension stock solution was obtained after anesthetizing the animals using modifications for the technique used by (MYRVIK et al. 1961) and (COFFIN et al.1968). The major differences were that the lungs were allowed to remain within the chest cavity during the lavage procedure (GARDNER et al., 1971), few drops of the stain gentian violet 1% were put on the saline solution, repeated washings of lungs for 6 times and washing of lavage by centrifugation.

Total cell count was accomplished on the cell suspension stock solution. It was aspirated into hemocytometer pipette and diluted (10 to 1) with normal saline containing few drops of gentian violet to stain nuclei. One drop was placed on the counting chamber, covered and the cell counted under binocular microscope. The mean count (cells/mm^3) was used for calculating total number of cells in the stock solution. The relative count (cells/100 gm. body weight) was obtained from absolute count.

The cell differential count was made from the stock solution. One drop of the stock suspension was spread over a glass slide. The smears were air dried, fixed immediately with formalin vapor for one minute and stained with Geimsa stain for about 30 minutes,washed thoroughly in distilled water, cleared with xylene and mounted with balsam. The different types of cells, Pulmonary Alveolar Macrophages (PAM) and Lymphocytes (L) were counted in each field and tabulated as the relative percentage observed. Statistical analyses were conducted after(SNEDECOR 1959).

RESULTS AND DISCUSSION

1. Relative Count of Pulmonary Cells After Exposure To Synthetic Dust

The mean count per 100 gm. body weight of the pulmonary cells in the control hamsters is more or less constant. Positive correlation was found between the total count of pulmonary cells and the body weight (correlation coefficient 0.96). This correlation is logical when considering the relationship between alveolar surface and body mass.

The results (Table 1) have shown an increase by 30% of the total cells after 12 hours exposure to the synthetic dust and 85% after 24 hours and more than 100% increase after 48 hours. After 72 hours the relative total count decreased in number than that shown after 48 hours for the same exposure, but is nearly double that of the control.

Analysis of variance showed that exposing hamsters to the synthetic dust for 12,24,48 and 72 hours affected significantly the number of pulmonary cells ($P/0.01$).In this connection (LA BELLE and BRIEGER 1961) noticed that a tenfold increase in macrophages 12 hours after injection of dust particles. Between 48 and 72 hours exposure, the relative total pulmonary cell count starts to show a drop indicating that the peak of the curve (of pulmonary cell count against period of exposure)- Fig. 1- lies somewhere between 48 and 72 hours. In an attempt to explain why there is a drop in the pulmonary cell count despite the continued exposure to dust, is that the pulmonary cells are continually reproduced as esposure to dust continues and that after 48 hours concomitant disposal of cells takes place (probably through rupture,resorption or expulsion through the respiratory passages) which would account for the drop in their number. This assumption is substantiated by the finding that after a resting period of 5 weeks, the count was still more than in the animals which were exposed for 48 hours then clearance for just one month (4 weeks) and after rest for 10 weeks the count drops steadily.

After exposing periods for 24 and 48 hours respectively, clearance periods for one or two months decreased pulmonary relative count than the animals sacrificed directly after the two exposing treatments. Animals exposed

for 72 hours then survived for 5 weeks showed a drop by about 31% and still double that the control. Hamsters lasted for 10 weeks after the same exposure period showed still further drop by about 51% from the exposed animals, thus bringing the count near the base line. (WATERS et al., 1974) concluded that the mode of death of alveolar macrophages may, in fact, have important implications in the clearance of particulate materials from the lung.

TABLE (1)

Relative count of pulmonary cells in control and after exposure for different periods and their clearance

Exposure period	Mean body weight gm.	Mean absolute count	Mean count/ 100 gm. body weight
Control	121	1842	1455
12 hours	80	1501	1883
24 hours	65	1714	2614
Clearance one month after 24 hrs. exposure	170	2627	1545
Clearance two months after 24 hrs. exposure	163	2466	1523
48 hours	80	2559	3185
Clearance one month after 48 hrs. exposure	93	1993	2141
Clearance two months after 48 hrs. exposure	115	1812	1575
72 hours	100	2857	2857
Clearance 5 weeks after 72 hrs exposure	100	2140	2140
Clearance 10 weeks after 72 hrs exposure	135	2095	1553

2. Differential Count of Pulmonary Cells After Exposure to synthetic dust

Table (2) shows that pulmonary alveolar macrophages consituted more than 99% of the pulmonary cells in the control hamsters and less than 1% were lymphocytes. Polymorphs and eosinophils were diffecult to find in any smears. In this connection (GARDNER et al., 1972) found, in the unexposed rabbits,99% of the pulmonary cells were mononuclear macrophages, small lymphocytes constitued 1% of the total observed, occasional polymorphonuclear leucocytes and eosinophils comprised the remaining 1%.

The lymphocytes in the differential count increased gradually from less than 1% in the control group to 1.8% then reached 16.5% after 12 and 24 hours respectively of dust exposure. After 48 hours of exposure the maximum increase of lymphocytes was reached then began to decrease to 11.3% after exposing for 72 hours. Pulmonary alveolar macrophage cells percentage was 99.34% for the controls, reached 98.2, 83.5, 81.52 and 88.7 after exposure for 12, 24, 48 and 72 hours respectively. The obvious increase in the lymphocyte count may bare some connection with the allergic manifestations known to result in some individuals from exposure to dust.

Clearance periods for one and two months after the exposure treatments for 24, 48 and 72 hours respectively decreased the lymphocyte cell percentage than the none-cleared animals, the prolonged clearance gave the lower lymphocyte percentages. On the other hand, the clearance periods previously mentioned increased the pulmonary alveolar macrophage percentages than the none-cleared animals. Values of pulmonary alveolar macrophage increased with increasing clearance periods.

The comparison of the two graphs (Fig. 2) of the total relative number of lymphocytes and pulmonary alveolar macrophage against time of exposure and clearance shows that the two curves are more or less parallel but that of the lymphocytes overshoots that of the pulmonary at 72 hours exposure.

TABLE (2): Differential Count of Pulmonary Cells In Control and After Exposure For Different Periods and Their Clearance

Exposure period	Pulmonary Alveolar Macrophage%	Lymphocyte %
Control	99.34	0.66
12 hours	98.2	1.8
24 hours	83.5	16.5
Clearance one month after 24 hrs. exposure	97.0	3.0
Clearance two months after 24 hrs. exposure	98.8	1.2
48 hours	81.52	18.48
Clearance one month after 24 hrs. exposure	95.3	4.7
Clearance two months after 24 hrs. exposure	96.8	3.2
72 hours	88.7	11.3
Clearance 5 weeks after 72 hrs. exposure	94.6	5.4
Clearance 10 weeks after 72 hrs. exposure	98.7	1.3

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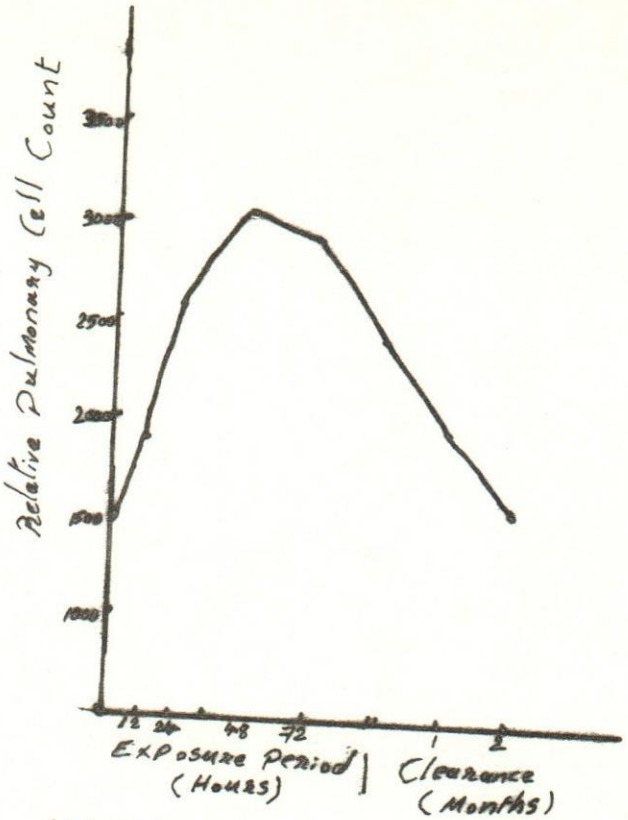

Fig. (1) Shows that the peak of the curve (of pulmonary cell count against period of exposure) lies somewhere between 48 and 72 hours.

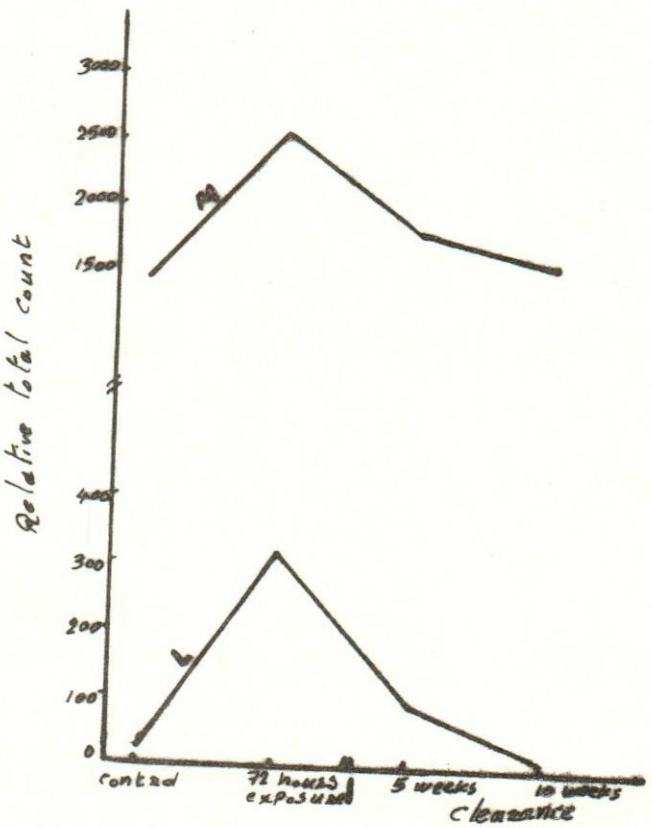

Fig. (2) Shows a comparison between the two constituents of the pulmonary cells (namely the PAM and Leucocytes).