

# دراسة جراثيولوجرام خلايا النيوتروفيل في الأرانب الهندية المحقونة بعترة السل البقرى وسل الطيور وعترة سل الانسان

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## الملخص العربى

استهدف هذا البحث دراسة جراثيولوجرام خلايا النيوتروفيل فى الأرانب الهندية المحقونة بعترة السل البقرى وسل الطيور وعترة سل الانسان بغرض استخدام هذه الطريقة كوسيلة لتصنيف هذه العترات معمليا .

قسمت مجموعة من الأرانب الهندية المتجانسة الى أربعة مجموعات ثم حقن أفراد المجموعة الأولى بوزن معلوم من عترة السل البقرى . وحقنت أفراد المجموعتين الثانية والثالثة بنفس الوزن من عترة سل الطيور والانسان كل على حدة . أما المجموعة الرابعة فتركت بدون عدوى لاستخدامها فى المقارنة .

وقد تم دراسة الجراثيولوجرام لعشرة أرانب من كل مجموعة أسبوعيا هذا بجانب اختبارها بالفرد المقارن للتيوبركيولين وعزل الميكروب من الفئد الليمفاوية المجاورة لمكان الحقن . واستمرت الاختبارات أسبوعيا الى حين ظهور نتائج ايجابية للتيوبركيولين . وقد تبين من النتائج وجود ظواهر تتميز كل ميكروب على حدة شوهدت قبل الحصول على نتائج ايجابية للتيوبركيولين .

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**STUDIES ON THE GRANULOGRAMME OF NEUTROPHIL  
CELLS IN GUINEA PIGS EXPERIMENTALLY INFECTED  
WITH BOVINE,  
AVIAN AND HUMAN STRAINS OF TUBERCULOSIS\***

(with one table and 3 figures)

By

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**SUMMARY**

The granulogramme of neutrophil cells was studied in three groups of guinea pigs inoculated with an equal dose of an avian, a bovine and a human standard strains of tuberculosis, respectively. A 4th group was served as non infected controls. Ten animals from each of the infected groups were examined weekly. Result revealed some characteristic findings which could be taken as criteria for differentiating the three types of TB. bacilli. Such findings were observed before the development of tuberculin reaction.

**INTRODUCTION**

The severity of the infection as well as the reaction of the patient may be gauged by the magnitude of the leukocyte count, the degree of left shift and the presence of toxic cytoplasmic granulation, WINTROBE (1961). It was also recorded by SCHALM (1965) that the severity of the infection is measured by the occurrence of toxic changes in the neutrophils as basophil and cytoplasmic granulation.

In 1949 BENDA AND URQUIA examined blood of human patients with tuberculosis. They recognized three types of neutrophils based on the color of the cytoplasm and the toxic granulation. These types were, the normal type (gN), the intermediate (g±) and the frankly pathological (g<sup>+</sup> and g<sup>++</sup>) these types were also observed by the same authors in tuberculous guinea pigs). A differential neutrophil count was made using this classification. The authors call it a granulogramme; departure from the normal granulogramme, provides a very useful aid for diagnosing tuberculosis. REMLINGER et al. (1949) applied the technique of BENDA AND URQUIA while demonstrating the tubercle bacilli in sputum and gastric washings of human patients. The authors claimed that this test is an absolute proof of the infection. It was negative in normal people and in many cases of various diseases in which it was used.

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As the guinea pigs vary in their susceptibility to bovine, human and avian strains of tuberculosis WILSON AND MILES (1957). Thus, the present investigation is devoted to study the granulogramme of neutrophil cells in guinea pigs experimentally infected with bovine, human and avian strains of tuberculosis aiming to aid the laboratory differentiation of these pathogenic mycobacteria.

### MATERIALS AND METHODS

Two hundred and eighty female *albino* guinea pigs were used for studying the granulogramme of neutrophil cells in TB infection. The animals were divided into 4 groups. Three group of 80 animals each, were the test. The remaining 40 guinea pigs represents the control. Animals of the 1st test group were injected subcutaneously in the inguinal region with 1 ml saline containing one mg of 4 weeks old culture of *Mycobacterium avium* (D<sub>4</sub>). Individuals of the other 2 test groups were injected with the same amount with *M. bovis* (local strain) and *M. tuberculosis* (PN), respectively. Weekly, cardiac blood samples of 10 guinea pigs were collected from each of the infected groups. A similar number from the control guinea pigs was examined every other week. After sampling the guinea pigs were tuberculin tested using the simple intradermal comparative tuberculin test. Results of the tuberculin reaction were read within 72 hours. The animals were then sacrificed. From the lymph nodes near the site of inoculation, smears were made, stained with Ziehl-Neelsen and cultures were performed on Lowenstein Jensen media.

The examination within each of the infected groups was continued weekly till the evidence of positive tuberculin reaction.

The granulogramme of neutrophil cells of the control and infected guinea was determined after BENDA AND URQUIA (1949). The blood smears were stained with May-Grunwald Giemsa GRADWOHL (1948) using neutral buffered distilled water. One hundred neutrophil cells were counted classified into 4 groups; the normal type (gN), the intermediary type (g±) and the frankly pathological types (g<sup>+</sup>) and (g<sup>++</sup>) and the percentage of each type was calculated.

### RESULTS

Animals infected with either human or bovine strains of tuberculosis reacted positively to the mammalian tuberculin at the fourth week after infection. On the other hand, the reaction of guinea pigs injected with *M. avium* to the avian tuberculin appeared on the 5th week. The assessment of the infection based on bacteriological examination of lymph nodes at successive weeks after infection revealed presence of the previously injected strain in each group. The control animals revealed negative tuberculin testing and bacteriological examinations.

Since the granulogramme of the control animals was not varied significantly during the time of the experiment, an average value was drawn representing the 4 control groups.

Results of the granulogramme studies were presented in the table below. Figures 1-4 show the different types of neutrophil leucocytes.

The granulogramme of control and tuberculous guinea pigs at different weeks after infection.

| Weeks | Variables % | Significance of mean values / infected |                      |                      |                     |
|-------|-------------|--|----------------------|----------------------|---------------------|
|       |             | Control c                              | Avian a              | bovine b             | human h             |
| I     | gN          | 76.20±6.31                             | +c<br>3.90±1.76      | +c<br>0.00           | +c<br>1.90±1.64     |
|       | g±          | 23.80±6.31                             | +c,b,h<br>81.90±5.41 | +a<br>18.40±5.37     | +a<br>19.10±3.96    |
|       | g+          | 0.00                                   | +c,b,h<br>14.20±3.89 | +c,o<br>54.70±7.36   | +c,a<br>74.70±4.88  |
|       | g++         | 0.00                                   | +b,h<br>0.00         | +c,a,h<br>26.90±4.93 | +c,a,b<br>4.30±1.41 |
| II    | gN          | 76.20±6.31                             | +c<br>0.00           | +c<br>0.00           | +c<br>0.00          |
|       | G±          | 23.80±6.31                             | +c<br>4.10±2.47      | +c<br>0.00           | +c<br>0.00          |
|       | g+          | 0.00                                   | +c,b<br>95.90±2.47   | +c,a,h<br>16.30±4.29 | +c,b<br>88.60±5.73  |
|       | g++         | 0.00                                   | +b<br>0.00           | +c,a,h<br>83.70±3.29 | +b<br>11.40±5.73    |
| III   | gN          | 76.20±6.31                             | +c<br>0.00           | +c<br>0.00           | +c<br>0.00          |
|       | g±          | 23.80±6.31                             | +c,b,h<br>59.90±5.03 | +c,a<br>0.00         | +c,a<br>0.00        |
|       | g+          | 0.00                                   | +c,b,h<br>40.10±5.03 | +a<br>9.20±4.14      | +a<br>5.30±2.28     |
|       | g++         | 0.00                                   | +b,h<br>0.00         | +c,a<br>90.80±4.14   | +c,a<br>94.70±2.28  |
| IV    | gN          | 76.20±6.31                             | +c<br>0.00           | +c<br>0.00           | +c<br>0.00          |
|       | g±          | 23.80±6.31                             | +c,b,h<br>64.40±3.90 | +c,a<br>0.00         | +c,a<br>0.00        |
|       | g+          | 0.00                                   | +c,b,h<br>35.60±3.90 | +a<br>5.10±3.73      | +a<br>3.40±2.84     |
|       | g++         | 0.00                                   | +b,h<br>0.00         | +c,a<br>94.90±3.73   | +c,a<br>96.60±2.84  |
| V     | gN          | 76.20±6.31                             | 0.00+                | —                    | —                   |
|       | g±          | 23.80±6.31                             | 69.40±4.03           | —                    | —                   |
|       | g+          | 0.00                                   | 30.60±4.03           | —                    | —                   |
|       | g++         | 0.00                                   | 0.00                 | —                    | —                   |

+ P < 0.01

## DISCUSSION

Results of the granulogramme of neutrophil cells in guinea pigs injected with different strains of tuberculosis (avian, bovine and human) are in accordance with the scheme proposed by BENDA AND URQUIA (1949), concerning the proportion of neutrophil types in health and disease. In normal individuals, the normal cells predominate, while in pathological conditions the intermediary and pathological types increased. Their proportion depends, however, on the severity of the disease's process. The (gN) neutrophils in our study were found to be decreased in all infected guinea pigs, one week after injection. Moreover, these cells disappeared completely on the following weeks of the experiment. Simultaneously, the intermediary and frankly pathological cells were elevated. It is to be noted that, because of the severity of the human and bovine bacilli, the (g±) cells disappeared from the granulogramme of the human and bovine TB infected animals from the 2nd week after infection till the end of the experiment. Their pathogenicity, however, were expressed by the predominance of frankly pathological neutrophils, but not the intermediary cells. On the other hand, as the avian bacilli were mild for guinea pigs, the predominance in this case was for the (g±) cells. These cells were increased largely at the 1st week after infection with the avian bacilli, however, they decreased severely at the 2nd week, then started to increase gradually. An explanation to this behaviour could be offered that the 2nd week was the severest period in the course of infection with the avian bacilli. This is true, when regarding the highest increase of the frankly pathological neutrophils at that period in this group. The return of the (g±) cells in a gradual increase after the 2nd week and the simultaneous decrease of the frankly pathological types refers to the control of infection by the guinea pigs. It may be the natural unsusceptibility of guinea pigs to the avian bacilli which suppresses the severity of the disease. The submitted explanation is in accordance with BENDA AND URQUIA (1949) when stated that "the beginning of primary infections and attenuated or cured tuberculosis is expressed by the intermediary type granulogramme".

The frankly pathological type neutrophils (g+ and g++) were mainly dominated in bovine and human TB infected animals. The more severe the condition, the more increase is the count of (g++) cells. This criteria was clearly seen in guinea pigs with bovine tuberculosis where the (g++) neutrophils appeared in a considerable number from the 1st week after infection (26.90%) then reached to a level of (83.70%) at the 2nd week and continued to increase further on. On the other hand, in a rather severe agent to guinea pigs as the human TB bacilli, the (g+) cells predominated at the last two weeks, there after, the (g++) cells were increased from the 3rd week till the end of the experiment.

In conclusion, the granulogramme studies could differentiate the 3 types of TB bacilli in the present experiment. At the 1st week after infection, absence of (g++) cells beside the significant increase of (g±) cells denoted infection with avian tuberculosis. Prevalence of the frankly pathological neutrophils (g+ and g++) characterized the bovine bacilli infection. On the other hand, human TB infection was marked by the higher increase of (g+) cells.

From the 2nd by the 4th week after inoculation, absence of ( $g\pm$ ) neutrophils characterized bovine and human TB infected animals. During the 2-nd week higher values of ( $g+$ ) cells with absence of ( $g++$ ) neutrophils occurred only in those guinea pigs with avian tuberculosis. The increase of ( $g++$ ) cells accompanied with the decrease of ( $g+$ ) types was observed in animals infected with the bovine bacilli. The contrast was the character of human TB infections.

At the 3rd and the 4th weeks of the experiment, avian TB infection was differentiated by the presence of ( $g\pm$ ) and ( $g+$ ) cells in increased numbers and the absence of ( $g++$ ) cells. Bovine and human tuberculous guinea pigs were characterized by elevation of ( $g++$ ) neutrophils.

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GRANULOGRAMME OF TB



Fig. 1. Normal type neutrophil (gN). The granules appear as a fine dust set in transparent cytoplasm (x1500).

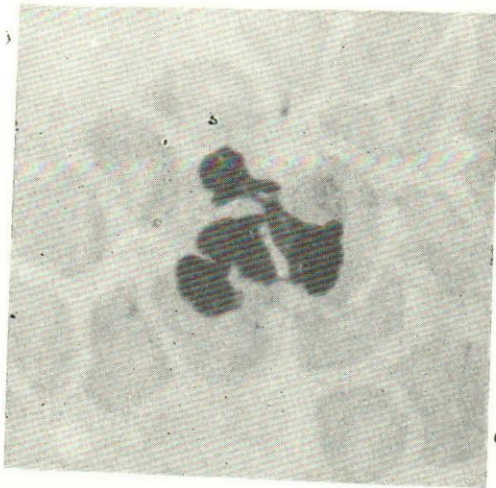


Fig. 2. Intermediary type neutrophil (g+) Simple numerical increase of the granules against dirty, opaque cytoplasm (x1500).



GRANULOGRAMME OF TB

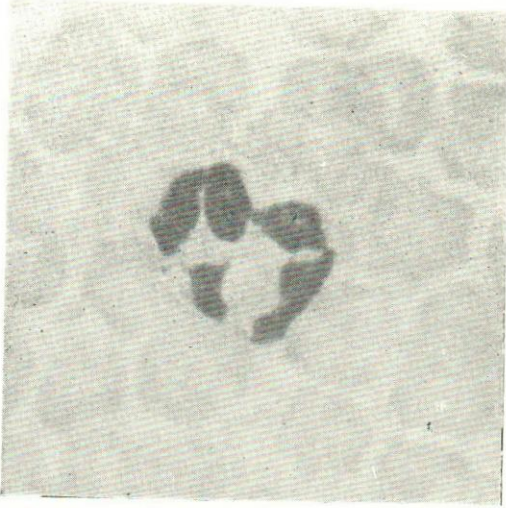


Fig. 3. Neutrophil of the Frankly pathological type (g++) Inequally sized, irregular granules (x1500).

