

دراسة سيتولوجية لمركبات الدم في الأرانسب الهنديية  
المحقونة بعترات السل البقرى وسل الطيور وعتره سل الانسان

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تم في هذا البحث دراسة بعض مكونات الدم في ثلاثة مجموعات من الأرانسب الهنديية ، حققت الاولى بعتره سل الانسان ، والثانية بعتره سل الابقسار والثالثة بعتره سل الطيور . هذا بالاضافة الى مجموعة رابعة غير محقونة أعتبرت المجموعة الضابطة .

اجريت الفحوص اسبوعيا على عشرة حيوانات من كل مجموعة ، واستمرت الى حين ظهور نتيجة ايجابية لاختبار التيوركيولين في المجموعات المحقونة . وقد أظهرت النتائج بعض التغيرات المميزة التي تساعد في تصنيف العترات المحقونة معمليا . جدير بالذكر ان هذه التغيرات الدموية قد ظهرت قبل حدوث نتيجة ايجابية لاختبار التيوركيولين .



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HAEMATOLOGICAL STUDIES FOR DIFFERENTIAL DIAGNOSIS  
BETWEEN THE BOVINE, AVIAN AND HUMAN STRAINS OF TUBERCULOSIS  
IN EXPERIMENTALLY INFECTED GUINEA PIGS  
(With 5 Tables)

By

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SUMMARY

Some blood elements of guinea pigs which are widely used as experimental animals for diagnosis of tuberculosis were studied aiming to find out the possibility of the differentiation between the bovine, avian and human bacilli of tuberculosis. Three groups of guinea pigs were infected with an equal dose from an avian, a bovine and a human standard strains of tuberculosis, respectively. In addition, a 4th group was served as non infected controls. Blood of ten animals from each group was examined weekly and the animals were tuberculin tested. The examinations were continued till the 1st evidence of positive tuberculin reaction within each group. Results revealed some characteristic findings which could be taken as criteria for differentiating the three types of TB bacilli. Such findings were observed earlier than the 1st signs of a positive tuberculin reaction.

INTRODUCTION

Guinea pigs are highly susceptible animals for the bovine, human and the avian TB bacilli, though the bovine bacillus is rather more virulent while the avian bacilli are of the least pathogenicity. This distinction has been used in practice as a mean for differentiating these organisms WILSON and MILES (1957). Injection of the TB bacilli in guinea pigs produces pathological lesions characteristic for the injected

agent. These lesions varied from a local reaction in case of avian bacilli to a generalized with the human or the bovine organisms. A reflection of tissue reaction, changes in blood elements may follow depending on the degree of tissue involvement and the severity of the invading agent. It is aimed, therefore, to study these blood changes which may be further used as an aid for type differentiation of these mycobacteria.

#### MATERIALS AND METHODS

Hematological studies were performed on 280 female albino guinea pigs of about 300 gm. each. The animals were selected from a colony proved to be free from tuberculosis. From the total sum, 40 guinea pigs were considered as controls. The rest were divided into three groups of 80 animals each. One ml saline containing 1 mg. of 4 weeks old culture of *Mycobacterium avian* (D4), *Mycobacterium bovis* (local strains and *Mycobacterium tuberculosis* (PN) was injected at the subcutaneous sublingual route in each animal of the first, second and third groups, respectively. The infected and control guinea pigs were kept separate under similar conditions. Ten guinea pigs from the control group were examined every other week, while the same number from each of the infected groups was investigated weekly. For hematological studies, 1ml of blood was collected by cardiac puncture while the animal is slightly anaesthetized by ether inhalation. The guinea pigs were then tuberculin tested using the simple intradermal comparative tuberculin test (avian and mammalian). Results of the tuberculin reaction were read within 72 hours MERCHANT and PACKER (1961). At the end of that time the animals were sacrificed. From the lymph nodes near the site of inoculation, direct smears were made and stained with Ziehl-Neelsen

stain. Cultures were also made on Lowenstein-Jensen media with and without glycerine, incubated at 37°C for weeks and examined at intervals. The examination within each of the infected groups was continued weekly till the evidence of positive tuberculin reaction within that group.

### 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 5<sup>th</sup> 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 blood values of the control animals at the 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> weeks were not differed significantly, an average value was drawn representing the control groups. Mean values of blood picture of the three infected groups at 4 successive weeks compared with the average value calculated for the control animals, (Tables 1 - 5).

### DISCUSSION

There are many evidences that hematological studies are of use in diagnosis of tuberculosis MULLER(1943), STANICEWICZ *et al.* (1965) and AL-AMROUSI *et al.* (1972). However, records dealing with the use of blood investigations for differentiating bacilli of tuberculosis could not be traced from the literature.

Examination of guinea pigs blood infected experimentally with avian, bovine and human strains of tuberculosis showed

some significant variations which could be considered as criteria for differentiating the three types of tubercle bacilli. Of importance was the occurrence of leukocytosis and neutrophilia in bovine TB infected animals, one week after infection, and at the 3rd and 4th weeks in those guinea pigs inoculated with the human bacilli. Where as leukocytosis due to increase of lymphocytes occurred at the 5th week in case of avian TB infection. The timing of these changes reflects indeed variation in virulence of these mycobacteria, i.e. the bovine strain followed by the human then the avian strain. WINTROBE (1961) mentioned that the virulence of the invading organism, the reaction of the patient, and his general resistance are concerned in determining the magnitude of the neutrophilic increase. Moreover, neutrophilia will be absent when the infection is very mild.

Lymphocytosis was observed in animals infected with the human and avian strains of tuberculosis at the 4th and 5th weeks of the experiment, respectively. PEARMAIN et al. (1963) and LING (1968) were of the opinion that increase of lymphocytes indicates resistance.

Of importance also was the increase of PCV and MCV and the decrease of MCHC in the avian TB group at the 4th week of the experiment which may be due to the appearance of immature erythrocytes in the circulation at that time.

The experiment showed some significant criteria which may aid differentiation of the three types of mycobacteria. These criteria are specially valuable as they were detected early before appearance of positive tuberculin reaction.

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**Table 1 - Blood picture in control and tuberculous guinea pigs, one week after infection**

Variables	Significance of mean values/infected		
	Avian a	Bovine b	Human h
RBC $10^6$ /cmm	4.87±0.62	5.11±0.48	5.15±0.42
Hb gm%	12.57±0.68	13.30±0.40	13.80±0.81
PCV %	32.70±3.54	30.50±1.69	33.00±2.32
MCV cu	67.63±7.68	59.70±2.39	64.29±6.00
MCH uu gm	26.05±2.26	25.41±1.38	26.84±1.66
MCHC %	35.28±3.48	43.69±1.99	41.95±3.02
SR mm/h	0.45±0.50	0.20±0.40	0.90±0.54
WBC $10^3$ /cmm	6.17±0.99	13.59±1.29	7.41±1.79
Differe- ntial	Neut. 2435.40	7568.60	3329.40
count	Eos. 83.35	13.70	31.50
absolute values	Bas. 36.70	370.00	22.20
	Lym. 3161.65	5050.60	3439.70
	Mon. 149.75	426.40	128.70
	Kurloff 303.15	160.70	458.50

+ P < 0.05

**Table 2 - Blood picture in control and tuberculous guinea pigs, two weeks after infection.**

Variables	Significance of mean values/infected			
	Control <sup>c</sup>	Avian <sup>a</sup>	Bovine <sup>b</sup>	Human <sup>h</sup>
R.B.C. $10^6$ /cmm.	4.87±0.62	5.06±0.30 <sup>+h</sup>	4.94±0.34	4.86±0.26
Hb gm%	12.57±0.68	12.10±0.37	13.35±0.63	13.20±0.33
PCV %	32.70±3.54	33.20±3.35	30.50±2.29	33.90±2.84
MCV cu	67.63±7.68	65.83±7.80	61.78±4.89	70.02±7.75
MCH uu gm	26.05±2.26	23.97±1.46	27.06±1.93 <sup>+c</sup>	27.23±1.33
MCHC %	35.28±3.48	36.71±2.98	43.89±2.27	39.20±3.17
SR mm/h	0.45±0.50	0.70±0.64	1.40±0.80	0.80±0.75
WBC $10^3$ /cmm	6.17±0.99	6.18±1.98 <sup>+b,h</sup>	7.67±1.04 <sup>+a</sup>	6.29±0.74 <sup>+a</sup>
Differential				
Neut.	2435.40 ±595.82	798.40±627.14	3442.50±473.07	2712.80±329.10
Eos.	83.35±69.20	102.90±142.48	134.40±122.28	6.80±20.40
Bas.	36.70±48.13	17.40±35.93	62.50±48.91	13.50±27.01
Lym.	3161.65±534.69	4579.80±1425.10	3609.20±554.81	3443.20±462.17
Mon.	149.75±91.90	23.50±47.12	224.20±116.40 <sup>+a</sup>	31.50±32.14 <sup>+a</sup>
Kurluff	303.15±636.48	643.50±5.04 <sup>+b,h</sup>	197.20±131.99	82.20±65.47

+ P < 0.05

**Table 3** - Blood picture in control and tuberculous guinea pigs, three weeks after infection.

Variables	Significance of mean values/infected			
	Control c	Avian a	Bovine b	Human h
RBC $10^6$ /cmm	4.87±0.62	5.02±0.34	4.96±0.41	5.73±0.81
Hb gm %	12.57±0.68	14.30±0.60	12.70±0.50	13.65±0.32
PCV %	32.70±3.54	34.90±1.04	31.40±2.15	34.30±3.03
MCV cu	67.63±7.68	69.75±3.16	63.63±6.39	60.54±6.88
MCH uu gm	26.05±2.26	28.57±1.43	25.70±1.73	24.22±3.01
MCHC %	35.28±3.48	40.96±1.08	40.65±3.40	40.07±3.30
SR mm/h	0.45±0.50	1.40±0.66	1.50±0.81	1.60±0.80
WBC $10^3$ /cmm	6.17±0.99	8.82±2.05 <sup>+h</sup>	5.75±0.73 <sup>+h</sup>	19.36±3.75 <sup>+c,a,b</sup>
Differ- ential count	2435.40±595.82	2214.20±885.95 <sup>+h</sup>	1959.20±287.78	11031.80±1226.70 <sup>+c,a,b</sup>
Eos.	83.35±69.20	62.00±55.77	180.40±71.91	65.60±80.64
Bas.	36.70±48.13	111.00±184.23	18.40±28.62	34.80±69.62
absolute values	3161.65±534.69	5741.80±1222.40	3379.40±560.63	7620.70±2788.90
Mon.	149.75±91.90	115.30±116.02	97.40±45.72	418.50±338.53
Kurluff	303.15±636.48	570.70±257.07	115.20±120.59	188.60±105.83

+ P < 0.05

**Table 4 - Blood picture in control and tuberculous guinea pigs, four weeks after infection.**

Variables	Significance of mean values/infected			
	Control <sup>e</sup>	Avian <sup>a</sup>	Bovine <sup>b</sup>	Human <sup>h</sup>
RBC $10^6$ /cmm	4.87 $\pm$ 0.62	5.08 $\pm$ 0.56	4.86 $\pm$ 0.28	5.33 $\pm$ 0.49
Hb gm %	12.57 $\pm$ 0.68	13.30 $\pm$ 0.64	12.65 $\pm$ 0.55 <sup>+h</sup>	14.60 $\pm$ 0.49 <sup>+c,b</sup>
PCV %	32.70 $\pm$ 3.54	39.10 $\pm$ 1.81 <sup>+b,h</sup>	13.10 $\pm$ 1.82 <sup>+a</sup>	32.10 $\pm$ 2.43 <sup>+a</sup>
MCV cu	67.63 $\pm$ 7.68	77.78 $\pm$ 7.63 <sup>+c,h</sup>	64.22 $\pm$ 6.22	60.43 $\pm$ 2.91 <sup>+a</sup>
MCH uu gm	26.05 $\pm$ 2.26	26.42 $\pm$ 2.07	26.07 $\pm$ 1.62	27.58 $\pm$ 1.79
MCHC %	35.28 $\pm$ 3.48	34.03 $\pm$ 0.85 <sup>+b,h</sup>	40.78 $\pm$ 2.75 <sup>+a</sup>	45.63 $\pm$ 2.30 <sup>+c,a</sup>
SR mm/h	0.45 $\pm$ 0.50	0.90 $\pm$ 0.54	0.80 $\pm$ 0.75	1.40 $\pm$ 0.80
WBC $10^3$ /cmm	6.17 $\pm$ 0.99	8.24 $\pm$ 1.89 <sup>+h</sup>	5.49 $\pm$ 0.85 <sup>+h</sup>	13.97 $\pm$ 1.92 <sup>+c,a,b</sup>
Differential count				
Neut.	2435.40 $\pm$ 595.82	2134.30 $\pm$ 771.43 <sup>+h</sup>	1749.90 $\pm$ 304.95 <sup>+h</sup>	7064.10 $\pm$ 1270.90 <sup>+c,a,b</sup>
Eos.	83.35 $\pm$ 69.20	53.00 $\pm$ 56.46	46.10 $\pm$ 44.17	27.70 $\pm$ 55.56
Bas.	36.70 $\pm$ 48.13	17.90 $\pm$ 35.85	36.80 $\pm$ 41.51	13.10 $\pm$ 39.29
absolute values				
Lym.	3161.65 $\pm$ 534.69	5419.90 $\pm$ 1188.20	3318.70 $\pm$ 608.60	6387.20 $\pm$ 1416.30 <sup>+e</sup>
Mon.	149.75 $\pm$ 91.90	148.70 $\pm$ 108.59	239.50 $\pm$ 80.10	300.10 $\pm$ 286.85
Kurtuff	303.15 $\pm$ 636.43	462.80 $\pm$ 238.26	103.20 $\pm$ 81.66	172.80 $\pm$ 162.97

+ P  $\leq$  0.05

Table (5)

Blood picture of control and infected guinea pigs with the avian bacilli, five weeks after infection

Variables	Significance of mean values	
	Control	Infected (avian)
RBC $10^6$ / cmm	4.87±0.62	5.10±0.36
Hb gm %	12.57±0.68	12.25±0.46
PCV %	32.70±3.54	37.70±1.27
MCV cu	67.63±7.68	74.24±5.70
MCH uu gm	26.05±2.26	24.10±1.78
MCHC %	35.28±3.48	32.50±1.02
SR mm/h	0.45±0.50	1.50±0.50
WBC $10^3$ /cmm	6.17±0.99	11.36±0.61 <sup>+</sup>
Differen- tial count	2435.40±595.82	2841.70±688.99
Neut.	83.35±69.20	145.80±96.19
Eos.	36.70±48.13	55.80±75.97
Bas.	3161.65±534.69	7658.60±750.53 <sup>+</sup>
Lym.	149.75±91.90	206.30±74.67
Mon.	303.15±636.48	451.80±346.14
Kurluff		

+ P < 0.05

