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Growth Rate and Lectin Mediated Agglutination Tests in Three *Leishmania major* Strains

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ABSTRACT. Growth rate in culture media and lectin mediated agglutination tests were done to study three *Leishmania major* Strains. These strains were the Senegallese strain MHOM / SN / OO / DKI (SN), the Sudanese strain MHOM / SD / UG8 (SD) and the Saudi strain NHOM / SA / 84 / JISH (SA). The *in vitro* behavior of the three *L. major* strains was traced in biphasic blood agar media. The three strains grew well on the media, but the peak of maximum growth differed from one strain to another. The lectin study showed that Con A and RCA are not useful in differentiating strains while Ulex I, Ulex II, SBA and PNA can be used in differentiating various strains as they react differently.

Keywords: *Leishmania major*, Growth Rate, Agglutination Tests.

Introduction

Leishmania major the causative agent of rural leishmaniasis of man is endemic in many countries of the Middle East, Africa and Central Asia[1]. It is a zoomotic parasite primarily infecting wild rodents[2]. It has been isolated from dogs[3] and numerous human cases of cutaneous leishmaniasis. Different sandfly species serve as the vector in various geographical areas[1]. The species and subspecies of *Leishmania* are usually differentiated by a combination of geographical, pathological, serological and biochemical differences. Each geographical region appears to have its own particular combination of vector and type of disease[4].

Lectins are sugar binding and cell agglutinating proteins. Certain lectins interact with complex carbohydrate structures such as those occurring in glycoprotein or in cell surface. They can be used in an agglutination test to identify different cell populations[5].

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The aim of this study is to differentiate the three *Leishmania major* strains according to growth rates in culture media and lectin-mediated agglutination tests.

Material and Methods

***Leishmania* Strains:** Senegalese strain MHOM / SN / 00 / DK1 and Sudanese Strain MHOM SD / 89 UG8, both were obtained from Royal Tropical Institute, Amsterdam. The Saudi Strain MHOM / SA / 84 / Jish 118, was provided by King Faisal Research Center, Riyadh.

Assessment of growth rate in culture media: Quantitative count of promastigotes using Neubauer improved haemocytometer was assessed in biphasic media (USAMRU)[6,7].

Preparation of *Leishmania* promastigotes for lectin agglutination [8,9]**:** Amastigotes of the three strains were inoculated in a fresh USAMRU media then subculture into fresh media after one week. Promastigotes were taken on the 5th, 7th and 13th days after subculture, collected and washed twice in PBS pH 7.2, then mixed with equal volume of 2% formaldehyde. The fixed promastigotes were kept at 4°C.

Lectins and their specific inhibiting sugars: lectins were: concanavalin A (con A), Ricinus 120 (RCA), Peanut (PNA), Soybean (SBA), Ulex 1 and Ulex II. All lectins and sugars were supplied by Sigma Company UK. Lectins were dissolved and diluted in PBS pH 6.8 in different concentrations. The specific sugars were dissolved in distilled water.

Procedures for lectin agglutination[8]**:** 50ul, of parasite suspension was added to 50ul, of the different lectin concentrations in microtiter plates and left for one hour at 22°C. It was examined on a slide under microscope for presence of agglutination. The specificity of lectin reactions was then established by the use of inhibiting sugars. The promastigotes were left with the specific sugar for 15 minutes before the addition of the lectin then the reaction was examined wet using the light microscope to check for agglutination.

TABLE 1. The sugar specificities of lectins and their concentrations (after Schnur and Jacobson 1989)[10].

Lectin	Sugar specificity	Inhibiting sugar	Conc
Concanavalin A (con A)	α-D-mannose	Methyl -α mannopyranoside	500 mM
	α-d-glucose	Glucose	500 mM
Ricinus 120 (RCA)	β -D-galactose	Lactose	500 mM
	N-acetyl galactosamine	N-acetyl galactosomine	500 mM
Peanut (PNA)	Galactose	Galactose	500 mM
Soybean (SBA)	N-acetyl-D galactosamine	N-acetyl galactosomine	100 mM
Ulex I	L-fucose	D-fucose	100 mM
Ulex II	N-acetyl-D chitobiose	N, N-diacetyl chitobiose	100 mM

Results and Discussion

Growth rate pattern in USAMRU media: The results (Table 2 and Fig. 1) showed that the behaviors of the three strains in blood agar media was different from each other. The growth reached its peak on the 7th day (SD strain), 10th day (SN strain) and13th day post infection for SA strain. The strain was the only one which showed a clear stationary phase.

TABLE 2. Mean of promastigote number in USAMRU Media for the 3 *Leishmania major* strains.

Days post inoculation	Strain		
	SN	SD	SA
2	75×10^6	25×10^6	31×10^6
3	106×10^6	39×10^6	61×10^6
4	130×10^6	77×10^6	85×10^6
5	151×10^6	134×10^6	148×10^6
6	201×10^6	178×10^6	202×10^6
7	255×10^6	212×10^6	292×10^6
8	288×10^6	182×10^6	350×10^6
9	386×10^6	160×10^6	404×10^6
10	480×10^6	167×10^6	500×10^6
11	440×10^6	165×10^6	590×10^6
12	400×10^6	156×10^6	741×10^6
13	275×10^6	145×10^6	760×10^6
14	-	-	650×10^6

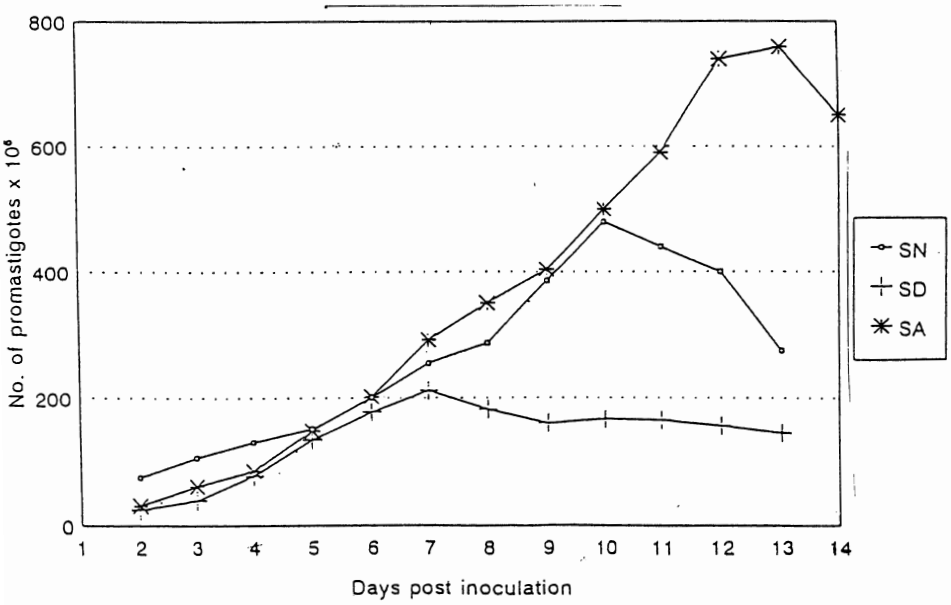

FIG. 1. Growth rate patterns of the SN, SD & SA strains in blood agar media (USAMRU).

Lectin-mediated agglutination test: As shown in Tables 3 and Figs. 2, 3, & 4, Con A lectin agglutinated moderately with the three strains. This agrees with Schottelius[11] who reported that con A agglutinated a group of *Leishmania* strains from the new and old world.

TABLE 3. The reaction of the various lectins at variable concentrations with the 3 *L. major* strains.

Strain	Lectin in ug / L																							
	Con A				R.C.A				P.N.A.				S.B.A.				Ulex I				Ulex II			
	250	125	50	10	250	125	50	10	250	125	50	10	250	125	50	10	250	125	50	10	250	125	50	10
SN	++	++	+	-	+++	+++	+++	+++	-	-	-	-	+	+	-	-	-	-	-	-	++	++	+	+
SD	++	++	+	-	+++	+++	+++	+++	-	-	-	-	+++	+++	+++	+	+++	++	+	-	+++	+++	+++	++
SA	++	++	+	-	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	++	++	++	+	-	++	++	+	+

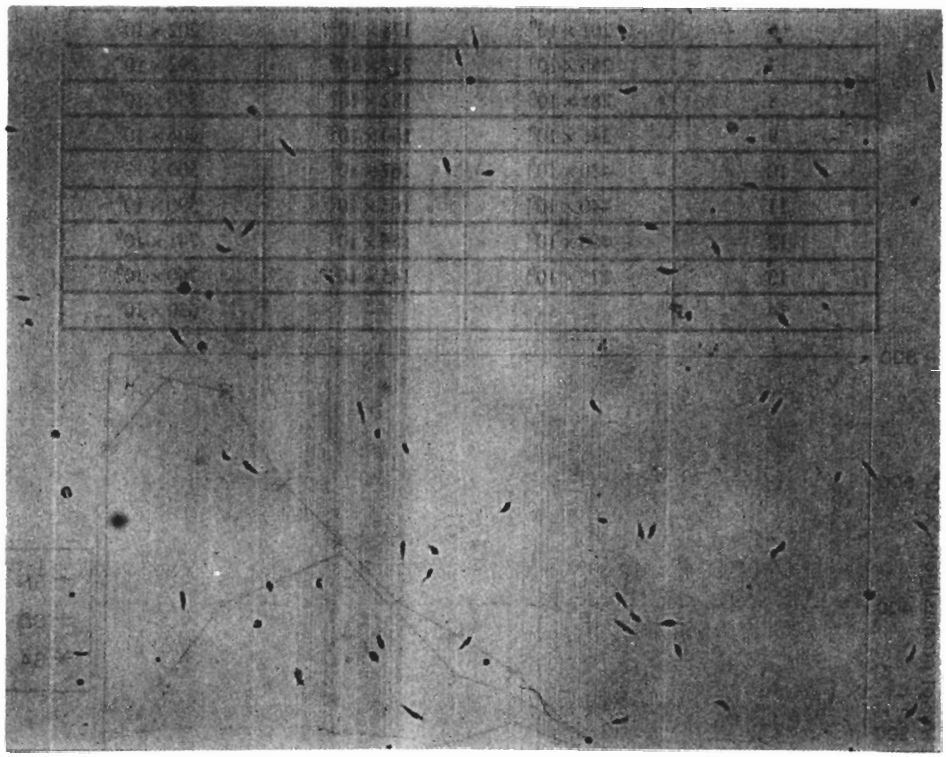

FIG. 2. Negative control showing no agglutination (× 10).

Ricinus 120 (RCA) lectin agglutinated the three strains strongly. This coincided with the results of Wilson and Pearson[12]. They stated that almost all *leishmania* strains except *L. mexicana amazonensis* agglutinated in the presence of RCA.

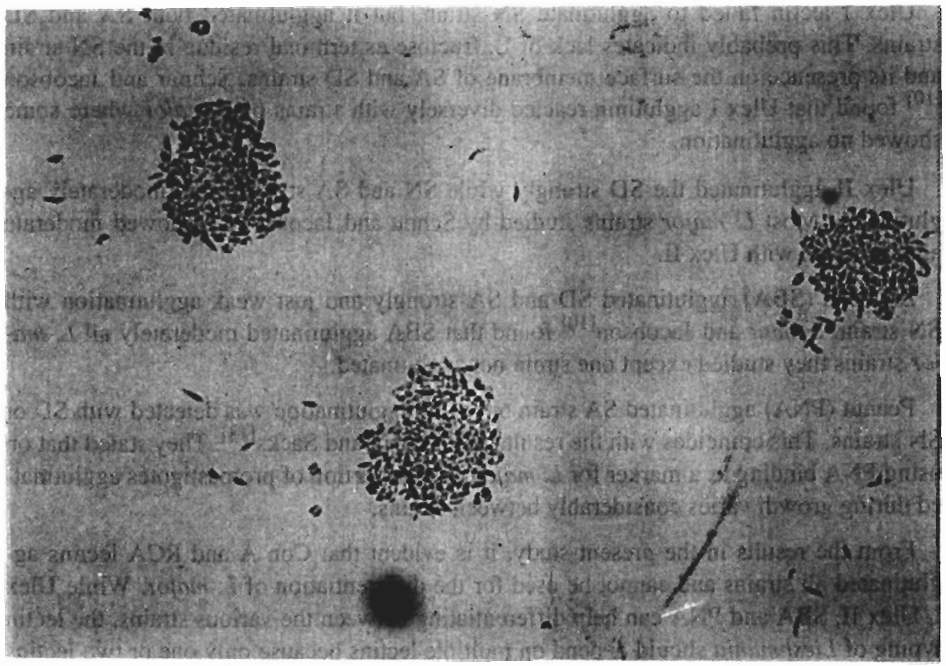

FIG. 3. Lectin-mediated agglutination test showing strong agglutination of promastigotes by lectins (× 10).

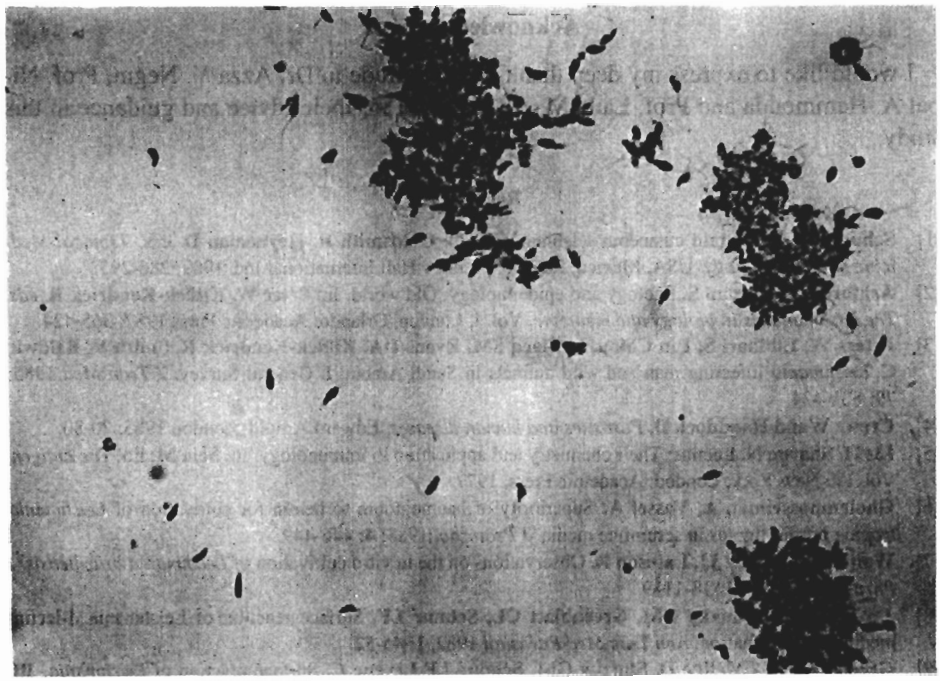

FIG. 4. Lectin-mediated agglutination test showing promastigotes agglutinated by lectins at a higher magnification (× 40).

Ulex I lectin failed to agglutinate SN strain, but it agglutinated both SA and SD strains. This probably indicates lack of L. fructose as terminal residue in the SN strain and its presence on the surface membrane of SA and SD strains. Schnur and Jacobson [10] found that Ulex I agglutinin reacted diversely with strains of *L. major* where some showed no agglutination.

Ulex II agglutinated the SD strongly while SN and SA strains were moderately agglutinated. Most *L. major* strains studied by Schnu and Jacobson[10] showed moderate agglutination with Ulex II.

Soybean (SBA) agglutinated SD and SA strongly and just weak agglutination with SN strain. Schnur and Jacobson[10] found that SBA agglutinated moderately all *L. major* strains they studied except one strain not agglutinated.

Peanut (PNA) agglutinated SA strain only. No agglutination was detected with SD or SN strains. This coincides with the results of Da Silva and Sacks[13]. They stated that on using PNA binding as a marker for *L. major,* the proportion of promastigotes agglutinated during growth varies considerably between strains.

From the results in the present study, it is evident that Con A and RCA lectins agglutinated all strains and cannot be used for the differentiation of *L. major.* While Ulex I, Ulex II, SBA and PNA can help differentiating between the various strains, the lectin typing of *Lieshmania* should depend on multiple lectins because only one or two lectins are not enough to give reliable results.

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References

[1] **Schnur LF.** Old world cutaneous leishmaniasis. In: **Goldsmith R, Heyneman D.** eds. *Tropical Medicine and parasitology.* USA, Mexico, Canada, Prentice-Hall International Inc. 1989; 286-295.

[2] **Ashford RW, Bettini S.** Ecology and epidemiology; Old world. In: **Peter W, Killick-Kendrick R.** *eds. The leishmaniasis in biology and medicine*, Vol. I, London, Orlando: Academic Press 1987; 365-424.

[3] **Peters W, Elbihari S, Liu Ching, LeBlacq SM, Evans DA, Killick-Kendrick R, Smith V, Baldwin C.** *Leishmania* infecting man and wild animals in Saudi Arabia, I. General Survey. *J Trop Med* 1985; **79:** 831-424.

[4] **Crewe Wand Handdock D.** *Parasites and human diseases.* Edward Arnold; London 1985; 70-80.

]5] **Lis H, Sharon N.** Lectins: Their chemistry and application to immunology. In: **Sela M.** Ed. *The antigen,* Vol. IV. New York, London: Academic Press, 1977.

[6] **Gholamhosseinian A, Vassef A.** Superiority of haemoglobin to hemin for cultivation of *Leishmania tropica* promastigotes in serumfree media. *J Protozool* 1988; **4:** 446-449.

[7] **Walton BC, Shaw JJ, Lainson R.** Observations on the in vitro cultivation of *Leishmania braziliensis. J Parasitol* 1977; **6:** 1118-1119.

[8] **Javobson RL, Slutzky GM, Greenblatt CL, Schnur LF.** Surface reaction of Leishmania. I-lectin-mediated agglutination. *Ann Trop Med Parasitol* 1982, **1:** 45-52.

[9] **Greenblatt CL, Meline D, Slutzky GM, Schnur LF Levene C.** Surface reaction of *Leishmania*, III. Ulex europaeus II Lectin affinity for excreted factor (EF) scrotype A Strains. *Ann Trop Med Parasitol* 1984; **2:** 99-107.

[10] **Schnur LF Jacobson RL.** Surface reaction of *Leishmania* IV. Variation in the surface membrane carbohydrates of different strains of *Leishmania major. Ann Trop Med Parasitol* 1989; **5:** 455-463.

[11] **Schottelius J.** Lectin typing of *Leishmania* strains from the new and old world. In: **Bog-Hansen TC.** *Lectins: biology, biochemisrty, clinical biochemistry;* Vol. 2. Berlin, New York; Walter de Gruyter 1982: 531-541.

[12] **Wilson Me, Pearson RD.** Stage -specific variation in lectin binding to *Leishmania donovani. Infect Immunol* 1984; **46:** 128-134.

[13] **Da Silva R, Sacks DL.** Metacyclogeneses is a major determinant of *Leishmania* promastigote virulence and attenuation. *Infect Immunol* 1987; **55:** 2802-2806.

دراسة معدل النمو واحتياجات تجلط اللكتينات في ثلاث سلالات من الليشمانيا الكبيرة

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المستخلص . تمت دراسة معدل النمو واختبارات تجلط اللكتينات في ثلاثة سلالات من الليشمانيا الكبيرة : السلالة السنغالية MHOM/SN/CO/DKI والسلالة السودانية MHOM/SD/89/UG8 والسلالة السعودية /MHOM/SA 84/JISH118 . تمت دراسة معدل نمو الطور السوطي للسلالات الثلاث في وسط USAMRU وتبين أن السلالات الثلاثة تنمو جيداً في هذا الوسط غير أنها تختلف عن بعضها في معدل النمو . بدراسة الكربوهيدرات الموجودة على الغشاء السطحي للسلالات الثلاثة باستخدام اللكتينات أمكن استنتاج الآتي : كوتكنا فلين (أ) وريسنص كميونس : لا يمكن الاعتماد عليهم في التفريق بين سلالات الليشمانيا حيث أن نتائج التجلط معهم متشابهة إلى حد كبير . يولكس ٢٢١ وكذلك لكتين فول الصويا والفول السوداني يمكن الاعتماد عليهم للتفريق بين السلالات المختلفة لليشمانيا حيث أن نتائج ا لتجلط معهم تختلف .