RESULTS

R E S U L T S

Inhibitory effect of S. mansoni immunosuppressive substance on non-adherent and pure non-adherent spleen cell proliferation :

1. Our results in table (1) showed that the prepared ISS has an inhibitory activity on the non-adherent spleen cells proliferation of group B (non-adherent to plastic Petri dishes, 2 hrs), when cultured with mitogen con A. It was noticed by examining the thymidine uptake that the inhibited response started at day 4 of the experiment and remained till day 6, then the cells gradually regained their power of activity to a certain extent but did not go back to the normal response observed in the control group (A) as shown in Fig. (1) .

2. Pure non-adherent spleen cells free of adherent cells as well as suppressor T cells of group B (spleen cells first depleted of plastic adherent then purified on nylon wool column) showed relatively inhibition in their proliferation as indicated by thymidine uptake shown in table (2). This inhibition although some how less than that of the former experiment yet at day 4,

there started a slight stimulation followed by a small increased in thymidine uptake at day 6 and remained nearly the same till day 10 , if compared with the normal response of the control group (A) this is still at a lower level Fig. (2). So from these results we can say that the inhibitory response observed in group B is due to a suppressive property of the injected (ISS) .

Result

Table 1: Immune response to mitogen con• A after injection with the immunosuppressive substance (ISS) in normal rats (before removal of adherent supressor cells)•

Group	Non-adherent lymphocyte culture *					
	Day 4		day 6		Day 10	
	Unstimulated control	+ con•A	Unstimulated control	+ Con•A	Unstimulated control	+ con• A
A	2102 ±368	4381 ±294	3048 ±37	10668 ±170	2897 ±214	6519 ±527
B	1400 ±129	1053 ±234	1828 ±128	1370 ±156	1115 ±175	3851 ±643

* = Spleen cells from rats after platting on plastic surface of Petridish cultured with or without con•A (0•5 ug)• Results expressed as Cpm of H^3- thymidine incorporated in the cells ± standared error of mean, Results of 3 experiments with triplicate cultures•

A- Normal control group•
B- Injected group with ISS•

Table 2: Immuno response to mitogen con. A after injection with the immunosuppressive substance (ISS) in normal rats and after removing the adherent suppressor cells.

Group	Pure non-adherent lymphocyte culture *					
	Day 4		Day 6		Day 10	
	Unstimulated control	+ con.A	Unstimulated control	+ con.A	Unstimulated control	+ con. A
A	5167 ±215	44732 ±5607	3774 ±104	68196 ±1025	2135 ±159	41095 ±674
B	1456 ±56	2322 ±21	1261 ±6	4695 ±370	1146 ±325	4515 ±181

* = Spleen cells purified by platting on plastic surface and passing through nylon wool column, then put into culture with or without con. A, results expressed as cpm of ^{3}H-thymidine incoparated in the cells ± standared error of means. Results of 3 experiments with triplicate cultures.

A - Normal control group.
B - Injected group with ISS.

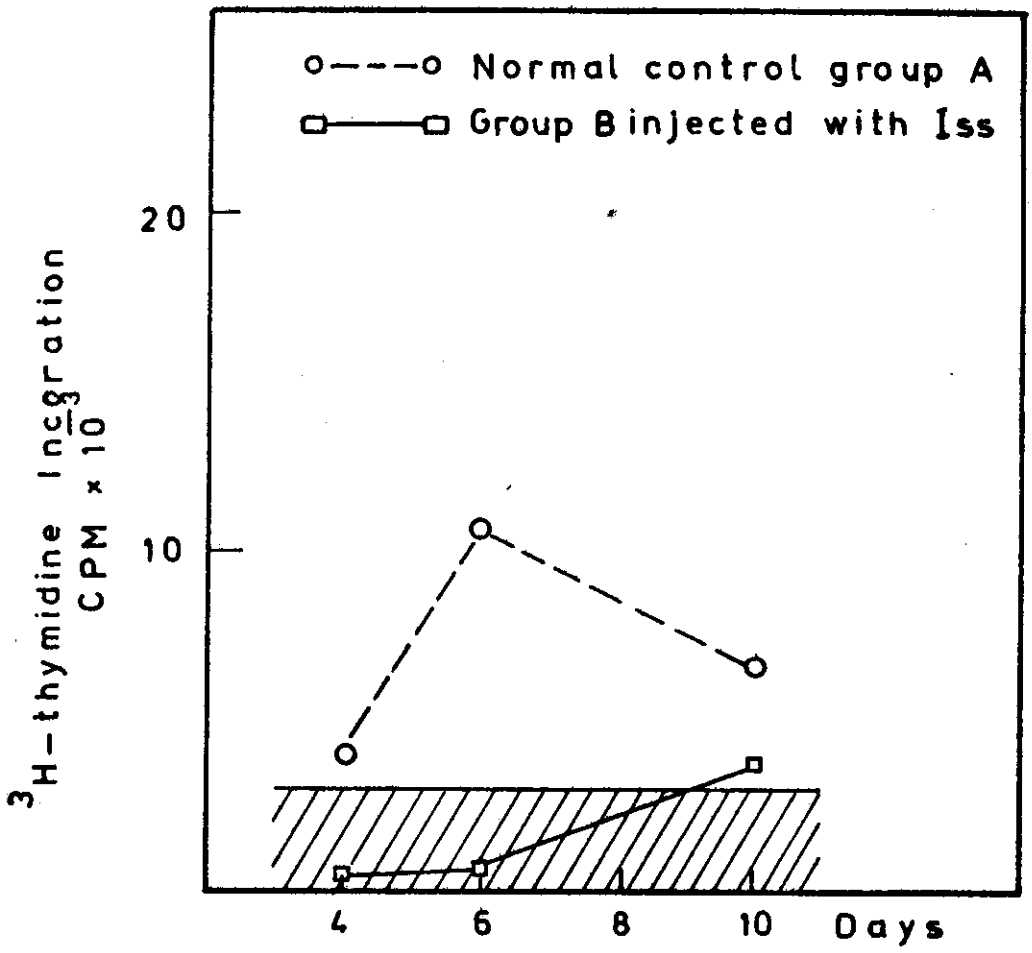


Fig.(1) Immune response to mitogen con. A. after injection with the immuno-suppressive substance (Iss) in normal rats (before removal of adherent supressor cells).

Note. shaded area in all curves corresponds at the range of thymidine incorporation into unstimulated rat spleen cells cultured with the media alone.

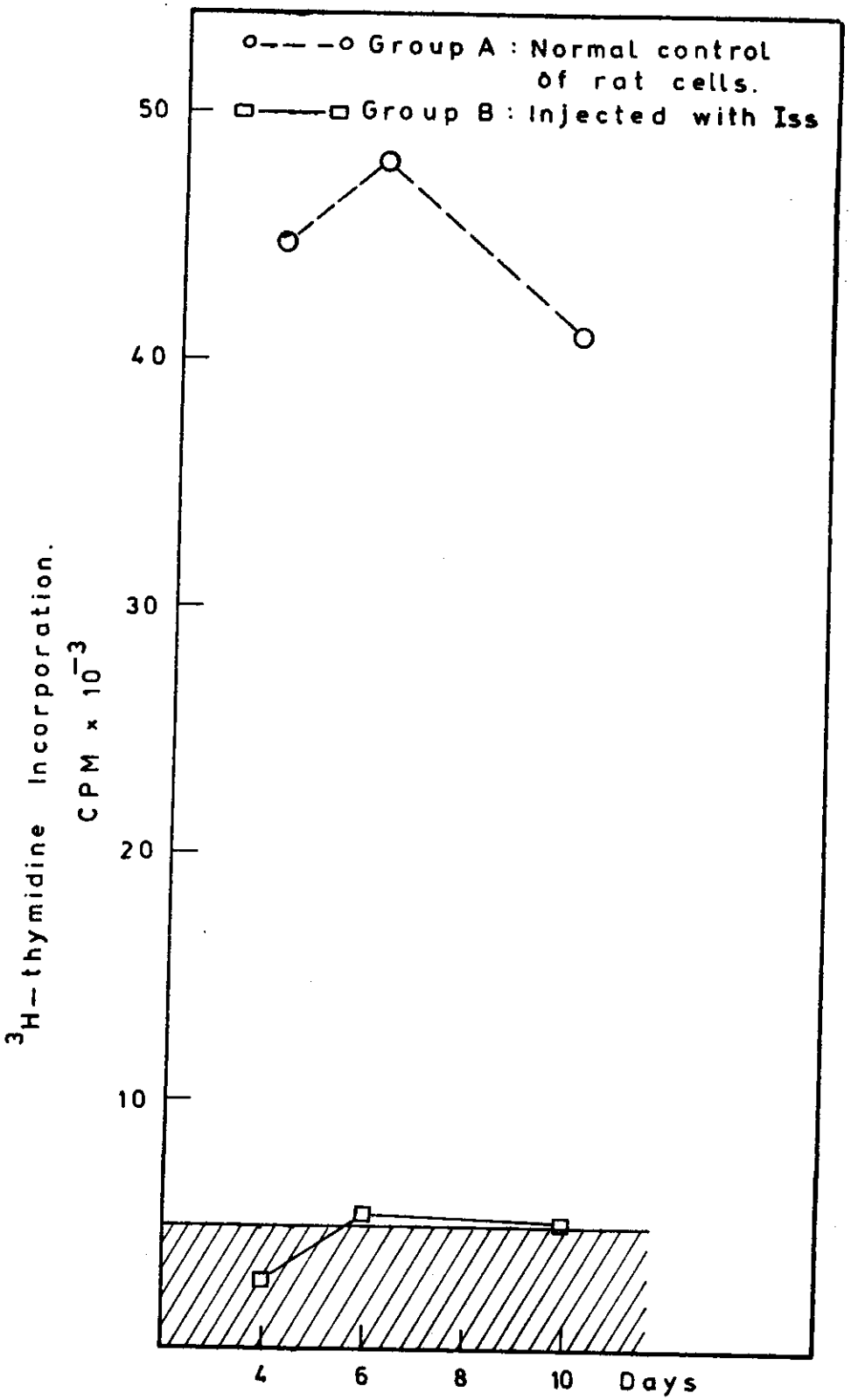


Fig.(2) Immune response to mitogen con. A. after injection with the immuno_ suppressive substance (Iss) in normal rats, after removing the adherent suppressor cells.

Inhibitory effect of S. mansoni immunosuppressive substanceon non-adherent and pure non-adherent spleen cells proliferation after infection with S. mansoni :

3. Our data in table (3) shows that the lymphocytic response of infected Fischer rats (group D) has a characteristic two stage phenomena when cultured during infection with con A. compared to non-infected control group (A). The first stage is of enhanced lymphocyte reactivity during the first two weeks and the second stage is of lowered lymphocyte reactivity at the fourth and fifth week after infection (Fig. 3). It is observed that the proliferative response of group (D) reaches the maximum stimulation at day 10 Fig. (3A) then decreased gradually to give a lower, stimulation at day 14 followed by great inhibition reaching a minimum value beyond that of the normal group (A) (Fig. 3B). If we compared this result with group (C) (infected and ISS injected) the same behaviour is displayed with a pronounced reduction in both the maximum and minimal values, i.e. a remarkable depression in lymphocytic response is observed with group (C) at day 10 always less than that obtained with group (D) also at day 35 a more pronounced inhibition is effected in group (C) compared with that of group (D) (Table 3) and (Fig. 3) .

Table 3: Inhibitory effect due to the immunosuppressive substance on the proliferation of infected rat spleen cells, before removing the adherent suppressor cells.

Lympho-cyte cells	Non-adherent lymphocyte culture*									
	Day 8		Day 10		Day 14		Day 28		Day 35	
	+media	+con.A	+media	+con.A	+media	+con.A	+media	+con.A	+media	+con.A
A	4238 ±306	18108 ±3883	2193 ±42	16994 ±3221	2897 ±214	16126 ±626	1972 ±247	14235 ±597	1241 ±96	13695 ±15
C	4293 ±746	10080 ±100	2260 ±350	34864 ±7664	0978 ±104	1387 ±35	1248 ±402	2825 ±179	1467 ±40	1030 ±205
D	4696 ±516	9142 ±674	3881 ±19	68059 ±3410	2776 ±102	27393 ±2460	1099 ±204	3093 ±182	1549 ±189	1460 ±134

* Spleen cells of Fischer rats cultured after platting on3 plastic Petri dish with or without con.A (0.5ug). Results expressed as cpm of (H) thymidine incorporated in the cells ±standared error of mean value, results of 3 experiments with triplicate cultures.

A Group of non-infected control rats.
C Group of rats infected with 800 of S.mansoni cercariae at day zero, then injected with the prepared ISS at day 4.
D Group of rats infected with 800 S.mansoni cercariae at day zero.

Table 3 **A** : Stimulation of non-adherent lymphocyte proliferation when cultured with different concentration of con A at day 10 after infection.

Con.A concentration ug/well	Cell culture group (a)		
	A	C	D
Control culture	1193 ±43	2260 ±350	3881 ±19
.01	4506 ±93	7499 ±179	12091 ±186
.05	5013 ±990	9601 ±407	16245 ±245
.1	5029 ±137	13382 ±934	38757 ±641
.5	16994 ±3221	34864 ±7664	68059 ±3410
1.	15160 ±216	9238 ±1401	38551 ±9167

(a): Spleen **cells** of Fischer rats cultured after platting on plastic petri dish. Results expressed as cpm of ^{3}H-thymidine incorporated in the cells ± standard error of mean, results of 3 experiments with triplicate cultures.

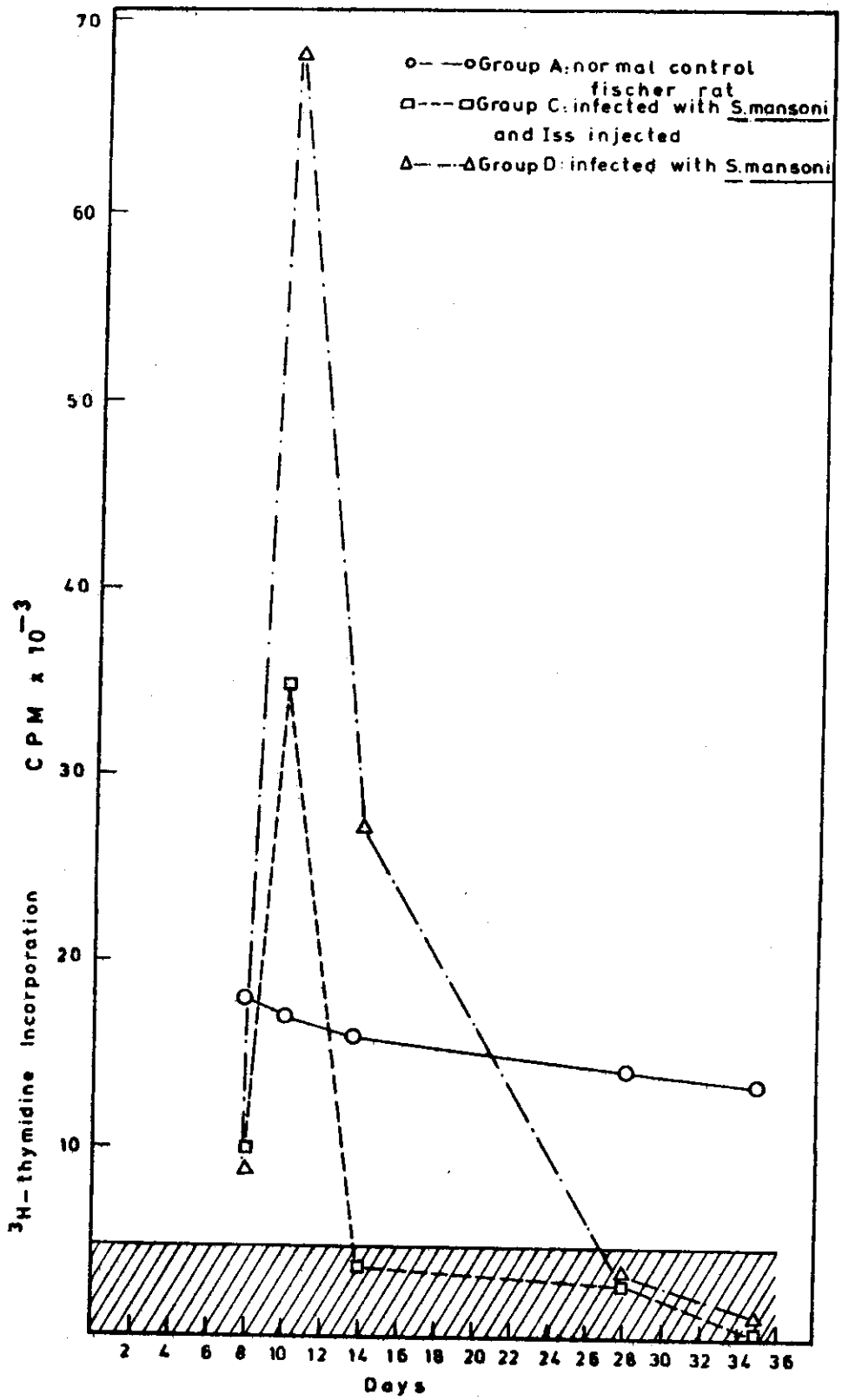


Fig. (3) Inhibitory effect due to the immunosuppressive substance on the proliferation of infected rat (group C) spleen cells, before removing the adherent suppressor cells.

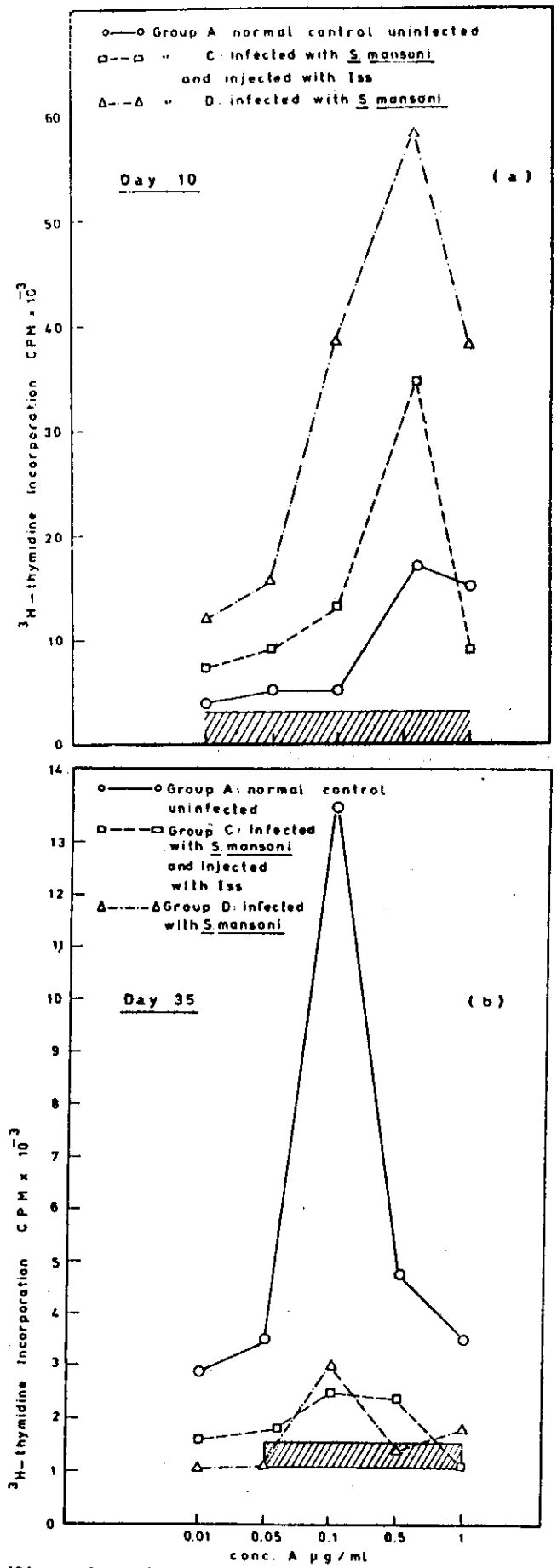


Fig.(3)(a&b) Inhibition of cell proliferation of infected rat spleen cells (group C) due to the immunosuppressive substance, at the maximal stimulation response (day 10) fig 3 a and at maximal inhibition (day 35) (fig. 3 b) when cultured with different concentrations of con.A. before removing the adherent suppressor

4. In preliminary experiment to investigate the role of suppressor cell, the pure non-adherent cells from group (C, D & A) cultured with different concentrations of con. A (Table 4), we found that removing of the adherent T suppressor cells from the culture medium affected the stimulating response by reinforcing it. This is evidenced by more reactivity was shown in both group (C & D) if compared with group (A). Besides, we noticed that the lymphocytic response of group (C) was less than that of group (D) Fig. (4) .

On the other hand at the unresponssiveness stage where the inhibition takes place the removal of the T-suppressor cells had no much effect on the proliferative cells indicated by almost no change in the inhibited response. However group (C) always being more inhibited than (D) (Fig. 4). From Figs. (3a) and (4a) one can deduct that the optimum stimulation was affected at 0.5 ug/ml. concentration of con. A when using the crude non-adherent cells (Fig. 3A). On passing the cell population through Nylon wool column we got a pure T-lymphocyte culture which could give us an optimum response at 0.1 ug/ml

concentration of con. A (Fig. 4a). (i.e. pure lymphocyte culture could stimulate better and at less concentration of con A) .

As shown in Figs.(3b & 4b) neither the non-adherent nor the pure non-adherent lymphocyte could stimulate a response to different concentration of mitogen con A.

Table 4: Spleen cell proliferation of infected rat after removing the adherent suppressor cells.

Lympho-cyte cells	Pure non-adherent lymphocyte culture *									
	Day 8		Day 10		Day 14		Day 28		Day 35	
	+media	+con.A	+media	+con.A	+media	+con.A	+media	+con.A	+media	+con.A
A	5167 ±215	64732 ±5607	3774 ±104	57576 ±5502	2135 ±159	41095 ±674	1013 ±215	27096 ±4700	1679 ±52	21930 ±11
C	2941 ±396	100701 ±2019	2415 ±251	156272 ±7606	1620 ±40	38765 ±3100	1738 ±108	9639 ±99	1284 ±99	8038 ±15
D	3754 ±325	140763 ±8658	2178 ±93	151664 ±5791	2311 ±400	55973 ±1556	2006 ±97	15989 ±127	1664 ±63	12071 ±35

* Spleen cells purified by platting on plastic surface of Petri dish and passing through nylon wool column then put into culture with or without con.A(0.1 ug)Results expressed as cpm of $(H)^3$ thymidine incorporated in the cells ± standared error of mean value results of 3 experiments with triplicate cultures.

A Group of non-infected control rats
C Group of rats infected with 800 S.mansoni sercariae at day zero and injected with the prepared ISS at day 4.
D Group of rats infected with 800 S.mansoni cercariae at day zero.

Table 4 A: High stimulation of pure non-adherent lymphocyte proliferation when cultured with different concentration of can A, at day 10 after infection.

Con. A concentration ug/well	Cell culture group[(a)]		
	A	C	D
Control culture	774 ±104	757 ±240	2178 ±93
.01	30985 ±2135	36009 ±2134	57197 ±5017
.05	57576 ±5502	58455 ±7435	73035 ±2624
.1	160446 ±3899	156272 ±7606	186663 ±5791
.5	68196 ±1025	42775 ±266	151664 ±6136
1.0	60274 ±1328	22331 ±998	62206 ±3622

(a): Spleen cells purified by platting on plastic surface of petri dish and passing through nylon wool column then put into culture. Results expressed as cpm of ^{3}H-thymidine incorporated in the cells ± standared error of mean, results of 3 experiments with triplicate cultures.

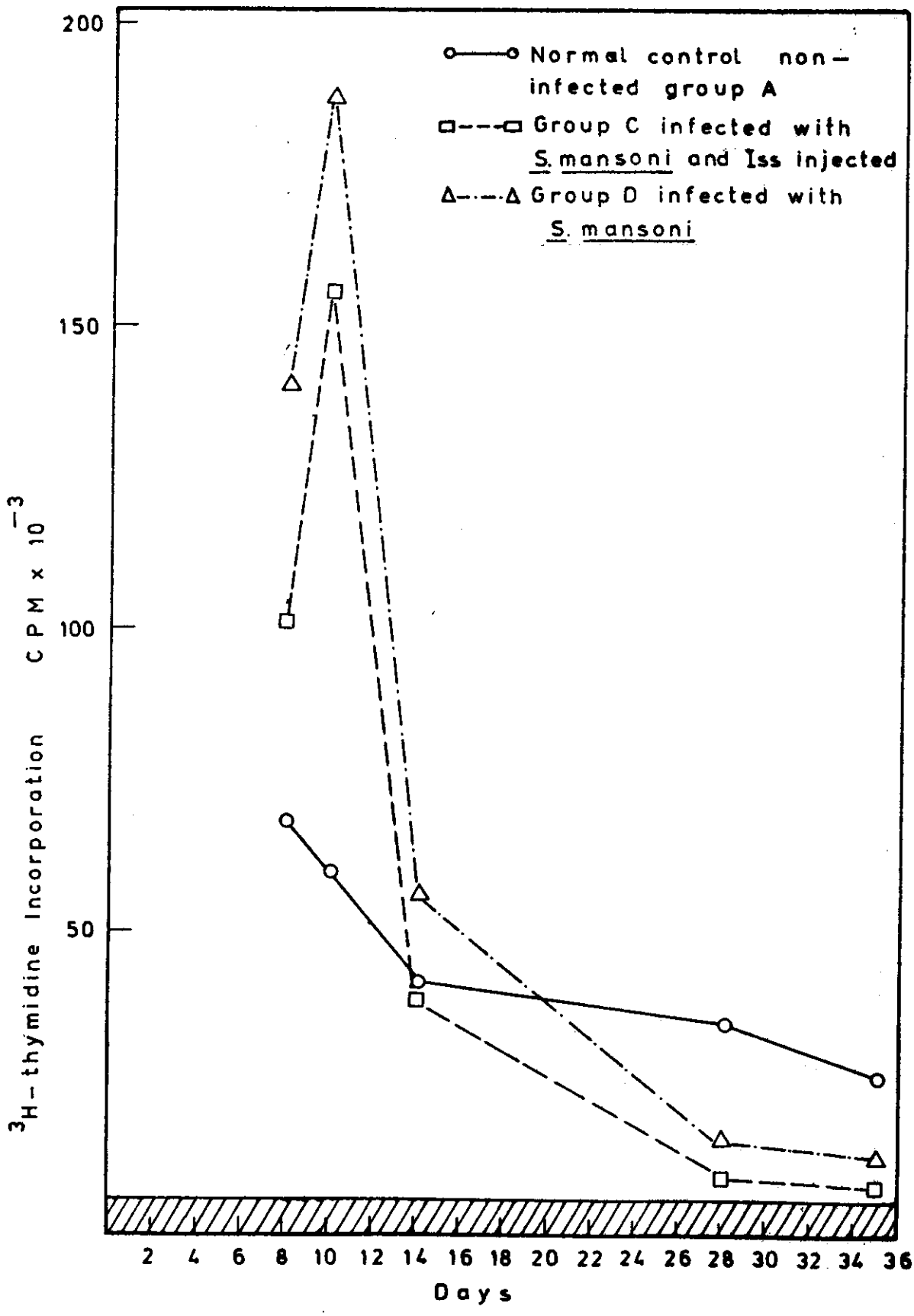


Fig.(4) Spleen cell proliferation of infected rat after removing the adherent suppressor cells.

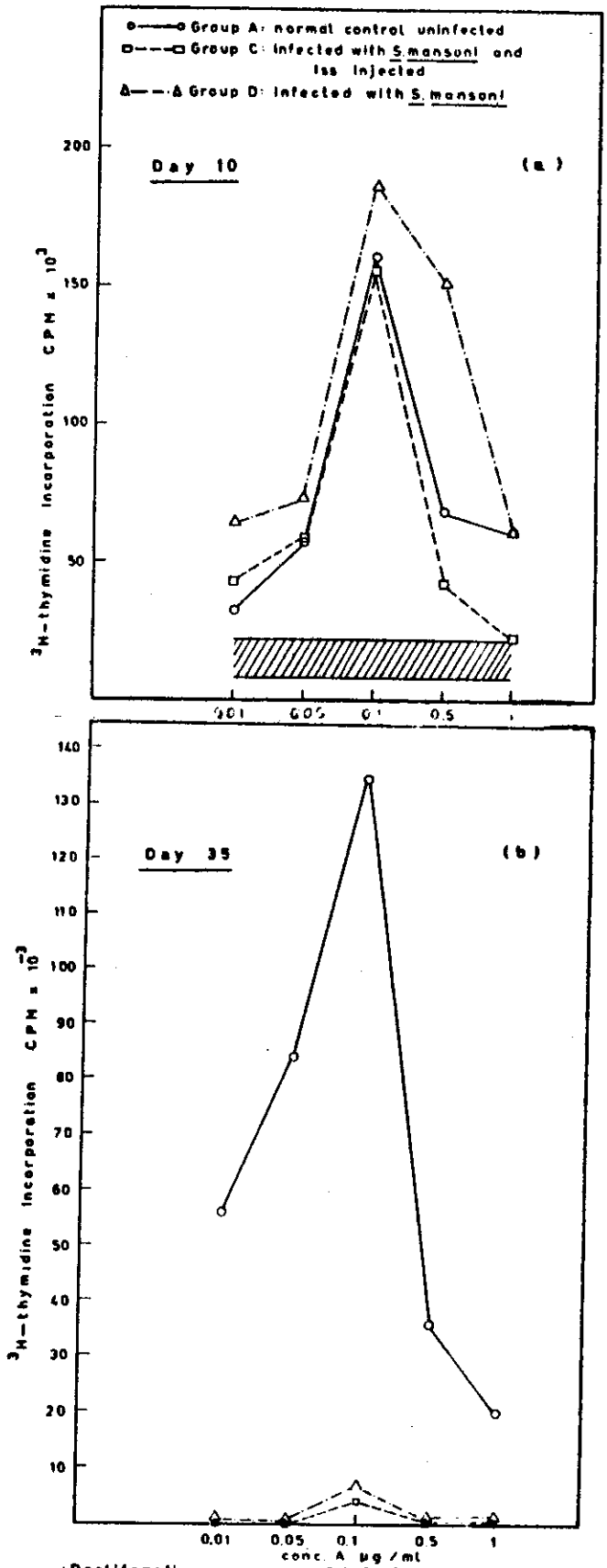


Fig. 4. (a&b) Proliferative response of infected rat spleen cells after removing the adherent suppressor cells at the maximal stimulation response (day 10) (Fig 4 a) and at the maximal inhibition response (day 35) (Fig. 4 b) when cultured with different concentration of con A

Specific inhibitory effect of the immunosuppressive substance in S. mansoni infected rats:

Studying the specific response of 14 day infected group D and infected ISS injected group (C) spleen cells of rats, showed clearly that cells which have been fully responsive of day 14 after infection (table 5 and Fig.5) became totally unresponsive at day 35. This depression in the specific response to S. mansoni antigen does not appear to be related to increased specific suppressor cell activity.

As shown in table (6) and Fig. (6) at the unresponsive stage, the nylon wool depletion of day 35 infected spleen cells do not restore lymphocyte response to S. mansoni antigen (Fig. 6). When we compare the specific response of infected group (D) and group (C) injected with the ISS beside the infection with S. mansoni we found that at the maximum of response day 14 group (C) could not reach the same reactivity to stimulate as group (D). This pronounced reduction in response may be due to the injected ISS. The same result could be detected at the inhibitory stage (Figs. 5 & 6) where the inhibition of group (C) is higher than that

obtained by group (D). Thus it is presumed that the injected ISS may have a specific immunosuppressive effect as well as non specific immunosuppressive effect which could decrease the lymphocytic immune response of rat schistosomiasis.

Table 6: Specific response of infected rat spleen cells after removing the adherent suppressor cells and cultured with S.mansoni antigen at day 14 and day 35.

	Lymphocyte culture *			
	Day 14		day 35	
Group	Unstimulated	±Sch.Ag	Unstimulated	± Sch.Ag
C	2340 ±62	16170 ±127	1663 ±162	2064 ±108
D	2756 ±37	29608 ±974	1790 ±688	2421 ±534

* = Day 14 and day 35 pure non-adherent cells (mean cpm ± s.e.) cultured with medium alone (unstimulated or with 40 ug/well of schistosoma antigen.

C = Infected rat with s.mansoni and ISS injected

D = Infected rat with s.mansoni only.

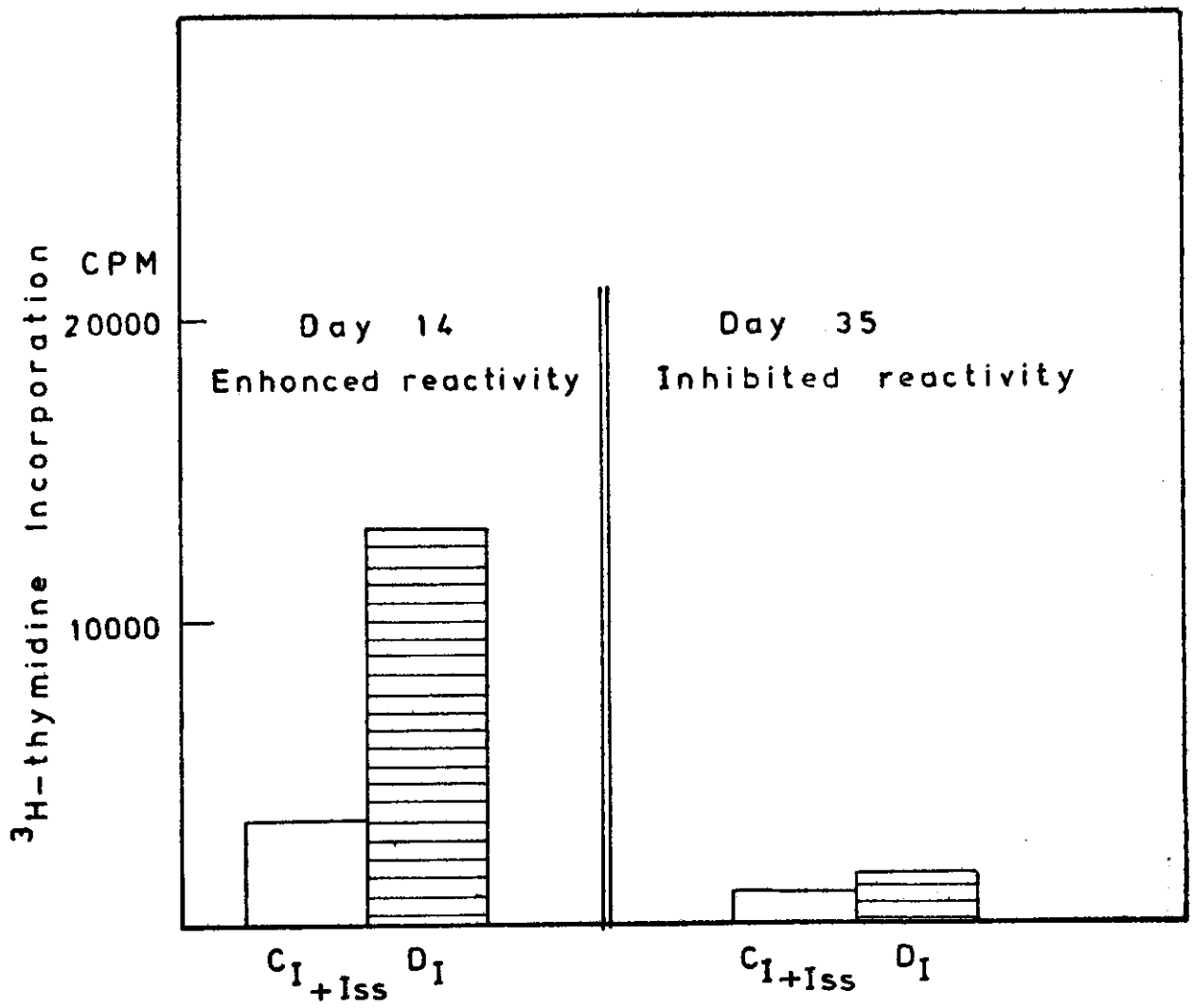


Fig. (5) Specific response of infected rat spleen cells when cultured with S. mansoni antigen at day 14 and day 35 and before removing the adherent suppressor cells.

C_{I+Iss} : Group C infected with S. mansoni and Iss injected

D_I : Group D infected with S. mansoni only.

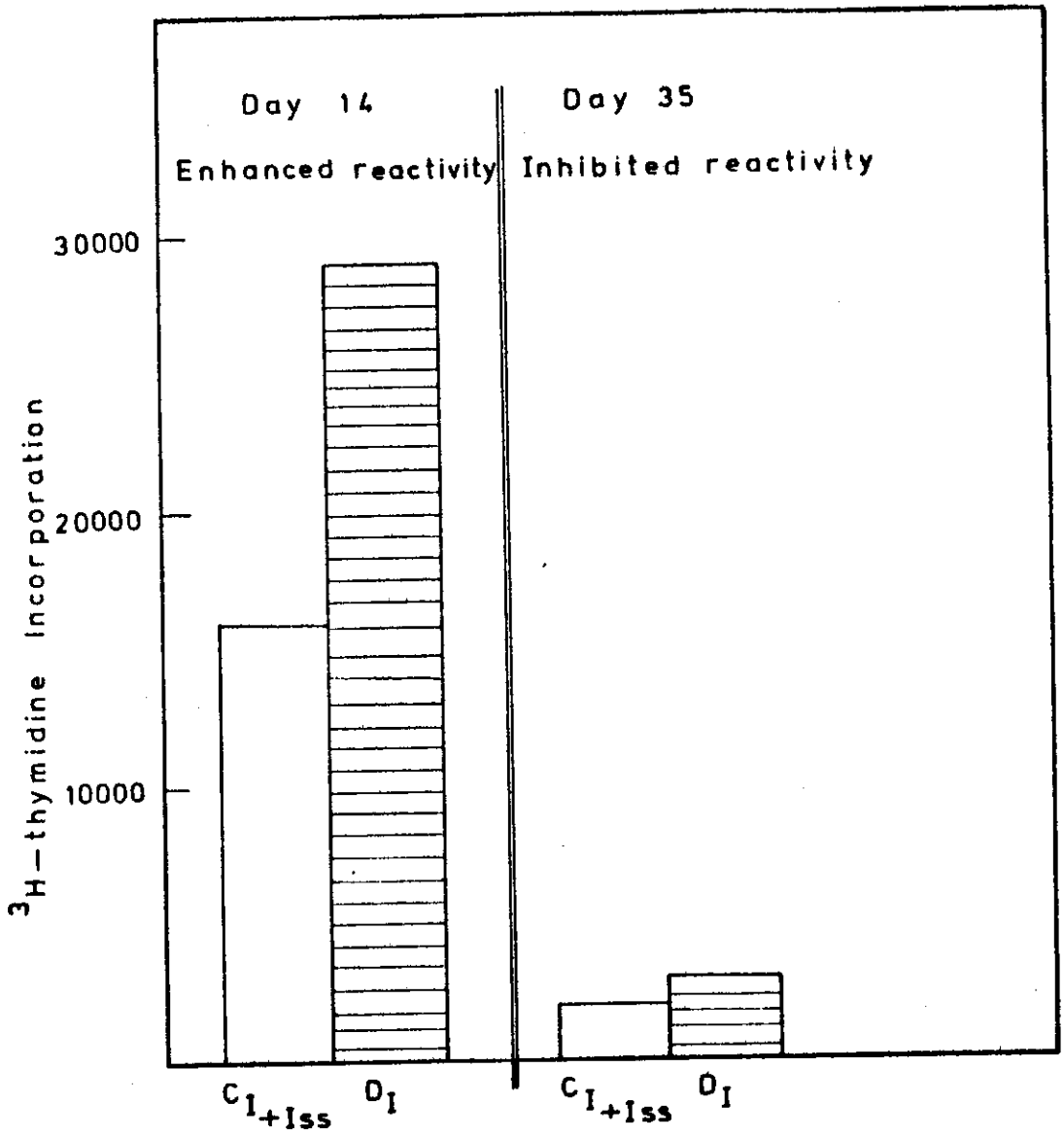
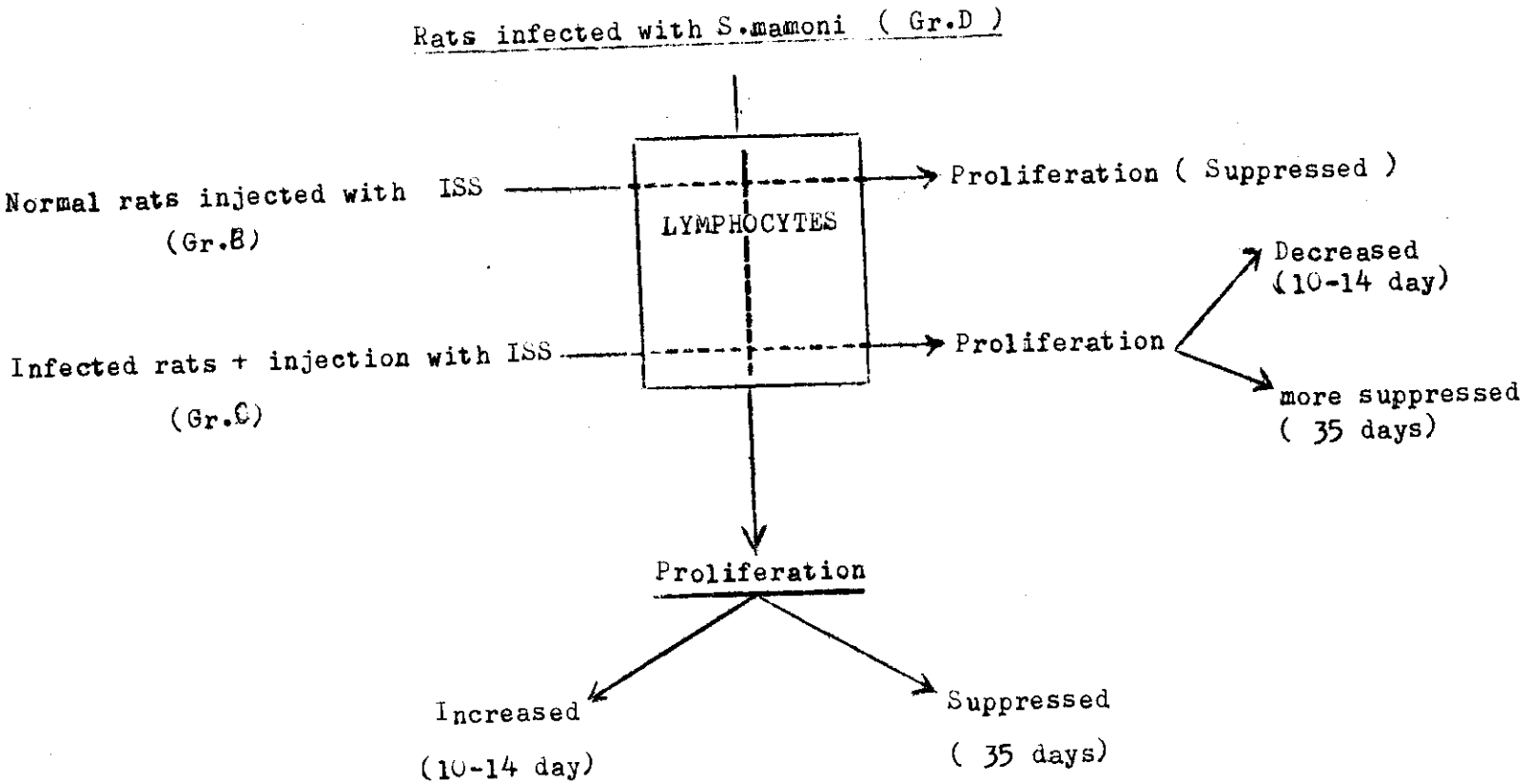


Fig.(6) Specific response of infected rat spleen cells after removing of adherent suppressor cells and cultured with S.mansoni antigen at day 14 and 35.

C_{I+Iss} : Group C: infected with S.mansoni and Iss injected

D_I : Group D: infected with S.mansoni only


Rats infected with S.mamoni (Gr.D)
Normal rats injected with ISS
(Gr.B)
Infected rats + injection with ISS
(Gr.C)
LYMPHOCYTES
Proliferation (Suppressed)
Proliferation
Decreased
(10-14 day)
more suppressed
(35 days)
Proliferation
Increased
(10-14 day)
Suppressed
(35 days)