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CLEARANCE KINETICS OF HETEROLOGOUS ANTIGEN AND ANTIGEN-ANTIBODY COMPLEXES IN *SCHISTOSOMA MANSONI* INFECTED MICE

by

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Summary — The clearance from the circulation of mice of ^{125}I-HSA (human serum albumin) and of high affinity ^{125}-HSA-anti-HSA complexes consisting of 30 per cent 11S and 60 to 70 per cent > 11S complexes was investigated in mice with single-sex and bisexual *S. mansoni* infection and in uninfected control mice.

The removal of radiolabeled Ag and Ag-Ab complexes from the circulation of mice was characterized by three exponential phases. Mice infected with *S. mansoni* showed an impaired clearance of the injected larger (> 11S) complexes which was attributed to the decreased capacity of the liver to clear the complexes from the circulation. Fifteen minutes after injection, a reduction of 66.5 per cent and 76.6 per cent was found in the uptake of preformed complexes per gram of liver tissue in single-sex and bisexually infected mice respectively as compared to uninfected controls. It is likely that the early clearance phase is significantly affected by the intra- and extravascular equilibration process of the injected material. In bisexually infected mice, the early clearance of Ag was found to be more rapid than that of Ag-Ab complexes. Organ localization outside the liver represented a minor fraction of the injected material. The absolute amounts of radioactivity retained by the kidneys at 96 hours after injection were small in each group but significantly higher in mice which had received Ag-Ab complexes than in mice treated with Ag alone.

KEYWORDS : Clearance; Antigen-antibody complexes; *Schistosoma mansoni*.

1. Introduction

Circulating immune complexes (CIC) have been observed in many parasitic diseases. In schistosome infection, the presence of circulating antigen (Ag) and antigen-antibody (Ag-Ab) complexes has been demonstrated in mice (Santoro, Vandemeulebroucke & Capron, 1979), rats (Santoro *et al.*, 1978) and hamsters (Deelder, Van Dalen & Van Egmond, 1978) while Ag-Ab complexes have been observed in man (Hiatt *et al.*, 1980; Lawley *et al.*, 1979). The role of « immune complex formation » is beneficial in facilitating the removal of antigens from the circulation, mainly through the reticulo-endothelial system (RES) of the liver. An excessive quantity of CIC can lead however to saturation of the RES (Haakenstad & Mannik, 1974) resulting in enhanced glomerular deposition of large-latticed (> 11S) soluble IC (Haakenstad, Striker & Mannik, 1982). The role of CIC in the pathogenesis of glomerular disease in experimental schistosomiasis has been studied in mice (Digeon *et al.*, 1979) and hamsters (Carlier, Bout & Capron, 1980).

In a previous study an impaired clearance of preformed and intravenously injected heterologous Ag-Ab complexes (HSA-anti-HSA) has been observed in *S. mansoni* infected mice (Kestens, Van Marck & Gigase, 1983a).

This initial study has been extended in the present work to obtain a more detailed description of the clearance profiles of heterologous Ag and Ag-Ab in *S. mansoni* infected mice.

2. **Material and Methods**

2.1. *Animals*

Female outbred 6 to 8 week old Swiss albino mice were used throughout the experiments.

The mice were subdivided into six groups of 25 to 35 mice each. The mice of groups one and two were injected with Ag (^{125}I-HSA) and Ag-Ab complexes (^{125}I-HSA-anti-HSA) respectively and served as controls. The animals of groups three to six were percutaneously infected with *S. mansoni* (Puerto-Rican strain) cercariae by the ring method. Mice of the third and fourth group were infected with *S. mansoni* worms of a single sex (unisexual infection) for 10 weeks, using an infective dose of 30 cercariae per mouse. The mice of groups five and six were infected with *S. mansoni* worms of both sexes (bisexual infection) for 10 weeks, using 30 cercariae per mouse. Ag alone was injected into the mice of groups three and five while Ag-Ab complexes were injected into the mice of groups four and six. The animals of the six experimental groups were caged, 15 mice per cage and maintained on water and standard pellets for the duration of the experiment. At least 24 hours before the administration of the radiolabeled Ag or Ag-Ab complexes, sodium iodide was added to the drinking water of each cage.

Nine mice of each group were used to study the blood clearance. The same nine animals of groups two, four and six were also used to determine the nonspecific blood activity remaining within the organs as described below.

2.2. *Preparation of the Ag-AB complexes*

The Ag-Ab complexes were prepared using ^{125}I-labeled human serum albumin (^{125}I-HSA, spec. act. 40 μCi/mg, IRE, Belgium) as antigen and goat anti-human serum albumin (GAHu/alb, Nordic Laboratories, the Netherlands) as antibody. The antibodies were characterized by high affinity for Ag ($K = [Ag\text{-}Ab] / [Ag][Ab] = 3.4 \times 10^8$ litres per mole (Kestens *et al.*, 1983a). Ag and Ab dilutions were prepared with borate buffer (0.2 M borate; 0.15 M NaCl; pH 8.3). Ab (6.15 mg/ml) was added to an equal volume of Ag solution (60 μg/ml) to obtain 11S ($\pm$ 30 per cent) and heavier than 11S (60 to 70 per cent) complexes as previously described (Kestens *et al.*, 1983a). The mixture was kept overnight at 4 °C before use. The size distribution of each freshly prepared batch of Ag-Ab complexes was reassessed by linear sucrose density gradient (SDG) ultracentrifugation.

2.3. *Blood clearance studies*

Ag or Ag-Ab complexes were administered to the mice by injecting 0.3 ml of the solution containing 1 mg of protein in a lateral tail vein. Twenty μl blood samples were taken from the retro-orbital plexus at defined intervals after injection (1, 2, 5, 10, 15, 30 min and 1, 2, 5, 8, 24, 48 and 96 h) using calibrated heparinized capillary tubes (Bilbate LTD, England). The blood samples were dispensed in 12 $\times$ 75 mm plastic test tubes containg 1.0 ml of 0.1 M HCl for measurement of the radioactivity in an automatic gamma scintillation counter (Intertechnique CG-4000 provided with a 2 $\times$ 2 inch well type NaI crystal). The radioactivity in the blood at time zero was estimated by nonlinear curve fitting (see statistics) and was considered as 100 per cent of the injected dose.

2.4. *Distribution of radioactivity in the organs*

The mice were sacrificed with an overdose of ether at 15 and 30 min and 1, 2, 5 and 96 h after injection and bled by cardiac puncture. Liver, spleen, kidneys and lungs were removed, weighed and assessed for radioactivity under fixed geometry to avoid count rate differences. The specific activity retained in each organ was determined by subtracting the nonspecific contribution of blood activity remaining, from the total organ activity. The nonspecific activity was determined by injection of ^{51}Cr-labeled autologous red blood cells into the circulation between 30 and 15 min before the mouse was killed. Red cell chromium labeling was accomplished as follows: 20 μl of blood were taken from the orbital plexus of the mouse, dispensed in 1 ml of physiological saline (0.14 M NaCl; pH 7; 4 °C) and washed three times using the same solution. Ten μl radioactive chromium (Na_2 $^{51}CrO_4$, vol. act. 0.5 mCi/ml, IRE, Belgium) were added to 100 μl red cell suspension and incubated at 37 °C for 30 min. After incubation the cells were washed three times with phosphate buffered saline (pH 7.3; 4 °C). The successive manipulations of the red cells were done as gently as possible to avoid deterioration. The blood cells ($\pm$ 10^5 CPM) were injected in 0.2 ml volume in a lateral tail vein of the same mouse they had been taken from.

2.5. *Size determination of remaining circulating complexes*

The size of the complexes was assessed by ultracentrifugation in a linear sucrose density gradient (SDG) (Martin & Ames, 1961). Briefly, 100 μl serum samples were layered on top of a 4 ml borate buffered linear SDG (10-40 per cent). The gradient was centrifuged for 16 h at 100,000 $\times$ G in a swing-out rotor. Fractions of 175 μl were harvested from the top and the radioactivity in each fraction was measured. The examined serum samples of mice had been obtained at sacrifice from 15 min to 5 h after injection.

2.6. *Statistics*

The results obtained from the blood clearance study were further analysed by computer program on an Apple II microcomputer using the

linearization method for nonlinear curve fitting (Draper & Smith, 1966). The program was previously tested by comparison with results obtained from the BMDP P3R nonlinear regression program (1977). Nonlinear correlation coefficients of the regression curves were calculated. The goodness of fit was investigated by comparison of pure error and lack of fit estimates, obtained from repeat observations (Draper & Smith, 1966). Tests of significance were carried out using the two sample t-test of Student, considering a difference as significant when $p < 0.05$.

The confidence intervals indicated on the graphs are the 95 per cent confidence limits obtained by $t_{95} \times$ SEM where the t-value was obtained from the Student's distribution. The values in the tables and on the histogram are given as mean $\pm$ standard deviation.

3. **Results**

The clearance of heterospecific Ag and preformed Ag-Ab complexes from the circulation of mice was studied until 96 h after the intravenous injection. The mathematical analysis of the blood clearance of Ag and Ag-Ab complexes revealed that their removal from the circulation of mice occurred in three exponential phases. The Ag and Ag-Ab clearance could not satisfactorily be described using two exponentials due to a significant lack of fit. By using three exponentials, the lack of fit became negligable as compared to the pure error estimate indicating that the curve fitting was acceptable.

The Ag and Ag-Ab clearance from the circulation of mice can be expressed as follows :

Percentage of the activity remaining in the blood = A1 Exp ($-$ 0.693 t/B1) + A2 Exp ($-$ 0.693 t/B2) + A3 Exp ($-$ 0.693 t/B3).

In this equation the An coefficients represent the proportion of the injected radioactivity cleared from the blood during the respective clearance phases while the Bn coefficients can be considered as the corresponding halflives (Table 1). In each group a nonlinear correlation of 96 per cent or higher between elapsed time after injection and the percentage of remaining activity was observed.

3.1. *Clearance of Ag*

The decay half-life of the injected Ag in the initial exponential clearance phase was about 5 min in the examined groups. The percentage of the injected Ag amount removed from the circulation during this initial phase was similar in the uninfected controls and in mice with a single-sex *S. mansoni* infection but was surprisingly larger in bisexually infected mice (Table 1). As a consequence, an enhanced initial removal of the injected Ag from the circulation of bisexually infected mice as compared to uninfected controls and single-sex infected mice was observed (Fig. 1a). The half-lives (time constants) of the second exponential phase varied between 2.5 and 3 h in the three examined groups but did not differ significantly. Neither did those of the third and final exponential phase

TABLE 1

Clearance of ^{125}I-HSA (Ag) and of ^{125}I-HSA-anti-HSA (Ag-Ab) complexes from the circulation of mice

Experimental groups		1st phase[1]		2nd phase[1]		3rd phase[1]	
		$t^{1/2}$ (min) (B1)	% (A1)	$t^{1/2}$ (hr) (B2)	% (A2)	$t^{1/2}$ (hr) (B3)	% (A3)
Uninfected mice							
1	Ag	6.09 ± 0.89 c	28.90 ± 1.70 c	2.88 ± 0.35 c	30.40 ± 1.80 c	22.20 ± 0.80 c	40.70 ± 1.90 c
2	Ag-Ab	3.09 ± 0.53	43.62 ± 2.89	1.79 ± 0.36	24.32 ± 1.94	24.61 ± 0.80	32.06 ± 1.50
Single-sex infected mice							
3	Ag	4.22 ± 0.31 a	28.77 ± 0.83	2.55 ± 0.29 c	25.66 ± 1.45 a	22.32 ± 0.66	45.57 ± 1.62 a, c
4	Ag-Ab	3.68 ± 1.50	26.61 ± 4.49 a	1.28 ± 0.68	23.78 ± 4.70	21.80 ± 1.33 a	49.61 ± 3.59 a
Bisexually infected mice							
5	Ag	4.45 ± 0.59 a	38.61 ± 2.36 a, c	2.87 ± 0.58 c	23.64 ± 2.23 a	21.11 ± 0.69 c	37.75 ± 2.05 a, c
6	Ag-Ab	4.38 ± 2.14	23.68 ± 4.46 a	1.15 ± 0.45 b	21.09 ± 4.01 b	19.20 ± 0.80 a	55.24 ± 1.92 a

[1] Each exponential clearance phase is represented as the percentage of the injected activity cleared from the circulation with decay half-life $t^{1/2}$. The number of mice was the same in each group (n = 9). The values are given as mean ± SD.

— Values marked with a and b differ significantly from the values of the corresponding control groups (uninfected mice).
a = $p < 0.001$; b = $p < 0.05$.

— values marked with c and d indicate significant differences between Ag and Ag-Ab clearance in mice of corresponding groups.
c = $p < 0.001$; d = $p < 0.05$.

which approximated to 22 h. The quantities of Ag leaving the circulation during the second and the third exponential phase were variable but in such a manner that the differences of the initial clearance phase between the examined groups gradually disappeared.

3.2. *Clearance of Ag-Ab complexes*

The initial clearance of the injected Ag-Ab complexes from the circulation of mice is presented in Figure 1b. A significantly decreased initial clearance of the injected material was observed in the *S. mansoni* infected mice as compared to the uninfected controls. These differences are quantified in Table 1. The percentages of the injected Ag-Ab complexes removed from the circulation during the first exponential phase are seen to be significantly smaller in *S. mansoni* infected mice than in uninfected controls whereas their corresponding time constants are comparable. In

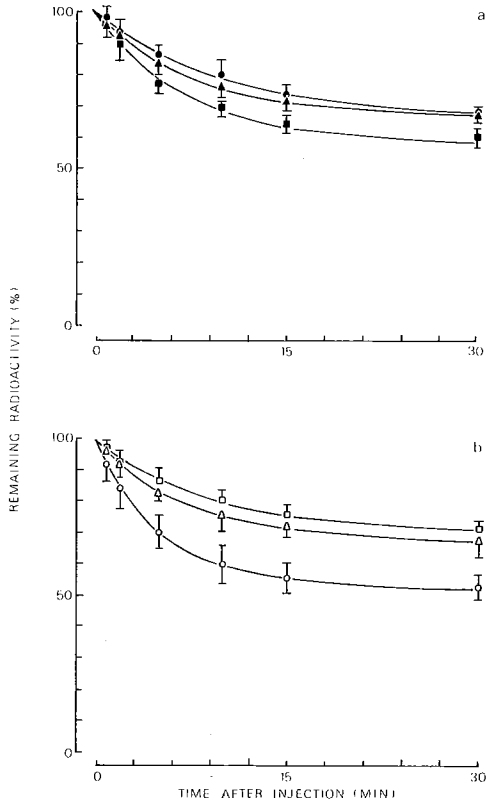

Figure 1.

Initial ^{125}I-HSA (a) and ^{125}I-HSA-anti-HSA (b) clearance from the circulation of mice with a 10 week old single-sex *S. mansoni* infection (30 cerc./mouse), (△), of mice with a 10 week old bisexual *S. mansoni* infection (30 cerc./mouse), (□), and of uninfected controls (O). The statistical limits indicated on the graphs are the 95 % confidence intervals calculated with the t_{95}-value of the Student's t-distribution.

the second clearance phase, time constant and associated percentage were found to be smaller in infected mice than in the uninfected controls, but the difference was significantly only for the bisexually infected group. In the final (third) clearance phase, time constants and corresponding percentages in both *S. mansoni* infected groups differed significantly from those of the controls.

The initial removal of the injected Ag-Ab complexes from the circulation of uninfected control mice was more pronounced than when Ag alone was injected (Fig. 2a). In mice with a single-sex infection however, the initial clearance of the injected Ag-Ab complexes was almost identical to that of the injected Ag (Fig. 2c). In bisexually infected mice, the Ag clearance was more marked than the initial Ag-Ab clearance (Fig. 2e). The entire clearance profiles of the injected Ag and Ag-Ab complexes are given in the Figures 2b, d and f. When present, the differences between both Ag and Ag-Ab disappearance curves gradually decreased during the final clearance phase.

3.3 *Size distribution analysis of the remaining circulating complexes*

The results were essentially the same as those from preliminary studies (Kestens *et al.*, 1983a). Briefly, SDG analysis of serum samples from mice of various groups, 15 min after injection of the Ag-Ab complexes, revealed a significantly decreased removal of $>11S$ complexes in *S. mansoni* infected mice as compared to the uninfected controls whereas the initial clearance of 11S complexes was comparable in all groups. The significant difference in size of the remaining circulating complexes among the examined groups observed at 15 min after injection became smaller with time but was still obvious at 5 h.

3.4. *Tissue localization of the injected Ag and Ag-Ab complexes*

Of all the organs studied, the liver was found to retain the largest amount of the injected activity in all groups with the only exception of the mice with Ag-Ab complexes examined at 96 h in which the kidneys had accumulated slightly more activity than the liver (Tables 2, 3 and 4). The percentage of the injected activity (corrected for the remaining blood activity) trapped in the liver was also calculated per gram of tissue (Fig. 3) to study the effect of the significant hepatomegaly (increase of 58 $\pm$ SD 11 per cent) in bisexually infected mice on the blood clearance of the injected material. It confirmed that the initial uptake of the injected Ag-Ab complexes by the liver was significantly reduced in *S. mansoni* infected mice as compared to the uninfected controls. A reduction of the global clearance capacity of the liver of 60.0 $\pm$ SD 8.5 per cent in the case of a single-sex infection and 64.0 $\pm$ SD 7.8 per cent in the case of a bisexual infection was found 15 min after injection, increasing to 66.5 $\pm$ SD 8.1 per cent and 76.6 $\pm$ SD 5.0 per cent respectively when the clearance capacity of the liver was calculated per gram of tissue. The difference in Ag-Ab uptake between the livers of *S. mansoni* infected mice and those of uninfected controls decreased with time and became insignificant at 5 h after injection.

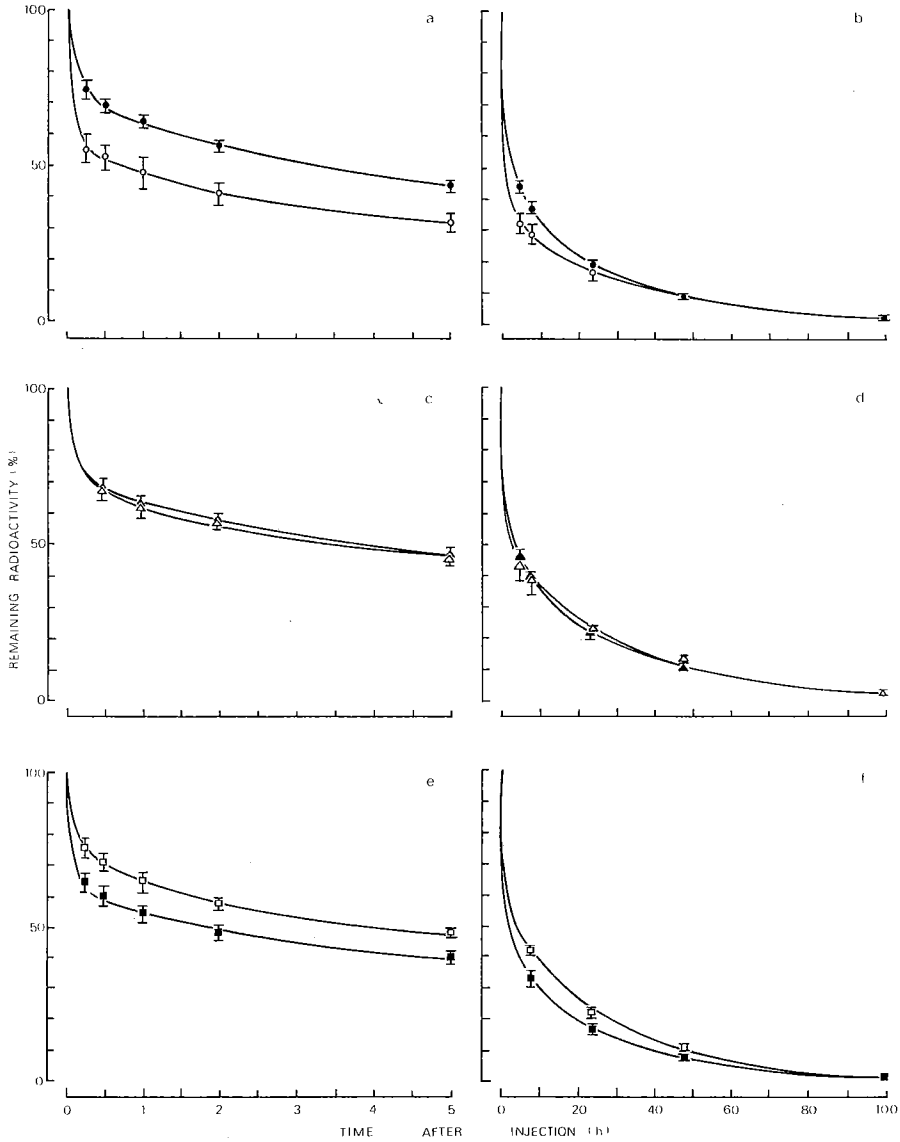

Figure 2.

Comparison of early and total ^{125}I-HSA (black symbols) and ^{125}I-HSA-anti-HSA (white symbols) clearance from the circulation of uninfected control mice (a and b), of mice with a 10 week old single-sex *S. mansoni* infection (30 cerc./mouse) (c and d) and of mice with a 10 week old bisexual *S. mansoni* infection (30 cer./mouse) (e and f). The statistical limits indicated on the graphs are the 95 % confidence intervals calculated with the t_{95}-value of the Student's t-distribution.

TABLE 2

Relative tissue distribution of the injected ^{125}I-HSA (Ag) and ^{125}I-HSA-anti-HSA (Ag-Ab) complexes in uninfected control mice

	Time after injection					
	15 min	30 min	1 hr	2 hr	5 hr	96 hr
Ag	(n = 3)[d]	(n = 3)	(n = 3)	(n = 2)	(n = 3)	(n = 3)
Liver[a]	4.72 ± 0.45	3.94 ± 0.65	3.76 ± 0.42	3.38 ± 0.78	2.25 ± 0.22	0.12 ± 0.03
Spleen[a]	0.115 ± 0.052	0.097 ± 0.037	0.18 ± 0.02	0.15 ± 0.01	0.015 ± 0.026	0.004 ± 0.004
Lungs[a]	0.20 ± 0.07	0.32 ± 0.03	0.41 ± 0.07	0.48 ± 0.06	0.25 ± 0.10	0.025 ± 0.013
Kidneys[a]	1.70 ± 0.43	1.46 ± 0.21	1.52 ± 0.06	1.29 ± 0.05	0.82 ± 0.13	0.051 ± 0.012
Blood[b]	74.3 ± 0.8	68.0 ± 0.5	63.4 ± 0.5	57.0 ± 0.5	43.9 ± 0.6	2.06 ± 0.15
Total[c]	81.0 ± 1.8	73.8 ± 1.4	69.3 ± 1.1	62.3 ± 0.5	47.2 ± 1.1	2.26 ± 0.21
Ag - Ab	(n = 6)	(n = 6)	(n = 3)	(n = 3)	(n = 3)	(n = 9)
Liver[a]	28.7 ± 4.5	19.6 ± 2.3	11.2 ± 1.1	5.57 ± 0.39	3.01 ± 0.31	0.21 ± 0.018
Spleen[a]	0.23 ± 0.10	0.56 ± 0.10	0.36 ± 0.11	0.11 ± 0.01	0.06 ± 0.06	0.12 ± 0.07
Lungs[a]	0.17 ± 0.17	0.074 ± 0.052	0.13 ± 0.11	0.13 ± 0.07	0.13 ± 0.12	0.021 ± 0.018
Kidneys[a]	1.49 ± 0.24	1.44 ± 0.49	1.40 ± 0.21	1.14 ± 0.20	0.60 ± 0.22	0.29 ± 0.13
Blood[b]	55.4 ± 1.0	51.7 ± 1.0	47.7 ± 0.8	41.5 ± 0.9	31.4 ± 0.7	2.21 ± 0.12
Total[c]	86.0 ± 6.0	73.3 ± 3.9	60.8 ± 2.3	48.6 ± 1.6	35.2 ± 1.4	2.85 ± 0.41

[a] = Percent of the injected activity recovered in the whole organ ± SD.
[b] = Total remaining blood activity obtained from the clearance curve fitting ± SD (n = 9).
[c] = Sum of the recovered activity in the examined organs and total blood.
[d] = Number of mice used.

TABLE 3

Relative tissue distribution of the injected ^{125}I-HSA (Ag) and ^{125}I-HSA-anti-HSA (Ag-Ab) complexes in mice with a single-sex *S. mansoni* infection

	Time after injection					
	15 min	30 min	1 hr	2 hr	5 hr	96 hr
Ag	(n = 6)[d]	(n = 6)	(n = 6)	(n = 3)	(n = 3)	(n = 9)
Liver[a]	5.65 ± 0.62	4.37 ± 0.31	3.26 ± 0.75	3.16 ± 0.32	2.27 ± 0.24	0.14 ± 0.06
Spleen[a]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lungs[a]	0.22 ± 0.12	0.23 ± 0.17	0.41 ± 0.25	0.06 ± 0.10	0.19 ± 0.05	0.026 ± 0.018
Kidneys[a]	1.49 ± 0.18	1.35 ± 0.28	1.04 ± 0.29	1.12 ± 0.39	0.87 ± 0.04	0.059 ± 0.02
Blood[b]	71.7 ± 0.3	67.5 ± 0.1	63.7 ± 0.2	57.7 ± 0.3	45.6 ± 0.4	2.38 ± 0.14
Total[c]	79.1 ± 1.2	73.5 ± 0.9	68.4 ± 1.5	62.0 ± 1.1	48.9 ± 0.7	2.61 ± 0.24
Ag - Ab	(n = 6)[d]	(n = 5)	(n = 3)	(n = 3)	(n = 3)	(n = 9)
Liver[a]	11.46 ± 1.65	10.10 ± 1.95	7.10 ± 1.27	4.55 ± 0.84	3.74 ± 1.07	0.21 ± 0.07
Spleen[a]	0.44 ± 0.30	0.66 ± 0.24	0.57 ± 0.04	0.29 ± 0.06	0.29 ± 0.09	0.19 ± 0.07
Lungs[a]	0.10 ± 0.10	0.06 ± 0.05	0.05 ± 0.05	0.08 ± 0.05	0.18 ± 0.04	0.021 ± 0.014
Kidneys[a]	1.72 ± 0.27	1.35 ± 0.28	1.16 ± 0.07	N.D.[e]	1.17 ± 0.28	0.39 ± 0.12
Blood[b]	71.6 ± 1.6	67.1 ± 1.9	61.9 ± 1.8	54.6 ± 2.5	43.9 ± 1.8	2.42 ± 0.32
Total[c]	85.3 ± 3.9	79.3 ± 4.4	70.8 ± 3.2	59.5 ± 3.5	49.3 ± 3.3	3.23 ± 0.59

[a] = Percent of the injected activity recovered in the whole organ ± SD.
[b] = Total remaining blood activity obtained from the clearance curve fitting ± SD (n = 9).
[c] = Sum of the recovered activity in the examined organs and total blood.
[d] = Number of mice used.
[e] = Not done.

TABLE 4

Relative tissue distribution of the injected ^{125}I-HSA (Ag) and ^{125}I-HSA-anti-HSA (Ag-Ab) complexes in mice with a bisexual *S. mansoni* infection

	Time after injection					
	15 min	30 min	1 hr	2 hr	5 hr	96 hr
Ag	(n = 6)[d]	(n = 5)	(n = 6)	(n = 3)	(n = 3)	(n = 5)
Liver[a]	11.73 ± 1.32	9.68 ± 1.37	9.46 ± 1.49	6.89 ± 0.66	5.05 ± 1.49	0.23 ± 0.04
Spleen[a]	0.50 ± 0.36	0.45 ± 0.30	0.29 ± 0.29	0.13 ± 0.07	0.11 ± 0.10	0.014 ± 0.006
Lungs[a]	0.12 ± 0.12	0.11 ± 0.11	0.06 ± 0.05	0.21 ± 0.13	0.06 ± 0.11	0.016 ± 0.003
Kidneys[a]	1.21 ± 0.37	1.22 ± 0.23	0.76 ± 0.23	0.72 ± 0.11	0.59 ± 0.18	0.067 ± 0.005
Blood[b]	63.4 ± 0.7	58.5 ± 1.0	55.1 ± 0.8	49.9 ± 0.7	39.1 ± 0.9	1.67 ± 0.10
Total[c]	77.0 ± 2.9	70.0 ± 3.0	65.7 ± 2.9	57.9 ± 1.7	44.9 ± 2.78	2.00 ± 0.15
Ag - Ab	(n = 5)	(n = 6)	(n = 3)	(n = 3)	(n = 3)	(n = 5)
Liver[a]	10.31 ± 1.52	7.96 ± 0.56	7.71 ± 0.39	6.84 ± 2.12	4.71 ± 1.34	0.25 ± 0.09
Spleen[a]	0.40 ± 0.35	0.62 ± 0.23	0.91 ± 0.49	0.39 ± 0.17	0.26 ± 0.26	0.11 ± 0.04
Lungs[a]	0.08 ± 0.10	0.17 ± 0.10	0.25 ± 0.12	0.08 ± 0.03	0.12 ± 0.07	0.026 ± 0.011
Kidneys[a]	1.35 ± 0.28	1.25 ± 0.19	1.00 ± 0.12	1.06 ± 0.02	0.93 ± 0.11	0.26 ± 0.12
Blood[b]	75.0 ± 1.4	69.9 ± 1.7	64.7 ± 1.3	57.7 ± 1.3	47.1 ± 0.8	1.79 ± 0.21
Total[c]	87.1 ± 3.7	79.9 ± 2.8	74.6 ± 2.4	66.1 ± 3.6	53.1 ± 2.6	2.44 ± 0.47

[a] = Percent of the injected activity recovered in the whole organ ± SD.
[b] = Total remaining blood activity obtained from the clearance curve fitting ± SD (n = 9).
[c] = Sum of the recovered activity in the examined organs and total blood.
[d] = Number of mice used.

From 15 min to 5 h after injection of the Ag in bisexually infected mice, the percentage of the injected activity retained by the liver, even after correction for remaining blood activity, was significantly larger than in single-sex infected mice and uninfected controls (Fig. 3).

The accumulation of the injected activity in the lungs was small in each group at all times and never exceeded 0.3 per cent of the injected dose of Ag-Ab complexes and 0.5 per cent of the injected dose of Ag alone. A maximum of 1 per cent of the injected activity was captured in the spleen after injection of Ag-Ab complexes and no more than 0.5 per cent when Ag alone was injected. The spleens of single-sex infected mice did not retain detectable amounts of Ag at the examined time periods. Less than 2 per cent of the injected activity was found in the kidneys with only minor differences between the groups. Though the absolute amounts of activity retained in the kidneys at 96 h after injection were small in each group, the mice which had received Ag-Ab complexes accumulated significantly more tracer than when Ag alone was injected.

4. **Discussion**

The present study describes and quantifies the modified clearance of heterospecific Ag and Ag-Ab complexes in *S. mansoni* infected mice with reference to uninfected controls. In agreement with earlier studies on rabbits (Mannik *et al.*, 1971) and mice (Haakenstad & Mannik, 1974), we found that Ag-Ab complexes were eliminated from the circulation in three exponential phases. According to those authors, the initial phase was associated with the clearance of the larger (> 11S) complexes whereas the second and the third phase were thought to represent the equilibration and catabolic phases respectively. Although Ag clearance was described as biphasic, our data strongly suggest that Ag clearance develops also in three exponential phases. SDG analysis of the injected Ag preparation always revealed a sharp peak at 4.6 S.. The possibility that aggregated antigen had been injected could therefore be excluded. The first two blood samples, 1 and 2 min respectively after injection, appeared to be very important to determine the presence of an initial clearance phase with an half-life of only a few minutes. The possibility of overlooking the existence of such components must therefore be considered when blood sampling is not started immediately after injection. The fact that Ag alone is also cleared in three phases suggests that the early clearance phase does not solely reflect the disappearance of > 11S complexes from the circulation but also includes the equilibration of the injected material between the intra- and extravascular spaces. In this equilibration process the clearance of Ag might be more rapid because of its smaller molecular size. The total recovery of activity after 15 min in the groups where Ag had been injected, tended indeed to be smaller than in the groups with Ag-Ab complexes. Which of those mechanisms, the preferential and fast uptake of > 11S complexes or the accelerated disappearance of the small sized Ag in the equilibration process is more important in the early clearance depends on several factors. In uninfected controls, the early clearance is probably mainly determined by the presence

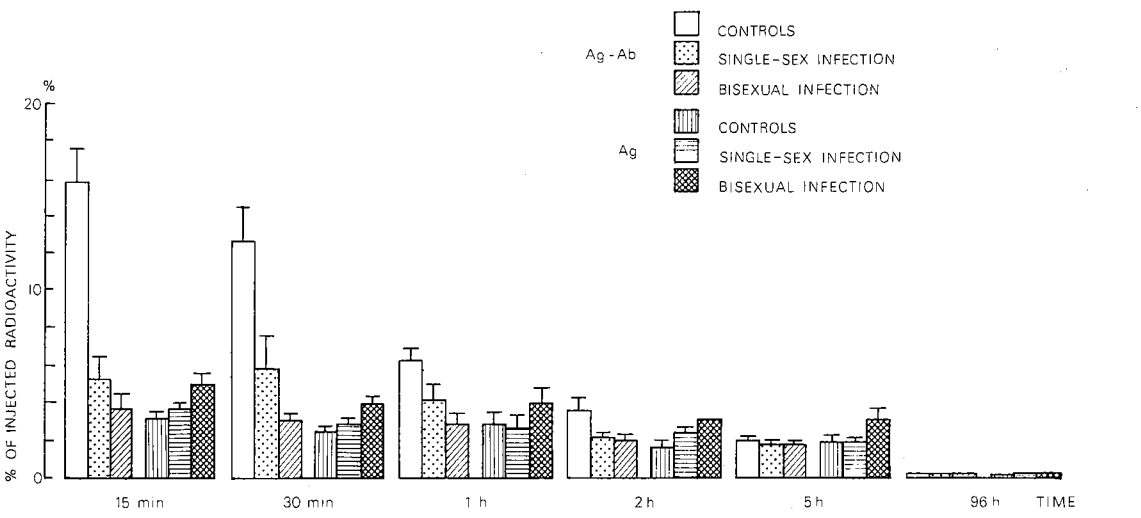

Figure 3.

Uptake of ^{125}I-HSA-anti-HSA complexes (Ag-Ab) and ^{125}I-HSA (Ag) per gram mouse liver tissue ± SD in uninfected controls, in 10 week old single-sex *S. mansoni* infected mice (30 cerc./mouse) and in 10 week old bisexually *S. mansoni* infected mice (30 cerc./mouse).

of > 11S complexes so that the clearance of Ag-Ab complexes is more important than the early clearance of Ag. In single-sex infected mice we might assume that both mechanisms were in balance while in bisexually infected mice the equilibration process of the injected Ag was more important than the clearance of the injected complexes. In our experiments, 1 mg of protein was injected which is still less than the saturation dose (Haakenstad & Mannik, 1974). An increase of the injected dose beyond the saturation dose could result not only in an impaired clearance of > 11S complexes but also in an initial removal of small-latticed (11S) complexes that exceeds that of large-latticed (> 11S) complexes (Haakenstad *et al.*, 1982).

The impaired clearance of > 11S complexes in *S. mansoni* infected mice mentioned in a previous paper (Kestens *et al.*, 1983a) has been confirmed by the present study. It suggests, together with the decreased liver uptake of > 11S complexes in infected mice, that the mechanisms which mediate the uptake of complexes by the liver have become less effective in a single-sex infection and ineffective in a bisexual infection. We assume that this state of ineffectiveness was reached when Ag-Ab uptake by the liver decreased to the level of Ag uptake in the corresponding group. In rats, a reduction or even loss of Fc and C3 receptor activity on the Kupffer cells, has been observed following the injection of heterospecific Ag-Ab complexes (Nishi *et al.*, 1981). The importance of complement and C3 receptors in the Ag-Ab removal from the circulation seems however questionable since earlier studies in rabbits (Arend & Mannik, 1971) and mice (Miller *et al.*, 1975) using also heterospecific Ag-Ab complexes indicated that their clearance was not affected by complement depletion. Haakenstad and Mannik (1974) suggested that the impairment of liver RES after HSA-anti-HSA injection was caused by saturation of IgG receptors on Kupffer cells. It is most likely that the decreased clearance of such complexes in *S. mansoni* infected mice results from saturation of these receptor sites e.g. by immune complexes derived from parasite antigens and host antibodies. The presence of parasite antigen in Kupffer cells of *S. mansoni* infected mice has indeed been demonstrated (Van Marck, 1975; Deelder *et al.*, 1980) and in an immunofluorescent study (Kestens, Van Marck & Gigase, 1983b) we were able to demonstrate an impaired uptake of i.v. injected HSA-anti-HSA complexes by Kupffer cells of *S. mansoni* infected mice. Since the hepatic uptake of large-latticed immune complexes has been found to alter the mononuclear phagocyte function in contrast with small-latticed complexes (Jimenez, Haakenstad & Mannik, 1983), the decreased liver uptake of the injected Ag-Ab complexes in *S. mansoni* infected mice is probably the result of liver RES saturation by large-latticed immune complexes.

Some factors, determining the deposition and localization of Ag-Ab complexes in the renal glomerulae have already been described. Koyama *et al.* (1978) demonstrated that high and moderate antibody affinity for antigen favoured the glomerular deposition of Ag-Ab complexes. More recently, Haakenstad *et al.* (1982) demonstrated that large-latticed circulating complexes lead to glomerular deposition whereas small-latticed complexes do not. Our findings indicate on the other hand that the clearance of high affinity > 11S complexes from the circulation of *S. mansoni* infected

mice is impaired. More insight on the pathogenic role of circulating complexes might therefore be obtained from future clearance studies of large-latticed complexes in parasitic infections, e.g. schistosomiasis.

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Clearance d'antigènes étrangers et de complexes antigènes-anticorps hétérospécifiques chez la souris infectée par *Schistosoma mansoni*.

Résumé — La clearance d'albumine sérique humaine marquée (^{125}I-HSA) et de complexes albumine humaine — anti albumine humaine de chèvre, obtenus in vitro et injectés par voie i.v. a été étudiée chez les souris porteuses d'infection unisexuée ou bisexuée à *S. mansoni* et chez des contrôles non-infectés. Ces complexes de haute affinité sont constitués pour 60 à 70 p. cent de complexes lourds (> 11S).

La disparition de l'Ag et des complexes Ag-Ab se déroule en trois phases exponentielles. Une clearance réduite de complexes lourds (> 11S) a été observée chez les souris infectées. Le captage de complexes par gramme de tissu hépatique était significativement réduit chez les souris infectées par rapport aux témoins. Cette première phase de la clearance est probablement influencée par les processus d'équilibration intra- et extravasculaire du matériel injecté. La clearance précoce de l'antigène était plus rapide que celle des complexes Ag-Ab chez les souris porteuses d'infection bisexuée. La rétention de complexes Ag-Ab en dehors du foie ne représentait qu'une petite fraction du matériel injecté. Les quantités de matériel radio-actif retenues par les reins 96 heures après l'injection étaient toujours minimes, mais néanmoins plus importantes après injection de complexes que d'antigènes seules dans les trois groupes.

Clearance kinetiek van heterospecifieke antigeen en antigeen-lichaam complexen in muizen besmet met *Schistosoma mansoni*.

Samenvatting — Antigeen (^{125}I-HSA) en antigeen-antilichaam complexen (^{125}I-HSA-anti-HSA) bestaande uit 30 ten honderd lichte (11S) en 60 tot 70 ten honderd zware (> 11S) complexen werden in vitro bereid en intraveneus ingespoten bij muizen. De clearance van antigeen en antigeen-antilichaam complexen werd bestudeerd bij muizen die unisexueel en bisexueel besmet waren met *S. mansoni* en bij onbesmette controle dieren.

Het verdwijnen uit de perifere bloedsomloop van zowel Ag als Ag-Ab complexen verliep in drie exponentiële fasen. In muizen besmet met *S. mansoni* werd een vertraagde clearance waargenomen van de zwaardere (> 11S) complexen die geassocieerd werd met een verlaagde clearance capaciteit van de lever. Vijftien minuten na injectie werd een reductie van respectievelijk 66,5 ten honderd en 76,6 ten honderd gevonden van accumulatie van de ingespoten complexen per gram leverweefsel in unisexueel en bisexueel besmette muizen en dit vergeleken met de onbesmette controles. Het is aanneembaar dat de initiële clearance fase sterk beïnvloed wordt door het intra- en extravasculaire equilibratieproces van het ingespoten materiaal. In bisexueel besmette muizen verliep de initiële clearance van het antigeen sneller dan deze van antigeen-antilichaam complexen. De hoeveelheden van het ingespoten materiaal die teruggevonden werden buiten de lever waren in verhouding onbelangrijk. De absolute hoeveelheden die teruggevonden werden in de nieren 96 uur na injectie waren klein maar nochtans significant hoger in muizen ingespoten met antigeen-antilichaam complexen en dit vergeleken met muizen ingespoten met Ag alleen.

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