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Short Persistence of Trichomonas Vaginalis in Reinfected Immune Mice. (20129)

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The chronic tissue infection of mice with *Trichomonas vaginalis*(1) produced by intramuscular injection of the parasites into the hindleg was found(2) to render the animals immune to a reinfection into the leg muscles of the opposite body side. A marked degree of immunity was observed in 70-100% of the animals for periods up to 10 weeks during which the primary lesion persisted. No anatomical lesions and no parasites were found at the site of reinfection in protected animals examined 12 days after the reinoculation. Experiments of this type seemed suitable also to study the fate of the parasites during the early phases of the reinfection. Technically this was facilitated by the strictly localized character of the infection of mice with *T. vaginalis* and because the fate of the parasites could be followed both by microscopical examination and by culture. The results obtained in a group of reinfection experiments are given in this report.

Method. The technic used in the present experiments corresponded closely to that described earlier(2): Adult white mice, from one colony, were infected intramuscularly in the left hindleg with 500,000 parasites contained in 0.5 ml of an overnight culture of *T. vaginalis* in CPLM medium(3). Three to 4 weeks later when, according to earlier experience, a high degree of immunity was present, the immunized mice were reinfected with the same dose of *T. vaginalis* into the muscles of the right hindleg. A few drops of sterile India Ink were added to the culture dilution in order to mark the site of the infection in the tissues. Groups of normal mice received the same infection. At different intervals after the infection, 4 hours, 8 hours, 1 day, 3, 6, 7, 8, and 10 days, groups of 5 to 10 immune and normal mice were sacrificed and the site of the infection was examined microscopically for the presence or absence of motile trichomonads. Cultures in CPLM medium were taken at the same time from the tissues of both the immune and normal groups and examined after 48 hours incubation. The primary lesions of the immunized mice were also examined.

Experimental. A series of 5 experiments, all giving comparable results, was carried out. The data, which are given in Table I, are based on 2 experiments for the intervals of 4 hours and 1 day and on 3-4 experiments for the remaining intervals of 8 hours, 3, 6-8, and 10 days.

All mice of the immune groups were carriers of large abscesses at the site of the primary immunizing infection. The pus of these abscesses contained numerous active parasites which grew abundantly in culture. The fate of the parasites of the reinfection is indicated by the outcome of the cultures taken at the different intervals after the reinfection. In groups no. 2 to 6, covering the intervals from 8 hours to 10 days, the majority of the immune mice did not contain viable parasites at the site of the reinfection. This is demonstrated by the fact that 70-100% of the cul-

TABLE I. Fate of Reinfection with *T. vaginalis* in Muscles of Immune and Normal Mice.
Immunization: 500000 parasites intramusc. left hindleg
Reinfection: 500000 parasites intramusc. right hindleg
Interval between 1st and 2nd infection: 21–29 days
Autopsy: 4 hr to 10 days after reinfection

Group No.*	Interval, days†	No. of mice	Parasites of reinfection			
			Motility	No. of cultures		% negative cultures
				Negative	Positive	
1 I	.17	20	0	4	16	20
1 N	.17	20	0	0	20	0
2 I	.33	30	0	22	8	73.0
2 N	.33	30	0	9	21	30.0
3 I	1.0	20	0	20	0	100.0
3 N	1.0	20	±‡	0	20	0
4 I	3.0	25	0	21	4	84.0
4 N	3.0	30	+	2	28	6.7
5 I	6–8	33	0	27	6	82.0
5 N	6–8	35	+	2	33	5.7
6 I	10.0	20	0	20	0	100.0
6 N	10.0	25	+	0	25	0

* I = immune group; N = normal group.
† Between reinfection and autopsy.
‡ ± signifies that 50% of the normal mice contained motile parasites.

tures failed to show growth of the parasites while in the corresponding groups of normal control animals trichomonads were recovered by culture in the majority of cases. The differences between immune and normal groups were in all these instances significant with 'p' values of 0.01 and less. In the group 1 I, however, sacrificed and examined 4 hours after the reinfection, viable trichomonads were demonstrated in 16 out of 20 mice and the difference between the immune group and the normal group was not significant.

The observation of motility of *T. vaginalis* in direct smears from the infected tissues had essentially the same result, but this method had the disadvantage that marked differences of the immune and normal groups could only be noted after 24 hours and later because motile parasites were also not detected in the smears of the normal controls during the first 8 hours. Marked anatomical lesions could only be expected in the groups 6 I and 6 N examined 10 days after the reinfection. In accordance with earlier observations parasitized abscesses were found in the control group, but were absent in the group of immune mice.

Discussion. The experiments seemed to indicate that the immunity which accompanies the chronic intramuscular infection of mice with *T. vaginalis* exerted an appreciable effect on the persistence of the parasites at the site of the reinfection. The inoculum survived in the tissues for 4 hours but parasites could not be recovered in the majority of instances after 8 hours. The disappearance of viable parasites from the site of the reinfection was a permanent one; the percentage of negative cultures remained on the high level of 70-100% for the entire period of observation up to 10 days after the reinfection. This frequency of immunity under the conditions of this type of active immunization is in good agreement with our earlier observations(2) which were based on the absence of parasitized abscesses in reinfected immune animals.

The findings confirm the general experience in protozoan immunity in which, according to Culbertson(4), the persistence of parasites in immune animals is considerably shortened. In case of *T. foetus* it was shown by Nelson(5) that in experimentally immunized rabbits the duration of a vaginal reinfection was reduced from 23 days to 2 days. The intramuscular trichomonad infection of mice which may persist in normal animals for 12 weeks and longer (1) seemed to be limited in immune animals to a period between 4 to 8 hours.

Summary. In mice immunized by intramuscular infection with live *T. vaginalis* the parasites of a homologous reinfection were found to survive in the tissues of the host for

4 hours. At later intervals from 8 hours to 10 days after the reinfection, viable parasites could no longer be recovered by culture from the majority of animals.

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Alpha Cell Damage and Blood Sugar Changes in Rabbits after Administration of Cobalt.* (20130)

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In a preliminary report it has been shown by us that in the rabbit a single intravenous injection of cobaltous chloride causes both transitory hyperglycemia and severe selective damage to the pancreatic alpha cells(1). Although the blood sugar returned to the pretreatment level within a few hours after cobalt administration, the alpha cell damage was still present after 48 hours. Despite these persistent anatomical changes, a second dose of cobaltous chloride administered at 48 hours induced a second hyperglycemic episode. These observations suggest two possible mechanisms for the action of cobalt. On the one hand it might be assumed that the alpha cells on being damaged by cobaltous chloride released a factor with hyperglycemic properties. This hypothesis would be in conformity with numerous reports which give evidence for the presence of a hyperglycemic glycogenolytic factor (HGF) in the pancreas, and which suggest that the alpha cells are the site of its production(2-4). The absence of a persistent hypoglycemia after the initial hyperglycemia and the repeated hyperglycemic episodes elicited by additional injections of cobaltous chloride may indicate simply that alpha cells sufficient to maintain blood sugar homeostasis and to cause a hyperglycemic phase on readministration of cobaltous chloride have escaped the damaging effects of the initial injection. On the other hand, the alternate hypothesis has to be considered that the alpha cell damage and the hyperglycemic episodes are independent effects of cobaltous chloride, the latter being of extrapancreatic origin. The experiments to be reported in this paper were undertaken to study the interrelationship between the anatomical and functional changes caused by cobaltous chloride.

Material and methods. Cobaltous chloride as 0.5% solution in saline was administered intravenously to normal and alloxan diabetic rabbits of both sexes weighing from 2000 to 3000 g. Alloxan diabetes was produced by the intravenous injection of 100 mg/kilo of alloxan monohydrate (Eastman). Those animals which maintained blood sugar levels over 300 mg for at least one week were utilized. Blood samples for glucose determination by the Nelson modification of the Folin-Wu Micro Method(5) were withdrawn from the marginal ear vein. Animals were sacrificed by intracardiac air embolization and portions of the pancreas and liver were removed for histological examination, which included formalin fixed paraffin sections of both organs stained with Haematoxylin and Eosin, Bouin fixed pancreas prepared by Gomori's Method (6), and alcohol fixed liver stained by the MacManus technique(7).

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