

(50 ml) was applied to a Sephadex G-75 column (5 × 80 cm) after the addition of $^{113}\text{Cd}^{2+}$ (1.5 mg) to replace zinc in MT and eluted with Tris buffer solution (10 mM, pH 8.6). The MT fractions (monitored by atomic absorption analysis of cadmium) were combined and concentrated by ultrafiltration on a Diaflo UM-10 membrane. The concentrated solution was then put in a DEAE Sephadex A-25 column (1.5 × 28 cm) to separate the 2 forms (MT-I and MT-II) by gradient elution with Tris buffer (pH 8.6) between 10 mM (100 ml) and 300 mM (400 ml). The separated MT-I and MT-II were concentrated to about 1 ml as above. The contents of cadmium, zinc, and copper in MT-I and MT-II were: 2.48, 7.9×10^{-3} and 44.8×10^{-3} mg/ml; 3.43, 27.2×10^{-3} and 10.7×10^{-3} mg/ml, respectively.

Results and discussion. Cadmium-113 high resolution NMR-spectra of each MT in 10 mm NMR-tube are shown in figures 1 and 2. Cadmium-113 peaks were observed between 610 and 670 ppm downfield from aqueous $\text{Cd}(\text{ClO}_4)_2$ solution (0.1 M). The chemical shifts were in accordance with the reported shifts of cadmium-sulfur complexes and indicated that cadmium in MT was coordinated with mercapto groups⁷. The number of signals can be summarized as follows: MT-I, 614.9 (1), 624.7 (2), 648.9 (1) and 671 (2); MT-II, 612.2 (1), 627.2 (1), 631.3 (1), 648.2 (1), 656.0 (1) and 669.6 ppm (2). Although the primary structure and the numbers of metals in rabbit liver MT are not known, the numbers of metals in the rabbit MT might be 7 as in the case of both forms of mouse liver MT⁴. 2 ^{113}Cd peaks may be present at 648 ppm (corresponding to 7 metals in MT-I) but broad signals prevented to clarify this. The similarity of both spectra indicated the close resemblance of amino acid sequences and chemical environments in MT-I and MT-II. Although the line width of

inorganic cadmium is very narrow⁸ (e.g., 0.49 Hz for 2 M CdSO_4), the broadening of line width was observed in the spectra of MT (more than 120 Hz) as observed in the case of Cd-alkaline phosphatase⁹ and it severely reduced the high sensitivity of the nuclei. ^{13}C -enriched potassium cyanide was added to the MT solution and the ^{13}C NMR-spectra were recorded to observe separate ^{13}C signals with ^{13}C - ^{113}Cd coupling. However, the spectra gave only a single ^{13}C peak which was shifted from a ^{13}C peak in the same solution without MT (unpublished observation). The result indicated that cadmium was strongly coordinated with sulfhydryl groups. 20 cysteinyl residues among the 61 amino acids in MT can be the primary coordinating groups to the metal, and it is considered that cadmium requires 1 (or more) coordinating group, including water, for the usual tetrahedral structure in MT.

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Experimental immunity against trypanosomiasis

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Summary. 9 groups of 6 female rats were used in an experiment using fraction 3 of *Trypanosoma rhodesiense*. 500 µg gave 100% immunoprotection and 1000 and 1500 µg gave 66% immunoprotection when challenged with 5×10^2 *T. brucei*. 2 groups of 10 female rats were tested for a short period inoculation immune response. In this, 750 µg of fraction 3 of *T. rhodesiense* gave 70% immunoprotection when challenged with *T. brucei*.

It has been demonstrated that subcellular particles or antigens of trypanosomes stimulate the formation of IgG antibodies, and that these particles display a common antigenicity across species²⁻⁴. Previous experiments have demonstrated higher IgG activity and greater immunoprotection from a centrifugal fraction termed fraction 3, when

challenged with homologous and heterologous strains in mice and rats⁴⁻⁷.

This report presents the results of 2 sets of experiments on inoculation with fraction 3 of *T. rhodesiense* in rats subsequently challenged with *T. brucei*. Fraction 3 of *T. rhodesiense* was isolated as previously described and alum was used continuously as adjuvant⁷. The rats were inoculated in the foot pads^{7,8}. Blood was taken from the tail daily from day 3 after-challenge to day 30 to gage infection.

In the 1st experiment, 9 groups of 6 female rats of an average weight of 200 g were used. 1st group was kept as

Table 1.

Total dose of fraction 3 (µg)	Number of animals	Number with trypanosomes	Survival time (days)		
			6	12	Beyond 30
Control	6	6	1	0	0
0.1	6	6	1	0	0
1.0	6	6	4	0	0
33	6	6	2	0	0
100	6	6	6	6	0
500	6	0	6	6	6
1000	6	2	6	6	4
1500	6	2	6	6	4
2000	6	6	6	0	0

Table 2.

Experimental group	Number of animals	Number with trypanosomes	Survival time (days)		
			6	12	Beyond 30
Control	10	10	7	0	0
Fraction 3 Inoculated (750 µg)	10	3	10	10	7

control. The other groups were inoculated with a total of 0.1, 1.0, 33, 100, 500, 1000, 1500 and 2000 µg protein of fraction 3 of *T. rhodesiense* respectively, in 4 injections on days 0, 7, 14, and 21⁸. On day 28, the rats were challenged with 5×10^2 *T. brucei*. 500 µg gave 100% protection while 1000 and 1500 µg gave 66% protection (table 1).

In the 2nd experiment, 2 groups of 10 female rats of an average weight of 200 g were used. 1 group was kept as control. The other group was inoculated with a total of 750 µg of fraction 3 *T. rhodesiense*, in 2 injections on days 0

and 10⁸. On day 13, the rats were challenged with 5×10^2 *T. brucei*, this being one of the shortest periods of inoculation for a secondary response^{8,9}. As can be seen from table 2, 70% of experimental animals exhibited no infection.

These results strongly suggest that trypanosomal fraction 3 has potentialities as an immunizing preparation against heterologous strains. Further investigations are being carried out to determine the scope of these potentialities.

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Endogenous control as a possible basis for varying migratory habits in different bird populations¹

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Summary. In handraised experimental groups of 4 populations of *Sylvia atricapilla*, it was found that the development of migratory restlessness under constant conditions was a fairly good reflection of the different (typical, partial, and non-) migratory habits of the populations. Thus, the different migratory habits seem to be endogenously preprogrammed and genetic in origin. The results of a preliminary cross-breeding experiment are at least compatible with the assumption that partial migratory habit in the blackcap is due to polymorphism.

In many bird species, migratory habits are differentiated according to age, sex, or populations. For example, populations of migratory species of higher latitudes are often more typically migratory than those of lower latitudes, which often are only partial migratory or even resident². Such an example is realized in the blackcap *Sylvia atricapilla*: the north and middle European populations are exclusively, or almost exclusively, migratory, the south European ones are partial migrants, and those of the Canaries and Cape Verde Islands are considered to be resident³⁻⁵. In 1976 and 1977, 17 blackcaps from southern Finland, 25 from southern Germany, 25 from southern France and 24 from the Canaries were handraised, and from their 50th day after hatching kept in constant experimental conditions (light/dark ratio 12.5 h/11.5 h, 22 °C). In the development of migratory restlessness (which in this species is shown to be a fairly good reflection of actual migration⁶) the birds showed conspicuous population differences which were in agreement with the migratory habits of their populations of origin: all Finnish and German birds exhibited migratory restlessness in contrast to only 80% of the French and 20% of the birds from the Canaries. Thus the graduated migratory habits of the different populations are at least on principle also expressed in birds kept in constant conditions. These results suggest that the different migratory habits of the populations investigated are not so much direct responses to various environmental conditions but rather endogenously preprogrammed and genetic in origin. If this conclusion is true, according to the results obtained it is also probable that the different migratory habits within partially migrating populations such as in the blackcaps from southern France, are based on polymorphism. Polymorphism has formerly already been discussed by Lack⁷ as a possible basis of partial migration, according to ringing recoveries.

The supposed polymorphism can be proved by cross-breeding experiments. If the assumption proves right, then, for instance, sedentary individuals from partially migrating populations should also produce migratory offspring (provided that in populations such as these, migrants and nonmigrants do not form isolated subpopulations, for which no indications exist in the blackcap). A first preliminary cross-breeding experiment was conducted. A male and a female blackcap from southern France which, in constant conditions, did not develop migratory restlessness and which derived from 2 different nests, were bred 1977 in an aviary at Radolfzell; 3 young birds from this breeding pair could be handraised and investigated in the same constant conditions as previously their parents: 2 out of 3 birds exhibited migratory restlessness, the 3rd one did not. This result is at least compatible with the assumption that, in the blackcap, partial migration is based on polymorphism. The investigations are to be continued and the quantitative aspects of the migratory restlessness of the experimental birds of the different populations are dealt with elsewhere.

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