were obtained from BioMérieux Laboratories (France). The cells were washed 3 times with phosphate buffered saline (PBS, pH 7.2) before use.

After 4 weeks of acclimatization at each temperature, 25 animals of each lot were immunized by an intracoelonic injection of $2.3 \cdot 10^6$ SRBC in 0.2 ml, 0.5 M NaCl; 5 animals received 0.2 ml 0.5 M NaCl (controls).

Plaque forming cell assay. 14 days after immunization, plaque forming cells (PFC) were detected using the method described by Jerne et al.[2]. *Dicentrarchus labrax* serum was used as complement source. After a 1st incubation (2.5 h, 25 °C) of the plaques[3], complement was added and plaques developped at 25 °C for 2 h. Viability of lymphoid cells was determined with the dye exclusion assay (0.2% trypan blue in PBS).

Results and discussion. Our results are summarized in the table. It can been seen that a peak of the primary response is observed when animals were bred at 18 °C. In a number of teleostean fishes it has been demonstrated that temperature affected antibody synthesis and secretion; a low temperature could decrease these functions. Our results show the effect of temperature on the SRBC response in *Dicentrarchus labrax* with an optimum at 18 °C[4-6]. In earlier work we have shown the presence of NK cells in this fish, and the activity of these cells was temperature-dependent[7]. This previous work proved the importance of breeding temperature on cellular immunity (T cell dependent). Moreover, because lymphocytes of fishes can be cultivated at low temperatures, we could study, in parallel, the immune response level and the structural and biochemical modifications of these cells which could explain this cooperation phenomenon.

Results for 10^6 recovered lymphocytes

Breeding temperature	13 °C	18 °C	21 °C
PFC mean ± Sm for		← * →	← * →
each temperature	33.04 ± 5.14	217.3 ± 18.8	39.6 ± 4.8
Number of assays	20	22	19

← * → Significative difference between results for 2 temperature (Student's t-test: $p < 0.001$).

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Studies on the Dd antigen-antibody system. III. Investigations on antigen Dd-reactivity in families

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Summary. This paper reports the results of observations on antigen Dd-reactivity in families, age of onset of the trait, and its distribution with respect to sex.

Antigen Dd, present in certain samples of human dandruff, has the ability to form precipitate with selected human sera. The frequency of antigen Dd-reactors varies widely in different populations[3-6]. The antigen is heat stable and prolonged storage at low temperatures does not seem to affect it.

The antibodies formed in response to antigen Dd appear to be a permanent characteristic of an individual and this indicated to us that antigen Dd-reactivity may have a genetic background. The present investigations were thus carried out to explore this possibility.

Materials and methods. Venous blood samples were obtained aseptically from 350 individuals belonging to 60 families from the major castes of Punjab. The families represented, in most cases, 3 generations. For certain comparisons, we had available with us, information on antigen Dd-reactivity in 300 adult Punjabi blood donors from a previous study (2nd paper in this series). The methods of analysis and other technical details were the same as described earlier (1st paper in this series).

Results and discussion. We have observed that human sera which react with antigen Dd, retain this ability for considerable lengths of time. Indeed, we have reason to believe that antibodies to antigen Dd may be a permanent feature in a reactor individual because all our sera found to be reactors in 1976 have remained reactors to date. Furthermore, an individual found to be a reactor by Shrivastava[3] in 1972, continues still to have antibodies to antigen Dd. These observations suggest that genetic factors may be involved in the immune response against antigen Dd. We started investigations in this direction by first analyzing 50 cord sera of Punjabi origin for the presence of antibodies against

Table 1. The frequency of antigen Dd-reactors and nonreactors in families with at least one reactor

Sample	Dd-reactor Number	%	Dd-nonreactor Number	%	Total offspring
Families with excess of Dd-reactors (n = 7)	25	65.79	13	34.21	38
Families with excess of Dd-nonreactors (n = 18)	32	27.83	83	72.17	115
Families with reactors and nonreactors in equal proportion (n = 6)	13	50.00	13	50.00	26
Total (n = 31)	70	39.11	109	60.89	179

Table 2. Distribution of antigen Dd-reactors and nonreactors in the control group and in reactor families

Sample	Dd-reactor Observed	Expected	Dd-nonreactor Observed	Expected	Total
Punjabi blood donors (control)	75	90.81	225	209.19	300
Reactor families	70	54.19	109	124.81	179

$\chi^2_1 = 10.564$; $0.001 < p < 0.01$.

antigen Dd in them. We did not encounter a single reactor serum in this sample. However, there are many traits which appear months or years after birth and sometimes make their appearance only after adulthood is reached. This fact prompted us to collect family data for our further investigations.

Of the 60 families analyzed, 31 had one or more antigen Dd-reactor individuals in them and only these families were considered for subsequent investigations. The distribution of antigen Dd-reactor individuals in these families enabled us to divide them into 3 categories, depending upon whether there was an excess of reactors or non-reactors in them or whether the 2 types were represented in equal proportion (table 1). In these families we found 70 reactors in a total of 179 individuals, giving a frequency of 39.11%, which differs significantly ($0.001 < p < 0.01$) when compared with the control group (table 2). The familial clustering of reactors, evident in this sample, does not lend itself to easy genetic interpretation. There seems little doubt, though, that the trait is not sex-linked, since its frequency in the two sexes is roughly the same (table 3).

To study the inheritance pattern, we further classified these families according to parental mating types, as shown in table 4.

We have recorded several cases where non-reactor offspring have been found in matings between 2 reactor parents. This may be sufficient proof against the autosomal recessive mode of inheritance for this trait, if we consider all reactor individuals to be recessive homozygotes. As against this, the autosomal dominant mode of inheritance would seem somewhat more likely, if we disregard the only case where in a mating between non-reactor parents, a reactor child has been found. To explain this lone anomalous case factors like illegitimacy or mutation may surely be invoked, but there is yet another fact that militates against the hypothesis of autosomal dominance for this trait. Our observation is that, both in matings between reactor parents and between reactor and non-reactor parents, there are far less reactor offspring than would be expected on the basis of Mendelian segregation (table 4). This discrepancy cannot be explained even if we assume that the product of the reactor gene, in the dominant homozygous condition, may not be viable. The ratios observed between reactor and non-reactor offspring would have been different in that situation.

The unexpected preponderance of non-reactors in these mating types may be attributed to the often late manifestation of this trait. The incidence of antigen Dd-reactivity in parents is significantly higher than in the offspring, as becomes clear from table 5. The offspring, particularly those from the 3rd generation, were often minor children. Although direct confirmation of it remains to be obtained, we have some evidence to suggest that antigen Dd-reactivity may be age dependent. We have found reactor individuals in younger age groups also, but the median age of reactors in our sample is 50 years for males and 42 years for females. In yet another sample, representing various diseases (data not shown), we have observed that 80% reactors were of age exceeding 25 years.

On the basis of all these observations the following conclusions emerge: 1. Antigen Dd-reactivity is present in both the sexes in about equal proportion. 2. If under genetic control, the gene responsible for the reactor condition may be dominant over its allele for the non-reactor or normal condition. 3. Since Mendelian segregations are not always observed (which can, to some extent, be explained on grounds of late manifestation of the trait), the possibility of non-genetic factors should be looked into. It may not be unlikely that antigen Dd-reactivity may be the immune response of an individual against a microbial antigen. Indeed, such possibility has been suggested also by Carleton Gajdusek (personal communication).

Table 3. Distribution of antigen Dd-reactors in families by sex

Sex	n	Dd-reactor Number	%	Dd-nonreactor Number	%
Male	106	44	41.51	62	58.49
Female	73	26	35.62	47	64.38

Table 4. Distribution of offspring in different parental mating types

Number of families	Mating types	Total offspring	Dd-reactor	Dd-nonreactor
11	$+\chi+$	33	11	22
20	$+\chi-$	50	13	37

+, Dd-reactor; −, Dd-nonreactor.

Table 5. Incidence of antigen Dd-reactivity in parents and offspring

Sample		Dd-reactor	Dd-nonreactor	Total
Parents	Number	46	50	96
	%	47.92	52.08	
Offspring	Number	24	59	83
	%	28.92	71.08	

$\chi^2_1 = 6.752$; $0.001 < p < 0.01$.

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