GENETIC RESISTANCE TO MAREK'S DISEASE (MD) AND LYMPHOID LEUKOSIS (LL) IN CHICKENS

Résistance génétique à la maladie de Marek et à la leucose lymphoïde

Resistencia genética a la enfermedad de Marek y a la leucosis linfoide

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Genetic resistance to classical MD has been well documented by HUTT and COLE in 1947. Breeding programmes for the selection of resistance stock have been proposed and practiced for the control of acute MD (COLE, 1968; BIGGS *et al.*, 1968). The nature of this resistance, however, has not been defined as it was for L/S group of viruses (CRITTENDEN, 1968). Although the symptoms of these two diseases are very similar in many respects and sometimes these were not separable by necropsy, it is well known that the mechanisms for genetical resistance to MD and L/S viruses are different (CRITTENDEN and BURMESTER, 1969).

In 1967, when we had the first severe outbreak of MD in Japan, we had serious discussion which step should be taken first to control the disease, selective breeding or the development of vaccine. The decision was made for both. Then, from 1968 we started our selection programmes for resistance to MD in one of our Governmental Poultry Breeding Stations at Shirakawa by setting a new isolated farm. For the first two years much efforts were made to collect rather basic information of the genetics of MD resistance and then a large scale breeding has been started since 1970. During these years, a great progress was made for vaccine development and the first vaccine was released for general use in 1972.

There is no doubt about the value of the vaccine because the use of either MDHV or HVT vaccine in many countries has reduced dramatically the incidence of the disease and in consequence majority of commercial breeders and even geneticists have lost their interest and enthusiasm in genetic programmes. We strongly believe still that genetic eradication is important as our long-term objective to control the disease.

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The present report concerns a brief review of our breeding programmes for resistance to both MD and lymphoid leukosis.

Part I. Breeding for resistance to MD

For the first two years since 1968 when our programmes had started, much efforts were made to accumulate basic knowledge of the disease, such as methods of infection, routes of infection, sex differences in susceptibility, and differences of infectious viral agents and so forth. Some attentions were also paid to find genetic differences among strains and families within strains.

A full scale breeding was started in 1970, since then 24 purebreds and 38 crosses have been tested so far for strain-cross differences against M. D. incidence and 11 strains for family differences by means of artificial infection. The last one is now incorporated with breeding for economic traits by use of an index.

The total number of chicks tested for MD tests was 53,300 for the past 5 years.

Since Line 01 is one of the most susceptible strain to MD infection and is assumed to be C/AB for L/S subgroup viruses, we decided to preserve the virulent viruses named SK virus for further experiments in this Line 01 and whole blood sample from the birds showing typical MD lessions by necropsy was injected intraperitoneally into one-day old chicks. Each chick received a dose of 0.2 ml of a mixture of one part natriumcitrate and four parts whole blood diluted to 1/10 by physiological saline solution. Chicks died during the experimental period and ones survived till the termination of the experiment were necropsied by experienced veterinarians.

Experimental results

1. *Artificial inoculation «versus» contact infection*

As was reported by many other workers (Cole, 1968; Biggs *et al.*, 1968), chicks inoculated MD agent into body cavity showed higher disease incidence than that of contact-infected group. In the former the first MD incidence or death was observed in 3-4 weeks of age, while it appeared in 7-8 weeks of age in the latter, the peak mortality of these groups occurrs 3-4 weeks apart. The percent mortalities due to MD were 70-80 % and 25-30 % for the artificial inoculated group and the contact-infected group, respectively. There was no apparent difference in diagnostic symptoms between these two groups. When the MD incidence in survived birds at the end of the test was included, the MD incidence raised up to 75-90 % and 50-75 % in the inoculated and contact groups, respectively.

2. *Route of MD infection*

Three routes of virus inoculation were tested if there was some difference in MD incidence and types of lession by intraperitoneal, intramuscular and subcutaneous routes of infection. None of difference was observed but the first MD incidence seemed to be earlier by intraperitoneal inoculation.

3. *Difference of viruses*

Three viral agents collected from different flocks were tested to know if there was any significant interaction between viral agents and strains of chicks in MD incidence. Four strains of chicks, 3 W.L. and 1 R.I.R., were used. The result is given in Table 1.

TABLE 1

DIFFERENCES IN MD INCIDENCE BY VIRAL AGENTS AND STRAINS OF CHICKS

Virus	Strain of chicks				Ave.
	01	02	05	06	
SK	71.7 %	78.0 %	78.7 %	37.8 %	66.7 %
O	56.7	54.8	57.0	19.5	47.2
NIAH	31.4	39.5	48.0	12.6	32.7
Uninoculated Control... ...	21.6	33.0	60.3	13.3	31.1
Ave.	43.2	51.1	60.6	20.1	43.6

There were striking differences in virulence of the agents and the SK virus induced higher lesisons both in nervous systems and various parts of viscera. No interaction of virus × strain of chicks was observed. Accordingly, the SK virus was chosen as the infectious agent in further experiments. The virus has been passaged in the Line 01.

This infectious agent is assumed to be a mixture of at least two kinds of viruses. The whole blood sampled from chicks which showed only nervous lessions and another blood sample collected from chicks showing typical viscera lessions but no gross nervous lession were inoculated separately into two groups of chicks from the same strain. Characteristic lessions similar to those in the donnors were observed. For our experiments whole blood was collected from chicks in which both nervous systems and viscera were affected with MD. Recently an isolate of SK virus has been made by Dr. Yoshio OHKT of National Institute of Animal Health.

4. *Sex difference in MD incidence*

Sex differences in MD incidence were universal in most works. Females are more susceptible than males. Despite of this fact, the interaction between sex × genetic group is the pertinent concern from a genetical point of view. From our experience, none of significant interaction, either sex × sire family or sex × strain, was detected. But sex difference depends sometimes on strains and ages of test. Higher susceptibility in females was not universal in some strains and in some ages. For the past two years sex difference in MD incidence has tended to be diminishing in our flocks. The reason is not clear however.

5. *Criteria for selection*

BIGGS *et al.* (1968) has reported that the age of death or incidence of MD gives higher heritability than the percent MD incidence. Two trials with 4 strains totalling 3,273 chicks were made to estimate heritabilities of cumulative percent incidence of MD and age of death caused by MD starting from 4 weeks to 120 days of age. Heritabilities differed among strains and ages. In one strain, age of death at the end of test yielded the highest value of 58 %, while in another strain the estimate for percent MD incidence till 11-12 weeks of age yielded the highest value of 25 % and that for the age of death was 5 %. One generation of two-way selection for these two criteria based on full-sib records resulted realized heritabilities of above 100 %.

Since there was a criticism however to use the age of death as selection

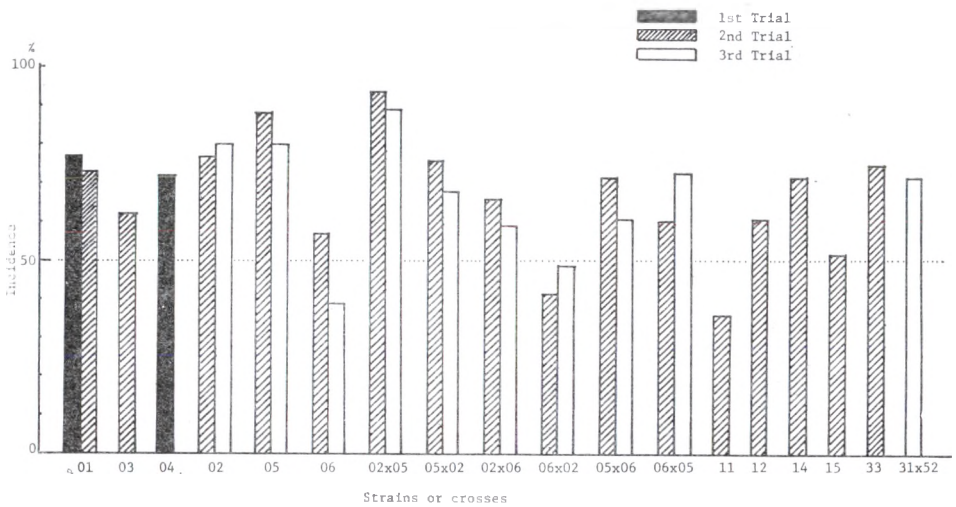

FIG. 1. MD incidence by strains and crosses.

criterion in our breeding because later death causes higher economic loss. Thus, we decided to use percent MD incidence of each full-sib family until 120 days of age. This criterion has been incorporated into selection indices as an additional information from full sibs.

6. *MD incidence in purebreds and crossbreds*

Seventeen purebreds and 38 crossbreds were tested so far for MD incidence by artificial inoculation. Strain differences were prominent and the results of 3 trials are illustrated in Figure 1.

Diallel crossings among three strains, one highly resistant, one intermediate

and another highly susceptible, were made to estimate relative importance of gene actions in the age of death caused by MD. The average age of death by strains and crosses pooled over three trials are shown in Table 2.

TABLE 2

AVERAGE AGES OF DEATH CAUSED BY MD IN 3 STRAINS AND CROSSES (DAYS)

Female lines	Male lines			Ave.
	05	02	06	
05	79.4	80.8	96.1	85.4
02	92.0	91.2	104.4	95.9
06	95.8	100.2	104.5	100.2
Ave.	89.1	90.7	101.7	93.8

It is very clear that general combining ability or additive gene action was most important and a little reciprocal effect was also seen, while specific combining ability or non-additive gene effect was negligible. This suggests that resistant crossbreds should be expected by crossing two resistant purebreds. Almost completely dominant effect observed by COLE (1968) was not confirmed. Later tests support the above finding without any exception.

7. *Association of MD resistance and economic traits*

Genetic correlations between MD resistance and either of growing or adult viability was the range of 0.1-0.3, which suggests breeding for MD resistance will bring about simultaneous improvement of growing and adult viabilities. Genetic correlations between MD resistance and either of egg production, egg weight or adult body weight were all not significant from zero. Thus, selection for resistance will result no detrimental effect to economic traits. Nevertheless, it must keep in mind that lesser selection intensities for each of economic traits by introducing additional trait may decrease the rate of total economic gain substantially.

8. *Effects of selection*

Whether or not we can improve a strain for resistance to MD combined with a total economic score is a pertinent objective. Although COLE (1968) has proved the effectiveness of selection for MD resistance, based on progeny testing, such idealistic selection for a single trait are not always feasible in most commercial operations in which several traits are incorporated into an index.

Comparisons of survivor's progeny and progeny from a breeding flock in which the result of MD inoculation test of each sib family was incorporated into a selection index were made in two strains. The results obtained for three generations are presented in Figure 2.

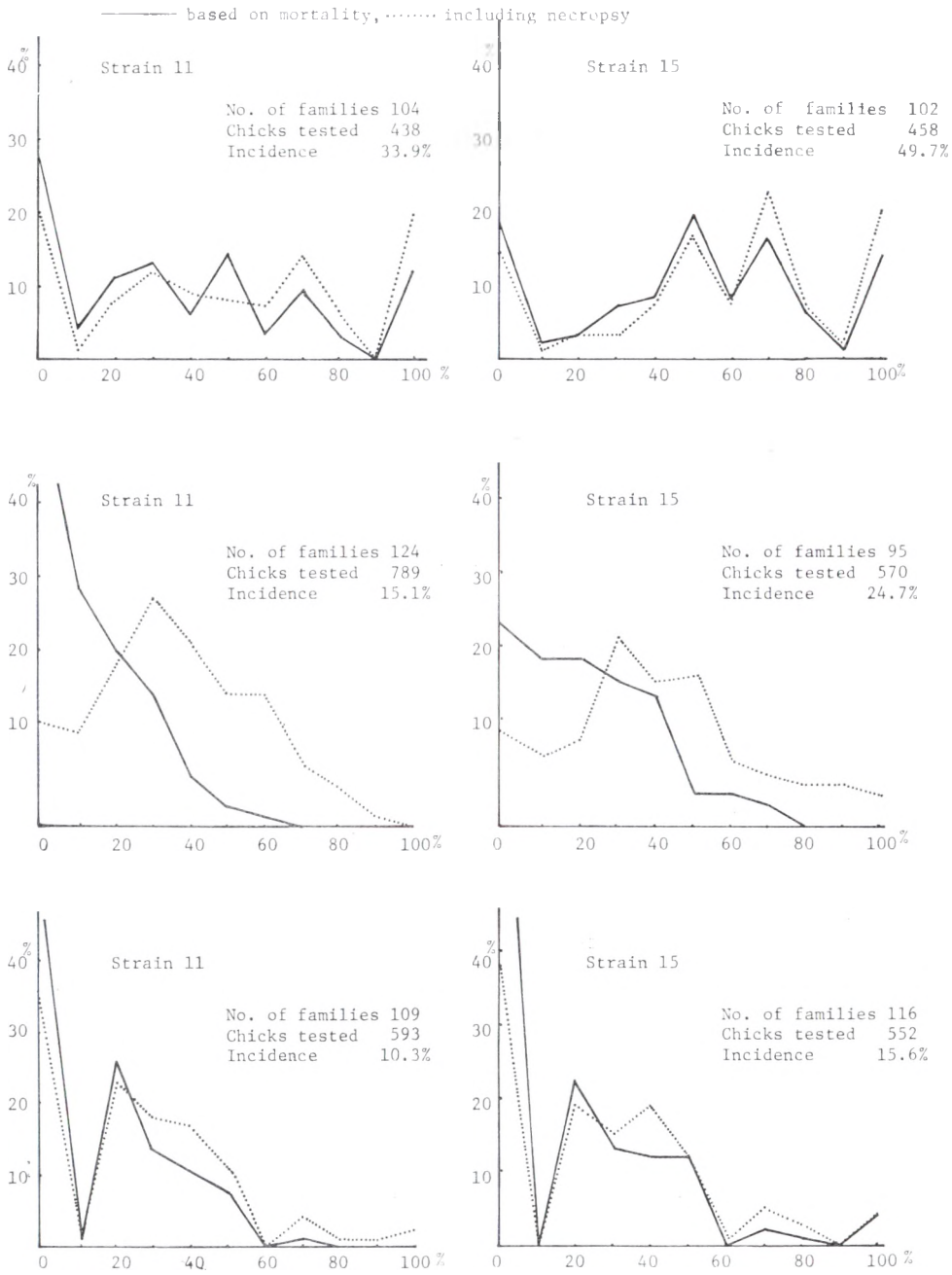

FIG. 2. Effects of selection for MD resistance in two flocks.

The distributions of full-sib familial incidence of MD in two strains in successive years have shifted toward lower incidence. This apparent tendency of shifting the distribution may not be attributable to merely selection effect, because the virulence of MD agents and antibody from parental generations may reduce the incidence to a certain extent. Progeny from survivors showed remarkable reduction within two generations of selection, which suggests the possibility of utilizing survivor's progeny for selection.

The natural MD incidence of these two strains in the breeding station were 5.6 % and 15.6 % in 1970 and those in 1973 reduced to 0.7 and 11.8 %, respectively. Under such a condition, breeding for resistance is no longer efficient without the information from the MD infection test from an isolated facility.

Our recent tests at several locations indicate that some crosses produced by these resistant strains in comparison with other vaccinated commercial stocks have performed as equally or even better without vaccination.

Part II. Resistance to L/S subgroup viruses

Studies on the relative occurrence of viruses of different subgroups indicate that subgroup *A* virus occurs more commonly than subgroup *B* virus and that *C* and *D* subgroups have been only identified in laboratory stocks (Calnek, 1968; Duff and Vogt, 1969; and Crittenden and Motta, 1969).

Mechanisms of genetic resistance to L/S subgroup viruses are well described by Crittenden (1968). The resistance is at the cellular level. The virus is unable to penetrate the cell membrane. Thus, the resistance is detected by challenge of either cell cultures, embryos, or chicks. A single pair of autosomal genes contols resistance to each subgroup virus, and resistance is recessive to susceptibility (Crittenden *et al.*, 1967).

Our breeding programmes for resistance to leukosis sarcoma subgroups viruses were first not arisen from a theoretical thinking. When we tested the frequency of the resistant gene for subgroup *A*, Line 01 which is the most susceptible to MD was almost 100 % homozygous for the resistant gene, while Line 06 which is the most resistant strain to both MD and leukosis in the field by natural infection was 100 % homozygous for susceptible gene. This suggests us an idea that a resistant line which possesses both cellular defence and tumor growth suppressing ability may be established. This seems to be not as simple as we expected.

Preparation of viruses: The RSV preparation was kindly supplied by Dr. Takehiko Shimizu of National Institute of Animal Health. The dose of the inoculum was adjusted to 100 PFU with 0.1 ml. The injection of the viruses was made subcutaneously into both sides of wing-webs of chicks at either 2 or 4 weeks of age. Whenever needed, each of RSV (*A*) and RSV (*B*) was inoculated into each side of wings to identify its genotypes for two *loci*.

Diagnosis of tumor growth: After inoculation all chicks were checked twice a week their tumor incidence and growth. The size of tumor was classified by the following standards: (−) no tumor incidence, (+) smaller than 0.5 cm × 0.5 cm, (++) larger than 0.5 cm × 0.5 cm but no necrosis and discoloration, and (+++) larger than 0.5 cm × 0.5 cm with necrosis and discoloration. Observations were continued for 8 weeks after inoculation or otherwise stated.

Incidence of retrogressive tumor was recorded at the end of the test period as a ratio of the number of chicks showing smaller size of tumor compared to its size in the previous week plus the number of chicks showing disappeared tumor to the total number of chicks once diagnosed as positive. Total number of chicks used were a little over 15,000 for 5 years.

Experimental results

The first preliminary trial using 453 chicks of 8 strains concerned with strain differences of tumor incidence due to RSV (*A*). A large difference of the susceptibility was noticed. As stated in earlier section, Line 06 was 100 % susceptible, while Line 01 was almost completely resistant. Other two W. L. lines were 97.2 % and 90.5 %, one R. I. R. 92,2 % and other two W. L. lines were intermediate being 45.8 % and 47.3 % susceptible. Theoretically, the chicks free from sarcoma incidence are assumed to be homozygous to the resistant allele.

No observation was made on the retrogressive tumor incidence since the experiment was terminated by 3 weeks after inoculation. Several chicks which had no tumor incidence from two W. L. strains were retained for breeding. The progeny from these resistant birds were all resistant, which proved that the wing-web test could be utilized for detecting the resistant genotypes to L/S subgroup viruses.

1. *Strain differences in tumor incidence by RSV (A) and RSV (B), and their retrogression (2nd and 3rd trials combined)*

The second trial concerned to detect strain differences in tumor incidence and retrogression of tumors induced by RSV (*A*) and (*B*). Four strains totalling 1600 chicks were used. Inoculation of the viruses were made on 2 weeks and 4

TABLE 3

Analyses of variance for the incidence of tumors by RSV and retrogression percentage

Source of variation	Tumor incidence			Retrogression		
	d. f	MS	Significance	d. f	MS	Significance
Age of inoculation (A) ...	1	0.99	*	1	4.20	**
Types of viruses (V)	1	7.24	**	1	0.36	NS
Strains of chicks (S)	3	4.08	**	3	20.70	**
A × V	1	0.00	NS	1	0.11	NS
A × S	3	0.25	NS	3	0.71	*
	3	15.40	**	3	0.00	NS
A × V × S	3	3.00	NS	3	0.01	NS
Error	1522	0.15		1132	0.10	

* Significant at 0.05.
** Significant at 0.01.

weeks after inoculation but in some strains observations were made every week for the first 4 weeks and then 6th and 8th weeks.

Two kinds of viruses were inoculated into the same chick simultaneously on both sides of wings, each received either RSV (*A*) or RSV (*B*). In order to check the difference of inoculation time, virus inoculation was made at 2 weeks of age in one group and at 4 weeks of age in another. The results of this trial is summarized in Table 3.

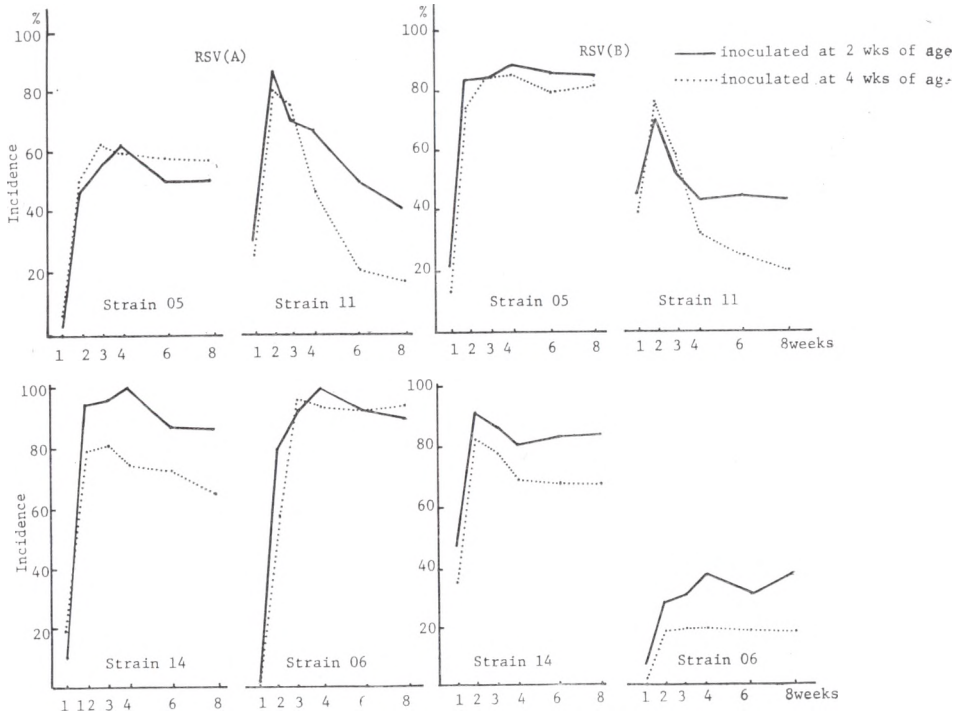

FIG. 3. Patterns of tumor incidence and retrogression by strains and viruses.

Fourteen strains inoculated at 2 weeks of age with RSV (*A*) and (*B*) yielded some correlation estimates. The incidences by two kinds of viruses gave non-significant correlation of -0.12. The incidences of retrogressive tumors induced by two viruses gave a significant positive correlation of 0.88.

The third trial consisted of 10 purebreds and 5 crosses tested for *A* virus, and 7 purebreds and 5 crosses for *B* virus inoculated at 4 weeks of age, and further 14 strains and 2 crosses for *A* virus and 5 strains for *B* virus inoculated at 2 weeks of age, totalling 4,288 chicks being used for strain-cross differences in tumor incidence and retrogression.

Correlation coefficient of retrogression of *A* and *B* types was 0.74, which is significantly high and positive. The difference of correlation coefficients obtained

in two separate trials, 0.88 and 0.74, may be due to sampling because the first one was obtained from the same chicks tested for both viruses, while in the second trial two viruses were tested in different chicks.

Patterns of tumor incidence and its retrogression by weekly examinations are illustrated in Figure 3, and percentage of regressed tumors induced by two kinds of viruses for 4 strains are given in Figure 4.

2. *Relationships between susceptibility to RSV (A) and economic traits*

Pedigree identifications of Line 03 test-crossed to Line 01 resulted 37 females out of 139 females tested and 4 males out of 24 males tested being homozygous to the resistant gene. Comparisons of economic performance of 35 C/A females and 39 C/O females whose tumor incidence of progeny were above 60 % were made. The results is presented in Table 4.

TABLE 4

ECONOMIC PERFORMANCES IN THE C/A AND C/O CHICKENS (LINE 03)

Phenotypes	Growing viability %	Egg wt. g	Body wt. kg	Age of lst egg day	Production rate %	Adult viability %
C/A	90.6	47.9	1.93	169	84.4	84.4
C/O	85.5	47.5	1.91	170	84.2	78.5

None of traits showed significant difference, although some indications of difference in viabilities in growing and adult stages were seen.

3. *Positive associations of MD resistance and retrogressive tumor incidence*

So far obtained, our results indicate that the resistance to MD by inoculation and percentage of retrogressive tumors induced by RSV show a very high correlation. Pooled estimate of correlation coefficient between MD resistance of a strain by inoculation and percentage of retrogressive tumor induced by RSV (*A*) and RSV (*B*) of a strain are 0.86 ($P < 0.01$) and 0.68 ($P < 0.05$), respectively. This evidence suggests strongly that some mechanisms at least for recovery from tumor growth after cells or tissues were penetrated by the viruses may be related in both MD and LL.

SHARMA and STONE (1972) has found that the MD resistant and MD susceptible chickens differed markedly in virus-neutralizing antibody response, although they had no striking differences in precipitating anitbody levels. It is likely that gene or genes controlling resistance to MD may act as pleiotropic to induce retrogression of tumor induced by RSV (*A*) and RSV (*B*) through the process related to the finding by SHARMA and STONE.

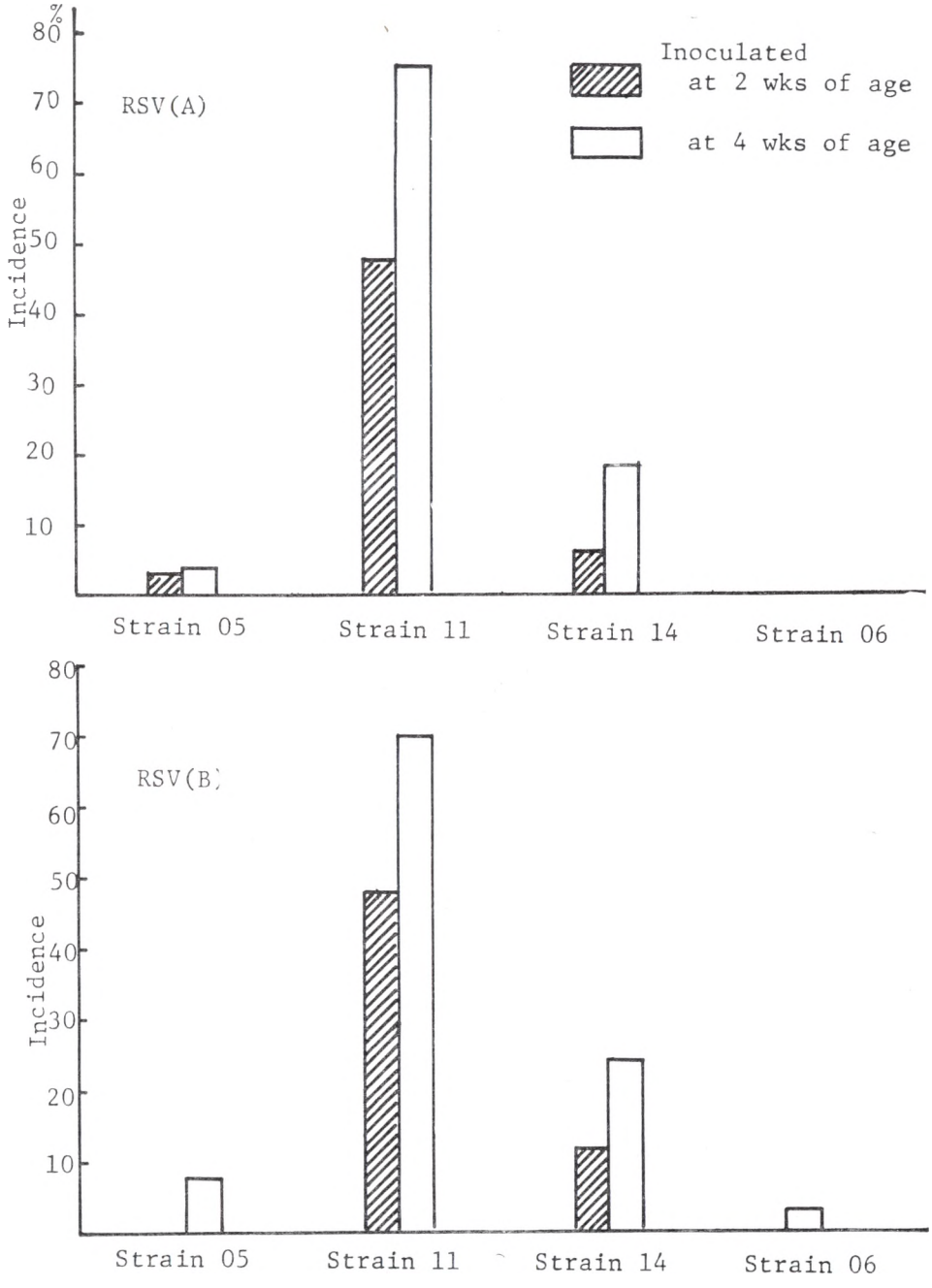

FIG. 4. Percentage of retrogressive tumor incidence.

SUMMARY

Breeding experiments for resistance to MD and LL were briefly outlined.

The most important findings from our experiments are: 1) a high correlation between MD incidence in the isolated farm and the breeding station and thus the familiy records from the isolated farm can be incorporated into selection indices in the breeding station, and 2) a remarkable association of MD resistance and the incidence of retrogressive tumors induced by Rous Sarcoma viruses *A* and *B* types.

The latter high correlation suggests that some mechanisms of resistance to MD and LL may be similar.

RESUME

On a réalisé une série d'expériences pendant l'élevage pour étudier la résistance à la maladie de Marek (MD) et à la leucose lymphoïde (LL).

Les découvertes les plus importantes qui ont été enregistrées sont les suivantes: 1) une corrélation élevée entre l'incidence de MD dans la grange isolée et la station d'élevage, et la possibilité conséquente d'incorporer les records familiers de la première aux indices de sélection de la deuxième, et 2) Une relation remarquable entre la résistance au MD et l'incidence de tumeurs régressifs induits à travers de virus du sarcome de Rous, types *A* et *B*.

Cette dernière corrélation suggère par sa fermété la possibilité de qu'il y existe une similitude entre quelques mécanismes de résistance au MD et au LL.

RESUMEN

Se ha realizado una serie de experimentos durante la cría para estudiar la resistencia frente a la enfermedad de Marek (MD) y a la leucosis linfoide (LL).

Los hallazgos más importantes que se han registrado son los siguientes: 1) Un alto grado de correlación entre la incidencia de MD en la granja aislada y la estación de cría, y la consiguiente posibilidad de incorporar los registros familiares de la primera a los índices de selección de la segunda, y 2) Una relación destacable entre la resistencia a MD y la incidencia de tumores regresivos inducidos a través del virus del sarcoma de Rous de los tipos *A* y *B*.

Esta última correlación sugiere por su firmeza la posibilidad de que exista una similitud entre algunos mecanismos de resistencia a MD y a LL.

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