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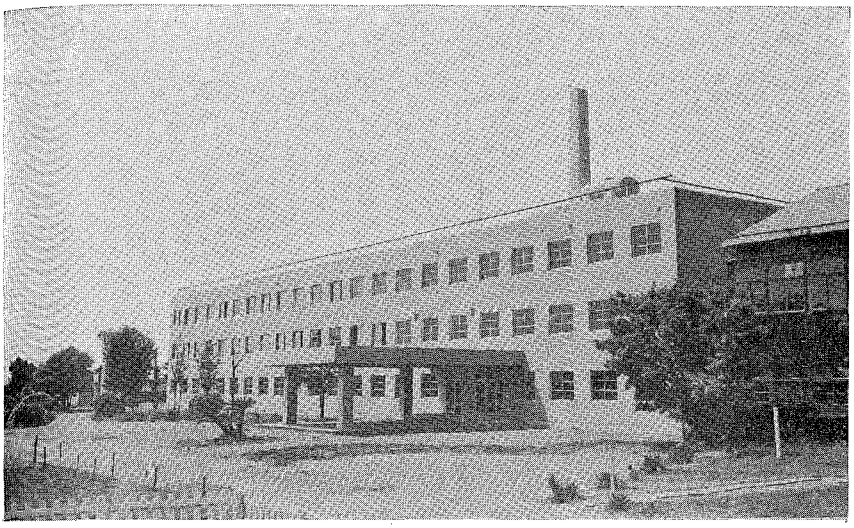

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GENERAL STATEMENT

According to the first construction plan for our main building, the erection of the remaining one third was started in August 1963. By the end of April 1964, the building will be completed. In addition, the construction of a γ-greenhouse was started at the end of the year.

As to the research activities, the second laboratory of the Department of Microbial Genetics was established in July. The main object of this laboratory is the analysis of cellular regulation mechanisms of gene action.

Dr. M. Kimura finished his two year work at the University of Wisconsin. He attended the XI International Congress of Genetics in Hague serving as vice president. He came back to the institute in September. Other members who were working in the U.S.A., Drs. S. Iyama, T. H. Yosida, Y. Yamada, T. Taira and S. Nawa, came back to the institute, and are continuing their researches.

At the XI International Congress of Genetics, Japan was given the honor to be the host for the next Congress in 1968. In order to organize and to operate the Congress, our members have to work hard during the coming years.

H. Kihara

ABSTRACT OF DIARY FOR 1963

Jan. 25. 111th meeting of Mishima Geneticists' Club.
Feb. 22. 112th meeting of Mishima Geneticists' Club.
Mar. 15. 113th meeting of Mishima Geneticists' Club.
Mar. 25. 114th meeting of Mishima Geneticists' Club.
Apr. 26. 115th meeting of Mishima Geneticists' Club.
May 15. 49th Biological Symposia.
May 24. 116th meeting of Mishima Geneticists' Club.
June 14. 117th meeting of Mishima Geneticists' Club.
July 5. 118th meeting of Mishima Geneticists' Club.
July 9. 51st Biological Symposia.
July 29. – Aug. 2. Summer Seminar of Genetics given by Junior Research Members.
Aug. 6. 52nd Biological Symposia.
Aug. 31. 119th meeting of Mishima Geneticists' Club.
Sept. 20. 120th meeting of Mishima Geneticists' Club.
Nov. 19. 53rd Biological Symposia.
Nov. 20. 54th Biological Symposia.
Nov. 21. 55th Biological Symposia.
Nov. 23. 121st meeting of Mishima Geneticists' Club.

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[1] Research member under grant from the Toyo Rayon Foundation.
[2] Research member under grant from the N. I. H. (U. S. A.).
[3] Visiting researcher.
[4] Research member under grant from the Rockefeller Foundation.

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Kanko YOKOTA

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Tanryû OTOMO, Head of Department
Toyotake MINAMIGUCHI, Chief of General Affairs Section
Seiichi KOIZUMI, Chief of Finance Section
Hiroko NAKANO, Ryôji OYAMA, Shigeru TSURUMI and Asakichi MANO
Clerks, Librarian, Typists, Chauffeur, Field Laborers and Janitors.....9

Misima Branch of Hatano Tabacco Experiment Station

Masao TANAKA, D. Ag., Head
Assistants 3

Association for Propagation of the Knowledge of Genetics

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Yataro TAZIMA, Managing Director
Seiji MATSUMURA, Manager
Yosito SINOTO, Manager

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Kempo TSUKAMOTO, Director of National Institute of Radiological Sciences
Bungo WADA, Professor of Sizuoka University

PROJECTS OF RESEARCH FOR 1963

Department of Morphological Genetics

Genetics of the silkworm (TAZIMA)
Chemical mutagenesis in the silkworm (TAZIMA, SADO and MURAKAMI)
Studies on dose-rate dependence of radiation-induced mutation rates (TAZIMA and ONIMARU)
RBE of radiations for induced mutation frequency in the silkworm (MURAKAMI)
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Genetical and embryological studies in insects (SAKAGUCHI)

Department of Cytogenetics

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Determination and differentiation of sex in higher plants (TAKENAKA and YONEDA)
Induction of abnormal mitosis and inhibition of growth by substances

extracted from certain plants (TAKENAKA and OGAWA)
Interspecific hybridization in *Nicotiana* (TAKENAKA)
Genetics of *Pharbitis nil* (TAKENAKA)
Origin of *Prunus Lannesiana* (TAKENAKA)
Cytogenetics of *Oryza* species (TAKENAKA, YONEDA and CHU)
Cytological studies on the yeast cell (YONEDA)

Department of Physiological Genetics

Genetical studies on insecticide-resistance in *Drosophila* (OSHIMA)
Physiological studies on eye-pigment formation in *Drosophila* (TAIRA and OSHIMA)
Population genetics of deleterious genes in natural populations of *Drosophila melanogaster* (OSHIMA, WATANABE and WATANABE)
The persistence of some natural lethal genes in experimental populations (OSHIMA and WATANABE)
Studies on chromosomal aberrations of natural populations of *Drosophila melanogaster* (WATANABE and OSHIMA)
Comparative gene analysis with reference to the origin of wheat (KIHARA, TSUNEWAKI and NISHIKAWA)
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Production of polyploids by N_3O treatment (KIHARA and TSUNEWAKI)
Genetic studies of wheat aneuploids (TSUNEWAKI)
Genetic basis of ecological differentiation in Agropyron (SAKAMOTO)
Collection and preservation of Oryza species (KIHARA)
Morphological study of *Oryza* (KIHARA and KATAYAMA)
Investigation of photoperiodic responses of *Oryza* species (KATAYAMA)

Department of Biochemical Genetics

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Embryological and biochemical studies in the silkworm (TSUJITA)
Genetical and biochemical studies of chromoprotein (SAKURAI)
Biochemical studies on the differentiation of muscle proteins in animals (OGAWA)
Biochemical studies on the mechanism of cell division in animals (OGAWA and KOBAYASHI)
Chemical research in anti-tumor substances (OGAWA)
Biochemistry of the mechanism underlying variations in flower color (ENDO)

Department of Applied Genetics

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Theoretical studies on plant breeding techniques (SAKAI and IYAMA)
Studies on competition and migration in plants (SAKAI and IYAMA)
Biometrical study of cytoplasmic inheritance (SAKAI and IYAMA)
Developmental genetics of quantitative characters in plants (SAKAI, IYAMA, SUZUKI and SHIMAMOTO)
Genetic studies on developmental stability in plants (SAKAI and SHIMAMOTO)
Studies on the effects of linkage disequilibrium in *Drosophila* population (IYAMA)
Comparison of evolutionary mode between series *Sativa* and series *Glaberrima* (MORISHIMA and OKA)
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Crossiong-experiments and observations of sterility of hybrids between wild and cultivated rice strains (MORISHIMA and OKA)
Analysis of sterility genes in *Oryza* (OKA and MORISHIMA)

Department of Induced Mutation

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Population genetics of *Drosophila* (MUKAI, CHIGUSA and YAMAZAKI)
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Department of Microbial Genetics

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Genetics of multi-drug resistance in bacteria (IINO)
Genetics of motility in bacteria (ENOMOTO)
Genetics of cellular regulatory mechanisms (SUZUKI and ISHIDZU)
Genetic fine structure analysis on microorganisms (ISHIDZU and IINO)

FOREIGN VISITORS IN 1963

Feb.	23.	Dr. N. E. JODON (Rice Experiment Station Crowley, Louisiana, U.S.A.)
Feb.	23.	Dr. S. BATRA (Bethune College Calcutta-12, India)
March	13.	Dr. Y. TANADA (Professor, Department of Insect Pathology, California U.S.A.)
April	29.	Dr. S. WORTMAN (Associate Director, The International Rice Research Institute, Los Banos, Laguna, The Philippines)
May	3.	Dr. W. R. SINGLETON (Blaudy Experiment Farm, University of Virginia, Charlottesville Virginia, U.S.A.)
May	14.	P. NARAHARI (Scientific Officer, Biology Division, Atomic Energy Establishment, Tronbay Byculla, Bombay-8, India)
May	15.	Dr. E. MAYR (Comparative Zoology, Harvard University, Cambridge, Mass., U.S.A.)
May	15.	Dr. D. D. KECK (National Science Foundation, Washington 25, D. C., U.S.A.)
May	24.	Prof. Dr. H. KAPPERT (Münster, i/Westf., Germany)
June	3.	Dr. J. E. O'CONNELL (Deputy Chief, Tokyo office, National Science Foundation)
July	8.	Dr. R. C. PIGHETT (Professor, Department of Agronomy, Purdue University Lafayette, Indiana, U.S.A.)
July	20.	Dr. M. T. HENDERSON (Professor of Agronomy, Louisiana State University, Louisiana, U.S.A.)
July	27.	Dr. A. B. JOSHI (Dean, Post-Graduate School & Deputy Director, Indian Agricultural Research Institute, New-Delhi-12, India)
Aug.	6.	Dr. H. GRÜNEBERG (Experimental Genetics Research Unit (Medical Research Council) University College, London)

Sept. 17. Dr. H. W. Li (Director, Institute of Botany, Academi Sinica)

Sept. 19. Dr E. M. Hutton (Assistant Chief, C.S.I.R.O. Division o Toropical Pastures. Cunningham Laboratry, Mill Road, St Lucia, Brisbane, Australia)

Sept. 27. Dr. A. Schenk (Director of National Sericultural Experimen Station, Ales, France)

Sept. 28. Dr. A. W. Nordskog (Professor of Iowa State University U.S.A.)

Oct. 13. T. J. Lee (Chungang University, College of Liberal Arts and Science, Department of Biology)

Oct. 19. Dr. D. Hamissa (Plant Nutrition Section, Giza, Egypt)

Oct. 21. T. S. Pattabiraman (Member of Parliament, Secretary of Congress Party in Parliament, New-Delhi, India)

Oct. 23. Dr. H. E. Brewbaker (Sugar Beet Consultant, United States Aid Mission to Iran, U. S. Embassy, Tehran, Iran)

Nov. 11. Dr. C. Stern (Univerity of California, Berkeley, California, U.S.A.)

Nov. 11. M. S. El-Balal (National Research Center, Cairo, Egypt)

Nov. 18. Dr. A. Abraham (Professor of Kerala University, India)

Dec. 1. Dr. A. G. Steinberg (Professor of Biology, Western Reseve University. U.S.A.)

Dec. 5. Prof. J.R.A. McMillan (Dean, Faculty of Agriculture, University of Sydney, Australia)

Dec. 9. J. N. Tepora (College of Agriculture, University of the Philippines College. Laguna, Philippines)

Dec. 25. Dr. D. Atsmon (The Weizman Institute of Science, Rebovoth, Israel)

RESEARCHES CARRIED OUT IN 1963

A. GENETICS, CYTOLOGY AND BIOCHEMISTRY OF ANIMALS

1. *Genetic variability of deleterious genes in a large natural population of Drosophila melanogaster*[1)]

(By Chozo OSHIMA, Takao WATANABE and Taishu WATANABE)

A great number of flies were collected on the lees of grapes in several graperies of Kofu and Katsunuma cities in the middle of October 1963. More than seven hundred second chromosomes were isolated individually from collected male flies and duplicated by using the complete multiple inversion method. After four generations, 666 strains were obtained, which were heterozygous for *Cy* chromosome and each second chromosome and their genetic backgrounds were substituted with isogenic chromosomes of the Samarkand strain.

The viability of flies, having such genetic constitutions, was examined and their second chromosomes were divided into four classes; lethal, semi-lethal, subvital and normal, by the degree of viability of all homozygous flies. The frequencies of the four classes were 17.06, 19.01, 27.40 and 36.53 per cent respectively.

The frequency of deleterious chromosomes in the large population (17.06+19.01 per cent) was remarkably higher than that in the small Suyama-Juriki population (13.14+5.60 per cent). On the other hand, the mean viability of homozygous flies for 415 quasi-normal chromosomes was estimated to be 77.22, when the viability of *Curly* and quasi-normal heterozygous flies was standardized as 100. This value was about ten per cent lower than that (88.77) for 330 quasi-normal chromosomes in the small population.

From the results, it may be concluded that the large population had a larger diversity in recessive deleterious genetic variability than the small population. However, it is generally considered that the diversity could have been reduced by increased chance of homozygosity in a small population, but the rates of reduction of the deleterious classes were not similar in our results. In the small population, the frequency of semi-lethal chromosomes was remarkably reduced, but that of lethal chromosomes has retained at a considerably high level.

[1)] This work was supported by the United States Public Health Service Grant, RG 7836.

2. *Mechanism of persistence of a lethal gene in a small natural population*[1)]

(By Chozo OSHIMA, Takao WATANABE and Taishu WATANABE)

Lethal second chromosomes had been isolated from a small population (Suyama and Juriki population) during the past four years (1959–1962) and allelism tests were performed repeatedly between lethal genes isolated in two successive years. From the results, the evidence was obtained that a special lethal gene had been maintained in the same population for a long period such as three years at least. The lethal strain has been maintained in the laboratory by the *Curly* balanced system, and when the salivary chromosome was observed, the lethal gene was found to be always associated with a paracentric inversion (In(2L)A:26A–33E) on the left arm of the second chromosome. On the other hand, its location on the chromosome could be estimated to be 53.2 by the crossing over values from definite loci of several dominant marker genes. The locus was very close to centromere on the left arm and it was presumed to lie between the centromere and the inversion.

The locations of other lethal genes isolated simulteneously from the Suyama-Juriki population in 1962 were determined. The majority were distributed in the central region, with a few found in both terminal regions. These findings may imply the existence, in the central and both terminal regions of the chromosome, of an epistatic gene complex which is made up of some closely linked loci, and behaves as a unit. If the paracentric inversion existing in both mid-arms of the second chromosome were heterotic, it could contribute to the establishment and maintenance of such a gene complex in a natural population.

3. *Location of lethal genes on the second chromosome*[1)]

(By Takao WATANABE and Chozo OSHIMA)

About one hundred lethal chromosomes were isolated from many flies caught in houses of Suyma and Juriki, and also those emerged from several traps kept there for a week. These lethal chromosomes were divided into 18 groups based on the results of allelism test. Six chromosomes belonging to three of these groups seemed to carry two lethal genes each allelic to a different lethal. Thirty-eight lethal chromosomes isolated from different flies were found to have one allelic gene in com-

1) This work was supported by the United States Public Health Service Grant, RG 7836.

mon. Such a high frequency of appearance of a certain lethal gene coul be caused by genetic drift.

The balanced strain with dominant marker gene (*Sp Bl L*) and *Curl* was crossed with fifteen balanced strains involving each a lethal an *Curly* gene. F_1 female flies (lethal heterozygotes) carrying dominant marke genes were back-crossed to the original *Cy*-lethal heterozygous males Non-*Cy* and *Cy* flies, having eight different phenotypes each, were obtaine in the F_2 generation. The crossing over values between these dominan marker genes were examined by the rate of recombinants among *C* offspring. The location of inversion, if the lethal chromosome had one was determined at the same time. The number of non-*Cy* offspring i these cultures was reduced; especially the number of some phenotype decreased due to the presence of the lethal gene. It could be inferre from this result, which one of the four regions included the lethal gene and the locus of the gene could be determined by the crossing over valu from the marker gene. The results are shown in Fig. 1.

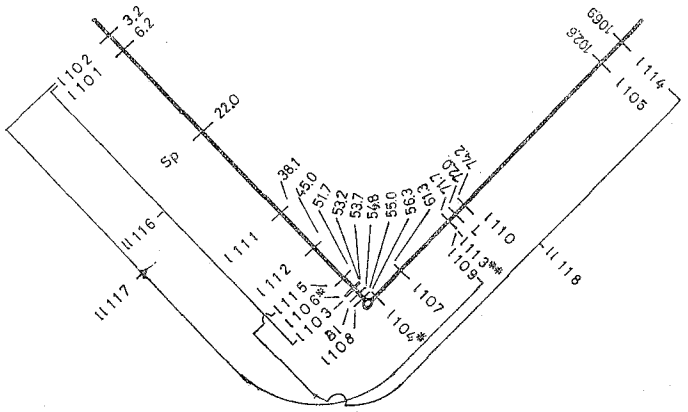

Fig. 1. The location of lethal genes in the second chromosome extracted from the Suyama-Juriki natural population.
* associated with In(2L)A: 26A - E
** 〃 In(2LR)D: 36F - 49B

The majority of lethal genes were distributed in the central region, and a few were located in both terminal regions. These lethal genes such as *l*(2)62j 104, 106 and 113 were associated with an inversion. However, no lethal genes seemed to be included within the inversion. Two loci of lethal genes in the double lethal chromosome were separated by more than 50 units in all cases.

4. *Frequencies of inversions in a large and a small natural population*[1]

(By Taishu WATANABE[2] and Chozo OSHIMA)

By the observation of salivary gland chromosomes, the chromosomal polymorphism was analized in a large population in Kofu and Katsunuma cities, Yamanashi Prefecture, and also in a small one in Suyama and Juriki villages, Shizuoka Prefecture.

One hundred larvae were sampled from a cage population, originated from the Suyama and Juriki population and kept in a population cage for eight months, and on the other hand, one hundred larvae were employed, developed from different female flies caught in the Kofu and Katsunuma population.

Ten kinds of autosomal inversions were observed; their break points are shown in Table 1 and their frequencies in the small Suyama and the large Kofu and Katsunuma population are given in Table 2.

Table 1. Autosomal inversions found in Japanese natural populations and their locations.

Chromosome	Inversion	Break points
2	In(2L)A	26A - 33E
	In(2L)B	22D - 34A
	In(2R)C	52A - 56F
	In(2LR)D	36F - 49B
3	In(3L)E	63A - 74C
	In(3L)F	66C - 71B
	In(3R)G	89D - 96A
	In(3R)H	92D - 100F
	In(3R)I	93D - 98F
	In(3R)J	96E - 98F

The large population carried more inversions on both arms of the two autosomes. No inversion was found in the X chromosome. A pericentric inversion (In(2LR)D) was only found in a lethal chromosome isolated from the Suyama population. In(2L)A, In(3L)F and In(3R)J were rather infrequent and seemed to be endemic. But In(2L)A was observed to have been maintained for at least three years in the Suyama-Juriki

[1] This work was supported by the Grant of the Japan Society for the Promotion of Sciences.

[2] Visiting researcher from Department of Biology, Kyushu University.

Table 2. Frequencies of inversions in the small and the large population.

Population	Suyama	Katsunuma	Kofu
Inversion & standard	Frequency (%)		
2L (standard)	84.5	68.0	67.0
In(2L) A	0.5	0.0	0.5
In(2L) B	15.0	32.0	32.5
2R (standard)	100.0	79.0	73.0
In(2R) C	0.0	21.0	27.0
3L (standard)	97.5	89.5	92.0
In(3L) E	2.5	8.5	6.5
In(3L) F	0.0	2.0	1.0
3R (standard)	82.0	61.0	63.5
In(3R) G	2.5	18.0	11.0
In(3R) H	15.5	10.0	14.0
In(3R) I	0.0	11.0	11.0
In(3R) J	0.0	0.0	0.5

population in association with the lethal gene (*l*(2)62j 106). Other common inversions, such as In(2L)B, In(2R)C, In(3L)E, In(3R)G, In(3R)H and In(3R)I seem to be in the state of equilibrium in the large population because the number of their hetero and homozygotes closely fitted the frequencies expected from Hardy-Weinberg's law.

5. *Cytodifferentiation in the fatbody of Drosophila melanogaster*

(By Toshifumi TAIRA and T. M. RIZKI)

In the wild type strain of *D. melanogaster*, the anterior region of the fatbody at the prepupal stage is clearly distinguishable from the posterior region due to a difference in autofluorescent cytoplasmic inclusions. The inclusions in the cells of the anterior region were identified as kynurenine and those of the posterior region were confirmed to be isoxanthopterin, on the basis of chromatographic and spectrophotometric examinations. In the *vermilion* mutant, the cells of the anterior region of the fatbody have no cytoplasmic kynurenine granules, but the posterior region has as many isoxanthopterin particles as the wild strain. The *sepia* (*se*) mutant remarkably differs from the wild type strain in that the anterior region containing kynurenine granules extends farther toward the

front area of the posterior region. The distribution of isoxanthopterin particles in the cells of the posterior region appears to be similar in both strains, but the number of particles per cell in the *se* strain is considerably smaller than in the wild type strain. The *rosy* mutant has many granules of 2-amino-4-hydroxypteridine instead of isoxanthopterin in the cells of the posterior region. In the *Henna-recessive* mutant, many particles of sepiapterin, which never were detected in other strains, were found among a smaller number of isoxanthopterin particles in the cells of the posterior region. These observations are in good agreement with the results from qualitative and quantitative analyses of those fatbody compounds.

Our observations show that specific enzymes releasing action of the relevant genes of the examined eye-color mutants appeared in the larval fatbody as early as even before the formation of the compound eyes.

6. *A drive element, Nomadic Hunter (NH), in a natural population of Drosophila melanogaster*

(By Yuichiro HIRAIZUMI and Kyôko NAKAZIMA)

About 600 males of *D. melanogaster* were collected from a natural population of Ohdate, Akita Prefecture. These males were then crossed

Table 1. The number of tested and *NH*-carrying males, and the time of fly collections in 1963.

No. of tested males	No. of *NH* males	Time of collection
12	0	Aug. 23—Aug. 28
120	2	Sept. 3—Sept. 7
63	1	Sept. 12—Sept. 15
230	2	Sept. 23—Sept. 27
73	1	Oct. 4—Oct. 10
57	1	Oct. 17—Oct. 20
48	1	Oct. 30—Nov. 3
0*	0	Middle of Nov.
1	0	End of Nov.
Total 604	8	

* Fly collections were continued but no *melanogaster* was captured.

singly to *cn bw* females. From the F_1 progenies of each cross, 5 males were collected to back-cross *singly* to *cn bw* females. In the F_2 generation the ratio of phenotypically *cn bw* to wild flies is expected to be approximately 1:1, and in fact this was so for almost of all tested males. There were however 8 exceptional cases where some of the wild second chromosomes behaved as if they carried segregation-distorter (*SD*) locus. This probably new drive element will be called, for the time being, as Nomadic Hunter (Symbol *NH*). The numbers of tested males, *NH*-carrying males, and the time of fly collections are presented in Table 1.

Examination of salivary gland chromosomes revealed that all of the 8 *NH*-bearing chromosomes carried, in their right arms, a inversion complex which was apparently different from those in *SD-5* and *SD-72* chromosomes. Further studies on this *NH* element are in progress.

7. *Further evidence supporting a high spontaneous mutation rate of polygenes controlling viability of Drosophila melanogaster*[1)]

(By Terumi MUKAI, Sadao CHIGUSA and Isao YOSHIKAWA

As previously reported (Mukai, Chigusa, and Yoshikawa, Annual Report 13) spontaneous mutant polygenes controlling viability have been accumulated by the following method: A single male *Pm/+* from the cross *Cy/Pm*×*+/+* (an isogenic stock of Burdick: W160) was sampled and multiplied by the cross *Cy/Pm* (♀♀)×*Pm/+*(1♂), and 104 lines of *Cy/Pm* ×*Pm/+*$_i$ (i=1, 2,....., 104) were established. In each line, the second chromosome has been maintained through a single male by the cross *Cy/Pm* (5♀♀)×*Pm/+*$_i$(♂). In several generations, homozygous and heterozygous viabilities of these chromosomes have been tested and the results from Generation 10 through Generation 25 were previously reported.

Table 1. Analysis of variance for homozygote viabilities of quasi-normal lines in Generation 32.

Source	Sum of squares	d. f.	Mean square	F
Between lines	4026.30	79	50.9658	8.81***
Error	3239.31	560	5.7845	
Total	7265.61	639		

*** significant at a low level.

1) This work has been supported by Grants GM-7836 and RH-34 from the Public Health Service, U. S. A.

In Generation 32, the homozygous viabilities of those chromosome lines were estimated with the aid of *Cy*-method for 80 quasi-normal lines. The result of variance analysis is presented in Table 1.

The genetic variance among quasi-normal lines was estimated from Table 1 to be $\hat{\sigma}^2_G=5.6477$. The reduction of the mean from the control was 4.76. This value was estimated by a similar method to that described in the paper presented in Annual Report No. 13. The average number of mutations was estimated by the following formula which was also given in a previous paper (Annual Report No. 13):

$$\frac{A^2}{B} \leq p \qquad (1)$$

where A is the reduction of mean viability and B stands for the genetic variance. The result is $4.0118 \leq p$, which implies that polygenic viability mutations occurred at the rate of 0.1254/second chromosome/generation. If interaction existed among loci, this figure might be a slightly biased estimate, but would not deviate much from the true value. The result shows a remarkable consistence with the previous estimate (0.1301/second chromosome/generation).

8. *Polygenic mutation rate and homozygous genetic load in Drosophila populations*[1)]

(By Terumi MUKAI)

In an equilibrium random mating population where frequency of Gene A is p and that of Gene a is q, q is approximately μ/hs (μ indicates mutation rate from "A" to "a" and h the degree of dominance of "a" gene). Thus, homozygous load becomes μ/h per locus under the "Classical" model.

If there is no complicated interaction among loci, the expected survival rate, when the population becomes suddenly homozygous without selection, can be expressed as follows:

$$S = e^{-\Sigma\frac{\mu}{h}} \qquad (1)$$

In the previous Annual Report (No. 13), we have estimated the total mutation rate in the second chromosome of *D. melanogaster* to be $\Sigma\mu = 0.1301$. Under the assumption of $\bar{h}=0.04$ (Stern, *et al.* 1952), calculated S becomes 0.03868. According to Greenberg and Crow (1960), the relative survival rate of homozygotes from equilibrium populations was estimated

1) This work has been supported by Grants GM-7836 and RH-34 from the Public Health Service, U. S. A.

to be 0.632 in comparison with that of random heterozygotes. These two figures are significantly different. The difference must have been caused by the simple assumption of the Classical Hypothesis. In conclusion, it can be stated that the Classical Hypothesis in its simplest form is inconsistent with the actual situation of random mating populations.

9. *Overdominance of spontaneous mutant polygenes controlling viability in homozygous genetic background in Drosophila melanogaster*[1)]

(By Terumi MUKAI, Sadao CHIGUSA and Isao YOSHIKAWA)

In Generation 32 of the experiment described in the foregoing article (7), dominance degrees of accumulated mutant polygenes were tested in heterozygous condition with a chromosome supposed, since it did not undergo any mutation, to be identical to the original chromosome. Line 92 (abbreviated as $+_0/+_0$) was chosen for this purpose on the basis of the result from the preliminary test in which its viability in homozygous condition was estimated as normal (viability index=32.08). The viabilities of heterozygotes were estimated by counting the number of offspring in the crosses $+_0/+_0$ (7♀♀)×$Cy/+_i$ (5♂♂). The relative viability was expressed by the percentage of phenotypically wild-type flies in one culture.

For the sake of simple presentation, the 80 quasi-normal lines were divided into 5 groups in the order of degree in homozygote viability. Thus, each group consisted of 16 lines. The relationship between the average of homozygote viabilities and that of heterozygote viabilities is presented in Table 1.

Table 1. Relationship between homozygote and heterogote viabilities.

Rank	1	2	3	4	5
Homozygote*	31.66	29.18	27.98	26.82	24.61
Heterozygote**	51.11	51.38	51.51	51.70	51.85

* Result of counting 427,886 flies. Expected viability of normal flies=33.3.
** Result of counting 392,077 flies. Expected viability of normal flies=50.0.

The genotypic correlation between homozygote and heterozygote viabilities was estimated to be $\hat{r}_{GG'}=-0.45$. These results clearly indicate that newly arising mutant polygenes show overdominance with respect to viability in homozygous genetic background at least when the mutant

1) This work has been supported by Grants GM-7836 and RH-34 from the Public Health Service, U. S. A.

polygenes are located only in one chromosome.

For reference, the control viability of the heterozygotes was estimated to be 49.91 by using viabilities of 5 heterozygotes whose corresponding homozygotes had shown the highest viabilities in the 80 homozygously quasi-normal lines.

10. *Deleterious heterozygous effect of spontaneous mutant polygenes controlling viability in heterozygous genetic backgrounds in Drosophila melanogaster*[1]

(By Terumi MUKAI, Sadao CHIGUSA and Isao YOSHIKAWA)

In Generation 32 of the experiment for spontaneous mutations, similar experiments to those described in the foregoing article (9) were conducted using two normal second chromosomes; one (abbreviated $+_W/+_W$) from the population from where the original chromosome ($+_0$) has been extracted, and the other (abbreviated $+_B/+_B$) from an entirely unrelated population.

The viabilities of heterozygotes were estimated by the same method as that for the heterozygotes with the original chromosome: Crosses $+_W/+_W$ (7♀♀) × $Cy/+_i$ (5♂♂) and $+_B/+_B$ (7♀♀) × $Cy/+_i$ (5♂♂) were conducted for each line. The percentage of wild-type flies in the offspring was employed as viability index for heterozygotes.

For the sake of simple presentation, the 80 quasi-normal lines were grouped into 5 in the order of degree in homozygote viability. Thus, each group consisted of 16 lines. The relationship between the average of homozygote viabilities and that of heterozygotes is presented in Table 1.

Table 1. Relationship between homozygote and heterozygote viabilities.

Rank	1	2	3	4	5
Homozygotes*	31.66	29.18	27.98	26.82	24.61
Intra-populational heterozygotes $+_W/+_i$**	50.92	50.22	50.29	50.06	49.87
Inter-populational heterozygotes $+_B/+_i$***	55.28	54.41	55.26	54.60	54.78

* Result of counting 427,886 flies. Expected viability of normal flies=33.3.
** Result of counting 390,152 flies. Expected viability of normal flies=50.0.
*** Result of counting 465,820 flies. Expected viability of normal flies=50.0.

The genotypic correlation between the homozygote and heterozygote

[1] This work has been supported by Grants GM-7836 and RH-34 from the Public Health Service, U. S. A.

viabilities was estimated to be $\hat{r}_{GG'}=+0.56$ (intra-populational) and $\hat{r}_{GG'}=+0.27$ (inter-populational). These results clearly indicate that newly arising mutant polygenes are heterozygously detrimental to their carriers in heterozygous genetic background.

For reference, the control viability of the heterozygotes was estimated to be 51.05 and 55.95 in intra- and inter-populational hybrids, respectively.

11. *Allelism frequency in spontaneous recessive lethal genes in Drosophila melanogaster*[1)]

(By Isao YOSHIKAWA and Terumi MUKAI)

The method for accumulating spontaneous mutations was described in the Annual Report No. 13. In those experiments, the tests for the detection of recessive lethal mutations in the second chromosomes were conducted together with the estimation of the mutation rate of polygenes controlling viability. Each lethal mutation has an almost equal probability to be established in each line. The twenty-five lethal chromosomes have been collected through the forty-first generation.

The allelism frequency among all the collected recessive lethals was estimated. For a series of lethal chromosomes all possible cross combinations were made. The experimental results are shown in Table 1.

Table 1. The result of allelism tests.

No. of lethal chromosomes	No. of crosses	Frequency of appearance 1	2	3	Frequency of allelism
25	300	21	2	0	0.0067

It has been reported that the allelism frequency among recessive lethal genes extracted from unrelated populations is 0.0025 (Prout 1954). The test of significance for the difference between this figure and the present estimate was carried out, but it was impossible to detect it.

On the basis of this finding and another estimate in the present experiment with respect to the recessive lethal mutation rate (0.0063/second chromosome/generation), which is almost equal to that of Wallace (1956), it may be concluded that spontaneous recessive lethal mutations occurred at random in the second chromosome loci which are mutable to the recessive lethal.

[1)] This work has been supported by Grants GM-7836 and RH-34 from the Public Health Service, U. S. A.

12. *Spontaneous mutation rate of polygenes controlling developmental time in Drosophila melanogaster*[1)]

(By Terumi MUKAI and Tsuneyuki YAMAZAKI)

As a by-product of the experiment of Generation 32 for the estimation of mutation rate of polygenes controlling viability, we have estimated the developmental time of 80 quasi-normal lines in homozygous condition. Using *Cy*-method, two types of flies, i.e., phenotypically *Cy* flies and wild-type flies, segregated in one vial. The difference between the average developmental time of these two types $[(\overline{+})-(\overline{Cy})]$ was employed as index.

The analysis of variance was conducted for the trait in question, and the result is given in Table 1.

Table 1. Analysis of variance for developmental time in Generation 32.

Source	S.S.	d. f.	M. S.	F
Between lines	41.9997	79	0.5316	4.24**
Error	63.0222	560	0.1254	
Total	105.0219	639		

** significant at the 1% level.

The genetic variance was estimated as $\hat{\sigma}_G^2=0.05078$. The delay of the average developmental time in comparison with the control was 0.5785 days. The control was estimated as the average developmental time of the five lines. The criterion of selecting these five lines was described in the foregoing article (9). The high negative genotypic correlation between viability and developmental time described in the following article (13) justifies this method.

Mutation rate of polygenes controlling the trait in question was estimated by using Formula (1) [Mukai *et al.* (7)]. The result is as follows:

$$\frac{(0.5785)^2}{0.05078} \leq p$$

$$6.5892 \leq p$$

where p indicates the average number of mutations per second chromosome. From this result, it can be said that the mutation rate of polygenes controlling developmental time is 0.2059/second chromosome/generation. This rate is of the same order as that of viability mutations.

1) This work has been supported by Grants GM-7836 and RH-34 from the Public Health Service, U. S. A.

The reliability of this estimate might be lower than that of viability mutation rates, because the estimated control value has larger variance in the former than in the latter.

13. *Correlation between viability and developmental time in Drosophila melanogaster*[1)]

(By Tsuneyuki YAMAZAKI and Terumi MUKAI)

In the present experiment, correlation between viability and developmental time was examined. Study of this kind was first carried out by Dobzhansky and Spassky (1942).

As a by-product of the experiment for the estimation of polygenic viability mutations (7), we have estimated the developmental time of homozygotes and heterozygotes produced by random combination of homozygote lines in Generation 32. Using *Cy*-method, two types of flies, i.e., phenotypically *Cy* and wild-type, are found in one vial. The difference between the average developmental time of these two types $[(\overline{+})-(\overline{Cy})]$ was employed as index.

The result of the experiment is given in Table 1 which shows that the average developmental time is negatively correlated with viability, i.e., both phenotypic and genotypic correlations between viability and developmental time are negative.

Thus, the mutant polygenes control both developmental time and viability pleiotropically, and it can be concluded that more viable lines develop faster than less viable ones at least when these lines have not experienced any natural selection.

Table 1. Characteristics of the average developmental time of each genotype in Generation 32.

Item	Homozygote	Ramdomly combined heterozygote
No. of lines tested	80	77
Average viability index	+0.56(day)	+0.49(day)
Genetic variance	0.05078	0.07838
Phenotypic correlation with viability	−0.2565**	−0.4238**
Genotypic correlation with viability	−0.7358**	−0.8947**

** significantly different from zero at the 1% level.

1) This work has been supported by Grants GM-7836 and RH-34 from the Public Health Service, U. S. A.

14. *Persistence of two recessive genes in experimental populations in Drosophila melanogaster (III)*[1]

(By Sadao CHIGUSA and Terumi MUKAI)

Two cases of heterosis associated with the recessive genes *sepia* and *expanded wing* have been examined as to whether or not these phenomena have been caused by overdominance.

Four artificial populations (Pop. A-1 and Pop. A-2 for *se*, and Pop. B-1 and Pop. B-2 for *ew*) were established, and the gene frequencies of Pop. (A or B)-1 and Rop. (A or B)-2 in the starting generation were 0.5 and 0.1, respectively. The homozygosity of the genetic backgrounds in A-populations is extremely low, while that of B-populations is relatively high. Each population consisting of 8 sub-populations has been maintained by so-called Pearl's method, and the frequencies of recessive homozygotes were estimated in some generations. Four populations seem to have reached equilibria since Generation 20-25, which indicates the superiority of the heterozygote over either homozygote with respect to fitness. The gene frequencies that were estimated in the latest generations of these populations are given in Table 1.

Table 1. Gene frequencies in populations.

Population	Generation	Population count (recessive homo.) (%)	Progeny test* +/+	+/*x*	*x*/*x*	Gene freq. (*q*) (%)
A-1 (*se*)	54	4.87	184	76	13	18.7±1.7
A-2 (*se*)	44	7.45	98	46	12	22.4±2.4
B-1 (*ew*)	50	1.01	122	68	2	18.8±2.0
B-2 (*ew*)	46	3.90	73	40	5	21.2±2.7

* *x* indicates *se* or *ew*.

From Table 1, it can be seen that the recessive genes in question have been maintained in high frequencies in populations. This might have been caused by overdominance with respect to fitness. Further analysis is now in progress.

15. *Interruption of "sex-ratio" condition by double infection with two SR agents of different origin*[2]

(By Bungo SAKAGUCHI and Kugao OISHI)

As to the nature of the so-called SR agents, it was demonstrated by

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[2] This work was supported by U. S. Public Health Service Research Grant GM 10238 from the Division of General Medical Sciences.

Poulson and Sakaguchi (1961) that they are minute filamentous microorganisms belonging to the order of treponema-like spirochetes with 10μ long and 0.1μ wide filaments. They occur in high concentration in the hemolymph of adult females of *D. willistoni* as well *D. nebulosa*.

Recently, Sakaguchi and Poulson (1961) found that they can be easily transferred from either of the two species, *willistoni* and *nebulosa*, to the Oregon strain of *D. melanogaster* where they become stable and persistent.

In order to find the difference, if any, between the *willistoni* and the *nebulosa* spirochetes, an attempt was made to combine by superinfection both kinds in the same host. For this purpose two lines of the Oregon strain were established by artificial transfer, one hosting the *willistoni* and the other the *nebulosa* spirochetes. The females of these two preinfected Oregon lines were superinfected, so, as to bring together the *willistoni*, SRB-3, and the *nebulosa*, NebSR, spirochetes.

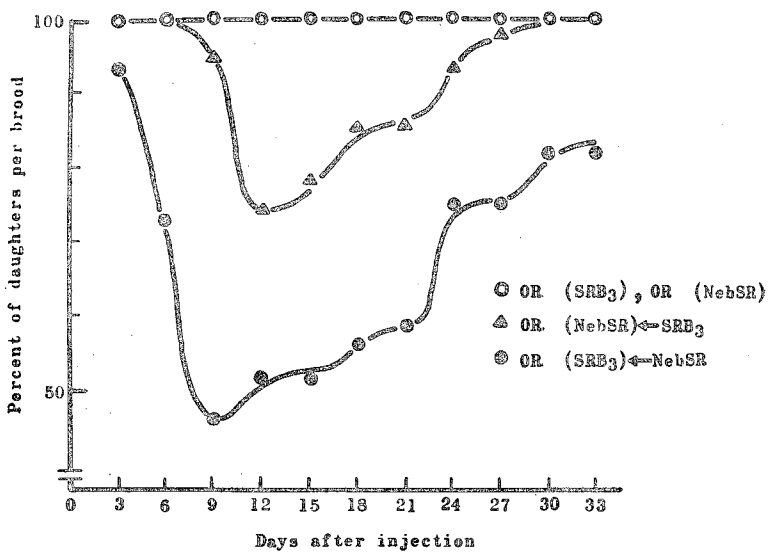

Fig. 1. Interruptions of SR condition in *D. melanogaster* by superinfection with SR agents from *D. willistoni* and *D. nebulosa*.
OR(SRB-3) : SR line of *D. melanogaster* hosting *willistoni* SR agent.
OR(NebSR) : SR line of *D. melanogaster* hosting *nebulosa* SR agent.

In detail, 14 females of the Oregon strain, hosting *willistoni* SRB-3 were superinfected with the original *nebulosa* agent (NebSR) and the percentage of females in the progeny was examined. Figure 1 shows a striking effect in that the percentage of females was reduced from 94%

in the first brood to 45% at the third brood (after 6 to 9 days from the time of injection), but it increased again gradually and finally reached upwards to 80% in the tenth or eleventh brood. In the control superinfected by the same SRB-3 spirochetes (to which it was the host), or left without superinfection no males were produced (Fig. 1)

The reciprocal infection was carried out in similar fashion, namely 12 females of the Oregon line, preinfected with NebSR agent, were superinfected with *willistoni* SRB-3 spirochetes. The examination of the sex relation revealed the same tendency, except that the percentage of females was considerably less decreased than in the other direction (Fig. 1). In the pertinent control no males at all were produced.

The interruption of the SR condition is assumed to be due to interference between two genetically different kinds of SR spirochetes. The mechanism of this phenomenon was demonstrated by *in vivo* and *in vitro* experiments described in another article of this Annual Report.

16. *Nature of the interference phenomenon between the "sex-ratio" agents from D. willistoni and D. nebulosa*[1]

(By Bungo SAKAGUCHI, Kugao OISHI and Susumu KOBAYASHI)

So-called maternally inherited "Sex-Ratio" (SR) condition in *D. willistoni* and *D. nebulosa* is caused by the presence of *Treponema*-like spirochetes in the female bodies of the species hosting them (Poulson and Sakaguchi 1961).

The SR condition is interrupted when the two kinds of SR spirochetes, *i.e.* from *D. willistoni* and from *D. nebulosa*, are combined in the same host (as described in an other article of this Annual Report).

In order to analyse the phenomenon of interference between the two kinds of SR spirochetes, phase- and electron-microscopic examination of hemolymph taken from superinfected females hosting both was carried out. Many clumps were found in the hemolymph taken from such females. Their formation was observed from three to six days from the time of superinfection, and the number of free spirochetes was distinctly reduced. Furthermore, the clumps gradually disappeared from the hemolymph of the infected females after 9 to 12 days and the number of free SR spirochetes gradually increased again. However, no clumps were observed in the hemolymph taken from the control which was superinfected with the same kind of SR spirochetes to which it was the host.

[1] This work was supported by U. S. Public Health Service Research Grant GM 10238 from the Division of General Medical Sciences.

Clump formation between the two kinds of SR spirochetes was also examined *in vitro*. Hemolymphs of females of *willistoni* SR and *nebulosa* SR strain were separately removed with micropipette and were then mixed on a hollow-slide. The mixed hemolymphs were allowed to stand for some time and were examined by phase- and electron-microscope. Formation of clumps of SR spirochetes began after one hour from the time of mixture and the size of clumps gradually increased. The clumps were usually of irregular round shape and measured about 20 to 50μ in diameter. Electron microscopic observation revealed that the clumps after one hour from the time of mixture consisted of many filaments which could be clearly distinguished. However some filaments were partially obliterated 6 hours later. As a control experiment, hemolymphs were mixed, each kind among themselves. It was found that no clumps were formed in the preparation.

Extract freed from SR spirochetes was prepared from *nebulosa* SR females by ultra-high speed centrifugation and was added to *willistoni* SR spirochetes, and the effect was examined by electron microscopy. No clumps were observed in the preparation but partial obliteration of filaments was seen.

Another experiment was carried out to detect the effect of the extract freed from SR spirochetes of *nebulosa* females on *willistoni* SR condition. The extract was injected into SR females of *willistoni* and the sex proportion in the progeny from the injected females was examined in each brood. It was found that the proportion was 100 per cent females in an early brood but gradually decreased and finally reached to about 50 per cent, *i.e.* normal sex-ratio, in 9 to 12 day broods, and SR condition was never restored.

These results indicate that SR spirochetes of *nebulosa* may produce a substance which kills those of *willistoni*. It is unknown at present whether the latter also produce a substance which has an influence on the spirochetes of *nebulosa*.

17. *Low temperature effects on abnormal "sex-ratio" condition in Drosophila*[1]

(By Kugao OISHI and Bungo SAKAGUCHI)

It has been demonstrated by Poulson and Sakaguchi (1961) that the phenomenon of almost exclusively female progeny in *D. willistoni* and *D. nebulosa*, referred to as maternally transmitted "Sex-Ratio" (SR)

[1] This work was supported by U. S. Public Health Service Research Grant GM 10238 from the Division of General Medical Sciences.

condition, is caused by treponema-like spirochetes present in the body of the females. The spirochetes of *D. willistoni* and *D. nebulosa* injected to the Oregon-R strain of *D. melanogaster* have proved to be stable and persistent (Sakaguchi and Poulson, 1962). However, some males usually appeared in the earlier broods (1 to 8 days after mating) in every generation.

To test the effect of low temperature on the SR condition, young flies of OR (SRB-3) strain which was established in Oregon-R by infection with *willistoni* spirochetes, were raised at first at 15°C for 46 days and then maintained at 24°C. The sex proportion in the first generation hatched from those cultures at low and high temperature were examined as shown in Table 1. It was found from the results that the percentage of the females in both cultures was 100 showing complete SR condition (Table 1).

Table 1. SR condition in first and second generation derived from parents treated with low and high temperature.

Parents culture kept at	15°C								24°C		
Brood,	a	b	c	d	e	f	g	h	i	j	k
days after mating	1-4	5-8	9-12	13-16	17-20	21-24	25-28	29-46	47-51	52-56	57-
No. of females and males in first generation	3:0	26:0	9:0	10:0	3:0	9:0	5:0	5:0	11:0	25:0	11:0
No. of first generation flies tested	0	25	6	8	3	9	5	4	11	24	11
No. of first generation flies that produced progeny	0	10	4	2	1	3	1	1	11	18	6
No. of SR	0	2	0	0	0	0	0	0	11	18	6

The females of the first generation hatched from both cultures were mated with normal Oregon-R males. All were culture at 24°C and the proportion of the sexes in the second generation was examined. The SR condition in the second generation derived from the parents cultured at low and high temperature showed a different behavior. The condition almost disappeared in the second generation derived from low temperature culture, except for 2 flies in the early broods. But, when the culture was kept at 24°C after staying at 15°C for 46 days, stable SR condition was transmitted through first to second generation (Table 1). The cause for the disappearance in the the former case of SR condition in the second but not in the first generation is now investigated.

18 *Morphology of "sex-ratio" agents of D. willistoni and D. nebulosa studied by electron microscope*[1]

(By Susumu KOBAYASHI and Bungo SAKAGUCHI)

Poulson and Sagakuchi (1961) were able to show that "sex-ratio" (SR) agents are small spirochetes, presumably *Treponema*, present in each of the two species studied, *D. nebulosa* and *D. willistoni*. In order to make clear whether there is any morphological difference among the SR agents of those species, electron microscopical observations have been undertaken.

The morphology of the spirochetes in adult *D. willistoni* is very similar to that of *D. nebulosa*. The size of the spirochetes of the two species can be estimated to be of the order of about 0.15 to 0.08μ in diameter and 8 to 16μ in length. In most instances the spirochetes showed irregular undulation running along the filaments as represented in the photographs (Fig. 1 in page 30). It seems that in *D. nebulosa* the undulation is more pronounced that in *D. willistoni*. Occasionally, the filaments are branched.

A striking morphological feature are knob-like bodies ranging from 0.8 to 1.2μ in diameter and showing a weak electron density. Those bodies may occur at any point along the filament, *i. e.* they may be found either in an intercalary or a terminal position. Furthermore, they form one or two minute granules (buds) which may develop into young spirochetes.

In the spirochetes so-called "protoplasmic cylinder" and "cell envolope" can be distinguished. The cylinder is 8 to 12mμ in diameter and the envelope is about 2*m*μ wide. In some spirochetes the protoplasmic cylinder appeared to be double coiled, each coil approximately 5*m*μ thick. Occasionally the cell envelope could not be distinguished from the cylinder by negative contrast technique.

19. *Cellular studies of antibody formation I. Proliferative capacity of antibody-forming cells*

(By Toshihiko SADO and Martha R. LEONARD[2])

An understanding of the process of cellular differentiation is of fundamental importance in biology. We feel that the one approach in understanding this complex cellular process is to study the cellular changes

[1] This work was supported by U. S. Public Health Service Research Grant GM 10238 from Division of General Medical Sciences.

[2] Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA.

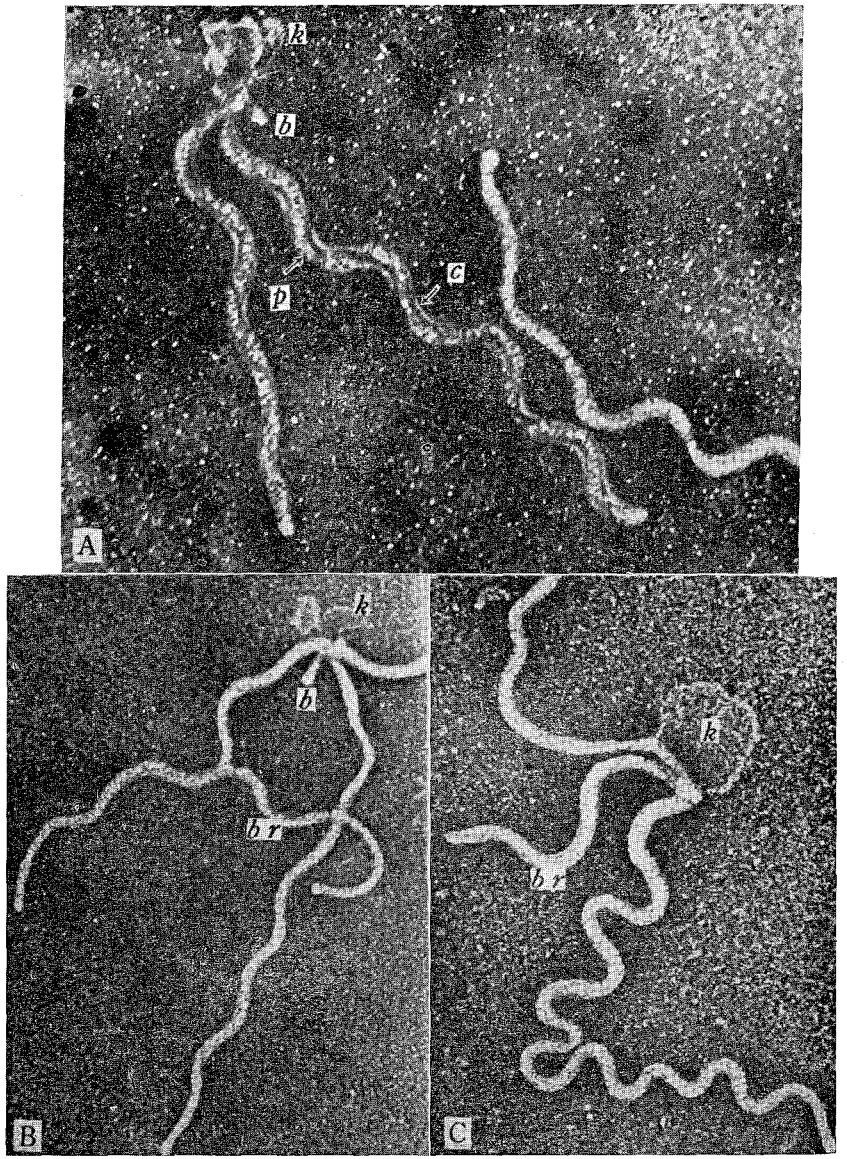

Fig. 1. Electronmicrographs of SR agents of *D. willistoni* and *D. nebulosa*. Negative contrast technique.
A and B: SR agent of *D. nebulosa* (A: × 28,000, B: × 18,000). C: SR agent of *D. willistoni* (× 21,000). b: Bud, br: Branch, c: Cell envelope, k: Knob-like body, p: Protoplasmic cylinder.

that take place in an immune response because past studies have shown that lymphoidal cells of the spleens and lymph nodes undergo functional and morphological changes when stimulated by a specific antigen. Moreover, this alteration may persist for many years, and, hence, raises the question of how the information to synthesize a specific protein is transmitted through generations of cells. In view of this consideration, the cellular aspects of antibody formation is, in a sense, a problem of somatic cell genetics.

It is known that somatic cellular division is characteristic of the immune response. However, little is known about the proliferation of cells that are actually synthesizing antibodies. In order to obtain better insight into this problem, the following experiments were carried out.

Spleen cells from rabbits preimmunized against bovine serum albumin (BSA) were cultured together with the test antigen in 0.1μ-porosity diffusion chambers which were implanted into sublethally irradiated mice. At various intervals after culture, the proliferative capacity of antibody-forming cells were studied by combined H^3-thymidine (H^3-Tdr) labeling and immunofluorescence techniques. Antibody-synthesizing cells were classified into two types on the basis of size and appearance: a large, or *immature,* cell type and a small, or *mature,* type. The results show that prior to the differentiation of antibody-synthesizing cells (day 3) there was a very rapid proliferation of blast cells. During the log phase of antibody production (day 5) large antibody-synthesizing cells were very active in DNA synthesis. Very few such cells are capable of synthesizing DNA on days 9 and 11. On day 5 some of the mature cells incorporated H^3-Tdr but no such cells were labeled on day 6 or later. This indicates that these cells are non-dividing, differentiated, terminal cells. These data suggest that antidody response is a reflection of proliferation of antibody-synthesizing cells.

20 *Cellular studies of antibody formation II. The cell cycle of blast cells involved in secondary antibody response*

(By Toshihiko SADO and Takashi MAKINODAN[1])

Studies on antibody response to suboptimal, optimal and supraoptimal doses of antigen clearly indicate that cytokinetically the blast cells are one of the most important cell types involved in an immune response in a lymphoidal organ. The previous observations have suggested that blast cells are the precursor of antibody-synthesizing cells and they do proliferate very rapidly during the early phase of antibody response.

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For this reason, we have focused our attention to the cell cycle of blast cells involved in secondary antibody response. When spleen cells from primed rabbits are cultured with the test antigen in *in vivo* diffusion chambers which are implanted in the irradiated mice, the number of blast cells reaches a state of dynamic equilibrium between three and four days after culture. The cell cycle of blast cells was, therefore, analysed during this period by determining the frequency of labeled mitoses as a function of time after a single pulse injection of H^3-Tdr to the recipient mice. From this experiment each phase of the cell cycle was estimated as follows: G_2, 0.7hr; M, 0.5hr; G_1, 0–1hr; S, 5–6.8hr; and the generation time, 8–9hr. To our knowledge this is the shortest generation time observed among differentiating mammalian cells. In spite of this short cell cycle, the estimated time for the S phase of 7hr is comparable to that of other mammalian somatic cells with a longer generation time. Our data thus support the current view that the S phase is relatively constant independent of the cell type and generation time among mammalian cells (Defendi and Manson 1963, Cameron and Greulich 1963). It is of interest to note that if we accept 7hr as the length of S phase, then the estimated generation time with use of H^3-Tdr index (percent of labeled cells after one hour exposure to H^3-Tdr) is also ~9hr (G.T.=7hr/H^3-Tdr).

Insight into the problem of DNA metabolism of blast cells during the S phase was gained by estimating the mean grain number per metaphase as a function of time after pulse injection of H^3-Tdr. The results showed that the relative uptake of H^3-Tdr is maximal during the latter third of the S phase. These data are comparable to those reported by Humphrey *et al.* (1963) in their study with Chinese hamster cells cultured *in vitro*. They concluded that data of this nature reflect the rate of DNA synthesis by cells at different stages of the S phase. We, on the other hand, feel that the availability of H^3-Tdr with respect to the target DNA synthetic site is variable with time, and, therefore, consider data of this nature as reflections of the diffusion rate of H^3-Tdr, the relative pool size of Tdr and the DNA synthetic rate of cells in the S phase.

21. *Cellular studies of antibody formation* III. *Suppresion of appearance of antibody-synthesizing cells with supraoptimal dose of antigen*

(By Toshihiko SADO and Carol CHADWICK[1])

It is known that competent cells appear in reduced numbers or do not

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appear at all when nonprimed lymphoid cells are stimulated with either suboptimal or supraoptimal doses of antigen. Previous studies in this laboratory showed that suboptimal anti-RBC response can be induced in a similar manner even with use of lymphoid cells from primed donors. However, we do not know whether the suboptimal response is due to (a) decrease in the efficiency of antibody synthesis per functional cell, (b) decrease in the number of functional cells or both (a) and (b). In an attempt to have better insight into this problem, we have used soluble BSA antigen in this study. This permits us to assess immunohistochemically the number of antibody-containing cells in relation to the amount of antibodies synthesized.

Spleen cell donors were either primed adult mice or albino rabbits. Generally 24×10^6 primed spleen cells were cultured in 0.1 μ-porosity diffusion chambers with varying doses of BSA ranging from 5×10^{-6} mg to 30 mg per chamber. Anti-BSA titers were determined by Stavitsky's passive heamagglutination method. The number of antibody-containing cells per chamber was estimated by multiplying the number of cells per chamber by the percentage of antibody-containing cells determined with Coon's immunohistochemical method.

The results showed that the optimal dose of BSA is approximately 5×10^{-2}mg per 24×10^6 primed spleen cells. Furthermore, a response of approximately 5% of the maximum was obtained with an antigen dose either 100-fold more than or less than the optimal dose. As regards the number of antibody-containing cells produced, two points are noteworthy. (i) The appearance of these cells was suppressed remarkably with supraoptimal doses of antigen, i.e., an approximately 75 and 97% decrease in the number of competent cells was obtained by increasing the antigen dose 100-fold and 600-fold, respectively. (ii) The efficiency of functional cells to synthesize antibodies was reduced markedly when these cells were cultured in presence of excess antigen. From these observations we can conclude that suppressed secondary antibody response caused by excess doses of antigen is due first to decrease in the number of functional cells and secondly to decrease in the efficiency of functional cells. These results indicate that on the cellular level the problem of immunological responsiveness and unresponsiveness resides not only on the cells in the multipotent, primary compartment but also on the cells in the unipotent, secondary compartment, both of which require antigenic situmulation before they are transformed into functional cells (Makinodan and Albright, 1963).

22. *Change of a somatic character by DNA in Ephestia*[1]

(By Saburo NAWA and Ernst CASPARI[2])

This work has been designed to test the possibility of somatic transformation by extracted DNA in a higher organism. The wild type of *Ephestia* has a pattern of black scales on its forewing, while a recessive mutant *ml* has a uniform type of scales without pattern. There are some advantages in using this gene for transformation work: it is possible to observe a large number of cells, since a single wing contains thousands of scales each corresponding to one cell. It is, moreover, easy to detect a black scale among a large number of light-colored scales.

Fibrous DNA was prepared either by the sodium lauryl sulfate and chloroform method or by the phenol method from larvae, pupae or adults. Experiments were mainly carried out by using larval DNA, since it has less impurities than pupal or adult DNA and since it is easy to collect larvae.

DNA from normal larvae was injected into *ml*/*ml* larvae on the eighth day of the fifth instar. The wings of adults developed from the larvae treated with DNA were scored for black scales. The frequency of the appearance of black scales was variable in different experiments, as was the survival of treated animals. In the best case, three black scales were found on the wings of twenty-five adults in an experiment in which individual larvae were treated with 3.2 μg of DNA.

This phenomenon may correspond to transformation observed in some microorganisms, although further experiments, now in progress, are necessary for drawing valid conclusions.

23. *Genetical and biochemical studies of chromogranules contained in the larval hypodermis of the silkworm*

(By Mitsuo TSUJITA and Susumu SAKURAI)

In our previous paper it was reported that the presence of yellow chromogranules which contained sepiapterin combining with a specific protein in the hypodermal cells gives a yellow color to the larvae. It has been shown by several investigators (Jucci 1932, Hatamura 1943, Shimizu 1943, Doira and Chikushi 1962) that the opaque skin of the silkworm is due to the occurrence of a large amount of uric acid in the

[1] This work was carried out at The University of Rochester, *U.S.A.*, under Contract AT (30-1)-2902 with the U.S. Atomic Energy Commission.

[2] Department of Biology, The University of Rochester, Rochester, New York.

hypodermal cells. On the contrary, it was found from our investigation that there is an intimate relation between larval color or oily skin character and the shape, color or amount of chromogranules in the hypodermal cells. Therefore, to confirm whether the chromogranules contain a large amount of uric acid or not, experiments were carried out and the following results were obtained.

The chromogranules contain soluble proteins enclosed in an outer membrane or pellicle, which is mainly composed of proteins and lipids.

By the purification procedure described in our previous paper (Tsujita and Sakurai 1963) the chromogranules were isolated. After adding distilled water, the chromogranules were homogenized and centrifuged. The supernatant containing soluble proteins was loaded onto the DEAE (diethyl-amino-ethyl) cellulose column, and was eluted by phosphate buffer solution at pH 8.0. It could be fractionated into the following three parts.

i) 1st fraction contained proteins combining with yellowish brown pigment.

ii) In the 2nd fraction of the normal strain sample proteins, among them the protein combining with sky-blue fluorescent substance,

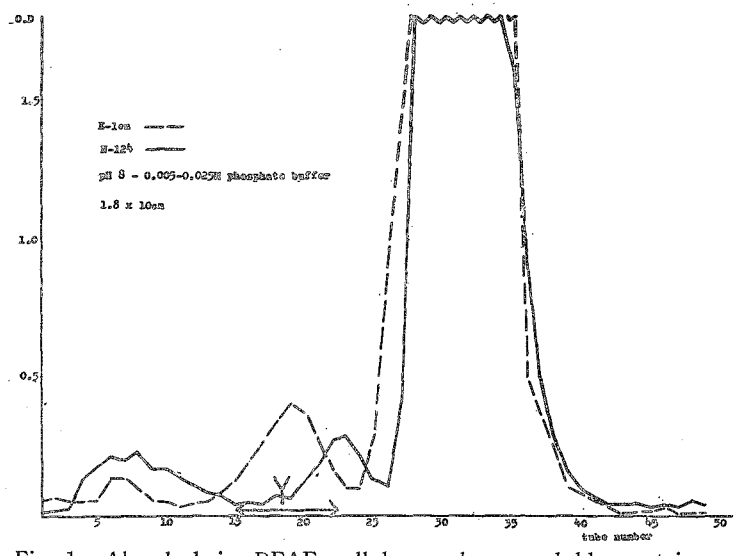

Fig. 1. Absorbed in DEAE cellulose column, soluble proteins of chromogranules were eluted by gradient elution method with 0.005–0.025 M phosphahe buffer at pH 8.0 and were collected all in the same amount of 4 ml in each of the test-tubes. Y band: the yellow pigment-protein complex.

were eluted, while in the same fraction of the lemon strain sample the protein combining with sepiapterin was collected. The latter protein was eluted before the former as shown in Fig. 1.

iii) 3rd fraction contained the protein combining with uric acid and that combining with isoxanthopterin.

A fairly large amount of uric acid and isoxanthopterin could be also detected in the insoluble protein which is a major component of the pellicle of chromogranules.

0.1 M sodium citrate solution and 0.1 N hydrochloric acid were mixed in proportions, 1:9, 2:8, 3:7, ····· . The pH values of each of the mixtures are given in Table 1.

Table 1. Relationship between precipitates and pH of sodium citrates-hydrochloric acid solution.

Precipitates			Yppt			Wppt										
pH value	1.10	1.20	1.53	2.83	3.25	4.48	4.52	4.69	4.82	5.11	5.35	5.70	6.15	6.45	6.78	7.85

Yppt: yellow precipitates Wppt: white precipitates

A few drops of the soluble proteins squeezed out from the chromogranules was added into 2 ml of each of the above-mentioned mixtures. Specific precipitates could be produced at definite pH intervals; in the interval between pH 2–3, flattened crystals containg sky blue fluorescent substance could be produced from a normal larval sample, and similar crystals containing sepiapterin were produced from the lemon larval sample, while in the interval between pH 4–5, white colored powdery precipitates were produced from both samples, which contained isoxanthopterin and uric acid (Table 1). Therefore, it seems that the former crystals were derived from the protein of the 2nd fraction and the latter precipitates from that of the 3rd fraction among the three which were fractionated using DEAE cellulose.

It was concluded that a number of genes inducing oily skin character of the silkworm larvae are concerned directly or indirectly with the formation of chromogranules.

24. *Chromogranules in the hypodermis of normal and several oily skin mutant larvae of the silkworm*

(By Mitsuo TSUJITA and Susumu SAKURAI)

Using normal and several oily skin mutants of the silkworm, the

chromogranules in the cytoplasm of larval hypodermis were studied. The results are as follows:

i) *E-lem*: The skin color is yellow and non-transparent. The chromogranules obtained by purification method from the cytoplasm either of hypodermal cells of the lemon larvae or those of the dilute lemon larvae had almost the same shape, that is, they were irregularly spherical measuring 2-3μ in diameter. The granules obtained from the hypodermis of larvae with the genotype *lem/lem*; $+^{d-lem}/+^{d-lem}$ had much darker yellow color than those obtained from larvae with the genotype *lem/lem*; *d-lem/d-lem*.

ii) Normal larvae (C124): The larval skin is opaque. Similar spherical granules were obtained from the hypodermal cells of normal larvae by the same purification method as that used for the lemon larvae. The diameter of these granules was 1.5-2.5μ, being shorter than that of the granules from *E-lem*. They were white.

iii) Lemon semi-oily mutant: The skin color is yellow and semi-transparent. In the hypodermal cells the amount of chromogranules is smaller than in the normal lemon larvae. The diameter of the granules is 1.5-2.0μ and they are often joined like beads in a string. It was difficult to isolate them from the cytoplasm.

iv) the *od* oily mutant: The skin exhibits strong transparency. A small amount of chromogranules can be observed in the cytoplasm. Their shape is somewhat different from those in the hypodermal cells of normal larvae. Since the chromogranules are so few, it is difficult to isolate them from the cytoplasm.

v) The w^{ox} oily mutant: This mutant larvae have the strongest skin transparency among the several mutant strains used in the present experiment. The chromogranules can be scarcely observed in the cytoplasm of the hypodermal cells.

vi) The *w-3* semi-oily mutant: The larval skin is almost opaque; it is weakly translucent. In the cytoplasm very light-brown chromogranules are found. The size and amount of chromogranules are somewhat smaller than in the hypodermal cells of normal larvae. They can be isolated from the cytoplasm.

It may be said from our observations that non-transparency or transparency of larval skin depends on the presence or absence of chromogranules contained in the hypodermal cells of the larvae.

When the larvae were fed on mulberry leaves painted with 1% neutral red solution the skin all over the larval body became reddish purple. It was found in our observations that this color change is due to staining of the hypodermal granules with neutral red. However, when the stained larvae fed on mulberry leaves painted with 1% melamine acetate

solution, the chromogranules gradually broke down and the pigment, neutral red, which stained the granules became released and was lost from the hypodermal cells.

Not only the chromogranules in the larval hypodermis but also various types of granules in the cytoplasm of the epithelial cells of several tissues such as mid-gut, Malpighian tubules, silk-glands and so on can be stained with neutral red. It may be considered, therefore, that the mechanism of staining with neutral red of the chromogranules in the hypodermal cells is somewhat different from that of absorption and maintenance of pteridine compounds as well as uric acid within the granules.

25. *Genetic analysis of growth of early chick-embryos*

(By Takatada KAWAHARA)

Three purebreds: White Leghorns (WL), Barred Plymouth Rocks (BPR), Nagoyas (NG), and the reciprocal F_1's, F_2's, and back-cross hybrids between WL and the two others were investigated. The number of somite pairs was examined in embryos at thirty-six and forty-eight hours after incubation. As preliminary experiments conducted before suggested that early embrynic growth in chickens showed seasonal variation, special attention was paid at the same time to between-strains comparison. Results of this investigation are summarized as follows: Growth rate estimated from the number of somite pairs in early embryonic stages varied among strains, indicating that it is a genetic trait. The number of somite pairs was 16.51, 17.24 and 17.90, in purebred WL, BPR and NG respectively, while it was 15.96, 18.47, 14.30 and 18.08, in WL ♀ × BPR ♂, BPR ♀ × WL ♂, WL ♀ × NG ♂ and NG ♀ × WL ♂, respectively. Marked differences were found between breeds in the number of somite pairs when examined 48 hours after incubation, NG and BPR showing a larger number than WL. The number of somite pairs in WL was 1.06, which was 1.04 less than that in NG or BPR. Significant differences were found between reciprocal F_1 hybrids (48 hours embryo). Namely, F_1 hybrids of WL dams with NG or BPR sires produced less somite pairs than the reciprocals (3.78 pairs in the WL-NG cross and 2.51 pairs in the WL-BPR cross). The reciprocal differences were also found in F_2 and back-crosses, as follows: WL-BPR F_2: 0.84, WL-NG F_2: 1.31, F_1(WL-BPR) ♀ × WL ♂: 0.98 and F_1(WL-NG) ♀ NG ♂: 1.04 pairs. The heritability values, h_s^2, h_d^2, h_{s+d}^2 and c^2, for the number of somite pairs estimated in the embryos 48 hours after incubation were 0.07, 1.11, 0.59 and 0.26, respectively. These results suggest that early

growth of chick embryos is strongly influenced by maternal factors, and that the differences between reciprocal F_1 hybrids may be partly due to maternal cytoplasm-hybrid genotype interaction.

26. *Heterosis and asymmetry in testis weight in domestic fowl*

(By Takatada KAWAHARA)

A comparison between purebred and crossbred strains was made regarding testis weight at various developmental stages. The materials used were cockerels of White Leghorns, Barred Plymouth Rocks, Nagoyas, and F_1 hybrids between WL and two other breeds. Testis weights were examined 0, 5, 12 and 43 weeks after hatching. The results obtained are summarized as follows:

1) Correlation coefficients between testis weights and body weights were always positive ranging between 0.307 and 0.595 over all four stages of observation.

2) Weight of testis, either absolute or expressed in percentage of total body weight, was larger in the F_1 hybids than in their parents, indicating heterosis, especially in the later stages of development.

3) Generally, the left testes of cockerels were heavier than the right ones. Asymmetry was shown by an index, namely |Left testis weight-Right testis weight|/Left testis weight+Right testis weight, in percent. The asymmetry index of F_1 hybrids was 9.82%, while that of purebreds was 8.55%. 0, 5, 12 and 43 weeks after hatching F_1 hybrids had 3.51%, 1.93%, 0.56% and 0.78% larger indices than the purebreds, respectively. Crossbred cockerels were more asymmetrical in testis weight than the purebreds, especially when young; as they continued to grow, the asymmetry of crossbreds was approaching that of the purebreds.

27. *Chromosomal study of mouse lymphocytic neoplasm, P388, growing in vivo and in vitro*[1)]

(By Tosihide H. YOSIDA, Lloyd W. LAW[2)] and Robert A. ROOSA[3)])

Karyotypes of several lines of mouse lymphocytic neoplasm, P388, growing *in vivo* and *in vitro* were examined. Parental P388 ascites line was characterized by stem-line cells with 41 telocentric chromosomes. Modal chromosome number of another ascites subline, P388/L, was 44,

1) This study was carried out in the National Institutes of Health, Bethesda, Md., U.S.A. in 1962-1963.

2) National Cancer Institute, Bethesda, Md., U.S.A..

3) Wistar Institute, Philadelphia, Penn., U.S.A..

all of telocentric type. Although all chromosomes in these two lines were telocentric, their length was considerably different from that of normal somatic chromosomes. On other hand, P388/P line, adapted to *in vitro* culture was remarkable owing to a drastic change of karyotype which consisted of telocentric, median and submedian metacentric chromosomes. Majority of cells of this line had 34 telocentrics, 11 median metacentrics and 4 submedian metacentrics. Four single-cell clones derived from P388/P line had all similar karyotypes to that of the parental line, but number, length and arm index of metacentric chromosomes were slightly different. R-26 line which developed by reinoculation of culture adapted cells had also a diversified karyotype like the parental culture line, but the chromosome number, especially that of metacentrics, was lower.

It may be concluded from the present study, that chromosome alteration may occur frequently in tumor cell populations growing *in vivo* and *in vitro*. Thus, tumors are usually in heterogeneous condition. The most vigorous cell type, however, may control the mode of a cell population under certain conditions.

28. *Karyotypes of various drug-resistant sublines of P388 neoplasm growing in vitro*[1)]

(By Tosihide H. YOSIDA, Lloyd W. LAW and Robert A. ROOSA)

The chromosome pattern was examined in a sensitive and several 8-azaguanine (AZG)- and amethopterin (AMT)- resistant lines of lymphocytic neoplasm, P388, growing *in vitro*. Karyotype of sensitive P388/P line was characterized by the modal chromosome number 49 and included 15 metacentrics. However, the karyotypes of various drug resistant lines were quite different from that of the sensitive line. A remarkable increase in chromosome number was found in resistant lines 102B AZG, 102H AZG and 146D AMT, developed by treatment with fairly low drug concentrations. A secondary decrease in chromosome number was observed in AMT- resistant lines 102J AZG and 146J AMT treated with higher drug concentration. In AZG-resistant lines, the decrease in the number of submedian metacentric chromosomes was striking and in the AMT-resistant line that of telocentric chromosomes was remarkable.

From the observation of chromosome number distribution in the sensitive and resistant lines and in several single cell clones it was

1) This study was carried out in the National Institutes of Health, Bethesda, Md., U.S.A. in 1962-1963.

considered that the parental P388 and the resistant lines were a mosaic of various karyotypes, although the type of the stemline cells was the most frequent in a cell population. A resistant cell or cells which have different karyotypes may be selected from such a cell population by treatment with drugs as selective agents. Therefore, resistant cell lines may have different karyotypes from those of the sensitive or formerly resistant lines.

29. *Karyological analysis of the mouse lymphocytic neoplasm, P388, growing in synthetic culture medium*[1]

(By Tosihide H. YOSIDA, Virginia J. EVANS[2] and Lloyd W. LAW)

To examine whether different conditions of the culture medium produce different effects on the karyotypes of cultured cells, synthetic and serum media were used in *in vitro* cultivation of mouse lymphocytic neoplasm, P388. P388 ascites and two *in vitro* lines, NCTC 4299 maintained in the serum medium and NCTC 4366 cultured in the synthetic medium, showed 41 as the modal chromosome number. Distribution of chromosome numbers, however, was different, NCTC 4366 showing the widest variation. In *in vitro* adapted P388 sublines, however, variation of chromosome numbers in NCTC 3749, maintained in the synthetic medium, was rather narrower than in P388/P line kept in the serum.

The number of metacentric chromosomes of NCTC 3749 was less than in the P388/P line. P388/DEP, an *in vitro*-derived acites tumor cell line, also showed a decrease by one metacentric chromosome as compared with the parent, NCTC 3749. Based on the above investigation, it is considered that under conditions *in vivo* as well as *in vitro*, the most adaptive karyotype will control the mode of a cell population representing its stem-line.

30. *Karyological study on mouse leukemias developed by virus infection*[3]

(Tosihide H. YOSIDA, Lloyd W. LAW and A. PRECERUTTI[4])

Chromosomes of 19 mouse leukemias induced by Moloney virus in-

[1] This study was carried out in the National Institutes of Health, Bethesda Md., U.S.A. in 1962–1963.

[2] National Cancer Institute, Bethesda, Md., U.S.A..

[3] This study was carried out in the National Institutes of Health, Bethesda, Md., U.S.A. in 1963.

[4] National Institute of Health, Buenos Aeres, Argentina.

fection were observed. They were examined in spleen, thymus, and mesenchymal lymph nodes. Among 19 leukemias, 15 (78.9%) had the modal chromosome number 40, all characterized by telocentric centromeres. No difference in karyotype could be found from that of normal somatic cells. Further, 3 leukemias were characterized by the mode at 41, and the remaining one had bimodal distribution of chromosome numbers with modes at 40 and 41. Also in these last four leukemias, all chromosomes were characterized by telocentric centromeres. Relation between the various leukemic mouse strains and their neoplasmic karyotypes are shown in Table 1. Almost all neoplasms examined here were characterized by lymphocytic leukemias.

The original leukemia developed by P-LLV virus (Precerutti and Law 1963)[1] was characterized by the modal chromosome number of 41 and

Table 1. Frequency of several mouse leukemias (Moloney) with 40–41 chromosomes

Strains / Chrom. No.	C3Hf/LW	C3Hf/Gs	C3Hf/Fg	BALB/c	RFM	Total
40	7	2	2	2	2	15(78.0%)
41	0	1	0	1	1	3
40, 41	0	1	0	0	0	1
Total No. of mice	7	4	2	3	3	19

by telocentric centromeres. The viruses obtained from this leukemia were injected into C3Hf/Bi and BALB/c mice which developed typical lymphocytic leukemias. Their stemline had 40 chromosomes like most of Moloney leukemias. Based on the above investigations it can be said that the karyotypes of the great majority of mouse leukemias induced by virus infection had apparently normal karyotype.

31. *Relation between chromosome constitution and protein specificity in mouse plasma cell leukemias*[2]

(By Tosihide H. YOSIDA, Michael POTTER[3], Yoshinori KURITA and Kazuo MORIWAKI)

Chromosomes of 7 strains of mouse plasma cell tumors were observed

1) Precerutti, A. and Law, L. W. 1963. Nature 198: 801–803.

2) A part of this study was carried out in the National Institutes of Health, Bethesda, Md., U.S.A. in 1963.

3) National Cancer Institute, Bethesda, Md., U.S.A.

in relation to the production of specific myeloma protein. As Table 1

Table 1. Karyotypes and protein specificity of plasma cell tumors in mouse.

Strain	Total no. of chrom.	Karyotype**	Protein specificity
RPC-6A	51–78 (77)*	75T+1M+1m	β-2A euglobulin
RPC-6C	70–96 (95)	90T+5M	β-2A urinary protein
RPC-20	62–86 (84)	82T+1M	Bence Jones unusual protein
MOPC-9	98–80 (80)	79T+1M	Bence Jones unusual protein
MOPC-31B	55–83 (81)	81T	γ-2′ myeloma globulin***
X5563 solid	62–84 (84)	82T+1SM+1ST	γ-2 myeloma globulin
X5563 ascites	33–92 (84)	80T+2SM+1ST+1m	γ-2 myeloma globulin

* In parenthesis, modal chromosome number.

** Telocentric, metacentric, subtelocentric and minute chromosomes are denoted by T, M, ST and m.

*** Position of electrophoresis band was slightly different from γ-2 myeloma globulin of X5563.

shows, all plasma cell neoplasms examined were characterized by near-tetraploid chromosome constitution. The majority of the neoplasms had marker chromosomes such as metacentrics, subtelocentrics or minutes.

An ascites developed from the solid type X5563 neoplasm. It produced the same γ-2 globuline, but its karyotype was different from that of the solid type. No constant relationship between karyotype and protein specificity could be found at the present time.

32. *Chromosomes of normal and dwarf cattle*[1]

(By Tosihide H. YOSIDA and Edward J. LAMONTAIN[2])

Chromosomes of dwarf female cattle were observed by skin tissue culture in comparison with those of normal females and males. The animals were 2 years old Herefords obtained from "Bay-Manor-Farm" Delaweare, U.S.A.. Normal as well as dwarf female cattle had 60 chromosomes, namely 29 telocentric autosome pairs and one submedian metacentric X pair. There was no difference in the karyotype between normal and dwarf females. Male normal cattle had 29 telocentric autosome pairs and in addition a submedian X and a median metacentric Y chromosome. From the above investigation, it was concluded that the

[1] A part of this study was carried out in the National Institutes of Health, Bethesda, Md., U.S.A. in 1963.

[2] National Cancer Institute, Bentesda, Md., U.S.A..

character of bovine dwarfism is not reflected in the change of karyotype being due to a single gene mutation.

33. *Karyological observations on a Misima subline of hyperdiploid Ehrlich tumor and its three clones*

(By Tosihide H. YOSIDA)

The hyperdiploid Ehrlich tumor (ELD) used in the present study was obtained from Children's Cancer Research Foundation, Boston, in December, 1958. Its origin goes back to Dr. Klein's stock at the Karolinska Institute in Stockholm. Since that time, this tumor has been maintained in Misima by serial animal passages using mice of a Swiss strain.

Chromosome number and karyotypes of the Misima subline of ELD tumor and three single cell clones derived from that subline were examined. The stemline cells of the Misima subline were characterized by the modal chromosome number of 44 including one A-chromosome (telocentric with secondary constriction), two B-chromosomes (large metacentrics), one Sa-chromosome (short arm) and two or three minutes. One small metacentric was remarkable, especially as it appeared at first after the 61st transplant generation. The three clones had almost the same karyotype as that of the stemline of the parental cell population. All ELD sublines reported until now by several investigators were characterized by 45 or 46 chromosomes including one A and one B chromosome. Based on the above investigation, it may be concluded that the Misima subline has a remarkablly different karyotype from the other sublines of ELD tumor.

34. *Induction of specific protein synthesis in tumor cells by the addition of exogenous RNA*

(By Kazuo MORIWAKI)

Genetical information is assumed to be transferred sequentially from DNA to protein through RNA. To reveal the role of RNA in this sequential pathway, the inducing ability of exogenous RNA in a specific protein synthesis of tumor cells was studied in the present experiment.

RNA fraction, containing soluble RNA, ribosomal RNA and probably larger molecular RNA, was extracted from plasma cell tumor (X5563) of C3H mouse by phenol method, slightly modified from that described by Kirby (1956). The plasma cell tumor strain employed was kindly

supplied by Dr. M. Potter of NIH, U.S.A.; its cells could produce w high efficiency the specific protein gamma myeloma globulin. Ehrl ascites tumor cells, which can not produce gamma globulin were tre ed for 15 hours with higher concentration of RNA ($O.D._{260m\mu}=50$/ml) 0°C. After contact with RNA, Ehrlich tumor cells were shaked for hours at 37°C with a suitable medium containing radioactive amino a (C^{14}-leucine). During this incubation, oxygen consumption of the ce was determined by manometer. Then, the tumor cells were disrupt by freezing and thawing. To the soluble protein extracted from d rupted cells, anti-myeloma grobulin rabbit serum was added. RN induced myeloma protein synthesis could be estimated by radioactivi of amino acid in the precipitate.

Although protein synthesis of Ehrlich tumor cells was apparent inhibited on the whole following the treatment with higher concentrati of plasma cell tumor RNA, induction of gamma globulin synthesis cou be observed in the tumor cells, when the ratio of gamma globulin sy thesis to total protein synthesis was compared. RNase digestion ar boiling of RNA caused loss of this ability.

These results indicate that a foreign protein synthesis can be induce in intact tumor cells by incorpolation of exogenous RNA.

35. *Electrophoretic analyses of esterase isozymes in various strains of mice*

(By Zenichi OGITA[1], Sachio OGITA[1], Yoshinori KURITA and Kazuo MORIWAKI)

Enzymes having multiple molecular forms but the same substrat specificity were named "isozymes" by Markert and Moller (1959). I order to study whether or not any differences in isozyme patterns exis among various strains of mice, the zymograms of liver esterases wer compared using thin layer agar electrophoresis. The details of th technique have been already reported by Ogita (1962). Liver esterase of various strains of mice were separated into 34 bands, and the position of the bands on the agar plate were designated as E_1, E_2, $\cdots$ E_{24} ir order of faster migration towards the anodal side.

In the present analyses, 10 inbred strains, AKR, C57BL, C57L, CBA, CFW, D103, DBA/2, dd, NH, SL, and 8 mutant strains, *ap*, A^y, C^{ch}, *Pc* hr^{rh}, *se*, *t*, W^v, were examined. Among them, C57BL and C57L lacked E_2 band and the rest lacked E_1 band. In the hybrids between C57BL (E_1 type) and CBA (E_2 type), enzyme activities appeared at both posi-

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tions, E_1 and E_2, through they were appreciably weaker. Similar result has been obtained in the case of hybrids between NH and *se*. NH mutant strain lacked E_{13} enzyme and the activities at E_{11} and E_{12} positions were apparently strong. On the other hand, *se* strain lacked E_{11} and showed strong activities at E_{12} and E_{13}. In the hybrids between them, the esterase activities could be observed at three positions, E_{11}, E_{12} and E_{13}. Also in this case, the enzyme activities at E_{11} and E_{13} in the hybrids were weaker than in either parent.

These findings suggest that the protein specificities in mouse liver esterases are controlled by genes, and that a gene dosage effect exists in the synthesis of the enzyme proteins.

36. *Cytological analysis of the mechanism of induction of chromosome aberrations by 4NQO*

(By Kazuo MORIWAKI, Yoshinori KURITA and Tosihide H. YOSIDA)

The present study was attempted to find out which phase is the most sensitive to 4-Nitroquinoline N-oxide (4NQO) during the division cycle of mammalian cells with respect to chromosome aberrations, and to reveal the mechanism of its mutagenic action.

Five days after intraperitoneal transplantation of Yoshida sarcoma cells to wistar rat, 0.8 ml of 10^{-3} M 4NQO solution was injected into the ascites fluid. Final concentration of 4NQO in the ascites fluid was estimated as at the order of 10^{-5} M by determining the optical density of the fluid at 365 mμ. The metaphasic chromosome figures were examined at 0, 4, 8, 12, 24 and 48 hours after the injection.

The first peak in the frequency curve of chromosomal aberrations, representing mostly chromosome elongation, appeared from 0 to 3 hours after the treatment. The second peak represented chromatid breaks and could be seen from 4 to 12 hours after injection. The third peak appeared from 24 to 48 hours after the injection, and represented predominant chromosomal translocations.

The chromosomal aberrations of the first peak were probably caused by the direct action of 4NQO on metaphase chromosomes. If it is assumed that one-generation time for those tumor cells is 19 hours as already reported by Ferymann (1956) and the division delay of several hours occurred following treatment with 4NQO, chromosomal aberrations at the second and third peaks may be considered to be induced by structural changes of chromatin threads caused by 4NQO in G_2 phase cells.

These results probably indicate that the chromatin materials in G_2

and M phase cells are remarkably sensitive to 4NQO and that distortions of chromosomal structure caused by this drug induce secondarily certain gene mutations, as suggested by Endo (1962). On the other hand, the disappearance of chromosomal aberrations during 13 to 23 hours after 4NQO injection shows perhaps that S phase cells may be less sensitive to this drug or that DNA synthesis itself might not be affected directly by 4NQO.

37. *An unusually Y chromosome found in a strain of the mous?*

(By Akira NAKAMURA and Akira TONOMURA)

For the past two years, a detailed analysis of the somatic metaphase chromosomes of cultured embryonic cells has been carried out in genetically different mouse strains and it was found that one of the inbred strains characterized by post-axial polydactyly (gene *Po*) has an usually long Y chromosome as compared with other strains, such as Swiss, DM/Ms, C3H and A/HeMs. The presence of a similar long Y chromosome has also been found in an inbred strain, C57Bl (Levan, Hsu and Stick; 1962).

In the present paper the results are reported of a comparative analysis of the relative length of the Y between the polydactylous strain, C57Bl and DM/Ms. Since the length of metaphase chromosomes depends on the state of contraction of the individual cells analysed, the relative length of the Y in each strain was expressed as the ratio between the length of the Y and that of the smallest autosomal pair, No. 20. The results obtained are summarized in Table 1.

Table 1. Comparison of the relative length of Y chromosome of the polydactylous strain with that of C57Bl and DM/Ms strains.

Strains	Relative length of Y*	Mean of Y	No. of cells examined
Polydactylous	1.2 1.5 1.3 1.3 1.4 1.2 1.1 1.1 1.1 1.1 1.3 1.2 1.2 1.1 1.1 1.1 1.2 1.3 1.1 1.1 1.5	$\bar{X}_1$=1.21	21
C57Bl	1.2 1.2 1.4 1.3 1.3 1.5 1.1	$\bar{X}_2$=1.28	7
DM/Ms	1.0 0.9 1.0 0.9 0.9 0.7 0.9 0.8 1.0 1.0 0.9	$\bar{X}_3$=0.90	11

* Length of the Y/mean length of No. 20 pair.

There was no significant difference between $\bar{X}_1$ and $\bar{X}_2$ ($t_{26}=1.2$, $0.20<P<0.30$), but between $\bar{X}_1$ and $\bar{X}_3$ the difference was highly significant ($t_{30}=6.7$, $P<0.001$). From these results it is clear that the Y chromosomes of Swiss, DM/Ms, C3H and A/HeMs mice are slightly shorter than the smallest autosomal pair, No. 20, while in C57B1 and the polyactylous strain here examined the Y chromosomes are intermediate between Nos. 19 and 20. Since the Y chromosome of the mouse does not form a regular pair or chiasma during pachytene, the greater variation in the appearane of the Y may suggest its indepentent evolution.

38. *Electrophoretic analysis of water-soluble proteins in Triturus embryos on cellulose acetate*[1]

(By Yoshito OGAWA and Izumi KOBAYASHI)

Water-soluble proteins in *Triturus* embryos immediately after fertilization were examined by means of electrophoretic analysis on cellulose acetate.

Embryos from 5 to 35 hrs. after fertilization and unfertilized eggs were mushed with the same volume of ice-cold distilled water in a glass homogenizer. The homogenate was allowed to stand for one hour at 0°C. under constant stirring and then was centrifuged at 2,600 g for 10 minutes. The clear supernatant was analysed using veronal buffer (pH 8.6, 0.07 M) at room temperature, 0.6 mA/cm width of strip. The quantitative analysis was carried out after staining with Ponceau 3R[2] or Nigrosine[3] solution.

The analytical results are given in Table 1. Eleven fractions were divided into four groups according to their mobility by analysis and their changes in amount during the development of embryos. Group I moved fastest to the anode side; it commanded 31.68% in total soluble proteins of unfertilized eggs, and was composed of fractions A, B, C and E. Fractions A and E were found only in the unfertized eggs. This group decreased in amount immediately after fertilization and disappeared 35 hrs. after fertilization. Group II, fractions D, G and H, account for 74.97–77.67% of the material. The total quantity of this group was practically constant but the amount of the individual fractions changed remarkably during the development of the embryos. Group III,

[1] This work was supported by a Grant-in-Aid for Fundamental Scientific Research (No. 710273) of the Ministry of Education in Japan.

[2] Y. Ogawa: Med. and Biol. **66**: 234, 1963.

[3] M. Ortega: Nature **179**: 1086, 1957.

Table 1. Results of electrophoretic analysis of water-soluble proteins in *Triturus* embryos.

Hrs. after fertilization	A	B	C	D	E	F	G	H	I	J	K
Control (Unfertilized egg)	1.07	4.42	10.78	14.10	15.41	17.52	21.82	9.62	1.97	1.72	1.57
5		2.15	5.70	13.62		9.83	38.73	23.79	3.80	1.40	0.98
20		0.30	3.56	16.53		12.57	23.28	37.86	3.58	1.10	1.22
35				26.93		16.69	31.59	16.45	4.65	2.02	1.67

fraction I, J and K, migrated fatest to the cathode side and no fraction in this group showed any significant change in the amount. The last group IV, fraction F, remained at the point of application on the cellulose acetate strip. This fraction showed a temporary decrease immediately after fertilization.

These results showed the same tendency as the results of electophoretic analysis of water-soluble proteins in the eggs of sea urchin[1] or medaka (*Orysias lapites*)[2] when agar or filter paper was used. Group I is equivalent to Nos. 3 and 6 in sea urchin and fraction A in medaka (phospho-protein), Group II to Nos. 1, 2 and 2a in sea urchin and fractions B, C and F in medaka (phopholipo-protein). Group III to Nos. 4 and 5 in sea urchin and fraction D in medaka (glyco-protein), and Group IV to fraction E in medaka.

The analytical results on cellulose acetate seems to be more detailed than that on agar or filter paper.

39. *Changes in the soluble proteins of mouse skin following the application of sweet Orange Oil, a tumor promoting agent*[3]

(By Yoshito OGAWA and Izumi KOBAYASHI)

Sweet Orange Oil is a component of the peel of *Citrus sinensis* and is used as an additive to soft drinks, in confectionery, bakery and drug trade in Europe and United States. The main constituents are limonene (about 90 percent) and decanal. Roe (1959)[4] belives that this oil shows

1) A. Monroy: Exptl. Cell Research **1**: 92, 1950.

2) Y. Ohi: Embryologia **7**: 208, 1962.

3) This work was supported by a Grant-in-Aid for Fundamental Scientific Research (No. 95625) of the Ministry of Education in Japan.

4) Roe, F. J. C.: Brit. J. Cancer **13**: 92, 1959.

co-carcinogenic activity in the skin of mice by combined treatment with 9, 10-dimethyl-1, 2-benzanthracene (DMBA), and Meek (1963)[5] suggested the possibility of its acting as a promoting agent in experimental skin carcinogenesis. In the present paper the changes in water-soluble proteins of mouse skin (dd strain) treated with Sweet Orange Oil (B. P. C. grade) are described.

In the treated group, Sweet Orange Oil was applied to the dorsal skin of mice six times with two day intervals until a total per animal of 2.4 g was reached. Some skin inflammation was found at the site of application immediately after treatment, and hair loss and considerable thickening of the epidermis without ulceration were recognized on the 7th day. 4 days after the last treatment, the treated part of the skin was cut off and mushed with the same volume of distilled water in a glass homogenizer. The homogenate was centrifuged at 1,500 g for 10 minutes. Electrophoresis of the clear supernatant was carried out at roon temperature on cellulose acetate membrane by using veronal buffer (pH 8.6, 0,07 M), 0.7 mA/cm width of strip for two hours. Nigrosine staining was used for the estimation of the analysed proteins. The non-treated group was prepared for control.

The results are shown in Table 1. The soluble proteins extracted

Table 1. Results of electrophoretic analysis of water-soluble proteins in mouse skin treated with Sweet Orange Oil. (%)

	A	F	G	B	C	H	D	E	I
Control (Nontreated)	17.54			46.61	12.70		23.41	0.7	
Treated (Normal components only)	22.11 (34.62)	14.50	10.30	18.40 (29.03)	7.72 (12.16)	11.43	14.89 (23.49)	0.45 (0.70)	0.20

from the treated group were separated into nine fractions (A, B, ···I), but that from the control group could be divided only in five fractions (A, B, ···E). Therefore, four more fractions (F, G, H and I) resulted from the treatment with Sweet Orange Oil. In the treated group, fraction A was increased and fraction B was considerably decreased as compared with the control. But no significant difference was found in fractions C, D and E.

The biochemical and cytochemical examination of the relation between the above findings and the carcinogenic activity of Sweet Orange Oil must be furtuer investigated.

[5] Meek E. S.: Exptl. Cell Research 29: 389, 1963.

40. *Selection for larval growth in Tribolium under two nutritional levels*

(By Yukio YAMADA and A. E. BELL)

Selection for large and small larval weight in *Tribolium castaneu* was investigated for 16 generations to evaluate the effectiveness of var ous selection methods and the importance of genotype-environment inte action under two levels of nutrition. Chemical composition of the tw rations is listed below.

Ration	Protein	Fat	Ash	Water	Others	Total
Good	18.3	8.5	2.8	10.4	60.0	100
Poor	13.2	4.2	2.2	9.9	70.5	100

The character for selection is the 13-day larval weight. Genetic par meters of the base population for the character were 0.25 ± 0.03 i heritability under both nutritional levels and the genetic correlation b tween the character under the two rations was 0.62 ± 0.12.

The experimental populations are listed below together with th respective selection methods.

Symbol of population	Selection method
GL	selected for large under Good level every generation,
PL	selected for large under Poor level every generation,
$\overline{\text{GPL}}$	selected for large on average under both levels,
GPL	selected for large under Good and Poor in alternating generations,
GS	selected for small under Good level every generation,
PS	selected for small under Poor every generation,
$\overline{\text{GPS}}$	selected for small on average under both levels,
GPS	selected for small under Good and Poor in alternating generations,
C	unselected control consisted of 20 pair-matings, each contributing one mal and one female to next generation.

The mating and selection were made in such a way that each pair c parents produced fertile eggs in the creamer which contained standar wheat media for the first 48 hours after removal from the matin creamer, in which they stayed for approximately a week after eme gence, and then the parents were transferred to two Good rations, eac

for 24 hours, two Poor rations, each for 24 hours.

Once the families for selection were chosen, the sibs raised in the standard wheat media were picked up in their pupal stage and sexed for mating to produce the next generation without weighing.

Each population consisted of 40 single-pair matings and best eight families were selected out of all fertile matings. Five males and five females in each selected family were drawn from the standard media and mated at random with the restriction that full sib mating was avoided.

All populations were raised in the climatic chamber of 33°C and 70% R. H. The whole experiment was run at thirty-five days interval per generation throughout from generation 0 to 16.

Average gain, in each population, per generation for the entire period of selection adjusted to the unselected control is as follows:

Population	Under Good	Under Poor
GL	66.0± 3.8	59.1±9.4
PL	53.6± 2.9	98.7±9.6
$\overline{\text{GPL}}$	52.6± 3.4	72.5±7.4
GPL	59.2± 1.4	83.4±8.4
GS	−106.0± 8.0	−53.2±3.3
PS	− 87.8±10.5	−75.3±4.7
$\overline{\text{GPS}}$	− 85.0± 5.9	−61.0±4.0
GPS	− 94.9± 8.7	−60.3±4.1

This shows very clearly that direct selection responses are generally larger than indirect selection under both Good and Poor nutritional levels. That is, GL and GS were both extremes in performance when they were tested under Good ration, while PL and PS were extremes when they were tested under Poor ration. This shows in turn that strain-environment interactions are quite obvious in later generations of selection. On the contrary, direct selection gains in the populations selected for the average of the two environments —the gain in average for GPL and GPS— do not give the highest response, probably because the amount of information for selection in those two populations was relatively smaller than in the other populations.

It is quite interesting from the view point of animal breeding that selection under Good environment has parallel response in the population means under two nutritional levels when the population has been selected for large (*i.e.* GL), while the population selected for large under Poor (*i.e. PL*) shows that the two response lines under Good and Poor levels

approach gradually each other as the generations of selection proceed. The response regression line under Poor is definitely steeper not only than that on Good of the same population but also than those in GL population on either environment. On the other hand, selection for small size was exactly reverse in selection response in that PS had parallelism but GS not. As an extreme, one of GS lines showed completely reverse ranking under two environments after generation 12 inclusively.

Asymmetrical selection responses in opposite direction were observed. One of the interesting aspects of the asymmetry in this study is the dependency of the asymmetry on the tested environment. Downward selection was favored under Good, while upward selection gave larger response under Poor.

Average realised heritabilities for two directions of selection gave 0.329 for large and 0.444 for small size. This suggests that in the processes of selection toward these opposite directions, gens that responded to selection for larger body and those involved in selection for smaller body may not belong to the same loci. (Supported by NSFG 15824)

B. GENETICS, CYTOLOGY AND BIOCHEMISTRY OF PLANTS

41. *Discovery of three cytoplasms inducing male-sterility in wheat*

(By Hitoshi KIHARA)

I have started my work on nucleus substitution in wheat only for its scientific interest. However, some of the results seem to have given rise to considerable research activities among the American wheat breeders, aiming at the development of a new breeding system for this important crop. They consider the male-sterile lines to be a promising means in establishing hybrid wheat. Therefore, a brief description will be given here on 3 hitherto known cytoplasms which induce male-sterility in wheat.

(a) *caudata* cytoplasm: A male-sterile line with plasma of *Aegilops caudata* and the genome of *Triticum vulgare* var. *erythrospermum*, initiated in 1935 by the production of the F_1 hybrid, retained its cytoplasmic sterility until the present time (1963). Eighteen male-sterile lines (10 of hexaploid and 8 of tetraploid wheats) were derived from this male-sterile wheat. So far 2 fertility-restoring genes have become

known, one in *T. compactum* var. No. 44 and the other in strain P 168 (6x-wheat with a pair of *caudata* chromosomes).

(b) *ovata* cytoplasm: Nucleus-substitution lines of tetraploid wheats with the cytoplasm of *Aegilops ovata* were all male-sterile. No restorers were discovered among 8 tetraploid wheat varieties which I have studied. According to Fukasawa, however, *T. dicoccoides* var. *kotschyanum* restores pollen fertility lost in *ovata* cytoplasm.

(c) *timopheevi* cytoplasm: In my previous paper (Kihara 1959), I mentioned briefly that *T. dicoccum* var. Hokudai with *timopheevi* cytoplasm was fertile. Later it was revealed that *T. durum* var. *reichenbachii* with *timopheevi* cytoplasm produces highly sterile pollen. Therefore we may say that the *timopheevi* cytoplasm induces male sterility and that *T. dicoccum* var. Hokudai has the ability to produce functional pollen grains in *timopheevi* cytoplasm.

42. *Effect of caudata cytoplasm on the development of 4 nucleus-substitution lines of common wheat*

(By Hitoshi KIHARA)

Using 4 advanced nucleus-substitution lines of 6x wheat, effects of an alien cytoplasm introduced from *Aegilops caudata* have been studied in 1963 in respect to several important characters. Performance of the substitution lines on those characters was compared with that of the respective normal lines and their difference was statistically tested. The results are summarized in Table 1. Pollen and seed fertilities are taken from the 1962 record, because of extraordinary wet ripening season in 1963. For estimating the frequencies of haploids and twin seedlings, data of earlier substitution-backcross generations were combined with those of 1963.

Substitution line of *T. compactum* var. No. 44 was the most male-fertile among 4 substitution lines and no haploid or twin seedlings have been found so far. Neither was its performance as to 3 other agronomic characters different from that of the normal line. On the other hand, the substitution line of *T. vulgare* var. Salmon behaved quite differently from its normal line. It was completely male-sterile and the frequencies of haploids and twins were extremely high. Also its performance concerning 3 other characters deviated significantly from that of the normal line. Responses of 2 other varieties to the same alien cytoplasm were, in general, between those of the above two. From those results it can be said that the degree of influence of an alien cytoplasm

Table 1. Performance of normal and nucleus-substitution lines of 4 hexaploid wheats.

Strain	Pollen fertility (%)	Seed fertility (self) (%)	Freq. of haploids (%)	Freq. of twins (%)	Heading date (day)	Plant height (cm)	Dry matter weight (gm)
T. vulgare var. *erythrospermum*							
Normal line	96.7	60.5	0.3	0.2	199.3	135.4	179
Substitution line	0.2**	0.0**	3.1**	3.9**	199.3	131.0	178
T. vulgare var. Salmon							
Normal line	89.4	75.1	0.0	0.3	197.8	112.2	95
Substitution line	0.5**	0.0**	27.9**	10.7**	192.2*	120.2*	125*
T. spelta var. *duhamelianum*							
Normal line	88.2	79.4	0.0	0.0	203.0	120.0	257
Substitution line	0.0**	0.5**	2.0	2.0	204.4	114.3**	194*
T. compactum var. No. 44							
Normal line	98.0	80.2	0.0	0.0	198.3	110.3	181
Substitution line	84.7**	35.3**	0.0	0.0	199.4	106.6	139

* and **: Substitution line is significantly different from the corresponding normal line at the 5% and 1% level, respectively.

on the manifestation of a wheat nucleus is mostly determined by its whole genotype.

43. *Production of tetraploid rice by N_2O-treatment**

(By Hitoshi KIHARA and Koichiro TSUNEWAKI)

Treatment of pollinated florets with nitrous oxide (N_2O) was demonstrated in wheat to be a very effective means for production of polyploids (Kihara and Tsunewaki 1960). The same treatment was applied last year to a cultivated rice variety in the following way. All florets of potted plants, except those which flowered just one day before the treatment, were removed from every panicle. The plants were placed in a steel tank and the air was sucked out by a vacuum pump, until the pressure inside the tank became 1/3 atm. Then it was filled with N_2O gas up to the pressure of 5 atms. Plants were kept in this condition for 12 hrs. After the treatment they were grown under natural condition. The seeds were sown and the seedlings were examined for their chromosome number. The result of this experiment, as shown in Table 1, indicates that N_2O-treatment is useful for the production of tetraploid rice.

Table 1. Frequency of tetraploids in the offspring of N_2O-treated rice; treatment was given for 12 hrs. at 5 atms., from one day after pollination.

Panicle No.	No. florets treated	No. seeds set	% seeds set	No. seeds germinated	% germination	No. tetraploids (including aneuploids)	% tetraploids	
							per germinated seed	per treated floret
1	36	34	94	23	68	7	30	19
2	36	35	97	24	69	7	29	19
3	27	26	96	20	77	3	15	11
4	25	25	100	23	92	3	13	12
5	37	35	95	17	49	9	53	24
6	22	21	96	14	67	3	21	14
Total	183	176	96	121	69	32	26	18

* This work was supported by Grant RF 62027 from the Rockefeller Foundation.

44. *Frequency of aneuploids in the offspring of 7 synthesized 6x wheats*

(By Koichiro TSUNEWAKI)

More than 30 6x wheats have been synthesized from different varieties of Emmer wheat and *Aegilops squarrosa* (MacFadden and Sears 1944, Kihara *et al.* 1950, Tanaka 1961, Sears unpubl.). Investigation of their genetic stability has a great significance in the considerations on the origin and evolution of common wheat. Among those synthetics, one strain, produced from *Triticum persicum* var. *stramineum*×*Ae. squarrora* var. *typica*, was studied by Tabushi (1957). He reported high frequency (about 27%) of aneuploids in its offspring. In order to obtain a general picture of the genetic stability of synthesized 6x wheat, chromosome numbers in the offspring of 7 synthetics, produced by Kihara *et al.*, Sears and Tanaka, were investigated. The result is summarized in Table 1 in which the frequency of apparent aneuploids with more or less chromosomes than 42 is indicated. However, plants with 42 chromosomes are not necessarily assumed to be euploid; they might be deficient for one chromosome and have another one duplicated.

This result clearly indicates that all synthesized 6x wheats are more or less genetically unstable. In total, 28.2% of the offspring were aneuploids, the majority being hypo-aneuploids. No clear-cut difference in aneuploid frequency was found among the 7 strains, though 2 of them, whose Emmer parent was *T. dicoccum* var. Vernal or *T. dicoccoides* var. *spontaneo-nigrum*, seemed to be slightly stabler than the others.

45. *Genetic studies of a 6x-derivative from an 8x Triticale**

(By Koichiro TSUNEWAKI)

A 6x strain named "Salmon" (2n=42) was obtained in the progeny of a hybrid between two strains of 8x *Triticale* (2n=56). In order to elucidate the genetic make-up of this derivative, several genetic investigations were undertaken.

(1) Salmon was crossed to *Triticum aestivum* var. Chinese Spring. The F_1 hybrid showed 21_{II} in a majority of PMC's. The extent of meiotic irregularities was definitely comparable to that observed in inter- or intrasubspecific hybrids of *T. aestivum*.

(2) A detailed analysis of chromosome configurations in the F_1 hy-

* This work was supported by Grant RF 62073 from the Rockefeller Foundation.

Table 1. Chromosome numbers in the offspring of 7 strains of synthesized 6x wheat.

Strains*	No. progenies	No. plants examined	Somatic chromosome no.						% aneuploids
			44 or more	43	42	41	40	39 or less	
T. orientale var. *insigne*	1	15	0	1	10	3	1	0	33.3
T. persicum var. *stramineum***	3	112	1	8	76	22	3	2	32.1
T. durum var. Golden Ball	1	16	0	0	11	4	1	0	31.3
T. durum var. Carleton	10	353	3	20	253	58	16	3	28.3
T. durum var. Pentad	1	19	0	1	14	4	0	0	26.4
T. dicoccum var. Vernal	1	18	0	0	15	3	0	0	16.7
T. dicoccoides var. *spontaneo-nigrum*	1	17	0	0	16	1	0	0	5.9
Total		550	4	30	395	95	21	5	28.2

* Strains of the synthesized 6x wheat were indicated by their Emmer parent. In all cases, *Aegilops squarrosa* var. *typica* was used as the *squarrosa* parent.

** Including the result of Tabushi (1957).

brid and a comparison of karyotypes of its parents in root-tip mitosis revealed that the satellited arm of one of the two wheat Sat-chromosomes was replaced in Salmon by an arm of a rye chromosome.

(3) Monosomic analysis of Salmon on waxiness indicated that *W* gene responsible for waxiness of common wheat is located on chromosome XIII. Non-waxy character of Salmon is attributable to the deficiency of a chromosome segment comprising the *W* locus.

(4) Nucleus substitution lines of Salmon and three other 6x wheats, all of which were produced by Dr. H. Kihara, were compared with the the respective normal lines. The substitution line of Salmon was superior to Salmon itself, while those of the other wheats were in general inferior to the corresponding normal lines.

From these results it is concluded that 8x *Triticale* was converted to the 6x wheat by elimination of chromosome complement of rye. However, the remaining wheat chromosome complement was slightly modified by integrating some chromosome segments of rye and losing some of wheat. This modification seems to be the main factor responsible for the peculiar developmental behavior of Salmon's nucleus substitution line.

46. *Transmission of monosomes and trisomes in an Emmer wheat, T. dicoccum var. Khapli*

(By Koichiro TSUNEWAKI)

As reported earlier (Kihara and Tsunewaki 1960, 1962; Tsunewaki, 1962), several aneuploids of an Emmer wheat, *T. dicoccum* var. Khapli were produced among polyploids by N_2O-treatment of artificially pollinated spikes. Three monosomics and 6 trisomics were obtained so far. Transmission of the monosomes and trisomes to their offspring has been studied. The result is summarized in Table 1.

No monosomics were recovered in the offspring of all 3 monosomics, although 2 trisomics were found. No transmission or a very low one of monosomes seems to be due to the low fertility of chromosome-deficient female gametes, because seed fertility of the monosomics was very low (about 30%) even at artificial pollination with pollen grains from disomic Khapli. This fact indicates that monosomics of, at least, this Emmer wheat are not suitable for genetic investigation, except that they might serve as a source of trisomics.

On the other hand, many trisomics (24% on the average) were obtained in the offspring of trisomics. A single tetrasomic was also found. Transmission rates of the trisome through female and male gametes

Table 1. Fertilities of mono- and trisomics of *T. dicoccum* var. Khapli and the frequency of aneuploids in their offspring.

Plant	% seed set (selfed)	No. of offspring				
		Total examined	Mono-somic	Tri-somic	Tetra-somic	Haploids
Monosomics						
K-mono-1	20.0	8	0	1	—	—
〃 2	35.0	5	0	—	—	—
〃 3	39.3*	11	0	1	—	—
Total	33.1	24	0	2	—	—
Trisomics						
K-tri-1a	100.0	36	—	9	0	—
〃 1b	92.5	38	—	6	1	—
〃 1c	83.3	35	—	9	0	1
〃 2	82.5	35	—	10	0	—
〃 3	62.5	24	—	6	0	—
〃 4	73.3	37	—	9	0	—
Total	82.3	205	—	49	1	1

* Seed fertility by artificial pollination with normal pollen.

were estimated to be 23% and 2%, respectively. Transmission rate of the trisome in this Emmer wheat is much lower than that reported for common wheat but is almost the same as in barley. Trisomics of the employed Emmer wheat, therefore, may be convenient for genetic study.

47. *An Emmer wheat with 15 chromosome pairs*[1)]

(By Koichiro TSUNEWAKI)

Since the work of Sakamura (1918) and Sax (1918), the chromosome numbers in *Triticum* are known to be 2n=14, 28 and 42 for 2x, 4x and 6x species, respectively. No exception to this polyploid series has been reported. During the course of a cytological investigation of the 4x wheat, the present author found 15 instead of the expected 14 chromosome pairs in a strain of *Triticum durum* Desf. var. *melanopus* (Al.) Körn. Thirteen pairs consisted of median or submedian chromosomes, while those of the other two pairs were telocentrics. Both telocentric pairs showed some meiotic irregularities. In a great majority of PMC's,

1) This work was supported by Grant RF 62073 from the Rockefeller Foundation.

however, they formed bivalents in diakinesis (Fig. 1) and metaphase (Fig. 2) and segregated in 1 : 1 fashion in anaphase. Karyotype analysis (cf. Fig. 3) indicated that the two telocentric pairs must have arisen by fragmentation of a submedian chromosome in the normal complement of Emmer wheat.

Figs. 1-3. Chromosomes of a strain of *Triticum durum* var. *melanopus*. ×700. Fig. 1. Diakinesis with 15_{II}. Fig. 2. Metaphase I with 15_{II}. Fig. 3. Mitotic metaphase in a root-tip cell of haploid spontaneously occurred in this *durum*.

48. *Further proof that Type 1 necrosis is controlled by the Ne-genetic system*

(By Kôzo NISHIKAWA)

It was shown in an earlier paper (Nishikawa 1962) that Type 1 necrosis found in hybrids from certain Emmer-*squarrosa* crosses could be attributed to *Ne*-genetic system. The new data confirm the earlier interpretation in showing that Ne_3, which causes necrosis when present together with Ne_1 and Ne_2, is responsible for the expression of Type 1 necrosis in the hybrids involving Khapli (*T. dicoccum* var. *arras*), and that necrosis genes carried by Khapli, which interact with Ne_3, are the same as Ne_1 and Ne_2 of common wheat.

Chinese Spring monosomic series was crossed with Khapli. The F_1's were scored for chromosome number and occurrence of necrosis (Table 1).

All F_1 plants were necrotic except those nullisomic for 3D. Comparable data were already presented by Tsunewaki and Kihara (1961) in crosses involving *T. macha* var. *subletshchumicum* ($Ne_1Ne_1Ne_2Ne_2ne_3ne_3$) instead of Khapli.

A hybrid between *T. macha* var. *sublet.* and Khapli was normal but did not segregate any normal plants when crossed with Prelude ($Ne_1Ne_1ne_2ne_2Ne_3Ne_3$) or Jones Fife ($ne_1ne_1Ne_2Ne_2Ne_3Ne_3$).

These results indicate that Khapli carries Ne_1 and Ne_2 on chromosome 5B and 2A, respectively. In other words Type 1 necrosis found

in certain Emmer-*squarrosa* hybrids is controlled by the *Ne*-genetic system established by Tsunewaki and Kihara (1961).

Table 1. Occurrence of necrosis in F_1 between Chinese Spring monosomics and Khapli.

Strains	No. of chromosomes	No. of plants		
		normal	necrotic	total
cm- 1A × Khapli*	34	0	5	5
〃 2A × 〃	35	0	4	10
	34	0	6	
〃 3A × 〃	34	0	10	10
〃 4A × 〃	35	0	2	10
	34	0	8	
〃 5A × 〃	34	0	7	7
〃 6A × 〃	35	0	3	10
	34	0	7	
〃 7A × 〃	35	0	2	8
	34	0	6	
〃 1B × 〃	35	0	1	10
	34	0	9	
〃 2B × 〃	35	0	2	8
	34	0	6	
〃 3B × 〃	35	0	2	10
	34	0	8	
〃 4B × 〃	35	0	2	10
	34	0	8	
〃 5B × 〃	35	0	4	10
	34	0	6	
〃 6B × 〃	35	0	5	10
	34	0	5	
〃 7B × 〃	34	0	8	8
〃 1D × 〃	35	0	2	10
	34	0	8	
〃 2D × 〃	35	0	2	10
	34	0	8	
〃 3D × 〃	35	0	2	10
	34	8	0	
〃 4D × 〃	35	0	2	10
	34	0	8	
〃 5D × 〃	35	0	1	10
	34	0	9	
〃 6D × 〃	35	0	3	10
	34	0	7	
〃 7D × 〃	35	0	2	9
	34	0	7	

* cm: Chinese Spring monosomics.

49. *Distribution of necrosis genes in Emmer wheat*

(By Kôzo NISHIKAWA)

There are 2 genetic systems for necrosis in wheat hybrids, namely *Ne*

and *Net* systems. In Emmer wheat, 4 genotypes, namely $Ne_1Ne_1Ne_2Ne_2$, $Ne_1Ne_1ne_2ne_2$, $ne_1ne_1Ne_2Ne_2$ and $ne_1ne_1ne_2ne_2$ are possible in *Ne*-system but only one gene and consequently only 2 genotypes occur in the *Net*-system, namely Net_1Net_1 and net_1net_1. In order to get an information on the frequency of those genotypes, hybrids were produced between 4 test varieties on one hand and 40 Emmer varieties on the other. Occurrence of necrosis in the F_1's was observed and the pertinent genotype for each Emmer variety was formulated. As previously shown by the present author (1962), Type 1 is epistatic over Type 2 necrosis. Khapli, that carries Net_1 in addition to Ne_1 and Ne_2, produced hybrids showing Type 1 necrosis, when crossed with ABD No. 1, the carrier of Ne_3 and Net_2. Therefore, it could not be determined from F_1 phenotype whether Khapli carried Net_1 or net_1. The same applies to 4 varieties of *T. dicoccoides*, i.e. *kotschyanum*, *straussianum*, P. B. I. and *aaronsohni*.

The result is summarized in Table 1 showing the relative frequencies of all possible genotypes in 8 Emmer species. It is remarkable that genotype Ne_1Ne_2 is confined to *T. dicoccoides*, with the exception of a *dicoccum* variety. This suggests that the dominant alleles Ne_1 and Ne_2 were the prototypes and in the evolutional course have mutated to the recessive alleles, ne_1 and ne_2. The most widely spread genotype in Emmer is Ne_1ne_2, followed by ne_1ne_2 and Ne_1Ne_2. It is noteworthy that ne_1Ne_2 has never been found in Emmer varieties so far tested. As to *Net* system both Net_1 and net_1 are almost equally distributed among the 8 Emmer species.

Table 1. Relative frequencies of genotypes of *Ne*- and *Net*-genetic systems in Emmer wheat.

Specie	*Ne*-system					*Net*-system		
	Ne_1Ne_2	Ne_1ne_2	ne_1Ne_2	ne_1ne_2	Total	Net_1	net_1	Total
T. dicoccoides	5	5	0	3	13	3	7	10
T. dicoccum	1	0	0	2	3	4	1	5
T. durum	0	13	0	2	15	7	10	17
T. orientale	0	1	0	0	1	0	2	2
T. pyramidale	0	1	0	0	1	0	1	1
T. turgidum	0	0	0	2	2	1	1	2
T. polonicum	0	2	0	1	3	2	1	3
T. persicum	0	3	0	0	3	0	3	3
Total	6	25	0	10	41	17	26	43

50. *Frequencies of Ne_1 and Ne_2 genes in Emmer and common wheats*

(By Koichiro TSUNEWAKI and Kôzo NISHIKAWA)

Three complementary genes, Ne_1, Ne_2 and Ne_3, cause in combination a progressive necrosis in common wheat. Ne_1 gene is located in B, Ne_2 in A and Ne_3 in D genome. Among them, Ne_1 and Ne_2 were extensively investigated by Caldwell and Compton (1943), Heyne, Wiebe and Painter (1943), Hermsen (1957, '59, '62, '63), Schmalz (1959), Tsunewaki (1960) and Tsunewaki and Kihara (1962). The number of examined varieties amounts to 520. For 4 of them, a contradictory result was reported by different authors. Those two genes are also found in Emmer wheat and their distribution among 41 varieties was investigated by Tsunewaki and Kihara (1962) and Nishikawa (1962a, b). Excluding the contradictory cases, frequencies of 4 possible genotypes in two groups of wheat are summarized in Table 1. From these results, frequencies of individual alleles were calculated and are listed in the same table.

In Emmer wheat, frequencies of 4 genotypes are in good agreement with those expected from random assortment between two pairs of alleles. Concerning each allelic pair, the dominant allele predominates in the Ne_1 pair, while the recessive is prevalent in the Ne_2 pair. In common wheat, frequencies of 4 genotypes deviate from those expected from random assortment. Frequencies $Ne_1Ne_1ne_2ne_2$ and $ne_1ne_1Ne_2Ne_2$ are much higher

Table 1. Frequencies of 4 genotypes and alleles for necrosis in Emmer and common wheats.

	Emmer wheat		Common wheat	
	Actual	Expected*	Actual	Expected*
Freq. of each genotype (No. of varieties)				
$Ne_1Ne_1Ne_2Ne_2$	6	4.53	1	35.38
$Ne_1Ne_1ne_2ne_2$	25	26.47	87	52.85
$ne_1ne_1Ne_2Ne_2$	0	1.46	206	171.53
$ne_1ne_1ne_2ne_2$	10	8.54	222	256.23
Total	41	41.00	516	515.99
Freq. of each allele (Relative freq.)				
Ne_1	0.756		0.171	
ne_1	0.244		0.829	
Ne_2	0.146		0.401	
ne_2	0.854		0.599	

* Expected from random assortment between two pairs of alleles.

than the expected ones from randomness. In this group of wheat, the recessive allele is predominating in Ne_1 allelic pair, while the frequencies of the 2 alleles of the other pair, were not markedly different from each other.

Those results clearly indicate that: (1) two genotypes, $Ne_1Ne_1ne_2ne_2$ and $ne_1ne_1Ne_2Ne_2$, are preferentially selected in common wheat against the two others, and (2) a shift in the gene frequencies must have occurred at the hexaploid level in favor to Ne_2 and, especially, ne_1.

51. *A polyhaploid plant of Agropyron tsukushiense var. transiens Ohwi found in a state of nature*

(By Sadao SAKAMOTO)

A. tsukushiense var. *transiens*, a common weed growing in fields along road-sides, is widely distributed in Japan, China, Manchuria and Korea. This species is hexaploid ($2n=42$).

In a valley of the hilly neighborhood of Misima, spontaneous polyhaploid plant ($2n=21$) of the species was found. It was smaller than the hexaploid specimens and had slender spikes, but its tillering was very vigorous. In natural condition it yielded two seeds which were

Table 1. Chromosome pairing at MI of PMCs of the polyhaploid and a monosomic of *A. tsukushiense* var. *transiens*.

Polyhaploid:

		No. of cells observed	%
	21_I	369	83.5
	$1_{II}+19_I$	61	13.8
1_{III}	$+18_I$	2	0.5
	$2_{II}+17_I$	8	1.8
1_{IV}	$+17_I$	1	0.2
	$3_{II}+15_I$	1	0.2
Total		442	100.0

Monosomic:

	$20_{II}+1_I$	164	97.0
	$19_{II}+3_I$	5	3.0
Total		169	100.0

assumed to have been produced by pollination with the pollen of hexaploid plants growing in its proximity. The two seeds gave two plants, one a disomic ($2n=42$) and the other a monosomic ($2n=41$).

Pollen fertility of the polyhaploid, the monosomic and the disomic was 0.001, 28.5 and 78.5% and seed fertility was 0, 53.3 and 76.6%, respectively. The polyhaploid was crossed by the disomic and by two *A. tsukushiense* var. *transiens* strains, and the crossing success amounted to 1.0~1.6%.

Chromosome pairing at MI of PMCs of the polyhaploid and the monosomic is given in Table 1. Of 442 cells of the polyhaploid 83% showed 21_{I} and 14% showed 1_{II} (terminal pairing)$+19_{I}$. In a few cells 1_{III} or 2_{II} were observed. In the majority of cells of the monosomic, chromosome pairing was $20_{II}+1_{I}$. This result indicates that *A. tsukushiense* var. *transiens* is an allohexaploid whose genome constitution comprises three different genomes.

52. *Preliminary studies on the relationship between diploid Eremopyrum distans Nevski and E. triticeum Nevski (Gramineae)*

(By Sadao SAKAMOTO and Mikio MURAMATSU*)

E. distans was crossed as the female parent with *E. buonapartis* Nevski var. *buonapartis*, *E. triticeum* and *Heteranthelium piliferum* Hochst. Only seeds from the cross *E. distans*×*E. triticeum* germinated. The F_1 hybrid showed vigorous and uniform growth. Most of the characters of the F_1 hybrid were intermediate between the parents. The F_1 offspring set no seeds. Chromosome pairing at MI of the F_1 was $0\text{-}3_{II}$ with 0_{II} as the mode. All bivalents were loosely associated by a terminal chiasma. It is concluded that the genomes of *E. distans* and *E. triticeum* are different.

53. *Spodogram analysis of leaf-blade in the genus Oryza***

(By Hitoshi KIHARA and Tadao C. KATAYAMA)

The surface structure of the glume is an important taxonomic character in the genus *Oryza*, as was recognized by Roschevicz. In order to examine the details, the present authors have studied the glume of 18 *Oryza* species by SUMP method (1960) and by histological observations (1962). Exploring other taxonomic characters similar to glume characteristics, ash figures

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of leaves, with special consideration of the silica cells, were examined this year, using 30 strains of 21 *Oryza* species.

On both ends concave silica cells were found in the species of section *Sativa*, while those with flat ends were observed in the sections *Granulata*, *Coarctata* and *Rhynchoryza*. The silica cells were classified into 5 morphological types.

(1) *Officinalis* type: Peanut-shaped (or dumbbell-shaped) silica cells concave at both ends were found in *O. officinalis*, *O. sativa*, *O. sativa* var. *spontanea*, *O. perennis*, *O. barthii*, *O. glaberrima* and *O. breviligulata*.

(2) *Minuta* type: Cross-shaped silica cells were observed in *O. minuta*, *O. latifolia*, *O. alta* and *O. grandiglumis*.

(3) *Australiensis* type: Silica cells are of slender peanut-shape, concave at both ends as observed in *Officinalis* type. This type was found only in *O. australiensis*.

(4) *Granulata* type: Silica cells of many different shapes, elliptic-~peanut-shaped, were found in this type. Their arrangement and size were also irregular. This type was detected in *O. granulata*, *O. meyeriana*, *O. abromeitiana*, *O. coarctata*, *O. ridleyi*, *O. longiglumis and O. subulata*.

(5) *Brachyantha* type: The silica cells of this type were a mixture of two kinds, namely *Granulata* type and modified wider *Granulata* type. Found in two species, *O. brachyantha* and *O. tisseranti*.

It is concluded that the morphology of silica cells could be used as a good taxonomic character in the genus *Oryza*.

54. *Occurrence of indica and japonica types among Sikkimese rice varieties**

(By Hitoshi KIHARA and Tadao C. KATAYAMA)

Kihara and Nakao (1960) reported *indica* and *japonica* types in Sikkimese rice based on the examination of morphological characters, phenol reaction and seed fertility. The most important key for the classification of cultivated rice into *indica* and *japonica* types is hybrid sterility between strains. Therefore, in order to confirm our classification of Sikkimese varieties, pollen fertility of F_1 hybrids with *indica* and *japonica* tester strains was studied.

Fourteen strains of Sikkimese rice were selected and crossed with two testers, namely an *indica* strain from Formosa, and a *japonica* strain from Japan. Phenol reaction of those Sikkimese strains was reexamined.

In confirmation of our classification, pollen fertility of F_1 hybrids

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clearly indicated the existence of both *indica* and *japonica* types in Sikkimese rice. Of 14 strains studied, C7716, C7725 and C7729 are of typical *indica* type, while C7707, C7719 and C7735 represent the typical *japonica* type.

Considering the present results obtained in pollen fertility, it is assumed that the differentiation of *indica* and *japonica* types in cultivated *Oryza sativa* might have occurred in the Indian sub-continent, particularly in Sikkim.

55. *Photoperiodic responses of Oryza species, VI**

(By Tadao C. KATAYAMA)

Several factors are responsible for different photoperiodic sensitivities among various *Oryza* strains. The acceleration degree, which was expressed by the differential heading acceleration under short day condition, was considered in a previous paper. In this report, the acceleration degrees of several strains distributed in high altitudes is reported. Nineteen strains belonging to *Oryza sativa* var. *spontanea* Roschev. and *O. perennis* Moench collected at Sikkim and Assam were used in this study. Two cultivated strains, i.e., No. 124 from India and KA from Japan were also used for the sake of comparison.

The acceleration degree was expressed by (1) the coefficient of linear regression of growth period on sowing time (denoted by LRC-1), (2) the coefficient of linear regression of growth period on day length at the time of flower bud formation (LRC-2), and (3) the angle of the slope of the regression line used in (2) (Angle). The results are summarized in Table 1.

All strains of high altitudes were highly sensitive having 0.85 to 1.00 in LRC-1, 2.55 to 29.50 in LRC-2 and 68.6 to 88.1 in Angle, respectively. Their heading dates strongly depended upon day length. They showed a rather unusual deviation of their critical day length from the general tendency, which was found in the correlation between critical day length and latitude. This phenomenon was explained as follows: Since their native places are very high (ca. 5,000 ft.), temperature rather than day length seems to have played the essential role in their adaptation.

It is assumed that rice plants growing in such high altitudes as in Sikkim and Assam might complete their life cycle during a short period when the natural temperature is suitable for their growth. This result clearly indicates that at high altitudes the predominating strains respond to a small change in day length which gives them a selective advantage in their natural conditions.

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Table 1. Acceleration rates of heading dates.

Strain	Heading date when sown on		LRC-1	LRC-2	Angle
	Apr. 17	June 16			
W0157	10-20*	10-29	0.85	2.55	68.6
W0635	10-22	10-26	0.93	6.22	80.9
W1079	10-25	10-26	0.98	19.89	87.1
W1081	10-24	10-26	0.97	11.60	85.1
W1082	10-26	10-26	1.00	—	—
W1083	10-24	10-26	0.97	11.60	85.1
W1084	10-26	10-26	1.00	—	—
W1085	10-26	10-26	1.00	—	—
W1090	10-16	10-17	0.98	19.89	87.1
W1091	10-15	10-17	0.97	11.60	85.1
W1092	10-16	10-17	0.98	19.89	87.1
W1095	10-25	10-25	1.00	—	—
W1098	10-21	10-26	0.92	4.58	77.7
W1099	10-22	10-24	0.97	14.50	86.1
W1100	10-19	10-25	0.90	4.15	76.5
W1102	10-23	10-24	0.98	29.50	88.1
W1104	10-24	10-24	1.00	—	—
W1105	10-24	10-26	0.97	11.60	85.1
W1107	10-19	10-22	0.95	8.14	83.0
W1108	10-19	10-22	0.95	8.14	83.0
W1115	10-19	10-21	0.97	14.50	86.1
W1116	10-20	10-21	0.98	29.50	88.1
W1117	10-20	10-21	0.98	29.50	88.1
W1121	10-22	10-22	1.00	—	—
W1134	10- 9	10-10	0.98	29.50	88.1
124	9-25	10- 7	0.80	1.92	62.5
KA	9- 7	9-26	0.65	0.96	43.8

* Headed on October 20.

56. *Characteristics of Oryza officinalis Wall. found in Borneo*[**]

(By Tadao C. KATAYAMA)

During the collection tour of *Oryza* species in Borneo made in 1963,

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46 strains of *O. officinalis* were found at different localities in North Borneo, Brunei, Sarawak and Kalimantan. These strains were conspicuously different from those collected in other tropical Asiatic regions in the following characteristics.

1) Native localities of 46 strains of *O. officinalis* were widely scattered over the whole island of Borneo, whereas those of strains found in other regions were rather limited to narrow areas. Population size of each strain found in Borneo was much larger than that of the strains found in other regions of tropical Asia.

2) In Borneo *O. officinalis* was found not only in shady places under the trees but also in open fields. Sometimes it was growing sympatrically with *O. sativa* in the paddy fields. On the contrary, the natural habitats of most strains of this species collected in other regions were shady spots in the forest.

3) High variation was found in size and shape of grains, leaf length and plant height of *O. officinalis* collected in Borneo.

4) The ratio of photoperiodically sensitive vs. insensitive plants among 46 strains of *O. officinalis* collected in Borneo was 7 to 5. The value indicates a considerable difference from the ratio, 1 sensitive to 3 insensitive, obtained for the strains of this species found in other regions. It is noteworthy that many sensitive strains were detected even in Borneo, an equatorial island, situated between 7°N to 4°S latitude.

Judging from those characteristics of *O. officinalis* found in Borneo, it is assumed that *O. officinalis* might have originated in Borneo or its adjacent islands, and that photoperiodically sensitive strains of this species might be the original form from which insensitive strains would have derived.

57. *Studies on the intercellular spaces of an Assamese floating rice**

(By Tadao C. KATAYAMA)

Intercellular spaces in leaves of *Oryza sativa*, *Zea mays* and *Vicia faba* were examined by the present author in the 1961. This year the leaves of an Assamese floating rice (strain No. C7795) were investigated. The seeds were sown and seedlings were grown both in paddy and upland conditions. The leaves were cut off with sharp scissors and divided into pieces each 3 cm long. The intercellular spaces were measured at different growth stages. Table 1 shows an example of total intercellular spaces in leaf blade and leaf sheath of 3rd, 4th and 5th leaf in a given stage.

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The sheath of a fully expanded leaf had always a wider total intercellular space than the blade. The total intercellular space in leaves of plants grown in paddy condition was larger than that of plants in upland condition. This difference was recognized in any growth stage of the plant. The most interesting result was that the development of air spaces in the leaf was markedly influenced by cultivation conditions, especially by the water content of the soil. The present result is an experimental evidence for the well-known opinion that the floating rice is very well adapted to deep water condition.

Table 1. Percentage and standard deviation of total intercellular spaces in leaves of an Assamese floating rice strain (C7795).

Growth stage \ Cultivation condition		Paddy	Upland
3rd leaf	blade	12.80±0.02	8.73±4.16
	sheath	13.22±8.79	9.07±6.62
4th leaf	blade	18.83±3.78	5.18±1.71
	sheath	31.75±1.96	9.07±6.62
5th leaf	blade	14.07±4.36	3.40±0.41
	sheath	20.67±0.19	6.32±3.18

58. *A genetic study of developmental instability in tobacco leaf*

(By Kan-Ichi SAKAI and Yoshiya SHIMAMOTO)

Diallel crosses were conducted with four varieties of tobacco. Two of the four were unstable in leaf formation while the remaining two were stable. Instability in leaf formation was observed in three ways: bilateral asymmetry, within-leaf variation in the distance between adjoining veins on the mid-rib and vein-deviation index measured by the degree of non-parallelity between two adjoining veins. The three measures of instability were in close association.

Analysis of variance of the data shows that inter-line variation was statistically significant for each of the three measures of instability. Dominance deviation associated with specific parents was significant for vein distance and asymmetry but not for vein-deviation index. Reciprocal differences were not detected. It is thus concluded that the instability is due mainly to the additive effect of genes, but in vein distance and in asymmetry, partial dominance effect seems also to be operative in some parental strains.

The summary of our data is presented in the following table.

Table 1. Genetic behavior of developmental instabilities in leaves in F_1 hybrids of *Nicotiana tabacum*.

	Vein distance			Asymmetry			Vein-deviation index		
	Mid-parent	F_1	Recip. diff.	Mid-parent	F_1	Recip. diff.	Mid-parent	F_1	Recip. diff.
A×C	6.75	6.90	0.45	177	159	9.7	4.97	5.09	0.72
A×S	7.63	8.12	0.12	165	119	7.3	5.32	5.02	0.60
A×T	8.92	9.88	0.23	207	193	−20.3	6.38	6.27	0.60
C×S	5.13	4.93	−0.16	125	113	0.3	4.04	3.95	−0.03
C×T	6.42	5.77	0.05	167	187	2.2	5.10	4.71	−1.29
S×T	7.30	7.37	0.52	155	129	7.9	5.45	5.63	0.32
Total	42.15	42.97		996	900		31.26	30.67	

59. *A developmental-gentic study of panicle characters in rice*

(By Kan-Ichi SAKAI and Yoshiya SHIMAMOTO)

Forty-six X_4 lines and the same number of control lines, each derived from the same rice variety, Norin No. 8, were examined for five panicle characters. The five characters were lengths of the main rachis, basal internode, and second, third and fourth rachillas from the base. Measurements were taken on a single panicle basis for all panicle-bearing tillers of a plant. Number of plants examined per line was 12.

The present study consists of two parts, one being the estimation of pleiotropic effect of main rachis genes over the other four characters, and the other, the inference of developmental relationships between them on the basis of their developmental instability. The estimation of pleiotropic parameters was made by the method of Sakai and Suzuki described in our companion contribution to this Annual Report. The inference of developmental relationships among different characters on the basis of their developmental instability is based on the following principle: If two given characters are found to be pleiotropically controlled by the same gene, they may either take their own developmental path separately at an early stage of development or they may start developing together in a single process until differentiation sets in at a later stage.

If developmental instability of a full-grown character is the reflection of accumulated effects of developmental noise occurring in the process, then it is expected that late differentiated characters may have a good share in instability, while early differentiation may make their instabilities independent from each other. Conversely speaking, genetic correlation

coefficients of developmental instability between two characters may reveal their developmental relationship which is genetically controlled.

Results of the present study have led us to conclude that: (1) Forty-six lines in the X_4 as well as in the control group involved genetic variation in all five characters investigated. (2) Genes responsible for length of the main rachis are pleiotropically effective on the length of the other four characters, though the intensity of pleiotropy varies among the latter. (3) It is found that the pleiotropic influence of the main rachis genes is less pronounced on the lower than on the higher rachilla. Basal internode is also partly affected by the same genes. (4) Forty-six lines of the control group were found to involve genetic variation in developmental instability of the five characters. (5) Computation of genetic correlations among instabilities of the five characters have shown that the main rachis and the fourth rachilla are developmentally very closely related, while the second rachilla is remotely related to it.

Details of the present study will be published before long.

60. *Density response and competitive ability in barley*

(By Kan-Ichi SAKAI and Shin-ya IYAMA)

In 1962, the density response was examined with twelve varieties of barley. Spaces assigned to each plant were 2×2, 4×4, 8×8, 16×16 and $32\times32\,cm^2$. Examined were on an individual plant basis plant height, number of tillers, number of panicles, length of the longest panicle, dry plant weight and seed weight. Analysis of variance showed that the effect of density was statistically highly significant for all characters except plant height.

It was found that the number of tillers increased linearly in proportion to widening the interplant spacing, while seed yield per plant behaved somewhat differently. The increase in seed yield was linear up to $16\times16\,cm^2$, but at wider spacing, it appeared to attain a plateau in some varieties. Plant weight was intermediate between tiller number and seed yield so far as density response was concerned. This is natural because dry plant weight consists of seed and straw weights, the former representing a good part of it. It was concluded from this experiment (1) that the spacing at which the increase in seed yield attained a plateau was different among genotypes, (2) that the overall response to plant density was different among genotypes, and (3) that attaining a plateau in seed yield at wider spacing was not necessarily associated with the vegetative growth of the given plant. It was considered the non-parallelism between reproductive and vegetative growth could be due to intraplan

competition in seed setting for the limited supply of available assimilation products.

In 1963, competitive ability of the same 12 varieties was examined with the aid of one variety as a tester. The experiment was conducted with four replications. The results proved that competitive ability was significantly variable among the 12 varieties.

Combining the two years' data mentioned above, it was examined, whether or not competitive ability and density response were related. The correlation coefficients between density response and comptitive ability were 0.548 for plant weight, 0.410 for number of tillers and 0.145 for seed yield, all falling short of the 5% level of significance.

There is a general tendency among ecologists and agronomists to confound the effect of density increase with the effect of competition. The present experiment, however, indicates that the two should not be mixed, but should be considered separately, one as density response and the other as competitive reaction.

61. *Survey of F_1 sterility relationships between strains of Oryza perennis*

(By Hiroko MORISHIMA and Hiko-Ichi OKA)

Oryza perennis is distributed throughout the tropical countries of the world. It comprises various geographical races. A survey of F_1 sterility relationships between Asian strains was reported previously (Jap. J. Genet. 37: 314–328). The same work was extended to South American and African (*barthii*) materials. The American strains, forming an intra-fertile group, generally showed a high F_1 sterility in crosses with Asian and African strains. The F_1 hybrids between African and Asian strains showed a wide range of pollen fertility. In some of those crosses, crossability was low, or the F_1 seeds did not germinate well because of the poor development of the embryos. These phenomena are still under observation.

62. *Characters relative to the breeding system in Oryza species*

(By Hiroko MORISHIMA and Hiko-Ichi OKA)

Wild rice species, *O. perennis* as well as *O. breviligulata*, are partly cross-pollinated. With the view to looking into their breeding system, a number of strains belonging to these species were investigated regarding 1) time interval from spikelet openning to pollen emission, 2)

longevity of pollen grains emitted from the anther, 3) number of pollen grains per anther, 4) anther length, and 5) the size of stigmas. In addition, regenerating or ratooning ability was estimated by measuring the development of adventitious roots from stem-cuts with the first or second node placed in moist sand for three days. In each character, a wide variation was found among the strains. The characters supposedly promoting cross-pollination (1–5) were correlated with one another, and also with regenerating ability.

63. *Change of population genotype of rice hybrids in response to different propagating methods*

(By Hiko-Ichi OKA and Hiroko MORISHIMA)

Hybrid populations between wild (*Oryza perennis*) and cultivated (*O. sativa*) strains were propagated until F_7 by three different methods, selfing in pedigrees, open-pollination (seedlings raised in pedigrees were mix-planted in bulk), and mix-harvesting in bulk. The plants propagated in mix-harvesting bulk rapidly approached cultivated forms and had a relatively small heterozygosity, possibly because of increasing selfing rate. Those selfed in pedigrees did not approach cultivated forms so rapidly. These facts suggest that a wild population containing a sufficient amount of genetic variability can change toward cultivated forms in response to selection pressure under cultivation. This work is still under way.

64. *Observations of wild and cultivated rice species in West and Central Africa*

(By Hiko-Ichi Oka)

From October 1963 to January 1964, together with Dr. Wen-Tsai Chang of the Academia Sinica, Taiwan, I visited Sierra Leone, Guinea, Mali, Niger, Dahomy, Nigeria, Cameroun and Tchad for studies of wild and cultivated rice species. Our main tasks were to investigate variations in *Oryza perennis* subsp. *barthii*, hybrid swarms between *O. breviligulata* and *O. glaberrima*, and other *Oryza* species, and to study basic problems underlying rice breeding in Africa. The results of observations have been described in a mimeographed report submitted to the Institute. To our great sorrow, almost at the end of the expedition Dr. Chang lost his life in a car accident.

65. *Polyploidization and RNA and protein content of wheat seeds*

(By Toru ENDO)

Table 1. Protein and RNA content of wheat seeds

Species and Genome	Prolamin (60% EtOH)	Albumin (H_2O)	Gloublin (2% NaCl)	Glutelin (.2% NaOH)	Total	RNA-P
T. spelta AABBDD	3.32 mgN/g (37%)	0.54 mgN/g (6%)	0.32 mgN/g (4%)	4.72 mgN/g (53%)	8.92 mgN/g	229μg/g
T. durum×*Ae. squarrosa* AABBDD	1.93 (31%)	0.58 (9%)	0.56 (9%)	3.20 (51%)	6.27	208
T. durum AABB	2.21 (36%)	0.48 (8%)	0.29 (5%)	3.16 (52%)	6.14	212
T. dicoccoides AABB	1.98 (33%)	0.71 (12%)	0.39 (7%)	2.91 (49%)	5.98	241
T. monococcum×*Ae. squarrosa* AADD	2.84 (32%)	0.34 (4%)	0.44 (5%)	5.34 (60%)	8.92	311
Ae. squarrosa 4x DDDD	2.38 (30%)	0.71 (9%)	0.61 (8%)	4.16 (53%)	7.86	190
T. monococcum AA	2.76 (34%)	0.65 (8%)	0.54 (7%)	4.15 (51%)	8.10	176
Ae. squarrosa DD	3.34 (37%)	0.92 (10%)	0.28 (3%)	4.46 (50%)	9.00	178

Abbr.: *T.*; *Triticum* and *Ae.*; *Aegilops*

Ribosomal RNA (rRNA), which represents 80 to 90 per cent of total RNA, is synthesized in conjuction with the nucleolar-associated DNA and then probably moves into the cytoplasm. The purpose of this work was to test whether an increase in DNA content due to auto-and allo-polyploidization has any effect on the rRNA and protein content of wheat seeds.

Materials used were selfed seeds of *Triticum* and related naturally ocurring polyploids as well as artificial amphidiploids. In each experiment, 400 to 800 mg of seeds was used. For the estimation of protein contents, seeds were macerated in cold acetone before extraction. Differential extraction of the proteins were made with the solvents listed in Table 1. Protein content was estimated by the phenol method and calculated in terms of serum albumin. Determination of RNA content was carried out by the Ogur-Rosen method.

There is positively a similarity in the relative concentration of each specific protein (Table 1). Also, no significant correlation was observed between the RNA content and polyploidization.

The present results seem to show that the increase of DNA content by polyploidization has in general no appreciable influence on the controlling mechanism of rRNA synthesis except for AADD type. Considerably larger amount of RNA in AADD than in the other employed tetraploid was observed.

66. *Secondary association in Lillium auratum*

(By Yō TAKENAKA and Y. E. CHU)

Secondary association in PMCs and karyomorphology of somatic chromosomes of *Lillium auratum* were studied. For karyological analysis the somatic chromosomes were divided into four groups (A, B, C, D) by their length, place of constriction and other chracters, and the occurrence of each type was expressed; $A_1A_2B_1B_2C_1C_2C_3C_4C_5D_1D_2D_3$.

The investigation of secondary association at diakinesis showed 12(1)*, 1(2)+10(1)** and 2(2)+8(1)*** figures with frequencies of 16.3% 30.4% and 27.5% respectively. At MI 12(1), 1(2)+10(1) and 2(2)+8(1) figures were observed with frequencies of 50.1% 30.6% and 11.7% respectively. According to the above data, the frequencis of secondry association at MI were less than those at diakinesis. This is assumed to be due to

* 12(1) indicates 12 bivalents.

** 1(2)+10(1) indicates 1 secondary association of 2 and 10 bivalents.

*** 2(2)+8(1) indicates 2 secondary association of 2 and 8 bivalents.

the decrease of attraction between the chromosomes, during the progress of meiotic stages.

If the phenomenon of secondary association is due to specific affinity between homologous chromosome segments, secondary association should occur always between the same chromosomes. At diplotene, it was found that there was high frequency of association between A_1A_1 and D_1D_1 and also between A_2A_2 and B_2B_2 or B_1B_1 (it was very difficult to distinguish B_1 from B_2 chromosome). From this finding, it could be assumed that there were some homologous segments between non-homologous chromosomes. At pachytene, it was found that D_1 always touched the short arm of A_1 and the short arm of B_1 or B_2 always touched the short arm of A_2.

From the above results, it is assumed that this species could have derived by hybridization between two closely related varieties.

67. *Cytological studies on a Nicotiana tumorous hybrid and its parents*

(By Yoshiaki YONEDA and Yô TAKENAKA)

Cytological and growth characters of a tumorous hybrid, *N. glauca*×*N. plumbaginifolia*, and its parents were studied *in vitro*. The materials used were as follows: "Normal" and "tumor" tissues were isolated from normal stems and tumor tissues of the hybrid respectively, and callus tissues were obtained from parental hypocotyle.

As control, we first observed mitotic cells of small, young *in vivo* tumors produced on the stem of hybrid. They had almost all 22 normal somatic chromosome. Polyploid and aneuploid cells were rarely found.

Chromosome number of the above four tissues cultured on White+yeast extract+2,4-D agar medium were counted. Though the frequency of normal diploid cells was high, polyploid and aneuploid cells were also frequently found, especially in the callus of *N. glauca*.

In order to reveal the relation of the variation in chromosome number to medium components, the tissues were cultured on basic media containing auxins, kinetin, single or in combination. In the "normal" tissue of the hybrid cultured on media containing both auxin and kinetin, the frequency of diploid cells was higher than that of polyploid ones, while in the "tumor" tissue polyploid cells became more frequent on the same media. In kinetin (2 ppm) medium, mitotic cells of the "tumor" tissue were predominently polyploid. Thus "normal" and "tumor" tissues were assumed to respond differently to kinetin concerning the occurrence of polyploid cells. The cultured tissue of *N. plumbaginifolia* had

cosistently a very high frequency of diploid cells. On the other hand, only polyploid cells were found in the callus of *N. glauca* at the time of this experiment.

In "normal" and "tumor" tissues of the hybrid and in the callus of *N. glauca*, anaphase bridges were found in high frequency on the various media used. A few such bridges were also found in the callus of *N. plumbaginifolia*. As most of the bridges were not accompanied by chromosome fragments, these might have been sticky bridges. Such bridges were demonstrated in the "tumor" tissue of the hybrid and the parental calli cultured on White's basic medium. As the mitotic cells were very few, such sticky bridges were not yet recognized in "normal" tissue cultured on the basic medium.

The "normal" and "tumor" tissues of the hybrid grew slowly on White's basic medium. The calli of parental species increased in fresh weight and some mitotic cells were also recognized when they were cultured on the basic medium. This hormone-independent growth of the callus tissues is considered to represent the so-called habituation under cultured conditions. The growth of *N. glauca* callus was stimulated by the addition of kinetin, auxin or both to the basic medium, whereas the callus of *N. plumbaginifolia* showed the stablest growth on White+2,4-D+kinetin+yeast extract.

68. *Tumors in Japanese morning glory*

(By Yô TAKENAKA and Yoshiaki YONEDA)

We found tumorous outgrowths in a strain, 056, of Japanese morning glory, which was supplied to the senior author by Mr. Omori in 1960. Since then many tumor-bearing plants have been cultivated in this institute. We succeeded in obtaining *in vitro* cultures of the tumor tissue in 1962. The present report is concerned with morphological and cytological characters of both *in vivo* and *in vitro* tumor tissues.

The tumor-producing plants retained the cotyledons throughout their whole life. Their leaf development was very variable. Some plants had curled and crooked leaves with sharp-pointed lobes. These phenotypic characters are assumed to be due to the genes "Blown", "delicate" and "feathered". They often bloomed, but the flowers were completely sterile. In other plants after the cotyledons no leaves appeared, only clumps of abnormal shoots. Such a plant had the appearance of teratoma. The small, swollen shoots had many leaflet-like protuberances below the apex. We often found a spheroidal lump of tumor tissue on such an

abnormal shoot. It is unknown at present how to explain these various phenotypes. One of the causes might be the complexity of the genetic background system in the original horticultural stock.

Histologically the tumor showed much disturbances in tissue organization. Meristematic tissues were distributed at the surface of the tumor and procambium strands ran through the parenchyma. In the interior, various types of vascular nodules were found. In general, the vascular tissues were developed strongly, but their distribution was irregular.

In cultured condition, the tumor tissue could grow on White's basic medium, while normal callus isolated from the wild type of morning glory showed no growth on this medium. This result indicated that the tumor growth was hormone-independent; it was promoted by yeast extract. The tumor tissue was yellow-brown and had very hard texture. Histologically a great amount of vascular tissues developed in its interior and the meristematic tissues were found only on the surface. For constant growth of normal callus, addition of auxin, kinetin and casein hydrolyzate or yeast extract to the basic medium was necessary; it was yellow and very soft in texture and mostly consisted of meristematic and parenchymatous tissues. Thus the vascular development in the tumor tissue was very different from that of the normal callus.

In cytological studies a difference was found; namely, polyploid cells were recognized in the tumor tissue cultured on White+2,4-D+yeast extract medium, while normal callus contained only diploid cellss on the same medium.

Tumor formations in Japanese morning glory are considered to be related to some genic systems (or genic combination). In the present case, at least three genes were identified in the tumorous plants. From the result, we have assumed that the tumor production in this plant may be included in the category of genetic tumors.

69. *Spring- and Winter-sown varieties of sugar beets, with special reference to photothermal induction*

(By Seiji MATSUMURA)

Induction of flowering in sugar beets (*Beta vulgaris* L.) has two important practical aspects. Development of seedstalks, *i.e.* bolting, is objectionable when the crop is grown for sugar production, but when the crop is grown for seed production initiation and complete development of seedstalks is desirable. All investigated varieties are longday and biennial plants.

Experiments with Spring-sown (Hon-iku 192, GW 359, *etc.*) and Winter-

sown varieties (Kleinwanzleben-AA, Cesena-P, *etc.*) confirmed that treatment of 5~6 month old beets with the temperature of 1~4°C for 1~2 months (July and August) increased the tendency to bolting; one month treatment was not effective in inducing bolting in Winter-sown varieties, especially in polyploids, but was effective in Spring-sown ones. The vernalized beets could be devernalized by subsequent treatment even at 20°C day and 19°C night temperature in an air-conditioned greenhouse with continuous lighting. Subsequent treatment at about 10°C in a growth cabinet at natural day length was markedly more effective than that in field cultivation. It is assumed, therefore, that the climatic conditions in Hokkaido district are suitable only for seed production of Winter-sown varieties, which are promising for sugar production in Southwestern districts of Japan, where (for instance, islands of the Inland Sea, Kagawa-ken) even seeds of Spring-sown varieties, mostly cultivated in Hokkaido for sugar production can be produced.

C. MATHEMATICAL GENETICS

70. *The mutational load in small populations*

(By Motoo KIMURA)

It is customary, in the calculation of the mutational load, to assume that the population is infinitely large and the gene frequency is exactly determined by the balance between mutation and selection. However, in a finite population, the factor of random drift becomes involved and both the gene frequency and the load become random variables.

Applying Wright's formula for the gene frequency distribution in finite populations, the mean value of the mutational load was calculated numerically by the use of an electronic computer.

The study showed that in a small population the load is considerably larger than in a large population. Futhermore, a rather unexpected result emerged: Namely, for a wide range of population sizes, a mutant that is slightly harmful is more damaging to the fitness of the population than a mutant with a much greater harmful effect.

The variance of the mutational load has also been computed. The results show that intergroup selection is ineffective in reducing this load.

The present study was done in collaboration with Dr. J. F. Crow and Mr. T. Maruyama, while the author was visiting the University of

Wisconsin. For details, see Kimura, M., J. F. Crow and T. Maruyama (1963) Genetics 48: 1303-1312.

71. *The stepping stone model of population structure*

(By Motoo KIMURA)

In Number 3 (1953) of these annual reports, the author proposed a new model for the breeding structure of populations which he called stepping-stone model. In this model it is assumed that the entire population is subdivided into colonies and the migration of individuals in each generation is restricted to nearby colonies. At that time, no satisfactory mathematical treatment of the model was obtained.

Recently, while the author was visiting the University of Wisconsin, the complete solution of the model concerning the decrease of genetic correlation with distance was obtained in collaboration with Dr. G. H. Weiss of the University of Maryland.

It has been shown that the decrease of genetic correlation with distance depends very much on the number of dimensions:

In one dimension, the correlation decreases approximately exponentially with distance:

$$r(\rho) \propto e^{-A\rho}$$

where ρ is the distance and A is a positive constant. In two dimensions, it falls off more rapidly and if the migration is isotropic in X and Y directions, we obtain for a large value of ρ, the relation

$$r(\rho) \propto e^{-B\rho}/\sqrt{\rho},$$

where B is a positive constant. Finally in three dimensions, it falls off still more rapidly and asymptotically,

$$r(\rho) \propto e^{-C\rho}/\rho,$$

where C is again a positive constant.

The quantity $1-r_1$, where r_1 is the correlation coefficient between two adjacent colonies, is also relevant when the tendency toward random local differentiation is discussed. It has been shown that the relation of this quantity to mutation and migration rates depends very much on the number of dimensions. This, together with the above results on the decrease of correlation at a large distance, clearly shows that the tendency toward random local differentiation is strongest in one dimension and becomes weaker as the number of dimensions increases. The details will be published in the April 1964 issue of Genetics.

72. *Effect of maternal inheritance on the genetical variance in a segreating population*

(By Shin-ya IYAMA)

In the Annual Report No. 10, the author reported on the effect of maternal inheritance on the variance in a hybrid population of autogamous plants. More general formula is now presented and maternal effect in a random mating population is discussed.

According to Sakai's model (1960),

$$P_n=(1-m)G_n+mP_{n-1} \tag{1}$$

where P_n and G_n stand for the genotypic values with and without maternal effect in the n-th generation respectively, and m represents the proportion of maternal effect in the total hereditary effect. In case of allogamous organisms, the maternal parents are to be traced back.

Formula (1) may be written by repeated substitution as follows:

$$P_n=(1-m)\{G_n+mG_{n-1}+\cdots+m^{n-1}G_1\}+m^nP_0 \tag{2}$$

Then, the mean and the variance in the n-th generation are,

$$\bar{P}_n=(1-m)\sum_{i=1}^{n}m^{n-i}\bar{G}_i+m^n\bar{P}_0 \tag{3}$$

$$V_{P_n}=(1-m)^2\sum_{i=1}^{n}m^{2(n-i)}V_{G_i}+(1-m)\sum_{i<i}2m^{2n-i-j}W_{G_iG_j}$$
$$+2m^n(1-m)\sum_{i=1}^{n}m^{n-i}W_{P_0G_i}+m^{2n}V_{P_0} \tag{4}$$

where V_{G_i} and V_{P_0} stand for the variances of G_i and P_0 respectively, and $W_{G_iG_j}$ and $W_{P_0G_i}$ stand for the covariances between G_i and G_j and between P_0 and G_i respectively.

Assuming random mating and no change of gene frequency, we may obtain the following relationships:

$$\bar{G}_i=\bar{G}_j=\bar{G},\ V_{G_i}=V_{G_j}=V_G,\ W_{G_iG_j}=W_{G_kG_l}\qquad if\ i-j=k-l,$$
$$W_{G_iG_{j+t}}=(\tfrac{1}{2})^tW_{G_iG_j}\ \text{and}\ W_{P_0G_{i+t}}=(\tfrac{1}{2})^tW_{P_0G_i}.$$

Then,

$$\bar{P}_n=(1-m)\bar{G}\sum_{i=0}^{n-1}m^i+m^n\bar{P}_0\qquad\sim\bar{G}\ (n\to\infty)$$

$$V_{P_n}=(1-m)^2V_G\sum_{i=0}^{n-1}m^{2i}+(1-m)^2W\sum_{j=0}^{n-2}(\tfrac{1}{2})^j\sum_{j=0}^{n-j-2}2m^{2i}$$
$$+2m^n(1-m)W_{P_0G_1}(\tfrac{1}{2})^{n-1}\sum_{i=0}^{n-1}(2m)^i+m^{2n}V_{P_0}$$

where W stands for convariance between genotypic values of the maternal parent and its offspring. Since $V_G=\sigma_g^2+\sigma_d^2$ and $W=\frac{1}{2}\sigma_g^2$, total genetical variance in a random mating population will approach to

$$V_{P_{n_\infty}}=\frac{(1-m)\ (2+m)}{(1+m)\ (2-m)}\sigma_g^2+\frac{(1-m)}{(1+m)}\sigma_d^2.$$

After sufficiently large number of generations, the maternal effect on the population mean will be lost and the genetical variances will reach a constant value, both the additive genetic variance and the dominance variance being smaller than when no maternal effect exists.

D. GENETICS AND BIOCHEMISTRY OF MICROORGANISMS

73. *Allelic repression in transductional heterogenotes of Salmonella**

(By Tetsuo IINO)

In *Salmonella-phage P22* system, a heterogenotic line occurring by transduction of an H_1-*fla* segment from a flagellated cell to a nonflagellated *fla-A*$^-$ mutant cell produces a trail of minute colonies on semisolid nutrient medium (Iino, Annual Report No. 9, 1958). Trail formation by *fla*$^+$/*fla*$^-$ heterogenotes is suppressed when specific antisera against H (flagella) antigens of their component cells are added to the semisolid media (Iino, Annual Report No. 13, 1962). This phenomenon was applied to identify H-antigen type expressed by H_1^i/H_1^b heterogenotes.

Transductions of H_1-*fla* segments were carried out from *S. typhimurium* (*i*:*1,2*) to a phase-1 culture of a *fla-A*$^-$ mutant of *S. abony* (*b*:*e,n,x*). Genotype of the resulting heterogenotes is expected to be $H_1^b fla^-/_{ex}H_1^i fla^+$. In this combination, trail production on semisolid media is suppressed by anti-*b,e,n,x* but not by anti-*i,e,n,x* sera. This result indicates that the $H_1^b fla^-/_{ex}H_1^i fla^+$ heterogenotes produce flagella carrying *b* antigen.

In transductions from *S. abony* to a *fla-A*$^-$ mutant of *S. typhimurium*, trail production by the resulting heterogenotes, $H_1^i fla^-/_{ex}H_1^b fla^+$, is suppressed by anti-*i,1,2* but not by anti-*b,1,2* sera.

A general rule deduced from these results is that heterogenotes of H_1 gene, resulting from the *P22*-phage mediated transduction between two *Salmonella* serotypes, produce flagella determined in their specificity

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by the endogenote, and the function of exogenotic H_1 is repressed by endogenotic H_1.

Allelic repression of endogenotic H_1 to exogenotic H_1 was also observed in transductions between *S. typhimurium* (*i:1,2*) and *S. dublin* (*g,p:-*).

When phase-2 cultures are used as recipients in the transductions described above, expression of both exo- and endo-genotic H_1 is repressed by the phase-2 antigen type determinant, H_2.

74. *Peptides pattern of flagellin of normal and curly mutant flagella in Salmonella abortus-equi**

(By Masatoshi ENOMOTO and Tetsuo IINO)

A curly flagellar mutant, SJ30, originated from a wild type strain of *S. abortus-equi*, SL23, has flagella of half wavelength compared with the wild type. It is attributed to a mutation in the structure gene of phase-2 flagellin, H_2. H-antigen type of the curly flagella is the same as that of the wild type, *enx*. Flagellins of these two strains, SL23, and SJ30, were crystalyzed (Enomoto and Iino, Annual Report No. 13, 1962), and two dimensional patterns of peptides from their tryptic digests were compared with each other. For the tryptic digestions of heat denatured flagellin, 2% flagellin and 0.04% trypsin in 0.05M-phosphate buffer (pH 8.0) were incubated for 20 hr. at 25°C with a drop of toluene. Two-dimensional separation of the produced peptides was performed by electrophoresis followed by chromatography. By electrophoresis at pH 6.5 in pyridine acetate buffer, peptides from both, SL23 and SJ30 flagellins, were divided into eleven groups based on charge differences. Distributions of these groups on the electrophoreograms were identical for the two strains. By subsequent chromatography with n-butanol-acetic acid-water system, 34-35 spots were separated further from eleven groups of peptides. Among them, the patterns of 32-33 peptides, among which 4 were histidine- and 8 were arginine-containing, were indistinguishable between SL23 and SL30. One peptide spot involved in group 4 of the electrophoreogram of SL23 was absent in SJ30. Instead, a new peptide spot appeared at a more distal point from the original line. The remaining peptide appeared at the same position in both strains but its amount was larger in SJ30 than in SL23. Together with the genetic data (Iino, Annual Report No. 9, 1958), these results support the previously proposed hypothesis that the morphological change of flagella by curly mutation is primarily caused by an alteration of an amino acid code in the structure gene of flagellin.

* This work was supported by a research grant from the National Institute of Allergy and Infectious Diseases (AI-02872), Public Health Service, U.S.A.

75. *Inhibition and regeneration experiments of the flagellar synthesizing system of Salmonella cells**

(By Michiko MITANI)

Phenol, lithium chloride and 44°C have been known as the specific agents which inhibit flagellar formation of growing bacteria (Iino & Mitani, Annual Report No. 13, 74, 1962). In an attempt to disclose a flagellar synthesizing system in bacterial cells, the search for specific inhibitors of flagellar synthesis of *S. typhimurium* was continued, and their effects were compared.

In addition to the above three agents, lithium carbonate and cresol were found to inhibit flagellar synthesis. The concentrations which inhibit flagellation of bacterial cells and yet minimize the growth inhibition are 0.12% and 0.20%, respectively, for phenol and lithium carbonate. Although final growth is diminished at those concentrations approximately by one tenth in contrast with the control, the number of flagella per bacterium is progressively decreased following cell divisions. But if the resulting deflagellated bacteria are transferred to synthetic regeneration medium, *de novo* flagellar synthesis is performed after a lag corresponding to the time requiring at least one bacterial division.

In the course of the regeneration experiment of flagella, the bacteria deflagellated previously by an inhibitory agent are found to regenerate their flagella in the regeneration medium containing certain other inhibitory agents. Namely, after preincubation in the medium with added 0.12% phenol, the deflagellated bacteria were transferred into broth containing 0.20% lithium carbonate or to a synthetic regeneration medium at 44°C. Then, flagellar regeneration of the cells was observed in the medium incubated at 44°C but not in the broth containing 0.20% lithium carbonate. When the cells were deflagellated by lithium carbonate treatment, flagellar regeneration was well observed both in the regeneration medium containing 0.12% phenol and in the medium incubated at 44°C. On the contrary, if the deflagellated cells were obtained by cultivation at 44°C, the cells did not regenerate their flagella either in the medium with added 0.12% phenol or with 0.20% lithium carbonate.

From these results it is assumed that each of the inhibitors affects a different step of flagellar synthesis; the most plausible order of sites inhibited by each agent is considered to be: 44°C→phenol→lithium carbonate.

* This work was partly supported by a research grant from the National Institute of Allergy and infectious Diseases (AI-02872), Public Health Service, U.S.A., to T. Iino.

76. *Mapping of the mutant sites in motility A-cisron of Salmonella typhimurium**

(By Masatoshi ENOMOTO)

With a few exceptions, mutant sites causing paralysis of flagella in *S. typhimurium*, TM2, are classified into two functional units, either *mot-A* or *mot-B* cistron, by means of the cis-trans test (Enomoto, Annual Report No. 13, 1962). From 48 mutants in *A*-cistron, 12 mutants showing no tendency to revert were chosen and transductions of the motility genes were carried out with phage P22 in all pairwise combinations. It was found that 10 out of 12 mutants failed to give motile recombinants with one or more mutants, and the remaining two recombined with all other mutants (Fig. 1). In order to map the reverting mutants, that is, those containing small alterations, further qualitative (yes-or-no) tests for recombinations were carried out between 12 stable mutants and all other members of *A*-cistron. To avoid confusions between recombination and reverse mutation, 12 stable mutants were used as recipients and the reciprocal tests were carried out only in crosses with relatively stable mutants. Out of 36 mutations tested, 15 mutations overlapped at least with one of the stable mutations including both SJ631 and SJ634

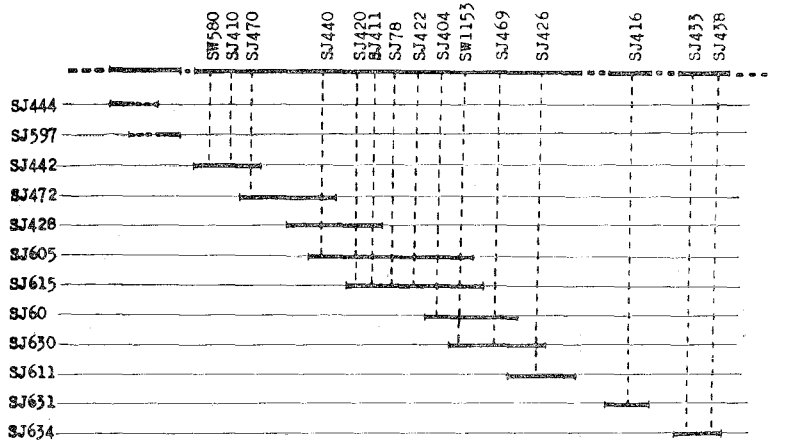


Fig. 1. Genetic map of *motility A*-cistron. Strains described vertically are extended (deletion) mutations. Horizontals represent the tentative alignment of mutation sites which overlap with extended mutations.

* This work was supported by a research grant from the National Institute of Allergy and Infectious Diseases (AI-02872), Public Health Service, U.S.A. to T. Iino.

which gave recombinants with all other stable mutants (Fig. 1). None of 36 mutations overlapped with the stable mutations, SJ444 and SJ597, which gave no recombinant with each other. These two mutants were found to revert at a very low frequency in repeated experiments, so that they are likely point mutants in closely adjacent sites. Except these two, stable mutants included relatively large alterations, so-called deletions, and their sites could be arranged in a linear sequence.

77. *Effect of phenol on the expression of multiple drug resistance of Shigella(R)*

(by Tetsuo IINO and Masatoshi ENOMOTO)

Shigella flexneri 2b-103 carries an episome, R, which confers on the infected cells resistance to four antibiotics, namely sulfonamide (Sa), streptomycin (Sm), tetracyclin (Tc) and chloramphenicol (Cm).

From growth experiments in media containing both 0.06% phenol and various concentrations of the antibiotics, it was found that the addition of phenol increased remarkably the degree of resistance of the 2b-103 cells to Cm and to less extent to Sm, and reduced the resistance to Tc (Fig. 1).

Preincubation of the cells in phenol containing media increases the degree of resistance more than 100 times to Cm as compared with the culture without preincubation in phenol broth (Table 1).

The modification of resistance is maintained only in the presence of phenol. When the cells are transferred from a phenol containing to a phenol free medium, the degree of resistance reverts to the original level.

Table 1. Effect of phenol on the resistance of *Shigella* 2b-103(R) to Cm.

pre-incubation	no. of surviving cells per ml of broth culture* containing		
	Cm (γ/ml) / phenol (%)	0	100
broth	0	2.6×10^9	$<10^3$
	0.06	2.8×10^8	3.4×10^4
broth+0.06%phenol	0	2.4×10^9	$<10^3$
	0.06	$4\cdot7\times10^8$	5.8×10^6

* 20 hr. incubation at 37°C; initial inocula: 10^4 cells/ml.

It has been inferred that infection with R-factors causes an alteration of the surface structure of the infected cells leading to reduction of permeability of the cell membrane for antibiotics. Thus, phenol may modify the surface structure of R-infected cells so as to favor the permeation of Tc and prohibit that of Cm and Sm.

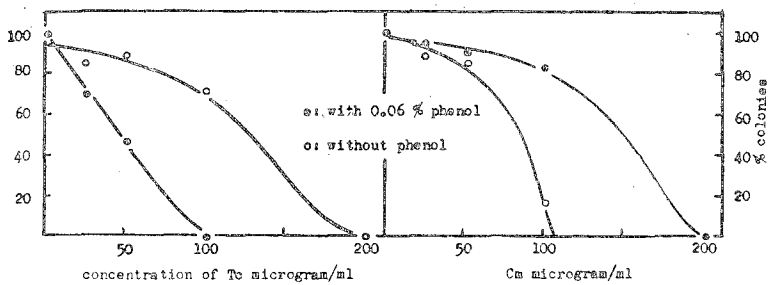


Fig. 1. Effect of phenol on drug resistance of *Shigella flexneri*. Colony number on nutrient agar (drug free) is taken as 100.

78. *Sensitization to phenol by infection of Salmonella with multiple drug resistance factors (R)*

(By Tetsuo IINO and Michiko MITANI)

It was found that the infection of R-factors to a strain of *S. typhimurium* TM2 results in sensitization of the infected cells to phenol as well as conferring resistance to four antibiotics, namely sulfonamide (Sa), streptomycin (Sm), tetracyclin (Tc) and chloramphenicol (Cm). TM2 cells are sensitive to all four at the concentrations of 10μg/ml of Sm, 10μg/ml of Cm, 5μg/ml of Tc and 50μg/ml of Sa. In the growth of broth culture this strain is reduced to 50% by adding 0.05% phenol to the media and is completely inhibited by 0.25% phenol.

An R-factor originally detected from *Shigella flexneri* 2b-103 was transferred via *Escherichia coil* W3102 to TM2. Resistance of the R-infected TM2, designated TM2(R), to antibiotics Sm, Tc, Cm and Sa, increased up to the level of 50 to 100μg/ml against the first three and to 1000μg/ml against the last one. While its resistance to phenol decreased after R-infection to about 50% in comparison with TM2. Complete growth inhibition occurred already in 0.12% phenol broth. Subclones of TM2(R) which spontaneously lost resistance to Tc and/or Sm still maintained the same level of sensitivity to phenol as the four drug resistant clones. Occurrence of this phenomenon in other bacterial strains is under investigation.

79. *Preliminary studies on an arginine-sensitive mutant of Salmonella typhimurium*

(By Jun-ichi ISHIDSU)

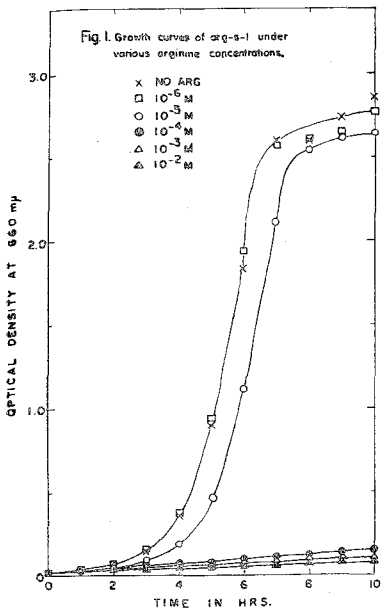
A new arginine-sensitive mutant (*arg-s-1*) was isolated from a wild-type strain of *Salmonella typhimurium* LT-2 by 2-aminopurine treatment. This mutant grows normally in synthetic minimal medium (K_2HPO_4, 10.5 g; KH_2PO_4, 4.5 g; $MgSO_4$, 0.1 g; $(NH_4)_2SO_4$, 1 g; sodium citrate, 0.47 g; glucose, 4 g; water, 1,000 ml) and can also grow almost normally in nutrient broth. But when the synthetic medium is supplemented with arginine (either hydrochloride or carbonate), its growth is specifically inhibited. The critical point of arginine concentration effective in growth inhibition of *arg-s-1* in liquid medium lies between 10^{-5} M and 10^{-4} M (Fig. 1). If the concentration of arginine is not too high but sufficient to inhibit at least the initial growth, the mutant suddenly becomes free from inhibition after a lag period, and starts growing at the same rate as it does in the absence of arginine. The lower is the original arginine concentration, the shorter is the lag period, and once growth starts, it continues until a similar level of stationary phase is attained having no relation to the original arginine concentration. This phenomenon suggests that arginine in the medium is being exhausted or inactivated in some way during the lag period even without significant growth of the bacteria. In addition, it was found that, if the glucose concentration is brought up to 1%, or higher, the inhibition period is shortened by the hour.

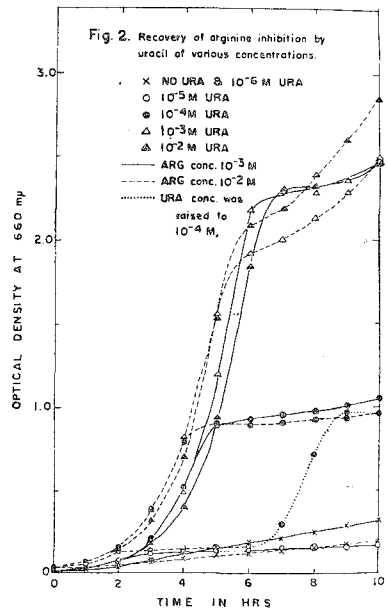
Among various common amino acids, vitamins, purines, or pyrimidines, so far as tested, only arginine can affect the growth of *arg-s-1*.

Most interestingly, this growth inhibition by arginine is removed immediately by adding a sufficient amount of uracil or some of its precursors or related substances, such as orotic acid, uridine, cytosine, cytidine, isocytosine, or uridylic acid. No other chemicals so far as tested could readily rescue *arg-s-1* from arginine inhibition. Tests with uracil indicate, as shown in Fig. 2, two facts: (1) When an equivalent amount of uracil to arginine, or more, is added to the medium, the growth inhibition is removed at once and the bacteria grow at the normal rate and attain the normal stationary level, and (2) when an insufficient amount of uracil, but higher than 10^{-5} M in concentration, is supplied, the bacteria start growing at the normal rate but the growth suddenly stops when a certain level, far below the normal stationary level, is attained. This stop is not brought about by arginine concentration but by uracil concentration only.

Since this mutant has no auxotrophic requirements, the damage of

its DNA is, most probably, not concerned with the parts controlling protein structure (structural genes), but those that are responsible for some kind of cellular regulation systems (regulator or operator genes).





80. *Comparative studies on mutagenic efficiencies of incorporated ^{32}P and γ-rays with Salmonella*[1]

(By Hiromi ISHIWA, Yonhon YAN[2] and Sohei KONDO)

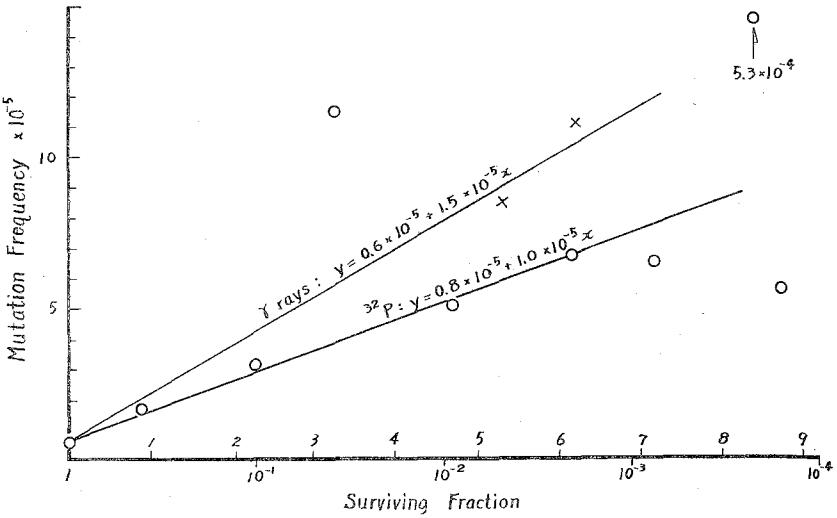
The mutation inducibility due to the decay of ^{32}P incorporated in *Salmonella* TL2 was compared with non-radioactive control *Salmonella* irradiated by gamma-rays. The forward mutation was observed from *gal*$^+$ to *gal*$^-$ colony plated on EMG-galactose complete agar plates (Lederberg), when the mutated colony showed pink or red color among many green wild type colonies.

The mutation frequencies (y) for two different kinds of mutagens, ^{32}P and gamm-rays, were plotted in Fig. 1 against survival fraction and lethal hits (x), respectively. As the figure shows, ^{32}P induced mutation

[1] This work was supported by Grant from the Toyo Rayon Foundation for the Promotion of Science and Technology.

[2] Department of Nuclear Science, Faculty of Science, Kyoto University, Kyoto.

frequency was rather lower than that caused by gamma-rays at the same survival fraction. From our experimental results, transmutation of ^{32}P atoms incorporated into the genetic substance seems to be little effective in inducing non-lethal mutations. Hence the mutations detected in this case may be mainly due to the internal as well as external effects of ^{32}P β-rays. Many contradictory results against this conclusion were reported by Dr. KAUDEWITZ *et al* (1958).



Comparison of mutation inducibility by disintergation of ^{32}P incorporated in Salmonella with that of γ-ray irradiation.

81. *Chemical modification of nucleic acids and their constituents*

(By Tomotaka SHINODA)

In order to investigate the aromatization of ribonucleic acid by TNBS and the specificity limitation of ribonuclease action towards the substrate, the effect of this reagent upon the individual constituents of RNA was determined. Under several conditions employed, the reaction with free base occurred only with guanine, and the others were not reactive towards the reagent. Up to the present, the following 12 derivatives of the constituents of RNA were obtained in monotrinitrophenyl form; adenosine, methyl adenosine, O-acetyl adenosine, guanine, guanosine, O-acetyl guanosine, cytidine, O-acetyl cytidine, adenylic acid and cytidylic acid, and in addition to these inosine derivative was also prepared. Funda-

mental physicochemical properties of these materials were observed in ultraviolet and infrared spectra and for electrophoretic and chromatographic behavior. A characteristic absorption of these derivatives in alkali medium was seen at 410 to 430 mμ, and in addition to that the absorption at 260 mμ which corresponds to that of bases. On the other hand absorption at 410 to 430 mμ seen in the alkali disappeared in acid medium while at 260 mμ it was unchanged or several of the derivatives showed an increase in absorption.

Preliminary studies of the effects of UV- and ionizing radiation on the derivatives were carried out by ultraviolet spectrophotometry and paper chromatography. In some cases liberation of picric acid was observed both in spectrophotometry and paper chromatography. Effects of chemical agents such as iodoacetate, dimethylsulfate, nitrous acid and periodate upon the trinitrophenylated derivatives of base were also studied.

Purified yeast RNA was used in the reaction, and the base composition of the material followed by the treatment with alkali was analyzed by paper chromatography. Based on the results, it has been determined that the reaction of RNA with the reagent occurred mainly in the sites of purine moieties of RNA. Ultraviolet absorption spectrum of modified RNA was very similar to that of modified guanosine, this observation being in good agreement with the result from base composition of modified RNA. Relative velocities of the action of ribonuclease upon intact and modified RNA were compared in the usual fashion, and a decreased velocity was observed in the latter. This is assumed to be due to the result of the reaction upon purine moiety of RNA.

E. RADIATION GENETICS IN ANIMALS

82. *Heterozygous effects of radiation-induced mutations on viability in homozygous and heterozyous genetic backgrounds in Drosophila melanogaster* (II)*

(By Terumi MUKAI, Isao YOSHIKAWA and Tsuneyuki YAMAZAKI)

To confirm the experimental results concerning the heterozygous effect of radiation-induced mutations on viability presented in the Annual Report No. 13, experiments were conducted in one homozygous and two

* This work has been supported by Grant RH-34 from the Public Health Service, U.S.A..

heterozygous (intra- and inter-populational hybrids) genetic backgrounds.

Two isogenic lines, A_1A_1 (W160S) and A_2A_2, extracted from the same population (W-1), and another isogenic line (BB) obtained from an entirely unrelated population were prepared for the irradiation with respect to the second chromosome. The X, the third and the fourth chromosomes of A_2A_2 and BB were substituted by those of A_1A_1.

After X-ray irradiation at the dose of 500 r, (irradiation is indicated by superscript (′)), heterozygotes A_1A_1', $A_1'A_2$ and $A_1'B$ were produced by the aid of *Cy/Pm Ubx/Sb* strains. The relative viabilities of irradiated heterozygotes with respect to the second chromosome were estimated in comparison with $Cy/+_{A_1}$ (in A_1A_1'), $Cy/+_{A_2}$ (in $A_1'A_2$) and $Cy/+_B$ (in $A_1'B$). The irradiated X and third chromosomes were completely substituted by the unirradiated ones of A_1A_1 before the estimation of viability. The control experiments were conducted in the same way without irradiation. The results are presented in Table 1.

From Table 1, it can be seen that radiation-induced mutations which occurred in homozygous individuals were heterozygously beneficial to their carriers, i.e., the average viability of A_1A_1' was larger than that of A_1A_1 by 0.0264 and this value is significantly different from zero and consistent with the previous result, while those that occurred in heterozygous genetic backgrounds, both in intra- and inter-populational hybrids

Table 1. Heterozygous effects of radiation-induced mutations on viability in *Drosophila melanogaster*.
(2nd chromosome, sperm, acute 500r)

Genotype	No. of chromosome lines tested	Total no. of flies counted	Average viability	(irradiated) −(control)
A_1A_1	292	222,446	1.0150	0.0264**
A_1A_1'	291	227,788	1.0404	
A_1A_2	308	283,633	1.0322	0.0116
$A_1'A_2$	305	271,479	1.0438	
A_1B	324	391,479	1.1474	−0.0051
$A_1'B$	312	368,877	1.1423	

In A_1A_1 and A_1A_1', viability of $\frac{Cy}{+_{A_1}}\frac{+_{A_1}}{+_{A_1}}=1.0000$.

In A_1A_2 and $A_1'A_2$, viability of $\frac{Cy}{+_{A_2}}\frac{+_{A_1}}{+_{A_1}}=1.0000$.

In A_1B and $A_1'B$, viability of $\frac{Cy}{+_B}\frac{+_{A_1}}{+_{A_1}}=1.0000$.

** significant at the 1 per cent level.

did not manifest their effects significantly.

After obtaining additional experimental results, the effect of irradiation on populations and the relation between heterozygous manifestation of the radiation-induced mutations and evolutionary history of the individuals will be discussed.

83. *Radiation-induced mutation rate and doubling dose in polygenes controlling viability in Drosophila melanogaster**

(By Terumi MUKAI)

As described in another paper of the present report, we irradiated the sperms of *D. melanogaster* with X-rays at the dose of 500r and tested the heterozygous effect of radiation-induced mutations in homozygous genetic background.

With the aid of variance analysis we have estimated the genetic variance caused by radiation-induced mutations as $\hat{\sigma}^2_{G(x)}=0.001161$. The increment of the mean viability was 0.03020 viability index.

Under the assumption that mutations are distributed on chromosomes according to Poisson distribution, the following formula can be obtained:

$$0 \leq \sigma_a{}^2 \leq \frac{B^2}{4A^2} \quad \cdots\cdots (1)$$

$$\bar{a} \leq \frac{B}{A} \quad \cdots\cdots (2)$$

$$\frac{A^2}{B} \leq p \quad \cdots\cdots (3)$$

where A is the increment of viability mean, B is the genetic variance caused by radiation-induced mutations, a is the heterozygous effect of a single mutation whose mean and variance are $\bar{a}$ and $\sigma_a{}^2$, respectively, and p indicates average number of radiation-induced mutations in the second chromosome.

Using Formulae (1), (2) and (3), the following estimates are obtained:

$$0 \leq \sigma_a{}^2 \leq 0.000369$$

$$\bar{a} \leq 0.0384$$

$$0.7860 \leq p$$

These results indicate that by 500r irradiation, at least 0.7860 mutations, on the average, occurred in the second chromosome. This result

* This work has been supported by Grant RH-34 from the Public Health Service, U.S.A..

is consistent with that obtained previously (Mukai 1961).

Doubling dose was estimated by employing the result of spontaneous mutation rate estimated for the same experimental material as that for the radiation experiment (0.1301/second chromosome/generation). The result thus obtained is 82.8r.

84. *Radiation-induced mutation rates of polygenes controlling the sternopleural bristle number in Drosophila melanogaster*[1)]

(By Terumi MUKAI, Isao YOSHIKAWA and Sadao CHIGUSA)

To obtain fundamental information on the genetic influence of radiation on human populations and on the estimation of spontaneous mutation rate, radiation-induced mutation rates of polygenes controlling the sternopleural bristle number in *Drosophila melanogaster* have been estimated.

An isogenic line extracted from a wild population of Erie, Pa. (U.S.A.) was employed. The males of the isogenic line were irradiated with X-rays at 250r and 500r. Immediately after irradiation, the irradiated males were crossed to the females of the same line in half pint milk bottles. The number of sternopleural bristles in females and males which had hatched on or before the 13th day after the mating were scored and the means and the variances were calculated to test the heterozygous effects of radiation-induced mutations. The results are summarized in Table 1.

Table 1. Means and variances of sternopleural bristle numbers.

	Control		250r		500r	
	♀	♂	♀	♂	♀	♂
Mean	17.0389	16.4057	17.0204	16.3750	17.0834	16.4252
Variance	1.4270	1.4106	1.4790	1.3253*	1.5548**	1.3309*
No. of irradiated genomes	6497	5411	5983	4827	5313	4306

* significantly different from the control at the 5% level.

** significantly different from the control at the 1% level.

1) This work has been supported by Grant GM-7836 from the Public Health Service, U.S.A..

The variances of the irradiated groups have approximately linearly increased with the dose in females (the rate of variance increase: 2.32×10^{-4}/r), but have decreased significantly in males as compared with that of the controls. This difference might be attributed to some change in the heterochromatic parts in Y-chromosomes. The means of sternopleural bristle numbers did not change, either in males or females. Thus, it may be suggested that heterozygously positive and negative mutations occur in equal frequencies and amounts. This might indicate that the trait in question is not strongly correlated with fitness.

85. *Further report on the increase in induced mutation frequency after fractionated irradiation of gonial cells of the silkworm*

(By Yataro TAZIMA and Kimiharu ONIMARU)

A marked increase in induced mutation frequency was revealed for both spermatogonia and oögonia when an X-ray dose of 1000r was given in two or three fractions separated by 24 or 48 hour interval (Tazima and Murakami, 1962, Ann. Rept. No. 13). This finding led us to examination, in a new experiment, of the most effective period for the second exposure and the length of duration during which the enhancing effect is observed.

Total dose of 1000r γ-rays was divided into two halves. 500r was administered to the larvae at the 7th day after hatching and the remaining 500r was given at several intervals, i.e., 2, 12, 24, 36 and 48 hours after the first exposure. The radiation dose-rate was 60r/min. For the estimation of mutation frequency the specific locus method for *pe* and *re* loci was used as usual.

The results are shown in Fig. 1.

It can be seen from Fig. 1 that fractionated irradiation enhances mutation frequency throughout the whole period examined, i.e., from 2 to 48 hours after the first exposure. The mutation frequencies showed their peak at 12 hours for spermatogonia and at 24 hours for oögonia. They were 4–8 times as high as those from single exposure, surpassing the level of average mutation frequencies obtained from chronic irradiation.

That mutation frequencies never fell below a single acute exposure level does not support the view that enhanced mutation frequencies were due to synchronization of the cell cycle, but indicates that sensitive cells were not killed but survived in a highly mutable state during a definite period of post-irradiation.

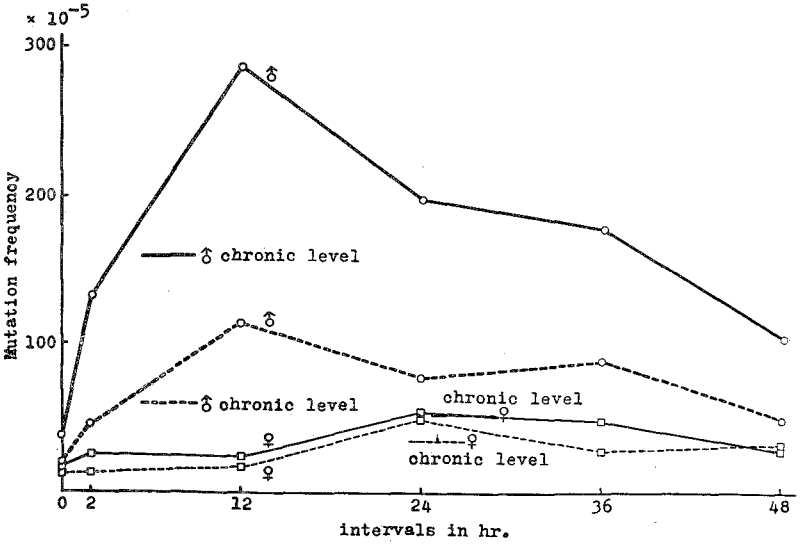


Fig. 1. Variation in induced mutation frequency with time interval between two exposures. Solid line represents the changes in mutation frequency for $+^{pe}$ locus and broken line those for $+^{re}$ locus.

This argument, however, does not necessarily rule out the selective cell-killing hypothesis. Because, even when sensitive cells are killed off, surviving resistant cells could react to fractionated irradiation in a similar manner as do the sensitive cells and give rise to analogous results.

86. *Independence of induced mutation-rate from radiation dose-rate in the germ cells of hibernating silkworm embryo*

(By Yataro TAZIMA and Kimiharu ONIMARU)

As a possible interpretation for the reverse type of dose-rate dependence of radiation-induced mutation rate in the silkworm gonia, the hypothesis of selective cell-killing was proposed by Tazima, Kondo and Sado (1961); namely, they assumed that sensitive cells at an advanced stage are killed by acute irradiation, whereas cells in dormant state can survive the irradiation and achieve repopulation afterwards, resulting in lower mutation frequencies of resistant cells.

According to this hypothesis, irradiation of gonads consisting of homo-

geneous dormant germ cells, if existed, should give rise to low mutation frequencies or at least equal to those for acutely irradiated gonads at the reverse type (type II) stage, regardless of dose-rate. Germ cells of hibernating embryos could be regarded as an nearly ideal model of such a homogeneous cell population.

Therefore, silkworm eggs, which had passed through hibernation but were still preserved in a refrigerator at 2.5°C, were chosen as the material for this experiment. Twenty batches of a wild type strain, C108, were divided each into two halves. One group was exposed to 1000r γ-rays for one minute (acute), while the other group was irradiated chronically with 1000r in total during 10 days (chronic). In both cases irradiation was carried out at 2°C. After irradiation, eggs of both groups were stored again in the refrigerator at 2.5°C for nearly one month until incubation. For the estimation of induced mutation frequency, specific loci method was used.

The results are given in Table 1.

Table. 1. Induced mutation frequencies obtained after γ-irradiation of hibernating eggs at different dose-rates.

Treatment	Dose-rate	male germ cells			female germ cells		
		No. obsd.	Mut. freq.		No. obsd.	Mut. freq.	
			pe	*re*		*pe*	*re*
			$\times 10^{-4}$	$\times 10^{-4}$		$\times 10^{-4}$	$\times 10^{-}$
Acute*	1000r/min	128,180	62.6	21.2	32,400	31.3	4.
Chronic**	0.0695r/min	139,340	64.2	25.8	48,600	21.5	12.

* ^{137}Cs-γ-rays. ** ^{60}Co-γ-rays.

The results were in complete contradiction to our expectation; namely, induced mutation frequencies were surprisingly high in both acute and chronic groups. In some F_1 batches mutation occurred in fairly large clusters. Furthermore, no significant difference in mutation frequency was observed between the two groups.

These results seem to indicate that germ cells of hibernating embryos are not similar to suposedly dormant germ cells in growing gonads at young larval stage, being far more mutable in the embryos. However before drawing a definite conclusion, it must be taken into consideration that the observed high frequencies might not be genuine, but could have been exaggerated to a considerable extent by cold storage as a result of disturbances in the repair process of premutational damages. T

answer this question, further experiments are in progress.

87. *Enhancement of radiation induced mutation frequency by post-treatment of silkworm gonia with 5-bromodeoxyuridine*

(By Yataro TAZIMA and Akio MURAKAMI)

As reported previously (Ann. Rept. Nat. Inst. Genet. No. 13), 5-bromouracil (BU) or 5-bromodeoxyuridine (BUdR), analogues to the normal base thymine or normal nucleoside thymidine of DNA, enhance radiation-induced mutation frequency to an appreciable extent, when administered after X-irradiation. As to the cause of this phenomenon, it was speculated that BU is incorporated into X-rayed DNA in the place of thymine and gives rise to a copying error during its replication. Whether the speculation holds true or not, has been examined as follows.

1) *Experiments to test whether post-treatment with thymidine increases radiation induced mutation frequency.*

Following a similar procedure to that used for BUdR, the effect of thymidine supplementation was studied. The chemical was administered to young larvae of a wild type strain by feeding them on leaves supplemented with thymidine (16 mg per 1500 larvae) for about one day after γ-irradiation.

Thus far, the experiment has been repeated four times at different stages, from hatching day to the second instar and more than four million F_1 eggs have been examined. No significant increase, however, has been confirmed. Hence, it may be concluded that thymidine, even in excess, does not increase radiation induced mutation frequency.

2) *Simultaneous feeding of BUdR and thymidine immediately after γ-ray irradiation.*

If an enhancement were due to replacement of thymine by BU, then it could be anticipated that the chance of its replacement is decreased by simultaneous administration of BUdR and thymidine. This would give rise to the decrease or disappearance of enhancing effect. In this respect, experiments have been conducted with silkworm gonia. Both chemicals were administered to young silkworm larvae in the moleular ratio of 1 : 1 (20 mg BUdR: 16 mg thymidine) and 1 : 2 (20 mg BUdR: 32 mg thymidine). Two series of experiments, one at first instar day 2 and the other at second instar day 1, have been performed. The γ-ray doses were 500r for the former and 1000r for the latter. The method used for the estimation of mutation frequency was similar to that previously used.

The results are, however, not conclusive, showing no definite increase or decrease in mutation frequencies among similarly treated groups.

Thus, it is doubtful in the silkworm whether the replacement of thymine by BU induces mutation or not.

88. *Relative biological effectiveness of fast neutrons in the induction of recessive visible mutations in the silkworm*

(I) *Effect of 14.1 MeV neutrons upon the gonia**

(By Akio MURAKAMI, Sohei KONDO and Yataro TAZIMA)

Effectiveness (RBE) of 14.1 MeV neutrons from $T(d, n)$ He reaction has been compaired with that of ^{137}Cs-gamma rays in the induction of visible recessive mutations upon gonia.

It has been reported by Tazima et al. (1961) that gonial cells of the silkworm respond quite differently to low LET radiations of high or low dose-rate at early and late developmental stages. In order to examine whether the same manner of response is observed for high LET radiations, this experiment has been undertaken

The exposure to 14.1 MeV neutrons was carried out at the Research Institute of Nuclear Medicine and Biology, Hiroshima University, by courtesy of Professor Haruma Yoshinaga. Young larvae of a wild type strain, C108, were exposed to fast neutron beams for 114 minutes by placing them in acryl tubes at several distances from 3.5 cm to 6.0 after hatching (abbreviated to early) and on the 7th day after hatching (abbreviated to late). Radiation dose-rate ranged from 6.7 rad to 19.6 rad per minute. The dose calculation was made by following the first collision dose method developed by Randolph, based on the analytical data of elemental composition. For the estimation of mutation frequency we applied the specific loci method with egg colour markers.

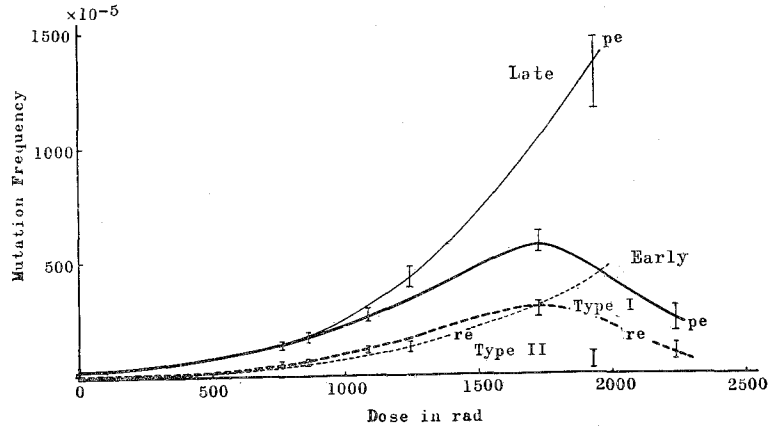
Mutation frequencies obtained for each locus were plotted against the dose of 14.1 MeV neutrons for spermatogonia and oögonia irradiated at early or late stages.

For the comparison of effectiveness, dose-response curves for mutation by ^{137}Cs-gamma rays were drawn from old (Tazima and Kondo, 1963) and new data.

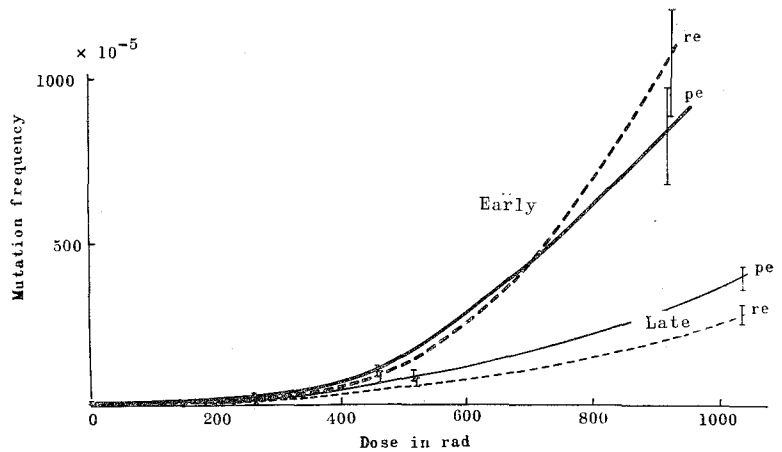
Those curves are shown in Figure 1 for spermatogonia and oögonia. In oögonia mutation frequencies are distinctly higher in groups irradiated at late stage than in those treated at early stage, throughout the whole dose

* This work was supported by Grant from Toyo Rayon Foundation for the Promotion of Science and Technology.

range examined. The results are in sharp contrast to those obtained after acute gamma-ray exposure, where induced mutation frequencies were higher at early stage. The phenomenon may presumably be due to dose-rate effect of neutrons. It may be noted that mutation frequencies from fast neutrons in the present experiment are clearly higher than those from chronic gamma irradiation, irrespective of the stage at irradiation.



Spermatogonia



Oögonia

Fig. 1. Dose-mutation frequency curves obtained after 14.1 MeV neutron irradiation at early and late gonial stages.

Since the dose-frequency curves do not follow linear relationship, it is hardly possible to make exact comparison of effectiveness between the two kinds of radiation for the whole dose-range. However, at an arbitrary level of mutation frequency, estimation is possible. The values estimated, for example, at the level of 100×10^{-5} mutation frequency are as follows.

At early stage RBE's of 14.1 MeV neutrons are 1.0 and 0.8 for spermatogonia and 1.2 and 1.2 for oögonia, while at late stage they are 1.8 and 2.1 for spermatogonia and 1.1 and 1.3 for oögonia at *pe* and *re* locus, respectively. Thus, RBE values of 14.1 MeV fast neutrons are higher in late than in early stage at the level of 100×10^{-5} mutation frequency. Though the values are fairly small at the lower level of mutation frequency, they become larger at higher mutation frequency levels. At still higher dose levels, however, RBE values decrease again.

89. *Relative biological effectiveness of fast neutrons in the induction of recessive visible mutations in the silkworm*

(II) *Effect of fission neutrons upon the gonia*[1)]

(By Akio MURAKAMI, Sohei KONDO and Yataro TAZIMA)

By the courtesy of AEC of United States and Dr. J.A. Auxier of Oak Ridge National Laboratory, an irradiation experiment of silkworm gonia with 1.5 MeV ^{235}U-fission spectrum fast neutrons has been conducted at Oak Ridge National Laboratory. The health physics research reactor (HPRR) was used. The neutrons generated from the reactor comprise 55 per cent neutrons within energy range from 0.1 to 1.5 MeV, 38 per cent within energy range from 1.5 to 2.5 MeV and 7.0 per cent within energy range from 2.5 to 6 MeV, the representative energy level being 1.5 MeV. The mean linear energy transfer (LET) is estimated as ca. 50 keV/μ.

The biological material and method are similar to those used for 14.1 MeV fast neutron experiments. The young larvae were placed at 100cm distance from the core of the reactor. Neutron dose-rates ranged from 229 to 259 rad per minute.

The results are given in Figure 1a and b. The mutation-response curves for 1.5 MeV fast neutrons rose more rapidly with the dose than

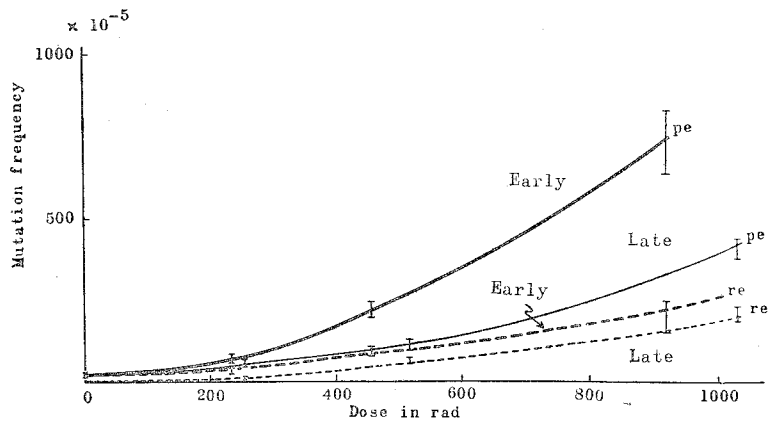
1) This work was supported by Grant from Toyo Rayon Foundation for the Promotion of Science and Technology.

for 14.1 MeV neutrons. The relation, however, can be regarded approximately as linear under 100×10^{-5} mutation frequency level. At this level RBE values of 1.5 MeV neutrons at early stage are 1.7 and 1.9 for spermatogonia and 2.1 and 2.4 for oögonia, while at late stage they are 2.8 and 3.0 for spermatogonia and 2.5 and 3.0 for oögonia at *pe* and *re* locus, respectively. Namely, the RBE values of 1.5 MeV neutrons in gonial cells are higher at late than at early stage at the 100×10^{-5} mutation frequency level. A similar tendency has also been observed for 14.1 MeV neutrons although the values were smaller (first report of this series). Combining both data, it may be pointed out that RBE from induction of visible recessive mutation increases with increasing LET within the energy range examined (Figure 2).

It has been known that in *Drosophila* the RBE values for fast neutrons in the induction of sex-linked recessive lethal mutations are about 1 but they are 4–6 times as high in the induction of dominant lethals.

The RBE values obtained in the present experiment are clearly higher than those expected from visible recessive mutation on *Drosophila*, indicating that the recovered mutations comprise not only recessive visible mutations, but also small deficiencies and some kinds of gross structual changes.

This conclusion is also supported by the finding that, in most cases, dose-response curves do not follow linear relationship, but are rather exponential.



Spermatogonia

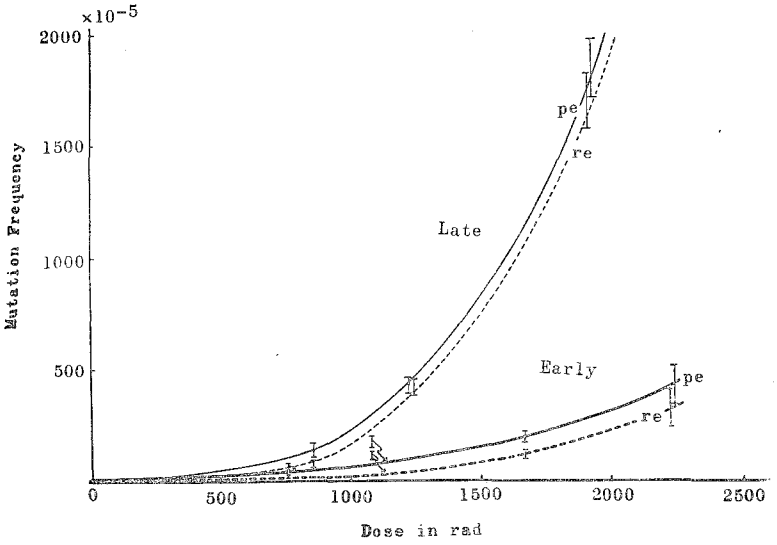


Fig. 1. Dose-mutation frequency curves obtained after fission neutron irradiation at early and late gonial stages.

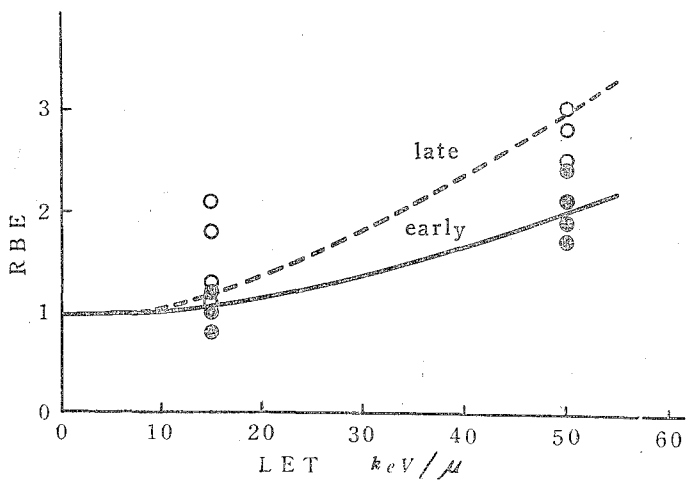


Fig. 2. Relation between RBE and LET in the induction of recessive mutation in silkworm gonia.

● indicates the early stage gonia.

○ indicates the late stage gonia.

90. *Relative biological effectiveness of 14.1 MeV neutrons in killing dormant silkworm eggs*[1)]

(By Akio MURAKAMI and Sohei KONDO)

Although killing effects of low ion density radiations, such as X- or gamma-rays, upon silkworm eggs have been studied by many investigators, scarcely anything is known about the effects of high ion density radiations. Our study of RBE in mutation induction has yielded much information in this respect. The need of examination of RBE of high energy neutrons for dormant silkworm eggs came from another consideration.

In this experiment biological effectiveness of 14.1 MeV neutrons has been compared to that of gamma-radiations concerning their killing effects on silkworm eggs at dormancy.

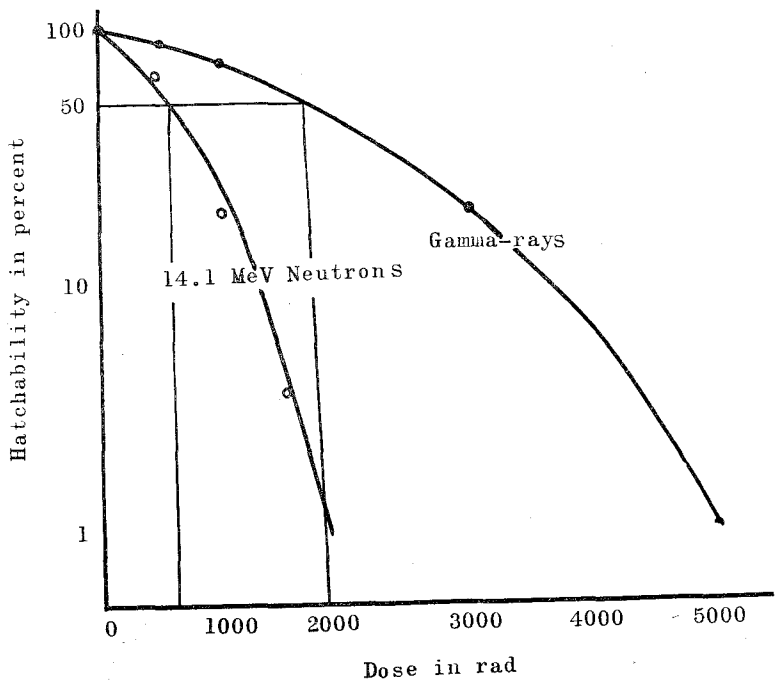


Fig. 1. Hatchability curves obtained after irradiation of dormant silkworm eggs with gamma-rays and 14.1 MeV neutrons.

1) This work was supported by Grant from Toyo Rayon Foundation for the Promotion of Science and Technology.

The exposure to 14.1 MeV neutrons was applied at the Biology Division, Oak Ridge National Laboratory, with use of Cockcroft-Walton accelerator. Dormant silkworm eggs were exposed to neutron beams at a distance of 6.3 cm from the tritium target for a variable length of duration, from 60 to 180 minutes. The neutron dose-rate was 5 rad per minute and total doses given were 500, 1000 and 1500 rad. In parallel to this, ^{137}Cs-gamma ray treatment was carried out at our Institute with the same material. Gamma-ray doses were 500, 1000, 3000 and 5000r at a dose-rate of 100r per minute.

The percentages of hatchability were plotted against doses given on semilogarithmic scale both for fast neutrons and gamma-rays (Figure 1).

The hatchability curve for 14.1 MeV neutrons declines more rapidly than that for ^{137}Cs-gamma rays. Calculated doses that allow 50 per cent hatchability (LD_{50}) are 600 rads and 2000 rads for 14.1 MeV neutrons and ^{137}Cs-gamma rays, respectively. That is to say 14.1 MeV neutrons are more effective than ^{137}Cs-gamma radiations with regard to their killing action. RBE thus calculated is 3.3.

91. *Mutations induced by ingested ^{32}P in silkworm*[1)]

(By Mituo IKENAGA and Sohei KONDO)

The mutagenic effect of decay of ^{32}P incorporated into the genetic substance could be due either to the effects of radiation accompanying radioactive decay or to some noxious events caused by nuclear transmutation of ^{32}P atom, such as chemical valence change (STRAUSS: 1958, STENT and FUERST: 1960). In order to elucidate the relative contribution of β-electron and transmutation effect to induced mutation, the mutagenic effectiveness of ^{32}P ingested by the silkworm on the third day of fourth instar was compared with that of corresponding doses of γ-irradiation from ^{137}Cs, using egg color mutants, *pe* and *re*. Since ^{32}P β-ray dose absorbed by the gonads is strictly proportional to the specific radioactivity of the silkworm, it was calculated in the present experiments by measuring the activity of all the pupae at middle pupal stage. The results obtained in both series, ^{32}P-treatment and γ-irradiation, are shown in Fig. 1. The mutants scored for mutation frequency are the total of *pe*, *re* and mosaic-*re*. As clearly seen from the graph, the mutation frequency induced by ^{32}P in males is quite the same as that induced by the corresponding dose of γ-rays, while ^{32}P-treatment in

1) This work was supported by Grant from the Toyo Rayon Foundation for the Promortion of Science and Technolegy.

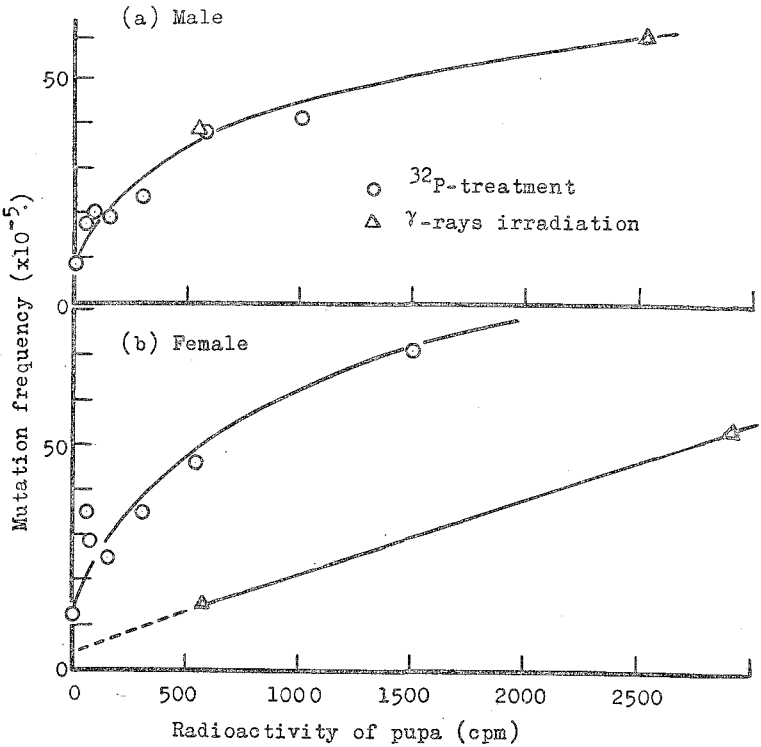


Fig. 1. Dose response curve for ^{32}P-treatment and γ-irradiation in silkworm: irradiation with 20r/day γ-rays corresponds to 500 cpm for male and 590 cpm for female pupae.

females induces mutations more efficiently than γ-irradiation. In other words, there seems to be observed a transmutation effect on induced mutation for female silkworm, but not for males. The observed results and the methods of calculating absorbed dose of ^{32}P will be published in detail elsewhere together with a discussion.

92. *Mutagenic effects of ethyl methane sulfonate on silkworm*

(By Sohei KONDO and Mituo IKENAGA)

It has been shown that ethyl methane sulfonate (EMS) induces mutations in lower organisms (LOVELESS: 1958) and in maize (NEUFFER and FICSOR: 1963). To interpret the mechanism of its mutagenic action it is supposed that this alkylating agent can ethylate the guanine moity in

position 7, thereby causing guanine to pair with thymine rather than cytosine during DNA duplications. However, EMS mutagenicity in higher animals was not yet reported. It was examined in the present experiment using the egg color mutant *pe*, *re* of the silkworm. For the estimation of mutation frequency the abdomen of both sexes of a wild strain was injected with 0.2 ml of EMS solution in late pupal stages (4 days before emergence) and then the insects were mated with the marker strain. The results obtained are given in Table 1. The

Table 1. The frequency of egg color mutants induced by injection with EMS at pupal stage.

Concentration of EMS (Mol.)	Total no. of eggs observed	*pe*				*re*			
		Whole		Fractional		Whole		Fractional	
		No. of detd.[1]	Freq. ($\times10^{-5}$)	No. of detd.	Freq. ($\times10^{-5}$)	No. of detd.	Freq. ($\times10^{-5}$)	No. of detd.	Freq. ($\times10^{-5}$)
a. *pe re* ♀ × + + ♂ (treated)									
Control	34,100	3	8.80	1	2.93	0	—	0	—
8.0×10^{-5}	28,657	4	14.0	0	—	0	—	0	—
8.0×10^{-4}	16,806	3	17.9	32	190	1	5.95	66	393
8.0×10^{-3}	15,507	1	6.45	83	535	0	—	206	1328
b. + + ♀ (treated) × *pe re* ♂									
Control	31,200	3	9.62	5	16.0	0	—	0	—
8.0×10^{-5}	13,540	1	7.39	6	44.3	3	22.2	1	7.39
8.0×10^{-4}	12,939	3	23.2	4	30.9	2	15.5	2	15.5
8.0×10^{-3}	5,287	1	18.9	21	397	1	18.9	19	539

[1] detected.

table clearly shows that EMS was effective. A remarkable difference between the relative frequencies of whole- and fractional-mutations was observed at high concentration of the chemicals (bottom rows for both sexes), and in most of the fractional-mutants a quarter of the egg or less is involved.

This abnormality of mutagenic action of EMS may be interpreted as follows; (1) EMS acts on dominant genes only during the developmental stages after fertilization. (2) The appearance of the mutation is somewhat delayed, since its stabilization requires one or two cycles of DNA duplication. (3) Most of the mutations are changes induced in one of the two strands of DNA molecule.

93. *Radiation response of actin synthesis in regenerating tissue of Triturus**

(By Yoshito OGAWA)

Synthesis of muscle proteins with respect to X-irradiation at various points of embryonal development of *Triturus pyrrhogaster* has been reported before[1]. From the same view point, the effect of X-ray irradiation on the synthesis of the contractile protein, actin, in the regenerating hind limb tissue of adult *Triturus* amputated at the knee was examined in detail and the findings are here reported. They may be helpful in the approach to the mechanism of chemo-differentiation of muscle tissue.

About 1,500 animals at different stages in the recovery progress after operation were exposed to single doses of X-rays with 20r., 50r., 200r., 500r., 1,000r. and 2,500r., and the examination for the first detectable trace of actin in regenerating limb tissue was carried out 3, 9, 15, 18, 19, 20, and 22 days after operation. The technique employed was essentially the same as in the experiments with early embryonal stages[2].

Pure G-actin was isolated from the skeletal muscle of adult *Triturus*, and the anti-serum was prepared by injecting it into rabbits. Before carrying out the precipitin reaction with saline extracts of granulated tissue of the amputated limb, the titer of anti-serum was adjusted to 1:512. In non-irradiated group, actin first became detectable in the regenerating tissue 20 days after operation[2]. The time of actin formation after operation in the X-irradiated group is represented in Fig. 1. When a dose of 20r. is given 3 days after amputation, the time of actin formation changes to the 18th day after operation. In the case of irradiation 19 days after operation with a heavy dose of 2,500r., the synthesis of actin was remarkably inhibited and the time of actin formation changed to the 30th day after amputation.

The curves in Fig. 1 show a similar response of actin formation to X-ray as in embryonal stages. These results may suggest the presence of two factors sensitive to X-rays in actin synthesis during tissue regeneration as well as in early development. One (R-Aa) suppresses the synthesis of actin and the other (R-Ba) markedly promotes it after X-irradiation. The inhibiting factor R-Aa is represented by the points found on the right of the vertical control line (Fig. 1, ○) and the promoting factor R-Ba by the points on the left (Fig. 1, ●). Factor R-Aa does not react to X-rays until doses over 500r. are applied, but factor

* This work was supported by a Grant-in-Aid for Fundamental Scientific Research (No. 710273) of the Ministry of Education in Japan.

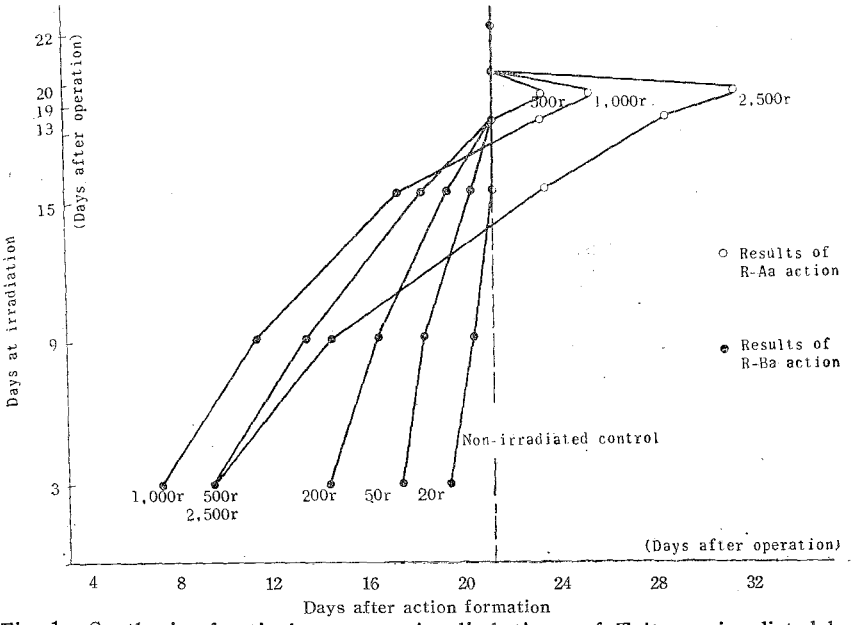


Fig. 1. Synthesis of actin in regenerating limb tissue of *Triturus* irradiated by X-rays with doses from 20 r to 2,500 r.

R-Ba is very sensitive to X-rays and is easily detectable with only 20r. dose. The factors R-Aa and R-Ba are detectable, respectively, at irradiations between 18th and 19th day and between 3rd and 15th day after operation. The most effective time of irradiation is the 19th day after amputation for factor R-Aa and the 3rd day for factor R-Ba under the above experimental condirtons.

1) Y. Ogawa: B. B. A. **54**: 397, 1961.
2) Y. Ogawa: Exptl Cell Research **26**: 269, 1962.

94. *Effects of L-gulonolactone and glucose on the synthesis of the skeletal muscle protein, myosin, in X-irradiated Triturus embryos**

(By Yoshito OGAWA)

Previously, the effect was reported of sodium-glucuronate on abnormal differentiation caused by X-rays of muscle proteins during early em-

* This work was supported by a Grant-in-Aid from Tokyo Biochemical Research Fundation.

bryonal development of *Triturus pyrrhogaster*[1]. This paper deals with the effects of L-gulonolactone and glucose on the synthesis of myosin in X-irradiated material of the same animal. These experiments are partly carried out in order to investigate the protective action of glucuronic acid and its derivatives on abnormal differentiation in early embryos induced by X-rays.

Pure myosin was isolated from skeletal muscle tissue of adult *Triturus* and the anti-serum was prepared by injecting it intravenously into rabbits. Immediately after fertilization, the embryos were raised in 0.01% solution of L-gulonolactone (most effective concentration for promoting the synthesis of myosin[2]) or glucose, at 18°C., and total body X-irradiation of embryos with 50r., 200r. and 500r. was performed 108 hrs. after fertilization. After irradiation, the treatment with L-gulonolactone or glucose was continued and the analysis of myosin was immediately carried out. Embryos from 108 to 204 hrs. after fertilization were mashed in ice cold potassium chlorid solution (0.6 M). The homogenate was allowed to stand for one hour at 0°C. under constant stirring and then was centrifuged at 2,000g. for 30 minutes. Before carrying out the precipitin reaction with the anti-serum, the clear supernatant was placed in a collodium membrane tube and treated with large amount of distilled water for 48 hrs. at 0°C. to eliminate the potassium chloride. Constant volume of anti-serum adjusting the titer to 1:512 and progressively decreasing amounts of antigen, ranging from 1:10 to 1:160 in embryo weight, were used for preventing a possible failure due to excess of antigen[1,3]. Non-treated and irradiated, treated and non-irradiated and normal (non-treated and non-irradiated) control groups were prepared.

In normal embryos, myosin became at first detectable 176 hrs. after fertilization[3]. In the present experiment, myosin formation in the normal group was first recognized 180 hrs. after fertilization, since the examination was carried out in periods of 12 hrs. In the case of non-treated and irradiated material, the time of myosin formation after fertilization was changed to 156 hrs at 50r., to 144 hrs. at 200r. and 132 hrs. at 500r.[4]. Moreover, the production of synthesized myosin was markedly promoted, and the acceleration was propotional to X-ray dosage (Figs. 1 and 2).

In the non-irradiated but treated with L-gulonolactone embryos, myosin appears 132 hrs. after fertilization. The development of this protein, therefore, was acclerated by 48 hrs. as compared with the control. But no significant difference was found in the amount of the synthesized protein (Fig. 1). In the irradiated and treated with L-gulonocatone group, the tendency to increasing the rate of formation as

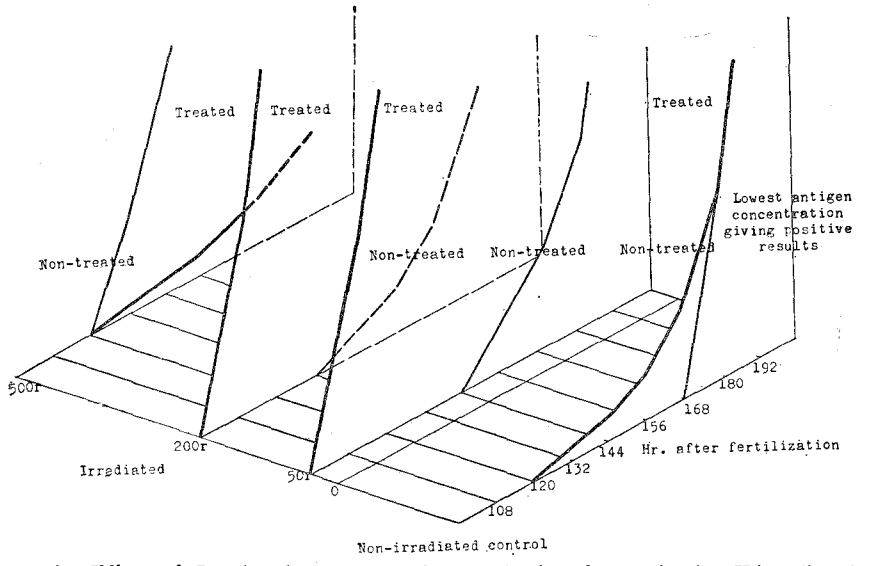


Fig. 1. Effect of *L*-gulonolactone on the synthesis of myosin in *X*-irradiated *Triturus* embryos.

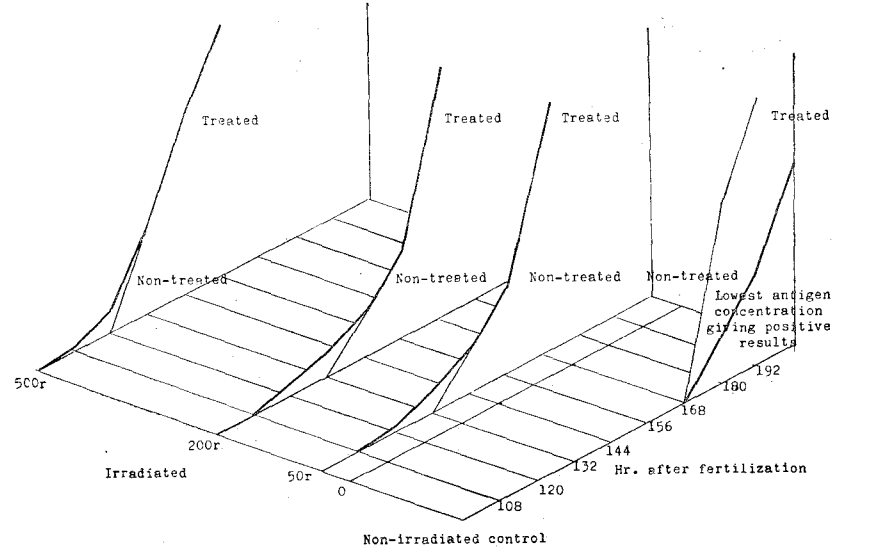


Fig. 2. Effect of glucose on the synthesis of myosin in *X*-irradiated *Triturus* embryos.

well as amount of myosin was remarkably strong when irradiated with 50r. and 200r. However, a heavy dose of 500r. showed no influence on the rate of myosin formation and suppressed its amount (Fig. 1). These results show the same tendency as found in the experiment with sodium-glucuronate[1].

In the non-irradiated but treated with glucose group, the rate of myosin formation was not influenced as compared with the control, but some reduction of the amount was recognized (Fig. 2). In the case of irradiated and treated with glucose material, the promotion of the rate of myosin formation by X-rays was accelerated by about 24 hrs. irrespective of the dose of X-ray, though no remarkable effect was found on the amount (Fig. 2).

Thus, it was found that L-gulonolactone as well as sodium-glucuronate shows a significant protective effect on the development of myosin in embryos irradiated with light X-ray doses (under 200r.) and the effects of both on myosin synthesis are clearly different from that caused by glucose.

1) Y. Ogawa: Reports of 7th. Symp. on Glucuronic Acid 128, 1961.
2) Y. Ogawa: Med. and Biol. **66**: 216, 1963.
3) Y. Ogawa: Exptl. Cell Research **26**: 269, 1962.
4) Y. Ogawa: Nature **186**: 77, 1960.

95. *Measurements of thymine dimers formed in ultraviolet irradiated bacteria and its related problem to photoreactivation*

(By Mituo IKENAGA and Sohei KONDO)

It is well known that ultraviolet radiation prevents colony formation in microorganisms. A reasonable explanation is that a large part of the inactivation of cells, after ultraviolet irradiation, results from the formation of thymine dimers in DNA polynucleotide chains. It is also known that the effects of ultraviolet irradiation on colony formation may be reversed by the action of visible light which splits thymine dimer into monomers by some enzymatic process (see recent reviews by RUPERT: 1963, WACKER: 1963). This phenomenon, photoreactivation, would be expected to occur when the visible light is administered at the same time as the ultraviolet treatment, unless long lag periods were involved. For instance in the preliminary experiment with *E. coli* $15T^{-}A^{-}U^{-}$, complete loss of photoreactivability was observed when the cells were held for 60 min after inactivation in a half-synthetic medium at 37°C.

The purpose of the present study was to obtain data accounting for the loss of photoreactivability.

E. coli $15T^-A^-U^-$ was grown in a half-synthetic medium in presence of 2 μg thymine-2-C^{14}/ml. Ultraviolet irradiation with 160 ergs/mm^2 was carried out at 254 mμ during the logarithmic growth of bacteria in synchrony. After a few different kinds of treatment (Table 1) bacterial suspensions were sedimented, dried and hydrolysed in 70% perchloric acid for 60 min at 90°C. The thymines and thymine dimers of the digest were separated by paper chromatography (86:14 n-butanol: H_2O). The radioactive regions were cut out and counted by a gas flow counter. The experimental results are shown in Table 1. Thymine dimers were

Table 1. The radioactivity of C^{14}-thymine dimers in irradiated *E. coli* 15 $T^-A^-U^-$ with various post-treatments.

	C^{14} activity in dimer region (cpm)*	Surviving fraction (%)
No ultraviolet irradiation	—**	100
Irradiated only	64.2	8.1
Irradiated and photoreactivated***	28.2	54
Irradiated and grown 60 min at 37°C in the darkness	10.2	9.1

* Sections of chromatograms containing dimers were cut into 1 cm strips, counted by windowless 2π gas flow counter.

** Apparent thymine dimer activity was observed with unirradiated bacteria. The values given in the middle column were subtracted according to this apparent activity.

*** Photoreactivation was carried out for 15 min at 37°C, with illumination from a 500 watt flood lamp at distance of 3 cm from the sample tube.

most frequent in irradiated cells without further treatments. Based on the obtained activity, 64.2 cpm, we were able to calculate the number of thymine dimers per lethal hit to be about 250 dimers/cell/hit. For the photoreactivation exposure the number of thymine dimers was changed by a factor of about 0.5, which is reflected in the increasing number of surviving cells. A considerable decrease in thymine dimers was observed when irradiated cells were allowed to grow for 60 min, when the complete loss of photoreactivality could be seen. Thus the loss of photoreactivability may be due to the decrease in photoreactivable damages, *i.e.*, in the number of thymine dimers, in spite of the fact that the surviving fraction at the time remains unchanged. A

possible molecular mechanism for the observed results is under consideration.

F. RADIATION GENETICS IN PLANTS

96. *Induced mutation and pleiotropy of genes responsible for quantitative characters in rice*

(By Kan-Ichi SAKAI and Akio SUZUKI)

Two groups of lines derived from the same variety of cultivated rice, but different in one having been X-rayed in 1959 while the other was left untreated, were comparatively investigated on an individual plant basis in respect of heading date, plant height, plant weight, tiller number, panicle number, length of the biggest panicle and grain yield. Comparison of genetic variance between the two has indicated that X-ray treatment succeeded in inducing mutations in those quantitative characters. The X-induced mutations were unidirectional being mostly in minus direction. Irrespective of the increase in genetic variation in X_4 lines, the error variance measured by block $\times$ line interaction appeared to be not different between X_4 and control lines.

By subtracting genetic covariance of the control population from that of the X-rayed group, genetic correlation due to pleiotropic effect of genes was estimated. The underlying principle for this estimation is as follows: If we assume (1) that mutation at one locus does not affect mutation in other loci which are located in the same or other chromosomes, (2) that different polygenes are evenly scattered over all chromosomes rather than forming assortative groups per chromosome, and (3) that X-ray induced deficiency of chromosome segments, if any, is deleterious for survival in rice and accordingly non-existent in the X_4 lines, then the genetic correlation in the X_4 population will involve extra correlation due to pleiotropic effect of newly mutated genes in addition to the effects of selection, gene linkage and pleiotropy involved in the genetic correlation of the non-irradiated population.

It was found that panicle length and number of panicles per plant were for the most part controlled by inversely operating pleiotropy of the genes, the p^G, that is the parameter of genetic correlation due solely to pleiotropic effect of genes being -0.91. Details of this study will be published in due time in Radiation Botany.

97. *Relation of radiation effects to dose rates of gamma-rays in diploid wheat*

(By Seiji MATSUMURA and Tomoo MABUCHI)

In order to investigate the relation of radiation effects to dose rates, dry seeds of *Triticum monococcum flavescens* were irradiated by gamma-rays at 4~15 kr. For acute and chronic irradiation the dose rates, 10,000 r/hr with ^{137}Cs and 35.6 r/hr with ^{60}Co, were used, respectively. Chronic irradiation was slightly more effective in inhibiting seedling growth than the acute treatment applied at the beginning of the chronic, and was clearly less effective than that applied at the end of the latter. Also, acute irradiation showed clearly a higher chlorophyll mutation rate than the chronic did, which were terminated just before sowing. The later was the irradiation at the same dose, the more pronounced was the inhibition of seedling growth and the increase in chlorophyll mutations, especially at acute irradiation. Thus, almost no intensification of radiation damage due to storage effects was found, as already reported in Ann. Rep. No. 13: 97-99.

A series of similar experiments with the same material were initiated to verify the earlier studies. Dry seeds were exposed to gamma-rays at the dose rates, 10,000 r/hr with ^{137}Cs and 26 r/hr with ^{60}Co, respectively. In general chronic irradiation was more effective inhibiting seedling growth and increasing chlorophyll mutations than the acute treatment, applied at the end of the chronic, especially at high dose (15 kr). It is supposed, against the earlier studies, that a slight intensification of radiation damage due to storage effects was involved.

98. *Relation of radiation effects to dose rates of X- and gamma-rays in paddy rice*

(By Seiji MATSUMURA and Tomoo MABUCHI)

Dry seeds of *Oryza sativa* (Nôrin 8) were exposed to X- and gamma-rays at 5~20 kr. For acute and chronic irradiation the dose rates, 10,000 r/hr with X-rays (173 kVp, 25 mA, 0.5 mm Al+0.5 mm Cu filter) or ^{137}Cs and 20 r/hr with ^{60}Co, were used, respectively, as shown in the diagram of Figure 1. The germination rate, seedling height, seed fertility in X_1 and chlorophyll mutations in X_2 were investigated (Tab. 1).

Chronic irradiation was more effective as to radiation damage of the above properties at the highest dose (20 kr) than the acute treatment applied at the end of the chronic radiation. Also, the earlier was the irradiation applied at the same dose in both treatments, the more reduced

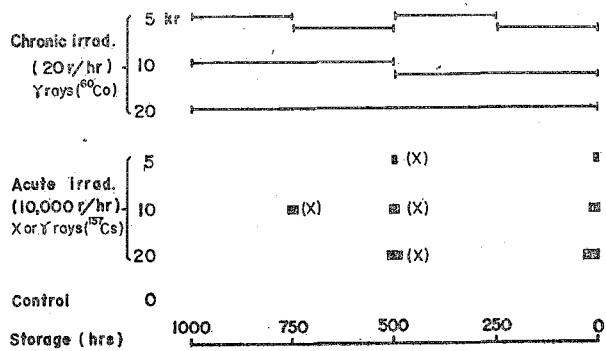


Fig. 1. Diagram of acute and chronic irradiations in rice (1962).

Table 1. Effect of acute and chronic irradiations on rice.

Dose (kr)	Dose rate (r/hr)	Storage (hrs)	Germination rate (%)	Seedling height (cm)	Seed fertility (%)	Chlorophyll mutation rate (%)
0			96.2	9.2	78.3	0.64
5	20	750	96.6	8.8	76.9	2.55
〃	〃	500	97.0	8.5	72.4	2.70
〃	〃	250	95.8	8.7	75.8	2.08
〃	〃	0	96.4	8.3	77.4	2.48
〃	10,000	500	97.8	8.5	74.8	2.93
〃	〃	0	95.8	8.4	74.3	2.17
10	20	500	96.8	7.0	56.5	5.44
〃	〃	0	96.0	8.3	67.6	6.05
〃	10,000	750	97.4	7.2	61.4	6.54
〃	〃	500	96.4	7.6	62.3	4.50
〃	〃	0	96.0	8.2	68.7	3.44
20	20	0	94.0	4.2	36.0	8.90
〃	10,000	500	92.4	5.5	45.6	7.06
〃	〃	0	95.6	6.9	48.5	8.19

was seedling growth and seed fertility especially at higher doses. Thus an intensification of radiation damage due to storage was taking place.

99. *Effects of acute and chronic irradiation on growing wheat*

(By Seiji MATSUMURA and Tarô FUJII)

Seedling of di-, tetra- and hexaploid wheats were transplanted into

the gamma-field. Six lots were placed in distances from 59 to 17 meters from the source. The total dosage was approximately 721.5 r at the minimum and 18,259 r at the maximum intensity being 4.9~124 r/20hr. Growing seedlings or plants of the same materials were also irradiated by acute gamma-rays in three different growing stages.

In the acute irradiation of the diploid wheat at an early seedling stage and of young plants in spring, LD_{50} seemed to be around 0.5~0.8 kr. But in chronic condition LD_{50} was around 2 kr. On the other hand, LD_{50} of tetra- and hexaploid wheats in spring was about 1.5 kr in acute condition, while that of the chronic condition was around 3~5 kr.

Young plants in early spring were more sensitive than in seedling stage during winter and this phenomenon was common to all three ploids when acute radiation was applied.

A similar behavior was observed in tetra- and hexaploid wheats when radiation was given to dormant seeds. But when growing plants were treated, the hexaploid seemed to be slightly more resistant than the tetraploid.

100. *Dose rate dependence of mutation rates of gamma-irradiated pollen grains in maize*

(By Tomoo MABUCHI and Seiji MATSUMURA)

In order to investigate the relation of mutation rates to dose rates, pollen grains of maize, which had the dominant gene *Su* against sugary endosperm, were collected from naturally dehisced anthers, or before dehiscence, and were irradiated by gamma-rays from a ^{137}Cs source with the doses of 500~1,650 r at two different dose rates of 917 r/min and 1.25 r/min. These acute and chronic irradiations were terminated almost simultaneously before dusting the female ears having the recessive gene *su* with the treated pollen.

The endosperm alterations from normal to sugary resulting from gamma-ray treatment of the pollen affected a large proportion of whole endosperms and, in addition, only their parts producing chimeras (Tab. 1). Acute irradiation was more effective in increasing the mutation rate than the chronic one, especially at higher doses. Namely, the mutation rates increased almost linearly with increasing doses in the latter, while increasing exponentially in the former (Fig. 1). The mutation rate per roentgen was $5.5\sim8.1\times10^{-6}$ (average 6.6×10^{-6}) in chronic irradiation and $12\sim23\times10^{-6}$ in the acute one.

Table 1. Pollination with gamma-irradiated pollen grains and mutation rate of *Su*-gene in maize.

Dose rate (r/min)	Dose (r)	Duration of exposure	No. of female ears pollinated	No. of seeds set	No. of endosperms mutated (rate)			% of endosperms wholly mutated	Mutation rate /r/locus
					Whole Obs. (%)	Chimera Obs. (%)	Total Obs. (%)		
	0		16	825	0 (0.00)				
1.25	500	6hr 40min	49	4,328	5 (0.12)	7 (0.16)	12 (0.28)	41.5	5.5×10^{-6}
	1,000	13 20	69	5,414	33 (0.61)	11 (0.20)	44 (0.81)	75.0	8.1×10^{-6}
	1,500	20 00	69	3,735	24 (0.64)	10 (0.27)	34 (0.91)	70.0	6.1×10^{-6}
917	550	36sec	a 34	2,716	12 (0.44)	3 (0.11)	15 (0.55)	80.0	12×10^{-6}
			b 37	4,142	28 (0.68)	3 (0.07)	31 (0.75)	90.3	
917	1,100	72	a 49	5,418	95 (1.75)	21 (0.39)	116 (2.14)	81.9	19×10^{-6}
			b 19	1,732	28 (1.62)	7 (0.40)	35 (2.20)	80.0	
917	1,650	108	a 29	3,337	101 (3.03)	19 (0.57)	120 (3.60)	84.2	23×10^{-6}
			b 32	3,180	113 (3.55)	17 (0.53)	130 (4.09)	86.9	

a: Anther irradiated before dehiscence, b: Pollen irradiated.

The endosperm alterations are due usually, if not always, to deficiencies rather than to gene mutations, as already STADLER and others reported. It is assumed that the frequencey of "two-hit" aberrations was relatively high, especially at acute irradiation, and it was dependent upon the intensity of radiation. The chronic irradiation was liable to give a high proportion of restitution from chromosome breakages effecting a linear dose-response curve of the mutation rate, as in "one-hit" aberrations.

101. *Relation between polyploidy and effects of gamma- and neutron-radiation on wheat*

(By Seiji MATSUMURA)

Exposure to 14 MeV neutrons obtained from (D, T) reaction was carried out in the Biology Division of Oak Ridge National Laboratory. For the calculation of given doses, based on RANDOLPH'S method, the analysed elemental compositions and the measured fast-neutron fluxes were used. The dose, applied to dormant seeds, ranged from 0.48 to 1.80 krad for *Triticum monococcum* and from 0.95 to 2.24 krad for *T. durum* and *T. vulgare*. At the same time gamma-radiations were used for comparison at 4.3~17.2 krad in ORNL.

After exposure to 1.80 krad of fast neutrons, the seeds of *T. monococcum* germinated but most of the seedlings died in an early stage, while in *T. durum* and *T. vulgare* slow growth of the seedlings continued even at 2.24 krad of fast neutrons. The higher was the dose of fast neutrons and gamma-rays, the more delayed were germination and growth of seedlings, the more reduced were survival rate and seed fertility, and the more increased were chlorophyll mutations, with the exceptions of mutations in *T. vulgare* (Tab. 1). In general, *T. monococcum* is the most sensitive to fast neutrons and gamma-rays. There is no significant difference between *T. durum* and *T. vulgare*. Also the relative biological effectiveness (RBE) of fast neutrons to gamma-rays was found to be 10~15 for those properties in *T. monococcum*. The RBE values for the these characters in polyploids were higher than in *T monococcum*, respectively.

Table 1. Effects of γ-rays and fast neutrons (14 MeV)

Dose	Germination (%)	Length of seedlings (cm)	Survial (%)	Fertility in X_1 (%)	Chlorophyll mutation rate in X_2 (%)
T. monococcum flavescens (2n=14)					
Control	70	11.07	75.71	55.00	0.00
γ-4.3 krad	75	10.03	56.00	41.57	4.47
γ-8.6	68	8.09	58.82	24.96	5.93
γ-12.9	57	5.96	54.39	24.24	4.59
N-0.48	72	9.45	52.11	38.34	1.85
N-0.95	61	5.96	50.82	25.53	16.16
N-1.43	50	3.85	32.00	10.45	6.06
N-1.80	38	2.04	0.00	—	—
T. durum Reichenbachii (2n=28)					
Control	72	10.21	72.22	38.37	0.00
γ-8.6 krad	86	11.00	83.72	44.07	0.00
γ-12.9	84	10.16	64.29	43.16	0.00
γ-17.2	76	9.92	81.58	44.64	4.10
N-0.95	74	9.37	54.05	32.62	8.16
N-1.43	84	8.58	40.47	14.62	4.76
N-1.80	74	8.01	29.73	24.45	10.00
N-2.24	72	5.41	8.83	14.03	0.00
T. vulgare erythrospermum (2n=42)					
Control	66	15.90	93.94	22.36	0.00
γ-8.6 krad	58	14.82	89.66	13.11	0.00
γ-12.9	68	14.36	89.66	7.92	0.00
γ-17.2	68	12.31	70.59	5.62	0.00
N-0.95	70	14.10	71.43	9.22	0.00
N-1.43	82	13.15	53.66	9.25	0.00
N-2.24	71	9.05	19.35	4.18	0.00

102. *Relative biological effectiveness of 14 MeV neutrons to ^{60}Co gamma-rays in einkorn wheat*

(By Tarô FUJII)

The RBE of 14 MeV neutrons to ^{60}Co gamma-rays by using the

specific loci method in einkorn wheat was investigated. F_1 seeds from the cross between the original normal strain and a chlorina mutant were used in this experiment. Dormant F_1 seeds were irradiated at 4.3, 8.6 and 12.9 krad of gamma-rays and 0.45, 0.95 and 1.43 krad of neutrons. Mutations from dominant normal green to *chlorina* occurred by both irradiations and appeared in the leaves and stems of the heterozygotic X_1 plants as longitudinal stripes.

Around 80 per cent of seeds germinated in the control lot and in the lowest dosage lots from both neutron and gamma-ray irradiation, and germination percentages were gradually decreasing with increasing dosage of both kinds of radiation. Moreover, a similar tendency was observed in early stages as to seedling growth which was gradually inhibited with increasing dosage. According to these results, neutron irradiation was about 13 times as effective as that of gamma-rays. Survival rate in the non-irradiated control was about 90 per cent and about 50~80 per cent of germinated seedlings survived in 0.45 and 0.95 krad lots from neutron irradiation and from all lots irradiated by gamma-rays. On the other hand, only about 4 per cent of germinated seedlings survived in the highest neutron lot.

No mutation was observed in the control lot, and the number of plants which contained striped tillers increased with increasing dosage of both kinds of radiation. The rates for striping were calculated per spike and 0.19, 0.35 and 0.48×10^{-6} per rad were obtained from 4.3, 8.6 and 12.9 krad gamma-ray irradiation, respectively. 9.65 and 7.12×10^{-6} per rad were obtained from 0.45 and 0.95 krad neutron irradiation. 1.43 krad neutron irradiation is too high for einkorn wheat. From the results, RBE value of 14 MeV neutrons to ^{60}Co gamma-rays was about 13.

103. *RBE in Arabidopsis treated with fission and 14.1 MeV neutrons to gamma-rays*

(By Tarô FUJII)

RBE values of fission and 14.1 MeV neutrons to gamma-rays in *Arabidopsis* were studied by specific loci method. The flower of this plant is small and it is somewhat difficult to obtain many F_1 seeds. Therefore, F_2 seeds from the cross between a single recessive hairless and a wild strain (Landsberg) were used.

Dry seeds were subjected to 1.1, 2.1 and 2.9 krad of fission neutrons in Oak Ridge National Laboratory and also to 0.7, 1.6, 2.3 and 4.0 krad of monoenergetic 14.1 MeV neutrons in Hiroshima University. 24 to 47 krad of gamma-irradiations from ^{60}Co and ^{137}Cs were

also carried out in each neutron experiment in Oak Ridge National Laboratory and National Institute of Genetics, respectively. About 1,000 seeds in each lot were sown in sterilized sand and experiments were carried out under continuous artifical light in an air-conditioned room at 25±2°C. Around 90 per cent of seeds germinated in the controls and all irradiated lots and almost all of seedlings survived. Irradiations up to about 50 krad of gamma-rays and 5 krad of neutrons had no effect on germination or survival rate.

During the growth of seedlings, segregation ratio of hairless homozygotes was examined. Hairless homozygotes segregated according to the ratio of 3:1 in all lots, as expected, and segregation fitted, according to chi-square test, the simple Mendelian ratio. Therefore, hairy plants must have consisted of homo- and heterozygotes, namely two thirds could be assumed to be heterozygotes. Should somatic mutation from dominant hairy to recessive hairless occur in heterozygotic seeds it had to appear as hairless sectors for which they were examined. Number of such plants with such sectors were counted. Mutations rates increased with increasing dosage in each kind of radiation and the relation was non-linear. Mutation rates were almost the same at 24 krad of ^{60}Co gamma-rays and 1.2 krad of fission neutrons and 0.70 per cent were obtained in both lots. From the results, RBE value of fission neutrons to gamma-rays was about 16. On the other hand, mutation rate in the lowest dosage lot of 14.1 MeV neutrons was somewhat high when it is compared with the other lots. But, RBE value of 14.1 MeV neutrons may be estimated as about 15 from the curve of mutation rates and dosages.

104. *Effects of gamma-irradiated pollen grains on pollen-tube mitosis*

(By Yozô IWANAMI* and Seiji MATSUMURA)

The effects of pollen grain irradiation on pollen-tube mitosis were investigated in *Lilium auratum* LINDL., *Impatiens Balsamina* L., *Camellia japonica* L. and *Camellia sazanqua* THUNB. The data were obtained from artifical culture media, sugar agar plates with 80% sucrose and 1.5% agar. For irradiation gamma-rays were applied with ^{137}Cs 6,000c at dose rate 55 kr/hr. The doses ranged from 0.5 to 100 kr.

Inhibition of mitosis increased with increasing dose; namely the higher the dose, the more delayed was the time of its occurrence and the more reduced was the frequency of nuclear division. Division of the generative nucleus was delayed at small doses, namely at 0.5 kr in *Lilium* and

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1 kr in *Camellia* and *Impatiens*.

In *Lilium auratum* the generative nucleus remained mostly undivided in showing chromosome elements even at 5 kr, while at 20 kr a few nuclei consisted of chromatin masses or chromosomes. The pollen grains of *Lilium* did not germinate smoothly on the media. In natural condition, therefore, their pollen-tube mitosis may be more radio-resistant. At 80 kr irradiation of *Camellia japonica* pollen the mitotic division of the generative nucleus was almost completely inhibited. In *Camellia sazanqua* the generative nucleus did not divide into 2 sperm nuclei above 25 kr, while relatively many generative nuclei with chromosomes were observed at 50 kr. The pollen grains of *Impatiens Balsamina* had the highest radio-resistance. At 50~80 kr the generative nucleus underwent complete mitotic division.

Radio-sensitivity of pollen grains varied widely according to the species. Doses estimated as inhibiting pollen-tube mitosis by 50 per cent are presented in Table 1 for the four species. In general, their pollen-tube mitosis was much more radio-resistant than the meiosis in PMC's and mitosis in root tips.

Table 1. LD_{50}'s doses (kr) for pollen-tube mitosis from gamma-irradiated pollen grains of 4 plant species.

Species	Formation of chromosomes	Prophasic condensation of chromatin threads
Impatiens Balsamina	60~70	60~70
Camellia japonica	50	26~60
Camellia sazanqua	11~24	5
Lilium auratum	6~ 9	0.5~1

G. HUMAN GENETICS

105. *Studies on selection in ABO blood groups*[1)]

(By Yuichiro HIRAIZUMI, Ei MATSUNAGA, Hisajiro IZUMIYAMA, Toshiyuki FURUSHO)

An extensive survey was carried out in the City of Ohdate in 1962 in order to investigate the selective mechanisms operating on ABO blood groups. The data available covered approximately 2,500 couples with wife's age ranging from 30 to 40 years and more than 6,000 children.

1) This work was supported by a grant (RF 61113) from the Rockefeller Foundation.

Not only the ABO blood groups of parents and children, but also fertility of the mothers and mortality of the children were determined. The data have been subjected to statistical analyses. Among the results obtained up to date, three points may be summarized as follows.

(1) There were no differences in the proportion of infertile as well as childless couples, in the mean number of living children, or in the proportion of prenatal deaths between compatible and incompatible mating groups (see Table 1).

Table 1.

	No. of matings	Proportion of couples as yet infertile	Mean no. pregnancies	Proportion of prenatal deanths per pregnancy	Proportion of couples as yet childless	Mean no. of children
Compatible matings	1389	0.022	3.189	0.108	0.030	2.590
Incompatible matings	1056	0.027	3.139	0.101	0.032	2.572

(2) The viabilities of children of various genotypes and the segregation ratio of O genes in the AO and BO fathers were estimated by means of maximum likelihood scoring method. Since a high speed computer is not available in our Institute we had to use hand computers. Because of this difficulty it was necessary to simplify somewhat the

Table 2.

Definitions of parameters

Viability of compatible AO, BO, $AB=1.00$

Viability of incompatible AO, BO, $AB=x$

Viability of AA, $BB=y$

Viability of $OO=z$

k_A (k_B): segregation ratio of O gene from AO (BO) fathers.

h_A (h_B): probability that a parent of type A (B) be $AA(BB)$

Group A

$x=1.79\pm0.28$ $y=2.51\pm0.69$ $z=1.02\pm0.08$

$k_A=0.65\pm0.07$ $h_A=0.166\pm0.021$ (Expected value of h_A is 0.166)

Group B

$x=1.74\pm0.50$ $x=3.41\pm2.49$ $z=1.00\pm0.08$

$k_B=0.66\pm0.08$ $h_B=0.135\pm0.034$ (Expected value of h_B is 0.135)

the procedures in numerical calculations. Hence, the mating types were divided into two groups; (A) matings involving h_A and/or k_A and (B) those involving h_B and/or k_B (for h, k and other parameters, see Table 2). The estimated viabilities are presented in Table 2.

All figures in this Table are those of the first approximation and since the deviations from the null hypothesis of $x=y=z=1$, and $k=0.5$ are very large, it is necessary to continue the calculations to obtain much better estimates. However it is interesting to note that, in both groups, there was no indication of reduced viability of incomptible heterozygous children. This result is consistent with (1). The *O* genes from *AO* and *BO* fathers are transmitted to their children in more than 50%.

(3) Based on the viabilities estimated in (2), the average viability of children from each mating type was computed. These were then compared with the actually observed proportions of prenatal deaths as shown in Table 3.

Table 3.

Group 1		Group 2		Group 3		Group 4	
♀×♂	Prop. of prenatal deaths	♀×♂	Prop. of prenatal deaths	♀×♂	Prop. of prenatal deaths	♀×♂	Prop. of prenatal deaths
$O\times O$	0.118	$A\times O$	0.120	$B\times O$	0.110	$AB\times O$	0.136
$O\times \begin{cases} A \\ B \end{cases}$	0.102	$A\times \begin{cases} A \\ B \end{cases}$	0.101	$B\times \begin{cases} A \\ B \end{cases}$	0.113	$AB\times \begin{cases} A \\ B \end{cases}$	0.089
$O\times AB$	0.100	$A\times AB$	0.100	$B\times AB$	0.078	$AB\times AB$	0.055

In order to eliminate the possible effect of mother's genotype on prenatal death, 16 mating types were divided into 4 groups, each consisting of 4 mating types with the same genotype of the mothers. In each group, the 4 mating types were placed in the order of the estimated viability of children, i.e., in each group the first mating type has the lowest estimated viability of children. There is a clear correlation between them, i.e., a mating type with higher average viability of children showed lower proportion of prenatal deaths. This agreement indicates that the survey made at Ohdate City was technically trustworthy.

As described above, the data obtained in Ohdate City so far indicated no evidence of elimination of children due to ABO incompatibility, which is in a remarkable contradiction to the previous results obtained by others. The reason for this is yet unknown. The distorted segregation ratio of *O* genes in the *AO* and *BO* fathers, however, agreed well with that found in the previous study.

106. *Down's syndrome in Japan*[1]

(By Ei MATSUNAGA)

Down's syndrome or "mongolism" is a well-known abnormality due to chromosomal aberrations. A superficial resemblance in appearance in European patients with this abnormality to normal Asiatic people has often been alleged, as is evident from the designation itself, but the clinical features found in typical cases in Japan are as remarkable as those described for the European patients. As to chromosomal conditions, it seems that more than 150 cases, including unpublished data, have been examined in different laboratories of this country since the first report made by Makino, Tonomura and Matsunaga in 1960. In the majority of cases standard 21-trisomy was revealed, while several cases have been reported to be due to translocation or mosaicisms involving the 21st autosome.

The incidence of this abnormality is about 1 per 500 total births in Europe and North America, but comparative figures from other parts of the world have so far been very limited. Regarding the figures for Japan, Neel (1958) found, from an extensive survey made by ABCC, 6 cases among 63,796 newborns in three cities of Hiroshima, Nagasaki and Kure, but he added further 12 cases among 16,144 infants who were re-examined at the age of 8–10 months, so that the overall frequency may be estimated to be about 1 per 1,200 infants. However, this figure appears to be an underestimate, since the clinical signs are not always wholly developed when the patients are under the age of one year.

As an indirect approach to estimate the frequency of Down's syndrome in this country, a nation-wide survey has been conducted by correspondence with 218 institutions for mental defectives belonging to the 6–18 age group. The number of institutions that have returned the completed questionaires is 77 (35%) up to the present time, providing various informations on 4,483 persons in all. Although the response rate is increasing, a preliminary result based on these data may be reported here. There were 340 individuals with Down's syndrome among 4,483 inmates, the frequency being 7.6%. Examination of the distribution of I.Q. revealed the striking finding that almost all (96%) patients with Down's syndrome showed I.Q. less than 50, whereas the portion of those having I.Q. less than 50, regardless of whether they were affected with Down's syndrome or not, amounted to 68% of all inmates. Thus, the frequency of Down's syndrome among the inmates with I.Q less than 50 may be

[1] This work was supported by a grant (RF 61113) from the Rockefeller Foundation.

calculated as about 10%. If one assumes that our subjects were representatives of mental defectives in a general population with respect to I.Q., and that, as the extensive survey carried out by Miki *et al.* suggested, approximately 0.7% of the general population belonging to the age group between 6 and 18 have I.Q. less than 50, then the frequency of Down's syndrome among Japanese in the corresponding age group is 1 per 1,400. This is of course a rough estimate, but, taking into account the high mortality rate of the patients in early childhood, the incidence of this abnormality per total births in Japan seems to be not so far from that reported for Europe and North America.

Table 1. Distribution of births of children with Down's syndrome according to maternal age

Maternal age in years at birth of children	Percentage distribution		Relative incidence
	321 cases of Down's syndrome	All births in population	
15–19	0.9	2.0	0.46
20–24	13.7	26.1	0.53
25–29	18.4	35.5	0.52
30–34	18.7	21.9	0.85
35–39	27.7	11.2	2.48
40–44	16.8	3.2	5.31
45–49	3.7	0.2	18.73
All ages	99.9	100.1	1.00
Mean age	33.2	28.2	—

It has been repeatedly demonstrated that the incidence of Down's syndrome increases with advancing maternal age. Results obtained in Europe and North America are generally consistent in that the increase of this abnormality with maternal age is at first rather slow until the mother reaches the age of 35, and then it becomes very rapid as the mother approaches the age of menopause. Compared with the overall frequency, a 2- to 4-fold increase for the age group of 35 to 39, a 5- to 10-fold increase for the age group of 40 to 44, and after the age of 45 a 10- to 20-fold increase was found (Lenz, 1959; Penrose, 1961). The question remains, however, whether there is any geographical or racial difference concerning the increase rate. The results of our study of 321 cases are summarized in Table 1. The control population is calculated on the basis of the distribution of births in census reports of the years

corresponding to the birth dates of cases with Down's syndrome. As is apparent from the Table, there is for this abnormality no indication for any difference between Japanese and Caucasians as to suppression or acceleration of the increase rate with advancing maternal age.

107. *Clinical conditions of patients with apparently normal chromosomes*

(By Akira TONOMURA)

Based on cultures of peripheral blood and/or skin biopsy specimens, patients with the following clinical conditions were found to have 46 chromosomes of apparently normal karyotype.

	Age	*Sex*	*Clinical conditions*
1)	6 months	M	Polydactyly; reduplication of left thumb
2)	3 months	M	Multiple congenital abnormalities; heart defect and low set ears
3)	5 months	M	Oligodactyly; split hands
4)	2 months	M	Multiple congenital abnormalities; Megalocytosis
5)	13 years	M	Laurence-Moon-Biedl's syndrome
6)	8 years	M	Fanconi's syndrome
7)	12 years	M	Gargoylism (Hurler's syndrome)
3)	8 years	F	Pterygium coli
9)	21 years	F	Primary amenorrhoea
10)	31 years	F	"
11)	21 years	F	"
12)	18 years	F	Hypoplastic ovary
13)	25 years	F	Testicular feminization
14)	20 years	F	"
15)	25 years	F	Female pseudohermaphroditism
16)	18 years	M	Male pseudohermaphroditism
17)	22 years	M	"
18)	4 years	M	"

108. *Variation in length of the Y chromosome in man*

(By Akira TONOMURA and Hiroshi ONO)

During our current survey of chromosomes in human subjects associated with various types of congenital disorders, we have found an unusually long Y chromosome in a 12-year-old mentally retarded boy and in his apparently normal father. Variations in the length of the Y chromosome in this family were investigated in comparison with those of two control series obtained from normal males. The well-spread metaphase cells were photographed and the chromosomes were measured. The relative length of the Y chromosome in each cell was expressed as the ratio between the length of the Y and the mean length of nos. 21 and 22. The results are summarized in Table 1.

Table 1. Comparison of the relative length of *Y* chromosome of patient and his father with that of normal males.

Person	Mean of Y ($\bar{X}$)	No. of cells examined
Propositus	$\bar{X}_1=1.46$	20
Father	$\bar{X}_2=1.45$	20
Control series 1. (a male)	$\bar{X}_3=1.185$	21
Control series 2. (1 cell from each of 53 males)	$\bar{X}_4=1.186$	53

The "t" test was applied comparing the means:
$\bar{X}_1$ with $\bar{X}_2$; $t_{38}=0.18$ $0.80<P<0.90$
$\bar{X}_1$ with $\bar{X}_3$; $t_{39}=5.71$ $P<0.001$.
$\bar{X}_3$ with $\bar{X}_4$; $t_{73}=0.05$ $P>0.90$.

The results of statistical analysis support the view that the Y chromosome in this family is significantly longer than in the control series and may be a heritable characteristic. The presence of a significantly long Y chromosome has been reported in several cases with phenotypic abnormalities as well as in their normal relatives. In general, variation in the relative length of a chromosome may reflect differences in chromatin content (duplication, deletion or translocation) or contraction states of heterochromatin (differential spiralization). Since the Y chromosome in man is highly heterochromatic, the increased length of the Y in the present family seems to be due to a difference in the degree of spiralization of the whole or a part of the Y chromosome rather than that in chromatin content.

109. *Drumsticks of polymorphonuclear leucocytes in newborn females*

(By Akira TONOMURA, Hiroshi ONO and Keiichi SOGA)

In has been generally accepted that the drumsticks found in polymorphonuclear leucocytes of female blood films are the counterpart of the sex chromatin in tissue cells, although the quantitative correlation between drumstick and sex chromatin is still complicated. In a previous paper (Tonomura, Toyofuku and Matsunaga; 1962) the authors demonstrated that the number of drumsticks found in 500 neutrophils decreases slightly, but significantly with age. On the other hand, Smith *et al.* (1962) reported a lowered incidence of sex chromatin in normal newborn females. Furthermore, it has been suggested by Taylor (1963) that the reduction of the amount of sex chromatin is a reflection of altered metabolic processes in the neonatal period, possibly due to extremely high levels of oestrogens during the first few days after birth. If this is so, it is very interesting to know whether the frequency of drumsticks in the newborn females is influenced by the metabolic changes of this period.

In the present investigation blood smears were obtained from 10 females from 1 to 12 days after birth. Five hundred neutrophil leucocytes were examined in each smear, and the demenstrable drumsticks were counted. All of the newborn females examined showed considerably high frequencies of drumsticks at the average of 32.0; the lowest number was 23 and the highest was 40 in the examination of 500 neutrophils. In the previous study the incidence of drumsticks in 250 females whose ages varied from 1 to 79 showed an average of 12.0 ± 0.4. Though the numbers here examined are too small to be conclusive, the results of our preliminary study may indicate that the drumstick situation of newborn females is somewhat different from that of sex chromatin.

110. *Function of the amino group in the molecular complex consisting of hemoglobin and haptoglobin*

(By Tomotaka SHINODA)

In the endeavor to elucidate the mechanism of haptoglobin action and the role of the amino group in its specificity and catalytic function, an attempt has been made to convert α or ε-amino groups of lysine into the corresponding trinitrophenyl-amino residues. Human haptoglobin, which was previously typed in starch gel electrophoresis and purified with column chromatography, was dissolved in physiological saline solu-

tion and treated with TNBS at 37°. The hemoglobin binding capacity (HBC) and electrophoretic behavior of the resulting materials were compared in the usual way with those of the control preparation. As to the HBC of three types of haptoglobin 1-1, 2-1 and 2-2 at the stage of introduction of 1.8 moles of trinitrophenyl residues into the protein molecule, no significant changes were observed in the first two while in the remaining the decrease in HBC amounted to 15%. On the whole, the HBC of haptoglobin showed the tendency to be inversely proportional to the amount of trinitrophenyl residues introduced into protein molecules. Using electrophoresis, a fine separation of a complex of modified haptoglobin with nemoglobin could be achieved from the unmodified one. The formation of a complex consisting of hemoglobin and modified haptoglobin, has been observed on filter paper by cross-interaction technique during the course of electrophoresis. These findings are in good agreement with the result expected from tne loss of amino residues of haptoglobin through the reaction with TNBS. On the other hand, when hemoglobin was in the same way previously treated with TNBS the formation of the complex consisting of modified hemoglobin and haptoglobin was also observed but with less peroxidase activity. So far about 70% of amino residues of hemoglobin molecule have been altered by the reagent, and there was no more ability to form a molecular complex with haptoglobin. These results may be considered to be the consequence of the loss of functional amino groups which play an essential role in the formation of the molecular complex between the two components as well as conformation of the protein molecule at the time of the formation of the complex.

Analysis of the results is now in progress by means of more rigorous procedures.

111. *Haptoglobin and G-6-PD deficiency in a Japanene population*

(By Tomotaka SHINODA)

During the summer season of this year, a field survey on glucose-6-phosphate dehydrogenase (G-6-PD) deficiency was carried out in the Shichikawa area of Wakayama prefecture in collaboration with a group of Wakayama Medical College. Blood samples were taken from 1,096 persons, and the activity of the enzyme was measured by means of "glutathione stability test". Throughout the survey, one male subject was found to be deficient (the amount of erythrocyte glutathione was less than 20 mg per 100 ml of packed erythrocyte after treatment of blood specimen with oxidating agent), thus the frequency of the trait

in Japan being roughly 0.1%.

In addition to the screening of G-6-PD deficiency, sera were separated from the blood samples and haptoglobin types were determined by starch gel electrophoresis. So far 171 specimens have been analyzed, and the frequencies of the types 1-1, 2-1 and 2-2 were 7, 77 and 87, respectively. Based on this result, the frequency of gene Hp^1 may be estimated as 0.266.

Further anslysis of the samples is now in progress.

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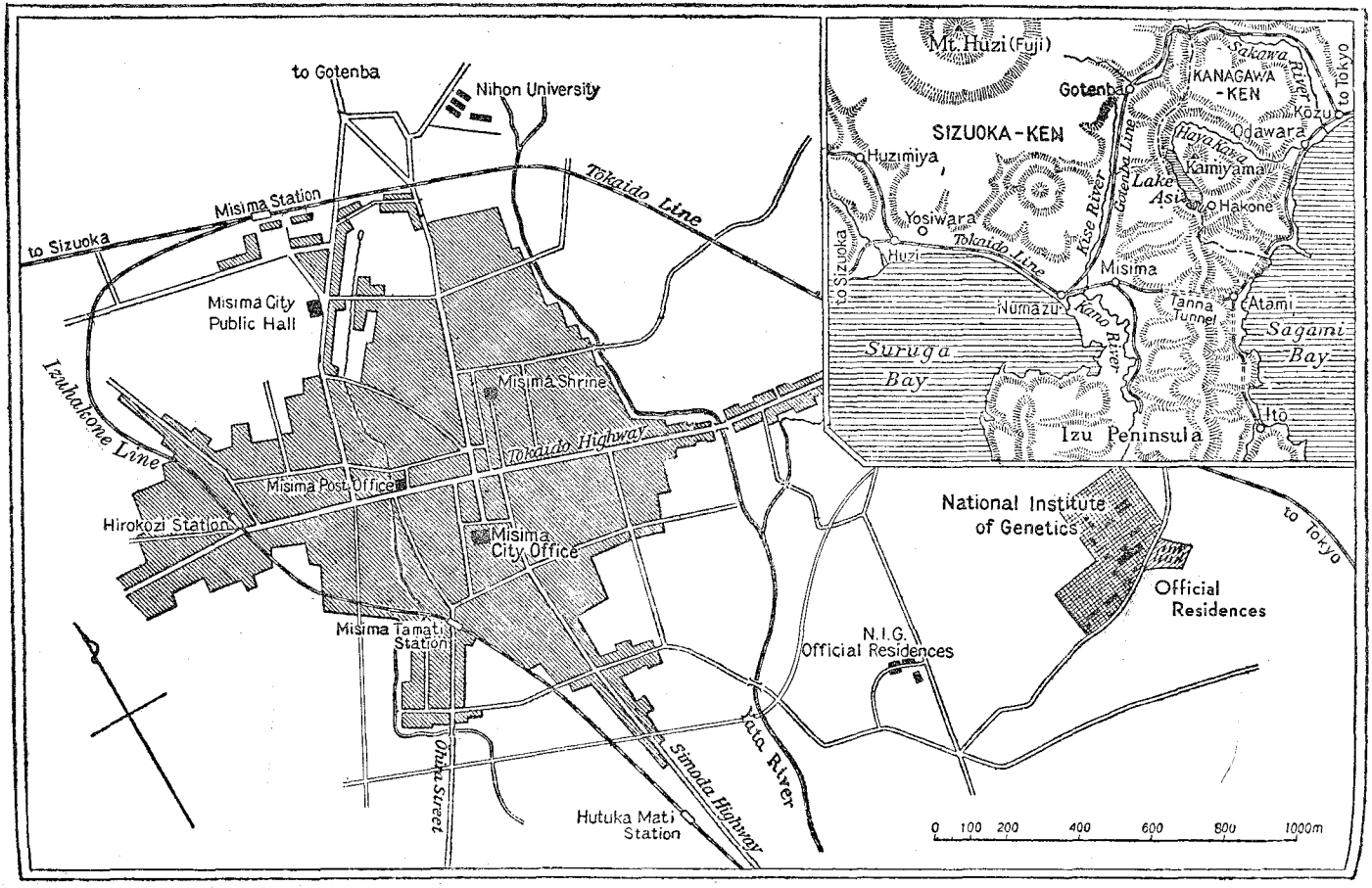
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