THE INHERITANCE OF SUSCEPTIBILITY TO YELLOW FEVER ENCEPHALITIS IN MICE

CLARA J. LYNCH AND THOMAS P. HUGHES

(From the Laboratories of the Rockefeller Institute for Medical Research, and the Laboratories of the International Health Division of the Rockefeller Foundation, New York)

Received October 2, 1935

INTRODUCTION

THE importance of host resistance as a factor in determining the incidence of infectious disease has been emphasized in recent studies. The methods developed in the study of genetics have provided a practical means of investigating the problems of host resistance and from the accumulated data there can be no doubt of the existence of heritable factors which affect the response of the individual to infection. A bibliography of the more important contributions in this field is given in a review of the subject by HILL (1934). The experiments to be reported here give evidence of the presence of such factors influencing the incidence of a virus disease, yellow fever.

The susceptibility of mice to the virus of yellow fever was demonstrated by THEILER (1930). SAWYER and LLOYD (1931) tested the suitability of mice as test animals in the study of yellow fever immunity and found that different strains of mice varied in susceptibility to this virus, ranging from 100 percent to 50 percent mortality with standard dosage of virus. This information was not only of practical value in the work on yellow fever immunity, but also indicated the operation of genetic factors in yellow fever resistance. On this basis the following investigation was undertaken.

METHOD OF TESTING

Since yellow fever infection occurs in mice only as an encephalitis, test inoculations were made by intracerebral inoculation while the mice were under ether anaesthesia. Susceptible mice when so inoculated with a virus "fixed for mice" show a typical paralysis which usually occurs on the fourth or fifth day after inoculation and terminates in death within one day from onset. Mice showing no paralysis in ten days are classed as survivors and are discarded.

The virus selected had been "fixed" by passing in mice from brain-to-brain for over 100 passages and had reached its maximum virulence for this animal. To obtain approximately equal dosages, a virus was used which had been preserved by drying while in the frozen state, in which form SAWYER, LLOYD, and KITCHEN (1929) have shown its virulence to be retained over a period of years. The dried virus was diluted with a

sufficient quantity of suitable diluent to yield a final concentration in which approximately 30 minimum lethal doses were contained in 0.03 cc., which amount is inoculated intracerebrally into the mice to be tested. However, for certain technical reasons concerned with the drying of virus, tubes opened at different times vary in virus content, and since the keeping quality of the virus is such that titrations cannot be completed before the virus activity is lost, the doses are not uniform. To overcome this difficulty the contents of several tubes are pooled for each test and to demonstrate viability of the virus a number of unselected Swiss mice are inoculated with each virus suspension used.

MATERIAL

The "Swiss" strain was chosen as a source of susceptible animals. The foundation stock consisted of 2 males and 7 females obtained in 1926 from Dr. A. de Coulon in Lausanne, Switzerland. The individuals used in these experiments were taken from lines, some of which were inbred brother by sister and others partly pen inbred. SAWYER and LLOYD (1931) had previously tested mice from a branch of this stock, observing 100 percent mortality in each of two lots consisting of 30 and 23 mice respectively.

TABLE 1

Mortality percentages of mice from the Swiss and Det strains. Preliminary tests

TESTED BY	GROUP	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	PERCENT DIFFERENCE	χ^2	P
Sawyer and Lloyd	Swiss (test 4)	30	30	100.0			
	Swiss (test 5)	23	23	100.0	18.5	10.83	<.01
	Det (test 3)	54	44	81.5			
Lynch and Hughes	Det	88	58	65.9	15.6	4.01	.05
	Swiss	138	134	97.1	31.2	40.91	<.01

The strain selected as a source of resistant stock had also been tested by SAWYER and LLOYD. Although in their tests the lowest mortality had been given by the "Rockefeller Institute Stock," certain practical considerations led to the choice of stock referred to by SAWYER and LLOYD as "Strain D" and designated by us as "Det." Five pairs were purchased and they and their descendants were inbred, usually brother by sister, but occasionally parent by offspring. Mice from the second to the sixth generations were used in these tests.

In the hands of SAWYER and LLOYD, the Det mice had a mortality of 81.5 percent (table 1), about 18.5 percent lower than the Swiss strain; however, the comparison may not be accurate since the two groups were

inoculated with different virus preparations. A preliminary test of the Det mice in our colony resulted in a distinctly lower figure: of 88 mice inoculated, 65.9 percent succumbed. Among 138 Swiss mice tested with the identical virus preparation, there was 97.1 percent mortality. The difference is 31.2 percent and the χ^2 test for the significance of the difference gives P as $<.01$. This result confirmed our choice of material since the divergence of the strains is sufficiently great to warrant a cross between them. Therefore matings were made between representatives of the Det and Swiss strains and the hybrids thus produced were crossed back to the parental strains, sometimes to the original parents. The resulting population was divided into two lots and was inoculated with virus. Variations in the rate of reproduction together with exigencies of time and laboratory space prevented a regular scheme of distribution into two groups so parents and their offspring were not always inoculated together. During the course of the experiment some animals died of intercurrent disease before their reaction to yellow fever virus could be determined. Not all individuals of the P_1 and F_1 generations contributed offspring to the experiment.

RESULTS

The first test included samples from all the generations under observation (table 2). The original strains were represented by 39 Swiss and 18 Det mice. Upon inoculation all the Swiss mice succumbed, while the

TABLE 2

Test I. Mortality percentages of representatives of the parental strains, F_1 and backcross progeny

GROUP	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	PERCENT DIFFERENCE	χ^2	P
Swiss	39	39	100.0	22.2	9.32	$<.01$
Det	18	14	77.8			
F_1 (Swiss×Det)	37	31	83.8			
Backcross (F_1×Swiss)	146	137	93.8	23.1	24.03	$<.01$
Backcross (F_1×Det)	99	70	70.7			

mortality rate of the Det mice was 77.8 percent. The difference between the strains, when measured by the χ^2 test is significant, $\chi^2 = 9.32$ and P $= <.01$. Thirty-seven individuals were available for examination in the F_1 generation obtained from the cross Swiss by Det. Of these 31, or 83.8 percent died—a mortality not much higher than that of the Det and significantly different from that of the Swiss strain ($\chi^2 = 6.86$; $P = <.01$). In the backcross generation somewhat larger numbers are dealt with, the two classes comprising 146 and 99 animals respectively. The descendants produced by crossing the hybrid to the more susceptible strain showed a heightened mortality (93.8 percent) while the mice resulting from the

backcross to the less susceptible stock showed a decreased mortality (70.7 percent). This difference is greater than would be expected on a basis of random sampling of the same population, having a χ^2 value of 24.08 and $P = <.01$.

The second test also included representatives of the five classes (table 3). Only 8 Swiss mice were available but again the mortality was 100 percent, while 61 mice from the Det strain showed only 50.8 percent mortality—the lowest rate yet observed. The F_1 had a mortality of 65.4 percent. Compared with the Swiss strain the difference in mortality is probably significant ($\chi^2 = 3.76$; $P = .05$). The backcross progeny produced by mating the F_1 hybrids to the more susceptible strain showed a mortality of 70.2 percent while in the backcross to the less susceptible strain the mortality was reduced to 50.0 percent. The difference between them is 20.2 percent, about the same as in the first experiment, and is also mathematically significant ($\chi^2 = 8.32$ and $P = <.01$).

TABLE 3

Test II. Mortality percentages of representatives of the parental strains, F_1 and backcross progeny

GROUP	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	PERCENT DIFFERENCE	χ^2	P
Swiss	8	8	100.0	49.2	6.96	<.01
Det	61	31	50.8			
F_1 (Swiss×Det)	26	17	65.4			
Backcross (F_1×Swiss)	114	80	70.2	20.2	8.32	<.01
Backcross (F_1×Det)	84	42	50.0			

In comparing the two tests certain similarities are apparent. In both cases there is a significant difference between the susceptible and partially resistant stocks, the hybrids are more like the resistant strain and the backcross groups have a heightened or lowered mortality depending on the degree of susceptibility of the parent stock to which the backcross was made. On the other hand, the dissimilarities are so marked that it becomes doubtful whether the figures of the two tests should be combined. Each class in the second test has a lower mortality than its corresponding set in the first test, with the exception of the Swiss in which only 8 individuals were used in the second test. The samples of the parental Det strain gave significantly different mortalities in the two tests and the backcross progeny from the susceptible strain showed no greater mortality in the second test than did the backcross progeny from the resistant strain in the first test. Since in all probability the quantity of virus in the inoculum varied between tests, the lower mortality rate in the second test may reasonably be referred to this difference in virus content. However, a further genetic analysis of the data is possible. Some of the hybrid parents were back-

crossed in the two directions. We find that, in the first experiment 4 F_1 parents, and in the second test 6 F_1 parents were mated with individuals from each stock. Their offspring are listed in tables 4 and 5. These tables

TABLE 4

Test I. Mortality rates of the progeny of the same F_1 individual backcrossed to both parental strains

IDENTIFICATION NUMBER OF F_1 PARENT	BACKCROSS PROGENY				SIGNIFICANCE OF DIFFERENCE
	FROM F_1×DET		FROM F_1×SWISS		
	NUMBER DEAD	NUMBER ALIVE	NUMBER DEAD	NUMBER ALIVE	
♂ 69.0	8	6	14	0	
♂ 70.0	5	2	22	1	
♀ 54.1	5	1	6	0	
♀ 54.3	4	0	5	0	
Total	22	9	47	1	$\chi^2=12.37$ P = <.01
Percent dead	71.0		97.9		

show in detail the degree of regularity with which these individuals produced offspring with unlike mortality ratios among siblings resulting from contrasted matings. The totals again indicate hereditary differences.

TABLE 5

Test II. Mortality rates of the progeny of the same F_1 individual backcrossed to both parental strains

IDENTIFICATION NUMBER OF F_1 PARENT	BACKCROSS PROGENY				SIGNIFICANCE OF DIFFERENCE
	FROM F_1×DET		FROM F_1×SWISS		
	NUMBER DEAD	NUMBER ALIVE	NUMBER DEAD	NUMBER ALIVE	
♀ 68.20	0	5	10	0	
♀ 55.40	1	2	3	0	
♀ 55.25	1	4	7	5	
♀ 61.46	0	11	10	2	
♂100.00	6	12	9	8	
♂101.00	1	0	6	3	
Total	9	34	45	18	$\chi^2=26.08$ P = <.01
Percent dead	20.9		71.4		

A more exact comparison may be made between the offspring of the various types of mating when the susceptibility of both parents is known. The animals for which this information is available are grouped in table 6. In the first test all of the immediate progeny in any generation resulting

from crosses between two susceptible parents may be compared with the progeny resulting from matings in which only one parent was susceptible. There were only 9 mice available in the second class but their mortality was 22.2 percent as contrasted with a mortality of 91.4 percent among those with two susceptible parents. In the second test the figures for the corresponding classes are 27.6 percent and 69.6 percent, again a significant difference. In addition there was a third class comprised of 15 individuals with two resistant parents. In this group there was but one death, a mortality rate of 6.7 percent; however, this figure is not significantly lower than that given by the offspring from unlike parents.

TABLE 6

Mortality percentages among mice classified as to whether both parents were susceptible, only one susceptible, or both resistant

TEST	PARENTAGE	NUMBER DEAD	NUMBER ALIVE	PERCENT DEAD	PERCENT DIFFERENCE	χ^2	P
I	S×S	74	7	91.4	69.1	29.47	<.01
	S×R	2	7	22.2			
II	S×S	16	7	69.6	42.0	9.10	<.01
	S×R	8	21	27.6			
	R×R	1	14	6.7	20.9	2.66	.10

S = Susceptible.
R = Resistant

By this classification also the values obtained in the two tests are not similar. In the first test the mortality in the population derived from susceptible parents was 91.4 percent while in the second test it was only 69.6 percent. Since it has not been shown that the parental matings were between homozygotes, the genetic expectation for these values cannot be computed. However, as a whole the data seem sufficient to justify the conclusion that the type of parentage and the susceptibility of the offspring are correlated.

On the assumption that the two tests may properly be treated as self-contained units, an inquiry into the homogeneity of the groups of which they were composed was undertaken. The Lexian ratio was used to test the dispersion of the probabilities of death among the litters in each group, although the numbers dealt with in some cases were small. In the preliminary study of the Swiss, the sibling relationships were known for only 96 mice but this number which contained the only four resistant animals of the group proved homogeneous (table 7). While the Det stock appeared uniform in the first test, all of the mice used as progenitors were not tested and the larger population used in the second test was not homo-

geneous. The F_1 hybrid generation gave different results in the two tests. The backcross progenies lacked uniformity but homogeneity was shown in the groups classified on a basis of parentage.

TABLE 7

Tests for the type of dispersion of the probabilities of death within various groups

TEST	GROUP	NUMBER TESTED	NUMBER OF FAMILIES	L	χ^2	P
Preliminary	Det	88	27	1.22	40.27	.04
	Swiss	96	35	1.10	42.59	.18
I	Swiss	39	—	—	—	—
	Det	18	6	1.11	7.39	.19
	F_1 (Swiss×Det)	37	7	1.41	13.85	.04
	Backcross (F_1×Swiss)	146	20	1.71	59.06	<.01
	Backcross (F_1×Det)	99	20	1.52	45.94	<.01
II	Swiss	8	—	—	—	—
	Det	61	25	1.35	45.60	<.01
	F_1 (Swiss×Det)	26	7	1.12	8.73	.19
	Backcross (F_1×Swiss)	114	22	1.35	40.27	.01
	Backcross (F_1×Det)	84	22	1.63	58.58	<.01
I	S×S	81	13	0.77	7.66	.81
	S×R	9	2	0.40	0.32	.59
II	S×S	23	5	1.16	6.84	.15
	S×R	29	8	1.01	8.14	.32
	R×R	15	3	1.20	4.29	.12

In table 8 the various groups are arranged according to sex. There is no indication that sex is a differentiating factor. The apparently greater susceptibility of females shown in the last backcross group where a test

TABLE 8

Comparison of mortality of the sexes within various groups

TEST	GROUP	SEX	NUMBER DEAD	NUMBER ALIVE
I	F_1 (Swiss×Det)	♂♂	15	4
		♀♀	17	1
	Backcross (F_1×Swiss)	♂♂	55	3
		♀♀	82	6
	Backcross (F_1×Det)	♂♂	30	16
		♀♀	40	13
II	F_1 (Swiss×Det)	♂♂	10	6
		♀♀	7	3
	Backcross (F_1×Swiss)	♂♂	44	19
		♀♀	36	15
	Backcross (F_1×Det)	♂♂	12	25
		♀♀	30	17

for independence gives χ^2 as 8.16 and P as <.01, is probably due to the fact that in several litters where resistance was high there were no sisters brought to test.

DISCUSSION

In attempting to demonstrate genetic factors, it becomes necessary to control as far as possible any environmental conditions which may affect the results of the tests. The immediate surroundings in the laboratory were kept as constant as was possible, but many variables exist which are beyond control. In this experiment the occurrence of acquired immunity may safely be ruled out, since the mice were sent to a laboratory remote from the breeding room for tests. No survivors were returned. There can be no question as to the freedom of the tested animals from previous contact with yellow fever virus.

No selection of strains was undertaken during this experiment. Rather, the investigation was planned to take advantage of strains from diverse sources in which some differentiation already had occurred. Although lack of known material for the various crosses precludes a final analysis, the existence of segregating factors for susceptibility and resistance is evident. This is clearly shown by the differential ratios given first by backcrosses and second by progeny from different sorts of matings. The latter criterion for inheritance is not of universal application. When in a genetically pure strain, the character in question, because of somatic fluctuation, does not manifest itself with absolute regularity, progeny from a mixed parentage do not differ significantly from offspring descended from like parents. If progeny groups are dissimilar, whether the parents do or do not present phenotypic differences, they must be different genetically. Consequently progeny are the most reliable basis for determining the constitution of parents. When differing groups of progeny are descended from unlike types of matings, as in the present case the genetic explanation is self-evident.

As to the mode of inheritance, since matings between like parents, either both susceptible or both non-susceptible, produced two types among their offspring either fluctuating variations or the joint occurrence of a number of genes are indicated. Neither of these alternatives can be ruled out by our data.

SUMMARY

1. Mice from two sources gave different mortality rates when inoculated with the virus of yellow fever. This difference was maintained in three separate tests.

2. When the strains were crossed, the hybrids showed a mortality less than that of the susceptible strain. In one test the difference was clearly significant; in another it was probably significant. By crossing the hybrid back to the susceptible strain the mortality rate was increased; by crossing back to a more resistant strain the rate was lowered. This relationship was demonstrated in two tests.

3. When the mice were classified according to parentage, offspring from two susceptible parents were more susceptible than were offspring with one or two resistant parents.

4. Susceptibility did not appear to be modified by sex.

5. It is concluded that hereditary factors for resistance to yellow fever encephalitis are present in mice.

LITERATURE CITED

HILL, BRADFORD A., 1934 The inheritance of resistance to bacterial infection in animal series. A review of the published experimental data. London: Medical Research Council, Special Report Series No. 196: 69–71.

SAWYER, W. A. and LLOYD, WRAY, 1931 The use of mice in tests of immunity against yellow fever. J. Exp. Med., 54: 533–555.

SAWYER, W. A., LLOYD, W. D. M. and KITCHEN, S. F., 1929 The preservation of yellow fever virus. J. Exp. Med., 50: 1–13.

THEILER, M., 1930a Susceptibility of mice to the virus of yellow fever. Science N.S. 71: 367.

1930b Studies on the action of yellow fever virus in mice. Ann. Trop. Med. and Parasit., 24: 249–272.