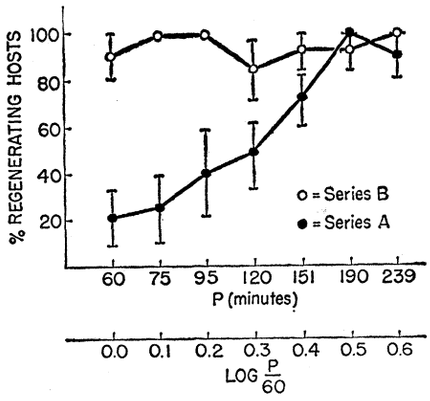

Fig. 2. Percent of hosts regenerating a basal disk (at mid-peduncle of the reassembled animal) as a function of length of the incubation period, P. Each point represents 8 to 17 experiments. Bars show the standard error of the estimate ($\sqrt{r(1-r)/n}$, where r is the proportion regenerating and n is the number of experiments).

A and not in B (*5*). The original polarity of the fragments was maintained in all experiments. The time elapsed between amputation of the host and completion of grafting was recorded, and 60 minutes were added as a correction for the time required for fusion of host and graft. The resulting time interval was used as an estimate of the period during which the host was free of tissue-mediated influence from the graft. This interval was designated the incubation period, P.

In series A, all grafts retained their basal disk. In series B, all grafts regenerated a basal disk at the proximal tip. In addition, disks appeared in the mid-peduncle of many of the reassembled animals (Fig. 1, B and C). Such animals sometimes retained a constriction at the graft line; in these cases the supernumerary basal disk always appeared immediately distal to the constriction, indicating that it had differentiated from the proximal tip of the host. All regenerating basal disks appeared between 24 and 48 hours after grafting. Whether derived from the host or from series-B grafts, they were easily recognized by their form and by their characteristic stickiness.

Nearly all hosts grafted with truncated half-peduncles (series B) regenerated a basal disk, regardless of the duration of P. The experimental treatment was thus sufficient to induce formation of basal disks in the series B hosts. In contrast, the majority of hosts grafted with half-peduncles bearing a basal disk (series A) failed to regenerate a disk, unless the incubation period exceeded 2 hours (Fig. 2). Induction of the basal disk in series-A hosts was thus either blocked or reversed by the disk of the graft (*6*). It is clear that the mechanism of disk induction must be sensitive to the presence of a basal disk.

With increasing duration of P, grafted basal disks were progressively less successful in inhibiting induction of disks in the hosts. Presumably, induction becomes irreversible after a variable period following amputation. Under our conditions, irreversible induction in 50 percent of the cases required that the host remain without a basal disk for a critical period of 2 hours. Three hours of incubation without a disk sufficed for irreversible induction in substantially all cases. This is much less than the time (24 to 48 hours) required for the formation of a morphologically recognizable disk.

We sought to determine how long a grafted disk has to be present to inhibit disk induction. Series-A experiments with P equal to 60 minutes were conducted as previously, except that the basal disk of the graft was excised at various intervals after fusion. If the disk had not been excised, only 20 percent of the hosts would have regenerated disks at mid-peduncle (Fig. 2). An increased incidence of regeneration indicated that there had been insufficient time for complete inhibition. The presence of the grafted disk for 5 hours was sufficient to reduce the incidence of regeneration to the background value of 20 percent, whereas removal of the disk after only 2 hours allowed 70 percent of the hosts to regenerate.

In our system, a grafted basal disk can inhibit the development of another disk if present during a period of induction, which appears to last less than 5 hours. During this period, removal of the grafted disk is sufficient to alleviate the inhibition. We wish to emphasize that we have defined inhibition operationally; the evidence on hand does not establish any particular mechanism. However, it is tempting to speculate that the effect is mediated by an inhibitory substance produced by the basal disk itself. Such a substance may also serve as a negative feedback in the intact animal, regulating the size of the basal disk.

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5. We did not attempt to make A and B grafts equal in size, since this would have resulted in a systematic difference between the groups with respect to the origin of the distal end of the graft. We felt that it was most important to standardize the nature of the graft tissue in direct contact with the assay site.
6. The difference between the two experimental series at P equal to 60 or 75 minutes was highly significant (probability is less than .05). The difference (70 to 75 percent) was large compared to the standard error of the estimate for each result (13 to 15 percent) and thus cannot be attributed to the systematic difference in size between grafts of the two series, which was small compared to the size variability within each group.
7. Supported in part by NSF grants GY-3124 and GB-4171. We thank Rick Stafford and Irene Sosnowski for assistance in photography and maintenance of cultures, respectively.

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Apparent Production of Two Types of Antibodies by a Single Cell

Abstract. *Immunization of mice with bacterial, sheep red cells coated with antigen resulted in apparent production of antibody-forming plaques with specificities for both hemolysis and bactericidal activity. The secondary response for production of these bifunctional types of plaques could be induced by red blood cells or bacteria alone. A significant mutual suppression of immune response between the two immunizing agents was observed.*

One of the most heated controversies in immunology centers around the potentiality of the antibody-forming cells. The dispute concerns whether a single cell can produce only one type of antibody at a given time. The experimental evidence has been equivocal, and available data support the possibility of production of one (*1*) or several types of antibodies per single cell (*2*). The major factor for the persistence of the controversy is the fact that, although methods utilized in the past for testing the potentiality of antibody-forming cells were elegant and precise, they were also quite complex and cumbersome. Development of simple and rapid tests for detecting antibody-forming cells provided an opportunity to extend and test the suggested alternatives (*3*).

Table 1. Formation of hemolytic (H) and bactericidal (B) plaques by spleen cells. PFC, plaque-forming cells.

PFC per spleen (No.)		
H	B	H and B
Sheep erythrocytes		
2×10^4	80	0
E. coli *0127 antigen*		
60	7.2×10^4	0
Erythrocytes and E. coli		
1.5×10^4	4×10^4	0
Erythrocytes coated with E. coli		
5×10^4	6×10^3	2.3×10^3

We used Jerne's agar plaque technique and examined the immune responses to sheep erythrocytes and bacteria of *Escherichia coli* 0127 in mice. We investigated the possibility of antibody production against the two different types of antigens by a single cell and also observed the competition between the antigens resulting in suppression of antibody formation. White mice (Swiss origin, 6 weeks old) were immunized intraperitoneally or intravenously by one of the following antigens: 0.2 ml of 10-percent sheep erythrocytes; 0.2 ml of 1-percent heat-killed *E. coli* 0127: B8; 50 μg of somatic antigen (*E. coli* 0127: B8) suspended in saline; and 0.2 ml of 10-percent sheep erythrocytes coated with heated somatic antigen of *E. coli* 0127: B8. Mice were killed on the 4th day after immunization, blood was removed by cardiac puncture, and spleens were tested for the presence of antibody-forming cells (*4*). Because antibody-forming cells to both erythrocytes and bacterial antigens were investigated, bacteria and red blood cells were both incorporated into soft agar. Spleen cell suspensions were added to the agar; after incubation for 1 hour at 37°C, fresh human cord serum, used as a source of complement, was poured over the plate surface. The plates were incubated for an additional hour, and the hemolytic plaques were marked and counted. The incubation at 37°C was continued for 6 more hours to allow sufficient bacterial growth. To distinguish between hemolytic and bactericidal plaques, the location of the former was first marked, and subsequently the hemolytic plaques were eliminated by the addition (to the agar surface) of a few drops of hemolysin against sheep erythrocytes. This caused lysis of all erythrocytes. The antibody-forming cells located in the centers of the plaques were observed with a dissecting microscope, and only plaques containing a single nucleated cell were scored in analysis. For testing of hemolytic and bactericidal titers, it was necessary to supplement mouse serum with fresh human cord serum as a source of complement. Absorption of mouse serum was carried out with 20 percent of packed cells (erythrocytes or bacteria) which were kept suspended in the serum for 2 hours at 4°C. Serums were cleared from the cells by centrifugation.

In the first series of experiments, groups of mice were immunized with one of the following antigenic preparations: sheep erythrocytes, *E. coli* 0127 bacteria, *E. coli* 0127 somatic antigen, or sheep erythrocytes coated with somatic antigen. Each preparation was injected into groups of three mice, and the reported values represent averages of each group. On the 4th day after immunization, bactericidal plus hemolytic antibody-forming cells of the animal spleens were examined. Animals immunized with a single type of antigen or with the mixture of antigens responded with the formation of antibody of single specificity per cell; immunization with sheep erythrocytes coated with bacterial antigen induced the appearance of plaque-forming cells which produced a high proportion of both hemolytic and bactericidal antibodies (Table 1). Frequency of cells with apparent double specificity amounted to 4.6 percent in relation to hemolytic plaques with single specificity and 38 percent in relation to bactericidal plaques with single specificity. The dual function of the antibody-forming cells could be explained by formation of cross-reacting antibodies. To examine this possibility, we tested serums of immunized mice for their bactericidal and hemolytic activity before and after absorption with sheep erythrocytes and *E. coli* 0127. We found that serum of animals immunized with bacterial red blood cells coated with antigen contained bactericidal and hemolytic antibodies in high titers (hemolytic titer 1:20,000; bactericidal titer 1:10,000). When this serum was absorbed with bacterial cells, the bactericidal activity was completely abolished without affecting the hemolytic titer. Absorption of the serum with sheep red cells removed the hemolysins, but the bactericidal titer of this serum remained unaltered. The serums absorbed with both erythrocytes and bacteria were not lytic for sheep erythrocytes coated with bacterial antigen.

The lymphoid cells are believed to retain memory of the antigen after the primary immunization; this becomes evident during secondary response when the immune response becomes rapid and intense. If some cells could produce antibodies of two specificities, this would be confirmed during a secondary response. Mice were immunized with the same antigenic substances as shown in Table 1; 12 weeks later, the animals were challenged either by sheep erythrocytes or *E. coli* cells. As shown in Table 2, mice given bacterial, antigen-coated erythrocytes during primary immunization produced bactericidal and hemolytic plaques when they were injected with erythrocytes or bacteria during the secondary challenge.

In the final series of experiments, we tested whether or not bacteria and erythrocytes affect each other's immune response. We injected bacteria and sheep erythrocytes intravenously with different time intervals between their administration. If intervals between injections were less than 6 hours, the antibody responses to both antigens was of the same order as for separate injections (3×10^4 hemolytic plaques and 1×10^5 bactericidal plaques). When the interval between injection of antigen was lengthened beyond 6 hours, the initially injected antigenic substance reduced the immune response to the second antigen. When the bacteria were injected first, the number of hemolytic plaques dropped to 4×10^2; when the erythrocytes were injected first, bactericidal plaques at 24 hours numbered 2×10^3.

Our studies showed the usefulness of Jerne's plaque technique in investigating the potentiality of antibody-forming cells. Although not tested, all evidence

Table 2. Formation of hemolytic (H) and bactericidal (B) plaques by spleen cells (number of plaque-forming cells per spleen) during a secondary immune response. The response was measured on the 4th day after immunization.

Primary immunization	Secondary challenge			
	Sheep red blood cells		*E. coli* 0127 antigen	
	H	B	H	B
Sheep erythrocytes	4×10^4	80	0	3×10^3
E. coli 0127 antigen	2.5×10^3	50	0	7×10^4
Erythrocytes and *E. coli* antigen	3×10^4	100	0	5×10^4
Erythrocytes coated with *E. coli* antigen	5×10^4	2×10^3	1×10^3	2×10^4

reported by others suggests that the plaque technique in our experiments detects only immunoglobulin M (*5*).

Our data suggest that two types of antibodies with different specificities were produced by a single cell. The alternate possibility is that a new cross-reacting antigen was formed between bacterial and red-blood-cell components and that this antigen stimulates production of cross-reacting antibodies which are both hemolytic and bactericidal. However, the available data do not support the latter conclusion, because all antibodies were absorbed specifically either by erythrocytes or by bacteria. The apparent antibodies with two specificities were formed only when the test antigens were closely associated; this was achieved by coating the erythrocytes with the somatic antigen. The antigens responsible for the production of bactericidal and hemolytic antibodies are quite complex and probably of polysaccharidic nature.

Several investigators obtained evidence that single cells can make antibody of only one specificity (*1*). With fluorescent antibody preparations of different colors and specificities, others determined the quantitative and qualitative distribution of heavy and light chains in antibody-forming cells. In general, single cells were found to contain only one kind of heavy chain and one kind of light chain (*6*). On the other hand, few reports suggest that an individual cell can produce antibodies of two or more specificities (*2*). Although the wide variations in the results can be ascribed to the use of different techniques, it is quite possible that an antibody-forming cell may recognize a limited number of structurally similar antigens. The surface antigens of red blood cells and several gram-negative bacteria may cross-react. Although this was not evident in our system, the relation between these two types of antigens is well proved (*7*).

The suppressive effect of initially administered antigen upon the substance later injected indicates the phenomenon of antigen competition (*8*); interpretation of this phenomenon varies widely. The mutual suppression may be the result of competition of antigens for pluripotential antibody-forming cells or for some early nonspecific products needed for antibody formation (*9*).

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Incidence of Gross Chromosomal Errors among Tall Criminal American Males

Abstract. *Chromosome studies on 129 tall men surveyed in four different institutions for the care of criminal males in Pennsylvania showed that 1 in 11 subjects displayed aneuploidy of the sex chromosomes; specifically, five cases of 47,XYY and seven cases of Klinefelter syndrome were identified. All the aneuploid subjects were mentally ill; none had been cytogenetically diagnosed.*

The prevalence of aneuploidy among criminal males who are mentally ill, mentally retarded, or criminally insane is a phenomenon well appreciated in Great Britain (*1*, *2*) but little recognized in the United States. In the course of a recent search for 47,XYY males among several criminal populations in Pennsylvania, we were impressed by the fact that 1 in 11 tall males displayed a gross chromosomal error but that all the affected individuals had gone undiagnosed despite frequent arrest and review (Table 1).

As a first step, inmates of four institutions for the detention of criminals were screened according to height, those 71 inches tall or over being selected for study. With the explicit permission of the subject, a buccal smear was made according to the method of Sanderson and Stewart (*3*) and 2 ml of venous blood was drawn into a heparinized syringe for the purpose of leukocyte culture (*4*). Two culture vials were set up for each subject. Chromosome counts were made of 25 well-spread metaphases; the unique morphology of the human Y chromosome makes the identification of XYY males by microscopic inspection quite satisfactory. Six clear metaphases were photographed on 4- by 5-inch (10- by 12-cm) film and karyotypes were constructed for each aneuploid subject. Individuals whose cultures failed were eliminated from the study. Mosaicism was not observed but cannot be ruled out as a possibility without parallel analyses of other tissues.

Individuals who displayed sex chromatin in the buccal smear or who demonstrated aneuploidy on chromosomal analysis were revisited for further study. The cytogenetic studies were repeated; in each instance the initial finding was confirmed. A physical examination was performed at this time and the prisoners' social, educational, and medical records were carefully reviewed.

As shown in Table 1, 1 in 11 subjects proved to be aneuploid. Seven of the 129 subjects were Klinefelter males with positive sex chromatin and palpably atrophic testes. Five others were 47,XYY males, including one Negro, apparently the first to be reported in the literature (*5*).

The incidence of gonosomal aneuploidy among tall American males in a facility for the detention of juvenile delinquents proved to be 1 : 14; in a

Table 1. Incidence of gross chromosomal errors among tall criminal American males, 71 inches or more in height. Abbreviations: *N*, number of subjects studied; JD, detention center for juvenile delinquents; MDDA, penal institution for mentally defective delinquent adults; UDA, penal institution for unselected delinquent adults; and CI, mental hospital for the criminally insane.

Type of facility	*N*	No. of subjects with chromosomal disorders		Overall incidence
		Klinefelter males	47,XYY	
JD	14	0	1	1:14
MDDA	30	2	0	1:15
UDA	35	1	2	1:12
CI	50	4	2	1:8
Total				1:11*

* Probability that this incidence is due to chance alone, $P = .001$.