Table 1. Effect of thymectomy on the waning of tolerance induced in adults, challenged with BGG adsorbed on bentonite, followed in 7 days by tracer I^{125}-labeled BGG.

Group*	No.	Day of challenge	Mean ADR (%) per day	
			Thymectomy	Control
A	10	20	25 (21–30)†	24 (23–25)
B	10	69	32 (31–33)	49 (33–92)
C	10	118	38 (20–93)	72 (36–97)
D	12	131	45 (27–64)	67 (53–92)

* Each group had an equal number of thymectomized and control mice. † Figures in parentheses represent the range in the elimination of the labeled BGG.

On the basis of a theory of cellular selection, we predicted that thymectomy in an adult animal tolerant to a specific antigen would delay or prevent the reappearance of reactivity to that antigen (*5*).

Newborn C_{57}BL/6J mice were injected intraperitoneally twice weekly with 1.2 mg of supernatant from bovine γ-globulin (BGG) centrifuged for 30 minutes at 59,000*g* in the center of the tube. Injections began during the first 3 days of life and continued for 5 to 10 weeks. One day after the last injection, the mice of each litter were separated at random; half were thymectomized by aspiration under pentobarbital anesthesia and half had sham operations.

One week after thymectomy, two litters were tested for tolerance by the intraperitoneal injection of 1.9 mg of BGG adsorbed onto bentonite followed 1 week later by a tracer dose of I^{125}-labeled BGG antigen. Tolerant mice tested by this technique have an "antigen disappearance rate" (ADR) of 15 to 25 percent per day, similar to the disappearance of tracer in untreated animals. The ADR for immune mice is 70 to 98 percent per day (*6*). In the two litters tested, the ADR fell within the tolerant range in all six mice (two sham-operated and four thymectomized).

Except for the two test litters, mice received no treatment until 130 to 160 days after operation. At that time each mouse was given 2.0 mg of BGG adsorbed onto bentonite intraperitoneally followed again in 1 week by I^{125}-labeled BGG. Although different litters were operated at different ages and had different periods of "antigen lapse," all the mice in a given litter were operated and later tested for tolerance on the same day. A total of 24 mice from eight litters was used.

In 11 sham-operated mice the I^{125}-labeled BGG was eliminated in rapid immune fashion with a mean ADR of 84 percent per day (range 68 to 99 percent). The 13 thymectomized mice remained tolerant, eliminating the radioactive BGG with a mean ADR of 39 percent per day (range 27 to 66 percent).

The role of the thymus was assessed in a different system. Forty-two adult male CBA/J mice were thymectomized or sham-operated at the age of 9 weeks (day 0). One week later, tolerance to BGG was induced by a single intraperitoneal injection of 1.9 mg supernatant BGG (*7*). At varying intervals after operation, groups of five or six each of the thymectomized and control animals were given 2 mg BGG-bentonite intraperitoneally followed a week later by a test tracer dose of I^{125}-labeled BGG. The results shown in Table 1 indicate that although tolerance in each group waned with time, it disappeared more slowly in the thymectomized animals.

These data give further evidence that the adult thymus is active in the development of immunologically competent cells. They support the hypothesis that long-standing acquired immunological tolerance occurs through irreversible inhibition or death of competent cells and that the waning of tolerance occurs through the development of newly arisen uninhibited cells. These new cells may arise in the thymus (*8*). The experiments do not eliminate the possibility that the thymus directs the development of these cells in the peripheral lymphoid tissue.

The waning of tolerance which eventually occurred in the thymectomized mice might be caused by incomplete thymectomy (to be analyzed at autopsy) or by very slow development of immunological cells by somatic mutation in peripheral tissues. The more rapid waning of tolerance in thymectomized adults given a single dose of BGG indicates that tolerance was less complete than in those mice repeatedly injected from birth.

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Heterogeneity of DNA in Density and Base Composition

Abstract. *Chromatography, on methylated-albumin columns, of DNA from calf thymus, mouse testis, and* Bacillus subtilis, *yielded, on elution by a sodium chloride gradient, fractions differing in density. The fractions eluted by higher sodium chloride concentrations had lower densities in a CsCl density gradient. Since DNA with higher guanine-cytosine content is eluted from the column with lower concentration of sodium chloride and has higher density, the density heterogeneity of DNA is best interpreted as a result of heterogeneity of base composition. An extra band observed in calf-thymus DNA had a higher density than that of the main DNA; it was eluted at a lower concentration of NaCl, indicating a higher content of guanine and cytosine. On the other hand, an additional DNA component in the mouse-testis DNA had a lower density and also it was eluted at a lower salt concentration, possibly an indication of an unusual base component in its structure.*

Heterogeneity of density of DNA molecules from an organism detected by density-gradient centrifugation has been interpreted as the reflection of heterogeneity of base composition (*1–4*), although other possibilities are not excluded (*5*). A methylated-albumin column can separate DNA into fractions differing not only in molecular size (*6*) but also in base composition (*7*). This technique provides an opportunity to investigate the relationship between heterogeneity in density and base composition of DNA. It also makes possible an examination of some properties of extra components of DNA in various organisms (*2, 8*).

The DNA was fractionated on the methylated-albumin column by applying a linear gradient of sodium chloride concentration. Elution with a gradient

increase of NaCl concentration improves the resolution over that obtained with stepwise elution (*7*). Fractionation of native DNA from calf thymus, mouse testis, and *Bacillus subtilis* by density as well as by molecular size is evident. The relation observed between elution pattern and density of DNA indicates that the heterogeneity of density in CsCl concentration gradient comes from the heterogeneity in composition of DNA. Thus, partial denaturation and complex formation of DNA with RNA or protein are not a likely cause for density heterogeneity of native DNA molecules of an organism. Denatured DNA has a higher density (*2*, *9*) and is eluted from the column later than native DNA (*7*), a fact which does not explain the present results. It was also found that the extra component of mouse DNA did not behave as expected in elution from the column, while calf-thymus DNA behaved normally.

Calf-thymus DNA (*10*) in a dry form was dissolved (400 μg/ml) in a saline citrate solution (0.15*M* NaCl plus 0.015*M* sodium citrate). Before it was used, the solution was kept at 4°C for 3 days or more with occasional shaking. Mouse DNA was prepared from the isolated testes of two adult mice. Testes were washed in a saline-EDTA solution (0.1*M* EDTA plus 0.15*M* NaCl, *p*H 8.0) once, resuspended in 10 ml of the same solution, macerated briefly in a tissue grinder and lysed with 2 percent sodium lauryl sulfate (final concentration). Subsequently, the DNA was isolated by the procedure described for the isolation of bacterial DNA (*11*). The DNA from *Bacillus subtilis* was obtained from cells grown exponentially in an enriched broth me-

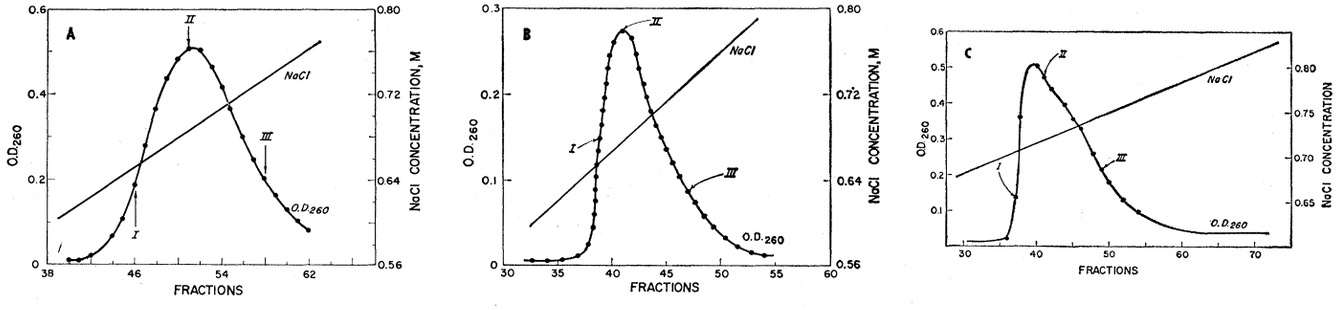


Fig. 1. Elution profiles of DNA from a methylated albumin column. *A*, Calf thymus DNA; 1 mg of calf-thymus DNA was dissolved in 50 ml of saline-citrate solution and charged on a methylated albumin column. Fractionation was made by a linear gradient of NaCl between 0.5*M* and 0.9*M* in 0.05*M* sodium phosphate buffer (*p*H 6.7). The volume of the starting and the final buffers was 100 ml, and 3-ml fractions were collected. *B*, Mouse testis DNA; 400 μg of DNA, isolated from mouse testes in 20 ml of saline-citrate solution, was fractionated. The starting and the final buffers were 60 ml of 0.5*M* and 60 ml of 1.0*M* NaCl in 0.05*M* sodium phosphate buffer (*p*H 6.7). The volume of each fraction was 2 ml. *C*, DNA from *B. subtilis*; 535 μg of purified *B. subtilis* DNA was dissolved in 32 ml of saline-citrate solution. The starting and the final buffers were 100 ml of 0.55*M* and 100 ml of 1.0*M* NaCl in 0.05*M* sodium phosphate buffer (*p*H 6.7). The volume of each fraction was 3 ml.

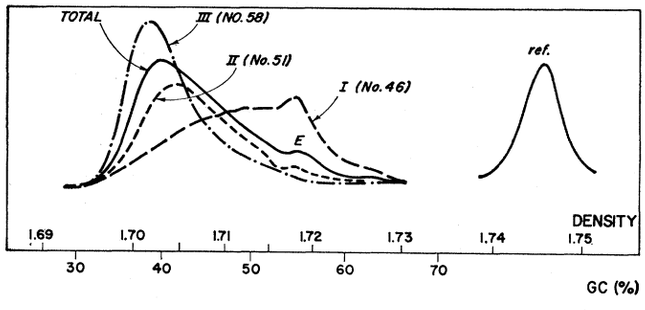
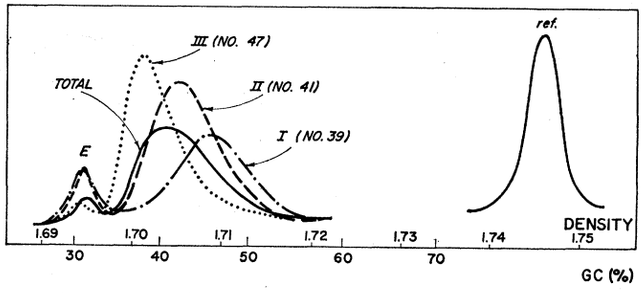


Fig. 2. Superimposed tracings of density gradient centrifugation pictures of fractionated DNA from calf thymus. Samples of DNA (1 to 2 μg) from front (*I*), middle (*II*), and rear (*III*) fractions (Fig. 1*A*) were centrifuged separately in 7.7 molal CsCl solution at 44,770 rev/min for 20 hr at 25°C. As a density reference, 1 μg of N^{15}—DNA from *Ps. aeruginosa* was added. Tracings of ultraviolet absorption pictures were superimposed by matching the DNA reference band. Distribution of unfractionated DNA (total DNA) was also added to the figure. *E*, extra component; *ref.*, reference DNA (N^{15}—DNA from *Ps. aeruginosa*).



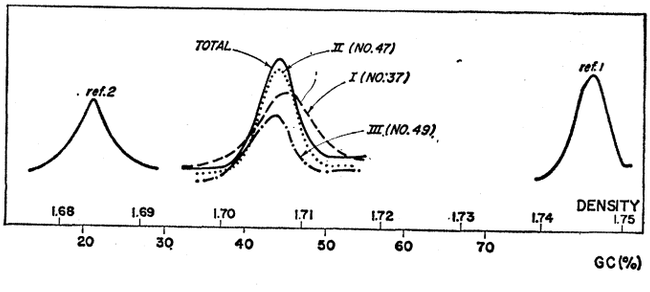


Fig. 3 (left). Superimposed tracings of density gradient centrifugation pictures of fractionated DNA from mouse testes. Three fractions chosen for the centrifugation are indicated in Fig. 1*B*. *E*, extra component; *ref.*, reference DNA (N^{15}—DNA from *Ps. aeruginosa*). Fig. 4 (right). Superimposed tracings of density-gradient centrifugation pictures of fractionated DNA from *B. subtilis*. Three fractions chosen for the centrifugation are indicated in Fig. 1*C*. Two reference DNA's were added for more sensitive comparison; *ref. 1*, N^{15}—DNA from *Ps. aeruginosa*; *ref. 2*, "light-DNA" from *Cancer borealis* (*16*).

dium (*12*). Cells were washed with saline-EDTA solution once, and incubated with 1 mg of egg-white lysozyme per milliliter at 37°C for 15 minutes. The lysis of cells was completed by adding sodium lauryl sulfate to the cell suspension (final concentration, 2 percent) with an additional incubation at 37°C for 5 minutes. The procedure for isolation of DNA was similar to the one described by Marmur (*11*). The column was prepared according to Mandell and Hershey (*6*) except that the column was half the scale described. Elution was performed by applying a linear concentration gradient of NaCl (*13*). NaCl was dissolved in 0.05*M* sodium phosphate buffer (*p*H 6.7).

Table 1. Analysis of distribution of calf-thymus DNA in CsCl gradient.*

Average density † (gm/cm³)	Average GC-content $(\bar{p})$ ‡	$10^{-2}\sigma$ § (p)	$S_{20,w}$ ||	10^6 MW ¶
		Total		
1.707	0.437	5.9	22.8	8.8
		Fraction I		
1.712	0.485	6.6	14.4	2.8
		Fraction II		
1.707	0.437	4.7	20.3	6.5
		Fraction III		
1.704	0.408	4.4	25.0	11.0

* See Figs. 1*A* and 2. † DNA samples were centrifuged in 7.7 molal CsCl solution at 44,770 rev/min at 25°C for 20 hrs with DNA labeled with N^{15} from *Ps. aeruginosa* for reference. The density of the reference DNA (1.746) was previously determined with reference to *E. coli* DNA, whose density was taken as 1.713 (*6*). ‡ Average mole fraction of GC content $(\bar{p})$ was calculated from the density profile (*17*). § Standard deviation (σ) of DNA distribution in CsCl density gradient field was calculated from GC content scale (p) (*17*). \|\| Sedimentation coefficients ($S_{20,w}$) were obtained by centrifuging DNA from the three fractions previously dialysed against 500 volumes of saline-citrate solution. The concentration of DNA in the centrifuge cell was 9, 22, and 10 μg per milliliter for fractions I, II, and III, respectively. ¶ Weight-average molecular weights were calculated from $S_{20,w}$ (*18*).

Table 2. Analysis of distribution of mouse DNA in CsCl gradient.*

Sample	Average density (gm/cm³)	Average GC-content $(\bar{p})$
Total	1.705	0.417
Fraction I	1.709	0.456
Fraction II	1.706	0.427
Fraction III	1.703	0.398

* See Figs. 1*B* and 3 and the legend of Table 1.

Table 3. Analysis of distribution of *B. subtilis* DNA in CsCl gradient.*

Average density (gm/cm³)	$(\bar{p})$	$10^{-2}\sigma$ (p)	$S_{20,w}$	10^6 MW †
		Total		
1.707	0.436	2.23	29.8	16.8
		Fraction I		
1.708	0.446	3.47	16.9	3.6
		Fraction II		
1.707	0.437	2.07	28.9	16.0
		Fraction III		
1.706	0.430	2.22	36.1	27.8

* See Figs. 1*C* and 4 and the legend of Table 1. † The concentration of DNA in the centrifuge cell for sedimentation coefficient measurement was 7, 23, and 10 μg per ml, for fractions I, II, and III, respectively.

Elution profiles of DNA from calf thymus, mouse testis, and *B. subtilis* are shown in Fig. 1*A*, 1*B*, and 1*C*. Samples of DNA (1 to 2 μg) from three fractions, leading, middle, and rear, were centrifuged in 7.7 molal CsCl solution (*14*). Equilibrated DNA distributions are shown in Figs. 2, 3, and 4 for calf thymus, mouse testis, and *B. subtilis*, respectively. Extra components in DNA from calf thymus and mouse testis were observed also. Since there is a positive correlation between the guanine-cytosine (GC) content and density of DNA (*1, 2*) our result is consistent with a property of the column, that is, DNA with higher GC content elutes first (*7*). The average molecular weight of DNA of each fraction was estimated from the sedimentation coefficient for DNA from calf thymus and *B. subtilis*. The fractionation by size is also clear (Tables 1 and 3). The quantitative analysis of the result is summarized in Tables 1, 2, and 3. In the case of calf thymus and *B. subtilis*, sedimentation coefficients of DNA fractions were also measured (Tables 1 and 3).

The extra component of DNA from calf thymus and mouse (Figs. 2 and 3) deserves some comments. These extra components have been reported (*3, 8*). Our results indicate that the extra band (heavy band) of calf thymus DNA behaves normally in the elution pattern; namely, it is eluted in a lower concentration of NaCl. This is most reasonably interpreted as a result of the higher content of guanine and cytosine of the component. On the other hand, the extra band (light band) of DNA from mouse testis behaves abnormally in this respect. It is eluted before the main DNA in spite of its lighter density. Since the band width of the light band indicates a molecular weight of approximately 19 million, the anomaly of elution behavior cannot be attributed to the smallness of its molecular size. One possible explanation is that the low density of the extra DNA from mouse may be due to the presence of an unusual base. For example, a high content of an unusual base like methylcytosine should lower the density because of the low density of the methyl group (*15*).

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Signal Duration as a Factor in Vigilance Tasks

Abstract. *Short signals in a vigilance test generated an inferior initial performance, as compared with longer signals, and resulted in a steeper decrement in performance. A short (1-minute) and a long (2-hour) vigilance task was undertaken by each subject.*

It can be hypothesized from the expectancy theory of vigilance (*1*) that the shorter the signal in a vigilance task, the poorer the initial detection performance and the steeper the decrement in detection performance. To test these hypotheses, 63 housewives served as the subjects in a clock-test on two occasions one day apart. The clock was 20 cm in diameter, with a plain white face and a black second hand with a radial sweep which completed a rotation once a minute. Sig-