Table 1. Ester content and boiling points of azeotropes in alcohol-borate systems.

Boiling point (°C)			Wt. ester in azeotrope (%)
Alcohol	Ester	Azeotrope	
	Methyl (4)		
64.7	68.7	54.6	75.7
	Ethyl (5)		
78.3	118.6	76.6	30
	i-*Propyl*		
82.4	140.8 (*12*)	82.0	5.4

If the three established azeotropes in alcohol-alkyl borate systems are compared, there is a marked decrease in ester content as the boiling points of the components increase (Table 1). It might be remarked in addition that boric acid is volatile in steam (*11*), behavior which is probably related to the ester-alcohol azeotropes.

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Specific Immunologic Unresponsiveness to Synthetic Polypeptide Antigens

Abstract. *The injection of two synthetic polypeptide antigens into adult rabbits treated with 6-mercaptopurine, or into newborn rabbits, resulted in immunological "unresponsiveness" to subsequent immunizations with these antigens. The "tolerant" animals were shown to be reactive toward the nonrelated antigen ovalbumin.*

The phenomenon of acquired specific immunological tolerance (unresponsiveness) toward nonliving antigens was reviewed recently by Smith (*1*). The various experimental models analyzed were mainly serum proteins of human and bovine origin. For the analysis of (i) the molecular requirements for the induction of tolerance, and (ii) the range of specificity of the tolerant state in molecular terms, more quantitative information concerning specific immunological unresponsiveness would be obtained if there were available well-defined chemical compounds capable of inducing immunological tolerance in experimental animals. Recent studies have shown that some synthetic polypeptides may be potent antigens of narrow specificity, and might be used to study further the chemical basis of antigenicity (*2, 3*). We report here the induction of specific immunological unresponsiveness to two synthetic polypeptide antigens.

The antigens used in this study, denoted p(Tyr,Glu)- -pLys and p(Tyr, Glu)-pAla- -pLys (*4*), are multichain copolymers of amino acids and were described previously (*3*). The multichain polymer p(Tyr,Glu)- -pLys is composed of side chains of peptides containing L-tyrosine and L-glutamic acid attached to the ε-amino groups of poly-L-lysine. Analysis of a sample (112) indicated an average molecular weight of 14,700 (*3*) and a residue molar ratio of Lys : Tyr : Glu of 1 : 1.1 : 2.3. In the second multichain copolymer, p(Tyr,Glu)-pAla- -pLys, side chains of poly-DL-alanine, attached to the ε-amino groups of poly-L-lysine, were elongated with peptides containing L-tyrosine and L-glutamic acid. Analysis of a sample (202) showed an average molecular weight of 33,000 and a residue molar ratio of Lys : Tyr : Glu : Ala of 1 : 1.7 : 2.4 : 19.

The induction of tolerance was attempted by exposing newborn rabbits to the antigens and by treating adult rabbits with 6-mercaptopurine and antigen (*5, 6*). The experiment of tolerance to p(Tyr,Glu)-pAla- -pLys in newborns was carried out with two litters. In one litter, four were test animals and four were controls. In the second litter there were two test and three control animals. The test animals were injected intraperitoneally with 40 mg of antigen within 24 hours after birth, and 45 days later they received another dose of 40 mg of antigen.

At 97, 120, and 140 days of age, the animals of the control and the test groups were challenged with an immunizing dose of 15 mg of antigen in Freund adjuvant (*3*). At various time intervals after each of these immunizations, the animals were bled and tested for immune response by precipitin reaction (*3*). The results (Table 1) were that none of the test animals showed any antibody, when tested after the third immunization at the age of 5 months. On the other hand, all the control littermates gave, after the third immunization, a typical precipitin reaction reflecting an immune response of at least 300 μg of antibody per ml of serum. The antibody response both in test and control animals was checked also by passive cutaneous anaphylaxis (*7*). All the controls gave positive reactions, while the test animals were negative. As little as 0.2 μg of antibody to p(Tyr,Glu)-p(Ala)- -pLys may be easily detected by this technique (*8*). It thus appears that the injection of p(Tyr,Glu)-pAla- -pLys at birth conferred immunological unresponsiveness towards this antigen.

Table 1. Unresponsiveness to p(Tyr,Glu)-pAla- -pLys, after exposure of newborn rabbits to antigen. On the 1st and 45th days, 40 mg of antigen was administered to the test animals and on the 97th, 120th, and 140th days, 15 mg was administered to the test and control animals. Antibody test and response are shown as indicated.

Litter 1		Litter 2	
Test	Control	Test	Control
	Antibody test on the 117th day		
4—	4—	2—	1+
			2—
	Antibody test on 129th day		
4—	3+		
	1—	2—	3+
	Antibody test on 148th day		
4—	4+	2—	3+

A second experiment was carried out in adult rabbits, aged 2 to 3 months. Ten experimental animals were each injected with 75 mg of antigen itnravenously and with 6 mg/kg of body weight of 6-mercaptopurine intramuscularly. The injections of 6-mercaptopurine were continued for 14 successive days. The animals were challenged with the first immunizing dose of 15 mg in adjuvant, 30 days after the initial injection of the antigen, and on the 52nd and 73rd day two subsequent challenges were made, each followed by antibody tests.

The results showed that seven out of ten animals were completely negative in precipitin tests, two were positive, and one doubtful. On the other hand, of nine control animals which were similarly immunized by three injections of antigen in adjuvant, eight were positive and one was negative.

Similar experiments were made with p(Tyr,Glu)- -Lys. Four rabbits were injected with 50 mg of antigen at birth, then at the 68th day with a further dose of 30 mg of antigen. At the age of 3, 4, and 5 months these four rabbits and four control littermates received three injections of 15 mg of antigen in adjuvant.

All four test rabbits remained unresponsive, whereas three out of four control animals showed a typical precipitin reaction (at least 200 μg of antibody per milliliter of serum). The unresponsive animals were then injected twice intravenously with 1.8 mg of antigen. The negative response persisted. The specificity of the unresponsive state was tested by injecting 25 mg of ovalbumin in alum; all four experimental animals and the four control rabbits gave a similar positive reaction to this antigen (600 to 2000 μg of antibody per milliliter of serum).

Tolerance to p(Tyr, Glu)- -pLys in adult rabbits treated with 6-mercaptopurine was attempted in four animals injected with 75 mg of antigen. All of the animals were unresponsive toward subsequent immunization with the antigen, whereas three out of four control animals showed a typical positive reaction.

Thus tolerance to synthetic polyamino acids can be conferred either by exposing newborn rabbits to the antigen or by treatment of adult rabbits with 6-mercaptopurine and antigen (*9*).

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Midbrain Reticular Influences upon Single Neurons in Lateral Geniculate Nucleus

Abstract. *The effect of electrical stimulation of the midbrain reticular formation upon patterns of discharge of single lateral geniculate neurons was studied. Data were processed by means of a 256-channel scaler analyzer. The rate of spontaneous discharge of geniculate neurons was raised by electrical stimulation of the reticular formation and their ability to respond to intermittent light was enhanced.*

Visual messages on their way from the retina to the cerebral cortex are known to be modified at the level of the lateral geniculate nucleus (LGN) by electrical stimulation of the brainstem reticular formation (*1*, *2*). This report, which is concerned with the influences of the midbrain reticular formation on single geniculate neurons, extends the earlier studies (*1*, *2*).

Cats were lightly anesthetized and paralyzed by a continuous intravenous infusion of a mixture of sodium pentobarbital and gallamine triethiodide in normal saline. Photic stimuli were delivered by means of a multibeam ophthalmoscope (*3*). Single unit spikes were picked up with tungsten microelectrodes and recorded on one track of a twin-track magnetic tape recorder. Recycle pulses, synchronous with the end of the light flash or with the stimulus applied to the midbrain, were recorded on the other track. Brief shocks (0.1 msec in duration, 3 to 5 volts) were applied by means of bipolar silver electrodes (*2*) to the midbrain reticular formation centered on Horsley-Clarke coordinates, $A = 2.0$, $LL = 3.0$ and $H = -1.0$.

The data recorded on tape were analyzed by means of a 256-channel scaler analyzer and displayed as poststimulus time histograms (*4*). These were constructed as follows. Spikes were sorted out by the scaler analyzer and stored in channels according to their time of occurrence after the stimulus as indicated by the recycle pulse. A relatively large number of stimulus cycles were used in order to obtain a reliable mean response pattern.

In these experiments units were held for at least 3 hours, which was the minimum time necessary for an adequate analysis of the pattern of the response.

Stimulation of the mesencephalic reticular formation by means of a train of four pulses (300 per second) delivered every 3.5 seconds increased the rate of the spontaneous discharge of a single geniculate neuron when the retina was adapted to low-level photopia.

The data for each histogram (Fig. 1, *A*, *B*, and *C*) required 20 minutes of recording time. Histogram *B* followed *A* immediately. Recording for *C* commenced 1 minute after intravenous injection of 32 mg of sodium pentobarbital (Sagatal).

The poststimulus time histogram constructed for 300 successive cycles of mesencephalic stimulation with 16 msec per channel in the analyzer (Fig. 1*B*) is to be compared with the spontaneous background discharge of the same unit without change in conditions except for the absence of mesencephalic stimulation (Fig. 1*A*). The excitatory action of the midbrain stimulation is evident, consisting of an early, sharp increase in firing, with a peak at about 135 msec after the shock, followed by a fairly well-maintained increase throughout the

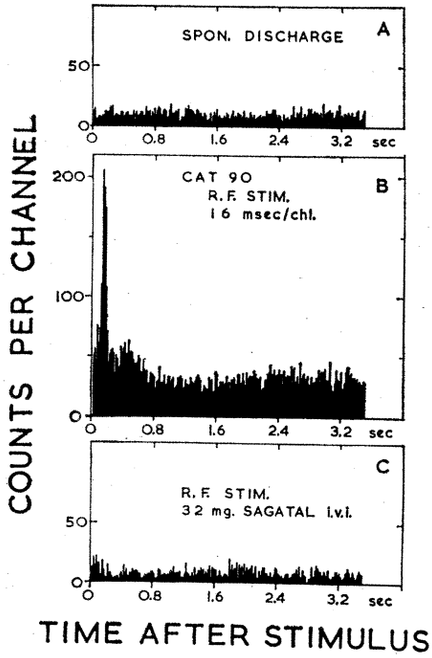

Fig. 1. Effect of electrical stimulation of midbrain reticular formation (R.F.) upon spontaneous activity of single lateral geniculate neuron. By means of a scaler-analyzer channel width of 16 msec, a total of 300 stimulus cycles were analyzed, each having a duration of 3.5 seconds. In each poststimulus time histogram, ordinates represent counts per channel and abscissae time after stimulus (recycle pulse). *A*, Spontaneous discharge under dim-light-adapted state without reticular stimulation. *B*, Midbrain reticular formation stimulated by a train of four pulses (300 per second, 0.1 msec, 3 volts). *C*, Thirty-two mg of sodium pentobarbital (Sagatal) injected intravenously under the same conditions as in *B*.