V region sequences of their L and H chains.

If identical idiotypic specificities indicate structural identity, then there are two indications that immunoglobulins of the S63 idiotype are present in normal BALB/c mice. First, immunization of normal BALB/c mice against the phosphorylcholine determinant yields immunocytes producing specific antibody that can be inhibited in the Jerne plaque assay by idiotypic antiserum of the S63 type (*17*). Second, immunoglobulins of the S63 idiotype are found in the normal serum of BALB/c mice (*17a*). These results suggest that the myeloma proteins with phosphorylcholine-binding activity are indeed an excellent model system for studying the relation between structure and function in bona fide antibody molecules.

Since the H chains, even from those proteins with differing idiotypic specificity, are identical for 36 residues, except for a single amino acid substitution, it will be particularly interesting to determine whether the entire H chains from proteins of differing idiotypic specificity are identical (for example, T15 and M603). If so, perhaps this H chain sequence, or one very closely related to it, is a prerequisite for all immunoglobulins with phosphorylcholine-binding activity. It has been shown that all myeloma proteins with α-(1 $\rightarrow$ 3)dextran activity have an identical lambda L chain (*18*). Proteins with β-(1 $\rightarrow$ 6)-D-galactan binding activity have very similar sequences in both L and H chains (*7*). Thus in some instances either L or H chain may play a dominant role in hapten binding, while in others a specific L and H pair may be rigidly required.

If immunoglobulins of the S63 idiotype are identical, this suggests that the corresponding V_L and V_H regions are coded by genes in the germ line of the BALB/c mouse, for it is unlikely that a random somatic recombination or somatic mutation process would generate multiple identical antibodies in different individuals. In this regard, it will be interesting to determine whether the H chain from M167 is identical to the others apart from a single substitution outside the hypervariable region, or whether other differences will be found. If a single substitution is present, it could be explained by a single base substitution, either in the germ line or in the soma.

The complete analysis of these proteins as well as those from groups with binding activity to other haptens should continue to provide insights into the structure, genetics, regulation, and evolution of immunoglobulin molecules.

P. BARSTAD
Division of Biology, California Institute of Technology, Pasadena 91109

S. RUDIKOFF, M. POTTER
National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

M. COHN
Salk Institute for Biological Studies, San Diego, California 92112

W. KONIGSBERG
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06510

L. HOOD
Division of Biology, California Institute of Technology

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Choline Content of Rat Brain

Abstract. *The free choline concentration in the rat brain was found to be 26.3 nanomoles per gram of brain tissue. This value was obtained through use of 6-second microwave irradiation for killing animals and inactivating enzymes, followed by a pyrolysis–gas chromatographic assay procedure. The identities of compounds measured from brain samples were verified by mass spectrometry.*

Knowledge of the concentration of free choline in the brain is important in studies of both the central cholinergic system and phospholipid metabolism. Controversies presently exist concerning the sources of free choline in the brain, for example, the contributions made by choline in the blood and the role of choline produced by the breakdown of brain phospholipids (*1*). Choline is considered a precursor for the synthesis of acetylcholine (*2*). Accurate assessment of acetylcholine turnover through the use of labeled choline depends on knowledge of the size of the endogenous choline pool (*3*). Choline is also a product of acetylcholine metabolism, and changes in the rate of acetylcholine release have been assessed by measuring choline in the cerebrospinal fluid (*4*).

The free choline concentrations reported recently vary widely (*5*). The values for rat brain range from 700 nmole per gram of brain tissue (*6*) down to 170 (*7*), 86.4 (*8*), and 39 nmole/g (*9*). While these values reflect both postmortem changes and differing analytical techniques (*10*), it has been speculated that the concentration of free choline in the brain is even lower than currently being reported. A

major obstacle to measuring concentrations of labile compounds in brain has been the length of time between death and enzyme inactivation. In an effort to arrive at a more correct value, Dross and Kewitz (*11*) calculated the in vivo concentration of choline in brain by extrapolation from the rate of increase of choline after death. Through the use of microwave irradiation to concurrently kill the animal and inactivate brain enzymes, we were able to directly measure the in vivo concentration of brain choline.

Table 1 shows the values obtained from 200-g male Sprague-Dawley rats. Rats in one group were killed by decapitation, and exactly 5 minutes later the brains were homogenized in 4 ml of a mixture of 15 percent 1*N* formic acid and 85 percent acetone containing butyrylcholine iodide as an internal standard. Animals in a second group were killed by 6 seconds of microwave treatment, which concomitantly inactivated enzymes (*12, 13*). Additional rats were killed by cervical dislocation, with brain enzymes inactivated by microwave irradiation 5 minutes after death. Immediately after microwave treatment of the latter two groups, brains were removed and homogenized as above. Another group of rats was killed by microwave irradiation followed by a 5-minute delay before homogenization. The homogenates were extracted as described (*13*), and choline was measured after further purification of the extract on a 50-mg column of Amberlite CG-120-Na^+ 200-400 mesh (Mallinckrodt), followed by derivatization to the propionyl ester by using 100 μl of acetonitrile and 300 μl of propionyl chloride for 20 minutes at 22°C (*14*). Choline and acetylcholine concentrations were measured by pyrolysis–gas chromatography. Mass spectral studies of brain samples verified the identities of choline and acetylcholine.

The value obtained for brain choline concentration by the use of rapid enzyme inactivation—26.3 nmole/g—is remarkably close to that predicted by Dross and Kewitz (27.5 nmole/g) (*11*). The generation of free choline after death was initially reported by Schuberth *et al.* (*15*). Dross and Kewitz (*11*) showed that the increase in brain choline concentration after death is fastest during the first few minutes after decapitation. Therefore, the values obtained depend on the interval between death and enzyme inactivation. We found brain choline concentration to be 148 nmole/g 5 minutes after the rat's death, which indicates an increase of 24.5 nmole per gram per minute. This is comparable to the rate of 20.5 nmole per gram per minute reported by Dross and Kewitz (*11*).

Table 1. Choline and acetylcholine concentrations in the rat brain when the means of killing and the time between death and enzyme inactivation were varied. All microwave treatments were 6 seconds. Each value represents the mean ± standard deviation for the number of animals shown in parentheses.

Method for killing and enzyme inactivation	Concentration (nmole per gram of brain)	
	Choline	Acetylcholine
Microwave; immediate homogenization	26.3 ± 1.6 (5)	24.8 ± 1.3 (5)
Microwave; homogenization 5 minutes later	25.4 ± 4.2 (6)	26.1 ± 0.7 (6)
Decapitation; homogenization 5 minutes later	156.7 ± 14.2 (3)	17.2 ± 2.1 (3)
Cervical dislocation; microwave 5 minutes later	148.3 ± 13.4 (3)	14.9 ± 2.8 (3)

When homogenization was delayed for 5 minutes after microwave irradiation, the choline concentration was 25.4 nmole/g, not significantly different from that obtained with immediate homogenization. Thus, the observed postmortem generation of free choline must result from enzyme activity that remains after death. Microwave irradiation eliminates this problem and allows ample time for dissection of the brain into discrete areas. To obtain these advantages, however, the microwave technique must be applied properly, since the benefits of this method arise primarily from the extremely short time required for irreversible enzyme inactivation. If the microwave irradiation is inefficient, and 20 to 40 seconds are required to kill the animal and inactivate enzymes, the results do not differ from those obtained by freezing the tissue or even by simple decapitation (*8*).

At 5 minutes after the rat's death, brain acetylcholine concentration was 14.9 nmole/g, having decreased at a rate of 2 nmole per gram per minute since death (Table 1). Choline and acetylcholine were found in the brain of microwave-killed animals in equimolar amounts. After death, much more choline was generated than could possibly come from brain acetylcholine. The source of this choline is probably the large phospholipid pool of the brain (*11*). Since the rate of decrease of acetylcholine concentration after death is much slower than the increase in choline concentration, acetylcholine values are not as sensitive to the speed of enzyme inactivation.

We found no significant differences in the values from decapitated animals as compared to those killed by cervical dislocation followed by microwave irradiation. Thus, the use of the microwave method does not cause any artificial changes in choline and acetylcholine concentrations. Since the acetylcholine concentration goes down after death, and our values are high, and since choline concentration increases after death, and our values are low, we feel we are closely approaching an accurate description of in vivo levels of choline and acetylcholine.

W. B. STAVINOHA
S. T. WEINTRAUB
Department of Pharmacology, University of Texas Health Science Center, San Antonio 78284

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