the respirometer to lure scavengers into the enclosure but to make the bait unavailable for consumption. After the RUM was lowered to within 30 m of the bottom, the respirometer was opened and flushed to obtain characteristic bottom water and then closed. A control measurement of oxygen consumption by the enclosed water and bait was made for 1 hour; after this time the RUM was lowered to the seabed and the respirometer door was opened again for flushing and for attracting a fish. The control showed negligible uptake of oxygen.

The first measurement was of *Coryphaenoides acrolepis* which was attracted and enclosed within 8 minutes. Except for occasional bumping of the trap sides, the rattail behaved like unrestrained rattails, as observed by the RUM television. It displayed little interest in the bait after initial capture. Oxygen consumption was monitored on the bottom for 3½ hours, after which the RUM was brought to the surface, and the weight and displacement volume of the fish were measured (Table 1). Increased respiration rates were correlated with relatively rapid movements at the beginning of the experiment. Dissolved oxygen concentration within the respirometer at the end of the experiment was 60 percent of the concentration of the surrounding seawater. There was no evidence of respiration changes with time, suggesting regulatory patterns of metabolism in *Coryphaenoides* at least to 60 percent of ambient oxygen concentration.

A subsequent lowering of the respirometer, following the procedure outlined above, yielded a respiration measurement of a hagfish *Eptratretus deani* (Table 1). The hagfish was captured 30 minutes after the respirometer door was opened. Upon entering the trap, the animal actively swam around and appeared excited by the bait. This period of excitement lasted 21 minutes, and then the animal intermittently swam around the trap and settled to the bottom. No significant changes in respiration rate were noted during the 13-hour recording period, which could be correlated with the intermittent periods of activity.

We compared our respiration measurements with rates of related shallow-water species. The macrourids are phylogenetically related to gadids which have shallow-water representatives such as the Atlantic cod (*Gadus morhua*), which normally inhabits cold water. The respiration of *Gadus* (*4*) is two orders of magnitude greater than that of *Coryphaenoides* (Table 2).

Table 2. Comparative respiration measurements of related shallow-water fishes of the same weight at comparable temperatures.

Species	Respiration (milliliters of oxygen per hour)		Temperature (°C)
	Total	Per kilogram wet weight	
C. acrolepis	4.4	2.4	3.5
Gadus morhua (*4*)	100.1	55.6	3.0
E. deani	0.2	2.2	3.5
E. stoutii (*5*)	0.9	9.4	4.0
Petromyzon marinus (*6*)	7.6	75.5	5.0

Shallow-water myxiniids include the hagfish *Eptatretus stoutii* and the lamprey *Petromyzon marinus*. Respiration of both of these species (*5*, *6*), for similar size animals and at similar temperatures, are significantly greater ($P < .05$) than the respiration of *Eptatretus deani* (Table 2).

Comparisons with related shallow-water species revealed that the respiration rates of the macrourid *Coryphaenoides acrolepis* and the myxiniid *Eptatretus deani* are significantly lower. These findings are consistent with other in situ studies which have shown decreased metabolic activity in the deep sea (*1*) and may be a synergistic function of food availability, pressure, and temperature. The respiration measurements of these fishes is an overestimate of their true respiration rate if it is true (as hypothesized) that these fishes may exist in a quiescent state until food is available (*7*). Even as an estimate of maximum respiration, our values show reduced rates of metabolic activity in deep-sea animals.

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Myelin Basic Protein: Location of Multiple Independent Antigenic Regions

Abstract. *Immunization of guinea pigs with homologous myelin basic protein induces antibodies that differ in their ability to bind specific peptide fragments of the protein. Antiserums with differing specificities made it possible to demonstrate at least three mutually exclusive antigenic sites in the protein molecule. One of these sites is located between residues 44 and 89, another between 90 and 116, and the third between 117 and 170.*

The present study was undertaken to determine the binding specificity of antibodies induced in guinea pigs by immunization with homologous myelin basic protein. Similar studies have been reported on other proteins of known sequence and structure, for example, ribonuclease (*1*) and lysozyme (*2*), but reports on the relation of structure to immunologic activity of myelin basic protein have dealt primarily with its encephalitogenic activity or its ability to induce delayed hypersensitivity reactions in vitro and have not been directed toward an analysis of antibody binding specificity. Binding of antibody to whole protein and to purified and chemically defined fragments was determined by radioimmunoassay (*3*). Two general immunization techniques were used, one induced antibody accompanied by a disease-resistant state, and the other induced antibody correlated in time of appearance with disease onset.

Guinea pig myelin basic protein (GPBP) and the smaller of the two rat myelin basic proteins (rat S) were

isolated and purified (*4*). The hydroxynitrobenzyl (HNB) derivative of GPBP was prepared by a modification of the technique described by Chao and Einstein (*5*). Fragments 1 to 116 and 117 to 170 (*6*) were prepared by cleavage of GPBP with a bromine adduct of 2-(2-nitrophenylsulfenyl)-3-methylindole (BNPS-skatole) at the single tryptophan residue (*7*). Fragment 1 to 20 was obtained by cyanogen bromide cleavage (*8*). Fragment 90 to 170 and a mixture of peptides from the first 89 residues of the molecule (1 to 37, 1 to 43, 38 to 89, and 44 to 89) were obtained by limited pepsin digestion (*9*). Fragment 44 to 88 was provided by Dr. Robert F. Kibler. A similar peptide (residues 44 to 89) has been isolated from the limited peptic digest of GPBP (*9*).

Adult, histocompatible strain 13 guinea pigs, weighing 450 to 600 g, were used for immunization. Animals were injected ten times with 0.1 mg of GPBP in incomplete Freund's adjuvant (IFA) over a 3-week period (*10*). They were then injected with 0.1 mg of whole GPBP in complete Freund's adjuvant (CFA) which contained 0.1 mg of mycobacteria ($H_{37}R_v$, Eli Lilly Co.). Neither multiple injections of antigen in IFA alone nor the single injection of antigen in CFA (which is encephalitogenic) induces significant levels of antibody. However, if the multiple injections in IFA are followed by the single injection in CFA, large amounts of specific antibody are produced, and the guinea pigs do not develop experimental allergic encephalomyelitis (EAE); that is, they are protected. The second method for production of antibody required only one injection consisting of 0.5 mg of GPBP plus 2.5 mg of mycobacteria. This injection induces severe EAE and a high level of antibody that is detectable at the onset of the disease.

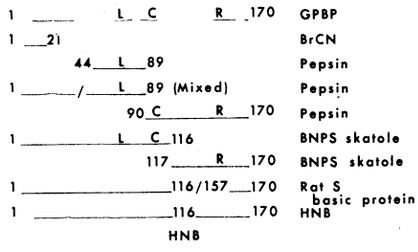

Fig. 1. Diagram of GPBP and fragments used for antibody specificity study. Residue numbers conform with those reported for bovine myelin basic protein (*6*). L, C, and R indicate location of three binding sites described in text.

The binding capacities of serums obtained by the two methods of immunization are shown in Table 1. The serums were tested not only for their ability to bind whole protein but also for their ability to bind equimolar quantities of the peptides (Fig. 1). Broad specificity serums (A and B) bound five of the six fragments listed. Narrow specificity serums, which bound some but not all of the five fragments, were obtained by one of the following modifications of either of the two immunization schemes. (i) Delaying the injection of antigen in CFA for at least 6 months after priming with GPBP in IFA yielded serum C. (ii) Preliminary immunization with rat S myelin basic protein in IFA followed by immunization with whole GPBP in CFA yielded serums D and E. (iii) A single injection with HNB-modified myelin basic protein in CFA yielded serum F. (iv) Preliminary immunization with fragment 117 to 170 in IFA and challenge with GPBP in CFA yielded serum G.

In order to analyze the binding characteristics of the various serums, the terms left, center, and right (L, C, R, Fig. 1) are used with reference to regions of the amino acid sequence as it is ordinarily printed. The left binding site is located on the amino terminal side of residue 90, the center site is located within residues 90 and 116, and the right site on the carboxyl terminal side of residue 116 (Fig. 1).

On the basis of the binding of serums A and B to specific fragments, we could infer the existence of only two sites on the molecule, the left site within residues 1 to 89 and the right site within residues 117 to 170. The existence of a third site (see below) could not be deduced from the binding data obtained with these fragments because of the common sequence (90 to 116) shared by fragments 1 to 116 and 90 to 170 and because both serums reacted with a second site in each fragment. The existence of the third (center) site was demonstrated with serums C and F, both of which bound only fragments 1 to 116 and 90 to 170 and failed to bind the mixture of fragments from residues 1 to 89 or fragment 117 to 170. This third site therefore was located in the region shared by the two larger fragments, residues 90 to 116.

Rat S resembles GPBP, except for a deletion of residues 117 to 156 in the carboxyl terminal portion of the molecule (*11*). When rat S was used for preliminary immunization the serums obtained (D and E) both failed to bind fragment 117 to 170. Serum D bound the left and center site, while serum E bound only the left site. Serum G, which was obtained by preliminary immunization with fragment 117 to 170, bound almost exclusively to the right site, as indicated by its high levels of binding to fragments 90 to 170 and 117 to 170, its low level of

Table 1. Binding specificity of antibody to myelin basic protein. Ten picomoles of ^{125}I-labeled GPBP or fragment were incubated with 50 μl of undiluted serum. Binding of ^{125}I-labeled GPBP to normal serum was 6.4 ± 0.4 percent. Binding of ^{125}I-labeled peptides to normal serum was always less than the binding of ^{125}I-labeled protein. The serums were prepared as follows. (A) Animals were injected ten times (three times a week) with 0.1 mg of GPBP in IFA, followed in 1 month by an injection of 0.1 mg of GPBP in CFA (0.1 mg of mycobacteria). (B) Animals were injected once with 0.5 mg of GPBP in CFA (2.5 mg of mycobacteria). (C) The procedure was that for (A) except that challenge with GPBP in CFA was delayed for 6 months. (D) Animals were injected ten times (three times a week) with 0.1 mg of rat S basic protein in IFA followed in 1 month by an injection of 0.1 mg of GPBP in CFA (0.1 mg of mycobacteria). (E) The procedure was as in (D), except that the challenge injection was 0.5 mg of GPBP in CFA (0.5 mg of mycobacteria). (F) Animals were injected once with 0.1 mg of HNB-modified GPBP in CFA (0.1 mg mycobacteria). (G) Animals were injected ten times (three times a week) with 35 μg of fragment 117 to 170 in IFA followed in 1 month by 0.1 mg of GPBP in CFA (0.1 mg of mycobacteria). Bleedings were made 1 month after the last injection.

Protein or fragment	Binding (percent)						
	Broad specificity		Narrow specificity				
	A	B	C	D	E	F	G
1–170	83	87	74	58	33	47	51
1–20	11	7	1	1	1		3
1–89*	34	33	5	21	19	3	
44–88	31	34	4		27	1	2
1–116	74	63	66	44	32	23	18
90–170	71	44	57	20	7	33	69
117–170	39	41	3	3	2	3	57

* Mixture of four peptides derived from region 1 to 89.

binding to fragment 1 to 116, and its failure to bind fragment 44 to 88.

Our conclusion from the data in Table 1 is that there are at least three mutually exclusive sites on the myelin basic protein molecule capable of binding antibody induced by homologous protein. We cannot conclude that there are only three since some of the fragments may contain more than one site. That some serums bind one or two of the fragments and fail to bind others indicates that there is no cross-reactivity among the specific sequences detected in the three regions.

Fragment 1 to 20 was not bound significantly by any of the serums listed in Table 1, with the possible exception of serum A. This region may contain a weak site, but the fact that only one of several dozen serums tested bound this fragment led us to conclude it was of little importance. This nonantigenic region of the molecule is probably at least 43 residues in length. No serum was found to have significantly greater binding for the mixture of fragments from residues 1 to 89 than for fragment 44 to 88.

The immunization schedules yielding serum A and serum C differed only in the length of time between priming with IFA and challenge with CFA. During the 6-month delay, only those cells capable of recognizing the center site survived. This fact, coupled with the frequent occurrence of serums capable of binding the center site suggests that this region is the most antigenic portion of the molecule. This is also the most reactive region of the molecule in other respects: it includes part of the site that induces EAE in guinea pigs (residues 114 to 122) (*12*), it contains the single methylated arginine residue 107 (*13*), and the single threonine residue 98 reported to be the receptor for glycosylation (*14*), and it is part of the region that binds most readily to isolated myelin lipid fractions (*15*).

The technique used in our study to demonstrate multiple sites within the molecule capable of binding specific antibody differs somewhat from that used in other studies (*1, 2*). By manipulating the immunization schedule, we obtained unique antiserums that differed qualitatively in their ability to bind fragments derived from the whole molecule. The success of our experimental approach with myelin basic protein suggests that at least some of its specific antigenic regions do not depend upon conformational characteristics of the intact molecule as was the case with lysozyme (*2*).

Although the role of antibody to myelin basic protein in pathogenesis of EAE was not investigated, it should be noted that the ability of basic protein to induce antibody appears to be unrelated to its ability to induce disease. The amount of mycobacteria present in the immunization mixture is critical for antibody production but not for disease induction—a single injection of basic protein with 2.5 mg of mycobacteria induces high levels of antibody, whereas an equally encephalitogenic injection of basic protein with 0.1 mg mycobacteria induces almost no antibody. Antigenicity of the latter mixture can be enhanced, however, by chemical modification of the antigen (Table 1, serum F) or by priming of the host with repeated injections of antigen in IFA (serum A). In both instances, enhanced antibody production is accompanied by loss of ability to induce EAE.

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Follicle-Stimulating Hormone Secretion in the Female Rat: Cyclic Release Is Dependent on Circulating Androgen

Abstract. *In adult cyclic female rats, intravenous injections of an antiserum to testosterone prevented the continued increase in serum follicle-stimulating hormone (FSH) which normally occurs during the early morning hours of estrus. This treatment did not prevent the initial increases in serum FSH (and luteinizing hormone) which occur during the critical period (1400 to 2000 of proestrus), nor did it interfere with subsequent ovulation. These observations indicate the existence of two separate mechanisms for preovulatory FSH release in the rat and implicate circulating testosterone as the stimulus for continued secretion of FSH during estrus.*

In the rat, the preovulatory increases in serum follicle-stimulating hormone (FSH) may be separated into two phases. The initial period of release occurs on the afternoon of proestrus (during the "critical period") and is characterized by rapid increases in serum concentrations of FSH as well as of luteinizing hormone (LH). The second phase of FSH release follows the first and occurs between midnight on the day of proestrus and 0600 of the following morning (day of estrus). During this second phase, serum LH has returned to baseline values while serum FSH is maintained at concentrations equal to, or greater than, those observed during the critical period (*1*). Thus, the greater part of FSH secretion in the female rat occurs in the absence of LH release. In this respect gonadotropin secretion in the rat differs from that of the human and the monkey in which both gonadotropins are released concurrently at midcycle (*2*).

The essential role of circulating estrogen in the stimulation of preovulatory LH release has been clearly established in several species (*3*). In has been tacitly assumed, in the absence of evidence to the contrary, that the "positive feedback" action of estrogen pro-