UCLA-593B was destroyed during the dating procedure, but sample UCLA-593A remained substantially intact, a triangular section of sufficient size and weight having been removed for testing from the underside of the shell, opposite the inner lip. While the early dates of the two samples came as a surprise, it was thought that the discrepancy between them might be attributable to the repeated use of the tomb over several generations, an explanation suggested by the large number of skeletons and piles of unarticulated bones found in the chamber. There is also the possibility, however, that several persons were killed to accompany the main occupant, or occupants, of the tomb.

The discrepancy between the early dates thus appeared to require further investigation. In the summer of 1964, while on a field trip in Mexico, I examined several conch shell trumpets found in West Mexican grave sites, including a large specimen from the region of Sayula, Jalisco, and a smaller one found near Comala, Colima. The large specimen was identified as *Strombus gigas* Linné, popularly known as the Queen conch, a fairly common shell native to the Caribbean, with a range including Southeast Florida, the West Indies, and Bermuda; the small specimen was identified as *Xancus angulatus* Solander, the West Indian chank, also a Caribbean shell with a range including the Bahamas, Key West, Cuba, Yucatan, and Bermuda (*9*). Neither species is native to the western American coast, indicating pre-Columbian cross-continental trade in conch shells or shell trumpets at a time equivalent to the late pre-Classic (*10*).

Because of the possibility that such movement of shells from the eastern coast might account for the surprisingly early dates registered by the two shell artifacts from the Etzatlán tomb, the remaining sample, UCLA-593A, and other artifactual and nonartifactual shell material from the same tomb were submitted to the Los Angeles County Museum for determination of species, genus, and native range (*11*). The large conch shell, sample UCLA-593A, was identified as *Strombus gigas* Linné, and therefore from the Caribbean; another, considerably smaller, shell trumpet turned out to be a *Xancus angulatus* Solander, again a Caribbean shell. The other shells, with one possible exception (*12*), were, however, positively identified as West Coast species, with a general range including the Gulf of California to Peru.

Because of the possibility of a considerable time lag between the harvesting of the *Strombus gigas* in the Caribbean and its eventual arrival and final burial in Jalisco, it was now deemed advisable to date a third shell (UCLA-593C) from the Etzatlán tomb, a shell unquestionably of West Coast origin. The shell selected was a small *Murex nigritus* Philippi, native to the Jalisco coast, with a range extending as far north as the Gulf of California and as far south as the north coast of Peru, and measuring 92 mm in length and 60 mm in height. This sample was dated several times to assure greatest possible accuracy. The final count, made 9 December 1964, yielded, with corrections, a date equivalent to approximately A.D. 254 $\pm$ 80. This age was computed on the basis of the surface ocean water in the region concerned having an initial C^{14} concentration of approximately -1 percent (or being -80 years) with respect to 0.95 percent of the National Bureau of Standards' oxalic acid radiocarbon standard (*13*).

It is hoped that some of the skeletal material from the tomb will soon be subjected to radiocarbon analysis in order to obtain another check on the present computations made from seashell artifacts (*8*). Pending additional radiocarbon dating, however, it is suggested that the present results be tentatively accepted as valid and a date of approximately A.D. 250 be assigned to at least one phase, possibly a late phase, of the Jalisco-Nayarit-Colima deep shaft-and-chamber tomb complex, and therefore to the large, hollow, polychrome figurines of the Ameca-Zacoalco or Etzatlán-Magdalena (Jalisco) and Ixtlán (Nayarit) types. Although the radiocarbon dates given here were obtained from samples from a Jalisco tomb, the Ixtlán area may be inferentially included, both because of the similarity of tomb architecture and because several figurines of a typically Ixtlán style were among the burial furniture in the Etzatlán tomb.

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11. The cooperation of Charles Rozaire, Curator of Archaeology, Los Angeles County Museum, in selecting and making sampling materials available for radiocarbon dating is gratefully acknowledged.
12. One shell was tentatively identified as *Strombus gracilior* Sowerby, native to the West Coast. However, a similar shell, *Strombus pugilis,* exists in the Caribbean. The condition of this artifact made specific identification impossible.
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14 December 1964

Polypeptide Chains of Antibody: Effective Binding Sites Require Specificity in Combination

Abstract. *Separated chains from antibody of different rabbits do not always give effective binding sites although combination of the chains does occur. There is a specificity of combination such that only chains from the same (or perhaps a related) rabbit form effective binding sites. There appears to be preferential combination between those chains which yield effective sites.*

Separated heavy (H) and light (L) polypeptide chains from a preparation of reduced and alkylated specific antibody have little antigen-binding activity, but activity can be largely restored by mixing the chains (*1, 2*). Restoration of activity is associated with a physical combination of H- and L-chains that results in a unit similar to native antibody, as demonstrated by radioimmunoelectrophoresis and ultracentrifugation (*2*) or by passage through Sephadex columns (*3*). There is a concomitant reformation of the antigen-binding region. Disulfide bonds do not participate in the combination since the sulfhydryl groups are alkylated.

Physical combination of L- or H-chains from normal γ-globulin with the complementary chains from antibody γ-globulin resulted in hybrids possessing little antibody activity. Apparently the

Table 1. Binding activity of mixtures of H- and L-chains and of untreated purified antibody. The chain mixtures in cold 0.01 *M* or 0.05 *M* propionic acid were dialyzed successively against cold 0.1 *M* propionic acid overnight, against 500 volumes of borate buffer for 1 hour, and against 500 volumes of fresh buffer overnight. No precipitates formed.

Preparation No.	Composition	Mole ratio of L- to H-chain*	Concentration of bound hapten (mole/liter × 10^6)†	Relative binding activity
		X_p Antibody		
1	Pool A		11.8	100
2	Pool B		14.9	100
		Mixtures of chains		
3	$H_1(X_p)_A + L(X_p)_A$	1.0	7.0	60‡
4	$H_1(X_p)_A + L(X_p)_A$	4.0§	7.5	64‡
5	$H_1(X_p)_A + L(X_p)_B$	1.6§	0.7	6‡, 5‖
6	$H_1(X_p)_B + L(X_p)_B$	1.0	8.8	59‡
7	$H_1(X_p)_B + L(X_p)_A$	1.0	1.1	7‡, 9‖
8	$L(X_p)_A$		0.3	2‖

* Mixtures made on the basis of OD units; extinction coefficient of H-chain is approximately 3 times that of L-chain. † All preparations run in the equilibrium dialysis experiments at a free-hapten concentration of 17.4 × 10^{-6} *M*. Protein concentration in the mixtures was equivalent to 1.33 mg of intact γ-globulin per milliliter, exclusive of excess L-chain. ‡ Relative binding activity is in relation to the antibody pool from which the H-chain was derived. § Mixtures examined in the ultracentrifuge. Excess specific L-chain does not significantly alter hapten binding compared with binding when one equivalent is used (compare preparations 4 and 3). ‖ Relative binding activity in relation to the antibody pool from which the L-chain was derived.

chains from the normal globulin used did not contribute as much to reformation of an active site as did the counterpart chains from the original antibody pool. Also, hybrids are even formed between H- and L-chains of globulins of different species (*4*). We have now compared hapten binding by mixtures of H- and L-chains from antibody directed against the same hapten in which the H- (or L-) chains are from one pool of serum and the L- (or H-) chains are from a different pool, the two pools being made up of serums from different rabbits. Such mixtures do not give good active binding sites although the chains do associate to give units showing physical and immunologic properties similar to those of native γ-globulin. This requirement of selectivity is very important in considerations of the mechanism of antibody formation.

Purified rabbit antibody against the *p*-azobenzoate group (X_p) was prepared by dissociation of specific precipitates (*5*). The antiserums were obtained after repeated injection of rabbits with a conjugate of *p*-azobenzoate and bovine γ-globulin. Two pools of serum were used: pool A consisted of serums from one or more bleedings of each of 24 rabbits, and pool B derived from several bleedings of one rabbit which did not contribute to pool A. Hapten-binding activity of antibodies and their derivatives was determined by the binding of *p*-iodobenzoate (I^{125}-labeled); binding was measured by equilibrium dialysis (*6*).

The H- and L-chains were reduced and alkylated in the manner of Fleischman, Pain, and Porter (*7*); 0.2 *M* mercaptoethanol was used for the reduction and 10 percent excess iodoacetamide for alkylation. The chains were separated by chromatography on Sephadex G-100 equilibrated with 1 *M* propionic acid. Representative tubes of each fraction were pooled, and the pools were dialyzed against 20 volumes of cold distilled water and pervaporated, with intermittent dialyses, against cold 0.05 *M* propionic acid. Sephadex G-100 gives a better separation of the H- and L-chains than does Sephadex G-75; moreover, it separates the H-chains into two peaks (*8*) which we designate as H_1- and H_2-chains and which we tested separately. Mixtures of either H_1- or H_2-chains with L-chains from the same pool show comparable antibody activity.

Concerning the relation between the source of the chains and the recovery of activity, H_1- and L-chains, from either the same pool or different pools of antibody to X_p, were mixed. L- and H_1-chains from pools A and B of antibody to X_p, which we designate $L(X_p)_A$, $L(X_p)_B$, $H_1(X_p)_A$, and $H_1(X_p)_B$, were mixed and the mixtures were studied. The binding of *p*-iodobenzoate by each original preparation of X_p-antibody was also measured (Table 1). Mixing H_1- and L-chains from the same pool of antibody (preparations 3, 4, and 6) led to about 60-percent recovery of activity of the intact antibody (*2*), but there was little recovery if chains from different pools were used (preparations 5 and 7). L-chains (preparation 8) [or H-chains (*9*)] alone showed little hapten binding.

The fractions of H-chains, $H_1(X_p)_A$ and $H_1(X_p)_B$, did not migrate upon immunoelectrophoresis, but when mixed with L-chains from either the common pool or a different pool they did migrate, showing combination. The combinations in mixtures in which the H_1- and L-chains both came from the same pool (preparations 3, 4, and 6) bound antigen, as judged by radioimmunoelectrophoresis (*10*), but the hybrid combinations in the mixtures of the H- and L-chains from different pools bound little antigen.

On ultracentrifugation, the sedimentation pattern of the mixtures of $H_1(X_p)_A$ with an excess of $L(X_p)_A$ or $L(X_p)_B$ (Table 1, preparations 4 and 5) showed combination of H- and L-chains in each case; the pattern was similar whether chains were from a common pool or from two different pools (Fig. 1). The rapidly sedimenting material (at 6.2*S*) in both preparations showed peaks similar in size and shape. The shoulder indicates some material with a slightly greater sedimentation coefficient, but neither mixture showed any heavier aggregate. The ex-

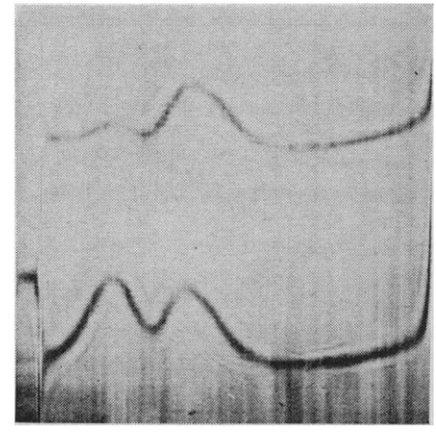

Fig. 1. Ultracentrifuge patterns of mixtures of H- and L-chains. Top, $H_1(X_p)_A + L(X_p)_B$; bottom, $H_1(X_p)_A + L(X_p)_A$. 59,780 rev/min, 50 minutes after speed attained. In mixing these solutions, 3.87 optical density (OD) units, the first of two portions of $H_1(X_p)_A$, was mixed with 2.34 OD units (1.6 equivalents) of $L(X_p)_B$; the second portion, 3.87 OD units, was mixed with 5.70 OD units (4.0 equivalents) of $L(X_p)_A$; the mixtures totaled 6.21 and 9.57 OD units, respectively. No subsequent precipitation occurred in either mixture; the optical densities of the solutions placed in the ultracentrifuge cells were 6.47 and 10.2, respectively. Thus, the concentration of $H_1(X_p)_A$ was the same in both cells because both solutions showed the same minor fractional increase in concentration during manipulation. Areas of the peaks for the heavier material are essentially equal, top figure: bottom figure being 1.1:1; for the lighter material the ratio is 0.22:1. The expected ratios were 1.0 and 0.2, respectively.

cess L-chain in either case appears as a peak at 3.1*S*.

The area of each peak shows that the amounts of recombined material are essentially equal, and the relative amounts of excess L-chains are as expected (Fig. 1). Therefore, the very low hapten-binding activity for $H_1(X_p)_A$ with $L(X_p)_B$ (Table 1, preparation 5) appears to be due not to a correspondingly low amount of combined material but rather to either a low hapten-binding constant for the material, or to hapten binding by only a small part of the material, or both. The same must also be true for $H_1(X_p)_B$ with $L(X_p)_A$ (Table 1, preparation 7).

Thus the H- and L-chains derived from a pool of specific antibody of several rabbits yield combinations giving good hapten-binding activity, as do those derived from antibody of an individual rabbit. Yet cross-mixing of the H- (or L-) chains from one source with L- (or H-) chains from the other source leads to combination but not to good binding activity. It therefore appears possible that the good hapten binding which results from mixing the H- and L-chains derived from the pool containing serum from the 24 rabbits really represents preferred recombinations between the H- and L-chains originally derived from the antibody of each rabbit contributing to the pool. The H- and L-chains of two or more of the 24 rabbits represented in this pool may possibly cross-combine effectively with one another. However, our results show that neither the H- nor L-chains of at least the one sole rabbit (not in the pool) would combine effectively with the L- or H-chains of an appreciable number of the 24 rabbits making up the pool. Such specificity of combination suggests individual differences among rabbits, and that these differences influence the hapten binding of the combined chains.

We have observed similar specificity of combination between the antibody chains derived from two individual rabbits where *p*-azobenzenearsonate was the hapten. Mixtures of the H- and L-chains derived from the same rabbit gave relatively good recovery of the hapten-binding activity, whereas cross-mixing of the chains of one rabbit with those of another gave little recovery of this activity. However, combination did occur, as judged by radioimmunoelectrophoresis.

Thus mixing H- and L-chains from a pool of antibodies from several rabbits can give a large recovery of sites, and H- and L-chains from different rabbits combine without forming effective sites. These two facts signify that the combinations of chains which give effective sites must be preferred over other combinations.

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Polysomes from Yeast: Distribution of Messenger RNA and Capacity to Support Protein Synthesis in vitro

Abstract. *Individual fractions of polysomes were isolated from yeast. Pulse labeling experiments in vivo show constant specific activity of messenger RNA in each polysome peak; this suggests a uniform density of ribosomes per unit length of messenger RNA. In the cell-free incorporating system, the amount of peptide per ribosome unit increased with the size of polysome.*

In rapidly dividing cells, many of the extracted ribosomes are found in clusters called polysomes. These clusters are of varying size, are apparently bound together by a strand of messenger RNA (mRNA), and serve as templates for protein synthesis. Since cell-free extracts appear devoid of free mRNA and contain an excess of free monosomes (which will rapidly bind added mRNA), the polysome classes in extracts may provide a useful guide to distribution of mRNA molecules with respect to size. Such a study requires high resolution of polysome classes under conditions which minimize mechanical shear and breakdown of RNA by nuclease action. This has been possible in yeast, where improvement in both the extraction of polysomes and analysis by density gradients affords separation and isolation of sufficient quantities of individual classes of polysomes to determine biochemical properties. A rapid turnover of P^{32} and S^{35} occurs in the polysome fraction, and polysomes isolated from sucrose gradients incorporate amino acids, whereas monosomes were inactive unless synthetic mRNA was added (*1*).

We report the size distribution and synthetic activity of the individual polysome classes. In particular an estimate of the ratios of messenger to ribosome to polypeptide was made throughout the polysome fraction.

The hybrid yeast *Saccharomyces dobzhanskii* × *Saccharomyces fragilis* was used. Since stationary cells contain few polysomes, cells were harvested during the log phase of growth by pouring the culture onto ice slivers. All subsequent manipulations were performed at 0° to 4°C to reduce ribonuclease activity. The cells were disrupted by grinding them for 4 minutes with glass beads, and the resulting extract was centifuged (10,000*g* for 10 minutes) to remove debris (*2*). Sucrose gradient sedimentation of this extract yields seven peaks of material heavier than the single 80*S* ribosomes (Fig. 1). Electron micrographs of material from these peaks contain in order, the dimer, trimer, tetramer, and so forth, up to the octomer and