cm below the bend of the right elbow, was measured by a thermocouple which had been soldered to a piece of silver (10 by 10 by 0.2 mm). The temperature of the stimulator was adjusted to equal that of the skin, and the stimulator was placed on the arm. The subject was instructed to adjust the temperature of the stimulator, by means of the lever switch, so that a just-detectable sensation of warm or cool was maintained throughout a 40-minute observation period. The experimenter changed the temperature of the stimulator toward neutral at 5-minute intervals and instructed the subject to readjust the temperature of the stimulator to produce the just-detectable sensation. This procedure insured the continued attention of the subject. Four measurements, on each of four subjects, were made of the temporal course adaptation to warmth and coolness. In every instance the temperature of the stimulator at which the measurement started was that of the skin of the forearm. The results are shown in Fig. 1.

Under the conditions of measurement, the range of temperatures to which complete adaptation could occur was much smaller than those found by previous investigators. The largest temperature range over which complete adaptation occurred in our study was 8.2°C (subject D.K.) and the smallest was 4.5°C (subject J.M.). Others have found the range to be 23°C (*3*), 27°C (*4*), 21°C (*6*), and even 40°C (*5*). Several factors may account for the smaller temperature range we found: better control of the stimulus and subject conditions, more stringent criterion for complete adaptation, and size and location of the area stimulated.

The rate at which adaptation occurs was very rapid for temperatures close to that of the skin and is markedly reduced for the more extreme temperatures. In practically all instances adaptation was substantially complete after 20 minutes and, in some instances, within 10 minutes. Also, it appears that adaptation to temperatures above skin temperature proceeds more rapidly than it does to temperatures below.

The final temperature to which each of the subjects could adapt was independent of the initial skin temperature. This is not as apparent from Fig. 1 as it was when the results of successive measurements on the same subject were plotted individually.

There are differences between individuals in the extent of the temperature range in which complete adaptation can occur. These differences appear to be characteristic of the individual rather than due to an error of measurement. When the data were plotted with temperature on the ordinate, rather than change in temperature, the individual differences were still apparent.

Kenshalo, Nafe, and Brooks (*11*) have reported measurements of the warm and cool thresholds as a function of the temperature to which the skin had been adapted. The adapting temperatures used by them ranged from 27° to 42°C. Within the range of adapting temperatures from about 30° to 36°C, measurements of the thresholds were fairly straightforward. The direction of the temperature change seemed to determine the quality of the thermal sensation. That is, a rise in temperature felt warm while a decrease in temperature felt cool. Outside this range, however, complications arose. The subjects reported a complex array of thermal sensations when measurements of the warm threshold were made at low adapting temperatures (below 30°C). An increase in the temperature of the stimulator first produced a sensation of "less cool" followed by "neutral" and, finally, "warm." In the measurement of the cool threshold at high adapting temperature (above 37°C) similar observations were encountered. A reduction in the temperature of the stimulator was reported as producing a "less warm" sensation followed by "neutral" and was finally reported as "cool." The extreme adapting temperatures used by Kenshalo, Nafe, and Brooks exceeded the temperature limits to which our subjects could adapt completely. At these extreme temperatures a residual sensation remained, and for experienced observers small increments or decrements in the extreme temperature altered the intensity of the residual sensation without altering its quality.

A statement concerning the nature of the stimulus events responsible for the qualities of warm and cool may be derived from the results of these two experiments. Only within the temperature limits to which complete adaptation has occurred does the direction of the temperature change determine the quality of the sensation. Outside of these limits or before complete adaptation has occurred the direction of small temperature changes only serves to increase or decrease the intensity of the persisting sensation.

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Primary-Type Antibody Response in vitro

Abstract. *Organ cultures of lymph nodes obtained from nonimmunized rabbits were incubated with bacteriophage ϕX174; as a result, 19S antibody was produced first, and then 7S antibody. Differences between this response and the secondary response to ϕX174 induced in vitro suggest that the former is a primary response.*

Secondary antibody formation can be induced and maintained in vitro (*1*), whereas primary antibody formation has not been detected under similar conditions of tissue cultivation (*1*). We now describe an apparent primary antibody response to bacteriophage ϕX174 induced and maintained in cultures of lymph nodes from nonimmunized adult rabbits.

Cervical and popliteal lymph nodes from nonimmunized albino rabbits (1.5 to 2 kg) were removed, cut into fragments, and cultivated in vitro in organ culture (*2*). Each culture initially contained 25 to 50 mg (wet weight) of tissues and 1.7 ml of medium which consisted of 20 percent normal rabbit

serum, 80 percent Eagle's minimal essential medium, and 100 international units of penicillin per milliliter of medium. Bacteriophage ϕX174 (*3*), in a final concentration of 10^7 to 10^9 plaque-forming units (PFU) per milliliter, was incubated with the node fragments for the first 2 to 48 hours of cultivation. The medium containing the phage was then removed; the fragments were transferred to fresh organ-culture dishes for further cultivation, after repeated washings with fresh medium to remove excess phage particles. The control cultures were not exposed to the bacteriophage. The medium was changed at regular intervals (1 to 4 days) for 2 to 3 weeks. The removed media were assayed for antibody to ϕX174 by incubation with 5×10^3 PFU of the phage per milliliter at 37°C, and the phage neutralization was measured by the pour-plate technique (*4*). Antibody activity was determined by either the percentage of phage neutralization after 24 hours of incubation, or by the standard assay of the rate of phage neutralization (*k*) (*4*). After incubation with ϕX174, portions of each culturing medium were treated with 2-mercaptoethanol (2-ME) at a final concentration of 0.1*M* for 30 minutes at 37°C prior to plating (*5*). Antibody activity that was inactivated by 2-ME was considered to represent 19*S* antibody, and that resistant to 2-ME treatment was assumed to represent 7*S* antibody (*6*).

In each of five experiments, neutralizing activity to ϕX174 appeared in the media of normal lymph node cultures after immunization in vitro with the phage. The specificity of the neutralizing activity that appeared in the media was demonstrated by the neutralization of the phage used for immunization, namely ϕX174; two other immunologically unrelated phages, T2 and PLT22 (*7*), were not neutralized. Also, ϕX174 was not neutralized by the media of unstimulated cultures or by the media of cultures stimulated in vitro by an unrelated antigen, namely flagellar antigen of *Salmonella adelaide* (*8*). In a typical experiment (Fig. 1), the medium was changed daily, and antibody to ϕX174 was first detected on day 4. Based on the criterion of susceptibility to inactivation by 2-ME, all of the antibody appearing from days 4 to 7 was 19*S*; 7*S* antibody was first detected on day 8 and remained at a low concentration throughout the cultivation period. The predominant type of antibody to ϕX174 formed during the 12-day cultivation appeared to be 19*S*.

Table 1. Primary-type and secondary antibody responses to bacteriophage ϕX174 induced and maintained in vitro. The value shown is the average of *k* values from three (triplicate) culture flasks.

Days in culture	Primary-type response		Secondary response	
	Without 2-ME	With 2-ME	Without 2-ME	With 2-ME
2nd to 4th	1.1×10^{-3}	0	0.7	0.9
4th to 6th	1.6×10^{-3}	0	1.5	8.0
6th to 8th	4.1×10^{-3}	1.1×10^{-3}	4.6	18.1
8th to 10th	2.7×10^{-3}	1.2×10^{-3}	5.1	28.9
10th to 12th	2.1×10^{-3}	1.2×10^{-3}	4.6*	22.2*

* The medium was removed on the 13th day when the experiment was terminated.

The antibody response induced in vitro by ϕX in nodes from nonimmunized rabbits (primary-type antibody response) was compared to that induced in vitro in nodes from rabbits immunized 3 months previously with 10^{11} PFU of ϕX174 (secondary antibody response). The *k* values of the media were determined to obtain quantitative measurements of neutralizing antibody. Three differences between these two responses are apparent from Table 1:

1) The *k* values obtained in the primary-type antibody response were approximately 1×10^{-3} to 1×10^{-4} of those in the secondary antibody response.

2) In the primary-type antibody response, 19*S* antibody formation preceded that of 7*S*, whereas 7*S* was present throughout the secondary antibody response.

3) The primary-type antibody response consisted predominantly of 19*S* antibody, whereas the secondary antibody response consisted primarily of 7*S* antibody. It is not known why the *k* values of the media from the secondary response became higher after treatment with 2-ME (*9*).

The differences between the two types of antibody response induced in vitro are generally similar to those observed between primary and secondary responses to the phage in vivo (*10*). Hence the specific antibody response induced and maintained in vitro in cultures of normal lymph nodes may be a primary one. However, since *Escherichia coli* C, the host bacterium for ϕX174, is ubiquitous, normal rabbits may have been naturally immunized with ϕX174. Serums from three of the five rabbits used in these experiments and from 10 additional nonimmunized rabbits were tested for antibody to ϕX174. None of the serums showed definite specific antibody activity (*k* values more than 0.001) but two of the nonimmunized group had trace amounts of neutralizing activity (*k* values of 4×10^{-4}; and 5×10^{-4}) which may represent specific antibody. The possibility has not been excluded, therefore, that immunization to ϕX174 which is not routinely detectable by the presence of serum antibody may have taken place in vivo and may have prepared the lymph nodes to respond to the subsequent antigen stimulation in vitro. Further studies of this cultivation system with lymphoid tissues from newborn and germ-free animals may provide more information concerning this critical point.

Two previous reports of elicitation in vitro of a primary antibody response (*11*) in cultured lymphoid tissues have not been confirmed. Fishman (*12*), using a two-step procedure, has described initiation of a primary antibody response in vitro, and this observation has been confirmed (*12*). In Fishman's studies, the antigen, bacteriophage T2, was incubated with peritoneal macrophages, and a cell-free extract of the antigen-macrophage mixture was used to stimulate fragments of normal lymph node in organ culture. No response was detected when the antigen alone was incubated with the fragments. In contrast, we have obtained an apparent primary response by incubating lymph-node fragments

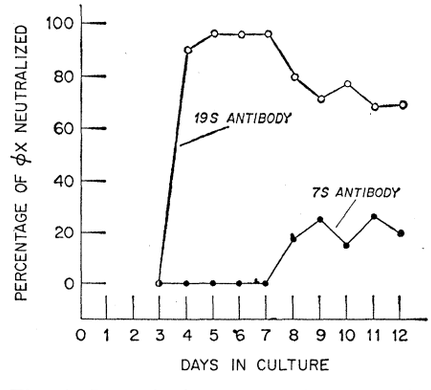

Fig. 1. Bacteriophage-neutralizing activity induced and maintained in vitro in cultures of normal lymph nodes after stimulation with phage ϕX174 for the first 2 days of cultivation. Each point represents the average value from two cultures.

with ϕX174 in vitro. This culture system preserves the morphological integrity of the tissues and supports cellular differentiation (*2*). Our results, therefore, may be explained by a maintenance in vitro of the various cell types participating in the antibody response as well as the excellent immunogenicity of ϕX174. From other experiments an apparent primary antibody response to flagellar protein of *Salmonella adelaide* can also be induced and maintained in vitro in the same manner (*13*).

Note added in proof: Globerson and Auerbach (*14*) have recently reported that spleen explants from mice treated with either phytohemagglutinin or adjuvant could be stimulated in vitro to form antibody to sheep red blood cells. No antibody formation occurred, however, following similar antigenic stimulation of explants of spleens from normal untreated mice.

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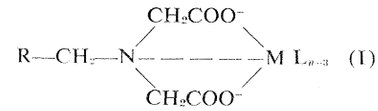
Concentration of Dissolved Amino Acids from Saline Waters by Ligand-Exchange Chromatography

Abstract. *Amino acids dissolved in salt solutions may be concentrated and removed from the solution by ligand exchange on copper–Chelex 100 resin. Competing inorganic ligands do not interfere, and ion exchange with cations does not occur; thus loss of metal ion from this column is avoided. To test the potentiality of ligand exchange for chromatography, the type and nature of the dissolved amino compounds in sea water were investigated. The data revealed that the bulk of the dissolved amino compounds is present in a combined rather than a free state.*

This study focuses attention on "ligand-exchange chromatography" for the removal of dissolved organic constituents from electrolyte solutions (*1*). The method is highly selective in concentrating a major portion of the organic substances from strong electrolyte solutions such as sea water. In this procedure a cation-exchange resin saturated with a complex-forming heavy metal (Cu^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, or Zn^{2+}) acts as a solid sorbent. Ligands which may be anions or neutral molecules such as ammonia, amines, amino acids, or olefins are removed from the liquid phase by formation of complexes with the metal attached to the resin and subsequent displacement of water or other liquids coordinated to the metal ion. The advantages of this type of ligand exchange lie in the high selectivity of the metal-resin ion exchanger for ligands that form complexes and chelates. This permits quantitative concentration of the organic ligand from very dilute solutions even in the presence of the competing inorganic ligands that are normally found in sea water.

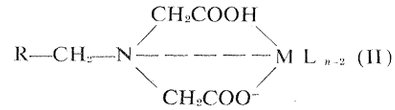
Previous uses of ligand exchange have involved resins of low specificity for the transition metals (*2*). As a result, only dilute electrolyte solutions could be used in extracting organic materials because competing cations would displace the complexed metal ion from the resin by ion exchange. This in turn would cause the loss of metal ion from the column (metal-ion bleed), lower the ligand-exchange capacity, and prevent a complete sorption of the organic ligands onto the resin.

We have used Chelex 100 resin (Bio-Rad Laboratories, Richmond, California) because of its extraordinary selectivities for the transition metals. The active sites of the resin are iminodiacetic acid groups similar to those found on ethylenediaminetetraacetic acid (EDTA). The structure of the complex formed in the resin is either:



(I)

or



(II)

where M is the metal ion, n is the coordination number of the metal, and L denotes the additional ligand bound to the metal ion (*3*). The chelating groups on the resin bind the transition metal so strongly that metal-ion bleed does not occur when saline waters are passed through the resin. Consequently, this resin extends the use of ligand-exchange techniques to strong electrolyte solutions such as sea water, brines, urine, or blood serum.

In the preparation of the metal resins, the following steps were taken. First, the resin (sodium form) was stirred with the appropriate metal chloride solution; we prepared Cu^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, and Zn^{2+} resins. Second, the excess metal chloride was removed by exhaustive washing with distilled water until the wash water was free of metal ions (*4*). Third, the resins were stirred with 3.0*M* NH_4OH and subsequently placed in columns (10 by 1 cm). Finally, the excess ammonia was removed by flushing the columns with distilled water until the *p*H of the effluent was between 8 and 9. After this, the columns were ready for chromatographic use.

The first group of organic compounds tested for their ligand-exchange properties was the amino acids; copper-Chelex resin served as the solid sorbent. To get some idea regarding the quantity of resin needed for an individual run, 1.25 μmole of each of the common amino acids (Beckman amino acid standard type 1) was added to 1 liter of artificial sea water. Five medicine droppers (2 ml each) filled with the copper resin were connected one after the other, and the solution of amino acid and salt water was subse-