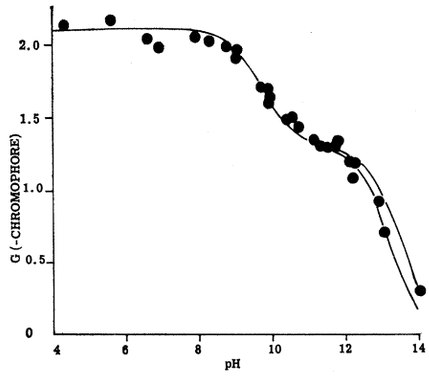

Fig. 1. Dependence of the disappearance yield of the chromophore of thymine on *p*H at an ionic strength of 1.5. Upper curve, $pK_2 = 13.5$; lower curve, $pK_2 = 13.2$. Air-saturated solutions. Units for G(—chromophore) are molecules changed per 100 ev absorbed.

Portions were chromatographed on paper (*6*) in one or two dimensions, and the distribution of radioactivity was determined by counting with a paper chromatogram scanner or by radioautography. Prior to chromatography, sodium hydroxide was removed from the 1.0 and 2.0*N* NaOH solutions by one of two procedures. In one, the samples were acidified to *p*H 1 with HCl and shaken with Norite A. Adsorbed thymine and irradiation products were eluted with a mixture of ethanol, water, and ammonium hydroxide (50:45:5 by volume), evaporated to dryness, and dissolved in water (*7*). Alternatively, solutions were neutralized by addition of the hydrogen form of the cation-exchange resin, Dowex-50.

Radioautographs of chromatograms of strongly alkaline irradiated solutions developed in two dimensions, first by a mixture of propanol and water (80:20) and then by a mixture of butanol, propionic acid, and water (92:47:61), revealed two products, neither of which is formed on irradiation of neutral air-saturated solutions. One accounts for more than half of the product radioactivity and absorbs light when illuminated by a long wavelength ultraviolet source; it moves precisely with authentic 5-hydroxymethyluracil (HMU) in cochromatographic experiments, and, when eluted from the paper, has an ultraviolet spectrum indistinguishable from that of HMU. Further evidence of its identity was obtained by chromatography with mixtures of *t*-butanol, methyl ethyl ketone, water, and ammonium hydroxide (40:30:20:10), and ethyl acetate, formic acid, and water (70:20:10). In each solvent the unknown behaved as expected for HMU, and there was no indication of uracil-5-carboxylic acid or 5-formyluracil. Formation of HMU is evidently initiated by attack on the 5-methyl group of thymine by the OH· free radical, or by $O^{\cdot -}$, the oxygen radical ion which results when OH· ionizes. 5-Hydroxymethyluracil has also been reported as a product of irradiation in the absence of air in solutions of neutral thymine (*8*) and as a product of thymine metabolism in liver slices and *Neurospora* (*9*).

The lesser product, which accounts for the bulk of the remaining product activity, moves precisely with urea in cochromatographic experiments (Table 1). It is probably formed by a series of reactions starting with addition of a free radical and possibly oxygen to the double bond in the ring and ending with alkaline hydrolysis. Supporting this idea are the observations that the saturated hydroxyhydroperoxide and dihydroxy derivatives of thymine give urea on standing in alkaline solution and that the reaction is accelerated by resin or charcoal. Ureido compounds were not found in appreciable amounts on any of the chromatograms of alkaline solutions.

In the range *p*H 10 to 13, the amounts of HMU and of compounds formed by addition to the double bond are intermediate between those found in neutral and in strongly alkaline solutions.

These results show clearly that as the *p*H is increased the major site of attack in the radiolysis of thymine in air-saturated aqueous solutions shifts from the double bond in the ring at neutral *p*H to the 5-methyl group in alkaline solutions. This shift can be correlated with the ionization of the thymine molecule or with other two-step changes in the system. Ionization is an important possibility for at least one of these steps because it leads to an increase in aromatic character of the pyrimidine ring and therefore to a decrease in reactivity of the 5,6 double bond activation of the methyl group. Free radicals and radical ions consequently would tend to react with the methyl group when thymine is ionized.

The possible significance of these findings to radiobiology should not be overlooked. The electron configuration around thymine in a DNA molecule is not known and radiation-induced reactions characteristic of the enol form may well occur and lead to formation of the 5-hydroxymethyl derivative. The presence of this group would probably not necessitate any change in the configuration of the DNA molecule, but might well interfere with or modify attempted replication (*10*).

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10. Supported by contract AT(04-1) GEN-12 between the AEC and the University of California.

29 March 1965

Immunoadsorbent for the Isolation of Purine-Specific Antibodies

Abstract. *An immunoadsorbent for the isolation of purine-specific antibody has been synthesized. The technique is applicable for isolating antibody to any purine or pyrimidine capable of being converted to a derivative which can be coupled to amine groups. The recovery of antibody from a serum sample is better than 82 percent, and the precipitability of the isolated antibody is as high as 89 percent.*

Since 6-halomethylpurines and pyrimidines have been synthesized (*1*), these bases have been coupled to carrier proteins, and antibodies specific to these groups which show cross-

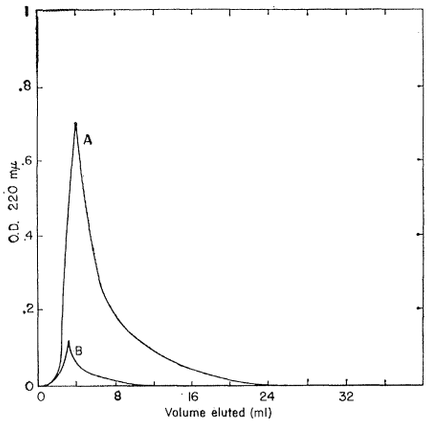

Fig. 1. Elution of proteins from 1 g of a purine immunoadsorbent prepared in a mixture of tetrahydrofuran and water (75:25) through which (*A*) 2 ml of rabbit anti-serum to a purine has been passed, and through which (*B*) 2 ml of normal rabbit serum has been passed. Both elutions were carried out with 0.1*M* phosphate-buffered saline, *p*H 2.3.

reactivity with deoxyribonucleic acids (DNA) have been obtained (*2*). Because specific antibodies to purines and pyrimidines cross-react with DNA, they have special importance for studies of nucleic acids and their antibodies as well as for studies of the mechanism of autoimmune diseases.

To conduct physical or chemical studies of these antibodies it is desirable to have effective methods for isolating and purifying them. Of the techniques available for the isolation of purified antibodies, one of the most successful depends on the use of immunoadsorbents (that is, insoluble polymers chemically bonded to antigens) for isolating antibodies from serum. The most common immunoadsorbents are insoluble derivatives of polyaminopolystyrene or *p*-aminobenzylcellulose. Both kinds of polymers are first diazotized and coupled to the antigen. Any remaining unreacted diazonium groups are then blocked with a "nonreactive" group such as β-naphthol. The blocking of the unreacted diazonium groups with β-naphthol is partially responsible for the nonspecific adsorption and release of serum proteins along with recovered antibody (*3*). The quantity of nonspecific protein released by this type of column ranges from 0.1 to 0.3 mg per milliliter of serum passed through a column consisting of 1 g of adsorbent (*4*).

We have synthesized new immunoadsorbents which do not require a diazotization step in their preparation (*4*). *N,N*-Dicyclohexylcarbodiimide induces the reaction of carboxylic acids and amines to form amides (*5*). In the presence of this reagent the acidic groups of carboxymethylcellulose couple to haptens and proteins having basic amino groups to form the desired immunoadsorbents. Columns composed of such specific adsorbents release one-tenth as much nonspecific protein as the columns made of diazotized aminoarylcellulose coupled to antigen (*3*).

Having found that the nonspecific protein isolated along with antibody can be decreased ten times if β-naphthol blocking groups are not required, we looked for a method for coupling purines and pyrimidines to cellulose which did not require blocking groups. We have now been able to synthesize a new immunoadsorbent for the isolation of purine-specific antibody. The technique may be used for isolating antibody to any purine or pyrimidine capable of being converted to a derivative which can be coupled to amine groups.

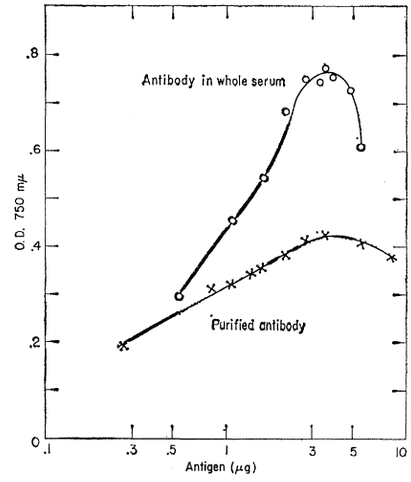

Fig. 2. Quantitative precipitin-determination curves for antibody to purine before and after purification.

A suspension of 5 g of aminoethylcellulose (*6*) was made in a mixture of tetrahydrofuran and water. To this suspension 1 g of 6-trichloromethylpurine (*1*) was added in small portions alternately with 0.5*N* NaOH, in order to maintain the *p*H at 10.0 to 10.5. The *p*H was checked periodically over a period of 6 hours and adjusted if necessary. The reaction mixture was stirred overnight, then filtered, washed exhaustively with acetone, and dried. Better yields were obtained with higher proportions of tetrahydrofuran in the solvent (Table 1). The volume of solvent was chosen so that the suspension could be stirred freely.

The antiserum was obtained from New Zealand white rabbits immunized with hemocyanin, from California giant keyhole limpets, which was coupled to 6-trichloromethylpurine by the method of Butler and co-workers for coupling this compound to bovine serum albumin (*2*).

One gram of immunoadsorbent suspended in 1-percent saline was poured into a 1.5-cm glass tube plugged at the bottom with glass wool. Pooled whole rabbit antiserum or normal serum was passed through the column and washed out with 1-percent saline until no more protein could be detected spectrophotometrically at 220 mμ in a 1-mm continuous flow-through cell. The antibody was eluted at *p*H 2.3 with 1-percent saline acidified with HCl or 0.1*M* phosphate-buffered saline.

Quantitative determination of precipitins, carried out by the procedure

Table 1. Coupling of 6-trichloromethylpurine to aminoethylcellulose. Five milliliters of normal rabbit serum were used in each case for the determination of nonspecific protein released.

THF*: water (v:v)	Purine coupled (mg)	Free aminoethyl groups coupled (%)	Nonspecific protein released (mg)
4:96	1.5	1.6	0.210
50:50	10.2	10.7	.140
75:25	14.0	14.7	.100
90:10	47.0	49.4	.016

* Tetrahydrofuran

Table 2. Retention, release, and purity of antibody isolated from immunoadsorbent.

THF*: water (v:v)	Serum passed (ml)	Antibody				
		Total in serum (mg)	Retained on column (%)	Released from column (%)	Precipitable † (%)	Theoretical purity‡ (% precipitable)
75:25	2	1.12	95	91	74	80
90:10	5	2.80	100	83	89	97

* Tetrahydrofuran. † Quantitative precipitin determinations were carried out with 6-trichloromethylpurine coupled to bovine serum albumin as the antigen. ‡ Calculated by taking into consideration nonspecific protein released by the immunoadsorbent.

of Kabat and Mayer (*7*), were made only on antibody eluted with the acidified 1-percent saline solutions. Protein was determined by the procedure of Lowry, Rosebrough, Farr, and Randall (*8*).

The purity and yields of antibody isolated with immunoadsorbents prepared in solutions consisting of tetrahydrofuran and water 75:25 and 90:10 are given in Table 2. In both cases at least 95 percent of the antibody in the serum was retained on the columns and better than 82 percent of the bound antibody was released on acidification with 1-percent saline acidified to *p*H 2.3 with HCl. The purity as measured by precipitability was close to theoretical, if nonspecific adsorption is taken into account. Figure 1 shows typical elution curves obtained with both antiserum and normal serum.

The precipitin determinations (Fig. 2) for both the whole serum and the purified antibody show a typical equivalence zone. However, the weight ratios of antigen to antibody at equivalence changed from 1:29 for immune serum to 1:19 for purified antibody. The same type of shift was noted for rabbit antibody to azophenylarsonate hapten isolated with carboxymethylcellulose coupled to *p*-aminophenylazophenylarsonic acid (*4*). The immunoelectrophoretic pattern of the isolated antibody to azophenylarsonate hapten was identical to that in the whole serum. The change in weight ratio may have been caused by release of a small quantity of remaining uncoupled hapten eluted with the antibody, by hapten released through hydrolysis of the column, or by some permanent change in antibody configuration induced by the isolation procedure.

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11 March 1965

Neurons of Insects: RNA Changes during Injury and Regeneration

Abstract. *A dense concentration of RNA appears in the perinuclear cytoplasm of nerve cell bodies in the third thoracic ganglion of the cockroach within 12 hours after their axons are cut. The basiphilic material of the perinuclear ring reaches maximum development 2 to 3 days after axon injury and returns to normal at about 15 days. Ribonuclease removes the basiphilic material, indicating that it is RNA. The perinuclear ring of RNA provides a marker for linking an individual central nerve cell body to a particular peripheral axon and the muscle it innervates.*

Changes in the distribution and concentration of RNA in the cytoplasm of vertebrate central neurons have been related to a variety of different functional states in these cells (*1*). In the cytoplasm of many vertebrate central neurons, RNA aggregates in large basiphilic masses, Nissl bodies (*2*), composed of quantities of ribosomes attached to densely packed endoplasmic reticulum (*3*). The dissolution (chromatolysis) and reformation of Nissl bodies in response to injury and regeneration of an axon have been extensively studied in the vertebrates (*4*). These investigations have clarified many basic neurological problems.

The arthropod ventral nerve cord provides a relatively simple system for investigating neurological problems (*5*) ranging from factors controlling neuronal growth (*6*) to mechanisms of learning (*7, 8*). Our purpose was to determine if changes in cytoplasmic RNA could be evoked in the central neurons of insects by injury and subsequent regeneration of their axons even though these cells lack distinct Nissl bodies (*9*). Our results show that marked changes in the distribution of cytoplasmic RNA can be detected in the soma of cockroach central neurons after their axons have been cut in the peripheral nerve trunks or interganglionic connectives. It is therefore possible to correlate alterations in RNA distribution with different functional states in these cells.

Sixty-four adult male cockroaches, *Periplaneta americana*, were operated upon within a period of 2 to 5 days after the last molt. Nine late-stage nymphs were also used, and no essential difference was seen between the experimental responses of nymphs and adults. The animals were lightly anesthetized with CO_2, and the entire ventral surface of the metathoracic ganglion was exposed by laying back a hinged flap of cuticle. The appropriate nerve trunks were cut on one side about 1 mm distal to the ganglion. The corresponding nerve trunks of the opposite side were exposed, but not cut, to serve as controls. Care was taken to minimize damage to the tracheal supply of the ganglion. The cuticular flap was then replaced, and the wound was sealed with wax. In 13 preparations, nerve 5 alone (*10*) was cut without exposing the ganglion. The nerve was reached through the transparent cuticle between the body wall and the coxa. The results were similar to those obtained when nerve 5 was cut after first exposing the ganglion. This indicates further that the evoked responses are not due to general interference with the tracheal system of the ganglion. Aseptic technique was observed.

At intervals from 6 hours to 40 days, the experimental ganglia were removed, fixed in Zenker's fluid for 2 hours, dehydrated, and embedded in paraffin. Serial sections were cut at 10 μ, and the tissue was stained for RNA with a new pyronine-malachite green stain (*11*). Six normal ganglia were also sectioned and stained.

The cytoplasm of nerve cells in either the normal ganglion or the control side of experimental preparation stains a uniform pink color. This confirms the rather homogeneous distribution of basiphilic material in the cytoplasm of these cells and the absence of discrete Nissl bodies (*9, 12, 13*). A slightly darker, thin, basiphilic ring around the nucleus was also confirmed in normal cells (*12*). Within 12 hours after cutting the axons, ganglion cells on the injured side show an increase in basiphilic material in the cytoplasm immediately surrounding the nuclear membrane. This perinuclear ring increases in density and width to reach a maximum between 2 and 3 days after the axon was sectioned and then declines gradually to normal at 15 days.

A detailed map of individual nerve-cell bodies above 20 μ in major diameter