My purpose has been to apply a more precise and objective cytotoxic technique, developed by Wigzell (*3*) for the H-2 system of mice, to the detection and measurement of antigen specific for Moloney tumor (tumor induced by Moloney virus) in cells and subcellular fractions. In this technique the target cells are labeled with $Na_2{}^{51}CrO_4$; after treatment of the cells with antibody and complement, cell death is assessed by measuring the isotope which is released into the supernatant.

Under appropriate conditions the technique is applicable to the Moloney tumor–specific system (Fig. 1). Moloney tumors are not uniformly sensitive to the cytotoxic action of antibody; the tumor routinely used in this study is an ascites form of YAC, a lymphoma originally induced in an A-strain mouse by neonatal injection of Moloney virus. The cells, suspended at a concentration of 5×10^7 per milliliter in saline containing 5 percent by volume of normal calf serum, are labeled for 30 minutes with $Na_2{}^{51}CrO_4$ (20 μc/ml) at 37°C, washed, and suspended in 5 percent calf serum at a concentration of 3×10^7 per milliliter. Labeled cells (20 to 40 μl) are incubated with equal volumes of diluted antiserum for 15 minutes at 37°C, and unfixed antibody is removed by centrifugation and replaced with an equal volume of 50 percent guinea pig serum as a source of complement. After further incubation for 45 minutes, a portion of the centrifuged supernatant is removed for estimation of liberated ^{51}Cr by means of a scintillation counter.

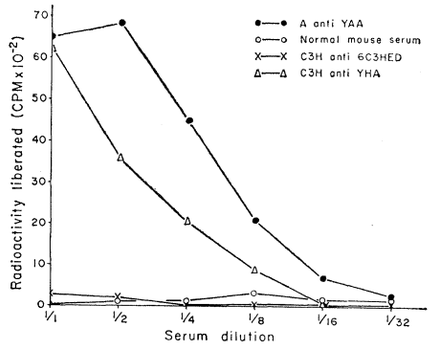

Fig. 1. Liberation of ^{51}Cr from labeled ascites tumor cells by antiserum specific for Moloney tumor in the presence of guinea pig complement. The target cells used were YAC, an ascites lymphoma induced by Moloney virus in A-strain mice. YAA and YHA are Moloney lymphomas of A and C3H strains, respectively; 6C3HED is an ascites leukemia of C3H mice unrelated to Moloney virus. CPM, count/min.

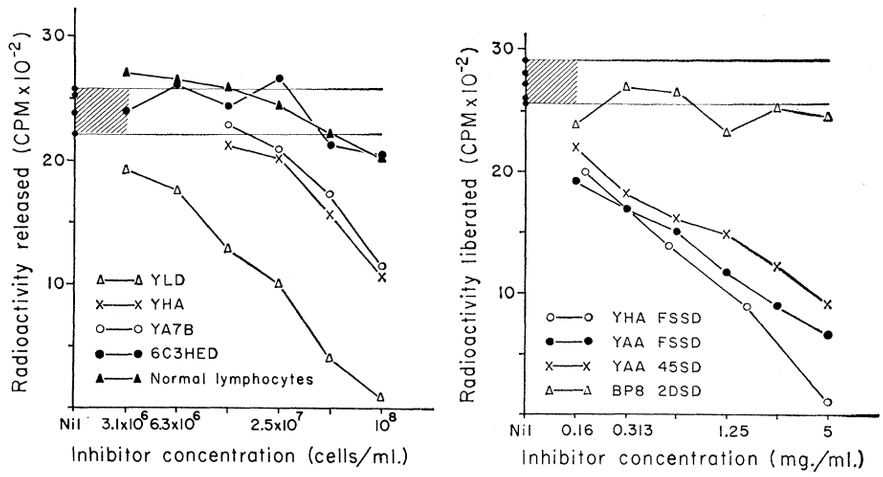

Fig. 2. Specific inhibition of cytotoxicity, as measured by ^{51}Cr release. Portions of antiserum to the Moloney tumor antigen were incubated with antigen and tested for residual cytotoxic antibody against YAC target cells labeled with ^{51}Cr. (Left) Whole cells used for inhibition; YHA, YLD, and YA7B are Moloney lymphomas of C3H, C57L, and AXC57B1 mice, respectively. 6C3HED is a leukemia of C3H not related to Moloney virus. (Right) Subcellular fractions used for inhibition. YHA FSSD was a freshly prepared material; the others were reconstituted after lyophilization (*4*). BP8 2DSD, known to be a highly active preparation of H-2 antigen, was prepared from an ascites sarcoma of C3H mice (*4*). CPM, count/min.

For measurement of tumor-specific antigen, the technique has been used as an inhibition test. Portions of antiserum, at a dilution which liberates about 25 percent of the total radioactivity from the target cells, are incubated for 15 minutes at 37°C with decreasing dilutions of antigen. Labeled target cells are then added, and the test is continued as already described. Antigen can be measured by this method either in whole cells or in separated subcellular fractions (Fig. 2).

The fractions used were prepared by the method described earlier for the separation of cell fractions rich in H-2 antigen (*4*); fractions rich in H-2 antigen are also usually rich in tumor-specific antigen.

This technique for measuring antibody and antigen specific for Moloney tumors offers (i) complete objectivity; (ii) a high degree of precision; (iii) lack of interference with the cytotoxic effect of residual antibody on labeled target cells by intact cells used as an inhibitor (such intact cells need not be removed before the test is continued); (iv) convenient assay of subcellular fractions which may be difficult to sediment; (v) greater accuracy and economy by avoidance of the absorption test, in which a relatively large amount of antibody is absorbed with antigen and residual activity is titrated after removal of the antigen-antibody complex.

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5. I thank Prof. Georg Klein for facilities and materials, Barbro Jansson for technical assistance, and the International Union against Cancer for awarding an Eleanor Roosevelt Fellowship. Work supported by grants from the Damon Runyon Memorial Fund (DRG-598), the Jane Coffin Childs Memorial Fund for Medical Research, the Swedish Cancer Society, and Lotten Bohmans Fund.

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Charge-Transfer Self-Complex Formed by 8-Azaguanine

Abstract. *The perpendicular distance between the planes of successive molecules of 8-azaguanine, in crystals of 8-azaguanine monohydrate, is 3.25 Å. This distance indicates intermolecular interaction of the charge-transfer type. 8-Azaguanine may act as a cell poison by forming a charge-transfer complex within the bacterial RNA.*

Brockman *et al.* (*1*) have shown that 8-azaguanine is a cell poison. Tracer studies with 8-azaguanine-2-^{14}C in vivo with *Streptococcus faecalis* have shown

Table 1. Interplanar separations between the molecules taking part in charge-transfer complexes.

Complex	Distance between molecular planes (Å)
Heterocomplexes	
Anthracene:sym-trinitro benzene	3.28(3)
Phenol:benzoquinone	3.33(3)
Quinol:benzoquinone	3.16(3)
Tetramethyl-*p*-phenylenediamine:chloranil	3.26(3)
Tetramethyl-*p*-phenylenediamine:bromanil	3.31(3)
Perylene:fluoranil	3.23(4)
Self-complex	
Potassium squarate monohydrate	3.24(5)

that 8-azaguanine is incorporated into the bacterial RNA. The cell-poisoning action appears to be associated with such incorporation, since strains of *S. faecalis* resistant to this compound cannot convert it to 8-azaguanylic acid. As a resut of this defect, the 8-azaguanine is not incorporated into the RNA of the resistant cells (*2*). These results indicate that 8-azaguanine exerts its cell-poisoning action only after incorporation into the bacterial RNA.

It is conceivable that the effect of the 8-azaguanine molecule on the RNA molecule arises from some secondary structural feature of the 8-azaguanine. In order to investigate whether 8-azaguanine had any unusual structural features an x-ray crystal structure analysis of 8-azaguanine monohydrate has been carried out. The results of this analysis will be reported in detail elsewhere. However, one observation was made which should be reported immediately.

The 8-azaguanine molecules lie almost exactly in the (102) plane of the crystal. A projection of one molecule in that plane onto a molecule in the plane below is given in Fig. 1. These molecules lie almost directly over one another. The distance between the molecular planes is 3.25 Å ($\sigma = 0.01$). The distances between molecular planes for a number of charge-transfer complexes are shown in Table 1.

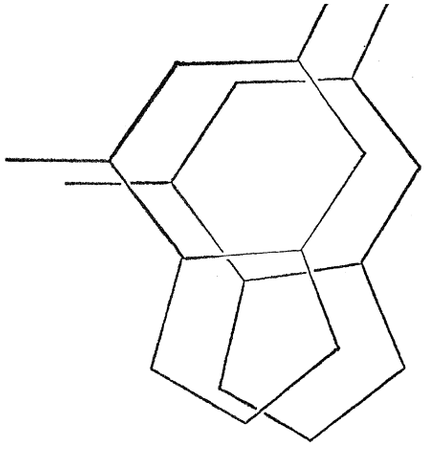

Fig. 1. Projection of a molecule of 8-azaguanine onto the plane of the molecule stacked below it in the crystal of 8-azaguanine monohydrate. The plane of projection is approximately the (102) crystallographic plane.

These complexes are characterized by a rather short intermolecular separation, normal to the molecular plane. The separation between the planes of aromatic molecules, in crystals held together by van der Waals forces only, is about 3.4 Å. As a rule, the formation of a charge-transfer complex is characterized by a reduction in this separation of 0.1 to 0.2 Å.

The distance between molecular planes of 3.25 Å, as in 8-azaguanine, falls into the range of distances in these charge-transfer complexes. This is strong evidence for the formation of a charge-transfer self-complex in the crystal of 8-azaguanine monohydrate.

A second feature of charge-transfer complexes is a slight displacement of the molecules from exact superposition. This is necessary to permit the p_z orbital overlap which provides the binding energy of the complex. The displacement of the two molecules (Fig. 1) is of the type required to permit complex formation (*3*).

A third feature of most charge-transfer complexes, a very strong absorption band in the visible region of the spectrum, is absent in 8-azaguanine monohydrate. The crystals are colorless. However, Mulliken has predicted that in self-complexes, where donor and acceptor molecules are the same, the charge-transfer absorption band should occur at frequencies higher than found in heterocomplexes (*6*). The crystals of potassium squarate monohydrate, which forms a charge-transfer self-complex, are colorless (*5*).

The charge-transfer absorption band for 8-azaguanine may be in the ultraviolet region. Unfortunately this hypothesis has not been checked owing to severe experimental difficulties in obtaining the necessary spectra. However, in potassium squarate monohydrate an appropriate absorption band has been found at 2500 Å (*5*).

The results of the structure analysis then suggest that 8-azaguanine forms a charge-transfer self-complex in the crystals of the monohydrate. At any rate, the close approach of 3.25 Å between neighboring 8-azaguanine planes is evidence of some unusual secondary interaction. This interaction provides a simple mechanism for the lethal effect on bacterial cells of the incorporation of 8-azaguanine into their RNA.

Protein synthesis appears to involve movement of the ribosome along the linear RNA chain which carries the amino acid code (*7*). If a molecule of 8-azaguanine is incorporated into the RNA molecule and forms a complex with another 8-azaguanine base in the same RNA molecule, or perhaps with a natural base, at some distance along the RNA chain, then the molecule will bend over on top of itself to form a loop. The points on the RNA molecule which are connected by the complex between bases can then form an obstruction which will prevent the ribosome from proceeding along the chain. Such an interference with the movement of the ribosome will inhibit protein synthesis, and so arrest the development of the cell.

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Cyclic Variations in the Digestive Gland and Glandular Oviduct of Chitons (Mollusca)

Abstract. *In* Cryptochiton stelleri *(Polyplacophora), variation in the size of the glandular oviduct parallels the cyclic variation in the size of the gonad. In* Katharina tunicata *the digestive gland reaches its maximum size in the fall then decreases to a minimum size in the spring when the gonad is at its maximum size. These variations are not due to changes in the water content of the glandular oviduct and digestive gland.*

Reproduction in the chitons *Katharina tunicata* and *Cryptochiton stelleri* is seasonal, an annual reproductive cycle being characteristic in both spe-