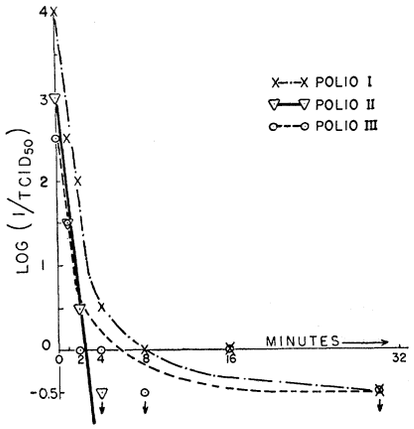

Fig. 1. Infectivity titers of polioviruses at intervals after exposure to chlorine at *p*H 7.0 at 25 to 28°C.

tenfold dilutions of sample and observing them for degeneration over a 6-day period. Residual chlorine was determined as the free available and combined available residuals, by means of the orthotolidine-arsenite method (*1*).

Infectivity titers after exposure to chlorine for various intervals are shown in Fig. 1. The change in titer of poliovirus type 2 was constant, resulting in an inactivation curve that is linear; the change in titer of polioviruses 1 and 3 was constant only during the first few minutes of exposure, resulting in a curve that is linear for the early part of the inactivation only.

The linear response of poliovirus 2 to chlorine in amounts that did not inactivate poliovirus 1 and 3 with similar regularity suggests that the latter are more resistant to chlorine than is poliovirus 2. This is indicated also by the time required for complete inactivation (infectivity titer < 0.0) of the three viruses. From 15 to 30 minutes were required for complete inactivation of polioviruses 1 and 3; 4 minutes were required for poliovirus 2—a time required, under similar conditions (*2*), to inactivate Coxsackie A_2. Whether these differences are characteristic of the virus types tested or

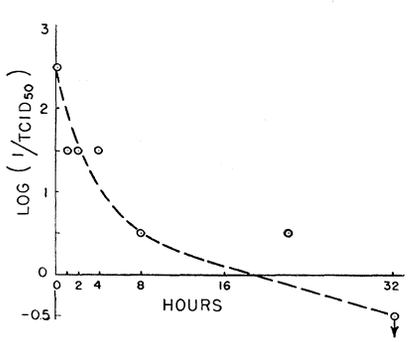

Fig. 2. Inactivation of poliovirus type 1 (Mahoney) by formaldehyde (1/4000 dilution) at *p*H 7.0 at 35°C in chlorine-demand-free water.

are dependent on the particular conditions chosen may be made clear by further studies.

The experiments described illustrate the rapidity (in minutes) with which chlorine inactivates viruses in a medium free of other oxidizable substances in comparison with time required (in hours) for inactivation under similar conditions by such common inactivating agents as formaldehyde (Fig. 2). They point out, conversely, and as recorded previously (*2*), that the chlorine residuals found to be sufficient (*3*) for bacterial disinfection are not similarly effective as viral disinfectants.

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1 July 1957

Binding of Histamine and Antihistamine to Bovine Serum Albumin by Mediation with Cu(II)

In the course of our work directed toward the evaluation of physical chemical data regarding protein-histamine-antihistamine interactions (*1*) we have been successful in forming a stable complex, *in vitro,* between copper(II)-bovine plasma albumin and histamine. The Cu(II)-bovine plasma albumin was also found to form a stable complex with the commercial antihistamine, Antistine (*2*).

The physiological role of histamine in allergy has been postulated (*3*) to involve the binding of histamine to a protein; however, recent *in vitro* experiments (*4*) have failed to show any measurable interaction between histamine and a series of purified animal proteins. The complexing of cupric ions to bovine plasma albumin has thus been interpreted as providing a site on the protein capable of binding histamine.

The technique of equilibrium dialysis was used to determine the extent of interaction between the Cu(II)-bovine plasma albumin and the binding molecule, histamine or antihistamine. The protein was crystalline bovine plasma albumin (*5*). The protein was dissolved in a phosphate buffer, placed in Visking 18/32 cellulose casing, and purified by out-dialysis at 0.0°C for 48 hours with the phosphate buffer. This buffer was prepared with reagent grade monobasic potassium phosphate and dibasic potassium phosphate. An ionic strength of 0.2 was maintained throughout the study. Buffer *p*H values of 6.95 and 8.90 could be obtained by using the proper proportions of the buffer constituents. Final protein concentrations were determined by drying aliquot portions of the purified protein solution at 105°C and correcting for the amount of buffer salt.

The copper-proteinate was prepared by adding the purified protein solution to a standard solution of cupric chloride and diluting it with buffer to a known volume. The concentration of copper in the standard solution was determined by electrolytic deposition.

The binding studies were conducted at 0.0°C. Ten milliliters of the copper-proteinate of known concentration was placed in the semipermeable membrane, which was then immersed in 20 ml of ligand solution. These cells were allowed to equilibrate for 24 hours. Simultaneously, blank cells were run in which the membrane contained only buffer, but they were immersed in the same ligand solutions. After equilibration, the difference in concentration between the blank cell and that containing the proteinate was directly proportional to the amount of ligand bound by the proteinate. Histamine concentrations were determined spectrophotometrically by means of a method developed in this laboratory (*6*). Antistine concentrations were determined spectrophotometrically at a wavelength of 240 mμ.

The calculation of the maximum number of moles of a given substance which

Table 1. Interaction of histamine and Antistine with Cu(II)-bovine plasma albumin at 0.0°C. (*A*) Represents the molar concentration of unbound ligand in equilibrium with the proteinate, and *r* represents the moles of ligand bound per mole of proteinate.

$(A) \times 10^5$	*r*	$(A) \times 10^5$	*r*
Histamine, pH 6.95			
4.94	0.273	18.0	0.671
7.06	0.347	19.0	0.980
9.22	0.454	22.4	1.351
13.8	0.621	42.5	6.250
Histamine, pH 8.90			
4.19	0.294	9.31	0.719
6.00	0.460	10.00	0.775
7.35	0.588	14.00	0.877
8.46	0.617	15.30	0.934
Antistine, pH 6.95			
3.03	0.155	10.25	0.357
5.67	0.191	12.14	0.529
7.46	0.253	19.96	0.689
8.04	0.315	22.57	0.917

can be bound to a protein molecule has been completely developed by Klotz (7). This method is based on the law of mass action and assumes that binding occurs in a stepwise fashion, with the first mole bound being held the most firmly. The expression relating moles of small molecules bound per mole of copper-protein complex, r, with concentration of unbound ion (A) is given by

$$r = \frac{m(A)}{K + (A)}$$

Here, K is the intrinsic dissociation constant for the system, and m is the maximum number of bound ions per molecule. In order to evaluate m and K, the equation is rearranged to

$$\frac{1}{r} = \frac{K}{m}\frac{1}{(A)} + \frac{1}{m}$$

A graph of $1/r$ versus $1/(A)$ will be a straight line with the intercept on the $1/r$ axis equal to $1/m$ and the slope of the line equal to K/m.

The results of the binding studies involving histamine at *p*H values of 6.95 and 8.90 and Antistine at a *p*H of 6.95 are shown in Table 1. It was found that these binding data obeyed the law of mass action; that is, the binding increased with an increase in concentration of unbound ligand in equilibrium with the Cu(II)-proteinate.

Extrapolation of the linear plot of the reciprocals of the amount bound versus the concentration of unbound ligand yielded values for the maximum moles of ligand bound per mole of proteinate. These values were 2.75 and 20.0 for histamine at *p*H values of 6.95 and 8.90, respectively. The Cu(II)-proteinate was capable of binding a maximum of 1.74 moles of Antistine per mole of proteinate at a *p*H of 6.95.

The equilibrium constants for the first mole of ligand bound, obtained from the slope of the linear plot, were utilized to determine the free energy change for the formation of the ligand-proteinate complex. These values were -0.871 kcal and -0.910 kcal per mole for histamine at *p*H values of 6.95 and 8.90, respectively, while the corresponding value of Antistine was -0.868 kcal per mole at *p*H 6.95.

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References and Notes

1. Supported in part by grant No. E-1354 from the National Institutes of Health, U.S. Public Health Service.
2. Antistine is the commercial name for 2(N-phenyl-N-benzyl-aminomethyl)-2-imidazoline hydrochloride, which was kindly donated by the Ciba Pharmaceutical Company, Summit, N.J.
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11 July 1957

Hereditary Ovarian Tumors in Drosophila melanogaster

A recent study has been published which describes normal oogenesis in the fruit fly, *Drosophila melanogaster* (*1*). During this study ovarian tumors were observed. However, the incidence was extremely low (two tumorous chambers among 39,900 developing eggs). It was shown subsequently that ionizing radiation (4000 r of Co^{60} gamma rays) increased the incidence of tumors by 26 times.

In *Drosophila* a developing egg consists of a chamber containing 16 cells. Fifteen of the cells function as nurse cells and nourish the 16th cell (the primary oocyte). All 16 cells arise from a single cell in the germarium, which undergoes four consecutive divisions. It was postulated therefore that, in the region of the germarium where 16 cell cysts are formed, an interaction takes place between cytoplasmic substances localized in this region and the genome of the cells. The stimulated genetic material is thought next to manufacture a substance which inhibits further cytokinesis in a precise fashion. Radiation might occasionally inactivate that portion of the genome of an oogonium responsible for the production of the inhibitor. This mutation would then be passed to the progeny of the oogonium. These cells would now divide in an uncontrolled fashion and produce the observed tumorous chambers which contain hundreds to thousands of mitotically active cells.

We set out to detect mutant genes which would cause such uncontrolled cell division; but we recognized that such genes would be difficult to obtain, since they would generally produce consequences which would be lethal at an early stage in the life-cycle. From our knowledge of oogenesis, it seemed reasonable to predict that some of the genes we were looking for might be found among the 60 or so nonallelic, recessive female sterile mutants of *Drosophila melanogaster,* because uncontrolled division in egg chambers would convert developing eggs to tumors and so sterilize the fly.

We therefore obtained approximately 20 female sterile mutants and proceeded to make Feulgen whole mounts of ovaries of females homozygous for the various female sterile genes (*2*). To our amazement, the first female sterile mutant examined turned out to be a case in point, and the first ovarian preparation contained more tumors than the total we had observed from all sources up to that time. The incidence of tumors was found to increase with the age of the female. In this strain, adjacent chambers in an ovariole often fuse together. If one such chamber is tumorous and the other is normal, there will be produced a compound chamber containing normal and tumorous cells. The actively dividing tumorous cells will subsequently invade the normal tissue of the compound chamber.

This mutant which produces tumors of one tissue at one particular stage in the life-cycle is *fused* (*fu*), discovered by C. B. Bridges in 1912. It is located at 59.5 on the X-chromosome. The allele in question is spontaneous in origin, but alleles induced by x-rays or chemicals have been frequently observed. In our stock (which was obtained from the Yale collection) *fu* is balanced over M5. Females heterozygous for *fu* show no tumors. On the other hand, females homozygous for fu^{ff} (an allele of *fu* induced by formalin treatment) also show ovarian tumors. It appears that *fu,* in addition to its many other bizarre effects (*3*), produces ovarian tumors and therefore represents excellent material for further studies of the mechanism of tumorous growth.

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26 June 1957

Fluorescence of Ethylenediamine Derivatives of Epinephrine and Norepinephrine

In 1952, Weil-Malherbe and Bone introduced a method for the chemical determination of total "epinephrinelike" substances in blood, which included separation of the catechol amines from other plasma components by adsorption chromatography and measurement of the separated fraction by fluorometry (*1*). Shortly thereafter, Persky and Ros-