must be inactivated before this activity is lost. Thus only one intact hemolysin unit per virus particle is necessary for the virus to be able to lyse rbc.

In Fig. 2, reduction of rate of hemolysis by NDV is seen to be proportional to dose of radiation and is single hit. Rate of hemolysis depends on number of intact hemolysin units per virus particle and although a virus particle with only one intact hemolysin unit can lyse rbc, it does so at a much slower rate than a virus with 15 intact hemolysin units.

The results of neutralization of the NDV hemagglutinin and hemolysin by antiserum (Fig. 1) indicate that either process is inactivated by adsorption of one antibody molecule per virus particle. Since the NDV hemagglutinin is presumed to reside in two sites on opposite sides of the virus, it is not surprising that one antibody molecule adsorbed to one virus particle renders it unable to agglutinate rbc. It is, however, surprising that the hemolysin unit is inhibited by only one antibody molecule per virus particle. It is possible that the 15 hemolysin units are clustered together in such a way that the attachment of one antibody molecule to any one hemolysin unit makes it impossible for the other 14 to function. This seems unlikely since it has been estimated that about 10-20% of the total surface area of the virus particle is covered by the hemolsyin units(4). It is also possible that the virus possesses a single attachment site for the rbc which is not associated with the hemolysin units. Thus adsorption of one antibody molecule to this attachment might then inhibit hemolysis by preventing attachment of the virus to the red cell surface. Rubin and Franklin(6) observed that NDV possesses between 2 and 3 such attachment sites for the host cell although only one antibody molecule adsorbed to the virus particle will inhibit the process of infection and plaque formation. It would seem that NDV has several different attachment sites which serve different purposes and act on different cell receptors.

Summary. The NDV hemolysin is shown to be inactivated by a single antibody molecule per virus. Electron irradiation of NDV indicates that rate of hemolysis of rbc depends on number of intact hemolysin units per virus, whereas ability to lyse rbc, given sufficient time, depends on the presence of only one intact hemolysin unit per virus.

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1. Granoff, A., Henle, W., *J. Immunol.,* 1954, v72, 322.

2. Burnet, F. M., Lind, P. E., *Aust. J. Exp. Biol.,* 1950, v28, 129.

3. Sagik, B. P., Levine, S., *Virology,* 1957, v3, 401.

4. Wilson, D., *Radiation Res.,* 1958, v8, 142.

5. Dulbecco, R., Vogt, M., Strickland, A. G. R., *Virology,* 1956, v2, 162.

6. Rubin, H., Franklin, R. M., *ibid.,* 1957, v3, 84.

7. Pollard, E. C., Guild, W. R., Hutchinson, F., Setlow, R. B., *Progress in Biophysics,* 1955, v5, 72.

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Enhancement of Tetracycline Serum Levels with Glucosamine Hydrochloride as Adjuvant. Cross-over Studies in Beagle Dogs. (24297)

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In the effort to achieve greater serum concentrations of antibiotics after oral administration, most research has centered on chemical modification of the antibiotic or the use of adjuvants. As an example of the latter, Carlozzi has shown that serum levels of tetracycline* in man were enhanced after oral administration by the use of the amino sugar, D-glucosamine as an adjuvant(1). Data on serum concentrations after oral administration

* Registered trade mark of Chas. Pfizer and Co., for tetracycline is Tetracyn.

TABLE I. Tetracycline Hydrochloride plus Glucosamine Hydrochloride and Tetracycline Hydrochloride. Comparison of serum concentrations after oral administration in a cross-over experiment in 40 beagle dogs.

Sample time	Biological activity* expressed in μg/ml after oral admin.† of Tetracycline hydrochloride	Tetracycline hydrochloride + glucosamine hydrochloride	% increase	Level of significance, %
1	1.123	1.972	76	1
3	1.114	1.512	36	5
5	.878	1.164	33	5
7	.566	.759	34	5

* Avg of 40 dogs. † 10 mg/kg.

of tetracycline hydrochloride plus glucosamine hydrochloride† as compared to tetracycline hydrochloride itself in a cross-over experiment in 40 beagle dogs will be presented here.

Methods. The beagles used in this study were pure-bred and were maintained under identical housing conditions, diet, and environment. Tetracycline hydrochloride plus D-glucosamine hydrochloride in a 1:1 ratio (10 mg plus 10 mg) was used as the test drug. Tetracycline hydrochloride of the same lot was used as the control drug. Dosage was 10 mg/kg administered by stomach tube. Blood samples were removed by jugular vein puncture at 0, 1, 3, 5, and 7 hours after drug administration. Immediately after drawing, the blood was permitted to clot at refrigerator temperature. The serum was then removed and assayed by standard microbiological procedures(2). Such biological activity in the serum has been expressed as μg/ml of tetracycline hydrochloride. The study was done with groups of 20 animals per experiment, that is, 10 on test and 10 on control drug. After a weeks' rest, the cross-over was initiated in which the previous test animals now served as controls and *vice versa*. A zero time sample was always assayed to exclude any possibility of carry-over from the previous week's experiment. The data were subjected to standard analysis of variance for a cross-over design experiment(3) and per cent increase in serum levels noted at one and 5% levels of significance.

† Trade mark of Chas. Pfizer and Co., for dosage form of tetracycline hydrochloride and glucosamine hydrochloride is Cosa-Tetracyn.

Results. The data have been summarized in Table I. Higher biological activity in the sera was observed at every time interval in the dogs receiving tetracycline hydrochloride plus glucosamine hydrochloride than in those animals receiving only tetracycline hydrochloride. Percentage increases in serum activity ranged from 76% at one hour, to approximately 34% at 3, 5, and 7 hours. An analysis of variance for such a cross-over experiment showed the levels of significance for these increases were one per cent at one hour, and 5% at 3, 5, and 7 hours. Although the exact mechanism for enhancement of serum levels after oral administration of tetracycline hydrochloride by D-glucosamine hydrochloride remains unclear, it is obvious that the greatest effect occurs in the early hours after administration.

Summary. Serum levels of tetracycline activity were enhanced by the presence of D-glucosamine hydrochloride from 76% at one hour to 34% at 7 hours over those obtained after oral administration of tetracycline hydrochloride alone in a cross-over experiment with 40 pure-bred beagle dogs.

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1. Carlozzi, M., *Antibiot. Med. and Clin. Ther.*, 1958, v5, 2.

2. Grove, D. C., Randall, W. A., *Assay Methods of Antibiotics, A Laboratory Manual.* New York, Medi-

cal Encyclopedia, Inc., 1955, 63.

3. Cochran, W. G., Cox, G. M., *Experimental Design*, N. Y., John Wiley and Sons, 1950.

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Distribution and Valence State of Radiochromium in Intracellularly Labeled Ehrlich Mouse Ascites Carcinoma Cells.* (24298)

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Gray and Sterling(1) reported a method for intracellular labeling of human erythrocytes with radioactive sodium chromate ($Na_2Cr^{51}O_4$). This method has been further studied and applied by several workers(2,3), resulting in adoption of the modified procedure as a standard method for study of erythrocyte life span and related investigations(4). Any procedure resulting in an intracellular label, stable for the life of the cell, has potentialities for the study of cellular biology. Thus, in addition to those used for erythrocytes, similar methods have been applied successfully to leucocytes(5) and platelets(6). Recently, the use of radiochromate for labeling cells of Ehrlich mouse ascites carcinoma has been reported(7). It was shown that the label was retained intracellularly until irreversible rupture of the cell occurred, and that incorporation of chromate ions in the cells did not impair their ability to produce subcutaneous tumors in mice, provided a certain maximum concentration of chromate ion was not exceeded. In their original paper, Gray and Sterling(1) studied the nature of intracellular label in erythrocytes, and demonstrated that the erythrocyte membrane allowed passage of the CrO_4'' ion, but was selectively impermeable to trivalent chromic (Cr^{+++}) ion. Radioactivity within the red cell was shown to be associated with the globin moiety of the hemoglobin molecule. It was also suggested, on the basis of indirect evidence, that the CrO_4'' ion was intracellularly reduced to the trivalent Cr^{+++} ion, and that the stability of the label was dependent on the firm binding of the Cr^{+++} ion to intracellular protein. Tumor cells are considerably more complex entities than the highly specialized erythrocyte. A study of the valence state and intracellular distribution of radiochromium in these cells was considered of interest, and is the subject of this report.

Methods. Propagation, harvesting and standardization of tumor cells. Ehrlich ascites tumor cells were propagated, harvested, freed of erythrocytes and standard suspensions prepared, by methods described elsewhere(8). *Labeling of tumor cells with radiochromate.* A suspension of 5 x 10^8 tumor cells in 10 ml 0.85% NaCl was labeled as previously described(7), using 0.9 μc of $Na_2Cr^{51}O_4$ (specific activity 39.38 mc/mg, Na_2CrO_4 concentration 0.02 mg/ml; obtained from Abbott Laboratories under the trade name Rachromate). Unincorporated radiochromate was removed by washing thrice with 0.85% NaCl (35 G, 0°C, 5 min). *Fractionation of tumor cells.* The labeled tumor cell suspension was fractionated by a method based on that of Hogeboom and Schneider(9). Cells were suspended in 0.25 M sucrose solution and disrupted by grinding in a Potter-Elvehjem homogenizer (60 min, 0°C). An aliquot of the total homogenate was removed for radioassay. The remainder was layered on 0.34 M sucrose solution and centrifuged (450 G, 0°C, 15 min). The supernate (SI) was reserved, the sediment suspended in 0.25 M sucrose solution, layered on 0.34 M sucrose solution and centrifuged (800 G, 0°C, 15 min). The sediment, consisting largely of nuclei, was dispersed in sodium desoxycholate solution, and aliquots prepared for radiassay; the supernate (SII) was combined with the original supernate (SI). The combined supernates,

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