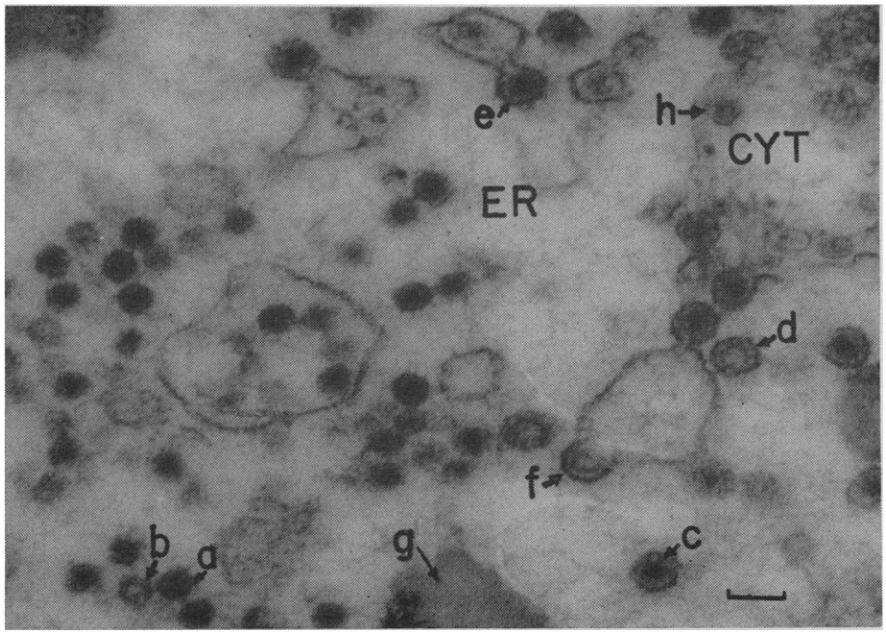

Fig. 1. Electron micrograph of cytoplasm of an epithelial cell from the jejunum of an infant mouse infected with EDIM virus, illustrating the different morphological forms of the virus described in the text. Most of the particles lie within a dilated vesicle of endoplasmic reticulum (*ER*), but some appear in the tongue of cytoplasm visible at upper right (*CYT*). The line at lower right represents 100 mμ.

thelial cells from the small intestine of infected mice. The particles varied in diameter from 65 to 75 mμ, and two distinct classes can be identified. In the smaller class (*a*, *b*) the particles measured about 65 mμ in diameter and were composed of a thick coat of medium density with an outer indistinct margin which did not have the sharply delimited characteristics of a typical membrane. This class of particle had an electron-dense center (*a*) or, less commonly, an electron-lucid center (*b*). The larger class (*c*, *d*) was similar to the first, with the exception that there was the addition of an outer sharply delimited membrane increasing the diameter to approximately 75 mμ. This class, like the first, had either an electron-dense center (*c*) or an electron-lucid center (*d*). Always associated with the virus particles within the dilated vesicles of endoplasmic reticulum were varying quantities of homogeneous, finely granular material of medium electron density, arranged in irregular masses (*g*).

The particles appeared to be most numerous within the vesicles of the endoplasmic reticulum and, in some cases, they appeared to be closely associated with the membrane of the endoplasmic reticulum in a manner suggesting possible formation at the membrane (*e*, *f*). Rarely, particles (*b*) were present outside the endoplasmic reticulum within the matrix of the cytoplasm at some stage in their development. In no case have the particles been found within the nucleus of the epithelial cells. No particles were seen in normal infant mouse intestine.

Transmission, infectivity, and purification studies (*2*) have yielded evidence that the agent of epidemic diarrhea of infant mice is a virus. The electron micrograph shown here reveals the presence of spherical particles that have the characteristic morphology of virus particles with dimensions compatible with filtration studies of EDIM virus. They have been tentatively identified as the EDIM virus particle (*5*).

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10 May 1963

Complement-Fixing Properties of Pepsin-Treated Rabbit and Sheep Antibodies

Abstract. *Rabbit antibody with a sedimentation coefficient of 7S was digested with pepsin to yield the 5S fragment. Washed specific precipitates of 5S antibody and egg albumin, when added to guinea pig serum, fixed up to 40 percent of the total complement. This fixation could not be attributed to contamination with 7S antibody. Absorption of guinea pig serum with washed precipitates of 5S antibody and antigen removed that portion of complement which was fixed by fresh 5S antibody-antigen aggregates, but not that fixed by 7S antibody-antigen aggregates. The presence of 0.001M ethylenediaminetetraacetic acid prevented this absorption of complement by the 5S aggregates.*

Pepsin digestion of rabbit antibodies with a sedimentation coefficient of 7*S* yields a smaller 5*S* fragment, which precipitates antigen, but supposedly lacks all of Porter fragment III (*1*). Fragment III can fix complement (C′) when it is aggregated, but fragments I and II cannot (*2*). The conclusion has been drawn that it is fragment III in antibody that fixes complement and that the 5*S* fragment, though precipitable, does not fix complement (*3*).

Reinvestigation of this problem for other purposes led to the finding that 5*S* rabbit as well as 5*S* sheep antibody does retain certain complement-fixing activity. Because these results are important not only to the theories of antibody structure, but also in the interpretation of experiments testing the biologic activity of 5*S* antibody, they are briefly reported here.

Antisera were obtained from sheep and rabbits immunized with human serum albumin and egg albumin in complete Freund's adjuvant. The 7*S* gamma globulin was isolated by sodium sulfate precipitation (*4*) and then dialyzed against phosphate saline buffer (*p*H 6.9, 0.17*M*). It was digested with 2 to 3 percent pepsin in acetate chloride buffer (*p*H 4.1, 0.12*M*) for 18 to 24 hours at 37°C. The smaller 5*S* fragment was isolated by sodium sulfate precipitation, and then dialyzed against phosphate saline buffer. In the ultracentrifuge it gave a single symmetrical peak with a sedimentation coefficient of 5*S*. There was no evidence of any component with a higher sedimentation coefficient. Antibody and antigen nitrogen

were determined by the micro-Kjeldahl method (*5*). Serial dilutions of antibody and antigen, in a fixed ratio at equivalence (*6*), were added to 100 fifty-percent ($C'H_{50}$) units of fresh-frozen (−20°C) guinea pig serum. Complement fixation was studied with antibody and antigen aggregating in the presence of complement, and also with washed precipitates of antibody and antigen. By itself, 5*S* antibody from rabbits had no detectable anticomplementary activity when the antibody nitrogen was $\leq$ 80 μg; rabbit 7*S* antibody, however, had no anticomplementary activity when the antibody nitrogen was $\leq$ 5 μg. The anticomplementary activity of sheep antibody was somewhat greater.

In certain experiments, samples of washed antibody-antigen precipitates were dialyzed first against 1000 volumes of 0.01*M* cysteine for 2 hours at 35°C, and then against 1000 volumes of 0.02*M* iodoacetamide for 24 hours at 4°C, then further dialyzed against phosphate saline buffer.

Figure 1 shows the results of testing the complement-fixing activity of 7*S* and 5*S* rabbit antibody to egg albumin both in solution and with washed antigen-antibody precipitates. Figure 1 is representative of results from six different preparations from three pools of rabbit antisera. It is evident that the 5*S* antibody solution on addition of antigen does fix complement to a small degree. The complement-fixing activity of the 5*S* washed precipitates is, however, 300 times greater. Others (*3*, *7*) did not use washed precipitates in testing for complement fixation, and this may explain in part why they did not detect complement fixation with the 5*S* antibody. Amirian (*8*) obtained about the same degree of complement fixation with 5*S* rabbit antibody to sheep erythrocytes, as reported here, but reported none with 5*S* rabbit antibody to pertussis.

All of the complement can be fixed with a sufficient amount of 7*S* antibody washed precipitate. However, as seen in Fig. 1, this differs from the behavior of precipitates containing 5*S* antibody where the amount of complement fixed is well below the total complement present. This maximum varied from 20 to 40 percent of the total complement added when different preparations of 5*S* antibody and different guinea pig sera were used. Since, apparently not all of the complement added can be fixed by precipitates containing 5*S* antibody, it was of interest to calculate the proportion of complement which was

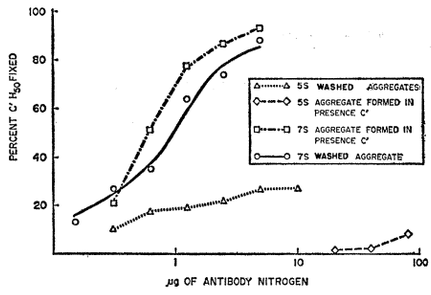

Fig. 1. Comparison of complement fixed by 7*S* and 5*S* rabbit antibody to egg albumin.

fixed by the 5*S* antibody, not in terms of the total complement added, but rather in terms of the maximum amount of complement which the 5*S* fragment could fix. This was done by calculating the ratio of the number of units of complement fixed by a given amount of 5*S* immune precipitate, to the maximum number fixable by the 5*S* immune precipitate (in the example given in Fig. 1, this would be 27 $C'H_{50}$ units). When this is done the curve of the percentage of the maximum amount of complement that can be fixed by the 5*S* antibody-antigen precipitate can be superimposed on that shown in Fig. 1 for the 7*S* antibody-antigen precipitate.

In further experiments guinea pig serum was absorbed for 18 hours at 4°C with a washed antigen-antibody precipitate containing 2 mg of 5*S* antibody nitrogen. The precipitate was removed by high-speed centrifugation. The guinea pig serum lost most, and in some experiments all, of that portion of complement which could be fixed by fresh 5*S* antibody-antigen aggregates; whereas 7*S* washed precipitates could fix complement in this serum to undiminished titer. When this absorption was performed in the presence of 0.001*M* EDTA, and then 0.001*M* calcium chloride was added to the absorbed guinea pig serum, fresh 5*S* washed precipitates fixed complement again, to the original degree.

Thus there are three independent pieces of evidence showing that the complement-fixing activity of the 5*S* preparations could not be due to contamination with 7*S* antibody: (i) the slopes of the lines (as plotted in Fig. 1) are different for the 7*S* and 5*S* precipitates; (ii) the maximum percentage of complement fixed by 5*S* washed precipitates is always considerably less than the total complement present; and (iii) absorption of guinea pig serum with 5*S* washed precipitate resulted in a loss of further complement fixation with fresh 5*S* washed precipitate, without any loss of complement fixation with 7*S* washed precipitates.

In addition, when 5*S* antibody-antigen precipitates were treated with cysteine they reduced their complement-fixing capacity by more than 90 percent, whereas 7*S* washed precipitates similarly treated fixed 20 percent less complement.

Thus it would appear that only a portion of total complement is fixed by 5*S* immune precipitates. This portion is fixed as efficiently by 5*S* immune precipitates as total complement is fixed by 7*S* immune precipitates. This portion can be absorbed out of guinea pig serum with 5*S* immune precipitate without affecting the fixation of the remaining complement by 7*S* antibody. The 5*S* antibody can no longer fix this portion of complement when 5*S* immune precipitates are digested with cysteine. We have no data at present in respect to which of the components of complement the 5*S* antibody fixes.

The relative complement-fixing capacities of both 7*S* and 5*S* sheep antibody are very similar to 7*S* and 5*S* rabbit antibody. However, washed 7*S* sheep antibody-antigen precipitates fix more complement than do antibody-antigen aggregates formed in the presence of complement (*6*). The same amount of complement was fixed by 5*S* sheep and rabbit antibody (compared by antibody nitrogen).

The information at hand is insufficient to interpret all of our results. We can list some of the areas of our ignorance, which these results have made us recognize. There is no good explanation for the fact that 5*S* washed precipitates fix complement to a far greater degree than do 5*S* aggregates formed in the presence of complement. We know neither the source nor the nature of the heterogeneity of the guinea pig complement in regard to its ability to be fixed by the 5*S* antibody. We do not know whether the complement-fixing behavior of the 5*S* antibodies here is completely general; it is possible that variation might even be encountered with antibody from the same species. It is unclear whether the action of pepsin and of papain are the same on the complement-fixing site of gamma globulin, and thus whether fragment III contains all or only a part of the complement-fixing site.

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27 May 1963

Herbicides: Combination Enhances Selectivity

Abstract. *The herbicides* N-*(3-chloro-4-methyphenyl)-2-methylpentamamide (solan) and 1, 1′-dimethyl-4, 4′dipyridylium dichloride (paraquat) were less toxic to tomato plants when applied together than either one alone. However, the combination was more toxic to crabgrass and eggplant. This phenomenon may result from the interaction of the herbicides with photosynthesis.*

The herbicide solan, *N*-3-chloro-4-methyphenyl)-2-methylpentamamide is a selective foliar herbicide for controlling weeds in tomatoes. It inhibits photosynthesis in intact plants (*1*). Herbicides of this type inhibit the Hill reaction of isolated chloroplasts (*2*). Another herbicide, 1,1′-dimethyl-4,4′ dipyridylium dichloride (paraquat), depends on photosynthesis for its phytotoxic action; treated plants show very little injury when placed in the dark. Apparently photosynthesis supplies the potential to reduce the paraquat molecule to a free radical. Formation of free radicals appears to be associated with phytotoxicity (*3*). Another photosynthetic inhibitor, 3-(*p*-chlorophenyl) 1,1-dimethylurea (monuron), delayed the action of 1,1′-ethylene-2,2′-dipyridylium dibromide (a relative of paraquat) (*4*).

We have investigated the effects of combinations of solan and paraquat on tomato (*Lycopersicon esculentum*) and crabgrass (*Digitaria sanguinalis*) in the greenhouse. The herbicides were sprayed on the leaves of 4-week-old tomato plants and 2½-week-old crabgrass seedlings. One week later, when the plants were either dead or recovering, they were weighed.

Table 1 shows the effect of solan and paraquat alone and in combination on the growth of tomato plants. Either herbicide was quite toxic if the rate was high enough. However, the addition of a small amount of paraquat reduced the effect of solan. Furthermore, the addition of 0.5 to 2 pounds per acre (0.55 to 2.2 kg/ha) of solan to 0.1 or 0.2 pound per acre (0.11 or 0.22 kg/ha) of paraquat also reduced injury. Hence either herbicide partially reverses the toxicity of the other. The statistically significant stimulation of growth by 0.025 pound per acre (0.03 kg/ha) of paraquat alone was not observed at higher rates. The importance of this observation cannot be determined without further work. In contrast, combinations of the herbicides resulted in greater injury to crabgrass seedlings (Table 2). Initially, a protective effect was also observed on crabgrass. However, by the end of the experiment this effect disappeared.

Table 1. Effect of solan, paraquat, and combinations of solan and paraquat on the growth of tomato. Data presented are averages of six replications.

Paraquat (kg/ha)	Fresh weight (g) with solan treatment, in kilograms per hectare, indicated				
	0	0.56	1.12	2.24	4.48
0	3.24	1.85	1.41	0.48	0.57
0.028	3.81	3.33	3.20	2.29	.85
.056	3.16	3.11	2.58	1.96	.87
.112	1.23	2.63	2.55	1.75	.72
.224	.25	1.11	.77	1.04	.39

Least significant difference at 5 percent, .28; at 1 percent, .37.

Table 2. Effect of solan, paraquat, and combinations of solan and paraquat on the growth of crabgrass.

Paraquat (kg/ha)	Fresh weight (g) with solan treatment, in kilograms per hectare, indicated				
	0	0.560	1.120	2.240	4.480
0	1.79	0.74	0.44	0.41	0.48
0.028	.85	.47	.42	.36	.31
.056	.36	.29	.37	.30	.23
.112	.35	.24	.22	.19	.22
.224	.24	.30	.24	.18	.20

Least significant difference at 5 percent, .17; at 1 percent, .23.

Table 3. Effect of solan, paraquat, and a combination of solan and paraquat on the growth of tomato and eggplant.

Herbicide (kg/ha)	Fresh weight (g)	
	Tomato	Eggplant
Control	8.7	3.40
Solan (0.560)	8.6	0.63
Paraquat (0.280)	2.5	.26
Solan (0.560) + paraquat (0.280)	5.6	.20

Least significant difference at 5 percent; tomato, 1.9; eggplant, .04,: at 1 percent; tomato, 2.8; eggplant, .06.

The different responses of tomato and crabgrass are difficult to interpret and are probably the result of complex interactions. The protective effect of solan against paraquat toxicity to tomato plants may be related to the inhibition of photosynthesis by solan. The protective effect of paraquat against solan is difficult to interpret. Greater injury by the combinations on crabgrass may be related to the smaller size of the crabgrass seedlings in relation to the tomato plants. Tolerance to solan in tomato plants increases rapidly with age (*1*).

To investigate these interactions further, eggplant (*Solanum melongena*), an extremely sensitive plant, was selected for comparison with tomato plants of the same age. The plants were 5 weeks old and there were three replications. The larger tomato plants used in this experiment had already become quite tolerant to solan (Table 3). Apparently, solan protected tomato but not eggplant against paraquat.

Toxicity increased on crabgrass and decreased on tomato if solan was combined with appropriate low concentrations of paraquat (*5*).

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1 May 1963