grown EE cells was not dependent on presence of xylose but was limited to a few cells in glucose-grown parental populations. Experiments presented here did not establish whether the selected xylose-capable cells were mutants or progeny of cells existing in the tissue source of the EE strain. Galactose capability by contrast appeared adaptive. Results of experiments with mixed sugars indicated that xylose and galactose capabilities were alternative rather than substitutive to glucose capability. Given interpretations of data are subject to the reservation that analyses were based on establishment efficiencies of less than 50%. It is not yet known what additional selective forces thus were imposed by conditions of experiment. Before undertaking determinative studies of representative numbers of clones separated from the parental population, it appears desirable to investigate possible effects of very small amounts of one sugar on capacity of cells to make primary use of another sugar for growth. The necessarily complex media used herein for propagation, as noted, were highly depleted but not totally freed of glucose.

Summary. Human esophageal epithelial cells (Minn. 55-12-1 strain) in serum medium microbiologically depleted of glucose were unable to grow with lactose, raffinose, trehalose, turanose, melezitose, melibiose or arabinose as sole carbon sources. Cultures in depleted medium grew well with maltose and occasionally with sucrose by production of reducing sugar through serum or cell-serum activity. Ability to grow in glucose-depleted medium containing fructose, xylose, and galactose was inherited. Acquired xylose capability of cultures appeared to result from selective enrichment of populations with constitutively xylose-capable cells. Expression of capability of cultures to grow in galactose medium was modified reversibly by prior exposure to galactose or glucose.

1. Chang, R. S., Geyer, R. P., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 336.

2. Eagle, H., Barban, S., Levy, M., Schulze, H. O., *J. Biol. Chem.,* 1958, v233, 551.

3. Chang, R. S., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 818.

4. Syverton, J. T., McLaren, L. C., *Cancer Research,* 1957, v17, 923.

5. Salzman, N. P., *Biochim. et Biophys. Acta,* 1959, v31, 158.

6. Winzler, R. J., *Science,* 1944, v99, 327.

7. Bradley, S. G., *Antibiot. and Chemother.,* 1958, v8, 282.

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Dissociation of Some Injected Antigen-Antibody Complexes *in vivo.* (25465)

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As a natural outgrowth of many studies (1-9) exploring the fate of antigens in normal, x-rayed or sensitized animals, recent investigations(10-12) have probed certain aspects of biological activity or metabolism of antigen-antibody complexes directly. Antigen-antibody (Ag-Ab) complexes prepared *in vitro* using radio-iodinated antigen or antibody(10, 11) were injected intravenously into rabbits and elimination from serum was traced as was their initial deposition and subsequent catabolism in various tissues(11). We previously found(11) that clearance from blood of intravenously injected, insoluble, suspended Ag-Ab complexes as indicated by trace labeled antigen proceeds more rapidly in sensitized than in control animals. It seemed to us that the circulating antibody in sensitized rabbits reduced the dissociation of injected complexes thus expediting their removal from the circulation. We also showed that in normal animals antigen-antibody complexes do, at least to some extent, dissociate. We present here further evidence for (a) dissociation of antigen-antibody complexes in sera of rabbits (normal and sensitized) *in vivo* and (b) rapid

equilibration of the labeled Ab moiety of the injected complex with the circulating Ab. This was demonstrated by comparing elimination from serum of Ab-labeled Ag-Ab complexes to elimination of Ag-labeled Ag-Ab complexes(11).

Methods. Rabbits were immunized by repeated(6-8) subcutaneous injections of 60 mg animal of beef gamma globulin at 3-4 day intervals. Eight to 10 days after final injection, rabbits showing qualitatively similar titers were exsanguinated by heart puncture, the blood allowed to clot and the serum collected. Preparation of beef gamma globulin (BGG) trace labeled with I^{131} (I*BGG), preparation of insoluble antigen-antibody complexes between I*BGG and anti BGG from the gamma globulin fraction of rabbits sensitized to BGG, and subsequent suspension in saline and intravenous injection of these complexes into normal or BGG sensitized rabbits have been described(11). The gamma globulin fraction of rabbits immunized against BGG was prepared(11) and iodinated by the method of Warren and Dixon(13). In a typical preparation approximately 550 mg of rabbit gamma globulin and 40 mc of I^{131} containing 5.6 mg of potassium iodide as carrier were used. The iodinated rabbit gamma globulin was immediately precipitated by addition of equal volume of neutralized saturated ammonium sulfate, centrifuged down, dissolved in a few ml of water and extensively dialyzed against saline in the cold. Although we found that this procedure gives only 3.7% of the theoretical yield in iodination of the protein (necessitating high activity used in the experiment), the titer of the iodinated antibody appears essentially unaffected. Complexes between unlabeled BGG and I* anti BGG were prepared in the manner described for labeled BGG and unlabeled anti BGG(11). Ag-Ab complexes in which the BGG is labeled are designated as I*BGG-anti BGG while those in which the antibody is labeled are designated as BGG-I* anti BGG. Rabbits sensitized to BGG or normal rabbits were intravenously injected(11) with 3.3 mg containing 2.6 x 10^6 cpm of BGG-I* anti BGG. Rabbits were bled periodically from ear marginal vein, blood was allowed to clot, and serum removed. Dry protein powders were prepared for counting(14) and the total sample weighed and counted in a scintillation well-counter.

Results of these experiments, and of those to which we wish to compare them(11), are presented in Fig. 1. We express results as relative specific activities (RSA) which have previously been defined(14) as:

$$\text{RSA} = \frac{\text{counts/min. in 100 mg protein}}{\text{counts/min. inj./g body wt}}.$$

RSA values allow direct comparison of data obtained from animals of the same species but of different weight.

Fig. 1 shows protein-bound serum activities obtained over 7 days when sensitized or normal rabbits are injected with BGG-I* anti BGG complex or with I*BGG-anti BGG complex intravenously *via* the ear marginal vein. Each point in Fig. 1 represents the average RSA value obtained from 4 similarly injected rabbits.

It can be seen that labeled antibody admin-

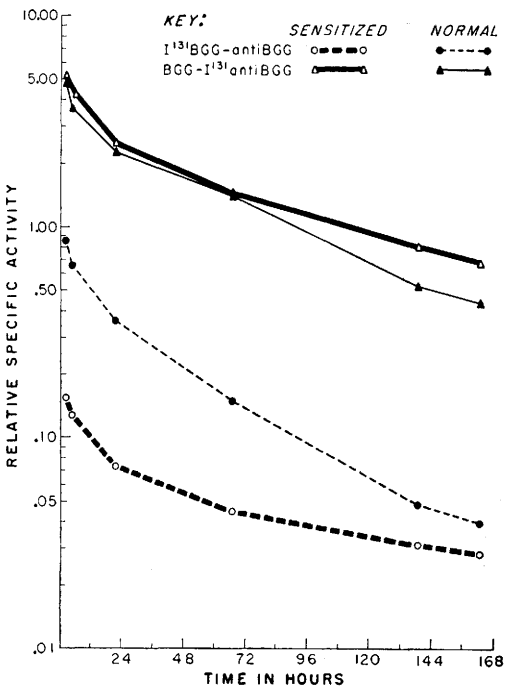

FIG. 1. Protein-bound relative specific activity of rabbit sera at various times after intrav. inj. with BGG-I^{131} anti BGG complex or with I^{131} BGG-anti BGG complex.

istered as part of an antigen-antibody complex is removed from the circulation of normal controls and rabbits sensitized to BGG at quite similar rates. On the other hand, when elimination of antigen-antibody complexes is traced by tagging the antigen moiety(11) (Fig. 1), disappearance of the label is much more rapid and, furthermore, is considerably influenced by presence or absence of circulating antibody in blood. It thus appears that, in blood circulation *in vivo,* there is dissociation of the injected complex and equilibration between labeled antibody administered in the complex and antibody in the circulation of sensitized animals. The small amount of labeled antibody in the complex must *rapidly* equilibrate with the large amount of antibody in the circulation since no difference is apparent in rate of clearance of the labeled antibody administered in the complex in sensitized and normal animals. In experiments in which the antigen component of the complex is labeled, excess circulating antibody assures that the label (Ag) will continue to form part of an Ag-Ab complex and be, therefore, more rapidly removed from the circulation.

The difference in levels of activity of labeled Ag and of labeled Ab in Ag-Ab-injected *normal* rabbits, apparent shortly after injection, is probably due to the initial clearance of injected Ag-Ab complex. Even complexes of Ab:Ag ratios smaller than those injected (*i.e.* partially dissociated complexes) are still very rapidly removed from the circulation.

Summary. When rabbits are injected with antigen-antibody complexes in which the antibody moiety is labeled with I^{131}, no difference is found in its clearance from blood between animals sensitized to the antigen and normal controls. This finding is quite different from previous results when similar complexes with the antigen moiety labeled are injected(11). In the latter case radioactivity is cleared much more rapidly in sensitized rabbits as compared to their controls. The dissociation of antigen-antibody complexes *in vivo* and the rapid equilibration of the labeled Ab of the injected complex with the Ab circulating in sera of sensitized rabbits are given as explanations for these findings.

1. Libby, R. L., Madison, C. R., *J. Immunol.,* 1947, v55, 15.

2. Talmage, D. W., Dixon, F. J., Bukantz, S. C., Dammin, G. D., *ibid.,* 1951, v67, 243.

3. Ingraham, J. S., *J. Infect. Dis.,* 1951, v89, 109, 117.

4. Haurowitz, F., Crampton, C. F., *J. Immunol.,* 1952, v68, 73.

5. Laws, J. O., Wright, G. P., *Brit. J. Exp. Path.,* 1952, v33, 343.

6. Crampton, C. F., Reller, H. H., Haurowitz, F., *J. Immunol.,* 1953, v71, 319.

7. Garvey, J. S., Campbell, D. H., *ibid.,* 1954, v72, 131.

8. Haurowitz, F., Reller, H. H., Walter, H., *ibid.,* 1955, v75, 417.

9. Francis, G. E., Hawkins, J. D., Wormall, A., *Biochem. J.,* 1957, v65, 560.

10. Weigle, W. O., *J. Immunol.,* 1958, v81, 204.

11. Walter, H., Zipper, H., *ibid.,* 1959, v82, 12.

12. Ishizaka, K., Campbell, D. H., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 635.

13. Warren, S., Dixon, F. J., *Am. J. Med. Sci.,* 1948, v216, 136.

14. Walter, H., Haurowitz, F., Fleischer, S., Lietze, A., Cheng, H. F., Turner, J. E., Friedberg, W., *J. Biol. Chem.,* 1957, v224, 107.

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