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Report on the Discussion of the Second Session

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Digitalis

DR. Peter Lauf from Duke University inquired about the number of molecules of digoxin bound per red blood cell. Dr. Butler estimated the number to be about 100, a number which approximates the figure of 200 to 250 obtained by measuring the binding of tritiated ouabain. Dr. Butler again emphasized that digoxin appears to be only in part bound to the membrane of the red cell and that there may be heterogeneity in the binding sites, while the remainder of the digitalis appears to be free in the red blood cell.

Dr. Haber expressed the opinion that the receptor on the red cell is probably more avid than that in the myocardium. Dr. Butler agreed and pointed out that it is difficult to arrive at a definite value in the absence of a dose-response curve. He emphasized that the concentrations used in studying red cell binding are much greater than those usually used in myocardial binding of digoxin. Dr. Spector from Roche and Dr. Pressman from Roswell Park addressed themselves to the question of the long half-life of the digitalis bound to antibody in the rabbit. According to Dr. Butler's data, the digoxin-bound antibody appeared to remain in the circulation for more than a year, a period much in excess of the normal half-life of antibody. Several possibilities were raised. Since the half-life of radioactively labeled purified antibody given to a rabbit is normal, the hapten may stabilize the antibody and thus prevent it from being degraded by normal catabolic mechanisms. Alternatively, since the amount of antibody is markedly in excess of the amount of digoxin in the circulation, Dr. Butler raised the possibility that antibody is being degraded but that the hapten can continue to bind new antibody molecules, thus accounting for the very long persistence of the digoxin in the immunized animals. Thus, he felt that it is possible that even after a year, there may be sufficient antibody left in the circulation to bind the trace amounts of digoxin that are present. Dr. Pressman inquired about other substances that might interfere with the binding of digoxin to antibody. Dr. Butler again pointed out that it is possible to find antisera that react specifically with digoxin and do not combine with other steroid hormones. When Dr. Pressman inquired about adverse effects that might result from the dissociation of the antigen-antibody complexes, Dr. Butler pointed out that the antibody is in marked excess and can neutralize the small amounts of digoxin at all stages of the study, thus precluding the appearance of digitalis toxicity. Dr. Lowenstein of New York University inquired about the possibility of determining the ratio of free and antibody-bound digoxin *in vivo*. Dr. Haber agreed that such data are not now available and would require studies with equilibrium dialysis. However, he felt that a rough approximation could be gained from studying the kinetics of antibody-antigen binding. Dr. Butler again emphasized that *in vivo* most of the digoxin is bound to antibody.

Renin, Aldosterone, and Catecholamines

Dr. Yalow from Mount Sinai noted that many of the Scatchard plots, though not

linear, were arbitrarily divided into two linear components. She cautioned against the use of such calculations for determination of equilibrium constants because they ignore the existence of two interacting systems occurring simultaneously. She expressed the opinion that unless one takes into account the fact that both types of antibody interact with antigens or both types of receptors interact with the ligand, it is not possible to calculate an accurate equilibrium constant for either phase. It became apparent that most of the calculations of the equilibrium constants did not consider this possibility and, therefore, may be slightly inaccurate and may have to be modified. Dr. Butler inquired whether all antisera to angiotensin protect the peptide against the activity of angiotensinase. Dr. Poulsen stated that he tested only a few but predicted that this will probably always be the case. He felt that the low molecular weight angiotensin will be protected in the binding site of the antibody by steric factors which will protect it from the angiotensinase. Dr. Lefkowitz emphasized the potential versatility of the use of receptors in the study of biologically active substances. He emphasized that the potential advantage of using receptors rather than antibody is their great biological specificity, while that of the artificially produced antibodies resided in their greater susceptibility to manipulations which permit the selection of high affinity antibodies. Dr. Blake from Maryland inquired whether any studies have been performed on the affinity of the catecholamine receptor after denervation since it is known that denervation results in supersensitivity to catecholamines. Dr. Lefkowitz said that such studies were in progress, but at this time, he was not certain that this supersensitivity was due to a change in the affinity of the receptor, and raised several other possibilities that might explain this observed hypersensitivity. Among these are an alteration in the binding site or at steps distal to it. Dr. Spector inquired why Dr. Lefkowitz' group had used only the microsomal fractions to look for the *beta* receptor. He pointed out that it might be distributed more widely in other parts of the cell but that because of various other substances that are present it might be degraded rapidly and be difficult to detect. Dr. Lefkowitz agreed with this possibility and said that probably it is distributed more widely. Both speakers agreed that use of a monoamine oxidase inhibitor might permit detection of the *beta* receptor in other subcellular fractions. Dr. Kirishma of the National Institutes of Health inquired about the relation between the binding of epinephrine and norepinephrine to the receptor and the activation of the adenylcyclase system. Both he and Dr. Lefkowitz agree that activation of adenylcyclase after binding is a very complicated series of steps and that it may require much larger amounts of catecholamines to cause activation of the system. Thus, when we examine the binding of the biologically active substance to its receptor, we may be studying only the first and least important step in the activation process. However, at the moment this appears to be the most easily dissectable part of the system. Perhaps activation of the system may require binding to more than one receptor through different structures on the effector molecule.