males. Reciprocal crosses of PHL or BALB/cWt females with males of other strains produce approximately equal numbers of male and female offspring. The BALB/cWt males produce about 30 percent morphologically abnormal sperm (*8*), which suggested the possibility that such sperm might activate the egg and initiate gynogenesis.

When SJL/J females were mated to BALB/cWt males 62.7 percent of the offspring were females (451 male : 757 female), thus providing an opportunity to test for parthenogenesis. Parthenotes would be hemizygous or homozygous for the maternal genotype and could be distinguished from the heterozygous hybrid offspring by phenotype if certain marker genes characterized the genotypes of the maternal and paternal strains.

Accordingly the blood from female offspring of SJL/J-Hbb^s/Hbb^s $Pgm\text{-}1^b/Pgm\text{-}1^b$ females, mated to BALB/cWt-Hbb^d/Hbb^d $Pgm\text{-}1^a/Pgm\text{-}1^a$ males, was examined for the type of hemoglobin beta-chain (*Hbb*) and the isozyme of phosphoglucomutase (*Pgm-1*) present in the erythrocytes (*9*). Fifteen females from four breeding pairs were examined and all proved to be heterozygous, that is, Hbb^d/Hbb^s and $Pgm\text{-}1^a/Pgm\text{-}1^b$. There were nine male siblings, so the sample contained 62.5 percent females which was typical of the population. If parthenogenesis had occurred at the rate predicted from the sex ratio, the probability of 15 heterozygotes out of 15 females examined would be less than 0.01.

A similar but smaller excess of females (53.8 percent, 342 male : 399 female) occurred in the offspring of 129/Rr-A^w/A^w c^{ch} p/c^{ch} p females crossed with BALB/cWt-A/A b/b $c+/c+$ males. In these animals normal hybrids are clearly distinguishable visually from those with the maternal gentoype. So far, 88 females and 72 males have been produced from four pairs and all were typical hybrids, that is, A^w/A $b/+$ c^{ch} $p/c+$. Again the sample reflected the excess of females (55.0 percent), and the probability of not finding parthenogenetic individuals, if present as predicted from the sex ratio, is less than 0.001.

From these findings I conclude that parthenotes from matings with BALB/cWt males occur rarely, if ever, and they are not responsible for the observed excess of females (*10*). Weir (*11*) has reached a similar conclusion for the offspring of matings with PHL males. The reason for the excess of females in the progeny of PHL or BALB/cWt males is still obscure.

Parthenogenetic adults have been reported in several vertebrate orders but Beatty considers the few reports of such mammals unproven (*12*). They were made upon noninbred rabbits, and, now that inbred strains of this and other species are becoming available, it may be appropriate to reexamine some of the claims. A low sex ratio may yet prove to be an indicator of spontaneous gynogenesis. The potential value for genetic analysis of parthenogenetic material particularly if it remains haploid has been pointed out (*13*).

W. K. WHITTEN
The Jackson Laboratory,
Bar Harbor, Maine 04609

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Immunogenicity of Glucagon: Determinants Responsible for Antibody Binding and Lymphocyte Stimulation

Abstract. *Bovine glucagon, a polypeptide of 29 amino acids, is immunogenic in rabbits and guinea pigs. The antigenic determinants of glucagon were investigated with isolated tryptic peptides of the hormone. Antibodies from virtually all of more than a dozen animals tested had specificity primarily for the amino-terminal heptadecapeptide. However, only intact glucagon and its carboxy-terminal dodecapeptide stimulated spleen or lymph node cells to synthesize DNA. It thus appears that glucagon was cleaved along functional lines into two parts, one of which contained the major antigenic determinant for serum antibody and the other of which was "recognized" by antigen-reactive cells.*

Recent investigations suggest that an antigen must be "recognized" by at least two functionally distinct lymphoid cells in order to elicit a humoral antibody response (*1*), and consequently must possess at least two antigenic determinants (*2*). We now report our study of a small immunogenic molecule of defined structure. The study was designed to resolve and characterize the determinants for the two cell types.

New Zealand white rabbits and randomly bred guinea pigs were immunized with 0.1 to 2.0 mg of crystalline bovine glucagon in complete Freund's adjuvant. Antiserums were assayed for binding of [^{125}I]glucagon (*3*). Each of more than a dozen animals immunized made significant responses. The preparation of [^{125}I]glucagon had a specific activity of 33 μc/μg. When an amount equal to 2000 count/min was used for each assay, 50 μl of serum from an immunized animal bound 50 to 80 percent of the total radioactivity, whereas the serum before immunization of the animal bound less than 5 percent. In addition, each animal developed specific delayed skin reactivity, which was assessed by induration and erythema 24 to 48 hours after intradermal injection of glucagon.

The antigenic determinants of glucagon were investigated by digesting the molecule with trypsin (*4*). An 0.085 percent solution of glucagon was incubated with trypsin treated with TPCK (a chymotrypsin inhibitor) (molar ratio 1 : 100) at 25°C, *p*H 7.8, for 2.5 hours with gentle shaking. The digest was then adjusted to *p*H 3.0, heated at 90°C for 2 minutes, and lyophilized. Glucagon contains one lysine residue at position 12 and two arginine residues at positions 17 and 18. The resulting three fragments and

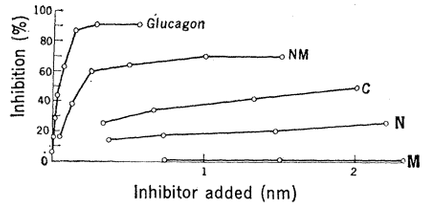

Fig. 1. Inhibition of binding between 2000 count/min of [^{125}I]glucagon and serum from an immune guinea pig by unlabeled glucagon and its tryptic fragments.

free arginine were resolved by high-voltage electrophoresis on paper at *p*H 3.5 and were further purified by chromatography on Sephadex G-25 and G-10. The amino-terminal dodecapeptide, middle pentapeptide, and carboxy-terminal undeca- and dodecapeptides have been designated N, M, and C, respectively. Amino acid analysis of C showed that the C peptide was a mixture, probably because the cleavage of arginine at position 18 was incomplete. In addition, an amino-terminal heptadecapeptide (NM) was prepared with the use of a reversible blocking agent, citraconic anhydride, for primary amino groups as described for insulin (*5*). The blocking group was easily removed with mild acid. Amino acid analyses of varying quantities of the isolated fragments indicated a degree of purity of at least 99 percent.

The capacity of the peptides to bind

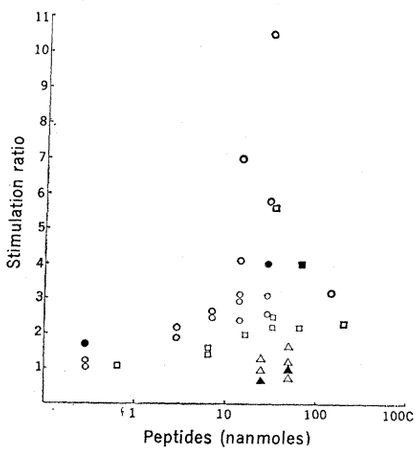

Fig. 2. Incorporation of [^{14}C]thymidine by lymphoid cells from immune guinea pigs cultured in the presence of glucagon (circles), C peptide (squares), or NM peptide (triangles). The open and solid symbols represent spleen and lymph node cells, respectively. The stimulation ratio is the ratio of counts per minute from cells cultured in the presence of antigen relative to cells from the same animal cultured in the absence of antigen. Data from five animals are shown.

serum antibody was determined by inhibition of the binding of [^{125}I]glucagon. With each of 15 antiserums, the order of effectiveness of the inhibitors was glucagon $>$ NM $>$ C $>$ N $>$ M. Data for a representative serum is shown in Fig. 1. While the order of effectiveness of the ligands did not vary between the species or between different members of the same species, the increments between them did. However, M was totally ineffective in all cases, and the ratio C : NM required for a given level of inhibition was never less than 5 : 1. The pronounced difference between N and NM may be due to a direct contribution of residues 13 to 17 to the determinant. On the other hand, x-ray diffraction studies indicate that glucagon is about 75 percent helical, with two sections of helix. The model which best fits the data and permits the most stable placement of hydrophobic residues predicts a helical segment encompassing residues 5 to 16 and another between residues 17 to 28 (*6*). Thus, the relative ineffectiveness of N may be due to an altered conformation of the determinant. It is of interest in this context that assays with a series of synthetic peptides initiated at position 16 disclosed that significant binding activity first appeared with peptide 5 to 16 (*7*).

The peptides were assayed for their ability to stimulate cultures of spleen and lymph node cells from immune guinea pigs whose antibody specificity had been determined, assessed by incorporation of [^{14}C]thymidine into DNA (*8*). Varying quantities of antigen were added to tubes containing 8 to 10×10^6 cells at the beginning of the culture period. After incubation for 48 hours, 0.1 μc of [methyl-^{14}C]thymidine was added to each culture tube, including controls which had received no antigen. After an additional 24-hour incubation, the cells were harvested and the radioactivity incorporated into acid-precipitable material was measured (Fig. 2). The variation between replicate samples seldom exceeded $\pm$ 5 percent. The maximum stimulation obtained with glucagon was 10.5-fold. Cells from this animal were stimulated 5.7-fold by C, but not significantly above the baseline by NM. The most effective antigen dose range was 10 to 100 nanomoles. Glucagon and C produced at least a twofold stimulation in this range with all the guinea pigs tested, whereas the values for NM fell between 0.7 and 1.7. Cells from normal animals were unaffected by the peptides. Thus, it appears that glucagon has been cleaved along at least partially functional lines into an amino-terminal heptadecapeptide which contains the major antigenic determinant for serum antibody and a carboxy-terminal peptide of 11 or 12 amino acids which can trigger antigen-reactive cells.

These results can be interpreted as supporting the view that humoral antibody and antigen-receptor molecules on the surfaces of immunocytes are qualitatively different, but it is also compatible with the data supporting cooperation of two functionally distinct cells in the immune response (*1*). In this latter instance, the cell which secretes antibody (AFC) is at best very inefficiently activated by antigen directly, but requires the intervention of another cell which is also antigen-specific, though not necessarily for the same determinant. This second cell (ARC) can be directly induced to proliferate by antigen (*9*). In the case of glucagon, we propose that the determinant recognized by ARC is in the C peptide, whereas the major haptenic determinant recognized by AFC is carried by the NM peptide. A basis is provided for understanding why haptens that are large enough to satisfy the binding requirements of receptor molecules, such as polyglutamic acid, are unable to stimulate lymphoid cells from animals which have made an intense antihapten response (*10*).

GEORGE SENYK, DANUTE NITECKI
JOEL W. GOODMAN
Department of Microbiology, University of California Medical Center, San Francisco 94122

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