higher in the GBL-injected animals than in the group injected with the combination.

Concentrations of these compounds were next determined at fixed times after an ethanol injection of 6.51 mmole/100 g or a GBL dose of 0.25 mmole/100 g. The animals were killed 30 and 90 minutes later, the approximate waking times of the two groups (Fig. 4). If one compares the effect of ethanol alone with that of the combination for each tissue and each dose, at 30 and 90 minutes, there is only one significant difference. The content of ethanol in the livers of rats injected with ethanol alone is significantly higher at 30 minutes than at 90 minutes ($P < .02$); that is, there is a significant decrease with time. There is no significant decrease in the ethanol concentration in the livers of the combination-injected animals from 30 to 90 minutes, and there is no significant difference between combination- and ethanol-injected animals at 90 minutes. Bustos *et al.* (*14*) have shown that in rats injected with or without pyrazole (a known inhibitor of alcohol dehydrogenase in vitro and in vivo) there is no significant difference in the concentration of ethanol in the blood until 4 to 8 hours after ethanol injections. It might also be added that in the work showing a prolongation of ethanol sleeping time after prior treatment with cortisone there were no differences in concentrations of ethanol in the blood upon waking (*3*).

We have confirmed the observations (*4*) that there is a peak corresponding to GBL in flor sherry (*15*). This peak cochromatographs with standard GBL and is present at approximately 1.5 mmole/liter. This is much less than the amount required for even the lowest level of synergy that we have studied in the rat. For a 70-kg man to ingest GBL to a dose of 2.5 mmole/kg he would have to drink 115 liters of wine.

It would appear therefore that GBL alone does not contribute significantly to the well-known pharmacologic effect of wine. However, we have observed a behavioral interaction of ethanol with GBL and GHB, known soporific components of the central nervous system. The metabolisms of both compounds require NAD, and thus there is the possibility of a competition for this cofactor. Such an antagonism has considerable precedent in the explanations "for many of the known acute effects of ethanol administration" (*16*). This increase in the tissue reductive capacity accompanying alcohol metabolism might tend to favor the conversion of succinate semialdehyde to GHB. There are similarities of actions that might also play roles in the synergy of these compounds. Acetaldehyde and GBL both cause increases in brain acetylcholine (*17*). Hahn and co-workers (*18*) have shown that there is a potentiation of ethanol-induced sleep time with an increase in brain dopamine; and GHB and GBL have been shown to cause increases in brain dopamine (*19*). There is the possibility of an active intermediate or intermediates formed when both substances are present simultaneously.

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Parthenogenesis: Does It Occur Spontaneously in Mice?

Abstract. *If parthenogenesis occurred in bisexual organisms, it would produce an excess of females and depress the sex ratio. The phenotypes of female mice, from matings that produce an excess of females, were examined for evidence of the presence of marker genes of paternal origin. All proved to be hybrids of the maternal and paternal strains, thus excluding parthenogenesis as the cause of the low sex ratio.*

Limited parthenogenetic development of mouse embryos has been observed after electrification of the oviduct (*1*) or treatment of ova in vitro with hyaluronidase (*2*). Edwards (*3*) found some gynogenetic development in eggs of mice inseminated with sperm that had been subjected to various treatments. Gynogenesis is a special form of parthenogenesis that follows activation of the egg by a sperm without contribution of genetic material. Individuals produced by parthenogenesis are called parthenotes (*4*). Successful development from haploid cells would be more likely in the inbred strains of laboratory rodents or their hybrids because recessive lethal genes have been eliminated by inbreeding. The related phenomenon, the development of spontaneous teratomas from male diploid primordial germ cells, is almost unique for mice of inbred strain 129, but teratomas can be induced experimentally in several other inbred strains and hybrids (*5*).

If numbers of parthenotes develop, one would expect to find an altered sex ratio with females predominating. The sex ratio of many inbred strains of mice have been examined (*6*) but only in PHL and BALB/cGnDgWt (*7*) is there a significant excess of females (58.2 and 60.8 percent, respectively). In neither case is the mechanism understood. The abnormal sex ratio appears regularly in the offspring from matings between PHL males and females from other strains but only in certain genotypes of the crosses that use BALB/cWt

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*).

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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