be analogous to model A, but with strong polarization. The calculated energy minima, which appeared at 2.1 A for XeF_2 and 2.4 A for XeF_4, are both somewhat larger than observed. In qualitative discussion, L. C. Allen [*J. Am. Chem. Soc.* **84**, 4344 (1962)] includes model C as a possibility, while K. S. Pitzer [*Science* **139**, 414 (1963)] and R. E. Rundle [*J. Am. Chem. Soc.* **85**, 112 (1963)] attribute the equivalent of type B bonding to the xenon fluorides. None of these authors have considered the implications of the vibration spectrum.

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8 February 1963

Antigens in Insulin
Determinants of Specificity of Porcine Insulin in Man

Abstract. *Porcine insulin, which is distinguished from human insulin only in the amino acid at the C terminal of the B chain, is antigenic in man. Even if the last amino acid or the last eight amino acids are removed from the C terminus of the B chain of insulin, the altered insulin still reacts with human antibodies to porcine insulin; thus, the antigenic determinant of porcine insulin is located in a part of the molecule where the amino acid sequence is the same as it is in the corresponding part of the human insulin molecule.*

As a result of experiments with insulin labeled with I^{131}, insulin-binding antibodies have been demonstrated in the serums of virtually all human subjects treated with commercial mixtures of bovine and porcine insulin (*1, 2*). Insulin-binding antibody is never observed in subjects who have never been treated with insulin. Although insulin antibodies react with insulins derived from a large variety of mammalian (*3, 4*) and piscine (*5*) species, the reaction is almost always strongest with bovine insulin (*3*). Porcine insulin (*6*) differs from human insulin (*7*) only in containing alanine instead of threonine as the C terminal amino acid of the B chain (B 30) whereas bovine insulin (*6*) shows additional differences in residues 8 and 10 of the A chain. Differences in immunologic reactivities of insulins from four different ungulate species (*3*) could be related to differences in the 8 to 10 region of the A chain (*6*) suggesting that this region of bovine insulin might constitute a site of antigenicity in man (*3*).

In the present study human subjects were immunized with pure porcine insulin in order to determine whether the C terminus of the B chain (B 30) is a site of antigenicity in man. Five newly discovered diabetic persons were treated with NPH (neutral protamine Hagedorn) porcine insulin at doses of 10 to 40 units per day. Insulin-binding antibodies were detectable in all subjects within 6 weeks to 3 months after institution of therapy. In three cases, antibody concentrations were sufficient to conduct these studies. Similar results were obtained in all subjects.

I^{131}-labeled insulin was prepared from crystalline bovine or porcine insulin or from desoctapeptide bovine insulin (insulin lacking the last eight amino acids of the C terminus of the B chain) according to methods described previously (*8*) or according to the method of Hunter and Greenwood (*9*). Mixtures of insulin and antiserum were prepared so that the concentrations of I^{131}-labeled insulin were always the same even though the concentrations of unlabeled intact insulin or insulin derivative from different species varied. After incubation for 2 to 3 days at 4°C, aliquots of the mixtures were applied to strips of Whatman 3-MM filter paper (*10*) which were used for electrophoretic or chromatoelectrophoretic separation of insulin-I^{131} bound to antibody, and "free" (unbound) insulin-I^{131} (*1*). Free insulin-I^{131} is adsorbed firmly to this paper at the site of application whereas insulin-I^{131} bound by antibody migrates with the serum proteins (*1*) in the region between β-and γ-globulin (*11*). The following insulin preparations were used: crystalline porcine insulin, desalanine porcine insulin (insulin lacking B 30), crystalline bovine insulin, desoctapeptide bovine insulin and crystalline human insulin (*12*). The ratio between insulin-I^{131} bound to antibody and free insulin-I^{131} is plotted as a function of the concentration of unlabeled insulin or insulin derivative in the mixture (Figs. 1–3). Insulin-binding antibodies in the serums of subjects immunized with porcine insulin reacted almost as well with desalanine porcine insulin, desoctapeptide bovine insulin, and human insulin as with the intact porcine and bovine insulins when competing against the binding of porcine insulin-I^{131} or bovine insulin-I^{131} (Figs. 1 and 2). The reaction with porcine insulin-I^{131} was slightly stronger than with bovine insulin-I^{131}. These results are in contrast to observations with antiserums from subjects treated with commercial mixtures of bovine and porcine insulin, in which human insulin (*3, 13*) and desoctapeptide bovine insulin (*2*) generally compete much less effectively than intact bovine insulin against the binding of bovine insulin-I^{131}. Binding of desoctapeptide insulin-I^{131} in antiserums to porcine insulin was also demonstrated directly by paper electrophoresis.

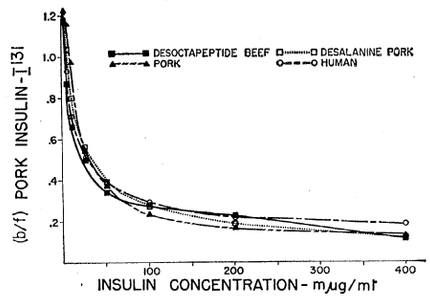

Fig. 1. Antibodies to porcine insulin. Plasma dilution 2:5. The ratio, b/f, of antibody bound (b) to free (f) bovine or porcine insulin labeled with I^{131} as a function of concentration of the unlabeled insulins in serum from a human subject treated with pure porcine NPH insulin for 7 months.

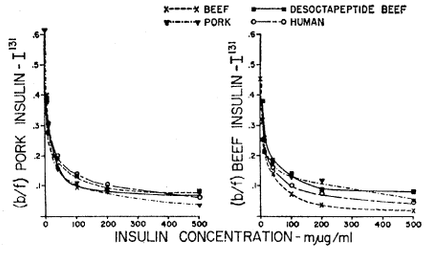

Fig. 2. Antibodies to porcine insulin. Plasma dilution, 1:5. Same patient as shown in Fig. 1 after 11 months of therapy with pure porcine NPH insulin.

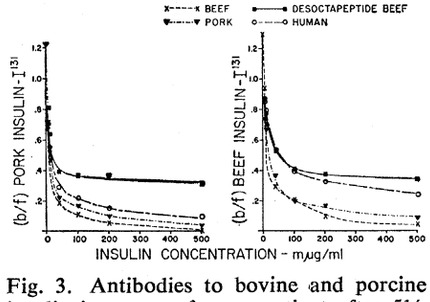

Fig. 3. Antibodies to bovine and porcine insulin in serum of same patient after 5½ months of further therapy with bovine NPH insulin. Plasma dilution 1:5.

One patient, treated only with porcine insulin for 11 months, was then treated only with bovine insulin for 5½ months. After treatment with bovine insulin, his antiserum reacted slightly more strongly with bovine than with porcine insulin and discriminated sharply between desoctapeptide bovine insulin and intact bovine insulin (Fig. 3).

Since porcine and human insulin differ only in the amino acid at the carboxyl end of the B chain, one might think that this grouping would be associated with the antigenicity of porcine insulin for man. However, since desalanine porcine insulin and desoctapeptide bovine insulin react well with antibodies to porcine insulin the C terminus of the B chain of porcine insulin is probably not the site of reaction with antibody. According to current theories of the mechanisms of immunity it is not reasonable to suggest that the site of reaction of an antigen with its antibody is quite distinct from the groupings which are responsible for its antigenicity. It thus appears that the specific antigenicity of porcine insulin resides in some region of the molecule that is identical with the corresponding region of human insulin in respect to amino acid sequence.

Several explanations of these findings may be considered. A small fraction of porcine insulin may have been altered during extraction and purification so that an antigenic specificity not present in the native protein was acquired or that the same effect may have been produced by its administration as NPH insulin. Either possibility suggests that human NPH insulin may also prove to be antigenic in man. Although Moloney (*14*) has reported the failure of antigenically potent animal insulins to induce formation of antibodies in the homologous species, the present case must be tested by attempted immunization of man with human NPH insulin (and with regular human insulin) when adequate supplies for this purpose become available. The occurrence of autoantibodies to γ-globulins and the ability to induce formation of antibodies to autologous γ-globulin that had been precipitated with ammonium sulfate and stored at −23°C (*16*) do not a priori exclude the possibility that human insulin may be antigenic in man.

An alternative interpretation is related to a possible difference in the 3-dimensional configurations of human and porcine insulins, this structural difference being responsible for the antigenic determinacy of porcine insulin. We know of no physicochemical evidence that supports this hypothesis but immunochemical evidence has been presented for the existence of conformational differences between porcine insulin and sperm whale insulin (*4*). These two insulins have identical amino acid sequences (compare *6*, *16*) but are readily distinguished immunochemically by certain human antiserums to bovine, porcine insulin (*4*). Such distinction can be attributed only to certain as yet unknown differences in three-dimensional configuration of the molecules (*4*). Removal of zinc by a salting out technique, and redissolving the insulin has no influence upon its immunologic reactivity (*5*) so that whatever conformational differences exist they appear to be fairly stable characteristics of the peptide structures.

In other experiments, we have observed that antiserums from rabbits immunized with porcine insulin in Freund adjuvant also react with desoctapeptide bovine insulin. Since leporine insulin differs from human insulin and porcine insulin only in containing serine at B 30 (*17*), these results would appear to be analogous to those observed in man. However, interpretation must be reserved in this case because of the possibility that new sites of antigenicity might result from a "denaturation" of insulin, after the relatively drastic emulsification procedure required for the preparation of adjuvant for immunization. In a limited series of trials we have been unsuccessful in immunizing rabbits with NPH porcine insulin without adjuvant.

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17 December 1962

Crystal Structures at High Pressures of Metallic Modifications of Compounds of Indium, Gallium, and Aluminum

Abstract. *X-ray diffraction shows that the high-pressure modifications (at 22 to 130 kilobars) of the antimonides of indium, gallium, and aluminum are analogous to white tin. The arsenide and phosphide of indium transform to NaCl type. The transformation of these semiconductors to their metallic states is empirically related to their energy gap under normal conditions.*

Both silicon and germanium adopt the white tin structure at the high pressures where they adopt metallic properties (*1*, *2*). In this report, the nature of the transition in InSb studied by Jayaraman *et al.* (*3*), and the transitions in GaSb, AlSb, InAs, and InP reported by Minomura and Drickamer (*2*) is described. Attempts to reach the transition in GaAs that was reported by the latter authors at 240 kb has failed.

All the compounds studied were of semiconductor grade (*4*). Each was diluted with the appropriate amount of amorphous boron to minimize absorption difficulties. As described previously (*1*), both flat and tapered pistons were utilized on each substance. In contrast to the behavior of the elements Si and