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

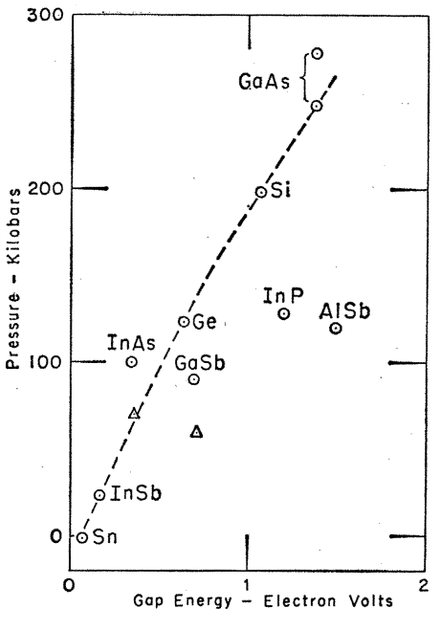

Fig. 1 (left). Transition pressure of group IV elements and group III–V compounds plotted against room-temperature gap energies.

Ge, these compounds showed few orientation effects, and the analysis of their high-pressure structures presented little difficulty.

The three antimonides gave patterns above their respective transitions which could be indexed as tetragonal with $c/a = 0.53$ to 0.55. The detailed crystallographic data and volumetric and nearest-neighbor distance changes are given in Table 1. The intensities were appropriate for the "white tin" structure; however, no additional lines appeared to allow placing the atoms as to ordering. It is reasonable to suggest that the ordering is the same as in the parent sphalerite structures—that is, a distorted tetrahedron of like atoms with an unlike atom at the center position. With flat pistons, two-phase patterns were obtainable for both GaSb and AlSb; but for InSb the transition was invariably complete after pressure was applied, and hence the pressures at which diffraction patterns were obtained for it are unknown. Smith and Martin report $\Delta V/V_o = 18.5$ percent for InSb from a two-phase pattern (*5*), and this figure appears in Table 1. Apparently "metallic" InSb is quite compressible (like Sn itself) since my pictures give a volume 85 percent of theirs.

Gallium arsenide was compressed twice to the point of piston failure. No transition appeared, and the final volume decrease attained gave $V_p/V_o = 0.888$. The transition(s) in GaAs have been reported to occur at 245 to 250 kb (*2*), apparently beyond the range of the present apparatus.

With flat pistons InAs and InP gave clear two-phase patterns, but with tapered pistons only one phase appeared. The high-pressure structure for both compounds was immediately found to be the same as that of NaCl, in opposition to the "white tin" structure of their electronic counterparts GaSb and AlSb. This is presumably an atomic size effect.

That there exist systematic relationships between the group IV elements, the group III–V compounds, and indeed the group II–VI compounds has been well known for many years. Figure 1 displays still another such relationship. Semiconductor gap energies (at room temperature) have been plotted against pressures of transition to the metallic phase. The gap energies are from various sources (*6*), while most of the pressures (in circles) are from the paper of Minomura and Drickamer (*2*); that for InSb is due to Jayaraman *et al.* (*3*). The triangle point for InAs is due to Paquin and Gregory (*7*), while that for GaSb is due to Jayaraman *et al.* (*8*). As can be seen, all transitions found to date lie to the right of a curve determined by the three elements, Sn, Ge, and Si and the compounds of two elements in the same row of the table, InSb and GaAs. InAs is an apparent exception, but there is at present disagreement about its pressure of transition. Although the points have not been plotted in Fig. 1, all of the group II–VI compounds studied by Samara and Drickamer (*9*) lie to the right of the same curve.

To appreciate the significance of Fig. 1, the approximate difference in Gibbs' free energy between the semiconducting form and the metallic form may be calculated (for zero pressure) as $\Delta G = -P\Delta V$. The results of such calculations are displayed in Table 2. The pressure for the transition in carbon to the metallic tin form is estimated roughly by extrapolating Fig. 1. The change in volume ΔV is obtained either from x-ray data previously given (*1*) or (for GaAs and C) is simply 20.9 percent of the zero pressure molar volume. It is seen immediately that for elements and iso-row group III–V compounds along the curve in Fig. 1, the relationship $P\Delta V = \Delta E/2$ is obeyed to better than the accuracy

Table 1. Structural parameters and dimensional changes in group III–V compounds.

Structure	a (Å)	c (Å)	c/a	V_p/V_o	$\Delta V/V_p$ (%)	$\Delta d/d_o$ (%)
		Aluminum antimonide				
"White Sn"	5.375	2.892	0.538	0.867	16.5	+5.2
		Gallium antimonide				
"White Sn"	5.348	2.937	.549	.903	16.9	+5.4
		Indium antimonide				
"White Sn"	5.537	2.970	.536		18.5	+8.9
		Indium arsenide				
NaCl	5.514			.926	18.8	+7.9
		Indium phosphide				
NaCl	5.710			.920	19.6	+7.4

Table 2. Gibbs' free energy differences for metallic versus semiconducting group IV elements and group III-V compounds.

Substance	Transition pressure (kb)	ΔV (cm³/mole)	$P\Delta V$/atom (ev)	$\Delta E/2$ (ev)
Sn	—0.7	4.3	0.003	0.03
InSb	+22	7.59	.09	.09
Ge	120	2.85	.32	.32
Si	200	2.73	.56	.54
GaAs	240	5.52	.69	.69
C	840	0.72	.62	2.80
GaSb	90	5.19	.22	0.35
AlSb	125	4.97	.32	.75
InAs	102	5.82	.31	.18
InP	133	5.45	.38	.60

of the data. For compounds formed from differing rows of the periodic table the only apparent relationship is $P < E/2\Delta V$. InAs is an exception which would be corrected by using the 70 kb transition pressure of Paquin and Gregory (*7*). As usual in these empirical relationships carbon fails to fit. A reasonably obvious explanation for this is that its electronic configuration is basically different from that of the other group IV elements. Carbon has only its 1*s* shell under its valence electrons, while the others all have a substantial core of complete *p*-shells and *d*-shells and so on. Hence any rule based essentially on interatomic distance is very apt to fail for carbon.

There is a very simple interpretation of the equality shown by the first five entries in Table 2. From the simplest model of a semiconductor, $\Delta E/2$ is easily shown to be the position of the Fermi level (*10*). Hence at a pressure of 1 atm the Fermi level of the (unstable) metallic modification lies at the same height as the bottom of the conduction band of the semiconducting elements and compounds obeying the rule $P\Delta V = \Delta E/2$. Considering the known complexity of the band structure of these materials, this is too good to be true, but the numbers speak for themselves.

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Botulinus Toxin: Effect on the Central Nervous System of Man

Abstract. *Electrical stimulation of multiple peripheral nerves, elicited "H" reflexes in a patient, 61 years old, with botulism. These reflexes are extremely suggestive of some central release or failure of inhibitory control of a monosynaptic or polysynaptic spinal reflex arc. This "central" action of botulinus toxin is similar to that suggested for tetanus toxin.*

Botulism is an extremely rare, yet deadly, form of food poisoning. The poisoning results from an exotoxin released from *Clostridium botulinum*. The toxin is one of the most lethal poisons known.

Previous knowledge of the physiological action of botulinus toxin is based primarily on laboratory experimentation in cold- and warm-blooded animals. The marked differences among species in response to toxin suggested the importance of investigating the effects of the toxin in man. It has generally been assumed that the toxin has its primary action at the nerve ending blocking the nerve impulse before it reaches the muscle, or glandular end organ. This toxic action has been attributed to an inhibition of the release of acetylcholine (*1, 2*), probably at the tips of the nerve endings (*2*), or to a block of the transmission of nerve impulses into final presynaptic arborization (*3*).

The concept of botulinus toxin as a pure peripheral neuromuscular toxin has been questioned by some Russian experimenters in the last decade. Mikhailov (*4*) has shown a defect in brain stem cardiac reflexes in botulinized animals. Erzina (*5*) and Matveev (*6*) note that botulinus toxin can attack non-neural tissue.

Recent studies in humans affected with severe botulinus poisoning have confirmed the severe peripheral neuromuscular effect of the toxin (*7*). Some evidence to support a simultaneous central effect of the toxin is described in this report.

The findings are based on studies of a 61-year-old man with severe botulinus poisoning and a severe progressive weakness, who was admitted to the hospital 48 hours after the ingestion of home-canned mushrooms. A son-in-law had milder symptoms.

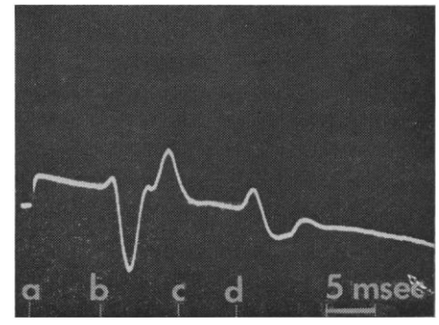

Fig. 1. "H" reflex from stimulation of ulnar nerve of a patient (at elbow). The nerve was stimulated for 0.1 msec at 150 v.

Figure 1 shows the result of stimulation of the ulnar nerve at the elbow with current pulses of 0.1 msec duration, with a voltage difference of 150 between the skin over the ulnar nerve at the elbow and an anode 1 cm away. The recording was taken from a surface electrode over the abductor digiti quinti (a partially affected muscle) in the hand. The indifferent electrode was 0.5 cm away.

The distance from the shock artifact *a* to the onset of the muscle response at *b* represents the time of conduction down the ulnar nerve, across the neuromuscular junction until the first muscle-fibers discharge. The period *b* to *c* represents the muscle discharge. At *d*, one sees a delayed response. This response was initiated at a lower threshold than the motor response *bc*. It was well developed by the time stimulation reached 80 volts and the muscle response first became apparent. It was only partially suppressed as the strength of stimulation increased up to 150 volts. The time the response appeared varied appropriately when the nerve was stimulated distally or proximally. This delayed response was recorded earlier, over the abductor digiti quinti muscle, when the nerve was stimulated at the elbow as compared to the wrist. This is the so-called "H" reflex, which was present and prominent in multiple recordings from different areas after stimulation of the peripheral nerve.

Figure 2 is a similar recording taken from a normal subject. Two successive stimuli are superimposed. The experiments were performed under identical conditions to those in Fig. 1. The markings in the background denote 5 msec. No "H" response is seen. The curve is characteristic of the simpler