is maintained at a pressure of 1 atm on one side, while the other side is kept evacuated causing flow of D_2 gas across the Pd foil stack. The Pd temperature is adjusted using an external electrical heater. Li reported measuring 6 W of excess heat over a duration of 9 h, with the experiment having been replicated six times.

Li and his collaborators have found that in Pd there is a resonant temperature T_r just above which both D_2 flow-rate and heat-conduction rate drop sharply, leading to a negative feedback mechanism. Li reported that this causes a heat 'ignition wave' to flow from the periphery towards the centre of the Pd foil, leading to the prospects of obtaining self-sustaining conditions.

The paper titled 'Low energy nuclear transmutation in condensed matter induced by D_2 gas permeation through Pd complexes' by Iwamura *et al.*, Mitsubishi Heavy Industries was considered as one of the most important papers of the Boston meeting. The experimental set-up is similar to that used by Li *et al.* described above. In the present experiments, the Pd complex comprised of a multilayer, thin film stack of Pd and CaO sandwiched between a thin Pd film in the front and bulk Pd foil substrate at the back. On the thin Pd film in the front side, Cs ions are implanted either by Cs-ion injection or electrochemical coating. The Cs-added side is exposed to D_2 gas, while the back side is evacuated by a turbomolecular pump. After several days of D_2 gas permeation, elemental analysis and depth profiling of the Pd film is carried out by Time of Flight–Secondary Ion Spectrometry. It is found that ^{133}Cs is transmuted to ^{141}Pr, a Z increase of 4 and A increase by 8! Before permeation there was no detectable Pr in the foil. A similar experiment with Sr ion implantation indicates that ^{88}Sr is transmuted to ^{96}Mo, again a Z increase of 4 and A increase of 8; clearly a case of four deuterons being absorbed by the Cs or Sr nuclei. Depth profiling indicates, however, that only the top 1 micron layer is active in the transmutation.

Akhito Takahashi's (Osaka University) paper entitled 'Studies on 3D fusion reactions in TiDx under ion beam implantation', reviewed their earlier work in the field. Takahashi also presented the results of the replication of the 'Iwamura effect' at his laboratory.

Hubler, Naval Research Laboratory, USA, is presently attempting to verify the Iwamura effect independently using the sophisticated accelerator mass spectrometer facilities.

Roussetski *et al.*, Lebedev Institute, Moscow reported the mesurement of highly energetic charged particles from hydrogen or deuterium-loaded foils made of Ti or Pd during glow-discharge experiments. ^{39}Cr detectors measured alpha particles in the range of 9 to 16 Mev. Similar high-energy alpha particles were also observed by them when a picosecond laser beam was focused on 30-micron thick TiH_2 or TiD_2 foils. At ICCF-9, this group had reported observing alphas in the 8 to 14 Mev range using dE-E SSB detector from previously loaded Au/Pd/PdO multilayer, thin films mounted in a vaccuum chamber during the exothermic desorption phase. Thus, emission of high-energy alphas, irrespective of the foil-loading technique or excitation technique or whether the loading is with hydrogen or deuterium, has been corroborated by different groups.

A noteworthy feature of the Boston meeting was the absence of any paper pertaining to the measurement of neutron yield, indicating the diminished importance of neutrons as a diagnostic tool in the new dispensation of CMNS physics.

The emphasis in the immediate future is clearly the understanding of the characteristics of the NAE and the theoretical basis of the phenomena. There is tremendous scope and challenges for the young researcher who enters the field at this juncture.

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RESEARCH NEWS

Pentacoordinated phosphorus in action

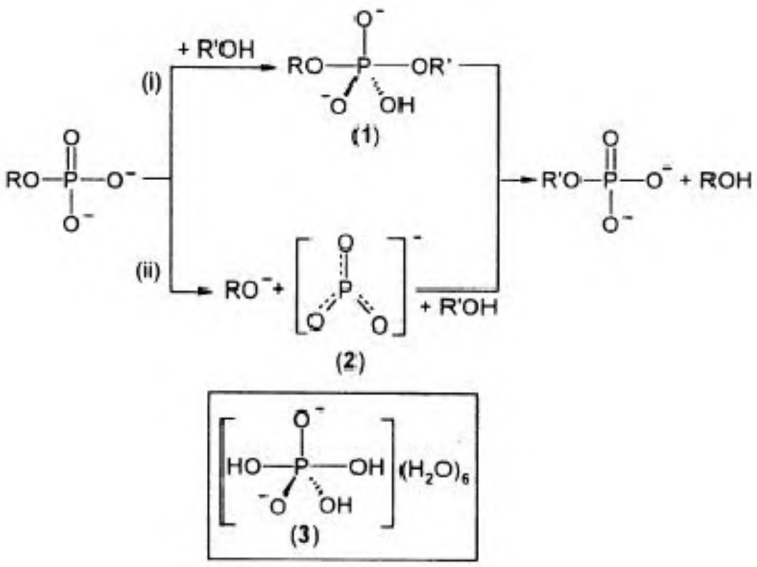
K. C. Kumara Swamy and N. Satish Kumar

The recognition that phosphates play a central role in the living world gave rise to extensive kinetic and mechanistic studies on solvolytic reactions of simple phosphoric acid esters as well as biochemical reactions catalyzed by enzymes like phosphatases, ribonucleases, mutases, etc.[1,2]. Nucleophilic displacements (e.g. solvolysis/transesterification) on phosphorus are involved virtually in every aspect of cellular energetics and many aspects of biosynthesis. Two basic pathways for these reactions involving phosphate monoesters are shown in Scheme 1. Variations in the intricate details of the mechanism are possible depending on the details we are interested in[1] but the focus is on whether the pentacoordinated species (**1**) or monomeric metaphosphate species (**2**) is involved. In contrast to carbon which can form only four stable covalent bonds, phosphorus is able to form five. Hence, while the nucleophilic attack on carbon leads to a transient five-bonded transition state, attack on phosphorus could produce a relatively long-lived pentacovalent intermediate. Numerous *neutral* and a few *monoanionic* pentacoordinated phosphorus compounds have been well characterized in the small molecule domain[3,4]. However, it is perceived that dianionic phosphorane intermediates like **1** do not exist as stable species in the gas phase, unless the – 2 charge is effectively delocalized[2,5]. Thus the solvated form of the latter with six water molecules (**3**) is predicted to exist as a TBP species in the gas phase. The metaphosphate (**2**) is stable in the gaseous phase, but not in aqueous solutions. It has been a dream for many biochemists to 'catch' species such as **1** or **2** in true bio-systems and structurally characterize them. This highlight refers

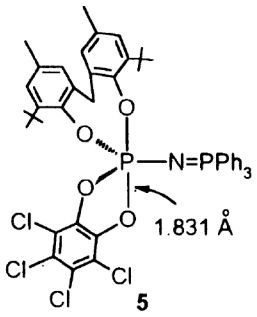
primarily to the recent report on 'pentacovalent phosphorus intermediate' of type **1** in a phosphoryl transfer process[6].

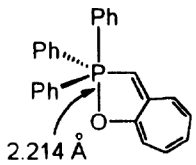
Phosphoglucomutase (PGM) catalyses the interconversion of glucose 1-phosphate and glucose-6-phosphate via glucose 1,6-bisphosphate and is an important reaction that bridges glycogen metabolism and glycolysis. In the first step, the phosphoryl group is transferred from Asp8-carboxylate end to the free hydroxyl of the hexose phosphate substrate to form the glucose 1,6-bisphosphate (Figure 1 *a*). What Lahiri *et al.*[6] reported is the X-ray structure of a stabilized pentacovalent phosphorane **4** (with a trigonal bipyramidal geometry) in this step (Figure 1 *b*). It is consistent with process (i) shown in Scheme 1. The structure is stabilized by a large number of hydrogen bonds and the phosphoryl oxygen coordination to magnesium, thus delocalizing the – 2 charge at this phosphorus centre. Thus this note reports a significant breakthrough in our understanding of these biochemical processes.

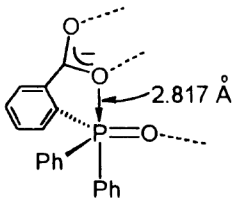
The apical P–O bond distances of 2.0–2.1 Å for the trigonal bipyramidal phosphorus in **4** are longer than those observed usually in neutral compounds and this has been the subject of some discussion[7]. In known structures of *penta*oxyphosphoranes the P–O distances fall in the range 1.57–1.76 Å (Cambridge data-base, November 2002). Examples of pentacovalent phosphoranes with longer apical P–O distances (e.g. **5** and **6**) do exist, however[4b,8]. This distance could still be longer, depending on the substituents and on the extent to which there is contribution from coordinate covalent character (cf. **7**)[9]. If the equatorial P=O oxygen is involved in strong hydrogen bonding or if it abstracts a proton (with lengthening of this bond; cf. hydrogen bonded structure **7**), the apical O → P coordinate bond length can approach the covalent bond distance with eventual reorganization of the surrounding protons. The reverse also could happen. The structure of **4** is *anionic* around phosphorus with a carboxylate oxygen as one of the apical atoms, and

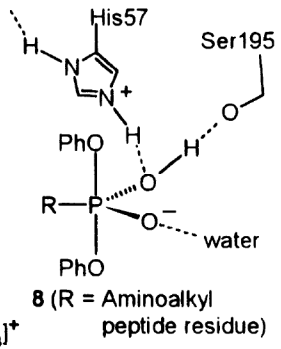


Scheme 1.









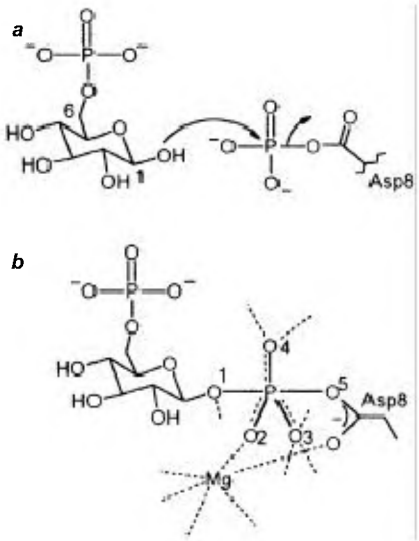


Figure 1. ***a***, First step in the interconversion of glucose-1-phosphate to glucose 6-phosphate. ***b***, A simplified drawing of the β-glucose-1,6-(bis)phosphate intermediate (**4**) structure in the active site of β-phosphoglucomutase at 1.2 Å resolution (data from ref. 6). The extra hatched-line bonds from O(1), O(3) and O(4) at the pentacoordinated phosphorus are hydrogen bonds; hydrogen bonds involving other oxygen atoms are not shown. Selected bond parameters: P–O(1) 2.0, P–O(2), P–O(3) and P–O(4) 1.7, P–O(5) 2.1Å. O(1)–P–O(5) 174°.

with significant hydrogen bonding. Thus the observed apical P–O bond lengths appear reasonable. Stabilization of penta-coordinated phosphorus intermediate through hydrogen bonding has also been demonstrated recently in the X-ray structure of the complex (**8**) of human α-thrombin with the inhibitor (α-aminoalkyl) phosphonate[10].

It is important to note that the first direct observation of metaphosphate **2** in a condensed aqueous phase in the active site of fructose-1,6-bisphosphatase is also reported early this year[11]. Fructose-1,6-bisphosphatase (FBPase) is a key regulatory enzyme that catalyses the hydrolysis of fructose 1,6-bisphosphate to fructose-6-phosphate and orthophosphate (PO_4^{3-}). An X-ray structural study by Honzatko and coworkers reveals that while crystals of FBPase grown at neutral pH have an orthophosphate at the active site, those grown at pH 9.6 (or higher concentrations of K^+) have metaphosphate and water (or OH^-) in equilibrium with each other.

It remains to be seen how the structural evidence thus obtained for both the transition state species (intermediates (?)) proposed in Scheme 1 in *pure biochemical processes* can be gainfully exploited in future.

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Transplanting the fish

T. J. Pandian

Cryopreservation of fish eggs and embryos has been unsuccessful[1]. Establishing a transgenic strain in a species of conservation and/or commercial importance demands time, labour and money, as the site of integration of a foreign gene in the chromosome of the host remains uncontrollable and this results in unpredictable expression[2]. To overcome these difficulties, Yoshizaki has achieved – what I proposed to call – transplanting the fish.

Primordial germ cell (PGC) is the progenitor of the germ cell lineage and is committed to differentiate into either spermatogonia or oogonia after the completion of gonadal differentiation[3]; as such the PGCs have the potential to develop into complete individuals. Hence they were extracted from the rainbow trout embryos, marked with green fluorescent protein (GFP) gene and transferred into host fry of the same species or closely related species. Because the *vasa* transcripts are restricted to germ cell lineage, a transgenic strain carrying *GFP* gene driven by the *vasa* regulatory regions was generated[4]. Consequently, the expression of *GFP* gene was limited to the PGCs alone[5,6]. The genital ridge, isolated from the transgenic embryos, was dissociated by trypsin and flow cytometrically sorted into GFP-positive and GFP-negative cells. On transplantation of these exogenous GFP-positive cells into the peritoneal cavity of the recipient hatchlings, the exogenous GFP-positive cells were incorporated into the genital ridge of about 20% host fry, very much like the endogenous PGCs. Subsequently, 4% of the exogenous cells proliferated, underwent meiosis and differentiated into eggs and sperm, in synchrony with the endogenous PGCs. Thus the donor-driven gametes produced the normal progenies through fertilization.

This new technique provides the scope for transgenesis, and *ex situ* conservation of PGCs, from which progeny of the concerned species can be derived. The introduction of foreign gene into the PGCs and selection of transformants, using selected markers, facilitate the *in vitro* selection of transformants. The method is advantageous over the conventional transgenic technique, as cells carrying foreign gene can be selected in a petri dish, instead of rearing hundreds of fishes and making a large number of DNA analyses to identify the suitable/desired candidate transgenic. The selected transformant can then be developed into an individual fish, using chimera technique[7].

Yoshizaki proposes to rapidly mass produce tuna sperm and eggs by transplanting its PGCs into *sterile* mackerels, which are cheaper to maintain, and to conserve fish species by cryopreserving their PGCs. Not only Yoshizaki, but also another group led by Strussmann is on the job. During the early nineties, he introduced sperm cells into *sterile* pejerrey *Odontesthes argentinensis* and found them to be alive until the 10th week after introduction. In view of the global warming and thermal pollution from nuclear plants, he was attracted to devoting more of his time on sex determination, differentiation and reversal; he is one, who has made several, impressive contributions on thermal impact on sex reversal in fishes. However, Robert J. Gold, a student of Strussmann is now engaged in deriving sperm from sterile testis of a recipient, into which bits and pieces of testis of the donor species have been