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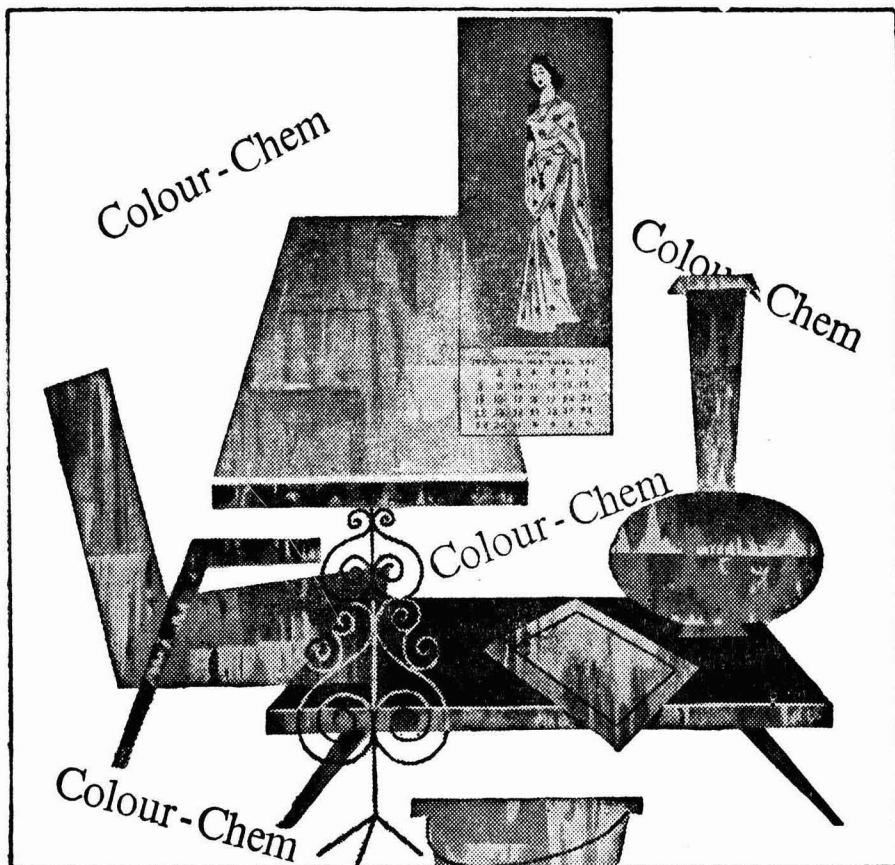
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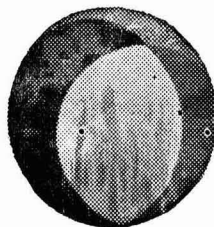
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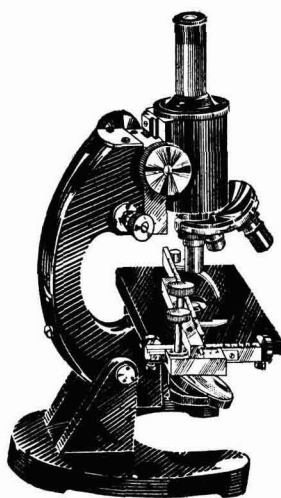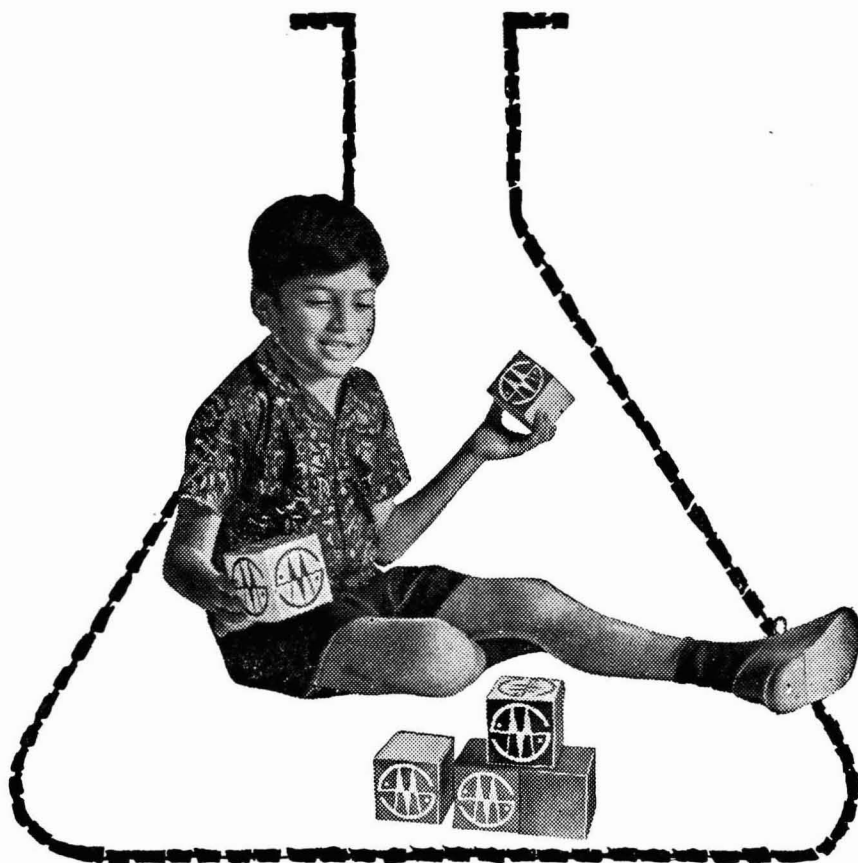
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Current Topics

Science, Technology & the Future*

THERE are two points I would like to make about science and technology. I always find it hard to convince people that the present state of affairs is not the end of the road. We all have an instinctive feeling that the present moment of time is the ultimate moment of time. We like to believe that our present knowledge is more or less complete and that the future world will not be much different from the world we know today. But this is an illusion. Nothing is more certain than that the future will be greatly different from the present.

This should be a matter of great encouragement to you here in India. If everything were known all that would be open to you would be to catch up with the leader — the United States of America — and merely catching up is never a very inspiring business. But if there is to be no end to scientific development you can already cast your thoughts ahead. In the long run those who organize themselves most sensibly will go farthest. Any nation that makes its decisions wisely today can be a leader in the world of tomorrow.

My second point is that technological development is not an ultimate end in itself. America has proved this to be so. Since the last war, America has carried the brunt of the technological load for the whole world. In doing so it has been shown that technology for its own sake, wealth for its own sake, is not a satisfactory goal in life.

Science is different here from technology. For science is basically an expression of our curiosity about the world. It is true that science leads to technology, but this is really as a byproduct; it is not the main aim. To find out about the world we live in is essentially a religious impulse. In fact the religions of the past have largely been derived from ideas about the structure of the world. Today we know far more than was known in the past, but our impulses are the same as those of our forefathers.

Perhaps this is why we in Britain have recently given special priority to the study of astronomy. This is in spite of the fact that very much like you in India there is a strong technological drive now in Britain. I think you will find it interesting that in the midst of this technological drive the Science Research Council has given its highest priority to astronomy. Already we are building the world's largest partially steerable radiotelescope, with the enormous area of 10 square miles. We hope to follow this at Jodrell Bank with a fully steerable radiotelescope even larger than the present 250 ft instrument. In Australia, in

cooperation with the Australians, we are constructing a large optical telescope — not quite so large as the Mt Palomar telescope, but not much smaller either. Again we hope to follow this venture by a corresponding instrument in the northern hemisphere. Taken over 10 years, investment on this scale in order to find out about the world is an expression of great confidence in the future, confidence that contrasts strongly with student riots and other social upheavals. It is gratifying to me at least to find that any clouds there may appear to be in our society have a silver lining — at any rate so far as astronomy is concerned.

It is also gratifying to me to be with you today to receive the Kalinga Prize. There is a particularly fitting connection between the hopeful future for astronomy, which I have just been describing, and being the recipient of this prize. The scientist, unless he is concerned with urgent practical problems, must be prepared to earn his keep in other ways — and perhaps the best way he can do so is by arousing the interest and curiosity of his fellowmen. I said interest and curiosity — not inspiration — for inspiration would be to go far beyond anything of which I myself am capable. Nevertheless science itself can be inspiring, not only to the scientist himself but to everybody.

I would like to repeat my thanks and appreciation both of this prize, and also of the warmth with which I have been received everywhere in India, by ending with just such an inspired picture. I have in mind the picture taken by the astronauts of the Apollo 8 mission to the moon. It is not the moon, however, that I am thinking about now. I am sure you will remember the picture of the earth as it appears from a great distance, a shimmering blue globe, half covered by constantly changing swirling patterns of white cloud.

If you were a traveller from outer space newly arrived in our solar system it would need but a glance to see that of the 9 planets of our system the earth is wholly outstanding, outstanding in its ceaseless varying activity, outstanding in its remarkable beauty. You would feel that those who lived on this glowing blue jewel should indeed count themselves immensely fortunate.

This is the thought I would like to leave you with. Our planet has spun on its way now for many hundreds of millions of years. Life in astonishing forms has emerged slowly and often painfully. In a very real sense we humans have become the trustees of life on this planet. It is important above all things that we should accept our responsibility and that we should not allow ourselves to become too preoccupied with the rivalries and passions of the present. We should always remember the long past. We should see our existence here as a privileged one, and we should see the future as an exciting adventure.

*Address delivered by Prof Fred Hoyle at the function held in New Delhi on 22 February 1969 for the presentation of the 1968 Kalinga Prize to him.

Quantum Mechanics & General Relativity—An Appraisal for Their Synthesis

B. S. MADHAVARAO

THE problem of bringing about a synthesis between general relativity and quantum mechanics is perhaps as old as quantum mechanics itself. Einstein himself was obviously the first to tackle this problem. He, however, did not attempt to quantize relativity, but to relativize quantum mechanics; in other words, he attempted to build up a unified field theory describing at the same time the gravitational and electromagnetic forces, which were the only ones known at that time. Following him, there were many others working on this problem of unification[1], but without success. In any case, from what we know today such attempts were premature, since no account was taken of many other interactions as significant as the two known at that time. After these early attempts interest in general relativity seems to have almost completely waned. During the last decade, however, there had been a resurgence of interest in the subject mainly due to the development of new mathematical techniques for obtaining solutions of Einstein's equations, tackling of problems relating to gravitational radiation and quantization of gravitation, devising new experimental tests of the null type and those based on the Mössbauer effect, and not the least to the interest taken by the builders of quantum theory, specially Dirac[2,3] in topics of general relativity.

Need for a Synthesis of the Two Theories

It might be pointed at the outset that doubts have been expressed in some quarters even about the possibility of bringing about a synthesis between the two theories from a strictly logical standpoint because of the quantum limitations of the concepts of general relativity[4,5]. But such limitations may well be due to the inadequacy of quantum mechanics itself, specially quantum field theory for which no consistent expression has so far been obtained. Again, others have questioned the need for such a synthesis in view of the negligible role played by gravitation in particle physics, for example, as pointed out by Feynman[6], an atom made purely by gravitation, in particular of two neutrons thus held, has a Bohr radius of 10^8 light years!

In spite of these criticisms and doubts, most physicists (including those who have raised these points) will agree, one can be sure, that a synthesis is a real need for the progress of theoretical physics. It is hard to reconcile oneself to the idea that classical gravitational fields, and quantum fields of several types can exist side by side without a unification being attempted between them. Further, there is the plain fact that such an investigation has real physical interest so that, in spite of other considerations, it is a topic worth pursuing. Although a complete fusion may be difficult to reach, an attempt at synthesis might perhaps indicate a meeting point of what appear to be two different views of physical reality.

Comparison of the Two Theories

Quantum theory is essentially linear in nature, while non-linearity is inherent in the theory of gravitation from the very nature of its build-up. Moreover, this non-linearity is of a very complicated nature, and compared to it the non-linearity of equations in other branches of physics, for example, the non-linearity of the Navier-Stokes equations of fluid mechanics, is of a comparatively simple nature, and its presence can be explicitly indicated. To give another example, we might consider the Born-Infeld electrodynamics which is non-linear just involving a square root. General action functions from which this theory can be deduced have been obtained[7,8], and it has been shown that the theory can be quantized in agreement with special relativity, but not general relativity[9,10], thus exhibiting the still deeper character of the non-linearity of general relativity.

Relation to microscopic and macroscopic phenomena— A second characteristic difference is the relationship to microscopic and macroscopic phenomena. Quantum mechanics taken in conjunction with special relativity has been able to initiate spectacular progress in the field of elementary particles as is well known. General relativity, taken in conjunction with Mach's principle, has made substantial contributions to cosmology which are, perhaps, not so well known. This principle asserts that, assuming the notion of relativity of motion, the inertial forces appearing in an accelerated laboratory have their origin in the distant matter in the universe, and is of much significance to cosmology. In fact, this principle can be used to obtain a generalization of Einstein's theory — which uses only a single tensor field — by postulating the existence of a scalar field also[11], a point which has also been emphasized by Synge[12]. Further, the suggestion first put forward by Dirac[13] that the gravitational constant G is decreasing with time is in consonance with Mach's principle, and of great significance to cosmology. Mention might also be made, in this connection, of the interesting result of Sciama[14] that G is related to the mass distribution in a uniformly expanding universe as $GM/Rc^2 \sim 1$, and of the interpretations of $(\hbar c/G)^{1/2}$, $(G\hbar/c^3)^{1/2}$ and $(G\hbar/c^5)^{1/2}$ as gravitational mass, and the invariant length and time units respectively[11]. Such cosmological considerations being macroscopic in nature lean heavily on general relativity, and do not obviously have any direct relationship to quantum mechanics. But the importance of quantum physics to astrophysics has been known for a long time, thanks to the important contributions of Saha, Chandrasekhar, Bethe, Gamow, and others. During

the last five or six years there have been signs of astrophysics and general relativity influencing each other profoundly, owing to the discovery and investigations relating to quasi-stellar radio sources, explosions in galactic nuclei, and X-ray emission from supernova remnants. These investigations have suggested that strong gravitational fields may, after all, play an important part in astrophysical phenomena. As an example, we might mention the recent work of Chandrasekhar[15] showing that relativistic gravitational forces cause radial instabilities in stellar models. Since, as mentioned above, the cosmological structure of the universe is governed by general relativity one should also be able to derive some cosmological aspects of the phenomena of high energy astrophysics[16,17]. Such phenomena include not only cases where large amounts of energy are involved, but also cases where the rate of energy release per second is very high. A predicted example of this nature[18] is the weak interaction leptonic process $e^+ + e^- \rightarrow \nu_e + \bar{\nu}_e$, which perhaps represents the first case of a weak current interacting with itself. This process appears to play a very important role in recent developments of ‘neutrino astrophysics’[17], round which is built a ‘neutrino cosmology’[19].

Role of experiments — A third comparison may be made of the role of experiments in the two theories. The situation regarding experiments in general relativity appears almost *orthogonal* to that in high energy physics governed by quantum phenomena. In this domain we have a plethora of experiments conducted to test the numerous theories built up, and conversely theories are built up to explain experimental facts. We had recently a remarkable example of this coordination between theory and experiment in the successful application of the $SU(3)$ group by Gellman and Ne’eman[20] to predict the existence of the Ω^- baryon, later confirmed by experiment. But, such an intimate relationship between theory and experiment is lacking in general relativity. It is not as if there were not enough types of theoretical results to be dealt with. On the other hand, an enormous number of different types of solutions of Einstein’s equations have been recently developed. But the difficulty has been to interpret and analyse them, and to investigate which of them can be brought within the range of experimental verification. Thus, for example, it has not been possible to fit even the simplest type of the flat space solution (with $T^{\mu\nu} = 0$) which has been proved unique by Lichnerowicz[21], into some kind of an experimental set-up. The situation is ironical in that the more one tries to investigate a particular theoretical aspect of general relativity, the more one gets into other more complicated theoretical situations. This aspect is clearly brought into focus by the question relating to the existence of gravitational radiation[22]. This is a typical example where, unlike in quantum mechanics, there is no experiment to guide the theorist. Consequently, there has been a large disagreement even on the very existence of gravitational radiation. Of the three famous experimental tests of general relativity, the most significant one is that relating to the red shift, and a recent experiment

of Pound and Rebka[23] based on the Mössbauer effect gives the best verification. In addition to another accurate null type test[11], besides the Eötvös experiment, we have other types[24] of a more sophisticated nature. In spite of all this, the situation regarding experimental verification cannot be considered quite satisfactory. General relativity is a beautiful theory, based on pure geometric intuition, and it would be a pity if, hypothetically, after another half a century of work by able theorists, not guided by the experimentalists, one would find that the theory is defective due to some basic incorrect assumption, and has therefore to be abandoned !

Role of group concepts — The last point to be considered here is one having a pure mathematical interest, namely the role of group concepts in the two theories. As is well known, group theory and the theory of group representations play a powerful role in quantum mechanics, specially in the field of elementary particles. A large number of concrete groups, like the $SU(3)$ mentioned earlier, are being used to explain particle symmetries, and derive more of them. General relativity, on the other hand, does not deal so extensively with several types of concrete groups, but is content with the use of the abstract notions of general group theory. Thus the group of general coordinate transformations may be called the Einstein group, but this would be a function group which is simple and hence would be of no avail in introducing quantum concepts. Barring the Lorentz and Poincaré groups of special relativity, there are just two special cases in general relativity where the group concept has some meaning. The first one is that relating to Riemann spaces of constant curvature[25], where one can introduce a group of motions different from that of flat space, but having the same number of parameters, and reducing to the Poincaré group in the case of limiting zero curvature. Some kind of a quantum mechanical formalism relating to elementary particles can be set up in this space by considering the representations of the group. Nevertheless, such a development would be only of limited interest.

The second example is that relating to another particular case of a so-called truncated theory obtained by imposing suitable boundary conditions at infinity so as to enable the theory to deal with the problem of gravitational radiation. One can introduce in this theory the generalized Bondi-Metzner group[26], study its several properties and those of its several subgroups, specially the one which is suitable to be considered as an *internal symmetry group* in consonance with the conclusions of O’Raifeartigh[27]. No definite results have, however, been obtained regarding the existence or otherwise of gravitational radiation by the use of these groups, probably because of the fact that no representations of this group have been employed.

Attempts at Quantization of General Relativity

Numerous attempts have been made in this direction under several headings like quantization of gravitation, of geometry, and of relativity. It

would take us far into this specialized field if we were to give a comprehensive account of all these theories. But the plain fact is that no successful theory has so far been developed for the general case. We can only classify the several theories into two categories which can be roughly described as (i) linearized theories and (ii) semi-quantized theories. The first type considers an idealized Lorentz space, and treats the departure of the actual metric from this flat condition as small, ie one linearizes the gravitational field equations and applies the standard methods of field quantization. Again, in other words, the gravitational field is treated within the framework of the ordinary Lorentz-invariant field theory as a zero mass, chargeless tensor field. Such theories, using perturbation methods, have been developed by Gupta[28,29], Rosen[30], Feynman[6], and Thirring[31]. Gupta's theory interprets the gravitational field as an ordinary tensor interaction, and is non-trivial, since it opens up the possibilities of other types of long-range interactions of interest in cosmology. Feynman's theory is of interest in regard to the method of perturbation employed, going from one order to a next higher one, and providing a historical example of algebra being done on a calculating machine. There have been some criticisms[32] of the linearized treatment that such a version throws out just those features which are physically new and interesting. But such criticisms lose force unless it is pointed out what exactly these features are.

As an analogue or alternative to the linearized version might be mentioned the theory based on the commutation rules satisfied by the β-matrices appearing in the Dirac type relativistic wave equation corresponding to spin two, and showing that by considering a particular representation of the algebra generated by the commutation rules one can obtain the equations corresponding to a weak gravitational field[33]. A similar result leading to the linearized gravitational field has also been found by Brulin and Hjalmars[34].

As regards type (ii), these are concerned mainly with methods of bringing the equations of the classical gravitational field into a form which will be amenable to the techniques of quantization, ie passing from a c-number theory to a q-number theory[22]. Notions of the phase space of general relativity, coordinate conditions, time-dependent solutions, formulation of the classical part in Hamiltonian form, devising the appropriate Poisson bracket expressions, and construction of the constants of motion are all developed as preliminary techniques before embarking on quantization. The important formulation in terms of a Hamiltonian is due to Dirac, and constitutes a powerful tool. After this formulation of the c-number theory, type (ii) theories just indicate the possible types of procedure of going over to the q-number theory, and specially in view of the uncertainty in constructing such a theory based on physical concepts, the actual problem of quantization is postponed. In addition to these two types of theories, there have been numerous contributions of pure mathematical interest relating to the topology of the Hilbert space formulation of quantum mechanics, and the topology in a Riemannian four-space, but these do not appear to have any real physical significance.

Gravitation and Theory of Elementary Particles

The gravitation is almost a foreigner in the hierarchy of elementary particles, thanks to its negligible interaction with other particles. Perhaps, this is too simplified a view, specially in view of the lack of a proper theoretical background to present-day particle theories. There have been many successes in the domains of hadron symmetries, conservation and violation rules, types of interactions and their explanations, hydrodynamics, and weak interaction dynamics, using abstract quantum field theory (which perhaps includes S matrix theory), and there also group theoretical methods, although there are still many unsolved problems relating to CPT, weak dynamics, and so on. We are, however, now in the stage of dealing with the central problem of the structure of the baryons and mesons themselves. It is also likely that the leptons too have an internal structure, and we may soon arrive at the stage of examining this problem also. It, therefore, appears desirable to take stock of the soundness of the several theoretical techniques now being used.

Let us consider, first of all, the use of group theoretical notions. These have played a fundamental role in explaining particle symmetries, but it is surprising to find that many of these symmetries cannot be explained in terms of relativistic covariant formalism, specially in view of the fact that the first new type of particle, viz the positron, was the outcome of Dirac's relativistic formulation of quantum mechanics. Looking at the current literature on the subject, it looks as if the number of types of symmetry groups used perhaps exceeds the number of elementary particles! Next, let us consider the Regge pole theory. These poles were discovered[35] mathematically in the non-relativistic Schrödinger equation, and we find that Regge pole phenomenology is still mainly non-relativistic. The same is *partly* true of current algebra since, in this case, it can be shown that it is not difficult to present in a covariant manner the relativistic representations of local current algebra for baryon and meson states[18]. Finally, let us consider the quark model giving the structure of baryons as consisting of three quarks, and the mesons of two (ie a quark-antiquark pair). This naive model (although quarks have not so far been observed) has been surprisingly successful in describing the quantum states, and the transitions between them. But, here again, the treatment has been both relativistic and non-relativistic. To explain the internal constitution of baryons and mesons as models of non-relativistic bound states, ie that the quarks inside can move in a non-relativistic manner is quite unsatisfactory. It has been shown that relativistic quark models[18] can be set up, but it is difficult to understand some cases where both the covariant and the non-covariant formalisms give the same result. Thus it will be seen that the present state of the theory of elementary particles is rather empirical in nature leaning heavily on the

extent of development of experimental technology. An experimentally established fact no doubt yields certainty, but this certainty is a limited one. For, there is always the possibility that a given explanation of such a fact, no matter how satisfactory it appears at present, may be superseded by another explanatory hypothesis in the future. Moreover, a theoretical formulation which possesses inner coherence, ie which is not self-contradictory, and which agrees with experimentally determinable facts as, for example, the quantum theory and the theory of relativity, has an absolute quality which the empirically grounded hypotheses can never claim. A proper theory has, therefore, to be built up to explain and predict subnuclear phenomena of which we know so little. It may well be that in such a theory, the structure of elementary particles is dominated at extremely short distances by gravitational effects. Whether a future comprehensive theory to explain the new phenomena will be a modification of the present relativistic quantum theory, or will rely more on general relativity is a matter for conjecture.

Non-linear Quantum Mechanics

As mentioned earlier, one of the major hurdles in synthesizing quantum mechanics and general relativity is the non-linearity of the latter as against the linearity of the former. An alternate way of synthesis would be the replacement of the present quantum theory by a non-linear one. This view has been expressed by Heisenberg[36], who has pointed out the essential role played by non-linear problems in physics, and expressed the possibility of the fundamental investigations of particle physics being of a non-linear character. The question of synthesis has, therefore, to wait for the building up of such a theory. But the question immediately arises as to what are to be the fundamental postulates and cardinal principles on which a non-linear quantum theory can be built. While linearity is unique, non-linearity can be of infinite types, providing literally an embarrassment of riches which we do not know how to utilize. In fact, even in present-day theoretical work in particle physics, non-linearities of several types appear, and are dealt with techniques suitable for the occasion. It appears, therefore, preferable to allow the teams of particle physicists to proceed with their numerous methods of investigation, however inadequate theoretically, until a comprehensive theory is evolved by intensive researches in several fields and their coordination.

Summary

The essential principles underlying quantum mechanics and general relativity have been compared and contrasted. Methods of quantization of general relativity are briefly discussed. Relationships of cosmology and general relativity to astrophysics are indicated. In view of the present state of the theory of elementary particles being of an empirical nature, it is suggested that a synthesis between the two subjects may perhaps be achieved in future by the setting up of a rational non-linear theory of quantum mechanics.

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Nucleoside Synthesis*

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PROGRESS in nucleoside synthesis has been extensive as evidenced by the significant research activity in this area. Over the past few years several books[1-6] and reviews[7-9] have appeared on nucleosides. The purpose of this article is to present a critical assessment of the usefulness of various methods of nucleoside synthesis and to cover all the developments in this field which have emerged since the appearance of our earlier review in this journal[9]. This review is essentially complete through 1967.

As in the earlier review, the methods of preparation of the nucleosides are classified under the following heads: (a) metal salt method; (b) Hilbert-Johnson method; (c) trimethylsilyl method; (d) direct condensation of the purines with various sugars; and (e) nucleoside interconversions.

Metal Salt Method

Synthesis of a glycosyl derivative of a purine, or pyrimidine or other heterocycles — The coupling of a poly-O-acylglycosyl halide with the heavy metal derivative of the purine or pyrimidine or other heterocycles generally gives nucleosides which have a C_1-C_2 *trans* configuration, irrespective of the relative configuration at C_1-C_2 of the original halo sugar[10]. A glycosyl halide in which the hydroxy or amino group at C-2 is masked with a group which does not participate in the displacement of the halogen atom at C-1 yields either C_1-C_2 *cis* nucleoside or a mixture of *cis* and *trans* nucleosides[11-14]. Generally sugars get attached at N-9 position. However, certain purines, viz 3-benzylhypoxanthine[15], 2,8-bismethylmercapto-6-dimethylamino-, and 6-dimethylaminopurines yield 7-glucosylpurine derivatives[16-19].

This procedure, however, suffers from several disadvantages such as low yields, contamination by mercury[20,21], and inability of certain purines, eg hydroxy-, mercapto- and aminopurines, to undergo coupling[22]. The latter difficulty has been overcome in part by acylation or benzoylation of the amino group[23] or by the use of alkylthiopurines[18,24,25].

This method has been successfully employed for the synthesis of the following nucleoside derivatives: (i) 9-(2,3,5-tri-O-benzoyl-2-C-methyl-β-D-ribofuranosyl)-6-benzamidopurine[26], (ii) 6-furfurylthio-2,3,5-tri-O-benzoylnebularine[27].

The structure of mercury derivatives of purines used for preparing nucleosides has been studied by comparing their UV, IR and PMR spectra with those of the corresponding sodium salts and N-7 and N-9 alkylpurines[28]. The results indicate that the mercury derivatives are covalent and that the

compounds which give 7-glycosides have the mercury atom attached to N-7 of the purine ring, and those which yield 9-glycosides at N-9. (iii) 3-substituted 7-(tri-O-acetylglycosyl)hypoxanthines[29]. 3-β-D-Arabinofuranosyladenine was obtained during a large-scale preparation of 9-β-D-arabinofuranosyladenine by the condensation of 2,3,5-tri-O-benzyl-D-arabinofuranosyl chloride with chloromercuri-6-benzamidopurine[30].

In view of the occurrence of amino sugars in several nucleoside antibiotics, there is considerable interest in the synthesis of amino sugar nucleosides. The 2,4-dinitrophenyl group continues to be used as a protecting group of the amino function in amino sugar[31,32], but the bis(phenoxy)phosphinyl[33], trichloroacetyl[34] and trifluoroacetyl[34] groups are invaluable. The synthesis of 9-(2-amino-2-deoxy-β-D-glucofuranosyl)adenine has been achieved[32].

9-(4-Acetamido-4-deoxy-β-D-ribofuranosyl)adenine has been obtained by the condensation of 4-acetamido-5-O-acetyl-2,3-di-O-benzoyl-4-deoxy-D-ribofuranosyl chloride with chloromercuri 6-benzamidopurine or by the titanium tetrachloride catalysed reaction of 1-O-acetate with chloromercuri salt, followed by deacylation with methanolic sodium methoxide[35].

The silver and mercury salts of pyrimidines yield different products with the same glycosyl halide. Unexpectedly, only the glycoside with an oxygen attachment (Chart 1) is formed in the reaction of 3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide with mercury salt of N-acetylcytosine[36] or thymine[37].
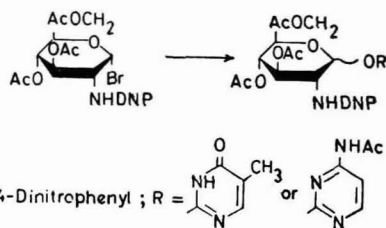
Chart 1 — O-Glycoside formation with mercury salts of pyrimidines

However, the condensation of the mercury salt of 4-(p-nitrobenzoyl)cytosine with 1-halo derivatives of D-glucosamine (I) gives the corresponding 4-(p-nitrobenzoyl)cytosine nucleoside derivative (II)[38]; the syntheses of 1-β-D-arabinofuranosyl-5-fluorouracil[39], 3-β-D-ribofuranosylorotic acid[40], orotidine[40], 5-fluoro-2'-deoxycytine[41], and 5-cyanouridine[42] have also been reported.

O→N-Glycosyl rearrangements in nucleosides catalysed by general Lewis acids have been further

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I

II

R = CH$_3$ or C$_6$H$_5$CH$_2$
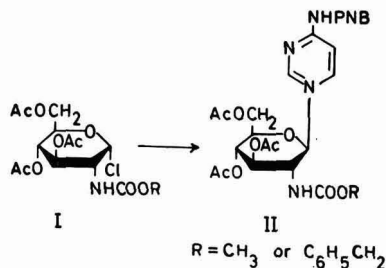
investigated. Garg and Ulbricht[36] found that the β-O-glycoside of N-acetylcytosine does not rearrange to the N-isomer. Reisser and Pfleiderer[43] have also reported the failure of rearrangement of the β-O-glucoside of 2-hydroxypyrazine to the N-isomer. The mechanism proposed earlier[44] is shown to be valid for these anomalies. The rearrangements of β-O-glucoside of 5-nitro-2-pyridone (III) to β-N-glucoside (IV)[45], O-glycoside of thymine (V) to N-glycoside (VI)[37] have been achieved.

III

IV

V

VI
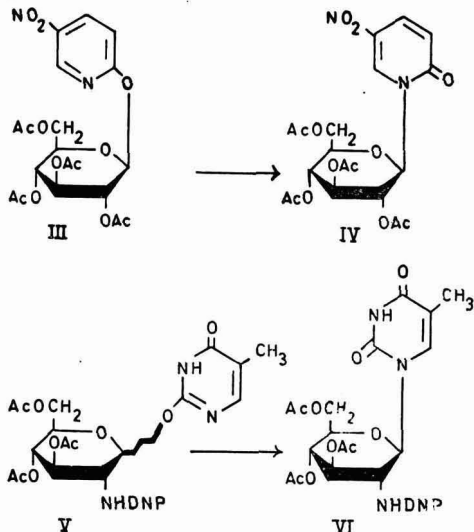
The order of activity of different reagents in bringing about the rearrangement of a pyrimidine O-glycoside is as follows: SnCl$_4$ ≫ HgCl$_2$ ≈ HgBr$_2$ ≫ HgI$_2$ > AgCl$_4$ (ref 46).

Hilbert-Johnson Method

This procedure[47] involving the condensation of 2,4-dialkoxypyrimidines with acetylglycosyl halides was employed by several workers[48-54]. The predominant (if not the sole) product, in the case of sugars bearing a 2-acyloxy function, is the C$_1$-C$_2$ trans anomer. However, it has recently been demonstrated that in certain cases the ' trans rule ' is not wholly operative and mixtures of anomeric nucleosides by this procedure have been obtained[55,56].

This method has also some practical limitations and is not applicable to certain pyrimidines. Condensation of tetra-O-acetyl-α-D-glucopyranosyl bromide with the ethoxy derivatives of 6-methyluracil and 6-methyl-2-thiouracil yielded, in each case, both α- and β-isomers of the glucosides but no N-glucosylamines (nucleosides)[57]. Neither glucosides nor N-glucosylamines were obtained when the ethoxy derivatives of 2-thiouracil and barbituric acid respectively[57] were allowed to react with tetra-O-acetyl-α-D-glucopyranosyl bromide. Similar failures are reported in the case of 2-methoxy-4-aminopyrimidine[58] and 6-carboxyuracil (orotic acid)[59]. Further, the method is also slow and requires a prolonged heating of the reaction mixture. Somewhat better results have been registered with protected glycosyl halogenides which are sufficiently stable at elevated temperatures[60,61] or in the reaction utilizing a solvent medium[62].

Utilizing the Hilbert-Johnson method Prystaš and Šorm, using 5-cyano-[42], 5-nitro-[42] and 5-acetamido-2,4-dimethoxypyrimidines[42] and 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride, obtained 5-cyano-, 5-nitro- and 5-acetamido-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-4-methoxy-2(1H)-pyrimidinone.

The reactivity of the substituted pyrimidines has been shown to be in the order: dibenzyloxy> di-(p-methoxybenzyloxy) > dimethoxy> diethoxy> di-(2,4-dimethoxybenzyloxy)>diisopropoxy[62a].

Anomeric 1-(2-deoxy-D-ribofuranosyl)thymines[63], 1-β-D-arabinofuranosylthymine[64], and 1-(3-deoxy-D-ribofuranosyl) derivatives of uracil, cytosine, thymine and 5-methylcytosine have been synthesized[65]. A synthesis of pyrimidine nucleosides of D-glucuronic acid has been reported[66].

Condensation of 2,3,5-tri-O-acetyl-4-thio-D-ribofuranosyl chloride with 2,4-diethoxy-5-methylpyrimidine followed by methanolysis produces the α- and β-anomers of 1-(4-thio-D-ribofuranosyl)thymine[67].

Trimethylsilyl Method

Trimethylsilyl derivatives of pyrimidine and purine bases are either distillable liquids or crystals with low melting points. Moreover, they are easily soluble in non-polar solvents such as benzene, toluene and carbon tetrachloride and are completely decomposed with alcohol or water. This method[68-72] gives a mixture of α- and β-nucleosides having both C$_1$-C$_2$ cis and trans configurations. C$_1$-C$_2$ cis type compounds have scarcely been obtained[13].

The easy removal of the protecting group (trimethylsilyl) by treatment of the reaction mixture with aq ethanol affords a convenient method as compared to Hilbert-Johnson's procedure.

The major disadvantage of this procedure is that the fusion of the silyl derivatives with halo sugars is necessary because attempts to condense the silyl bases and halo sugars in boiling benzene or xylene have proved unsuccessful[69]. The following nucleosides have been obtained using this procedure.

Wolfrom and Bhat[37] synthesized 1-(2-amino-2-deoxy-β-D-glucopyranosyl)-thymine by the fusion of bis(trimethylsilyl)thymine with 3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide or 3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-D-glucopyranosyl halide or 3,4,6-tri-O-acetyl-2-deoxy-2-trichloroacetamido-D-glucopyranosyl halide[34], followed by removal of blocking

groups. Condensation of 3,4,6-tris-O-acetyl-2-deoxy-2-N[bis-(phenoxy)phosphinyl]-amino-α-D-glucopyranosyl bromide (VII) with bis(trimethylsilyl)cytosine yields the nucleoside (VIII)[33].
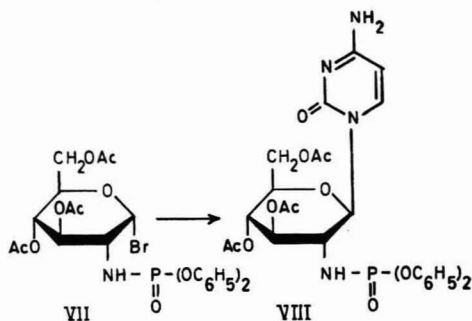
Bis(trimethylsilyl) ether of 5-(trifluoromethyl)-uracil[73] and 6-azauracil[74] have been condensed with 2-deoxy-substituted D-ribofuranosyl chlorides and 5-acetylmercapto-2,4-O-bis(trimethylsilyl)uracil with 3,5 - dichlorobenzoyl - 2 - deoxy - α - D - ribofuranosyl chloride[75].

Direct Condensation of Purines with Various Sugar Derivatives

A number of investigators[76-79] have reported the acid-catalysed fusion, under reduced pressure in the presence of an acidic catalyst such as sulphonic acid homologues or Lewis acids, etc, of an acyl sugar with various purines to provide the corresponding anomeric mixtures of nucleosides. In this procedure, neither acylhalo sugar derivatives nor heavy metallic salt of purine derivatives as starting materials are used. The overall operation is much more simple and in most instances the nucleosides are crystallized directly from the reaction mixture after removing the protecting groups from the sugar moiety. This method is, however, not applicable to all purines. Generally, in the case of purines having electron donating groups, eg $-OH$, $-SH$, or $-NH_2$, etc, the yields of resulting nucleosides are poor. On the other hand, some halogenopurines afford better results[79]. Except for these observations, no relationship between the reactivity of the purine derivatives and the electronegativity of the substituent groups in this reaction has been established. It may be attributed to the tendency of fusibility of the purine derivatives at suitable temperatures in the presence of a catalyst, which plays an important role in this reaction.

This reaction appears to take place via planar C-1 carbonium ion as the reaction intermediate. The reaction involves (i) initial attack of the acidic catalyst at 1-O-acyl group of sugar derivatives and (ii) the resulting carbonium cation of the sugar derivatives may suffer the attack of a purinyl anion. In these circumstances, two kinds of carbonium ions can be postulated as the possible reaction intermediates, viz planar C(1)-carbonium ion mentioned above and 1,2-*ortho*-ester type carbonium ion which has been regarded as the reaction intermediate in

the case of Fischer-Helferich's N-glycoside synthesis[80]. Its stability may be lower than that of the former in this reaction, according to the stereochemical consideration. Using this procedure, syntheses of several nucleoside derivatives have been achieved.

The synthesis of 3-(2'-deoxy-D-ribofuranosyl)adenine has been accomplished by reacting 1-chloro-3,5-di-O-(p-chlorobenzoyl)-2-deoxy-D-ribofuranoside with 7-pivaloyloxymethyladenine and deblocking the nucleoside (Chart 2)[81].
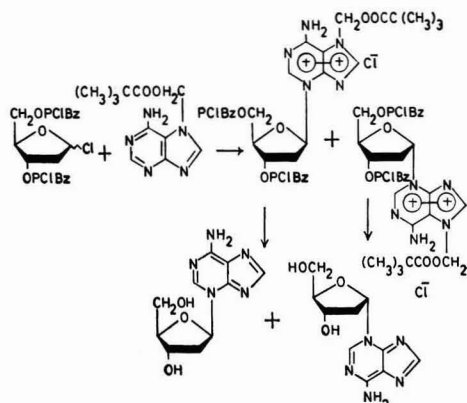
Chart 2 — Synthesis of 3-(2'-deoxy-D-ribofuranosyl)adenine

Onodera et al[82] have synthesized 1',2'-*cis* nucleosides using free 6-benzamidopurine and theophylline. The yields of 1',2'-*cis* nucleosides obtained are higher than those obtained by other procedures[83].

The synthesis of nucleoside (XI) by the fusion of 4-chloro-1-H-imidazo-[4,5-C]-pyridine (X) with 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (IX) and p-toluenesulphonic acid has been achieved[84].
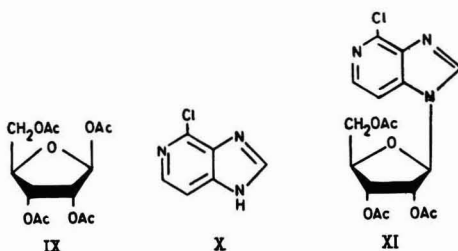
Rousseau et al[85] employed the fusion method for the synthesis of 3(4)-tri-O-acetyl-β-D-ribofuranosyl)-4(5)-bromo-5(4)nitroimidazole. Similar studies have been carried out to yield 9-(2,3,6-tri-O-acetyl-5 - deoxy- β - D - hexofuranosyl) - 2,6 - dichloropurine[86], nebularine[87] and 7-isonebularine[87].

Phosphorous pentoxide and sulphur trioxide are effectively used as dehydrating agents for the synthesis of nucleosides, but a few of the other common reagents, viz H_2SO_4, $ZnCl_2$, etc, are ineffective[88].

Nucleoside Interconversions

All the methods described above suffer from the disadvantage that all the purine or pyrimidine derivatives cannot undergo coupling in the prescribed manner. This difficulty has been surmounted by the interconversions of nucleosides. In this way conversions in the sugar moiety as well as in the base moiety have been achieved employing a number of techniques.

By oxidation — Several studies have appeared on the conversion of ribo- and gluconucleosides into 3-amino-3-deoxy-hexosyl nucleosides[89-92]. Periodate oxidation of 9-(β-D-ribofuranosyl) or 9-(β-D-glucopyranosyl)theophylline followed by condensation with nitromethane gives a mixture of isomers of gluco, manno and galacto configurations[91]. Hydrogenation of the nitro group with Pd/C gives the corresponding nucleosides (Chart 3)[91].

Extension of the reaction, whereby nucleoside dialdehydes are cyclized by nitroethane, produces branched chain nucleosides (Chart 4)[93].
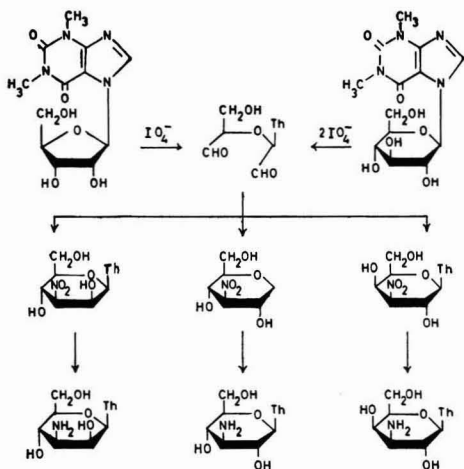
Chart 3 — Theopylline nucleoside of 3-amino-3-deoxy-β-D-glucose, mannose and galactose
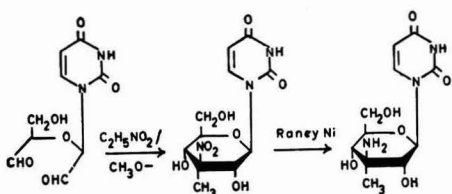
Chart 4 — Synthesis of branched chain uracil nucleoside

By anhydronucleosides — The utility of anhydronucleosides as routes for preparing other pyrimidine nucleoside analogues has been demonstrated by several workers[94-103]. The epimers of 2'-deoxyadenosine[94] and 3'-deoxyadenosine[94] have been obtained (Chart 5).
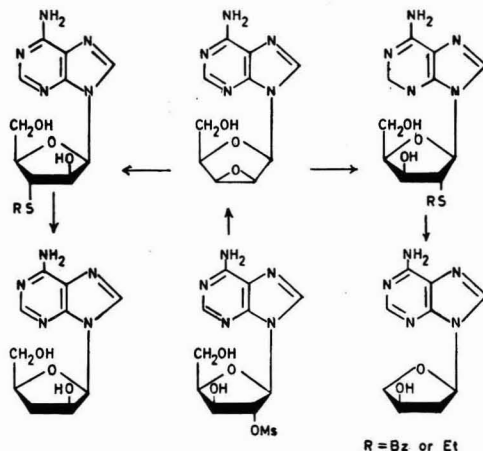
Chart 5 — Synthesis of epimers of 2'-deoxyadenosine and 3'-deoxyadenosine

N[4],O[3'],O[5']-Triacetyl-2,2'-anhydrocytidine (XII) has been postulated as an intermediate in a synthesis of 1-β-D-arabinofuranosylocytosine (XIII)[95].
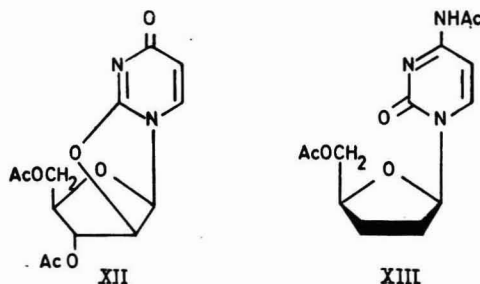
First synthesis of a nucleoside containing a 4',5' double bond, a structural feature that is present in nucleoside antibiotic angustmycin A (Chart 6)[96], has been achieved.
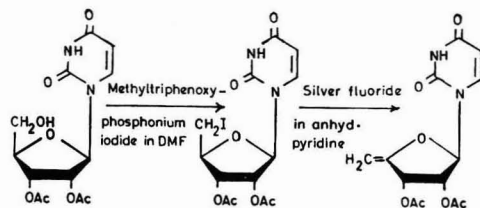
Chart 6 — Synthesis of 4',5'-unsaturated nucleoside

The use of base-catalysed elimination reactions has been successfully employed for the synthesis of 2',3'-dideoxyadenosine[99].

5'-O-Trityl-3'-O-tosyl-2'-deoxyadenosine has been converted to 3'-O-tosyl-2'-deoxyadenosine which in turn has been treated with ethanethiol to yield

115

6-amino-9-(3'-S-ethyl-3'-thio-2',3'-dideoxy-β-D-*threo*-pentofuranosyl)purine. Raney nickel desulphurization yields 2',3'-dideoxyadenosine also[97].

Treatment of 3'-O-tosyl-2'-deoxyadenosine with sodium ethoxide in ethanol affords a mixture of 6-amino-9-(2-deoxy-3,5-epoxy-β-D-*threo*-pentofuranosyl)purine and 6-amino-9-(2,3-dideoxy-2-*ene*-β-D-glyceropentofuranosyl)purine[98].

8,2'-Anhydro-8-hydroxy-9-β-D-arabinofuranosyladenine, the first purine cyclonucleotide having an O-anhydro linkage, has been reported.

Treatment of 5'-O-trityl-6-azauridine (**XIV**) with thiocarbonyldiimidazole furnished 1-(O²,2'-cyclo-5'-O-trityl-β-D-arabinofuranosyl)-6-azauracil[101] (**XV**).

XIV   **XV**
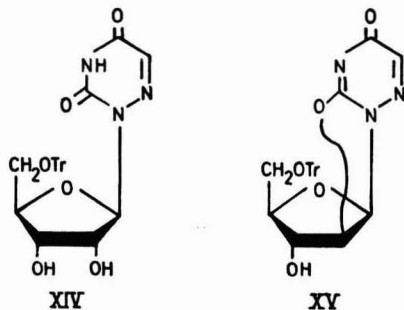
Horwitz *et al*[100] have reported the direct introduction of 2',3' unsaturation in the carbohydrate moiety of pyrimidine nucleoside, via base-catalysed elimination reaction (Chart 7).

Reaction of the 5'-O-trityl derivative of substituted uridine with thiocarbonyldiimidazole yields 2,2'-anhydro-1-(β-D-arabinofuranosyl)uracils. These derivatives have been converted into several other nucleosides[103].
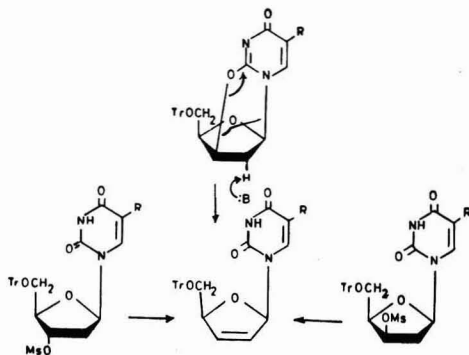
Chart 7 — Formation of 2',3'-unsaturated pyrimidine nucleoside via β-elimination reaction

By thiation of pyrimidine nucleosides — 2,4-Dithiouridine and 2-thiocytidine have been synthesized by Ueda *et al*[104].

The synthesis of 2'-O-methyl-2'-amino-9-β-D-ribofuranosyl-6-purinethione has been accomplished[105].

Ikehara and Muneyama[106], starting from 8-bromoguanosine, have synthesized 8-methylsulphonyl-, 8-dimethylamino- and 8-methoxyguanosines (Chart 8).
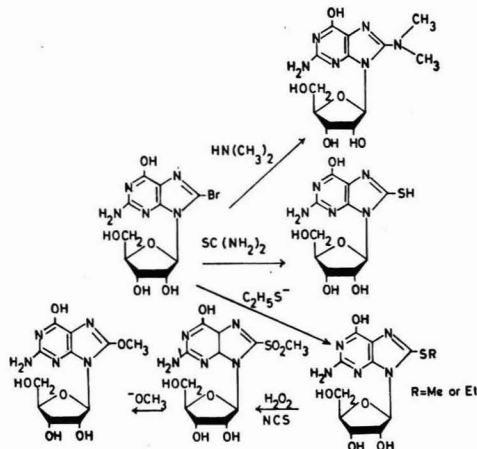
Chart 8 — Synthesis of 8-substituted guanosine derivatives

Other derivatives of nucleosides — A synthesis of purine nucleosides of D-glucuronic acid has been reported[107]. Synthesis of 9-β-D-glucopyranosyltheophyllines has been reported[108]. The synthesis of 9-β-D-ribofuranosyl-6-hydroxylaminopurine has been achieved by treatment of 9-β-D-ribofuranosyl-6-chloropurine with an excess of hydroxylamine[109].

Summary

An assessment has been made of the usefulness of different methods of nucleoside synthesis. Also the major developments in the field since the publication of the earlier review [*J. scient. ind. Res.*, **25** (1965), 404] are presented and discussed.

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Absorption & Transport in Insects

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THE absorption and transport of nutrients in insects, in so far as they have been studied up to date, present several differences in features from those observed in higher animals. Anatomical differences may account for some, but many more features still remain to be clarified adequately. Unlike the higher animals, insects are not endowed with the blood vascular system. The nutrients absorbed from the intestine pass directly into the haemolymph, which is constantly in circulation and which is in direct contact with the different organs and tissues like the muscle, fat body, tracheae, nervous system, etc. Another characteristic of this extracellular fluid is the relatively high amino acid and potassium content. The haemolymph, therefore, resembles closely the intracellular fluid in its osmotic properties. The insect tissues and organs function in a medium which closely resembles their internal environment, a situation not encountered in animals having a closed system of blood circulation. From this point of view, absorption and transport of nutrients in insects afford an interesting study.

The intestinal absorption of carbohydrates, amino acids and lipids was reviewed in detail by Treherne[1]; the same author[2] later reviewed the intestinal absorption of inorganic ions. Shaw and Stobbart[3] reviewed the role of active transport of water and ions in osmoregulation. An interesting aspect of the control of metabolism by active transport of potassium was discussed by Harvey and Haskell[4]. The comprehensive and excellent reviews on the metabolism of amino acids[5], carbohydrates[6] and lipids[7] in insects dealt with the aspects of their absorption and transport. The purpose of the present review is to present the salient features of the current status of our knowledge on the subject of carbohydrates, lipids and amino acids and to discuss some of the more recent contributions. Since this branch of study is still in its infancy, both as

regards knowledge on mechanisms as well as on the range of insects investigated, some of the ideas expressed in this review are more in the form of suggestions in need of further corroboration and provide a necessary nucleus for the crystallization of fresh thought and approach. Comprehensive citation of literature is not attempted in view of the earlier exhaustive reviews. Only literature having a direct bearing on mechanisms, especially the latest, is discussed.

Carbohydrates

Intestine — Insects are capable of efficient utilization of carbohydrates. The poly- and oligo-saccharides are broken down, presumably to mono-saccharides and these are absorbed from the midgut, almost completely. The digestion of carbohydrates has been studied intensively[8,9]. Appreciable contribution to the knowledge on carbohydrate digestion in insects was made by Saxena and his colleagues from India. Krishna and Saxena[10], Krishna[11], Saxena[12], and Saxena and Gandhi[13] have characterized the glucosidases and fructosidases from insect intestines. An additional significant finding was that the invertase of the gut of *Oxycarenus hyalinipennis* was a transglucosidase capable of transferring glucosyl units to sucrose, resulting in the formation of glucosucrose *in vivo*[14]. Since the investigators used crude extracts in the *in vitro* studies, the identity of the invertase with the enzymes synthesizing glucosucrose and other oligo-saccharides[15] is not established. The finding itself is, however, of interest and will be discussed later in this review.

The mechanism of absorption of glucose from the intestine was studied by Treherne in the cockroach *Periplaneta americana*[16] and in the locust *Schistocerca gregaria*[17-19] by using ^{14}C-glucose. In the cockroach, glucose absorption *in vivo* occurred mainly in the midgut caeca. The rate of absorption in this region was very fast, but the rate of crop emptying limited the passage of sugar to the

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absorptive region. Crop emptying was found to depend on osmotic concentration, the rate being slower at higher osmotic concentrations. Recently, Gelperin[20] made similar observations in the blow fly *Phormia regina* and showed that crop emptying was controlled by the osmotic pressure of the blood. In the locust, the absorption mechanism was studied in greater detail. The limitation posed by the slow rate of crop emptying was avoided by injecting the solution through the rectum. In concentrations ranging from 2 to 20 mM, the percentage absorption remained the same, but was reduced at 200 mM. Most of the absorbed glucose at 20 mM was traced to the blood sugar, the non-reducing disaccharide, trehalose. At 200 mM glucose level, its concentration in haemolymph showed an increase. The blood-lumen gradient diminished, thus decreasing the diffusion rate. Hence, a continuous uptake of sugar *in vivo* could be maintained by conversion to the comparatively impermeable disaccharide, trehalose. The absence of inhibition of transport with cyanide and iodoacetate confirmed the diffusion hypothesis. Also, the rates of absorption of glucose, fructose and mannose closely paralleled the insect's ability to convert them to trehalose[19]. Glucose, which was converted to trehalose most rapidly, was absorbed at a fast rate. From these investigations it was concluded that glucose is transported across the insect midgut by facilitated diffusion and not active transport.

The lack of a mechanism for active transport of glucose from the midgut of the silkworm *Bombyx mori* L. was demonstrated *in vitro* by a modified perfusion apparatus[21] using ^{14}C-glucose. *In vitro* techniques are clearly helpful in the study of the behaviour of isolated organs and concentration build-up of glucose could not be demonstrated in this insect as well as *Poecilocerous pictus* adults. However, appreciable fluid transport was found to occur from the surrounding medium into the lumen. This was inhibited by 2,4-dinitrophenol. Glucose transport, when unaccompanied by solvent flow, was not inhibited by dinitrophenol. *In vivo*, most of the orally administered ^{14}C-glucose was absorbed and converted to trehalose. Perfusion experiments carried out by Randall and Derr[22] on the grasshopper *Melanoplus differentialis* and by Gelperin[20] on *Phormia regina* also confirmed the absence of active glucose transport in insects.

If conversion to trehalose is taken as an essential step for the assimilation of glucose, absence of active transport in insects possessing low haemolymph trehalose but high glucose and fructose levels poses problems for assimilation. In such cases, perhaps, the more conventional form of carbohydrate storage via conversion to glycogen might be utilized. In fact, a close regulation of glycogen and trehalose levels is achieved even in insects having high trehalose levels[23] and several alternate mechanisms are available for the regulation of glucose, trehalose and glycogen levels[6,24].

Briefly stated, the main steps in the transport of dietary carbohydrate in insects consist of the diffusion of glucose from the intestine, its rapid conversion to trehalose in the fat body and release into haemolymph. In the blow fly, eg 50% of injected

[14]C-glucose was incorporated into haemolymph trehalose after 2 min and 90% after 10 min[25]. The back-diffusion of trehalose into the intestinal lumen is a much slower process and is further curtailed by intestinal trehalase. A regulation of the entry of dietary carbohydrate into haemolymph is suggested by the findings of Duspiva[26], Saxena and Bhatnagar[14] and Bhatnagar[15] on the conversion of disaccharides to oligosaccharides by the intestines. Such a conversion would slow down the entry of the oligosaccharide either as such or after breakdown to the mono- or disaccharide stage, thus providing ample time for the assimilation of glucose in the fat body at relatively high levels of dietary sugar. At low concentrations, the operation of this mechanism may not be warranted. Evidence for the absorption of di- and trisaccharides has been found by Maurizio[27].

Fat body — Although this tissue happens to be the major organ for carbohydrate metabolism, very little is known about the absorption and release mechanisms. It is not known whether glucose (or glucose-1-phosphate) necessary for the synthesis of trehalose and glycogen is actively transported or not. The extremely fast incorporation of glucose into these components suggests the ready availability of the precursor(s), but the comparatively low concentration in the haemolymph calls for a rapid transport mechanism. Whether diffusion is adequate to meet this demand or whether a specific mechanism is operating needs to be studied, but the study is rendered difficult by the high metabolic activity of the tissue.

The recent work of Wiens and Gilbert[28] showed that incubation of fat body from male adult *Leucophaea maderae* for 90 min with labelled glucose resulted in its incorporation into low molecular weight carbohydrates. Incorporation into glycogen also occurred at about one-tenth the rate. When this pre-labelled fat body was incubated in unlabelled medium for 90 min, 88% was released from the low molecular weight carbohydrate as compared to 23% from glycogen. Trehalose was identified as the major carbohydrate released. A hormonal influence on this release was indicated in view of the stimulation of trehalose release in the presence of corpus cardiacum extracts.

Central nervous system — The absorption of sugars by the central nervous system was studied by Treherne[29] in the cockroach *P. americana*. When ^{14}C-glucose was injected, the influx rate for sugar was found to be 1·09 mmoles/litre of nerve cord water/min. Glucose was found to move at a rate 2·5 times faster than the rate for trehalose. Substantial amount of radioactivity was incorporated into glutamine as well as glutamic acid.

Lipids

Lipids have received considerable attention in recent years and several reviews have appeared[7,30-32]. The digestion and absorption of lipids have been specifically discussed by Niemierko[33], Gilmour[5], Treherne[1] and House[9]. The latest and elaborate review by Gilbert[7] indicates, however, that the mechanisms are still incompletely understood.

The capacity for the digestion and absorption of fat depends largely on the feeding habits of insects. Insects which feed on lipid-rich diets, like the wax moth *Galleria mellonella*, are capable of assimilating substantial amounts of fat. In fact, *G. mellonella* not only assimilates the fatty acids of the beeswax but also the higher alcohols, presumably after oxidation to the fatty acid stage[33]. On the other hand, phytophagous insects need to digest very little fat in view of the low content in leaves.

Lipases which act on glycerides of long chain fatty acids appear to be essential for the assimilation of fat. Although esterases which act on the glycerides of short chain fatty acids are known to be highly active in intestinal extracts, these are not the true lipases, since the higher glycerides are not attacked. High esterase activity and negligible lipase activity were seen in intestinal extracts of the silkworm *Bombyx mori*[34] and *Hyalophora cecropia*[35], but appreciable lipase activity could be demonstrated in midgut extracts of *P. americana*[35] by a sensitive radioassay. Only 4-5% of orally administered ^{14}C-tripalmitin (carboxyl-labelled) was absorbed *in vivo* by the silkworm in 1 hr[36]. Eisner[37] found 23% digestion of labelled tripalmitin in *P. americana* 20 hr after feeding. The rest of the fat was not hydrolysed because of the accumulation of fat within the partly hydrolysed fat. The incorporation of lipids into crop epithelial cells was found to be enhanced in the presence of oleic acid, midgut extract or crop fluid. Treherne[38] found almost complete absorption of tripalmitin in the midgut of *P. americana* in 2 hr when fed as an emulsion in the presence of sodium carboxymethylcellulose and oleic acid. In the silkworm, the high content of unsaturated fatty acid in the mulberry leaf[39] might have ensured its presence in the gut contents, but the effect of oleic acid on the absorption of triglycerides needs to be elucidated. The high pH of the midgut seems to aid in absorption, since the absorption of linoleic acid-1-[14]C *in vitro* was found to be higher at pH 9·0 than at pH 8·0 (ref 36).

Niemierko[33] associated the high phospholipid content (47% of total lipid) of the intestine of *G. mellonella* with the assimilation of the digestion products of wax. Wlodawer[40] postulated that the products of the hydrolytic breakdown of wax during digestion penetrate the gut wall in a phosphorylated form. In the silkworm *Bombyx mori*, 50% of intestinal lipid represents phospholipid[39]. However, its role in absorption has not been investigated.

The partial digestion of fat and the increased deposition of fat in the presence of oleic acid led Eisner[37] to postulate that the rate of absorption of fat depends on the degree of hydrolysis and on the viscosity. Does this mean that micelle formation is an essential step in absorption? Although specific emulsifiers of the type of bile salts are not found in insects, the high phospholipid content of the insect intestine suggests that this might be a vehicle for the emulsification of the partially hydrolysed fat. This aspect needs to be explored further.

That absorption of sterols from the insect gut might occur after their esterification in tissue was suggested by Noland[41]. However, the inability of cholesterol esterase from the cockroach to esterify plant sterols[42] led Gilmour[8] to argue against Noland's theory. Sridhara and Bhat[43] studied the sterol esterifying and hydrolysing activities of the intestines of *Bombyx mori*. The esterifying activity was found to be 2-3 times more than hydrolysis and exhibited greater specificity for cholesterol than for sitosterol, stigmasterol or ergosterol. They suggested that other dietary sterols might first be converted to cholesterol (or some other closely related sterol) in the intestine and then esterified prior to absorption. The narrow specificity of the intestinal enzyme to esterify sterols would, therefore, impose a restriction on the absorption of sterols other than cholesterol.

Fat body — The insect fat body tissue serves as a fat reservoir. Fat absorbed or synthesized during the feeding stages is stored as triglyceride in the fat body. This fat is utilized during the later stages of metamorphosis and for egg formation and adult metabolism, especially for flight. The mode of transport of fat into the fat body cell is not understood clearly. However, it is well established that free fatty acids in the haemolymph are chiefly deposited as triglycerides in fat body, as is evident from *in vitro* incubation studies with this issue.

The release of fat from the fat body has been more thoroughly investigated and this mechanism is at present more clearly understood than the other phases of lipid absorption and transport. Tietz[44] was the first to observe a highly specific release of ^{14}C-labelled glycerides into haemolymph in the locust. This release did not occur into buffered saline and was inhibited by fluoride and cyanide. A considerable fraction of the released glyceride was found to be incorporated into the lipoprotein fraction of the haemolymph. Chino and Gilbert[45] found a similar release in *Hyalophora cecropia* and identified the glycerides involved. Incubation of the fat body with ^{14}C-palmitate resulted in the entry of the label predominantly into the triglycerides of the fat body. Maximum release of radioactivity from this pre-labelled fat body occurred into buffered saline containing haemolymph. The radioactivity in the released lipid was found mainly in the diglyceride fraction and was associated with a lipoprotein fraction. Analysis of the haemolymph showed that diglycerides constitute a major portion of the haemolymph lipid. It was concluded that lipid transport to the different tissues occurred predominantly in the form of diglycerides as lipoprotein complexes of haemolymph.

The significant findings on the lipases of flight muscle in *H. cecropia* indirectly pointed out the preferential utilization of diglycerides in this organ[35]. Diglyceride was found to be hydrolysed much more rapidly than triglycerides, the rate being five times faster with the former substrate. That the major glyceride of haemolymph should also be the preferred substrate at the site of fat utilization suggests that diglyceride is indeed the form in which fat is transported. Chino and Gilbert[45] also showed that preferential diglyceride release occurred into haemolymph in the locust and cockroach.

Subsequently, the high diglyceride content of insect haemolymph was confirmed in other species, by Nelson et al[46] in *P. americana*, by Wlodawer et al[47] in *G. mellonella* and by Tietz[48] in *Locusta migratoria*. Sridhara and Bhat[39], however, did not find a high diglyceride concentration in *Bombyx mori* haemolymph. Recently, Beenakkers and Gilbert[49] were able to throw more light on the mechanism of diglyceride release by using glycerides doubly labelled with ^{3}H-palmitate and ^{14}C-glycerol. The ratio ^{3}H/^{14}C was found to be more in the released glycerides, thus pointing to a reshuffling of the glycerides in the fat body. Based on this, two glyceride pools were postulated to operate in the fat body with only one pool in communication with the surrounding medium. Further corroboration of this interesting theory is awaited.

Investigations by Cook and Eddington[50] have raised some doubts regarding the universal role of diglycerides as transport lipid in insects. In *P. americana* adult fat body, the predominant forms of released lipid were triglycerides and free fatty acids, although diglyceride formed a substantial portion of the haemolymph lipid. This is at variance with the results of Chino and Gilbert[45]. Whether this discrepancy is due to differences in experimental conditions or whether such genuine differences are to be expected *in vivo* remains to be elucidated. Experimentation on other insect species will help in resolving this point. These variations perhaps reflect the differences in the enzymatic make-up of the fat body tissue and haemolymph samples used. In the investigations of Cook and Eddington, for example, the initial rate of diglyceride release was faster than that of triglyceride and a simultaneous release of fatty acid also occurred. It is, therefore, possible that an enzymatic conversion of diglyceride to triglyceride occurred in the haemolymph.

Recent work of Thomas and Gilbert[51] showed that fat body was a site for the synthesis of phospholipids from a variety of precursors — ^{14}C-labelled glycerol, palmitate, ethanolamine and inorganic ^{32}P. They observed that the synthesized phospholipid was released at a fast rate (though at a lesser rate than the release of neutral glycerides) into haemolymph. As with the release of diglyceride, the release was specific and occurred into haemolymph only. Metabolic inhibitors were found to affect release. A major portion of the released phospholipid consisted of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine and was found in conjugation with specific haemolymph lipoproteins. An uptake of labelled phospholipid from haemolymph into flight muscle was also indicated in these investigations. The comparatively low level of phospholipids in the fat body, in spite of the synthetic ability[39,51,52], also lends support to the contention that the synthesized phospholipids are transported to other tissues.

Amino Acids

In insects, amino acid levels in tissues are either slightly higher or equal to haemolymph levels[5,53-55], whereas in vertebrates, the tissue content is 5-10 times higher than the plasma levels[56]. Although the amino nitrogen content of tissues and haemolymph does not differ significantly, variations are encountered in the levels of individual amino acids[5,53-55,57-60]. In general, higher concentrations of glutamate, aspartate and, in some instances, tyrosine are common in tissues. The other amino acids remain at an equal or slightly lower level as compared to the plasma levels. Comparatively few investigations, however, have been forthcoming to explain the mechanisms underlying such concentration differences. Active accumulation of amino acids could provide a partial explanation.

Intestines — Treherne[1,61] has reviewed the literature on intestinal absorption of amino acids. The overwhelming support for the lack of active transport is apparent from all the investigations[62-65]. In the locust *Schistocerca gregaria*, uptake of ^{14}C-glycine and serine took place most rapidly from the caeca and the ventriculus. During absorption of ^{14}C-glycine, the total glycine concentration was found to increase in the gut lumen, while radioactive glycine was transported to the blood side. The intestine also exhibited a similar behaviour with glutamine. A rapid outward flow of water from the lumen to the haemolymph was recorded with ^{131}I-albumen. Amino acids were postulated to move along the concentration gradient thus created.

In the silkworm *Bombyx mori* L., histidine accumulation and transport was non-specific[66]. Concentration build-up and inhibition with dinitrophenol were not evident. No differences were revealed between the transport of D-histidine and L-histidine *in vitro*.

Concentration build-up did not occur with ^{14}C-glycine *in vitro*. However, uptake of dietary ^{14}C-glycine was almost complete within 1 hr.

The rates of transport of stereoisomers of amino acids by the midgut of the silkworms were not significantly different. D-Valine-1-^{14}C and L-valine-1-^{14}C from the racemic compound were transported to the same extent *in vitro*. This transport was slightly decreased in the presence of dinitrophenol (30% inhibition). Only a small quantity of the D-isomer was recovered from larvae which were fed DL-valine-1-^{14}C. An appreciable metabolic degradation was indicated *in vivo* and, to a lesser extent, *in vitro*. The reduced concentration of the D-isomer *in vivo* in spite of the demonstrated failure of the midgut to distinguish between D- and L-isomers for transport points to a limited diffusion of the latter in consequence of adverse concentration gradients. The faster metabolic mobilization of L-valine must have facilitated a faster diffusion[64-66]. The dependence of transport on metabolic utilization was also indicated in the investigations by Auclair[67] on *Blatella germanica*. Feeding of D-glutamic acid led to a slightly increased level of blood glutamic acid, whereas L-glutamic acid feeding resulted in large increase in the concentration of blood glutamine and alanine. Nuorteva and Laurema[68] could not find differences between the rates of absorption of D-glutamic acid and L-glutamic acid in *Dolycoris baccarum*.

Some degree of specificity is attributed to the transport of dietary amino acids to the haemolymph, in view of the relative constancy of amino

acids of the blood under different dietary regimes[69-71]. Also, the rate of absorption as measured by the disappearance of dietary amino acids from the alimentary canal shows wide variation for different amino acids[72]. On the other hand, Maltais and Auclair[73] showed a direct influence of dietary amino acid on the haemolymph amino acid in aphids. When pea leaves perfused with a single amino acid (proline or glutamine) were offered, an increase in the levels of aspartic acid, glutamic acid, serine and proline was registered. These could originate from the increased dietary amino acid, with suitable biosynthetic mechanisms which are generally encountered in different tissues. The role of the intestine itself in mediating specific transfer of amino acids can be studied only with the isolated tissue. *In vitro* experiments with the silkworm midgut showed its capacity for such a specific transfer[63]. When the intestine was perfused with solutions having varying concentrations of different amino acids, the variations were not reflected in the composition on the blood side. A fair degree of uniformity was found in the relative proportions. Tyrosine, alanine and the basic amino acids were transported to a greater extent than valine, methionine and leucine. Glutamic acid was the least absorbed among the amino acids. Here again, concentration build-up was not achieved by the midgut for any of the amino acids tested and dinitrophenol and cyanide had very little effect on either the transport or distribution. This situation suggests a facilitated diffusion mechanism[74] wherein membrane carriers might augment a diffusion of specific amino acids.

Fat body — Price[59] suggested a concentration build-up mechanism to account for the accumulation of glutamine in the fat body of the blow fly *Calliphora erythrocephala*. Glutamine was synthesized from aspartate and glutamate present in the external medium.

In the silkworm, fat body is a site for intense metabolic activity and protein synthesis[75-77]. The uptake of glycine was investigated in this laboratory in view of the importance of glycine in the metabolism of the silkworm during the fifth instar[78,79]. Using glycine-2-^{14}C, the ability of the fat body tissue to accumulate glycine was demonstrated[80,81]. More recent investigations have revealed the nature of the mechanisms (Chitra, C. & Shyamala, M. B., unpublished results) and these will be set forth in some detail.

Incorporation of glycine into the fat body exhibited saturation kinetics ($K_m = 0.03$ mM). Higher intracellular levels were encountered over a wide range of external glycine concentrations. Within the duration of the experiment, up to 90% of label was recovered as free glycine (TCA soluble) of the tissue. The rate of accumulation was fast, equilibrium being reached within 4-6 min. The fast rate necessitated a pre-incubation of the tissue with cyanide to demonstrate the almost complete inhibition elicited with this inhibitor. The accumulating ability was lost in mature larvae, pupae and moths[80]. Sodium and potassium ions were essential for transport. At low temperature (2-4°C), uptake was inhibited by about 50%. Some inhibition was

effected in the presence of aureomycin, chloramphenicol, Hg^{2+} and Cu^{2+}. Accumulation was sensitive to pH. Below pH 5.0, the system was inactivated. The optimum activity was between pH 6.5 and 7.5. Accumulation was not impaired at higher pH values with phosphate and boric acid-borax buffers, but with a more complex buffer system[82], pH dependence revealed an optimum value at pH 5.2.

Inhibition experiments with several amino acids as well as a close study of the kinetics of glycine transport revealed an allosteric or multivalent carrier system[83-85]. The more frequent substrate-velocity relation (Fig. 1) seen in a majority of experiments was occasionally replaced by a sigmoid curve (Fig. 2) ($K_m = 15-20$ mM) with fat body from certain batches of larvae. Multiple points of attachment on the carrier were also indicated by the effects of L-methionine on glycine transport. In the fat body tissue showing Fig. 1 type of response, a gradual increase in methionine concentration at a fixed concentration of glycine elicited an initial inhibition, a subsequent stimulation and ultimately a complete inhibition of uptake of glycine (Fig. 3). In tissue showing Fig. 2 type of response, the initial lag was followed by a region of stimulation and a subsequent inhibition. D-Methionine was only half as effective as L-methionine for either inhibition or stimulation. Similar results were also obtained with L-proline. In fact, L-proline, L-methionine and glycine showed a decreasing order of affinity for the allosteric site in stimulating uptake. Leucine and histidine were not found to inhibit glycine uptake.
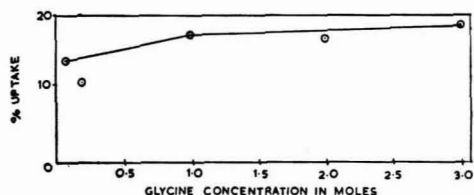
Fig. 1 — Effect of substrate concentration on glycine uptake of silkworm fat body: Type I response
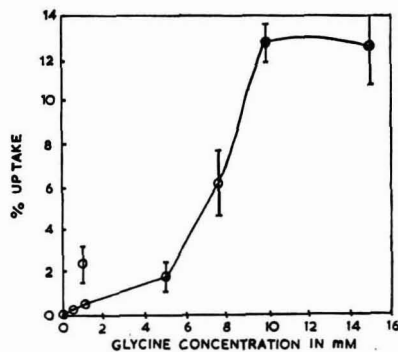
Fig. 2 — Effect of substrate concentration on glycine uptake of silkworm fat body: Type II response
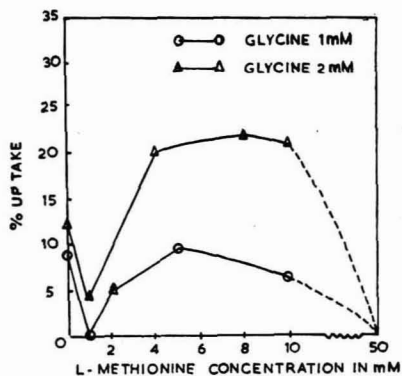
Fig. 3 — Effect of L-methionine on glycine uptake by silkworm fat body

Experiments using labelled methionine and glycine (unlabelled) showed that at concentrations of methionine which stimulate the uptake of glycine, methionine itself was not accumulated in the tissue. In fact, labelled methionine was not absorbed even in the absence of any external competing amino acid. Only a very low adsorption of the amino acid occurred at 10 μM concentration.

The typical allosteric effect may not be apparent in normal fat body either because of an overlapping carrier system with a low K_m (0·03 mM) or because of an activator amino acid, which brings about a significant lowering in K_m from 15 to 0·03 mM (cf Scarano, cited in ref 84). Also, high lability is characteristic of most allosteric systems. Loss of sigmoid response can easily result from mild temperature shocks, pH changes, action of metal ions, etc[86]. Murphy and Wyatt[87] reported a loss in the allosteric response of trehalose phosphate synthetase activity of Hyalophora cecropia fat body extracts in the presence of mercuric acetate. In fact, attempts to purify the enzyme by ammonium sulphate fractionation led to a loss in allosteric property. In view of the activation of the system by several amino acids and the instability of allosteric responses noted in general, it may not be essential to invoke the hypothesis of a separate carrier to account for uptake at low glycine concentrations. However, this alternative cannot be discarded, unless conclusive evidence to the contrary could be furnished.

Although the sigmoid curve is usually attributed to the presence of two or more interacting sites in an enzyme, Sweeny and Fisher[88] have pointed out that such kinetics could also be explained by models having a single independent catalytic site, which is accessible to multiple reaction pathways. If this concept is extended to transport carriers, the multiple pathways could be conceived as separate pools into which the substrate gains entry. In fact, Britten and McClure[89] observed a sigmoid response of pool size to substrate concentration in Escherichia coli and postulated different binding sites for transported amino acids. Recently, Beenakkers and

Gilbert[49] furnished evidence for the existence of more than one glyceride pool in Hyalophora cecropia fat body, based on an entirely different line of evidence. Some support for the operation of more than one glycine pool in silkworm fat body is gleaned from the nature of glycine release in this tissue.

Fat body incubated for 30 min in a medium which did not contain amino acids still retained appreciable quantities of endogenous glycine, which was partly released on subsequent incubation of the tissue with fresh medium. On the other hand, 80% of the labelled glycine accumulated in vitro was released on subsequent transfer to a medium not containing glycine. This release was attributed in part to diffusion, since cyanide and dinitrophenol did not completely inhibit it[80]. Also sodium and potassium ions had no effect on the release. These experiments indicated the greater accessibility of recently accumulated glycine to diffusion than endogenous glycine. Even though additional and more precise experimentation is necessary to establish the exact mechanisms, the evidence is sufficient to indicate the presence of more than one glycine pool in the fat body.

Separate glycine pools in the fat body need not be attributed to intracellular pools alone. Different pools may arise from the heterogeneity of cells and structures making up the tissue. Connective sheaths which encase insect tissues are suspected to exhibit semipermeability characteristics[90]. Walker[91] showed the presence of connective tissue in the fat body of the fifth instar larvae of Philosamia. If connective tissue acts as a barrier for the passage of amino acids, an intracellular accumulation would require that at least two permeability barriers be surmounted. However, very little is understood about the permeability of the connective sheath enclosing the fat body. Besides the connective tissue, fine tracheoles are enmeshed within the lobes of the fat body, and this would contribute to the inhomogeneity of the tissue.

Based on these investigations a tentative scheme has been proposed to depict the glycine absorption characteristics of silkworm fat body (Fig. 4). According to this scheme, glycine is transported
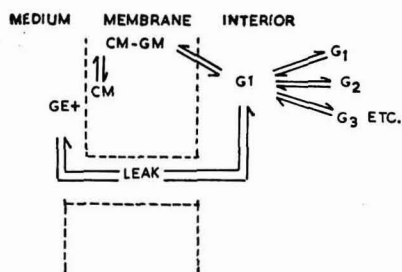
Fig. 4 — Tentative scheme for glycine transport in silkworm fat body [G_E, glycine in external medium; G_M, glycine in combination with carrier in membrane; G_I, glycine in cell interior which is freely accessible to diffusion; G_1, G_2, G_3, etc, different glycine pools; and C_M, multivalent or allosteric carrier]

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into the fat body cell by means of the multivalent carrier, C_M. The glycine which reaches the interior, G_I, is in communication with G_E and is also in equilibrium with glycine of the different pools, G_1, G_2, etc. Here, a multivalent carrier as well as more than one glycine pool are postulated, although sigmoid response, in itself, would have required either one of the postulates. Multivalent carrier is indicated from methionine uptake experiments. Methionine activates or inhibits the system, but is not taken up by the tissue at these concentrations. The action of methionine only at the membrane site (and not the interior of the cell) is thereby implied. Separate glycine pools are indicated from the different behaviours of endogenous glycine and recently accumulated glycine. The latter aspect needs more extensive experimental verification.

Exchange diffusion[86] was not indicated, since ^{14}C-glycine uptake was not stimulated in fat body, which had previously accumulated unlabelled glycine. Also, the release of accumulated labelled glycine was retarded at high external concentrations.

The metabolic significance of the glycine uptake mechanism of the fat body is not yet fully understood. In fifth instar larvae, amino nitrogen in fat body is equal to or slightly less than haemolymph amino nitrogen, on the basis of concentration per ml of tissue water. Hence, high glycine levels are not normally expected. In fact, injection or feeding of labelled glycine to silkworms resulted in its incorporation predominantly into the silk gland and not fat body. It appears likely that glycine uptake is directed towards maintaining and regulating its level in the fat body for synthetic activity rather than to maintain a reserve.

The strict control of the allosteric system that could be achieved in vitro with several amino acids affords an insight into the potentialities of this system for metabolic regulation of amino acid levels in the fat body tissue in response to changes in haemolymph concentrations. Some evidence for the operation of this system in vivo was sought for by injecting labelled glycine either alone or in combination with proline. After 5 min, the incorporation of glycine into the fat body was enhanced with a low level of proline (1 mM) and inhibited at very high levels (100 mM). Since extensive changes in haemolymph amino acids have been reported in the silkworm during the fifth instar[92-96], these changes could serve as regulatory mechanisms to control the amino acid levels of the fat body, which, in turn, would control the synthetic activity of the tissue.

Murphy and Wyatt[87] found an allosteric control of trehalose phosphate synthetase activity by trehalose and glucose-6-phosphate in the fat body extracts of Hyalophora cecropia. Some control was also noticed in vitro with whole tissue. Hence, allosteric mechanisms could be included among those responsible for metabolic regulation in insects. A thorough exploration for such carriers and enzymes might prove rewarding.

Integuments — Recent investigations on glycine uptake by this tissue of the silkworm were prompted

by the suspected role of this tissue as a storage site for nutrients[97]. Uptake of glycine was evident in early fifth instar larvae only, the ability being rapidly lost during the later stages. The mechanism is apparently quite different from that encountered in the fat body. The uptake is probably by adsorption, since the uptake did not show saturation kinetics when tested up to levels as low as 10 μM. The uptake was rapid. Cyanide, azide and dinitrophenol did not inhibit it. Requirement for sodium and potassium ions was noticed (Ravi Rao, U. & Shyamala, M. B., unpublished results). The physiological stage at which glycine uptake occurs appeared to synchronize with the period of post moult endocuticle deposition. Kojima et al[98] found a high ribosomal RNA content in the larval skin at this time. These ribosomes were shown to be associated with chromogranule formation and incorporation of ^{14}C-glycine was found to be predominantly into the proteins of these granules[99,100]. Adsorption of glycine on the cuticle perhaps forms the initial step for this protein synthesis. The interesting observation of Hackman[101] on the adsorption of glycine on chitin tempts us to envisage the adsorption of glycine on cuticular chitin. As protein synthesis proceeds, crosslinking of chitin-protein complexes would be completed. This completed structure might not possess the proper matrix for the adsorption of amino acids. Analysis of silkworms at different stages of the fifth instar showed that 30% of the total soluble nitrogen of the insect was found in the integuments in the early fifth instar. In the later stages, this was gradually reduced to about 15% (Ravi Rao, U. & Shyamala, M. B., unpublished results). Obviously, this hypothesis needs further probing and corroboration.

Malpighian tubules — Extensive work has been done on the absorption of water and ions[102]. Of particular interest to the present topic is the investigation of Ramsay[103] who showed a non-specific diffusion of amino acids and sugars across the tubule lumen of Dixippus morosus.

Summary

Mechanisms for the absorption and transport of organic metabolites in insects have been discussed. In general, active mechanisms are not available for intestinal transport of glucose and amino acids. Facilitated diffusion appears to mediate in the transport. Carbohydrate build-up within the inset is found to be achieved by conversion to trehalose and glycogen. The fat body brings about a rapid conversion of glucose to trehalose. Most of the glucose taken up by this tissue is released as trehalose into the medium.

Insects differ in their ability to assimilate dietary fat. The mechanisms for the intestinal absorption of fat have not been clearly elucidated. Phospholipids are envisaged to have a role. The fat body tissue constitutes a storage site for reserve fat. This tissue, although rich in triglycerides, shows a specific release of diglycerides into medium containing haemolymph. Since haemolymph contains diglycerides as the major form of neutral lipids, these have been postulated to be the transport forms

in insects. The diglycerides are found in the haemolymph as lipoprotein complexes.

Amino acid absorption into an amino acid-rich environment occurs in insects in the course of intestinal absorption. Since concentration build-up, either in or through this tissue does not occur, the mechanisms for the high free amino acid concentration of insect haemolymph cannot be adequately explained yet. The fat body tissue from silkworm possesses an accumulatory mechanism for glycine and presents such interesting features as multiple binding of substrate by carrier, metabolic regulation, etc. A tentative scheme is proposed to describe the transport characteristics of the fat body. Integument of silkworms indicates a transient ability to absorb glycine during early fifth instar.

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Studies on Hen Egg White Lysozyme: The Active Centre & Mechanism of Enzyme Action

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SOME reviews have appeared on the structure and function of hen egg white lysozyme (EC 3.2.1.17)[1]. It is only in the last few years, however, that the exciting data obtained by X-ray studies have revealed various interesting aspects of the specific spacial arrangement of amino acids participating in the conformation and biological activity of the enzyme molecule[2-14]. These studies define the three-dimensional features of the enzyme molecule; indicate the side chains of amino acids involved in maintaining its molecular conformation; map out the areas, probably in contact with substrate or inhibitor; and predict those amino acid residues which may direct the cleavage of the sensitive bond and have thus made available, for the first time, a probable model for the mechanism of enzyme action in its three-dimensional perspective[3,6,13]. Kinetic studies and the measurement of association constants using oligosaccharide substrates and inhibitors support the mechanism postulated from X-ray studies[15-32]. The main object of this review is to integrate the accumulated data from physico-chemical, kinetic and

X-ray methods pertaining to the enzyme activity, active centre and the mechanism of lysozyme action.

The literature has been covered up to August 1968, and the data are arranged in the following order: (1) substrates and enzyme activity; (2) inhibitors and enzyme activity; (3) synthetic activity of lysozyme; (4) enzyme conformation and activity; (5) role of amino acid residues in enzyme conformation and activity; (6) obtaining the active fragment of lysozyme; (7) comparative activity of lysozyme from hen egg white and other sources; (8) mechanism of enzyme action. In the following discussion, the 'active centre' or the 'active site' has been used in the sense discussed by various investigators[33-35].

Substrates and Enzyme Activity

The sensitivity of hen egg white lysozyme to various bacteria has been described in detail[36,37]. *Micrococcus lysodeikticus* cells[38], being most susceptible to the action of lysozyme, are commonly employed for the detection and the measurement of lysozyme activity.

Action of lysozyme on natural substrates — Salton and Horne[39] and Salton[40] have shown that the natural substrate of lysozyme is present entirely in the insoluble cell wall of *M. lysodeikticus*. Several methods

for the isolation and purification of the cell wall substrate are now available[41-47]. The search for a suitable soluble substrate has led to the further finding that lysozyme can hydrolyse chitin[48] and glycolchitin[48-52], though much more slowly than it does the cell wall. Chitin is a polymer of N-acetyl-D-glucosamine (GlcNAc) units, whereas the cell wall is composed of glycopeptides and oligosaccharides which contain GlcNAc and N-acetyl-muramic acid (MurNAc) alternately linked by β-(1-4) glycosidic bonds. Lysozyme does not hydrolyse chitosan[48] and glycochitosan[50]. It is now fairly well established that lysozyme specifically cleaves the β-(1-4) glycosidic bond between MurNAc and GlcNAc[44,53-56].

The cell wall peptidoglycans are present as complexes with various substances, such as proteins, polysaccharides, uronic acids, teichoic acids, lipopolysaccharides, etc, some of which are covalently linked in the cell wall mucopeptides. On removal of these complexing substances, the cell wall becomes susceptible to lysozyme. The peptide side chains attached to some of the muramic acid residues can vary both in their amino acid constituents and in the degree of cross-linking. These variations do not appear to contribute to the susceptibility, though removal of the peptide moiety results in increased susceptibility to enzyme action compared to intact cell walls. The positive charge on the free amino groups of lysine in the peptides may reduce susceptibility. The removal of either the lactyl or N-acetyl group, or the substitution of the acetyl group on oxygen in the hexosamine ring in the cell wall, confers resistance on the latter. Re-N-acetylation of the deacetylated cell wall restores its susceptibility to lysozyme. However, N-propylation does not restore cell wall sensitivity, suggesting that the N-acetyl group is of the optimum size for the activity of the enzyme[57-60]. Lysis of the cell wall is also affected by various environmental conditions. Thus, the pH optimum varies with the ionic species,

ionic strength, temperature and nature of the substrate[61-63].

Action of lysozyme on oligosaccharides — Small molecular weight substrates and inhibitors are of great help in investigating the molecular structural requirements of enzyme specificity, enzyme-saccharide complex formation, and the kinetics of the specific bond-cleavage. Neither such substrates nor inhibitors for lysozyme are commercially available, since the nature of the small substrates cleaved by lysozyme has become known only recently. The extensive use of lysozyme for understanding the chemical nature of bacterial cell walls[64,65] has led to chemical characterization of a number of oligosaccharide fragments susceptible to lysozyme[41-47,66-69]. Identification of the β-(1-4) bond in chitin cleaved by lysozyme has opened the way for the study of chitin oligomers[15,69-72].

The smallest oligosaccharide cleaved by lysozyme is the trimer of GlcNAc. It is hydrolysed to a dimer and to a monomer of GlcNAc, but the reaction is very much slower than cell wall hydrolysis, and requires a large amount of enzyme[70,71]. The rate of cleavage increases with increase in chain length up to the hexose oligomers and thereafter remains constant[15,71,72]. Similar observations have been made using cell wall oligosaccharides containing GlcNAc and MurNAc alternately joined by the β-(1-4) glycosidic linkage. Kinetic studies on oligosaccharides obtained either from cell wall or from chitin also suggest that in the hexamer saccharide, only the second or the fourth bond from the non-reducing end is sensitive to lysozyme action[15,71,72]. A hexamer oligosaccharide substrate is shown in Fig: 1.

Inhibitors and Enzyme Activity

Non-specific inhibitors — A variety of substances inhibit lysozyme activity non-specifically[73-79]. All the acidic polymers, viz hyaluronic acid, poly-γ-glutamic acid, pneumococcus polysaccharides,
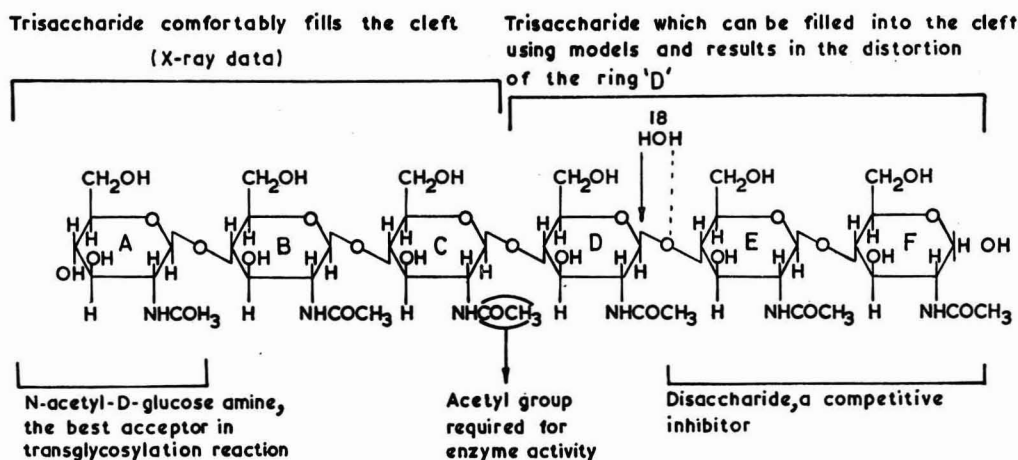
Fig. 1 — Hexasaccharide substrate of lysozyme [A-F denote each GlcNAc residue occupying the subsites, A-F, in the enzyme molecule as shown by X-ray studies[5,6,13]]

RNA, DNA and heparin, inhibit lysozyme activity[36,75,76], but then most basic enzymes are inhibited by these polymers. That these anionic polymers inhibit by neutralizing the charge on the basic protein molecule has been shown recently by Sela and Steiner[78]. Fatty acids, long chain alcohols and surface active agents, like sodium dodecylsulphate, etc, inhibit lysozyme irreversibly[73,74]. It is believed that the aromatic residues of lysozyme may be involved in its irreversible interaction with detergents[77]. The non-specific inhibition of cell wall lysis caused by imidazole and its derivatives has been interpreted on the basis of the formation of a charge-transfer type of complex between these compounds and the indole moieties and the tryptophan residue(s) present in the active site of the enzyme[79].

Oligosaccharide inhibitors and specificity — Oligosaccharides have been isolated both from hydrolysates of *M. lysodeikticus* cell walls and chitin. Some of these oligosaccharides inhibit lysozyme activity[6,15,71], those derived from chitin being particularly effective[69]. GlcNAc has been found by Wenzel *et al*[70] to competitively inhibit cell wall lysis of *M. lysodeikticus* as well as cleavage of trimer by lysozyme. There is, however, some evidence that the inhibition by GlcNAc may be non-competitive at least as regards trimer hydrolysis[31]. Di-GlcNAc is now known as a more powerful competitive inhibitor for lysozyme than GlcNAc[29,69]. Both these compounds are more active in this regard at pH 9·2 than at pH 6·2 (ref 29).

The cell wall oligosaccharides, MurNAc, GlcNAc-MurNAc and MurNAc-GlcNAc, are comparatively less inhibitory to lysozyme than chitin oligosaccharides; this is presumably due to differences between these two types of compounds in their binding affinity to the enzyme[69]. In all these instances, the β-anomers are stronger inhibitors than the corresponding α-anomers. Glucosamine, muramic acid and N-acetylglutamic acid do not affect lysozyme, despite their being products of hydrolysis of the cell wall. Sucrose at 0·05M concentration does not inhibit lysozyme activity, while cellobiose at the same level causes a 95% reduction in enzyme activity[29,69,70]. Generally such inhibition studies have been carried out using a very high concentration of the compounds involved. For instance, a 60% decrease in enzyme activity results in the presence of 0·15M GlcNAc, suitably buffered. Yet the normal 10 min assay period used for lysozyme hydrolysis of the cell wall never results in the liberation of such large amounts of GlcNAc. Consequently, it is difficult to envisage the inhibition of lysozyme by the small quantities of GlcNAc actually produced. It could, however, be that the mixture of cell wall products that arise may exert a cooperative additive effect on lysozyme activity.

Inhibition by oligosaccharide derivatives — Efforts have been made to find specific, synthetic substrates for and/or inhibitors of lysozyme, but so far only naturally occurring substances have been chemically modified.

The dimethyl ester of tetrasaccharide was prepared from di-GlcNAc-MurNAc and found to be cleaved by lysozyme at the same rate as the unmodified saccharide. Since the resultant methyl ester of disaccharide was not cleaved at all, it would appear that the negative charge on the carboxyl group of MurNAc is not essential for either the cleavage of the glycosidic bond or for inhibition of this process[69]. 6-Iodomethyl-GlcNAc inhibits the enzyme activity to the same extent as GlcNAc, and N-methyl-2-acetamido-2-deoxy-D-glucose has a weak inhibitory effect. The α- and β-methyl derivatives of GlcNAc were as effective inhibitors as their α- and β-anomers, whereas β-ethyl-GlcNAc was not inhibitory. 2-Formamide, 2-propionamide and 2-butyramide were also not inhibitory[29,69].

An attempt has been made to find a colorimetric procedure for assaying lysozyme activity. To this end p-nitrophenyl-tri-GlcNAc was synthesized and was found to be hydrolysed by lysozyme to p-nitrophenyl-GlcNAc and di-GlcNAc. The reaction was inhibited by GlcNAc and di-GlcNAc non-competitively and by tri-GlcNAc competitively[26,31].

Synthetic Activity of Lysozyme

The β-(1-4) glycosidic bond, which in an appropriate substrate is specifically cleaved by lysozyme, is also synthesized by lysozyme when it is incubated with the glycosidic moieties, both donor and acceptor molecules, under suitable reaction conditions[15,21,47,69,71,80-86]. Pollock *et al*[21], Chipman *et al*[47] and Sharon[69] have shown that such glycosidic bond formation is not a reversal of the cleavage reaction, but is a transfer reaction in which lysozyme carries a glycosidic moiety from the donor to the acceptor molecule.

Nature of the donor and the acceptor molecules — Using the tetrasaccharide of GlcNAc and the oligomer GlcNAc-MurNAc-GlcNAc-MurNAc as donors, it was shown by chromatographic and radioisotopic techniques that high order oligosaccharides, viz hexamer, octamer, decamer and dodecamer units, are formed by a transglycosylation reaction catalysed by lysozyme[15,21,47,69]. Various oligosaccharides obtained either from the cell wall or from chitin (di-, tri-, tetra- or pentamers) serve as donors as well as acceptors. GlcNAc, di-GlcNAc and MurNAc are good acceptor molecules, muramic acid, glucose, fructose and cellobiose have medium acceptor ability, and glucosamine and D-mannose are poor acceptors[21]. Pollock *et al*[21] and Chipman *et al*[47] have observed that during the transglycosylation reaction, lysozyme transfers a disaccharide moiety, such as GlcNAc-MurNAc, to the acceptor molecule, which may be anything from dimer to hexamer. Also, during the transfer process, the configuration of the transferred moiety is retained. The oligosaccharides synthesized by lysozyme are sensitive to its hydrolytic action and are cleaved just like cell wall oligosaccharides. The products of transglycosylation obtained with small molecular donors and acceptors compare well with cell wall products and have been found to be structurally identical[15,21,47,69].

Factors affecting transglycosylation — The transfer raction occurs in ammonium acetate buffer (pH 5·0-6·8) and at a temperature of 37°C. So far no cofactor requirements for the reaction,

whether as activator or inhibitor, have been shown. All known lysozymes show transglycosylation activity[85]. These investigations firmly establish the transglycosylation activity of hen egg white lysozyme and suggest the existence of a transgly-cosylation site on the enzyme molecule. However, more studies of a specific nature are needed to examine features shared in common by the hydrolytic site and the transglycosylation site on the enzyme molecule.

Enzyme Conformation and Activity

The conformational changes in lysozyme caused by various physical and chemical agents have been investigated extensively[7,87-144]. However, few attempts have been made to correlate the conformational changes with the concomitant alteration in the enzyme activity.

Effect of heat on enzyme conformation and activity — The bacteriolytic activity of heat-treated lysozyme appears to be unaltered[116-118]. Temperature-sensitization leads to changes in the conformation of the molecule dependent on the solution environments involved[106,115,119-122] and it has been found that lysozyme structure is stable to heat in the acid pH range. Aune *et al*[121] found by an optical rotatory dispersion technique (ORD) that acid and heat-treated lysozyme retains one-third of the ordered structure susceptible to further disruption by guanidine-HCl. Similar studies suggest that the stability of lysozyme molecule also depends on the number of disulphide bonds in the molecule and not on the hydrogen-bonded structure[119]. It has been shown by Jolles and colleagues[122] that the presence of four disulphide bonds greatly contributes to the heat stability of hen egg white lysozyme, and that lysozymes from other sources containing less than four disulphide bonds were more heat sensitive. Such heat stability may be either because the conformational changes brought about are confined to a particular segment(s) which may not alter the essential overall structure required for the enzyme activity, or alternatively because these conformational changes may be fully and readily reversible and, therefore, leave the enzyme activity unimpaired.

Effect of chemical agents on enzyme conformation and activity — Lysozyme activity is not affected by the concentration of urea up to $4M$; however, above this level, there is a progressive decrease in the enzyme activity with both *M. lysodeikticus* cells or glycolchitin as substrates[50,51,61,123-125]. Other small molecule substrates (tetra or higher oligosaccharides) have not yet been examined in this regard. The effect of urea on the enzyme activity also depends on the pH, temperature and ionic strength of the buffer used. Thus, lysozyme retains its maximum activity in the presence of $4M$ urea in $0.03M$ phosphate buffer[78], but the activity comes down to 20% in $0.1M$ phosphate buffer (Narayana Rao, D. & Shantoo Gurnani, unpublished data). Conformation of the enzyme molecule at high concentration of urea depends on pH and temperature. Extreme pH and high temperature-dependent changes in the conformation can be detected by the measurement of viscosity, helical content or UV

absorption. However, it appears that the activity of lysozyme is more sensitive than conformation of its molecule to the action of urea[50,51,106,126-128]; loss of enzymatic activity precedes any extensive disruption of the molecules. Thus, denaturing agents perhaps break side chain interactions, resulting in disruption of the alignment of those parts of the molecule which participate in the enzyme activity, before the stabilized structure, such as helices, β-structure, etc, are disorganized. Such observations indicate the importance of side chain interactions in enzyme activity.

The effect of guanidine-HCl on lysozyme conformation has been extensively investigated[114,115,129-183], on lines similar to those used for urea. Thus treatment of lysozyme with guanidine-HCl up to $3M$ has little effect on the organized structure of the enzyme, whereas in $6M$ guanidine-HCl, lysozyme is completely disorganized. The properties, such as viscosity, parameters, a_0, b_0, obtained from ORD studies, UV absorption, change between $3M$ and $5M$ guanidine-HCl. The change in structure of the enzyme molecule in guanidine-HCl solutions above $3M$ also shows that guanidine-HCl is a more powerful denaturing agent than urea, since changes occurring in $9M$ urea and in $3M$ guanidine-HCl are similar[131]. However, 90% of the enzyme activity disappears in $0.5M$ guanidine-HCl, pH 6.8 at 37°C (Narayana Rao, D. & Shantoo Gurnani, unpublished data). Thus, the loss of enzyme activity in $0.5M$ guanidine-HCl solution may be due to the solvent effect of guanidine ions, since at this concentration, as mentioned above, enzyme conformation remains unaltered. Like urea, the disruption of the organized structure of lysozyme molecule brought about by guanidine-HCl is pH, time- and temperature-dependent, and the structure and activity changes are reversed when the disorganizing agent is removed.

Effect of radiations on enzyme conformation and activity — Lysozyme exposed to ionizing radiations (γ-radiations, 26.6M rad dose) has about 37% of the original enzyme activity[134-137]. To detect conformational alterations, the rate of hard-to-exchange amide hydrogens was measured. Following irradiation, hydrogen exchange rate was faster than the rate of loss in enzyme activity, and it was concluded that only half the native conformation of lysozyme is essential for its enzyme activity. In our studies[138], when lysozyme was subjected to photo-oxidation, a 50% loss in the enzyme activity was accompanied by some changes in the physico-chemical parameters, suggesting that photo-oxidation had resulted in a slight alteration in the overall conformation of the molecule. At the same time, two tryptophan residues were oxidized. Whether the loss in enzyme activity is the result of modification of the tryptophan residues alone, or of modification of the conformation, or of the combined effect of both, remains to be explained. Similar observations have been made by other workers[139,140].

Effect of disulphide bond cleavage on enzyme conformation and activity — Progressive reducing of the disulphide bonds of lysozyme molecule appears to disrupt the conformation, the extent depending

on the degree of reduction. The reduction of all the four disulphide bonds leads to the complete inactivation of the enzyme. At least three disulphide bonds appear to be essential for full enzyme activity[122,141]. Lysozyme molecule, in which one disulphide bond between cystine residues at positions I-VIII was reduced, retained its total activity, whereas when only two disulphide bonds between cystine residues at III-V and IV-VI were retained, the molecule was completely inactive (Fig. 2). Completely reduced lysozyme (inactive) slowly regains its native conformation (on air oxidation) and 80-90% of its original enzyme activity[142,143]. It would, therefore, appear that the breaking of the two disulphide bonds at positions I-VIII and II-VII leads to partial disruption of the tertiary structure and perhaps also of the configuration of the active centre which is necessary for catalytic activity. In one study, when reduced lysozyme was treated with cystine, mixed disulphide-lysozyme was obtained, which was inactive. On removal of the cystine residues and reoxidation, two reoxidized lysozymes were isolated. One of these was native lysozyme, while the other was different in conformation and completely inactive[144]. It is interesting that goose egg white lysozyme has only two disulphide bonds, and is five times more active than hen egg white lysozyme; however, its conformation is more labile and more sensitive to heat and denaturing agents[122,141].

Technical difficulties usually hamper examination of the unique role of conformation in enzymatic activity. It is possible that up to a point disruption of conformation may not affect the specific configuration of the active site required for enzyme activity. Most physico-chemical methods employed for detecting the alteration in conformation do not provide information as to the nature of conformational change. It is also difficult to correlate data obtained from various experiments, because of the different agents used and the likelihood that they disrupt the conformations in different ways, even when the overall effect on conformation is still superficially similar. For example, urea at a particular concentration may disrupt interactions of certain side chains in one molecular region, while heat may affect interactions of some other side chains in another region. Measurement of a single parameter, such as viscosity, sedimentation or optical rotatory dispersion, cannot detect regional changes, and only indicates the overall effect on conformation. It was mentioned earlier that low concentrations of urea and guanidine-HCl, which do not affect the native conformation, do influence the activity of the enzyme by disrupting side chain interactions. The role of side chain interactions in maintaining the conformation is difficult to pinpoint precisely. Often efforts in this direction are based on chemical modification of the amino acid residues. These are accompanied by conformational changes and loss of enzyme activity. It then becomes ambiguous to conclude whether the loss of enzyme activity is due to the conformational disruption, or to modification of the particular amino acid residue(s) contributing to the active site. Except under carefully controlled conditions, where it is certain that only the secondary or the tertiary structure is altered, or the side chain interactions are disrupted, it will not be possible to establish with any certainty the extent and the necessity or otherwise of the conformation in enzyme activity.

Role of Amino Acid Residues in Enzyme Conformation and Activity

Since it would appear that each amino acid in a particular position in a protein chain has a specific role in the function of the molecule, it should be possible to elucidate the nature of this function by physical and chemical methods. Although apparently simple, the task seems to be beset with various methodological difficulties and unequivocal success has not so far been achieved, particularly with chemical methods. X-ray crystallography, however, has yielded a three-dimensional projection of lysozyme, though this must perforce be complemented and confirmed by chemical evidence.

The chemical procedures used for the purpose are complicated by the secondary effects of the reagents employed to modify the amino acid residues[33-35,145]. To date, chemical reagents which can specifically react with a particular side chain, of one particular amino acid residue only, are not available; often, more than one type of residue is altered, and secondary effects like the alteration of the overall conformation of the molecule occur. Theoretically, modification of one or more amino acids may not lead to the disruption of the native conformation, and it should be possible to modify residues without alteration in the conformation of the enzyme molecule, or vice versa, with or without concomitant loss of enzyme activity.

Basic Amino Acids

Lysine — The role of the lysine residues of hen egg white lysozyme either in conformation or enzyme activity is not yet clearly established. From titration studies on native, deaminated and guanidinated lysozymes it has been concluded that at least two carboxyl groups are involved in interaction with lysine residues within the molecule[129]. X-ray studies of lysozyme show that the ϵ-amino group of lysine-13 forms a salt bridge with the terminal carboxyl group[87]. Lysine-13 does not seem to be essential for enzyme activity[151], but has been hypothetically implicated in the antigenic determinant(s) of the molecule[146]. Carboxymethylation[147] and other chemical modifications[148-153] of lysozyme have shown that three lysine residues are easily available for reaction, while the other three residues become available only after the conformation of the molecule is drastically altered by treatment with denaturing agents. It has been suggested that at least three lysine residues are essential for maintaining the molecular conformation.

Deamination of the enzyme results in complete inactivation, whereas guanidination of all the residues has no effect on enzyme activity[129,154]. The acetylation of lysozyme is shown to result in progressive inactivation[155]. Acetylation of one-third of the total lysine residues causes no loss in the enzyme activity, whereas acetylation of all the
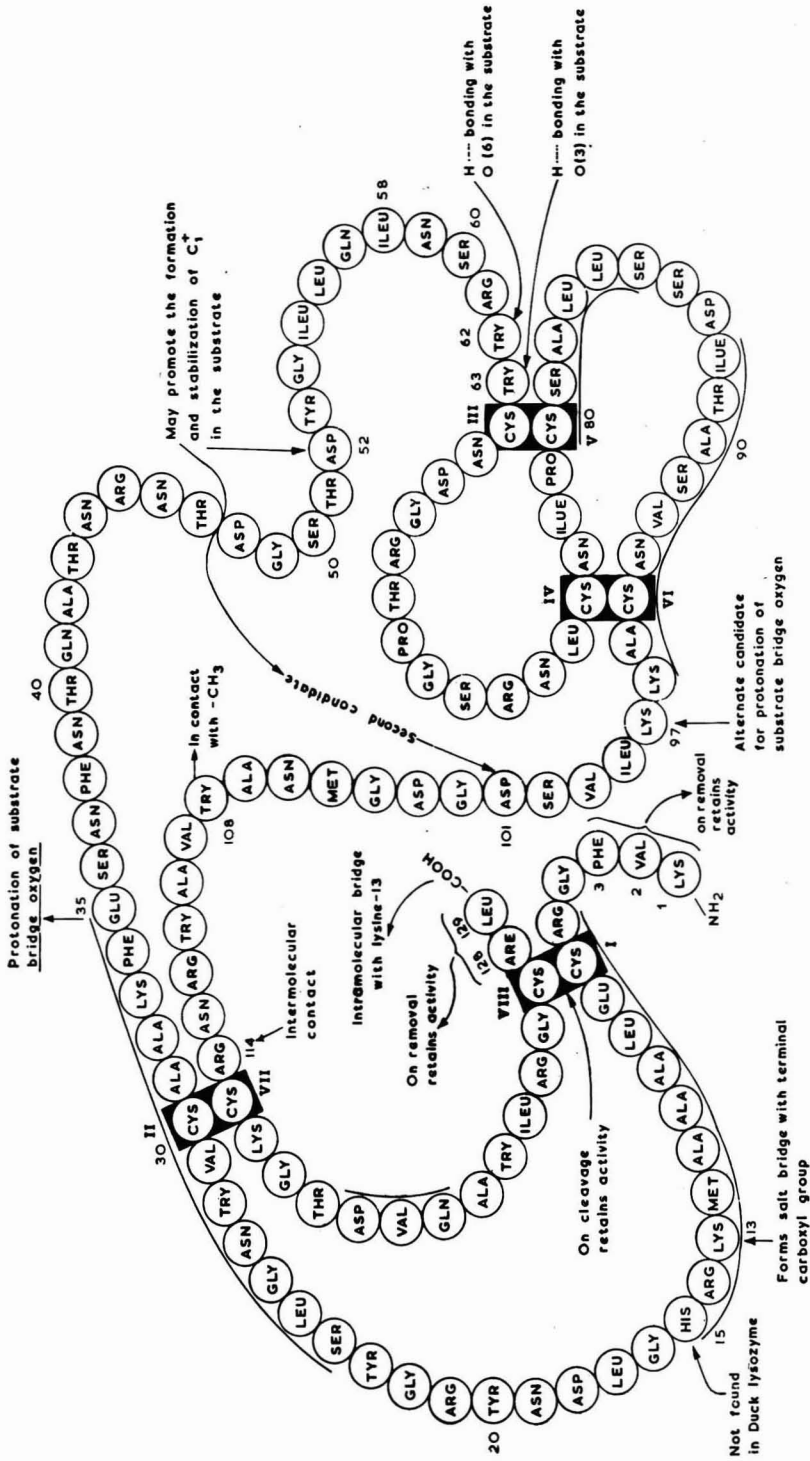
Fig. 2 — Structure of lysozyme[97] [Underlined residues are involved in helics[87]]

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residues results in a 75% loss[156]. These results have been interpreted as meaning that the positive charge on the lysine residues may be required for the interaction of the enzyme with the cell wall of the substrate, *M. lysodeikticus*, for the optimum enzyme activity[129,154]. However, this interpretation is at variance with other results[147] in which it is shown that carboxymethylation of three lysine residues does not lead to any loss in the enzyme activity.

The introduction of a new cross-link between two lysine residues by reacting lysozyme with phenolic disulphonylchloride does not appear to affect either the conformation or the activity of the enzyme[157-159]. Polyalanylation of lysozyme shows no effect on its conformation, but leads to a loss in the enzyme activity proportional to the number and the chain length of the polyalanine residues attached to the molecule; such losses could be the result of steric hindrances caused by the poly-peptide chains[152]. Recently it has been reported that lysozyme molecule shows a second pH optimum in the alkaline region (pH 9·2), and that various inhibitors inhibit enzyme activity more efficiently at pH 9·2 than at pH 6·2. These inhibition studies have led to the implication of lysine-97 in the lytic activity of the enzyme[29].

Studies to examine the actual role of lysine in the enzyme activity have been undertaken in our laboratory. Reaction of lysozyme with 2,4-dinitro-fluorobenzene (FDNB) was investigated at two different temperatures (15° and 26°C) and at pH 7·2 and 9·2 in 0·02M triethanolamine and in 0·1M bicarbonate buffers respectively. DNP-lysozyme prepared at pH 9·2 either at 26°C or at 15°C was completely inactive during the 10-15 min period of the reaction. The gel filtration and ultracentrifuge patterns, and the sedimentation coefficient data suggest that the introduction of DNP groups in lysozyme molecules leads to aggregation of the molecules (Narayana Rao, D. & Shantoo Gurnani, unpublished data). The two DNP-lysozymes, one fully and the other partially active, were obtained by reacting native lysozyme with FDNB at 15° and 26°C (pH 7·2). Some of their properties were studied in comparison with the native enzyme[160]. Amino acid analysis showed that in the fully active DNP-lysozyme one interior lysine reacted with FDNB, whereas in the partially active DNP-lyso-zyme, in addition, the N-terminal lysine and one or more tyrosines also reacted with FDNB. No significant alteration in the conformation of both the preparations was detected, and both preparations were found to be homogeneous by ultracentrifuga-tion and gel filtration. No significant difference in their sedimentation coefficients from the native enzyme was detected. Both the preparations were inhibited to the same extent in inhibition experi-ments, and the inhibitor, GlcNAc, did not protect the reactive amino groups when dinitrophenylation was carried out in its presence. These data suggest that the site occupied in GlcNAc does not involve lysine residue(s), as is also shown by X-ray studies[3,6,13]; however, the role of lysine in the sub-strate binding or cleavage is not excluded.

Arginine — Lysozyme contains eleven arginyl residues distributed in hydrophobic and hydrophilic portions of the molecule. X-ray studies show that all the arginyl residues are present on the surface of the molecule, and that arginyl-114 is involved in intermolecular contacts[87]. In a search for reagents to block specific arginine residues, it was observed that glyoxal reacts readily with arginine. However, all the eleven residues react with glyoxal and no selective reactivity could be detected. Neither enzyme activity nor conformation of the arginine-modified enzyme is known[150,161]. It is probable that arginine-114 which makes intermolecular con-tact (as seen from X-ray studies) may react more favourably and specifically with the modifying re-agent under suitable reaction conditions. It would also be interesting to determine whether the block-ing of the residue-114 (arginyl) would result in the disruption of the intermolecular contacts or cause other deleterious effects on the conformation and indirectly affect the enzyme activity. The role of the penultimate residue-128 (arginine) has been looked into by the tryptic cleavage method. The results suggest that arginine-128 may not be essen-tial to maintain either the conformation or the enzymic activity[141].

Histidine — The role of the single histidine resi-due in the enzyme molecule has been investigated by several workers[1,141,155,162-166]. It has been shown that at pH 5·5 histidine is not accessible to carboxy-methylation, though earlier studies using other methods of chemical modification of the proteins have indicated that histidine is more reactive in lysozyme than in other proteins[155]. At alkaline pH histidine residue is carboxymethylated without loss in enzyme activity[147]. It is now confirmed by X-ray and other methods that the single histidine residue present in lysozyme molecule is not involved in the active centre of this enzyme[13]. Evidence to support the non-involvement of the histidine resi-due in enzyme activity also comes from studies on various lysozyme preparations obtained from other sources. Thus, duck egg white lysozyme, which has the same lytic activity qualitatively towards *M. lysodeikticus*, differs in amino acid make-up from egg white lysozyme and is devoid of histidine residues; and interestingly enough it is 5-6 times more active in lytic activity[163]. A recent finding, however, suggests participation of histidine in dimerization of lysozyme[165]. It has been shown that lysozyme dimerizes between pH 5 and 9 with loss of one proton. Dimerization does not result in conformational change in the molecule as shown by the absence of changes in the net charge of the molecule, optical rotatory dispersion, viscosity and partial specific volume ($\bar{V}$)[93-95].

Since histidine does not seem to play any func-tional role in the active centre of lysozyme for bacteriolysis, it would be important to examine its role in the regulatory activity, antigenicity, and its role in species specificity or evolutionary identity. Some evidence for its role in esterase activity has just been reported[166].

Acidic Amino Acids

Glutamic acid and glutamine — In earlier titration studies, inaccessibility of the three δ-carboxyl groups of glutamic acid was observed. It was suggested

that the unavailable carboxyl groups might be involved in intramolecular interaction with lysine residues[129]. That esterification of the lysozyme leads to the distortion of the catalytic site has been reported, but the position of the esterified residues is not known[34,167,168]. X-ray studies indicate that the δ-amido group of glutamine-41 is intramolecularly hydrogen-bonded. A role for glutamic acid-35 in the lysis of the substrate has been suggested[3,13,87].

Aspartic acid and asparagine — X-ray data show that the γ-amido group of Asn-39 is in contact with various groups in the molecule. Involvement of aspartic acid-52 in the catalytic mechanism of lysozyme has been proposed[3,13,87].

Aromatic Amino Acids

Tyrosine — The accessibility of tyrosine residues to modifying chemical reagents such as iodine and for titration has been investigated. In an aqueous environment only two tyrosines readily react with the iodinating reagent and cyanofluoride. The third tyrosine residue is accessible to the reagents only after the protein has been denatured, or the reaction carried out in the presence of guanidine-HCl or urea. Neither enzyme activity nor alterations in the conformation of the derivatives are known[169-172].

Tryptophan — The role of tryptophan residues in the lysozyme molecule has been investigated extensively. With the solvent perturbation technique, it was found that all the tryptophans were exposed on the surface of the molecule; such exposure was not dependent on pH in the range 1·8-7·0, or on urea concentration up to $8M$ (ref 173). X-ray data indicate that tryptophan-62, tryptophan-63, and tryptophan-108 are situated on the surface of the cleft[6]. Photo-oxidation, iodination, oxidation by hydrogen peroxide, ozonization and N-bromosuccinamide (NBS) treatment of lysozyme suggest that tryptophan has some essential function in its biological activity. Recent studies show that tryptophan residue(s) is involved in the binding of substrate or inhibitor with the enzyme. The exposure of lysozyme to visible light in the presence of dyes such as methylene blue, eosine, FMN, resulted in the loss of enzyme activity and destruction of tryptophan and other amino acids[138,139,174,175]. The kinetic data obtained from photo-oxidation implicate one or two tryptophan residues in enzyme activity. Exposure of lysozyme to UV light also leads to the destruction of tryptophan[176-178].

Hartdegen and Rupley[16,179] and Rupley[71] have shown that treatment of lysozyme with iodine results in facile oxidation of residue-108 (tryptophan) to oxindole. X-ray studies on iodine-oxidized lysozyme show, in addition, that extensive oxidation leads to the oxidation of tryptophan-108 as well as tryptophan-62 (ref 180). Modification of the latter results in inactivation of the enzyme.

Hydrogen peroxide oxidizes tryptophan, although the position of the oxidized tryptophan is not known[181]. During ozonization, methionine is not oxidized nor are disulphide bonds cleaved, but the two indole residues (108, 111) are modified into formylkynurenine. Oxidation of these indole residues

did not affect the enzyme activity, pH optima, extent of inhibition by GlcNAc or heat stability of the enzyme leading to the conclusion that the essential conformation of the molecule is not affected by oxidation of tryptophan-108 and tryptophan-111, and that they are not essential for the maintenance of conformation and biological activity[182].

NBS has also been used by many workers in the hope that it may selectively oxidize the indole moieties of tryptophan residues[141,183-187]. Treatment with NBS resulted in progressive oxidation of tryptophan residues accompanied by a parallel decrease in enzyme activity. In completely inactivated lysozyme, tryptophan-62 is oxidized by NBS without any accompanying conformational change thereby suggesting its implication in enzyme activity[185,186]. However, it is now known that NBS is a non-specific reagent, and earlier data obtained with NBS would need a reappraisal[187]. X-ray data show that tryptophan-62, 63 and 108 interact with GlcNAc, di-GlcNAc and tri-GlcNAc[13].

The role of tryptophan-62 in the activity of the enzyme was further confirmed by the interaction of the enzyme with the cationic detergent, dimethylbenzylmyristylammoniumchloride (DBMA)[188]. The enzyme readily reacts with 0·05% DBMA at pH 5·6, and is completely inactivated without alteration in its conformation. NBS treatment of lysozyme (trypt-62 modified) weakens the interaction of the enzyme with the detergent and the acetylated enzyme (5ε-amino groups acetylated) interacts more readily with DBMA. The above study further shows that (1) certain carboxyl groups are involved in the interaction of the enzyme with the detergent and in the activity of the enzyme; (2) one or more carboxyl groups must be available in ionized form for the enzyme to be active; (3) the ionized carboxyl groups may help in the binding of the cationic detergent electrostatically to the protein, because titration of the carboxyl groups leads to the weakening of the detergent binding and acetylation of the enzyme facilitates the binding.

Data on the implication of amino acids in various functions of the enzyme are summarized in Table 1. The positions of amino acid residues in the molecule are shown in Fig. 2.

Obtaining the Active Fragment of Lysozyme

Some efforts have been made to understand whether the whole chain of amino acids is absolutely essential for the biological activity of lysozyme and whether a portion can be removed without loss of functional activity[141,189-191]. The ubiquitous presence of lysozyme in animals, plants, bacteria and virus has raised many questions with regard to the exact role of lysozyme in cells, tissues and body fluids. In addition to bacteriolytic and transglycosylation activities, its role in various metabolic reactions has been suggested[5]. It is also observed that lysozyme shows esterase activity like many proteolytic enzymes[166]. Efforts have been made to establish how much or which part of the molecule is absolutely essential for its bacteriolytic activity.

Removal of fragment(s) — It has been shown that the three amino acid residues, viz lysine-1, valine-2

TABLE 1 — ROLE OF AMINO ACID RESIDUES IN CONFORMATION AND ACTIVITY OF LYSOZYME

Amino acid	Functional implication	Position of the amino acid in the molecule	Methods employed for obtaining the information	References
		BASIC AMINO ACIDS		
Lysine	Proton donor for protonation of the glycosidic oxygen of the substrate	97	Inhibition studies at pH 6·2 and 9·2	29
	Forms salt bridge with the terminal carboxyl group	13	X-ray studies	87
	Antigenicity	13	Postulated	146
	Enzyme activity (not essential)	13	Reaction of lysozyme with 4-iodo-2-nitrofluorobenzene	151
	Intramolecular interaction with carboxyl groups	2 residues implicated; position not known	Titration studies	129
	Maintenance of conformation	3 residues implicated; position not known	Carboxymethylation with iodoacetate	147
	Maintenance of conformation and enzyme activity	Under investigation	Reaction of lysozyme with FDNB	160
Arginine	Intermolecular contact	114	X-ray studies	87
Histidine	Dimerization	15 (only one histidine residue in the molecule)	Heavy atom derivatives and sedimentation equilibrium studies	165
	Esterase activity	do	Kinetic studies	166
		ACIDIC AMINO ACIDS		
Glutamic acid	Proton donor for protonation of the bridge oxygen in the substrate	35	X-ray studies	3, 13
	Intramolecular contact, ionic interaction with lysine-13	129 (C-terminal)	do	87
	Interaction with interior lysines	2 residues involved; position not known	Titration studies	129
Aspartic acid	Promotion of formation and stabilization of C(1) carbonium ion in the substrate	52	X-ray studies	3, 13
	do	101	Inhibition studies at pH 6·2 and 9·2	29
		AROMATIC AMINO ACIDS		
Tryptophan	Enzyme activity	Position not known	Photo-oxidation	138
	do	do	Iodine oxidation	179
	do	do	H_2O_2 oxidation	181
	do	62	NBS treatment of enzyme	185, 186
	do	62	Interaction of protein with cationic detergent DBMA	188
	Substrate binding	108	Iodine oxidation	16, 71
	do	108, 62	Iodine oxidation and X-ray analysis	180
	do	62, 63, 108	X-ray-analysis	3, 13
	Maintenance of conformation	28, 62, 63, 123	Ozonization	182

and phenylalanine-3, can be removed from the N-terminal end without loss of bacteriolytic activity[189]; whether this affects the transglycosylation reaction is not known. Similar studies with other lysozymes have not been done. Jolles[141] found that two C-terminal amino acids of lysozyme can be removed without loss of bacteriolytic activity. A recent report, however, recorded that des-arginyl-leucine-lysozyme (C-terminal end) under certain conditions showed an activity pattern different from that of the native lysozyme[191]; this has been thought to be due to altered conformational properties arising from differences in the mechanisms by which neutral salts affect the organized structure of the proteins. It was interesting that des-arginyl-leucine-lysozyme was 15-20% less active when tetra-GlcNAc was used as the substrate. It would

be worth while examining whether such alterations as the above also affect its transglycosylation activity and esterase activity.

Synthetic Lysozyme

Only a single attempt appears to have been made, so far, in the direction of lysozyme synthesis. Naithani and Dhar[192] synthesized polypeptides having carboxyl groups in hydrophobic as well as in hydrophilic environments to examine the efficacy of unprotonated glutamic acid, which from X-ray studies appears to be involved in the fission of the polysaccharide chain.

Two polymers were synthesized: polymer-PG containing Phe: Glu: 1: 0·3 (mol wt 63650) and polymer-GcG containing γ-cholesterylglu: γ-benzyl-glu: glu: 1: 0·067: 0·6 (mol wt 12000). The ability of

these polypeptides to degrade the cell wall of *M. lyso-deikticus* in a manner similar to egg white lysozyme was investigated. Both polypeptides exhibited partial enzyme-like activity. On the basis of turbidity measurement the activity of 3·0 mg/ml of polymer-PG or 19·0 mg/ml of polymer-GcG suspension was equivalent to the activity of 0·1 mg/ml of the solution of crystalline egg white lysozyme. Glycopeptides obtained as cleavage products of the cell wall by hydrolysis with polymer-PG were identical with the products obtained from lysis by lysozyme[192]. These data are interpreted as supporting the role of glutamic acid in the mechanism of lysis by lysozyme.

Comparative Activity of Lysozyme from Hen Egg White and Other Sources

All lysozymes defined by Jolles[141] have the following properties. They are basic proteins of low molecular weight, heat stable at acid pH, and heat sensitive at alkaline pH; they hydrolyse the cell wall of *M. lysodeikticus* and release products which can be detected by the amino sugar reagents. They have been isolated from a variety of sources, viz hen egg white and hen lung; duck egg white; goose egg white; turkey egg white; papaya plant latex; human milk, saliva, tears, placenta, plasma, leucocytes and urine; bovine milk; rabbit spleen; dog kidney, spleen and *Nephthys bombergi* (annelid)[193-207]. Some aspects of the structure-activity relationships of certain lysozymes have recently been compared with hen egg white lysozyme[1,141,193,194,196,197,207]. All these lysozymes differ in amino acid composition and in conformational characteristics; where sequences of segments were compared with egg white lysozyme, these were found to be non-identical.

The lysozyme from duck egg white does not contain histidine and that from the goose contains only two disulphide bonds, while T4 lysozyme contains no disulphide bonds and instead has two free —SH groups. At least two disulphide bonds seem to be essential for maintaining the active centre configuration for catalytic activity of lysozymes from animal sources. These lysozymes, except that of the γ-bacteriophage, have leucine as the C-terminal amino acid. Lysine seems to be favoured as the N-terminal amino acid; however, papaya lysozyme has glycine and γ- and T_4-bacteriophage lysozymes have methionine. They also vary in their activity: human lysozyme is 3 times as active as egg white lysozyme, whereas goose egg white and bacteriophage-T_4 lysozymes are 6 times as active[194,197,198].

Arnheim and Wilson[199] isolated lysozymes from 17 bird species in the order Galliform and tested their immunological relationship with the anti-lysozyme from hen egg white. No differences between various lysozymes could be detected using immunodiffusion and immuno-electrophoretic techniques; however, micro-complementation techniques showed small differences among various lysozymes. It was concluded that these differences were due to small sequence differences among various lysozymes. Hen egg white lysozyme has been investigated more extensively than any other. Clearly more studies will be required to delineate various aspects of the structure-activity relationships of lysozymes. Since

these enzymes vary in their activity, amino acid composition, sequence patterns, and folding patterns, it will be of interest to investigate the amino acids involved in the active centre and in the mechanism of reaction. Like hen egg white lysozyme, lysozymes from other sources possess synthetic ability, but could vary with regard to rate of synthesis, donor and acceptor specificity; and substrate, and inhibitor affinity. The study of lysozymes from various sources shows that (1) conformational modes can vary and a single specific conformation is not essential for enzyme activity; (2) amino acid composition, sequence, C-terminal amino acids, N-terminal amino acids, and the number of disulphide bonds can vary without qualitative impairment of the enzyme activity, and that, in fact, variation has sometimes resulted in the improvement of enzyme efficacy; and (3) the absence of some amino acids and some disulphide bonds in certain lysozymes seems to preclude the possibility of their involvement in the active centre and the mechanism of enzyme action. On the other hand, those amino acids which are less prone to natural variation may play an important role either in maintaining overall conformation, or in contributing in some way to the maintenance of the active centre configuration. They may also directly participate either in binding the substrate or in the catalysis of the susceptible bond.

Mechanism of Enzyme Action

Lysozyme-saccharide complex — Lysozyme forms non-covalent complexes with a variety of saccharides, substrates and inhibitors. The complexing forces holding the two molecules together seem to be both specific and non-specific. Evidence for the existence of a fairly stable enzyme-substrate complex has been obtained by a variety of methods, such as ultraviolet absorption spectra, fluorescence spectra, NMR, circular dichroism, X-ray and kinetics. Employing the ultraviolet absorption method, Japanese workers for the first time observed that lysozyme complexes with chitin and with the dimer of GlcNAc (ref 52, 208). The complex formation resulted in a red shift. Lysozyme did not complex with the monomer, GlcNAc. NBS-treated lysozyme failed to complex with both chitin and di-GlcNAc. Since NBS treatment resulted in the loss of one tryptophan with loss of enzyme activity, it was concluded that tryptophan was involved in the complex formation. These observations were confirmed by other workers using smaller substrates obtained from chitin and cell walls. It was found that the red shift due to complex formation occurred up to hexamer substrates. Rupley *et al*[17], Butler and Rupley[19] and Dahlquist *et al*[209] determined the binding constants using this red shift and showed that the binding constant increased with increase in chain length up to tri-GlcNAc, and remained constant thereafter. The one-to-one equivalence was also confirmed by the equilibrium dialysis method.

The fluorescence technique has also been used to study such complex formation between lysozyme and its substrates. This method has an advantage over the ultraviolet method in that it provides an indication of the particular amino acid involved in

the binding process and further indicates the microenvironment around the residues involved in holding the bound molecule in position. The fluorescence emission spectrum of lysozyme at 385 mμ is almost entirely due to tryptophan. Complex formation with an oligosaccharide moiety is accompanied by a shift in the fluorescence maximum by about 10 mμ towards a shorter wavelength. Native lysozyme complexes with tri-GlcNAc, but not after iodine oxidation of the three tryptophan residues. It was, therefore, concluded that these three tryptophan residues were involved in the binding of tri-GlcNAc with the lysozymes of these two residues are in hydrophilic environment[20-23,30]. By fluorescence measurements one-to-one complex formation was observed and the binding constants agreed with those found by the UV method. Using circular dichroism and NMR, similar results of one-to-one complex formation and the involvement of one tryptophan residue in the binding of the enzyme with the substrate were obtained[25,77,210]. It now appears that lysozyme complexed with substrates of varying lengths can easily be crystallized. Crystallization time seems to increase with the increase in the chain length of the polysaccharide[211]. The availability of methods of preparation of cell wall and chitin oligomers has also stimulated recent kinetic studies by Sharon and coworkers[20,21] which have led to an understanding of the nature, specificity requirement and lysozyme substrate subsites.

Differential X-ray crystallography has revealed interesting features of the complex formed by lysozyme with GlcNAc, di-GlcNAc and tri-GlcNAc. In the crystal structure, various inhibitor molecules are bound within the cleft in the molecular surface, and occupy a number of sites. GlcNAc and di-GlcNAc are bound non-specifically. The trisaccharide substrate, lying in the cleft, is bound with a number of amino acid residues through specific contacts[3,6,13]. The α and β forms of GlcNAc bind to the enzyme molecule differently. The most specific interactions between GlcNAc and the enzyme molecule consist of hydrogen bonds between the NH group and the carboxyl oxygen of the N-acetyl side group, and the main chain CO and NH groups of residues 107 (alanine) and 59 (aspargine) respectively. Apart from these interactions, which are common to both the α and β forms, the oxygen atoms at positions 6 and 3 of β-GlcNAc molecule form hydrogen bonds with NH groups of tryptophan-62 and tryptophan-63. The CH_3 group makes contact with tryptophan-108; α-GlcNAc, on the other hand, appears to form hydrogen bonds between the oxygen atom at position 1 of the substrate and the main chain residue-109 (valine)[87].

A stable complex is formed between lysozyme and tri-GlcNAc (GlcNAc residues A, B and C, lettering from the non-reducing end). Its free reducing end residue (C) makes the same contacts as β-GlcNAc with the enzyme. The second residue (B) makes non-polar contacts with the enzyme and there appears to be hydrogen bonding between oxygen atom at position 6 and aspartic acid-101; oxygen at position 5 of the second GlcNAc residue (B) with oxygen of the OH group at position 3 of the third

GlcNAc residue (C) and oxygen of the OH group at position 3 of the residue (B) with the oxygen at 5 of the residue (A). The first GlcNAc residue (A) is attached by non-polar contacts and by its NH group with aspartic acid-101. Non-polar interactions stabilize the molecule. As a result of binding, conformation of the enzyme molecule changes slightly. Thus amino acid residue-62 (tryptophan) moves about 0·75 Å and tends to narrow the cleft, and there are small associated shifts, particularly in the part of the molecule to the left of the cleft, when tri-GlcNAc binds the enzyme[3,6,13,87]. Solution studies suggest that the interactions in the crystal phase are similar to those in solution[16,18,19]; X-ray data support this inference. Lysozyme molecules are surrounded by large fluid portions of mother liquor; each protein molecule is in contact with other protein molecules over a small area of its surface but its conformation is unaffected by neighbouring molecules[4,87].

Nature of the predicted model for enzyme mechanism — X-ray studies, hitherto generally employed to elucidate the structural details of proteins, have recently been extended to mapping the active centre also. Employing these studies, model building has yielded unexpected results, since it has provided a visual method for examining the various mechanisms of enzyme action at the catalytic site. A plausible model for the mechanism of enzyme action of lysozyme has thus been successfully assembled. It is important to examine whether this model explains other physico-chemical data obtained with lysozyme. Some alternate suggestions have already been offered[29].

Subsites — Model building shows that the cleft in the surface of the lysozyme can accommodate six GlcNAc molecules designated A, B, C, D, E and F. Amino acids surrounding GlcNAc residues A, B and C of the trisaccharide were located by X-ray data. Molecular areas surrounding the other GlcNAc residues D, E and F, the site of the cleavage between residues D and E and the amino acids and the probable mechanism involved in this cleavage were all inferred by attaching the three GlcNAc residues D, E and F to the residues A, B and C (in the model) from the reducing end and by adjusting the molecular spacial arrangements in the model of the hexamer substrate to the three-dimensional model of the enzyme. A fourth GlcNAc residue D when added at the free reducing end group of tri-GlcNAc also makes contacts with the enzyme molecule, except that carbon and oxygen atoms at position 6 of the residue D make much closer contacts with the main chain carbonyl of residue-52 (aspartic acid) and with tryptophan-108 and with the acetamide group of the GlcNAc residue C. This crowding is relieved by distortion of the normal chair conformation of GlcNAc residue D by putting the carbon atom at 6 in the axial position. Hydrogen bonding between oxygen atom at 6 and other CO group at 52 or glutamic acid-35 then becomes possible. Residues E and F were added without any difficulty and they made good contacts with the enzyme[3,13,87]. X-ray data and model building further showed that GlcNAc residue C could not be MurNAc, because oxygen atom of the OH group

at position 3 of GlcNAc directed into the cleft is hydrogen-bonded with tryptophan-63, leaving no room for the lactyl side chain. The same is true for residue E as well, so that in the cell wall co-polymer, only residues B, D and F can be MurNAc. Since it is known that lysozyme cleaves the bond between MurNAc and GlcNAc and not between GlcNAc and MurNAc, it follows that in the model, the bond between B and C and that between D and E can only be cleaved. Since GlcNAc residues B and C in tri-GlcNAc-lysozyme complex are part of the stable complex, hydrolysis between D and E is more likely in the hexamer-lysozyme complex.

The bond between D and E residues seems to be surrounded by glutamic acid-35 and aspartic acid-52 residues on either side of the β-(1-4) linkage between GlcNAc residues D and E. Both side chains have markedly different environments; glutamic acid-35 is found to be in the non-polar region near the bottom of the cleft, its neighbour being alanine-110, Cβ and CΥ of glutamine-57 and C(δ_1) of tryptophan-108. Aspartic acid, on the other hand, lies essentially in the polar region on the left side of the cleft, and appears to be involved in the complex network of hydrogen bonds that involve the amino acid residues, aspartic acid-46, serine-50 and aspargine-59. In this network, aspartic acid-52 is in the position of accepting hydrogen bonds from aspargine-46 and aspargine-59 and it is supposed that this environment may tend to hold aspartic acid-52 in the ionized state. The distance between oxygen at position 1 of the GlcNAc residue D and

$$glutamic\ acid-35—\overset{\overset{\textstyle O}{\|}}{C}—O\ is\ 3\ Å.$$ The distance between carbon atom at position 1 of the GlcNAc residue D

and aspartic acid-52—$\overset{\overset{\textstyle O}{\|}}{C}$—O is also around 3 Å. Although this precludes the possibility of a covalent linkage, nevertheless, it is close enough to promote the formation of a carbonium ion at carbon atom 1 of GlcNAc residue D and to stabilize it when formed[13]. Model building further reveals the probability that distortion of the GlcNAc molecule from chair conformation occurs; the ring collapses in its half-chair conformation in which the carbonium ion at carbon atom 1 would be stabilized by sharing its charge with the ring oxygen atom[212] and the molecular distortion also contributes to the carbonium

ion, with subsequent weakening of the –C_1–O–bond in the substrate.

Supporting kinetic evidence — This mechanistic model of lysozyme, the first of its kind, very neatly explains various aspects of substrate binding and its cleavage. Substantial data now available, especially those obtained from the kinetics of inhibitor and substrate binding, support the predictions made by X-ray studies. Chemical evidence as well as data obtained by fluorescence and equilibrium dialysis techniques confirm the one-to-one complex formation and the existence of subsites in lysozyme, and are all in general agreement with the above X-ray model.

Rupley[71] has shown that the bond between C_1 and bridge oxygen is broken when lysozyme is allowed to cleave tri-GlcNAc in the presence of $H_2{}^{18}O$. ^{18}O was found on C_1. Further, the same workers and others have established that lysozyme cleaves the second or fourth bond from the non-reducing end of the substrate[71,72]. These observations also support the results of X-ray studies.

The role of the amino acid tryptophan in binding to the substrate comes from binding studies using ultraviolet and fluorimetric techniques with the native lysozyme and lysozyme treated with various protein-modifying agents (cf section on amino acids). Thus, these studies support the X-ray evidence that tryptophan 62, 63 and 108 are involved in the binding of the oligosaccharides.

The role of the acidic amino acids, glutamic acid-35 and aspartic acid-52, participating in the cleavage of the β-(1-4) linkage between GlcNAc residues D and E, the former as proton donor to the ring oxygen and the latter promoting the formation and stabilization of the carbonium ion, is not yet supported by other experiments[213], although alternate amino acids have been suggested for the same functions[29]. Neuberger and Wilson[29], who studied the inhibition of lysozyme at pH 6·2 and 9·2 with various inhibitors, suggest lysine-97 as the proton donor and aspartic acid-101 as promoting the formation and stabilization of the carbonium ion. Other studies on the reaction of lysozyme with FDNB also suggest involvement of one or more lysine groups in the enzyme activity and/or the maintenance of the enzyme conformation.

Several mechanisms have been postulated for explaining catalysis by enzymes[214-216], but with little direct evidence. The new concept that emerges
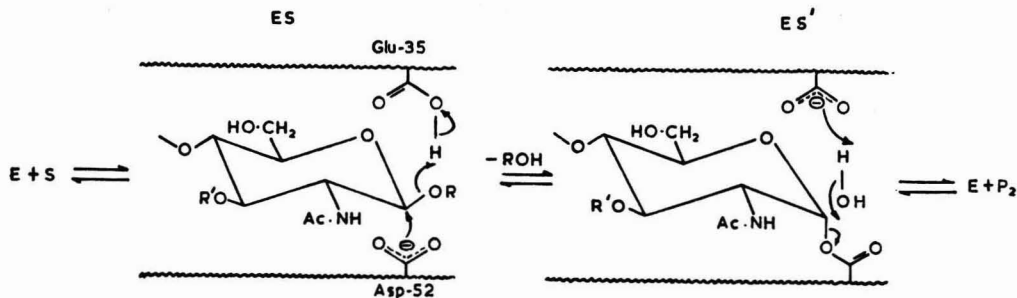

Fig. 3 — Mechanism of cleavage of β-(1-4) linkage proposed on the basis of kinetic data[26,27]

from studies on lysozyme, based on adequate evidence, is that an enzyme may activate its substrate molecule by distorting it, the process also involving distortion of the enzyme conformation itself. In other words, the acceleration in reaction rate by enzymes may be caused in part by the introduction of strain into that portion of the substrate which is to be cleaved. Presumably, the energy required for distortion can be derived from the energy of the initial binding of the enzyme-substrate complex and the attendant conformational changes in the enzyme itself. This mechanism, though speculative at the moment, is supported to some extent by kinetic evidence. Using dimers and trimers of sugars and their derivatives the mechanism presented in Fig. 3 has been proposed to explain the action of lysozyme and it appears to obey Michael Menten kinetics. Present evidence shows that the β-(1-4) linkage in the saccharide is cleaved by the concerted action of the acid (glutamic acid-35) and the base (aspartic acid-52)[26-28].

Summary

The progress made during the last few years in the understanding of the structure and function of hen egg white lysozyme has been reviewed. Based on X-ray studies, three-dimensional features of the enzyme molecule and the side chains of amino acids maintaining the molecular conformation and making contacts with substrate or inhibitor have been revealed. Model building has led to suggesting those amino acids which probably direct the cleavage of the sensitive bond and has provided a model for the mechanism of enzyme action in three-dimensional perspective. These studies on lysozyme support the concept that enzyme action involves change in the conformation of the enzyme as well as substrate molecule, and as a result some strain being introduced into that portion of the substrate which is cleaved by the enzyme.

Acknowledgement

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REVIEWS

AN INTRODUCTION TO FLUID DYNAMICS by G. K.
  Batchelor (Cambridge University Press, London),
  1968. Pp xvii+615. Price 75s

Prof Batchelor is a leading figure in the domain
of fluid mechanics, who can claim a great share
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What the author has achieved can be easily judged
by anybody who is acquainted with the existing
books dealing with the subject at undergraduate or
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that fluid mechanics deals with real situations
and consequently in writing this book he has taken
a point of view which maintains this aspect of the
subject. For example, after dealing with the funda-
mentals of the subject, describing the physical
properties of the fluids and establishing in perfect
generality the governing equations in the first three
chapters, in the next four chapters the author deals
with the motion of a uniform incompressible viscous
fluid which forms the major topic in the subject
due to its practical importance. There is no ideal
fluid existent in nature which is completely devoid
of viscosity and hence he treats this class of hypo-
thetical fluids at a much later stage, clearly explain-
ing under what circumstances the viscosity may
be assumed to be zero.

The author, throughout the book, lays emphasis
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The book consists of seven chapters spread over
615 pages and contains 24 plates representing the
visual nature of flows in important cases of interest.
The successive chapters are as follows: (1) The physi-
cal properties of fluids, (2) Kinematics of the flow
field, (3) Equations governing the motion of a fluid,
(4) Flow of a uniform incompressible viscous fluid,
(5) Flow at large Reynolds number: Effect of visco-
sity, (6) Irrotational flow theory and its applica-
tions, and (7) Flow of effectively inviscid fluid
with vorticity. At the end of the book, in addition
to the subject index and the bibliography of the
original papers which have been referred to in the
text, the author has included two appendices, one re-
cording the measured values of some physical pro-
perties of common fluids and the other recording the
expressions for some common vector differential
quantities in orthogonal curvilinear coordinates.

The reviewer has great pleasure in recommending
this book for prescription as the text-book in fluid
dynamics at M.Sc. level in all the Indian universities.

P. L. BHATNAGAR

INTERNATIONAL CONFERENCE ON SPECTROSCOPY,
  BOMBAY, JANUARY 1967, published by N. A.
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of Scientific Unions with the financial assistance

of Unesco, contains 29 invited talks and 6
contributed papers presented at the International
Conference on Spectroscopy held in Bombay during
9-18 January 1967. The conference was organized
with the laudable objective of bringing together
workers in different aspects of spectroscopy for a
fruitful interaction amongst them and discuss their
most recent investigations, even if the results are
not yet conclusive. From this point of view the
talks by Charlotte Moore-Sitterly, Herbig, Herz-
berg, Douglas and Dressler provide a valuable
account of the present state of our knowledge con-
cerning the spectra of rare earths and the spectra
of diatomic molecules. As has been pointed out
by Herzberg, laboratory spectroscopy may not
be an entirely fashionable pursuit today among
physicists, but one can easily realize from the papers
by Sitterly, Herbig, Herzberg and Mandelstam the
need for the analyses of more band systems, a search
for the spectra of new molecular ions both positively
and negatively charged, a measurement of photo-
ionization, and photo-dissociation cross-sections and
an identification of the ground states, before one
can hope to identify many of the diffuse interstellar
bands.

The article by Prof Coulson gives a very valuable
account of the theoretical principles which are
needed to understand the changes in shape and
size of a molecule when it undergoes electronic exci-
tation. Ordinary valence theory cannot be applied
to the shape of an excited molecule and the basic
concepts and principles which appear to be impor-
tant in treating this problem have been summarized
by him. The use of rotational fine structure and
the importance of the Franck-Condon principle
in the characterization of the electronic states of
nonlinear polyatomic molecules have been dealt with
by K. Keith Innes using the spectra of pyrazine,
tetrazine and diazine as examples.

Though infrared absorption and reflection spectra
have been studied extensively all over the world,
one of the important contributions from the group
working with Prof Lecomte in Paris has been their
work on the dispersion and optical constants of
liquids and solids. Using new methods, they have
determined the optical constants over a wide range
in the infrared and these have been proved to be
of great value in testing the accuracy of the disper-
sion formulae, especially in the regions of anomalous
dispersion. Prof Lecomte has shown how the direct
determinations of the refractive indices inside the
regions of anomalous dispersion for some crystals
show that the values of n can be much lower than 1
and pointed out the line of future investigations.
A very brief but useful discussion of the problems
of high resolution far infrared spectroscopy will
be found in the article by R. C. Lord. Pressure
induced infrared absorption and bound states of
van der Waals molecules have been dealt with by
Prof H. L. Welsh and it would have been helpful
to the reader if Prof Lecomte and Prof Welsh had

given some references to the papers from which one can obtain further information.

Resonance Raman spectra have been considered by Prof Shorygin and in this interesting review by a pioneer in this field are discussed the connections between Raman effect, fluorescence and absorption of light.

It is a pity that W. A. Anderson of Varian Associates could not send in his full paper on the indirect detection and line shape determinations of weak or obscured NMR lines. The present situation in the study of molecular motions in liquids by NMR has been reviewed very briefly by J. G. Powles. The utility of electron spin resonance spectroscopy for the investigation of the structure and properties of free radicals especially those with short lifetimes has been illustrated by C. A. McDowell by the recent work of his group on NF_2, HN_3 and CF_3 in various matrices at liquid helium temperatures. Max T. Rogers has dealt with the electron spin resonance studies of radicals in irradiated crystals. The paper by M. Balkanski on semiconductor spectroscopy contains a brief introduction to the optical spectra due to interband transitions and photon-phonon interaction followed by an account of the optical spectra of crystal imperfections, excitons, surface states, impurity states and localized modes. The other interesting topics covered in the conference report relate to plasmas by O. Theimer and A. von Engel and lasers by J. R. Singer.

Quite a few papers which are included in this volume do not serve any real purpose as the same authors have already published fuller papers elsewhere.

The present volume reveals a sad feature in two important respects: (1) The organizers did not invite greater attention to spectra of solids, conventional and laser excited Raman spectra, magnetic resonance spectroscopy and study of phonon spectra by inelastic scattering of neutrons. (2) Except for the two contributions presented at the special symposium in honour of Prof R. K. Asundi, which have been included in the present volume, there are no invited papers from Indian spectroscopists. This is a serious omission and would give rise to an impression that no active work is going on in spectroscopy in India. A discussion of the recent developments in instrumentation for spectroscopy would also have been profitable. In spite of these lacunae, the proceedings of the International Conference on Spectroscopy, Bombay, 1967, will be found useful by those working on the electronic spectra of diatomic and polyatomic molecules.

R. S. KRISHNAN

INTRODUCTION TO BIOCHEMISTRY by E. O'F. Welsh (English Universities Press Ltd, London), 1968. Pp vii+482. Price 45s

This paper-back addition to books in biochemistry is an introduction treatise. The author is a seasoned teacher who understands the requirements of the students of general biology. Modern biology like all life sciences demands a certain degree of proficiency in biochemistry. The students of classical biological disciplines, however, find it too tough to consult the standard text-books in biochemistry. A book which is written in a simple style and does not assume any previous knowledge of biochemistry or chemistry on the part of the student is in such cases helpful to initiate basic understanding of this subject.

The author has aimed to introduce the subject as a natural philosophy. In most chapters, a short historical background has been given, which traces the development of ideas and knowledge in that particular area, and evokes consequently a better appreciation of the current information. Some chapters are also original in their compilation, as for example, a separate chapter has been devoted to phosphorus and its role in the chemistry of life. There is a chapter on the evolution of life on earth. The book includes an important appendix of 62 pages, in which several of the commonly used chemical and biochemical terms have been explained clearly and at times in sufficient details. This glossary appears to be one of the most useful features of the book.

The book starts with an overall view of energetics in biological systems, the capture of solar energy through photosynthesis in the generation of nutrients and their utilization by biological systems. The next chapter examines the composition of living matter and introduces to the extent necessary the chemistry of constituents of cells. Minerals, the hydrogen-bonded structure of water (the most predominant of cellular constituents) are also discussed. Basic notions on pH are given. The next chapter is on enzymes, their basic nature and properties, which is followed by a chapter on digestion. The metabolisms of carbohydrates, lipids and proteins have been discussed in separate chapters. Cellular respiration and photosynthesis have been adequately and clearly reviewed. The chapter on enzymic activity in relation to biological adaptation should have received much more emphasis than has been given in the book. Sixteen pages have been devoted to the early notions on adaptation which bring the reader at best to the picture prevalent in 1950s. The current knowledge on this topic, which has advanced phenomenally in the last 8-10 years, does not figure at all in the book. Even the Jacob-Monod operon model has not been given. This is a serious lacuna. The argument can be extended further. The book is deficient in most that constitutes molecular biology today. The structure, function and biosynthesis of macromolecules have not been treated adequately.

The author has intended the book more specially for freshers in biology and medicine. It fulfils in part the expectations for the former type of students but it may not make up the mark for a recommended introductory treatise for students in medicine. The treatment of hormones, vitamins and blood constituents is fragmentary and does not have the appropriate orientation. Many other aspects of biochemistry desirable for students in medicine have either been omitted or discussed inadequately. The book has, however, its special features and would no doubt be of interest to students in general biology.

G.P.T.

INDUSTRIAL MICROBIOLOGY by L. E. Casida (Jr) (John Wiley & Sons Inc, New York and London), 1968. Pp xii+460. Price $ 14.50

This refreshingly new treatment of an introductory course of industrial microbiology by Prof Casida fills a void in the initiation and training of either industrial microbiologists or biochemical engineers.

The book has four parts consisting of an introductory chapter dealing with definition and scope, historical development of concepts and a very valuable chapter on description of fermentation equipment and use.

Part II includes broadly informative and useful chapters on screening, product detection, assay and culture handling methods. Chapters on media, scale up problems, phage and continuous fermentation are included. Information on biological waste treatment and patent laws and fermentation economics are most welcome additions, not usually found in industrial microbiology treatises.

Part III deals with ten different ' type ' fermentations, in such a way as to allow the reader to grasp the basic concepts of a group of similar fermentations without being lost in a mass of individual process variables, a useful and a novel approach indeed. The groups are: antibiotic and anaerobic fermentations, environmental and genetic control of metabolic pathways, microbial oxidative transformation of the substrate, hydrocarbon fermentations, microbial cells as fermentation products, vitamins and growth stimulants, enzymes as fermentation products, and organic acids.

Part IV is a projection into extended future use of microorganisms in the production of special metabolites, food production and agriculture, space travel and biochemical fuel cells, all exciting and hopeful fields of endeavour.

Industrial microbiology is, like medicine, a fully interdisciplinary science, where knowledge of engineering, economics, patent law, instrumentation and biochemistry is essential in addition to microbiology *per se*. This introductory text for the final year student or an industrial technician provides excellent broad-based information, both practical and conceptual, to whet his appetite for further specialization. The author is to be congratulated on a job thoughtfully conceived and excellently executed. The get-up, photographs and figures bear up to the usually high standards of Wiley publications.

The book should prove useful to both undergraduate and postgraduate students and to the technician/supervisor in a fermentation industry.

K. S. GOPALKRISHNAN

HANDBOOK OF EXPERIMENTAL PHARMACOLOGY: Band XXIII — NEUROHYPOPHYSIAL HORMONES AND SIMILAR POLYPEPTIDES by B. Berde (Springer-Verlag, Berlin), 1968. Pp vi+967. Price $ 48.00

The pharmacology of the neurohypophysial hormones has had intensive attention by a wide circle of investigators over the last decade and has achieved substantial progress. This volume makes the ambitious attempt to summarize the present state of knowledge, the interesting feature being that the presentations cover a wide front extending from anatomy to biochemistry and from zoology to clinical medicine.

Structure and function of the neurohypophysis; chemistry of the neurohypophysial hormones — oxytocin, vasopressins, vasotocin, isotocin, mesotocin and glumitocin; the influence of some oxidizing and reducing enzymes and proteolytic enzymes on neurohypophysial hormones and similar peptides; a comprehensive treatise on the metabolic effects of these hormones; bioassay procedures for neurohypophysial hormones and similar peptides, including immunological methods; production, release, transportation and elimination of the neurohypophysial hormones, the influence of these hormones and similar polypeptides on the kidneys, uterus and other extravascular smooth muscle tissues and on circulation; the milk ejection reflex and the action of hormones on mammary gland are titles of some of the chapters and highlight the nature of topics discussed.

' Phylogenetic aspects of neurohypophysis hormones ' and the ' Actions and functions of these hormones in lower vertebrates ' present a wealth of information of interest to comparative physiologists and endocrinologists.

' Antagonists of neurohypophysial hormones ' surveys the importance of this study in defining specificity of action of the neurohypophysial hormones and in elucidating the interrelations between various systems of humoral and nervous regulation.

' Basic pharmacological properties of synthetic analogues and homologues of the neurohypophysial hormones ' present the possible relationships between the chemical structure and the biological activities of these synthetic chemicals, utilizing the well-established pharmacological actions for evaluation purposes.

With its comprehensive coverage and extensive bibliography, the book will serve as a useful reference volume for all investigators interested in hormones in general and neurohypophysial hormones in particular.

M. SIRSI

NOTES & NEWS

A simple power output meter for laser beams

The wire resistance thermometry method has been made use of in the development of a simple power output meter for continuous laser beams at the Philips Laboratories, Eindhoven. Any device used for the measurement of output of laser beams has to fulfil the following requirements: (i) measurements at any desired time; (ii) negligible disturbance due to the introduction of probe; (iii) probe to be of an integrating type; (iv) accuracy coupled with reproducibility; and (v) an effective life longer than the effective life of a laser. These requirements are fully met with by the new device. The device consists of a pair of crossed wires (50 μm thick) not touching each other and connected to the Wheatstone bridge. The wires when positioned to intercept a negligible portion of the laser beam (diam 10-15 mm) have their resistance changed. Theoretical calculations made on the increase in resistance (ΔR) lead to the relation:

$$\Delta R = p\frac{W}{R} + qR^3$$

where W is the laser output; R, the radius of the laser beam; and p and q are constants. The output readings of the Wheatstone bridge, taken in the case of 120 W CO_2 gas laser, showed a close agreement with the observations made with a commercial infrared radiation meter [*Philips Res. Rep.*, **23** (1968), 375].

Stop-action holography

A new method of taking holograms relaxes the need for an absolutely motionless subject. Holograms of scenes undergoing periodic motions can now be formed with 'stop-action holography' which might be useful in commercial applications.

In an ordinary photograph the image may at most be blurred by the motion of the subject. But in the case of a conventional hologram the image would be completely destroyed even by a small motion of the subject. Stop-action holography alleviates this stability problem for objects that move repetitively. By using a laser as a stroboscope one can make the object appear stationary. When the pulse rate of mode-locked laser which has a controllable flash rate becomes equal to the repetition rate of motion of the subject the laser acts as a stroboscope and freezes the motion of the object.

To demonstrate this stop-action feature, an ultrasonic wave through a small tank of water was photographed. The laser was precisely synchronized with the sound wave and the wave appeared stationary. The acoustic wave appeared on the photograph as a column of interference fringes, spaced according to the acoustic wavelength in the water cell. The height of the column is equal to that of the acoustic transducer attached to the water cell.

This method can be useful in many electronic systems used in modern pulse-compression radars of optical deflectors in optical memories or an electronically steared array of transducers. In all these cases, a hologram of a sonic wave could lead to a better control over performance [*Bell Lab. Rec.*, **46** (1968), 274].

Total synthesis of penicillins

Two routes to the total synthesis of penicillins worked out by Dr A. K. Bose and his associates at the Stevens Institute of Technology, USA, hold promise of making it possible to prepare a wide range of variants of penicillin and thereby overcoming the limitations of the fermentation products, viz allergy and the resistance developed by bacterial strains. In the first synthesis, isobutyraldehyde is run through a series of reactions leading to a single ring intermediate thiazoline and then to a product isomer of penicillin V methyl ester. In the product obtained from iso-butyraldehyde the substituent group, CO_2CH_3, is at C-5 in place of C-3 in the usual compound (Scheme 1). The second synthesis involves transforming penicillamine in several steps to the new stereoisomer, 5,6-*trans*-penicillin V methyl ester.

The key discovery in the work of Dr Bose and his associates is the action of azidoacetyl chloride to convert the thiazoline intermediate to a bicyclic β-lactam. This compound is then changed to penicillin by reduction, hydrolysis, acylation and esterification.

In the synthesis of the stereoisomer, the penicillamine goes through formylation and ring closure steps to form an intermediate thiazoline. Thereafter, reaction with azidoacetyl chloride and triethylamine and subsequent reduction and acylation give the product penicillin [*Chem Engng News*, **46** (30) (1968), 15].
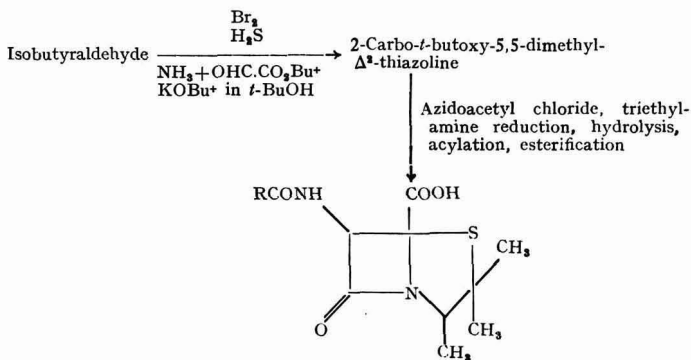
Scheme 1

A new method for the oxidation of aldehydes to carboxylic acids and esters

A new method has been developed for the conversion of alcohols into esters via aldehyde intermediates. The process involves the conversion of primary allylic alcohols into conjugated aldehydes in the presence of active manganese dioxide. The conjugated aldehydes thus obtained in the presence of HCN and CN- are converted into cyanhydrins which are further oxidized by manganese dioxide to acylcyanides. These in the presence of alcohol are converted into esters.

Following this procedure, benzaldehyde, cinnamaldehyde furfural, geraneal and farnesal have been converted into their respective esters and 85-95% yields have been obtained in all the cases. In the process, there is no *cis-trans* isomerization of the α,β-olefinic linkage, which is usually encountered in the oxidation of aldehyde to carboxylic acid by Ag_2O.

The present method is not applicable to non-conjugated aldehydes. Even after using a strong oxidizing agent, such as argentic oxide in place of MnO_2, in the presence of NaCN and methanol the product obtained is only cinnamic acid starting with cinnamaldehyde. Similarly, benzaldehyde is converted into benzoic acid and 3-cyclohexane carboxaldehyde (a non-conjugated aldehyde) into 3-cyclohexenyl carboxylic acid.

The conversion of non-conjugated aldehydes to acids can also be brought about by means of argentic oxide in tetrahydrofuran-water (9:1) at 25° under neutral conditions, though this process is not preferred for conjugated aldehydes [*J. Am. chem. Soc.*, **90** (1968), 5616].

A simple and rapid procedure for the preparation of phosphopyruvate hydratase

A simple method of preparing phosphopyruvate hydratase free of contamination from phosphoglycerate phosphomutase and pyruvate kinase has been reported [*Biochim. biophys. Acta*, **151** (1968), 298]. The method, which is based upon the finding that protracted incubation of dry yeast results in labilization of residual phosphoglycerate phosphomutase, particularly during alcohol fractionation in the presence of potassium chloride, can be carried out in less than 4 hr, with minimum equipment and thus is suitable for classroom experiments also.

Dry yeast (300 g) taken in 900 ml distilled water is incubated in a water-bath at 38°C with occasional stirring for 2 hr and then allowed to stand for 15 ± 1 hr at 38°C. The incubates are centrifuged at $5000\,g$ for 20 min and the supernatant is collected as 'crude fraction'. Acetone (54 ml) is added rapidly to the crude fraction while maintaining the temperature below 5°C. The reaction mixture is then centrifuged and to the supernatant thus obtained, acetone (38 ml/100 ml of the original crude fraction) is added. The reaction mixture is again centrifuged and the precipitate obtained is taken in water to about half the volume of the crude fraction. After centrifugation, the clear supernatant is adjusted to pH 4·75 and then 0·1 vol of 1·2M KCl is added. To each 100 ml of the preparation, ethanol (105 ml) is added, centrifuged and the precipitate discarded. The supernatant fluid is again fractionated with ethanol (65 ml/100 ml being fractionated) and centrifuged. This precipitate forms the 'ethanol fraction', which is taken into about one-tenth the volume of the crude fraction and centrifuged. The specific activity of the ethanol fraction compares well with that of the crystalline muscle enzyme preparation [*Biochemical preparation*, Vol XI (John Wiley & Sons Inc, New York), 1966, 31].

Molecular basis of chemical sensing

K. Owen Ash of Honeywell Corporate Research Centre, Hopkins, Minnesota, USA, has shown by ultraviolet spectroscopy the presence of fundamental biological molecular mechanisms that occur in chemoreception. When homogenized preparations of receptor cells pertaining to the refractory sense isolated from the rabbit nose were stimulated with aqueous solutions containing odorants like linalool or linalyl isobutyrate or both, molecular changes were initiated that could be detected in the form of a spectrometric record showing chemically induced specific decrease in ultraviolet absorbance at 267 nm wavelength. Although freezing and thawing did not affect the activity of the olfactory preparations, they rapidly became inactive when the temperature was raised to 68°C, implicating thereby the involvement of proteins or lipoproteins. The change in absorbance at 267 nm is probably owing to a change in macromolecular conformation, which is initiated by the formation of a complex as a result of an interaction between some specific ingredients in the olfactory preparations and the chemical stimulants. Such conformational changes might trigger the change in membrane permeability needed for chemical sensing by biological systems. The activity monitored at 267 nm is by no means unique to olfactory preparations, but occurs in varying degrees in other biological chemo-sensing tissues like brain and liver, although it is absent in muscle preparations. This shows that at the molecular level the mechanisms of olfactory receptors have much in common with other biological chemical sensors. The highest activity exhibited by the olfactory preparations in comparison with other chemo-sensing tissues might be due to the characteristic structure and location of the olfactory bipolar cells ideally suited to facilitate communication between the odor-containing atmosphere and the brain [*Science, N.Y.*, **162** (1968), 452].

Method for the routine preservation of microorganisms

For carrying out experiments with microorganisms over a long period of time, it is essential to ensure that the source material remains unchanged. The methods currently in use are cumbersome and in most of them the viability of the microorganisms suffers with period of storage. A new method reported is claimed to be free from these limitations. The method is based on a rapid freeze in liquid nitrogen followed by storage at −70°C.

A culture is prepared with the inoculum from a freeze-dried sample or some other suitable source. Using a pipette which delivers 36 drops/ml, drops of culture are allowed to fall into a Dewar flask containing liquid nitrogen. On freezing the drops sink to the bottom of the flask. When 300 or more frozen drops have formed, they are transferred to a container previously cooled at $-70°C$, and maintained at this temperature until required. The procedure is then to remove a single frozen drop with chilled sterile forceps and to put the drop into an empty sterile test tube. After rapid thawing, the suspension formed can be used either to inoculate a nutrient agar plate by stroking with a sterile loop, or to inoculate a growth medium direct. In practice, the plate method is preferable, because it enables the sterility to be checked and also a plate can be used for inoculation for 1-2 weeks.

Esch. coli K12HfrC and *Esch. coli* K12HfrH have been successfully stored for nearly a year by the above method. If a $-70°C$ refrigerator is not available, solid carbon dioxide or a liquid nitrogen storage container can be used. Care should be taken with CO_2, however, because unless the pellet is well sealed, CO_2 dissolves into the frozen pellet and can cause a severe change in pH, especially on thawing [*Nature, Lond.*, **220** (1968), 1139].

National Chemical Laboratory, Poona

The annual report of the laboratory for 1967-68 reveals that 37 sponsored projects (as against 31 in 1966-67) were in progress during the year. Six schemes were at pilot plant stage. A new pilot plant set up for the preparation of organic intermediates was used for developing know-how for the manufacture of a number of industrial organic chemicals. During the year, plants were being established by industry for the production of the following chemicals based on know-how developed by the laboratory: benzoic acid (500 tonnes/annum); titanium tetrachloride (150 tonnes/annum); acetanilide (2000 tonnes/annum); and

calcium hypophosphite (12 tonnes/annum). A plant is being established for the production of 200 units of vapour phase chromatographs per year.

The release to industry of processes in respect of rubber reclaiming and blowing agents, di-*o*-tolylbiguanide, aniline, dimethylaniline, phenacetin, phenylacetic acid, tri(nonylphenyl)phosphite, *n*-nitrophenol, *p*-menthane hydroperoxide and potentiometric strip chart recorder was approved.

During the year, 126 research papers were published. Five new patents were filed, bringing the total number of patents in force to 76.

In the fields of solid state and molecular physics, a number of basic interaction processes involving electrons, phonons, spins and external agencies such as photons have been studied. In continuation of earlier studies on the electronic properties of conjugated organic molecules, the correlation problem in many-electron atoms, considered as a consequence of the divergent nature of the existing Coulomb potential, was investigated. A modified Coulomb potential for interacting point charges was derived.

During studies on elementary excitations in magnetic solids, several new interaction mechanisms involving optical phonon modes were developed. One of the new processes arose from the direct coupling of various spin wave modes with phonons of the transverse optical modes in magnetic insulators. Studies on the effect of different variables on the transition temperature of superconductors have shown some specific two-body interactions to lead to enhancement of the transition temperature; one-body effects of various kinds tend to decrease it. A new mechanism of field dependent mobility in polar semiconductors was considered which accounts for the static and dynamic polarization of the ions induced by the electric field. Electron phonon interaction terms have been formulated in which the dependence of the dielectric constant on the deformation in the system by the application of an external electric field has been incorporated.

Studies on the sandwich system Al/CdS/Al have revealed that at

low voltage the conductivity is 'space-charge' controlled and at high voltage avalanche multiplication becomes the dominating factor. The dual negative resistance has been attributed to the presence of two special types of traps having different capture cross-section.

The structure of *m*-aminobenzoic acid has been refined with the help of three-dimensional data and it has been found that the molecules form dimers with short O...OH bonds; the two C-O bonds of the carboxylic group are nearly equal, suggesting that tautomerism of the type O-H...O $\rightleftharpoons$ O...H-O occurs.

Mössbauer effect studies have been made use of to identify the charge distribution in mixed oxide systems with spinel systems, viz $ZnMnFeO_4$ and $CoMnFeO_4$. The charge distribution has been found to be $Zn^{2+}Mn^{3+}Fe^{3+}O_4$ and $Co^{3+}Mn^{2+}Fe^{3+}O_4$ respectively.

Chromium(III) chelates of ortho-chloroacetoacetanilide, 2,5-dimethoxy-4-chloroacetanilide and acetoacetdiethylamide have been synthesized for the first time. Polarographic studies on copper chelates revealed that the nature of solvent influences the reduction of the chelates. An apparatus has been fabricated for making dry hydrogen fluoride by the thermal decomposition of potassium bifluoride.

A new method developed for the selective deposition of Co from a solution containing cobalt and nickel consists in depolarizing the cathode by introducing nitrate ions into the bath and complexing the Co(II) with malonic acid. Incorporation of perchlorate ions in the medium completely prevents the reduction of nickel ions. An interesting observation has been made in the polarographic reduction of the uncoordinated oxime group of the malonic acid oxime complex of cobalt [HON=C(COO)$_2$. $Co^{II}NO_2(H_2O)_3$]Na. The bound ligand is reduced at a more positive potential than the free ligand.

A tentative structure has been proposed for the gum of *Terminalia tomentosa*. The total polyester present in *Celastrus paniculatus* has been found to possess valuable pharmacological properties. A method has been standardized for

the isolation of cyclolandenol and cycloartenol from the opium marc.

The synthesis of the hitherto unreported ring system 3,4-phthaloyl-2-thiaquinolizine starting from 2,3-dichloro-1,4-naphthoquinone and 2-mercaptomethylpyridine has been achieved.

Synthetic studies were in progress in respect of a large number of compounds including bicyclic thiaxanthylium perchlorates, sulphur analogues of 3,4-benzophenanthrene, polycyclic nitrogen heterocycles, 4-arylcinnolines, bergamotene, etc.

A new method has been worked out for the solubilization of particulate hexokinase of brain and heart. The enzyme from brain was purified to 90% purity. A bacterial enzyme specifically hydrolysing inositol hexaphosphate has been discovered. A new method has been developed for the preparation of soluble acetylcholinesterase from ox brain. The subunit structure of the enzyme citrate-oxaloacetate lyase (citrase) has been determined and the conditions for the dissociation of the molecule into subunits in 3 distinct stages have been established.

An experimental assembly has been set up to study the performance of a packed tower in transferring heat between two directly contacted liquid phases. A new method has been proposed for estimating the Prandtl number. From studies on the mechanism of drop growth and mass transfer during drop formation in liquid-liquid extraction, an equation based on a dynamic similarity with the lines of force in a magnetic field has been proposed; the equation represents the experimental data with an average deviation of about 30%. Nomograms have been prepared for 30 equations which are used in the design of distillation columns. A correlation has been worked out for predicting the activity coefficient from a knowledge of the physical properties of the systems involved.

The product-oriented research projects in hand cover a wide range of products. Notable among these are: photoconductive cells, tin oxide resistor, ferroelectric materials, thermoelectric semi-conductor polycrystalline silicon, soft ferrites, catalyst materials, silicones and silicone intermediates, butyl titanate, chromatographic adsorbents, manganese perchlorate, ascarite, polytetrafluoroethylene, di-o-tolylbiguanide, dyes for synthetic fibres, triethyl phosphate reactive dyes, tri(nonylphenyl)phosphite (Polygard), potassium ethyl xanthate and potassium amyl xanthate, vanillin, synthetic glycosides and other synthetic carbohydrate materials, cellulose caprate, vitamin B_6, diethyl-m-toluamide, p-menthane hydroperoxide, colchicine, allantoin, ethylene brassylate, p-nitrophenol, acetyl sulphanilyl chloride, stationary liquid phases and solid support for gas liquid chromatography instruments, transformation products of citral, theophylline caffeine, 19-norsteroids and other modified steroids, chamazulene, styrene-DVB base cation exchange resin, acrylic base cation exchange resin, polystyrene foams, polyurethanes, styrenated alkyds, linseed oil emulsion, epoxy resin coatings, cement for diamond setting, adhesives for staple pins, self-sticking composition for paper labels, nitrile rubber, thiokol rubber, rubberized cork sheet, latex from backed coir, rubber reclaiming agents, shell moulding resin, 'Sinol' core binder, dry core binder, starch phosphate, double boiled linseed oil substitute, low temperature fluxes, methylamines, ethylenediamine, phenacetin, 2,4-dinitromonomethylaniline, ethyl acetoacetate and methylol stearamide.

Projects sponsored by industry, State and Central government institutions relate to benzoic acid, industrial chemicals from diketone, nonylphenol, alizarin, anthraquinonoid intermediates and dyes, chlorinated copper phthalocyanine, l-menthol, calcium hypophosphite, carbimazole, megimide, radioopaque dyes, sodium cyclamate, sorbide nitrate, sulphacetamide and its sodium salt, calcium silicate, silica-based tantalum pentoxide catalyst for butadiene synthesis, rayon grade pulp, β-phenethyl alcohol, perfumery grade geraniol, citronellal and citronellol from lemongrass oil, steroids and wax from sugarcane press mud, lac dye, titanium tetrachloride from ilmenite, and dissolving pulp from hard woods. The projects sponsored under PL-480 funds include (1) chemical and thermodynamic properties of refractory materials at high temperatures; (2) synthesis and properties of new type glycol monoalkyl ethers for the control of water evaporation to extend the industrial utilization of cottonseed oil; (3) wood phenolics, with special reference to their use in chemotaxonomy and their biosynthesis by tissue culture studies; and (4) effect of heat on tung oil and derivatives of tung oil and the characterization and identification of compounds resulting from heat treatment to extend the utilization of tung oil.

Pilot plants are in operation for studying the know-how for the production of (1) aniline, (2) tri- and tetrachloroethylenes and ethyl chloride, (3) phthalates, (4) dissolving pulp, (5) fine chemicals, and (6) organic intermediates.

Weather modification research

The control of weather enables man to reduce his losses incurred annually from the vagaries of nature in the form of hurricanes, hail, fog, drought, etc. Various research institutes in different countries are presently engaged in studies that will help man to understand the basic atmospheric processes that will enable him to modify weather. The Ninth Annual Report (for the year 1967) of the National Science Foundation, USA, describes the progress resulting from the increasing efforts undertaken by the different US research agencies in this endeavour.

The year under consideration has seen increasing use of computer mathematical models of small- and large-scale atmospheric processes like cloud droplet growth, hurricane, fog, etc. This modelling technique may ultimately prove to be an indispensable tool in attempts at large-scale weather modification.

As adequate knowledge about the mechanism required to modify weather on a large scale is lacking at present, greater emphasis was placed on attempts to modify weather within a 100-mile radius from the point of operation. Research on large-scale modification

had been largely restricted to the study of computer mathematical models of the atmosphere.

Computer models developed by the Pennsylvania State University were used successfully to determine the real time when and where to inject ice nuclei into building cumulus clouds. Different computer analyses have also been developed to study the feasibility of seeding experiments from aircraft.

Attempts have been made to disturb the energy balances of hurricanes by inducing artificial asymmetries or competing circulations. Modifications were attempted not to annihilate them completely but only to reap their benefits by moderating the hurricane severity. It has not yet been proved that modification of typhoons and hurricanes is feasible; new possibilities to this end are being explored both in field operations and mathematical modelling.

Studies on a mathematical model for a hurricane developed at the New York University have shown the importance of oceanic sensible and latent heat in the development and maintenance of a hurricane. The model is being programmed on the possibility of modifying the intensity through control of the water vapour supply from the sea surface.

Realizing that the atmospheric electricity plays a major role in the weather formation, various studies were undertaken to study the electrical forces in the atmosphere. These studies have shown that enhanced electrification accompanies the formation of ice in the clouds.

FORTHCOMING INTERNATIONAL SCIENTIFIC CONFERENCES

Date	Conference	Place
7-27 August	Eighth International Congress on Crystallography	New York
10-15 August	Sixth International Congress of Chemotherapy	Tokyo
11-14 August	International Symposium on Electron and Nuclear Magnetic Resonance	Clayton
11-15 August	Second International Conference on Medical Physics	Massachusetts
12-15 August	Third International Photo-Conductivity Conference	California
18-22 August	International Conference on Interfaces	Melbourne
20-27 August	Twenty-second International Congress on Pure and Applied Chemistry	Sydney
20-27 August	Twelfth International Conference on Coordination Chemistry	Sydney
24-31 August	Seventh International Conference on Soil Mechanics and Foundation Engineering	Mexico
24 Aug-2 Sept	Eleventh International Botanical Congress	Washington
26 Aug-1 Sept	International Scientific Symposium on the Geodesy and Geology of Mines and the Geometry of Mineral Deposits	Prague
28 Aug-5 Sept	Eighth International Congress of Nutrition	Prague
29 Aug-3 Sept	Third International Biophysics Congress	Cambridge
30 Aug-5 Sept	Eighth International Union for Quaternary Research Congress	Paris
31 Aug-3 Sept	International Wood Chemistry Symposium	Seattle
31 Aug-4 Sept	First International Conference on Calorimetry and Thermodynamics	Warsaw
31 Aug-5 Sept	Thirteenth International Congress of Association for Hydraulic Research	Kyoto
31 Aug-6 Sept	Fifth International Biometeorological Congress	Montreux
August	Fifth International Symposium on Neuro-secretion	Kiel
August	Seventh International Conference on High Energy Accelerators	USSR
2-5 Sept	International Symposium on Durability of Concrete	Prague
2-7 Sept	Ninth International Embryological Conference	Moscow
3-9 Sept	International Symposium on Macromolecular Chemistry	Budapest
5-10 Sept	Third International Clay Conference	Tokyo
7-14 Sept	Fourth International Congress on Metallic Corrosion	Amsterdam
8-10 Sept	International Symposium on Distillation	Brighton
8-10 Sept	International Colloquium on the Physical Properties of Solids under Pressure	Grenoble
8-12 Sept	Sixteenth International Symposium on Microscopy	London
8-13 Sept	Seventh International Congress on Clinical Chemistry	Evian & Geneva
8-13 Sept	Twenty-ninth International Congress of Pharmaceutical Sciences	London
9-12 Sept	International Symposium on Conformational Analysis	Brussels

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