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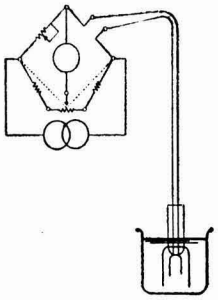

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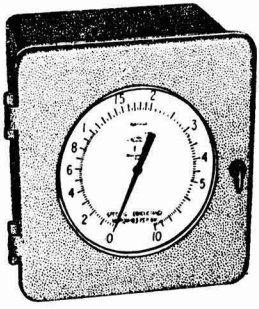

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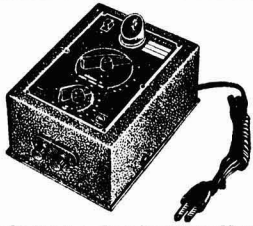

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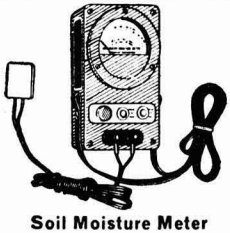

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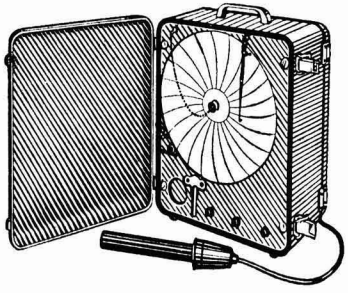

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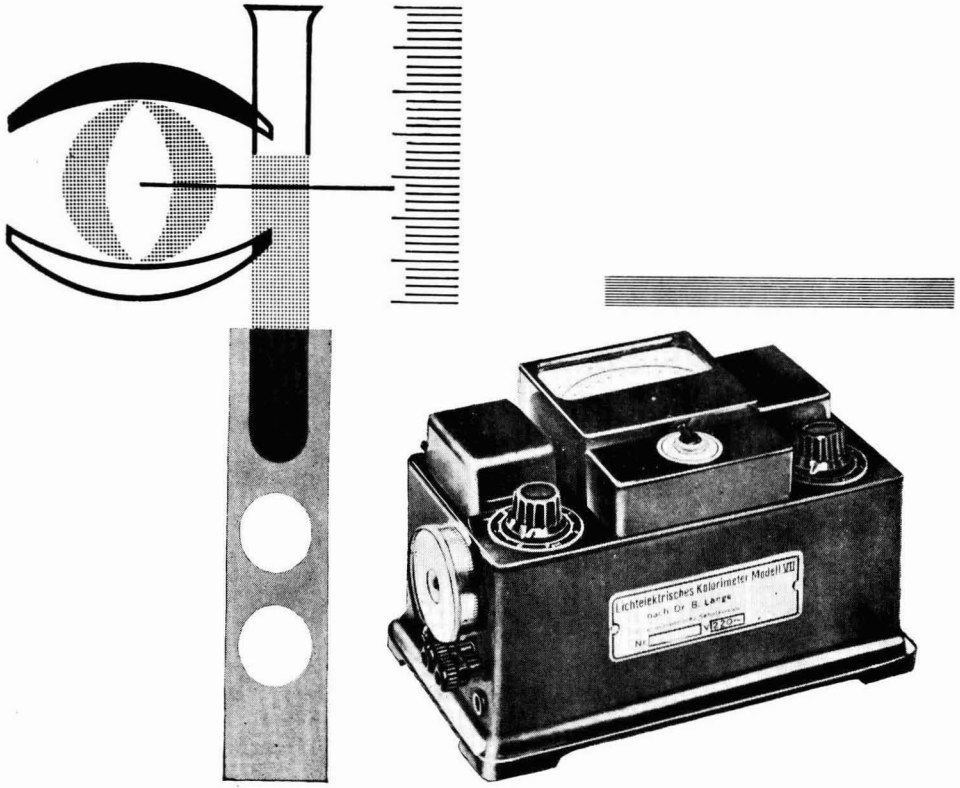

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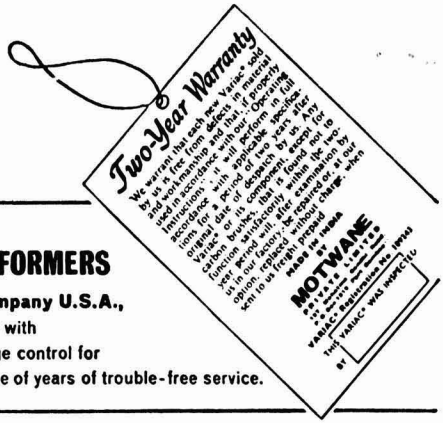

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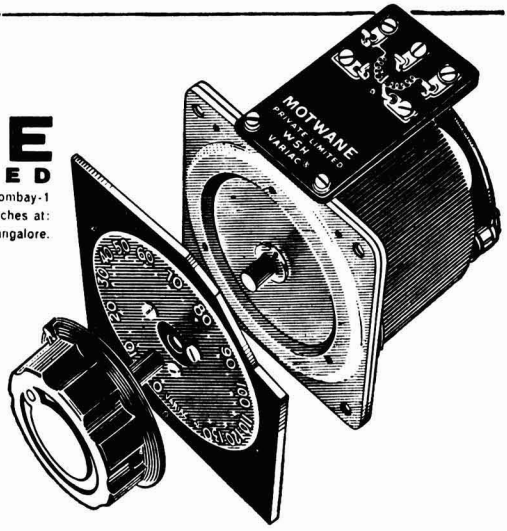

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Current Topics

Census of Scientific & Technical Personnel in India

ALONG with the decennial population census of India in 1961, a special census of scientific and technical personnel in the country as on 1 February 1961 was undertaken. The census data, collected through questionnaire cards, have been compiled by K. Ray, Head of the National Register Organization of the Council of Scientific & Industrial Research (CSIR), New Delhi, and published recently as a monograph* by the Registrar-General, India. The monograph provides, for the first time, a quantitative estimate of our scientific and technical manpower resources, and how these resources are deployed and utilized. The need for such a census arose because of the role of scientific and technical personnel assuming greater importance in the context of increasing emphasis on industrialization during the successive Five Year Plans. The census envisaged a wide coverage, and though a complete enumeration could not be achieved, it provided a large sample, collected all at once over the entire country, thus making it possible to project the findings over the entire scientific and technical population. The census covered 245,366 persons, roughly 60 per cent of total personnel.

Scientific and technical personnel have been defined as those having a recognized degree or diploma in science, engineering, technology or medicine, and are classified under 122 descriptions according to their qualifying subjects and academic levels. The studies are presented from different perspectives under 10 broad groups, viz. employment patterns; pay and earning; research personnel; professional personnel with additional degrees; scientific and technical personnel in non-technical work; women in science and technology; unemployment; interstate mobility; regional characteristics; and overseas trained personnel. The total enumeration grouped under three professional categories was 117,943 scientific, 83,116 engineering and technological and 44,307 medical personnel. Thus for every 100 science graduates there were 75 engineers; in advanced countries the number of engineers is greater compared to science graduates. Of the scientific personnel, 19 per cent held post-graduate degrees and the ratio between post-graduates and graduates was 1 : 4. In the engineering and technological category, 34,292 were graduates and 48,824 diploma holders. Medical personnel include 22,623 degree holders and 10,015 diploma holders.

A study of the employment pattern furnishes interesting facts. Two-thirds of the personnel were working in the public sector, a little less than a fourth in the private sector, and about one-tenth were self-employed. Teaching in schools and colleges, and technical work in and outside industry have been considered as technical employment. About a fifth of the personnel were in the teaching profession and a little less than a quarter were employed in industry. More technical jobs were outside the industrial sector and over one-third came under this category.

The overall non-technical employment of all categories was 18·6 per cent. It was nearly 40 per cent in the case of graduates in general science and 16·7 per cent for post-graduates (25·1 per cent for women post-graduates); out of 143,000 science graduates, 56,000 were engaged in non-technical occupation to which another 25,000 should be added as unemployed. Among the engineers and technologists about 4000 (6·5 per cent) engineers at the degree and diploma levels were engaged in non-technical pursuits. About 6·6 per cent medical graduates and 7·9 per cent diploma holders were reported to be similarly employed; about 6000 doctors were engaged in non-professional or semi-professional jobs and another 6000 were unemployed. Thus there is a considerable wastage of scientific and technical personnel as a result of drift to non-technical vocations and unemployment.

Unemployment among scientific and technical personnel was as high as 10·4 per cent of the total number enumerated, the break-up being science graduates 16·3 per cent, post-graduates 7 per cent, graduate engineers, technologists and diploma holders 3·7 per cent, and medical graduates 7·3 per cent and diploma holders 8·6 per cent. About 14 per cent of the persons qualified in indigenous systems of medicine were unemployed. Among post-graduates, the geologists and geophysicists recorded highest proportion of unemployed (10·7 per cent) followed by biologists (9·4 per cent), statisticians (7·7 per cent), mathematicians (7·2 per cent), chemists (5·7 per cent; chemical engineers 5·6 per cent of graduate engineers and technologists), physicists (4·8 per cent), and agricultural scientists (4·7 per cent).

A chapter devoted to overseas trained personnel, not forming a part of the census, is informative. Of about 20,000 overseas trained personnel, 10,000 are engineers, 4000 scientists and 6000 medical personnel. Of these only 20 per cent have assured employment on return to India and the rest have to look for employment.

The annual out-turn of science graduates (B.Sc.) rose to about 32,000 in 1963, indicating a fourfold increase since 1947-48. The out-turn of agricultural

Census of India 1961: *Scientific and technical personnel — Monograph series No.* 1 by K. Ray (Office of the Registrar-General, India, New Delhi), 1966. Pp. 88. Price Rs 1.50.

scientists also increased four times during this period. Post-graduates (M.Sc.) increased sixfold, i.e. to 6000, in 1963. Engineering graduates increased from 1300 in 1947-48 to 9000 in 1963-64, and diploma holders from 1440 to 13,000. Out-turn of medical graduates increased from 1566 in 1950 to 3256 in 1960.

An important conclusion that emerges from the census data — rather a disquieting one — is that our scientific and technical manpower resources are not properly and fully utilized. This is a needless and avoidable waste, and indirectly wastage of the resources of the country which could be put to better use. It is paradoxical that while planners forecast shortage of scientific and technical personnel at all levels for each Five Year Plan, out of 40,000 scientists and technologists, nearly 30 per cent were either employed in non-technical vocations or were unemployed at the beginning of the Third Five Year Plan. There is a manifest need for more qualified teachers, and it is difficult to reconcile oneself to the fact that a large number of scientists are engaged in non-productive employment. It is even more difficult to understand why doctors, engineers and post-graduate scientists have so drifted. There has been a considerable increase in our output of scientific and technical personnel during the Third Five Year Plan, and the situation needs careful review. Scientific and technical education is expensive; the cost of producing a graduate in science is estimated at about Rs 2500 and it would be more in the case of post-graduates, and the professional courses like engineering and medicine still more costly.

It appears that there is no realistic correlation between the projected out-turn of scientific and technical personnel of different categories and the actual demand. It is, therefore, urgently necessary to go deeply into the reasons contributing to the wastage of scientific and technical manpower trained at enormous cost. The factors which come in the way of these personnel finding suitable technical occupations have to be analysed and clearly understood and quick remedial measures initiated. Equally serious is the problem of overseas trained scientific and technical personnel. Their loss is more serious perhaps in terms of quality than numbers, and also represents considerable expenditure of foreign exchange. One of the complaints of industry, which should increasingly absorb our scientific and technical personnel, is that they do not measure up to their requirements. This problem has been investigated by the National Register Organization of CSIR, but has to be more thoroughly gone into. The requirements of the employer in various scientific and technical establishments and industry for different categories and levels of scientific and technical personnel have to be precisely established and our scientific and technical education pattern has to be suitably oriented to meet the quickly changing demands of the country. The colossal wastage of graduates in general science must be stopped. It is likely that these graduates before they took to the science degree courses were not aware of the openings and opportunities for them with just a degree in general science. Counselling services must be set up and strengthened in each university and higher technical institute to advise the prospective graduates what the future holds for them and help them to arrive at a worthwhile decision. The responsibility of universities and higher technical institutions should not stop with just imparting instruction; they should shoulder the responsibility of advising the future graduates in science what should be their best choice of scientific and technological disciplines to carve out a useful career for themselves. These agencies should also advise personnel during their career how to improve their qualifications so as to fit them to the needs and demands of the society. The acute shortage of science teachers has been voiced many times. One of the suggestions made in the monograph is that the graduates in general science should be absorbed as teachers. More opportunities should be made available for science graduates to equip themselves as teachers. Above all, scientific and technical education must be geared to the requirements of the country both in qualitative and quantitative terms. Scientific and technical education has been and is being expanded rapidly, and it is the responsibility of the educationists, both at the centre and the state levels, to maintain requisite standards.

The monograph is a valuable document and has brought to light many important features regarding utilization of scientific and technical personnel. The author of the monograph, who initiated the project, has done a signal service to the country by this timely publication, particularly when we are on the threshold of the Fourth Five Year Plan. It is to be hoped that this census will be a permanent feature of the decennial population census of the country. However, 10 years is too long a gap considering the rapid progress science and technology are making and equally rapidly changing economy of the country. It may be worth while for the National Register Organization to undertake a similar census by questionnaire once in five years so that it will help the planners to set realistic targets. It should also attempt to project, on the basis of the data collected, as to the future pattern of scientific and technical education, and employment opportunities. While doing so, the trends and developments elsewhere should be taken into consideration.

Gravitational Theories*

J. V. NARLIKAR

THE gravitational theories of Newton and Einstein may be regarded as classics since they have profoundly influenced the trends in physics. Newton's theory has the merit of being simple in its formulation. It could successfully account for so many observations on gravitation on the earth and the solar system. For two and a half centuries, Newtonian ideas dominated the thinking of the physicists.

Why give up such a simple and successful theory? By the beginning of this century physicists began to notice cracks in the logical foundations of Newton's theory. Special theory of relativity had shown the Newtonian concepts of space and time to be inadequate. A revision of these concepts was initiated. Moreover, Newtonian gravitation propagated with infinite speed — contrary to the ideas of special relativity. A more elaborate theory of gravitation was required. Einstein undertook this task and came up with general relativity.

Physicists usually discard an old theory for a new one when experiments and observations require such a change. On this ground alone such a change-over from Newton to Einstein was not justified. The so-called 'three classical tests' of general relativity do not really test the full consequences of the theory but only some of its superficial aspects. Gravitation being a weak force, laboratory tests are not easy to think of. For this reason progress in gravitational theories has proceeded more in the abstract manner of 'thought experiments' rather than in the 'trial and error' method of the rest of physics.

General relativity successfully incorporated the above necessary changes. Einstein, however, wanted to accomplish more. First, he wanted to incorporate Mach's principle in his theory — a task in which he only partially succeeded. Observation shows that the local inertial frame is one in which the distant parts of the universe are non-rotating. Does this follow uniquely from the cosmological solutions of general relativity? Counter-examples by Godel and others have shown that this is not so. This observation was the basis of Mach's principle. Moreover, Machian difficulties of a single particle in an otherwise empty space, which were present in the Newtonian theory, remain in the Einstein theory also. Einstein's second aim of a unified field theory remains as remote today as ever. Even if we manage to marry gravitation to electromagnetism, we still have to incorporate the weak and the strong interactions, and any other possible interactions that may be discovered in future. In this sense, Einstein's task has been left incomplete.

Two years ago Prof. Hoyle and I put forward a new theory of gravitation, which we now call the '*conformal theory of gravitation*'. The motivation for evolving a new theory did not come from experiments, for the reasons mentioned before, but from theoretical reasons. Earlier we were working with the generalization of the Fokker action principle of electrodynamics to Riemanian space. It was possible to do this in a satisfactory manner, showing that electrodynamics could be described entirely in terms of particle interactions as hoped for by Gauss in the last century. This method does away with the 'field' concept. If the particle-particle interaction could be made to work in the case of electrodynamics, it should be made to work in the case of gravitation also.

In electrodynamics the interaction is described by *bi-vector* Green's functions. The electromagnetic fields are not independent entities as in Maxwell's theory, but are derivable from the world lines of charged particles by means of Green's functions. The analogous quantity in the gravitational theory turns out to be mass. The mass can be defined by means of a *bi-scalar* Green's function in terms of particle world lines. Moreover, the electromagnetic equations are conformally invariant. The scalar Green's function chosen to define mass is also conformally invariant and this leads to a conformally invariant theory of gravitation. Hence the name chosen.

The theory incorporates Mach's ideas at the outset. The inertia of a particle is not its own property, but a property it *derives* from being in the presence of other particles. For there to be any theory at all, there should be at least two particles in the universe. The Machian is, therefore, avoided.

The equations of the theory simplify in the many particle case by the use of the smooth field approximation. Such an approximation works provided one is not too near a particle. The equations can then be transferred conformally to Einstein's equations, with the following two gains. The sign of the constant of gravitation is always positive and the x-term is absent.

McCrea has argued that our conclusion that the gravitation is always attractive in the conformal theory is based on the assumption of a positive sign of mass. This is not so. Even if the masses were all negative the observed phenomena would be the same. The change of sign for mass is a mathematical transformation that does not affect physics. We find it very satisfactory that the sign of gravitational constant should follow from such a general principle as conformal invariance. This also answers the criticism of Pirani and Deser.

The equations become similar to those of Einstein in the macroscopic case and so the theory predicts the same macroscopic results as general relativity. The equations become different, however, in the neighbourhood of particles where smooth fluid approximation does not work. The geometry in

*Based on a talk delivered by the author at the Seminar on Relativistic Cosmology and Gravitational Theories held at the National Physical Laboratory, New Delhi, during 21-22 February 1966.

this region is very strange, as has been shown by a later investigation. The nonlinearity of the equations arises as a result of the presence of the particles. Hawking, in a recent calculation, has computed Green's functions on the basis of the smooth fluid approximation being valid *everywhere*. Such a calculation ignores the inherent nonlinearity of the theory. Indeed the Wheeler-Feynman type calculation, which was possible in electrodynamics, is not possible here for the same reason.

Pirani and Deser argue that our theory is a ' non-local ' field theory for I_{ik} and not a particle theory. That particles are more fundamental than I_{ik} is shown by the fact that in the absence of particles there are no equations left. The I_{ik} themselves are not to be looked upon as fields but as geometrical entities. Even in general relativity the I_{ik} do not behave as pure fields (like the Maxwell fields) and this has led to difficulties in gravitational radiation and quantization.

The mathematical structure of the conformal theory resembles partially the theories of Dicke, Peres, Guisey, etc., but the central idea, viz. direct particle formulation, is quite different. Further consequences of the equations of this theory are under investigation.

Hydrogen Production & Utilization in Petroleum Refining & Petrochemical Industries—A Seminar

I. B. GULATI

Indian Institute of Petroleum, Dehra Dun

DURING the past ten years, significant advances have been made in the methods of production of hydrogen from liquid petroleum hydrocarbons. These processes are of particular importance in the context of large surplus of naphtha in India and the dire need of meeting the ever increasing demands of fertilizers. At the same time, availability of cheap hydrogen has given a big spurt to the application of hydrotreating techniques, such as hydrodesulphurization, hydrogenation, hydrocracking and hydrofinishing in petroleum refining industry. In view of the urgent need to upgrade the low grade kerosene from Assam crudes and produce more of middle distillates, for which the country's demand is large, active research work has been going on at various centres in India on a number of projects in this field to develop commercial processes and know-how. It was, therefore, appropriate and timely that the Indian Institute of Petroleum (IIP), Dehra Dun, organized a seminar on the subject during 19-21 March 1966, and created an opportunity for scientists and technologists in India, and also specialists from abroad to have a critical assessment of the research and development in the field and to focus attention on the recent techniques for production and application of hydrogen in petroleum refining and petrochemical industry.

The response to the seminar from the various research organizations as well as from the leading Indian and foreign firms was encouraging. As many as thirty papers were contributed by specialists and research organizations actively engaged in the field. The deliberations of the seminar were spread over three days and divided into five technical sessions of three hours' duration each. In order to make the discussions fruitful, the number of papers presented at each sitting was limited to 3-4.

The seminar was inaugurated by Shri O. V. Alagesan, Minister for Petroleum and Chemicals. In his inaugural address, Shri Alagesan referred to the commendable part played by the IIP in the advancement of petroleum technology and the amount of work it has put in by way of research, project studies, market surveys, etc. He pointed out that the production of hydrogen from petroleum and non-petroleum materials and its conversion into ammonia for nitrogenous fertilizers are the fields in which the country as a whole is vitally interested at the moment. He stressed the need for multiplying both the capacity and production of fertilizers, several times if we have to avoid large-scale import of food from other countries. The prevailing price of fertilizers in India is double that in the most advanced countries, and Shri Alagesan hoped that the seminar would discuss how hydrogen can be produced not only from naphtha but also from abundant raw materials like coal and lignite and that too at much lower cost than is the case today. Another area to which Shri Alagesan drew the attention of the scientists and technologists was the application of hydrogen processes to increase the production of middle distillates like kerosene and diesel oils, as the country will be faced with deficit in these products for several years to come. He desired the seminar to discuss these processes and advise on their utility in finding solution to the country's serious refining imbalances.

In his initiation paper, Dr M. G. Krishna, Director, Indian Institute of Petroleum, surveyed the status of hydrogen production and utilization in India, and drew attention to the fact that ammonia industry is, and will continue for a long time to come, the major consumer of hydrogen in India. At present, only about 35 per cent of the hydrogen used for fertilizer manufacture in the country is based on naphtha and refinery gas. This is expected to increase to over 75 per cent by 1970-71, when India is expected to produce 2·4 million tons of

nitrogen per annum. About 65 per cent of the world's ammonia production is from petroleum-based raw materials and less than 40 per cent from coal-based products. The use of hydrotreating operations increases correspondingly with the availability of hydrogen either as byproduct in refineries or by new reforming techniques. The hydrotreating capacity has increased by 90 per cent during 1964-66 in many parts of the world and is being extensively used for the removal of sulphur and other contaminants. Simultaneously, the hydrocracking technique for producing light and middle distillates has come into wide use. In USA, the hydrocracking capacity has increased from 3000 b.b.l./day in 1961 to 171,000 b.b.l./day in 1965. The large demand for kerosene and other middle distillates in India would justify consideration of hydrocracking technique. On the other hand, increased dependence on cheaper foreign crude with higher sulphur contents would need wide application of hydrodesulphurization and hydrogenation. It can be anticipated that within the next 5-7 years, large-scale expansion of hydrogen production facility would take place in the country and there is a great need for concerted efforts in the country to develop process know-how, design of plants and development of suitable catalysts.

Twenty-five papers were presented in the five technical sessions, which followed the inaugural session; all of them evoked useful and stimulating discussion and a free exchange of ideas.

Production of Hydrogen from Oil and Gas

At this session eight papers dealing with different aspects of production of hydrogen from naphtha were presented. The first paper by Dr K. K. Bhattacharyya and Siddapa Patil (IIP) discussed the basic aspects involved in the various processing schemes for hydrogen production from different materials and explained how the H/C ratio of the material affects the energy requirements and processing steps and thus finally the cost of production of hydrogen.

A comprehensive review of the developments in hydrogen production and its use for the synthetic and refinery processes in Russia was presented by Prof. A. T. Grinevich (Unesco Expert, IIT, Bombay). In the USSR, where 240 million tons of oil and 130 billion cu.m. of gas have been produced during 1965, the natural gas and byproduct oil gases provide about two-thirds of all hydrogen consumed. Because of the relative cheapness of hydrogen produced from natural gas, a number of installations for the production of hydrogen from coke have been replaced by new ones based on natural gas. The new catalysts developed for conversion, shift reactions and hydrodesulphurization described by the author evoked a good deal of discussion. Hydrocracking, according to Prof. Grinevich, is not yet sufficiently developed in USSR.

The salient features of the two important commercial processes for the production of hydrogen from naphtha were discussed in papers contributed from ICI and Messrs Heinrich Koppers. Presenting the first paper, Dr K. S. Gill (ICI) gave a lucid account of the development of ICI steam reforming naphtha. The method of addition of the endothermic heat of reaction is a major problem in the design of an oil gasification process. In the ICI process, the reaction is carried out continuously over a catalyst contained in a series of tubes held in parallel and suspended in a furnace from which the necessary heat of reaction is continuously transferred to the reactant stream. The development of a catalyst which is highly selective and active in the reforming of naphtha and stream, but which is completely inactive in all the reactions that are involved in the carbonization of naphtha, is the key step in the commercial success of the ICI process. The discussion that followed covered materials of construction, tube design and feed specifications. The Koppers' paper discussed the Koppers KONTALYT process for the production of hydrogenous gases from hydrocarbons. After hydrodesulphurization over Co-Mo catalysts, the steam-naphtha reaction is carried out over a nickel catalyst in the externally heated reformer tubes. The carbon monoxide in the crude mixture is converted in one or two stages and the carbon dioxide removed in washers. The paper also dealt with the production of hydrogen from bituminous coal and lignite.

Shri Viswanathan and Dr S. K. Mukherjee (FCI, Durgapur) discussed the metallurgy and design features of naphtha reformer furnaces as well as such aspects as corrosion, method of heat supply and burner design. During the discussion that followed, it was reported that the Fertilizers & Chemicals, Travancore (FACT), is now designing these furnaces under a collaborative agreement. The fabrication of the special alloy tubes as per their design will be done abroad.

Dr Desikan (IIP) presented the work done in the IIP on the production of hydrogen-rich gases by steam reforming of Ankleswar naphtha cut 30-130°C. at atmospheric pressure. Under optimum conditions, 100 per cent conversion of naphtha was obtained and by subjecting the gas to purification 94 per cent pure hydrogen could be produced without using any second stage reactor. The authors agreed that the economics of atmospheric pressure process cannot be decided without considering possible end uses of hydrogen.

There were two more papers in this session; 'Manufacture of hydrogen as a byproduct during the demethylation of toluene with steam' by Shri M. Ravindram and Dr N. R. Kuloor (Indian Institute of Science, Bangalore), and 'Production of hydrogen' by Shri K. J. Sethuraman and Shri Y. B. G. Verma (IIT, Madras). In the absence of the authors, these two papers were circulated for comments.

Production of Hydrogen from Coal and Lignite

In the context of large reserves of coal and lignite in the country and the present need for maximum utilization of indigenous resources, it was in the fitness of things that the second session was devoted to problems involved in the production of synthesis gas from these non-petroleum raw materials.

There were in all four papers on this subject contributed mainly from industry using coal or lignite as the raw material.

The essential features of the Koppers-Totzek process for the production of crude synthesis gas from ash-rich coals were discussed in the paper contributed by Dr Afred Jager (Koppers). It was observed that the process is good for slacks, but the cost of grinding is high. Dr M. Ramacharyulu (Regional Research Laboratory, Hyderabad), presenting experimental data on the gasification of the high ash and high fusion point coals of Andhra region, claimed that the work has established the operatability of an experimental atmospheric fixed bed slagging gasifier using solid fuel with an ash content of 30 per cent.

Of the various commercial processes available for oxygen gasification of lignite, the fluidized bed Winkler gasification process was chosen for India's first lignite gasification plant. The working of this unit was discussed in a paper presented by Shri C. R. Reddy (Neyveli Lignite Corporation).

It was felt that in an under-developed country like India with large potential market demand for fertilizers and chemicals, the large reserves of low grade coals and lignite are an important potential source of synthesis gases and there is need for continued research and development in this area.

Utilization of Hydrogen for Ammonia Manufacture

The papers (2) in this section dealt with problems involved in the manufacture and purification of synthesis gas for ammonia production. The various process routes for production of ammonia synthesis gas from coke oven gas were discussed in a paper from the Fertilizer Corporation of India (FCI), Sindri. Investment and production costs of three alternative schemes, including that adopted at Rourkela, were compared. The authors felt that if quantitative production of nitrogenous fertilizers has to be achieved concomitantly with economy in cost, coke oven gas should be allowed to figure more prominently as a feedstock for fertilizer production and necessary rearrangement of the fuel utilization pattern made.

In another paper from FCI (Sindri), the process routes for the conversion of primary gases of a steam catalytic reformer to ammonia synthesis mixture were discussed. The main consideration in selecting the process routes are the most economic use of energy and heat available from the reformer operations or recovered as steam from subsequent ammonia synthesis. While acceptable varieties of catalyst for water-gas shift reaction are already being manufactured by FCI (Sindri), they are also actively engaged in developing a low temperature CO-conversion catalyst of Zn-Cu-Cr type.

Several important points of equipment and process selection were raised by Shri N. D. Gopinath (FACT Engineering & Design Organization) in his paper on 'Modern developments in synthesis gas manufacture via steam naphtha reforming process'. A comparison of the economics of using motor driven reciprocating compressors versus steam driven centrifugal compressors showed an advantage of Rs 15 per ton of ammonia for the latter, but at the same time, manufacture and operation of the centrifugal type of compressors involve certain difficulties.

The salient features of the various processes available for the purification of synthesis gas were explained by Shri P. G. Menon (Neyveli Lignite Corp.). He discussed in particular the technique and economics of Vetro coke process for the removal of carbon dioxide from gases.

In the absence of the authors, a paper contributed from IIT, Madras, on various ammonia synthesis processes was circulated for comments.

Utilization of Hydrogen in Petroleum Refining and Petrochemicals

The largest number of contributions (12) was on the application of hydrogen in petroleum refining and petrochemicals and covered processes like hydrogenation, hydrocracking, hydrodesulphurization and hydrofinishing. The papers discussed processes and data, commercial applications and economics of plants and processes.

The opening paper by Mr Cessou and Shri Himmat Singh (Training Division, Indian Institute of Petroleum) was a comprehensive review and critical assessment of the role of hydrogen in modern refineries and outlined the tremendous potentialities that these processes hold. The authors discussed, in particular, the integration of hydrogen-based processes in a modern refinery. This paper was aptly followed by a contribution by Shri Sachdeva (IIP) on the economics of major hydrogen consuming processes; the particular cases studied were hydrocracking for maximum production of middle distillates, hydrodesulphurization of a gas oil, benzene hydrogenation for cyclohexane and hydrodealkylation of toluene for benzene production. In general, these units are economical only when hydrogen at equivalent fuel value is available, while the high cost of hydrogen from a supporting hydrogen producing plant may make the operation economically unattractive.

With this background of the overall scope of process techniques and economics of application of hydrogen, the seminar proceeded to discuss specific processes. The first case to be discussed was the Shell Trickle Hydrodesulphurization Process. Mr W. M. J. Ruedisulj (Shell Manufacturing Adviser) covered in a precise manner all the aspects of this process which has found wide acceptance in industry. During the discussion, it was pointed out by the author that the process is not designed to saturate aromatics and thus while treating a kerosene stock cannot give a simultaneous improvement of smoke point along with desulphurization.

The work done at the Indian Institute of Petroleum and Central Fuel Research Institute on the upgrading of Nahorkatiya inferior kerosene by hydrogenation was presented in the next two papers. The aim of these studies has been the development of industrial hydrogenation processes to replace the liquid sulphur dioxide extraction process used at present. The work done at IIP in this field was described by Shri Niyogi and Mr Duhaut. The aspects studied include improvement of smoke point and required chemical consumption of hydrogen at different improvement levels, development of a suitable catalyst for producing a superior kerosene and influence of process variables.

In the light of activity data of cobalt molybdate, nickel molybdate and tungsten-nickel type of catalysts, the feasibility of single-step hydrogenation or working in two successive hydrogenation steps was discussed. A contribution on the subject from CFRI, presented by Shri Mukherjee, covered both the hydrogenation of straight-run kerosene distillates and aromatic extract. Some of the runs with unsupported molybdenum sulphide have given, from straight run Nahorkatiya kerosene, a product with a smoke point as high as 28, but in the absence of complete data on the catalyst life, hydrogen consumption, etc., comparison of the two processes is not possible.

In view of the need to import large quantities of middle distillates, the possibility of hydrogenating neutral oils from low temperature tar fractions to middle distillates was discussed in a contribution from the Regional Research Laboratory, Hyderabad. The paper presented by Mrs A. Mirza gave the data obtained from investigations conducted on bench scale and pilot units using a sulphide catalyst. The product from the pilot plant compares favourably with a sample of marketed kerosene except that its density is high.

In recent years, one of the most notable applications of hydrogen in the refining industry has been in the hydrocracking processes. Three papers dealt with the hydrocracking processes. Shri Karnath and Shri Mohan Rao (IIT, Kharagpur) reviewed the developments in the hydrocracking technique and its possible application under conditions obtaining in India. The details of the hydrocracking process developed at the French Petroleum Institute and data on the economics of the production of maximum gas oil were presented by Mr R. Dutriau of IFP. The process can work in one or two steps, the choice depending on the characteristics of feedstocks and type of operation. The process which uses a fixed bed reactor with optimized quench system to eliminate the heat of reaction is specially useful for obtaining maximum production of diesel oil. A pay out period of 3·50 years for the two-step total conversion and 2·84 years for one-step without recycling was claimed.

In a paper on the hydrocracking of neutral oil fraction of low temperature tar, by Shri Janardana Rao and Dr M. G. Krishna, it was reported that cobalt molybdate and nickel sulphide on silica-alumina give higher yields of gasoline compared to silica alumina when working at 50 kg./cm.^{2} and hydrogen to a feed ratio of 500.

Another field of refining in which hydrogen is finding increasing use is the finishing of lubricating base stocks. The essential features of the well-established conventional method of finishing lube stock by clay treatment and the new hydrofinishing techniques were compared in a paper presented by Shri J. M. Sagar and Dr I. B. Gulati (IIP). A critical assessment of the two processes was made with respect to the characteristics of the treated stocks. This paper was followed by a presentation of the economics of the IFP hydrofinishing process by Mr Dutriau, who also dealt with the possible extension of the hydrotreating process for replacing solvent extraction for the refining of lube stocks.

In the interesting and long discussion following these two papers, the oxidation stability of treated oils, their additive response and, especially, the application of the two processes to the reclamation of used oils were discussed. It was pointed out that for the reclamation of used oils, a process which requires first a propane precipitation to remove the degraded additives, followed by hydrofinishing, has been developed. In this connection, an interesting remark was made by Mr Ruedisulj that in a number of instances, by rigorous control of processing step in some refineries of their group, they have not felt the necessity of any finishing step.

Two papers were contributed on the major hydrogenation process in the petrochemical field, viz. the manufacture of cyclohexane from benzene. Mr Gladel (Resident Director, IFP in India) presented the process and plant details and economics of the IFP process which has reached a final stage of development up to industrial application. Three critical features of this process are : very high purity cyclohexane, elimination of large quantity of reaction heat, and maximum hydrogen utilization. The author stressed the need of high purity of feed materials, hydrogen and benzene, and pointed out that coke oven benzene will have to be further purified by hydrodesulphurization for this use.

In the other contribution on the subject, some recent work done at RRL, Hyderabad, on the hydrogenation of benzene at atmospheric pressure was presented.

Conclusion

Summarizing the proceedings of the seminar, Dr M. G. Krishna emphasized that while process details and other technical know-how should be developed in India for conversion of liquid and gaseous hydrocarbon feedstocks to hydrogen, intensive efforts should also be made as a collaborative effort among institutions, to develop processes for the utilization of coal and lignite to the extent possible. The importance of developing know-how for catalyst manufacture and gas purification techniques was also stressed.

From the deliberations of the seminar it was evident that even under Indian conditions, there is full justification for the adoption of certain hydrotreating operations, e.g. hydrodesulphurization. On the other hand, the adoption of more intensive operations like hydrocracking will depend essentially on the demand for products and the availability of hydrogen in the refinery.

With regard to coal tar processing, it was felt that under the conditions of limited resources, finance and material obtaining in India, where it is economical and technically feasible, maximum utilization of indigenous resources should be aimed at and efforts should be made to process these liquid products into desirable liquid fuels of proper specifications. This work should be taken up as a national project.

At the concluding session, Dr K. S. Gill (ICI) thanked the Indian Institute of Petroleum for the very well-organized seminar and the excellent arrangements made for the delegates.

Regulatory Mechanisms—A Symposium

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BROADLY speaking, the functions of a biological regulator are twofold: (i) to maintain a constancy of the internal environment by counteracting processes that tend to upset the equilibrium and (ii) to confer upon the system a certain flexibility for adjusting to sudden special demands. A regulated system is orderly and purposeful. In contrast, a system which has lost the regulatory mechanisms is disorderly and wasteful. The entire area of regulation is in a fast stage of development. To assess the status of our knowledge on various aspects of the problem, a symposium on 'Regulatory Mechanisms' at body, cell and molecular levels was held at the All-India Institute of Medical Sciences during 28-30 December 1965. The symposium was organized jointly by the Indian Council of Medical Research and the Society of Biological Chemists, India. The symposium brought together on a common platform scientists from several disciplines for deliberation on a common theme. The various papers presented at the symposium are summarized below.

Regulation of Body Functions by the Nervous System

S. K. Manchanda [All-India Institute of Medical Sciences (AIIMS), New Delhi] and K. N. Sharma (St John's Medical College, Bangalore) discussed the neural mechanisms involved in the regulation of food intake. Dr Manchanda pointed out that two distinct areas of the hypothalamus provide the structural substratum for the regulation of food intake by the alternation of hunger and satiety. The lateral hypothalamic area and the ventromedial nucleus are the centres which control hunger and satiety respectively. Dr Sharma presented results of investigations pertaining to the role of energy balance in biasing the peripheral dual detector system, signalling sensory (taste, smell, texture, etc.) and nutrient (calories, glucose, amino acids, fatty acids, etc.) qualities of the diet. His results indicate that the state of energy balance controls the bias of this dual detector system. Thus an energy deficit shifts the critical cues from nutrient to sensory qualities of the diet.

Hormones

An important group of regulators in multicellular organisms are the hormones. The chemical constitution of different hormones varies considerably. Some, like insulin, are quite large molecules; others, like the antidiuretic hormone, are quite small. The mechanism of action of the various hormones is an area of great interest. J. R. Tata (National Institute for Medical Research, London) dealt with the involvement of ribosome synthesis and distribution in the action of growth and developmental hormones. Although nuclear RNA synthesis is stimulated very rapidly following the administration of either thyroid hormone, estrogen or testosterone, cytoplasmic protein synthesis is not stimulated for a long time until the appearance of newly formed ribosomes in the cytoplasm. The newly formed ribosomes and polyribosomes are more firmly bound to the membranes of the endoplasmic reticulum than pre-existing particles. It was concluded that whereas the biological specificity of some hormones may lie at the transcription of selective messenger RNA molecules, the synthesis of new ribosomes and their attachment to cytoplasmic membranes are required for the expression of the biological activity of the hormones.

G. P. Talwar (AIIMS, New Delhi) presented some exciting results regarding the mode of action of estradiol. A macromolecular factor has been purified about 10-fold from ovariectomized rat uterus. This factor, which has the ability to bind estradiol both *in vitro* and *in vivo*, has also the property of inhibiting purified *Esch. coli* RNA polymerase activity. However, when the factor is isolated from animals given estrogens prior to sacrifice, it no longer inhibits RNA polymerase. Partial reversion of inhibition is also achieved by combination of estradiol to the macromolecular factor *in vitro*.

The influence of insulin and of growth hormone on the incorporation of amino acids into proteins was discussed by F. G. Young (Cambridge University, UK). Growth hormone and insulin do not promote protein biosynthesis when they are added *in vitro* to broken cell systems in which the synthesis of protein can be demonstrated. Nevertheless, when a rat is treated with these hormones under suitable conditions and a broken cell preparation obtained from its tissues, the system exhibits evidence of a promotion of the activity concerned with the synthesis of protein.

I. S. Edelman (University of California, San Francisco) gave a fascinating talk on the molecular processes in the action of aldosterone on sodium transport. It seems that aldosterone regulates sodium transport in the isolated toad bladder via a series of reactions initiated by steroidal stimulation of DNA-dependent RNA synthesis. The primary receptor for aldosterone appears to be in the nucleus of the effector cells and the affinity of the various steroids for the specific nuclear receptor determines its effectiveness as regulator of sodium transport. It was suggested that the steroid-induced rise in sodium transport was mediated via effects on oxidative metabolism and that the aldosterone-induced proteins stimulate the tricarboxylic acid cycle at one or more steps between condensing enzyme and α-ketoglutarate dehydrogenase.

The paper of A. Farooq (AIIMS, New Delhi) was concerned with the mode of action of progesterone. The results indicate that the incorporation of glycine-^{14}C and uridine-^{3}H into RNA, lipids and proteins of uterus and vagina is lower in the

case of ovariectomized rats treated with both estrogen and progesterone as compared to the level of incorporation attained in animals treated with estrogen alone.

S. V. Bhide (Indian Cancer Research Centre, Bombay) suggested that testosterone may exert some kind of regulatory influence on the activity of tryptophan pyrrolase. The males of certain strains of mice have higher tryptophan pyrrolase activity than females of the same strain. Upon castration of males, the enzyme activity falls down and remains comparable to that of normal females. Lowered enzymatic activity in castrated males rises up with injections of testosterone propionate.

The occurrence of a natural inhibitor of gonadotrophin in the urine of monkeys was reported by N. R. Moudgal [Indian Institute of Science (IISc), Bangalore]. The inhibitor has been purified partially and its activity is associated with a non-dialysable heat-labile protein fraction. The inhibition is specific in its effect towards FSH. It does not inhibit LH activity as tested by the ovarian ascorbic acid depletion and ventral prostrate tests.

Neuroendocrine Interrelations

Regulatory mechanisms which involve an interplay between the nervous system and endocrine glands are known. H. Heller (Bristol University, UK) dealt with one such neuroendocrine system, namely the hypothalamo-neurohypophysial complex. The ultrastructure of the complex and the significance of some of its submicroscopic organelles were discussed. The 'elementary particles' have been isolated and some of their contents (hormones and their protein carriers) have been identified. It would seem that the neuroendocrine complexes share the same essential features in vertebrates and invertebrates. There are, however, many subtle variants in organization. In some systems, for example, the neurosecretory products are discharged into the blood to reach targets which may be remote, as in the case of the posterior pituitary hormones, or quite near, as in the case of the 'release factors' reaching the adenohypophysis by way of the hypophysial portal system.

It is believed that the steroid hormones elaborated by the gonads exert a feedback inhibition on the release of gonadotrophin. It is not known whether the steroid hormones exert their negative feedback effect at the level of the hypothalamus or at the level of the pituitary. K. N. Rao (AIIMS, New Delhi) reported on the uptake of labelled estradiol by the pituitary, hypothalamus, cortical areas of the brain and several visceral organs in ovariectomized rats. A similar report by K. R. Laumas (AIIMS, New Delhi) dealt with the uptake and retention of labelled progesterone in the brain, pituitary and other tissues of the ovariectomized rat previously primed with estradiol. Although the results indicate a significant uptake of radioactive steroids by both the hypothalamus and the pituitary, the precise site of feedback inhibition by these two ovarian hormones remains an open question.

The role of the central nervous system in the regulation of reproduction was discussed by G. S. Chinna (AIIMS, New Delhi). Estrogen excretion in the urine is increased as a result of lesions of the posterior and middle-medial region but destruction of the anterior area of the hypothalamus produces a decrease. The electrical responses of widely scattered areas of the limbic system in response to the stimulation of genital areas in mature and immature monkeys have indicated that genital afferents modify the electrical activity of some areas of brain in the limbic system and hypothalamus. The threshold of these responses is influenced by the gonadal hormones in the immature monkeys. It appears that hormones might play a role in the initiation of the activation of some brain regions concerned with sex behaviour by lowering their thresholds of excitation.

Control of Metabolic Processes

An obvious way to control the rate of any individual biochemical reaction would be to alter the total catalytic activity of the enzyme in question. Two types of regulatory mechanisms are known which achieve this objective: (1) mechanisms by which the actual synthesis of the enzymes can be either decreased (repression) or increased (induction) and (2) mechanisms which do not influence the synthesis of enzymes but cause either inhibition (negative feedback) or activation of the enzyme already synthesized.

M. Chakravorty (Banaras Hindu University) discussed the induction and catabolite repression of the enzyme L-arabinose isomerase in microorganisms. This is an inducible enzyme in *Salmonella typhimurium*. The enzyme is susceptible to catabolite repression since the addition of glucose and other catabolite repressors to the media leads to cessation of enzyme synthesis. It seems that catabolite repression may act at the permeability level and also by pushing out the pool of the inducer built up within the cell. The level of the enzyme already induced in *S. typhimurium* remains unaffected on infection with one of the 'C' mutants of the phage PLT-22.

The report of D. K. Biswas (AIIMS, New Delhi) dealt with the pattern of feedback control of aspartokinase in *Esch. coli* PA-15, a mutant which requires serine or glycine for its growth. L-Lysine consistently inhibits aspartokinase activity present in the dialysed sonic extracts. Under similar conditions, L-methionine and L-threonine inhibit aspartokinase to a much lesser extent, leaving the bulk of the enzyme non-inhibitable. The per cent inhibition obtained by adding L-lysine and L-methionine together is the same as that caused by L-lysine alone. On the basis of this and other results, it was concluded that whereas the inhibitory effect of L-methionine is of a non-specific nature, distinct lysine-sensitive and threonine-sensitive enzymes probably exist in *Esch. coli* PA-15.

V. C. Joshi (IISc, Bangalore) spoke on the regulation of the biosynthesis of cholesterol and coenzyme Q in the rat. Cholesterol and coenzyme Q are the two end products of isoprene metabolism, found in the liver. Both these compounds depend on the 'acetate-mevalonate' pathway for their isoprene units. It was suggested that excess cholesterol

inhibits its own synthesis at the primary level of mevalonate formation and also at the step of the mevalonate pool available for coenzyme Q synthesis. Excess coenzyme Q inhibits its own synthesis after mevalonate stage and also seems to block some step between acetate and mevalonate, common to both coenzyme Q and cholesterol.

Some interesting results were presented by O. Siddiqi [Tata Institute of Fundamental Research (TIFR), Bombay] regarding the genetic regulation of sulphatase synthesis in *Aspergillus nidulans*. A constitutive mutant, Sul^{reg}-1, is able to synthesize the arylsulphatases even in the presence of sulphate or thiosulphate. It is, however, repressed by methionine, cysteine and cystine. Constitutivity is recessive. The heterozygous diploid Sul^{reg}-1^{+} and the corresponding heterokaryon are repressed equally indicating that the domain of action of the repressor is not restricted to the nucleus.

B. P. Gothoskar (Indian Cancer Research Centre, Bombay) dealt with the interrelations of macromolecular synthesis *in vitro*. Exposure of growing liver cells to hydroxylamine inhibits protein synthesis very markedly in the cytoplasmic and nucleolar regions, non-nucleolar region showing less effect. RNA synthesis, however, seems to be stimulated during short exposures where inhibition of protein synthesis is not significant. Exposure of the cells to a very low concentration of acriflavine causes a rapid decrease in DNA synthesis. RNA synthesis in the nucleolar and cytoplasmic regions is decreased by dye exposure, but RNA synthesis in the non-nucleolar region seems to be resistant to short exposures. Hydroxyurea exposure causes a rapid decrease in DNA synthesis but has negligible effect on RNA and protein synthesis.

H. K. Jain [Indian Agricultural Research Institute (IARI), New Delhi] discussed the control of nucleolar organization in different organisms showing varietal variation in chromosome number. The role of different gene loci in the synthesis of nucleolar materials has been analysed by a quantitative study of the incorporation of tritium-labelled RNA precursors.

A genetic study of the *R. locus* in maize led G. R. K. Sastry (IARI, New Delhi) to suggest that while the operon hypothesis is very useful in the investigations with multicellular organisms, a direct extrapolation may not be justified.

B. B. Biswas (Bose Institute, Calcutta) presented some results concerning the possible role of histones as gene modifiers in germinating seeds of *Phaseolus aureus*. By treating the deoxyribonucleoprotein (DNP) fraction with sodium chloride of different molarity and *p*H, the suppressor capacity of that DNP fraction can be removed partly when assayed with RNA polymerase *in vitro*. Phosvitin can interact with histones and can derepress the repressor activity of lysine-rich histones.

N. S. Girija [Atomic Energy Establishment, Trombay (AEET), Bombay] discussed the *in vivo* effects of actinomycin-D in a *Esch. coli* vitamin B_{12}/methionine auxotroph after treatment of the cells with EDTA. Under these conditions, the incorporation of labelled uracil is immediately and completely stopped by actinomycin-D. However, the incorporation of labelled methionine into protein is stopped after approximately 15 min. of addition of actinomycin-D. EDTA treatment of cells also results in a reduced rate of synthesis of β-galactosidase upon induction.

M. D. Gadgil (Haffkine Institute, Bombay) reported that whereas calcium ions initiate exponential growth of virulent 195/p strain of *P. pestis* at 37°C., magnesium produces partial stasis for a period of 24-48 hr after which cell division resumes with increased growth rate and loss of virulence. It also appears that a shift in the temperature of incubation of *P. pestis* from 27° to 37°C. upsets the normal DNA/protein and RNA/protein ratio of *P. pestis*.

M. M. Bhargava (AEET, Bombay) discussed results which are consistent with the idea that there are two types of L-glutamic amino transferase in rat liver mitochondria. One type is firmly bound and does not show any change when the mitochondria is damaged by certain procedures either *in vivo* or *in vitro*. The second one, though latent in normal mitochondria, is activated by damage and gets simultaneously solubilized and released into the blood stream.

The studies of V. R. Naik (AEET, Bombay) indicated that pentose adaptation of homofermentative *Lactobacillus casei* shows a transition of the organism to heterolactic dissimilation of glucose. Growth of the organism on pentose induces phosphoketolase and glucose-6-phosphate dehydrogenase. This is accompanied by a significant reduction in the activity of phosphofructokinase and aldolase.

P. K. Maitra (TIFR, Bombay) reported that addition of glucose to a resting, aerobic suspension of the hybrid yeast, *S. fragilisx*, leads to its rapid utilization in an oscillatory manner. The kinetics of entry of glucose into the yeast cell is also oscillatory. It seems that the entry of glucose into the yeast cell is controlled by feedback through some product(s) of glucose metabolism.

The effect of inositol deficiency on glycolysis and respiration in *S. carlsbergensis* was discussed by A. Ghosh (Saha Institute of Nuclear Physics, Calcutta). The deficient cells respired at one-tenth the rate of normal cells when the cells were harvested from the early or mid-log phase. It appears that inositol deficiency might bring about the retardation of phosphofructokinase activity and thus interfere with respiration due to less formation of Krebs cycle intermediates.

D. S. Ghanekar (AIIMS, New Delhi) presented results which show that injections of phenobarbital to rabbits considerably increase the amounts of oxidation-reduction components of liver microsomes, as well as the capacity of microsomes to hydroxylate drugs. The possible relationship between the redox substances, mixed function oxidases and the role of the endoplasmic reticulum as a defence mechanism of the cell was discussed.

J. Ganguly (IISc, Bangalore) discussed some of the regulatory mechanisms which prevent the accumulation of the highly toxic retinoic acid, although the animal liver contains all the enzymes

for making retinoic acid from the stored retinyl ester. Firstly, the ester is stored in the Kupffer cells which makes it less mobile. Secondly, the hydrolysis of the retinyl ester in the liver is very poor. Thirdly, the equilibrium for the oxidation of retionol to retinaldehyde is far towards the side of the alcohol. Finally, any retionic acid formed is immediately detoxicated through the bile as a glucuronide.

P. M. Bhargava (Regional Research Laboratory, Hyderabad) dealt with the observed biochemical differences between liver cell suspensions and liver slices. The results are consistent with the view that these differences are probably due to a change in the permeability of the hepatic cell membrane following removal of the intercellular material during the preparation of liver cell suspensions. In addition, the permeability of liver cells in suspension to certain nutrients is found to decrease when contact between cells is increased. These observations suggest that both intercellular material and cell contact may be important for regulating the intracellular metabolic activity.

The regulation of each of the functions of vitamin A by a specific structural prerequisite was discussed by M. R. Laxman (IISc, Bangalore). Use of chemically synthesized 5,6-monoepoxyretinal leads to the formation of a new visual pigment possessing a two-banded spectrum. Studies on the role of 5,6-monoepoxyretinal in reproduction of rats show that in spite of giving rise to its corresponding alcohol, this compound fails to support the normal reproduction in female rats. Irreversible oxidation either in the β-ionone ring or in the side chain end group greatly affects the reproductive function of vitamin A.

The solubilization and extensive purification of the enzyme hexokinase from brain was described by M. D. Joshi (National Chemical Laboratory, Poona). The inhibition of the purified enzyme by glucose-6-phosphate and ADP was discussed in relation to the control of glucose utilization.

M. S. Kanungo (Banaras Hindu University) presented results concerning the effect of age on the isozymes of lactic dehydrogenase. The isozyme patterns of lactic dehydrogenase of the brain and the heart of rats seem to change in a manner that may decrease the capacity of these tissues to tolerate anaerobic condition with increasing age.

Model Systems and Molecular Conversion

G. Felsenfeld (National Institutes of Health, Bethesda, USA) presented results of investigations concerning the interaction of a number of biologically significant substances with DNA with particular emphasis upon the questions of alteration of DNA helix stability and the ability of these substances to recognize specific nucleotides or nucleotide sequences as preferred binding sites. Such studies indicate that some of these molecules possess properties which are relevant to the requirements of regulatory mechanisms that involve DNA as the primary site of regulatory action.

R. K. Mishra (AIIMS, New Delhi) dealt with the role of intra- and intermolecular forces in regulating the activity of heterogeneous molecular aggregates with particular reference to current notions regarding the structure of myelin. The possible mechanism of energy transfer in biological systems consisting of aggregates of lipids, proteins, lipoproteins and water molecules was discussed and a semiconductor model presented.

The properties of an acylase preparation which can hydrolyse both L- and D-isomers of N-acetylphenylalanine was discussed by R. V. Krishna [Central Food Technological Research Institute (CFTRI), Mysore]. The enzyme has been obtained from the bacterial isolate (P_{41}) got by enrichment culture technique, using N-acyl DL-phenylalanine as sole carbon and nitrogen source.

M. Ramakrishna (CFTRI, Mysore) reported that the oxalosuccinic decarboxylase (OSADC) activity of purified pig heart isocitric dehydrogenase (IDH) is selectively inhibited by semicarbazide under conditions which do not affect the IDH activity. In addition, purified preparations of IDH have bound pyridoxal phosphate and significant transaminase activity associated with them. The transaminase activity is totally lost upon sodium borohydride treatment. The IDH or OSDAC activity is, however, unaffected by the latter treatment.

Recent Advances in Thin Layer Chromatography

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THIN layer chromatography (TLC), though initiated as early as 1938 by Izmailov and Schraiber[1], developed mainly after 1958 and now is almost universally adopted as a useful analytical method in chemistry. Though the progress is a result of combined efforts of a number of workers, however, it was Stahl[2] who was mainly responsible for bringing out a standard equipment for preparing thin layers.

Just as an advance was brought about in partition chromatography by the change-over from the ' closed ' to the ' open column ' of paper chromatography, a similar thing happened in adsorption chromatography, i.e. from Tswetts columns to ' open columns ' of thin layer chromatography. This fact also explains why this process has been named as open column chromatography by some workers. Izmailov and Schraiber called it drop chromatography because they put a drop of a plant extract on a 2 mm. thick layer of alumina and let it dry, and when developed with alcohol resulted in a circular chromatogram. Some of the other names mentioned in the literature are strip, chromatoplate, spread layer and surface chromatography.

TLC not only combines the advantages of paper and column chromatography but in certain respects it is better than either of them, e.g. (i) it can be used for preparative, diagnostic and quantitative estimations; (ii) it is more sensitive; (iii) requires less amount of the substance which is under examination; (iv) developing period for the TLC is relatively short (15-60 min.); (v) aggressive reagents like sulphuric acid or nitric acid can be sprayed; (vi) the chromatoplate can be heated to higher temperatures; and (vii) different sorbents can be mixed if necessary.

TLC has its limitations and sources of error too which will be discussed later at the appropriate places. The process of TLC involves the following prerequisites: (i) preparation of thin layers; (ii) spotting of the substance; (iii) proper developing system; and (iv) identification, detection or visualization.

Preparation of Thin Layers

Till recent years when the full TLC equipment was not generally available, various workers solved their problems by designing their own applicators[3-8]. The important principles in most of them are similar and differences lie only in small details. Constant efforts have been made to improve the quality of layers. For example, if the upper level of the glass plates is not the same during the process of putting the slurry of the sorbent with the applicator, the thickness of the layers on the glass plates will be different. A pneumatic device presses the glass plates from below and they are held tight in such a way that all the plates are ensured same upper level and, therefore, even thickness of the layers. The same problem has been solved in a different way. The glass plates at a constant level and speed are being passed through underneath the applicator[9] which is fixed. Special devices for small strips or for micro slides[10,11] have been constructed.

If for any reasons the TLC equipment is not available the layers could be prepared by other methods. Aqueous slurries of silica gel has been poured on to a glass plate and with the help of a spatula[12,13] evenly distributed. Thin layers have been prepared either by spraying[14,15] suspensions of the sorbents or dipping[16,17] glass plates in them. Silica gel in ethyl acetate[18] was poured on a glass plate and spread by tilting the plate carefully from one side to other and then allowed to dry in a perfectly horizontal position. These layers are good but ethyl acetate suspensions do not give good layers with other sorbents. Moreover, the adherence of the layers to the plate was not enough to permit documentation of the plates. In our laboratories a simple method was found which gave layers practically with all sorbents[19]. Ethyl alcohol or ethyl alcohol-water was found to be the most suitable. Table 1 shows the necessary quantities of sorbents and solvents for a glass plate of 20×20 cm. size (thickness of film approx. 0·25 mm.). If the size of the plate is different, the amounts of solvent and sorbent should accordingly be changed. The quantity of the solvent mixture could be varied by ±10 per cent to suit individual needs.

Dry sorbents[20,21], particularly aluminium oxide, has been made use of by various workers for preparative work[21] but not for separating complex mixtures made up of substances with close R_f values. Moreover, spraying is likely to damage the layers. Alumina used for preparing loose layers has nearly the same grain size as that used for column chromatography. Layers are prepared by putting the dry powder on the glass plate and levelling it with the help of a glass rod[22]. The

TABLE 1 — QUANTITIES OF SORBENTS AND SOLVENTS NECESSARY FOR OBTAINING A FILM OF THICKNESS 0·25 MM. ON A GLASS PLATE 20×20 CM.

Sorbent	Quantity g.	Solvent*	
		Ethyl alcohol (96 per cent) ml.	Water ml.
1. Alumina Woelm basic	6		
2. Alumina Woelm neutral	6		
3. Alumina Woelm acidic	6		
4. Silica gel Woelm	6	13·5	1·5
5. Magnesium silicate Woelm	3		
6. Cellulose	2		
7. Polyamide Woelm	1	13·5	—

*Quantities are for binder-free sorbents.

greatest advantage claimed is that loose layers of a sorbent whose activity[23] has been predetermined can be prepared. The activity[24] can also be checked directly on the chromatoplate itself. A TLC of a mixture of azo dyes, viz. *p*-methoxyazobenzene, Sudan Yellow, Sudan Red and *p*-aminoazobenzene, was developed with carbon tetrachloride and the R_f values obtained compared with those obtained on alumina of known activities[23]. A knowledge of this property is particularly helpful when one is thinking of transferring chromatoplate resolution to column chromatography.

TLC resolutions have been done on glass rods[25], inside of a test tube[26], or Bunsen funnel[27] and on both sides[28] of the glass plate. The standard type and size of glass plate when not available were substituted by discarded photographic[29] plates, window[30] glass, frosted[31] glass and grooved[32] glass plates. Aluminium[33] plates have been particularly useful in preparative work.

Readymade layers of different sorbents either on glass or on poly(ethylene terephthalate) sheets[34] are available commercially.

Sorbents

The choice of sorbents a few years ago seemed to depend mainly on their physical properties. Silica gel was found to be acidic, alumina basic and kieselguhr neutral. In the last few years many new types of sorbents have been prepared and used.

Silica gel — Silica gel is available with or without binders like starch or the more preferred plaster of Paris. The presence of this binder which can be about 5-15 per cent is not always an advantage, since the Ca ions, from the binder, interfere in the chromatography of nucleotides[35] and some inorganic ions[36]. For the separation of unsaturated compounds silica gel with fluorescent indicator can be used. The usual commercial silica gel is slightly acidic (*p*H about 6). To suit special needs sorbent of more acidic or basic *p*H could be prepared by mixing slurries with aqueous solutions of oxalic[37] or acetic[38] acids. Small amounts of sulphuric acid[16,39] in chloroform-methyl alcohol (70: 30) slurries have been used, the advantage being that, if the developed chromatogram was heated, the organic substances got charred. The corresponding spots could then be located. Similarly, basic sorbent could be prepared by substituting alkalies[37] in place of acids. Silica gel layers with buffered solutions of boric acid, sodium citrate, sodium acetate, etc., can also be prepared. For purposes of partition chromatography silica gel layers could be impregnated with paraffin, silicon oil, triglycerides and like materials.

Aluminium oxide — Alumina was the first sorbent used in TLC. It is commercially available in acidic (*p*H about 4), basic (*p*H about 9) and neutral (*p*H about 7·5) types. It is important to note that real neutral alumina (obtainable from Firma M. Woelm, Eschwege, Germany) should not only have a neutral *p*H but should have non-exchanging properties. It has been observed that some of the so-claimed neutral aluminas have a neutral *p*H but have cationic and anionic exchange properties. Such pseudo-neutral aluminas are not suitable for separating substances which are sensitive to acidic or basic ions. As in silica gel, aluminium oxide is available with or without binder (usually plaster of Paris) and also with fluorescent indicator. A special type of fibrous alumina[40], though without binder, is claimed to give stable layers.

Kieselguhr (diatomaceous earth) — This sorbent, which has a comparatively neutral *p*H (about 7), is available with or without binders and is specially suited for partition chromatography. The capacity of resolving substances is lesser than either silica gel or alumina.

Magnesium silicate — This is comparatively a new sorbent and has a slightly basic property (*p*H 9). It has proved useful in the TLC of sugars[41].

Calcium silicate, calcium sulphate, hydroxyl-apatit — Calcium silicate layers are also useful for separating sugars and their phenylosazones[42]. Calcium sulphate gives very stable layers and is good for separation of steroids[43] and lipids[44]. Hydroxyl-apatit, which is actually a sort of complex calcium phosphate hydroxide, has been used for the separation of glycerides[45] and proteins[46]. This sorbent can be used with or without binder. Some of the other inorganic sorbents mentioned in the literature are magnesium oxide, talc, magnesium carbonate, calcium hydroxide, calcium carbonate, dicalcium phosphate and glass powder. Various workers have tried to use mixed sorbent systems, e.g. silica gel + magnesia, silica gel + calcium hydroxide, silica gel + kieselguhr, etc.

Cellulose and acetylated cellulose — These sorbents are available in various forms. Acetylated cellulose can be selected according to the degree of acetylation. If necessary, binders like starch or plaster of Paris can be added.

Polyamide — This is very useful for the separation of phenolic substances like flavanols, etc. If necessary, a binder like starch can be used.

Polyethylene powder — This has been used for separating fatty acids and their esters[47].

Dextran gels — Cross-linked dextran gels are available in various types and grain sizes and are particularly suitable for the investigations of proteins, nucleotides, etc.

Cellulose ion exchange powder — Various cellulose powders have been so modified in their structure that they act as ion exchangers, e.g. diethylaminoethyl cellulose (DEAE cellulose), epichlorhydrin linking triethanolamine cellulose (Ecteola cellulose) and polyethylenimin cellulose (PEI cellulose). These sorbents can be used with or without binders like collodion.

Ion exchange resins — These resins have been used alone or in admixture with cellulose powders. Other less used sorbents are urea[47], active coal[48], sugar and bentones[49].

Spotting of the Substance

For exact work pipettes of micro sizes are commercially available. Devices[50,51] which allow multiple applications simultaneously have been described. A simple method for spotting[52] non-bound layers is reported as well. A mechanism in which case the micropipette[53] moves at a regulated speed and at

the same time leaves streaks of the solution on the chromatoplate has been constructed. A normal micropipette[54] bent at 90° angle has been claimed to be very practical. One of the latest devices for spotting is a micropipette[55] made by attaching a sort of glass wool brush to a capillary.

Development of Thin Layers

Choice of a Solvent System

The choice of solvent or the mixture of solvents depends on whether the process is a case of adsorption or partition chromatography. In case of adsorption chromatography, the solvent or mixture of solvents (miscible) follows the rule of the eluotropic series. There are a number of suggestions but in principle they are similar. The more polar solvents producing the greatest migrations (giving higher R_f values) are listed at the bottom of Table 2. This property depends, of course, also on the ability of the solvent to take the particular material into solution. Three of such eluotropic series[56,57,57a] which are for oxygen containing sorbents are given in Table 2.

The following eluotropic series[58] has been suggested for polyamide for column chromatography which could be applicable for TLC too. Solvents are arranged in order of increasing eluotropic activity: water < ethyl alcohol < methyl alcohol < acetone < sodium hydroxide solution < formamide < dimethylformamide.

It is advisable to use pure solvents to avoid any unknown reactions or appearance of unknown spots on TLC. Purifying and dehydration[59] of some solvents can be achieved by passing through alumina columns. For acetone and ethyl acetate neutral alumina is used whereas for ether, benzene and chloroform basic alumina is suitable. Alcohol[60] from chloroform and peroxides[61] from ether are automatically removed in the above process.

The fact that the substance is not soluble in a particular solvent system does not necessarily mean that it will not separate on TLC, e.g. sucrose and glucose[62] can be readily separated on silica gel using methanol-ether solvent system in which they are apparently insoluble.

TABLE 2 — ELUOTROPIC SERIES OF SOLVENTS FOR OXYGEN CONTAINING SORBENTS

Strain[56]	Hesse[57]	Wohlleben[57a]
Light petroleum (30-50°)	Petroleum ether	*n*-Pentane
Light petroleum (50-70°)	Carbon tetrachloride	Petroleum ether (low boiling)
Light petroleum (70-100°)	Trichloroethylene	Petroleum ether (high boiling)
Carbon tetrachloride	Benzene	*n*-Hexane
Cyclohexane	Dichloromethane	*n*-Heptane
Carbon disulphide	Chloroform	Cyclohexane
Ethyl ether (anhydrous)	Diethyl ether	Carbon tetrachloride
Acetone (anhydrous)	Dimethylformamide	Trichloroethylene
Benzene	Ethyl acetate	Benzene
Toluene	Pyridine	Dichloromethane
Esters of organic acids	Acetone	Chloroform (alcohol free)
1,2-Dichloroethane	*n*-Propanol	Diethyl ether (absolute)
Alcohols	Ethyl alcohol	Ethyl acetate
Water	Methyl alcohol	Pyridine
Pyridine	Formamide	Acetone
Organic acids	Water	*n*-Propanol
Mixture of acids or bases	Glycol	Ethyl alcohol
Water, alcohols or pyridine	Glycerine	Methyl alcohol
		Water

Developing Chambers

It is preferable to work with saturated tanks; otherwise one might get the so-called edge effects which result in giving different R_f values at the edges than at the middle of the plate. To ensure saturation one can put a sheet of filter paper along broad sides of the tank and dipping in the solvent mixture. This problem of saturation has been tried to be solved by confining the developing chamber[63,64] to as small a size as possible.

Visualization

Spraying of a chromatogram with a reagent in most of the cases changes the structure of the substance. Sometimes even the colours so obtained fade away so fast that further work on the plate is not possible. Such problems have been tackled in different ways with varying degrees of success. A universal but simple method is that the developed chromatogram after drying is sprayed with distilled water[65]. As long as the plate is wet the substances show up as white spots against a translucent background.

The use of fluorescent indicators has been of great help in preparative, particularly in quantitative, separations. The use of organic or inorganic indicators like sodium salt of fluorescein, eosin, morin[22] or sodium salts of 3-hydroxypyrene-5,8,10-disulphonic and 3,5-dihydroxypyrene-8,10-disulphonic acids[66] or activated cadmium and zinc silicates[67,68] has been made. Though in most of the cases the indicators are mixed with the sorbent before preparing the thin layer, they can also be used as such by spraying them. The visualization is done by looking at the TLC under a UV lamp when the substances show up as dark spots against a fluorescent background. It is important to know that mostly unsaturated compounds, particularly having conjugated double bonds, can be identified with this method.

Special Techniques and Phenomena

Different Ways of Running a Thin Layer Chromatogram

The usual and easier way is the ascending method. But various other techniques to suit individual needs or problems have been tried. As in paper chromatography, it is possible to carry out descending[69,70] and circular[71,72] chromatography. The flow of the solvent from the reservoir to the layer is through a filter paper strip. The former could be applied to loose layers of sorbents also. The first example of circular TLC was, of course, from

Izmailov and Schraiber[1]. In circular TLC it is possible to put the glass plate in such a way that the sorbent layer is either facing upwards or downwards[73]. The use of centrifugal forces[74] for accelerating TLC has been tried. The wedge-strip[71] technique, which has been often used in paper chromatography, has been used in TLC with success.

Horizontal TLC — In this case also the sorbent layer could be facing upwards or downwards. The solvent is brought to the sorbent layer through a filter paper strip of the same width as the glass plate.

Continuous TLC — This is useful for substances having small and close R_f values. It could be carried out in S-[63], sandwich[64] or specially constructed chambers[75]. Continuous TLC could also be done on horizontal plates[76]. The principle is the same as with horizontal TLC with the difference that the solvent at the other end is allowed to evaporate. Continuous TLC using descending[70,77] technique has been accomplished too. A new method has been developed where the mixture which was to be separated was put near the apex on a thin layer prepared on a triangular glass plate[78]. Two different solvent mixtures were fed from different sides to the thin layer and fractions collected at the base.

Preparative TLC — In this case the size of the plates (20×100 cm.) so also the thickness of the sorbent layers (up to 5 mm.) are larger than in the normal TLC. Substances are applied not as spots but as streaks. Usually milligram amounts of substances[69,79,80] are separated but amounts as large as 100 g. have also been separated[79]. Special applicators have been constructed to prepare thick layers.

Stepwise and multiple development—For the separation of substances having smaller R_f values it is advisable to run the TLC plate more than once, drying the layer before it is developed again. If the solvents used for subsequent runs are the same as in the first case, it is called multiple or repeated development[80,81], but when the solvents are different in the first and later runs, it is called stepwise[37,82] technique. This method is specially useful for mixture of substances containing polar and non-polar substances. Therefore, polar solvent systems are usually employed for the first run and a non-polar solvent system for the later runs.

Multiple dimensional TLC — It is a variant of multiple development chromatography. For example, in one experiment the chromatoplate was developed with propanol-ammonia (2 : 1) which carried fatty acids, cholesterol and their esters to the solvent front and resolved lecithins and polar lipids. The second run with chloroform-benzene (3 : 1) separated fatty acids and free cholesterols and carried the esters to the solvent front. The TLC plate was turned at an angle of 180° and developed with carbon tetrachloride which resolved the cholesterol esters[83]. Using more than one plate and the same solvent for the first but different solvents for the subsequent developments after rotating the plates at an angle of 90°, a large number of amino acids[84] could be successfully resolved.

Separation-reaction-separation technique[85] — After letting the chromatoplate develop in one direction it is exposed to, for example, UV, X-rays or gases. The plate is then rotated by an angle of 90° and run again usually in the same solvent. In case a new reaction product has resulted it will show up on the chromatogram.

Elution — It can be done by scraping off the sorbent after marking the spot or sucking it into a microchromatographic column[86] which contains a cotton pad at the other end. This is then eluted as in normal column chromatography. For substances which can sublime, the following method may be useful. Almost directly over the developed plate is held another clean and cooled glass plate. The plate with the sorbent is warmed carefully. The substances sublime and get collected on the upper plate. Caffeine and theobromine[87] have been separated in this way.

Quantitative estimation — Usually the methods used in paper chromatography can be adapted too in TLC. Either of the following methods could be used: (i) planimetric[68,88,89] size of the spots are measured and then compared with a calibration curve drawn from known solutions; (ii) densitometric[90]; (iii) spectrophotometric[91,92]; (iv) colorimetric[93,94]; (v) polarographic[95]; (vi) fluorescence[96,97] measurement; (vii) substances could be eluted and titrated[98]; and (viii) radioisotopes can be directly[99] counted or estimated by autoradiography[100,101].

Multiple layers — TLC with different layers side by side have been reported by some workers. Stahl[102] calls his technique as three-dimensional or gradient TLC. A specially designed applicator permits the preparation of thin layers whose activity or *p*H or composition of the sorbents varies continuously and gradually from one side to the other. Multiple layers[103,104] of predetermined compositions have also been prepared.

Gradient elution[105,106] — This procedure helps in a continuous and gradual change of the composition of the developing system during the experiment. The chromatoplate is put on a sort of filter plate in the developing chamber which to start with contains less polar solvent. A constant feed of a polar solvent is maintained below the filter plate. With the help of a magnetic stirrer the solvent mixture is kept in motion. This is continued till, at the close of the experiment, the properties of the solvent in the chamber approach those of the added solvent. The height or the level of the solvent is kept constant by an outlet underneath the application point on the TLC plate.

Thin layer electrophoresis — Thin layer electrophoretic separations have been carried out though they are not so common as paper electrophoresis. Unidimensional or two-dimensional separation procedures have been adopted. Substances like amino acids[107], phenols[108] and inorganic substances[109] have been analysed.

Documentation — The simplest method would be a colour photograph which, however, especially under UV light, is not always very practical. Zinc oxide papers[110] have been used for recording chromatograms. Lately plastic polymer solutions[19] or suspensions[111] have been used for spraying the thin layers.

After the layers are dry they are peeled off and stored.

Multiple spots and tailing — Multiple spots, also called 'ghost spots', have been responsible for the prediction of so many new compounds or for the alleged presence of impurities which actually never existed. Whenever a compound can exist in one or more charged or uncharged ionic forms or is capable of forming complexes, ghost spots may appear. For example, adrenaline, noradrenaline or ephedrine, when separated on cellulose layers with butanol-acetic acid-water (4: 1: 5) with a little of trichloracetic acid, give double spots[112]. Using alumina or silica gel as sorbent one finds in the above case only single spots.

Tailing may be caused by overloading or the existence of the substance in more than one ionic forms. The addition of 1-2 per cent of acetic acid[113] to developing systems for fatty acids, and ammonia[114] or diethylamine[115] for TLC of bases and other nitrogenous lipids has been helpful. Addition of inorganic salts[116] like NaCl, NH_4Cl, $NaNO_3$, etc., to the alcoholic developing mixture is also claimed to reduce tailing.

Impurities in sorbents — Some of the sorbents, particularly silica gel, contain ferric salts which are sometimes a hindrance in the analysis of inorganic ions or in quantitative estimation with spectrophotometers. This difficulty could be overcome by letting the thin layers run full distance in a developing system of methanol and HCl, drying it and then using it for TLC. Silica gel[117] can be as well washed with a methanol and HCl mixture before spreading. For removing impurities it is preferable to pre-wash the thin layer plate with the same solvent mixture which is to be used later for developing. Washing with methanol or methanol-ether (80: 20) mixture removes some of the organic impurities present in the sorbents[118]. One sample of plaster of Paris containing silica gel[80] on analysis gave the following impurities for 50 g. of the sorbent: chloroform (6·8 mg.), benzene (5·5 mg.) and acetone (10·5 mg.) when eluted with 200 ml. of each solvent. Impurities[119] which have been derived by diffusion from plastic bottles, tubes or extracted from Soxhlet thimbles have been reported also.

The metals[120] present in the sorbents sometimes chelate with the functional groups of the organic substances under investigation. This may result in a tailing effect but could be overcome by adding in advance di-Na EDTA in an amount excess of that consumed by the water-insoluble components of the sorbents.

Artifacts — These are more common in column[121] chromatography than in TLC. There can be a number of causes for these changes in the substances. For example, it could be due to basic reaction of the sorbent, oxidation[122] reactions due to the presence of Fe(III) and Cu(II) ions or auto-oxidation due to contact with air. Sometimes even the developing systems are affected. The resulting products may appear on the chromatogram or influence the properties of the solvent system. Acetone could be converted to diacetone alcohol and ethyl acetate to acetaldehyde[123]. While steroid-β-esters[124] undergo reaction on alumina layers, ethylene ketals[125] get hydrolysed on silica gel layers. Sugars in ammonia solutions undergo an amination[126] reaction on silica gel, but when chromatographed on magnesium silicate[121] layers they are reported to get decomposed. Photochemical changes and UV illumination effects[127] are known. Solutions of substances under investigation should not be kept long as they may decompose or undergo other changes, e.g. it has been observed that lipids from human milk when left over with methanol for long periods resulted in the so-called methyl ester[128] artifacts.

R_f value (retention factor) — R_f value or the ability to reproduce it has been the subject of much discussions in recent times[129-133]. Though opinions have differed it can be easily concluded that some of the factors listed below influence the R_f values.

Quality of sorbents — It has been noted that sorbents are different from batch to batch[129]. It could be the grain size, *p*H, impurities or any other unknown factor which influences the R_f values. The smaller the grain size the larger[134] are the R_f values.

Thickness of the film[130,134] and technique of preparing it — The thicker the films, smaller are the R_f values. Different types of applicators for preparing thin layers are available. Some workers use manual methods. The shape, size and evenness of the plate influence R_f values.

Activity — Activity[135,136] of thin layers is influenced by the relative humidity. The longer it takes to spot the substance, the more are the chances for the change in activity. In one experiment on the separation of hexaphenyl on silica gel it was found that with relative humidity of 40 per cent, R_f was 0·15 and whereas at 65 per cent RH, the R_f value shot up to 0·72. Specially constructed chambers could minimize or even exclude the influence of humidity[135].

Chamber saturation — To ensure reproducible results it is advisable to use saturated developing chambers[37,137]. This gives constant, though smaller[137], R_f values and besides the 'edge effects' are avoided too[37].

Technique of Developing

TLC could be done either by ascending, descending, circular or horizontal methods. R_f values will be different in each case.

Developing systems — Only pure solvents are used to ensure reproducible results. Particularly, solvent mixtures consisting of multiple component systems should be changed frequently.

Solvent front — It has been shown that the speed of the solvent flow in the first 5 cm. of the plate is faster than in the second half of the layer[134]. Besides, if the solvent front is too long there are chances of demixing of solvents in multiple systems. The height of the starting point above the developing system is also important.

Nature and amount of the substance spotted — Higher amounts may give rise to tailing effects. R_f of a pure substance may be slightly different as compared to when it is present in a mixture. In such cases the substance should be eluted and run again and compared with an authentic sample.

Temperature — There are conflicting reports on the role of temperature in TLC. It is observed that higher the temperature, faster is the movement of the solvent. Higher temperature could adversely affect the adsorption capacity of the sorbent.

It can be seen from the above that when an experiment carried out in another laboratory has to be repeated elsewhere it is almost impossible to reproduce all the above conditions employed. It can, therefore, be concluded that R_f value is not a constant or absolute value like melting point or refractive index. The R_f value of a substance with particular reference to a solvent and sorbent and that of a reference compound should be used for comparison. It has been suggested that instead of reporting the average R_f values, the range[138] within which the R_f values vary should be provided.

Applications

There has been a large number of publications on the use of TLC in the last few years. Within the scope of this article it is only possible to include representative members of different groups of substances and for reasons of limited space only selected literature quotations have been included. In some cases sorbents containing binders like plaster of Paris have been used. These sorbents have been designated as silica gel P or alumina P, etc.

Alkaloids — Alkaloids can be separated on silica gel P, silica gel, basic alumina P, formamide impregnated cellulose[139], polyamide[140] and magnesium oxide layers[141]. Opium[139,141] and other synthetic morphin derivatives, *Belladonna*[139,140], *Rauwolfia*[139,142], strychnine[68,141], *Vinca rosea*[143,144], Cinchona[141,145], hashish[146], conessine[147], *Cissampelos*[148] and some habit forming drugs[149] and some synthetic xanthine derivatives[150] have been investigated.

Quantitative estimations of some alkaloids like strychnine[68], codein, thebain, narcotin[93] or reserpine[151] have also been reported.

Amines — Amines[152-156] could be separated on silica gel, silica gel P, cellulose, magnesium silicate, alumina P, kieselguhr, polyamide and electrophoresis on silica gel layers. A large number of amines, for example, adrenaline, noradreline, dopamine[153], serotonin and catecholamine[156], could be qualitatively or quantitatively analysed.

Alcohols, aldehydes and ketones — Alcohols[157-159] as such or in the form of their 3,5-dinitrobenzoates have been separated on silica gel P and alumina. Some long chain alcohols, particularly fatty alcohols, could be identified as their nitrates[160]. Polyhydroxy compounds[161] and *n*-alcohols[162] have also been investigated on silica gel or kieselguhr layers.

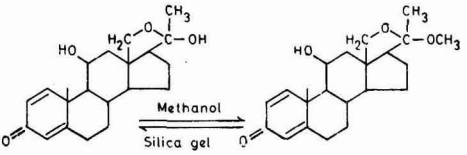
Aldehydes and ketones could be separated in the form of their 2,4-dinitrophenylhydrazones[163], semicarbazones[164] or phenylosazones[165] on silica gel P, or alumina P, magnesium oxide, kieselguhr, or basic zinc carbonate layers. Some aromatic aldehydes[166] and substituted diketones[167] have been investigated either on silica gel and calcium oxide layers. Ethyl methyl ketone[168] was identified in a sample of denatured alcohol.

Acids (excluding fatty acids) — The usual sorbent of choice is silica gel or silica gel P, though other sorbents have been employed. Acids like monocarboxylic[169], dicarboxylic[170,171], *cis-trans* dicarboxylic[172], keto acids[173] of biological interest and some cyclic acids[174] have been separated. Dinitrophenylhydrazone[175] and rhodamine derivatives of keto acids[176] have been identified. Benzoic and sorbic acids[68] using butanol-alcohol-ammonia (14: 1: 14) and silica gel as sorbent could not be separated, but by using cellulose separation was successful.

Amino acids, peptides and proteins — Amino acids could be separated as such or in the form of their derivatives like carbobenzoxy, phthalyl-, *p*-nitrocarbobenzoxy, trifluoracetyl, trityl, tosyl[177] or phenylthiohydantoin (PTH-amino acids), dinitrophenyl-[178], etc. Though silica gel with or without binders and cellulose are the sorbents of choice, separations have been done on a number of other sorbents[179,180]. In cases of DNP and PTH derivatives of amino acids it is preferable to do two-dimensional TLC. Silica gel P has also been used for the separations of dinitropyridyl, dinitropyrimidyl amino acids[181], methyl histidine and histidine[182]. Peptides[183,184] just like amino acids can be separated on silica gel or cellulose layers. The newest sorbent in this field is dextran gel[185,186]. DNP or Cbo derivatives[183] of peptides have also been separated on thin layers using two-dimensional TLC. The 'fingerprint' images[107] of peptides were prepared on silica gel using two-dimensional TLC, different solvent mixtures being used for running the chromatogram in each direction. Maps or 'fingerprints' were also prepared by using TLC in one direction and TL electrophoresis[187] in the perpendicular direction. A number of proteins, polypeptides and enzymes have been separated on dextran layers using gradient technique[185,188,189]. A linear relationship[185] between the R_f values on dextran layers with molecular weights of amino acids, small peptides and proteins is reported. By running the unknown substance with proteins of known molecular weight it could be possible to predict the approximate molecular weight of the unknown substance.

Steroids — Sterols, corresponding stanols and sterol esters[190,191], corticosteroids[192,193], pregnan derivatives, estrogenic hormones[194,195] and androstane derivatives[196,197] have been separated on silica gel P. The use of alumina for 17-keto steroids[198], polyamide for corticosteroids[199] and magnesium silicate for different types of steroids[200] has also been recommended.

When C-21 steroids from *Holarrhena antidysenterica* were chromatographed on silica gel layers with methanol or methanol containing solvent systems, they gave rise to a ketol[201] on C-20. It appears that in the presence of acidic silica gel methanol takes part in the reaction.



This ketal formation does not take place on alumina layers. Bile acids[202] are usually separated on silica gel P though alumina or Mg silicate[203] has

also been recommended. Silica gel layers have been proposed for use in the separation of sapogenins, e.g. from *Dioscorea*[204] and *Annemarrhena*[205], or steroidal alkaloids from *Holarrhena*[206] and *Veratrum*[207] or cardiac active glycosides[208-210] of *Digitalis*[209,210], Scilla, *Strophanthus*[210] or *Bufadienolides*[208], etc. Quantitative estimation of *Strophanthus*[211] and *Digitalis* glycosides[212] has also been reported. The use of talc[212] and alumina[213] as sorbents for the separation of above-mentioned glycosides has been tried.

Fatty acids and lipids — Silica gel is the sorbent of choice though other sorbents like kieselguhr or plaster of Paris have also been mentioned. Sorbents could be used untreated or after impregnation with undecane, silicon, sodium borate, sodium arsenite[214], etc. Close positional isomers of some hydroxy fatty acids and their esters[215], cholesterol fatty esters[216], model mixtures of epoxy, hydroxy, episulphido and normal fatty acids[217,218] and some stereo isomers of unsaturated hydroxy acids[219] have been separated on silica gel plates. Unsaturated fatty acids and their esters[217,220] and some long chain polyhydroxy[214], ketohydroxy[221] and hydroxamic acid derivatives[222] of some fatty acids have been separated using reverse phase TLC. Glycerides[223] from various plant oils like castor, corn, groundnut and soyabean have been examined with TLC. By converting unsaturated fatty oils into mercuric acetate adducts one can separate them on silica gel layers. The fact that mercuric acetate reacts faster with *cis* than with *trans*[224] was utilized for separating them on TLC.

The original compounds can be regenerated with hydrochloric acid. The use of silver nitrate in the silica gel as a complex forming agent for the unsaturated compounds has been extensively made.

Triglycerides[225,226] (according to their unsaturation and configuration of the double bonds), some waxes[227] and pine resin acids[228] have been successfully separated on silver nitrate containing silica gel and alumina layers.

Lipids — Generally sorbents free of binders[229] like plaster of Paris are preferred. Silica gel is again the sorbent of choice and can be used as such or after impregnation. The separation of phospholipids[229,230] and glycolipids[231,232], including sphingolipids and some plant lipids[233], has been done on thin layers. Quantitative estimations[234] have been successful. Gas and TLC techniques[235] have been combined for some investigations.

Carotenoids and chlorophyll — Because carotenoids are present in nature in small amounts, preparative TLC has been found useful to collect them in micro amounts[236]. Rhodoxanthin, zeaxanthin, cryptoxanthin, xanthophyll and carotin[237,238] were separated on layers of a mixture of silica gel P and calcium hydroxide (1: 6). Investigations on the chlorophyll and carotenoids[239] from sweet cherry trees have been done with the help of TLC. The cellulose layers have also been used for the separation of carotenoids, chlorophyll and pheophytins[240,241].

Terpenes and essential oils[242-245] — Terpenes, polycyclic terpenes and terpene alcohols can be separated on alumina P[242], silica gel[71], silver nitrate[243] containing silica gel, kieselguhr[244] and magnesium silicate[245] layers. Silica gel without binder has been used for the TLC of lavender oil[246] and chamomilla[247] extracts. TLC investigation of some official essences[248] has also been described.

Phenols and phenolic compounds — Phenols, phenolic aldehydes and phenolic acids could be separated on layers of silica gel[174], alumina[249], cellulose[250] and specially polyamide[174,251]. Some vegetable tannins[252] and depsides[253] have been investigated on polyamide and silica gel P respectively. Fluorescence indicators have been used for identification of some compounds.

Flavones, chalcones and anthocyanins — Though separations on silica gel[254] and cellulose layers have been done, polyamide[255-258] is being used more and more for the separation of flavones and flavonoids. The following relationship[257] between R_f values and glycosides of myricetin, quercetin and kaempferol has been described: (i) aglucone alone does not have any marked effect on R_f values; (ii) it is regulated by the place where the sugar molecule is attached; (iii) all 3-monosides behave almost identically; (iv) biosides move faster than monosides; and (v) 3,7-glycosides travel farther than others.

Anthocyanins[259,260] have similarly been successfully separated on polyamide, though sorbents like silica gel P and silica gel and cellulose mixture have also been used.

Isoflavones[261,262], biflavones[263], aurones, chalcones[264], thiolactones[265] and anthrachinones[266] have been separated on silica gel layers. Some coumarins, furocoumarins, lactones and substituted α-pyrones have been separated on silica gel[267,268], alumina[269] and polyacrylonitrile[270] layers.

Plant extracts — A large number of plants[271,272] containing different types of compounds have been investigated with the help of TLC. Adulteration[82,271,272] in drugs could be checked successfully in quite a few cases by comparing the thin layer chromatograms of the suspected and the real plants.

Carbohydrates — The newest in the TLC investigation of simple sugars are the sorbents magnesium silicate[41,273] and polyamide[274], though a variety of other sorbents have been used. Phenylosazones[275], 2-deoxy sugars[276], phenylhydrazones, dinitrophenylhydrazones[277], some ether derivatives[278] and amino sugars[279] have been analysed with the help of TLC. Sugars have been investigated on silica gel which contained a little sodium bisulphite[280] and also on porous ion exchange resins[281]. Dextran[282] and heparin[283] have also been identified on silica gel layers. Sugars in traces in hydrolytic products of flavonoids[255] have been identified on magnesium silicate layers.

Nucleotides — Cellulose, modified cellulose and ion exchange resins besides silica gel are recommended in the TLC of nucleotides. Polyphosphates[35] when separated with basic developing system cause difficulties, if the sorbents contain plaster of Paris. Using these modified cellulose layers, it is possible to separate nucleoside phosphates[284,285], nucleotide sugars[286] and oligonucleotides[287] from biological

materials. Ion exchange materials have helped in the TLC investigations of enzyme reactions[288] and quantitative estimation of nucleoside monophosphates and other nucleotide coenzymes[289]. Combination of TLC and TL electrophoresis techniques was found very useful in the separation of DNS bases, nucleosides and nucleotides[290].

Hydrocarbons — The TLC resolution of different hydrocarbons can be achieved on layers of silica gel[291], cellulose[292], acetylated cellulose[293] and alumina layers[135]. It is advisable to work in dark conditions for photosensitive components[127]. Aromatic hydrocarbons[294] of various ring systems and some atmospheric hydrocarbon impurities[292] have been identified. The presence of paraffin oil[295] as an impurity in vegetable oils could be controlled with the help of TLC. Olefines[296] have been separated on silica gel by converting them into their mercury complexes. The property of olefines[243] forming complexes with silver ions which is already known for column, paper and gas chromatography, has also been extended to TLC.

Food and allied industries — Saccharin, cyclamat and dulcin[297] could be separated on layers of a mixture of polyamide and acetylated cellulose. Flavour esters[298] as well as some flavour additives[299] which are added to vanilla extracts have been investigated. Preserving agents, e.g. benzoic, *p*-hydroxybenzoic, sorbic acids or their esters could be separated on silica gel[67], cellulose[68], cellulose and polyamide mixture[300] or silica gel and kieselguhr mixtures[301]. Further, the presence of preserving agents in galenicals[302] can be checked with the help of TLC. Antioxidants[88,303,304] could be separated on silica gel and other sorbents like alumina, polyamide and cellulose acetate.

Dyes — The food dyes like Azorubin, Brilliant Black, Cochineal Red A[305], or Ponceau BR, Amaranth[306], etc., could be separated on silica gel, cellulose or calcium carbonate layers.

Some natural dyestuffs like those from *Paprika* (Capsicum) and *Curcuma*[307] have also been identified on thin layers. Some fat-soluble food dyes[308] have been separated on silver nitrate containing sorbents like polyamide.

Vitamins — Most of the water-soluble vitamins, e.g. B_1, B_2, B_6, B_{12}, C, biotin, flavin-mononucleotide, calcium pentothenate and nicotinic acid amide[309,310] can be separated on fluorescence indicator containing silica gel layers. For those vitamins[311] (e.g. biotin, etc.) which are not visible under UV light in the above cases one can spray with specific reagents. Sorbents like alumina P, kieselguhr P and DEAE cellulose were also used. Fat-soluble vitamins[303,312,313] like A, D, E or K could be separated on silica gel P, alumina or impregnated silica gel layers.

Insecticides and pesticides — The investigation on insecticides and pesticides, particularly their residues left on food and other products, has been the subject of extensive researches in the last few years. Thiophosphoric acids[314,315] or their esters alone or in biological materials, chlorinated hydrocarbons[316,317] like aldrin, DDT, dieldrin and others could be separated on silica gel, alumina or kieselguhr layers. Pyrethrins[318] and some herbicidal compounds[319] have been identified on TLC layers.

Drugs and pharmaceuticals: Antibiotics — For the identification of antibiotics one can use bioautography[320] technique (microbiological) or resort to chemical spray reagents. Most of the antibiotics, e.g. penicillin[320], tetracycline[72,320,321], rifomycine[320], streptomycin[322], actinomycin[323], erythromycin[324], etc., could be separated on silica gel layers. Glycoside bound antibiotics like streptomycin, kanomycin, neomycin[325], etc., could be identified on layers of silica gel and alumina mixture. Neomycin[48] was separated on coal layers.

Sulphonamides — Qualitative or quantitative estimations of sulphonamides[326] have been done usually on thin layers of silica gel. Some sulphonamides having diuretic[327] or antidiabetic[328] properties were also separated on silica gel layers.

Phenothiazines and other related compounds[329,330], psychotropic drugs[331], barbiturates and other barbiturate-free hypnotic drugs[332,333] have been separated on silica gel, alumina or cellulose layers. TLC investigations of a number of analgesic and antipyretic drugs[68,334,335] have been carried out on alumina or silica gel layers. Separation of different types of drugs[336], e.g. stimulants of nervous system, depressors of central nervous system, analgesics of morphine group and the like, has been accomplished on silica gel layers. The presence of salicylsalicylic acid[337] (Salysal) as an impurity in salicylic acid could be checked on alumina layers. Silica gel as sorbent was used for the investigations of some 4-hydroxycoumarins[338] having anticoagulant properties and also for hydrolytic products of chloramphenicols[339]. Codéin, ethylmorphine[340] and some anaesthetics[341] could be successfully identified on thin layers.

Biological and clinical applications — Urine has been investigated for its contents of normal or diabetic sugars[275,342], barbiturates, non-barbiturate hypnotics[343], amino acids[344], peptides, acids like 3-methyl-4-hydroxymandelic acid[345], acetone and acetoacetic acid[346], homovanillic acid and related compounds[347,348]. A simple and rapid thin layer test for pregnandiol for the early detection of pregnancy[349] and for the control of female cycle has been worked out. Barbiturates and other hypnotics[350], cholesterols[351,352], amino acids[353], lipids and related compounds[352] in blood have been investigated. Squalene[354] has been confirmed in the human aorta. The presence of lipids in milk[355], liver[356] and nervous system[357] has been detected with the help of TLC. Similarly, serum bile acids[358] could be identified. Various biological materials were investigated on thin layers and it was possible to identify antihistamines[359], habit forming analgesics[360], central stimulants[361], organic acids[362], urinary estrogenes[363], some steroids or their metabolites[364]. The identification of sugars[365] and amino acids[366] in spermatic fluids, mucopolysaccharides[367] in sweat and *p*-aminobutyric acid[368] in brain extracts has been reported. Use of TLC in toxicological studies has been made to detect the presence of a number of compounds, e.g. barbiturates, narcotics, phenothiazines[369], meprobates[370] and doriden[371],

in viscera and other parts of dead bodies. Detection of doping[372] of race horses could be done by investigating their saliva.

Inorganic ions — The use of TLC in inorganic chemistry is not so widespread as in organic chemistry but it is becoming more and more popular. Silica gel which contains Fe and other inorganic impurities[117] is likely to be unsuitable in some cases but these impurities[117] can be removed. Metallic ions[373-376] like Ag^{+}, Cu^{+}, Cd^{4+}, Hg^{2+}, Al^{3+}, Cr^{3+} or Fe^{3+} and some other cations have been separated on cellulose, silica gel, ion exchange resins or alumina. Alkali metals[117], isotopes[377] like ^{234}Th and ^{238}U, metals like Ga, Al and rare earths[378] have been identified on layers of silica gel. Impregnated layers of silica gel were found suitable for the separation of ions[379,380] like Se and Te, Fe, Ni, Co, etc. Halogens[381] have been separated on silica gel or silver nitrate containing silica gel layers. Phosphates[382], mono and diphosphoric acids, sulphates and some polythionates[383,384] have been separated by TLC.

Metallo-organic compounds like dithiozonates[385] (diphenylthiocarbazone complexes) of Hg, Pb, Cu, etc., ferrocene derivatives[386] and some cobalt complexes[387] could be separated on silica gel layers. A TLC toxicological analysis of metals[388] has also been reported. The combined use of TLC and TL electrophoresis[389] has been found useful in some separations.

Miscellaneous compounds — The sulphur containing compounds like thioketones, thioamides, thioureas[390], thiophosphorous derivatives[391] and thiols[392] could be separated on chromatoplates. Some mustard oil glucosides[393], nitrogen containing compounds of pyridine or quinoline series[394], tetraphenyl porphyrines[395], polyphenyl ethers[396] and peroxides[397] were separated on silica gel layers. Benzoquinones, ubiquinones and some other natural and synthetic quinones[398,399] could be separated on silica gel or acetylated polyamide layers.

Summary

The constant improvements in TLC are responsible for its increasing applications in all branches of chemistry. This technique is of special help in the investigation of those substances which are present in very small quantities. Moreover, it can be combined with other physical or chemical methods of analysis. In future, besides its use in synthetic and phytochemistry, TLC will be of greater utility in biological and clinical investigations and in inorganic chemistry. The purities of various pharmaceutical products, foods and other articles of common use can be rapidly tested.

References

1. Izmailov, N. A. & Schraiber, M. S., *Farmatsevt, Sofia*, (1938), No. 3, 1; *Chem. Abstr.*, **34** (1940), 855.
2. Stahl, E., *ChemikerZtg.*, **82** (1958), 323.
3. Miller, J. M. & Kirchner, J. G., *Analyt. Chem.*, **26** (1954), 2002.
4. Barbier, M., Jaeger, H., Tobias, H. & Wyss, E., *Helv. chim. acta*, **42** (1959), 2440.
5. Machata, G., *Mikrochim. acta*, (1960), 79.
6. Vioque, E., *Grasas aceit.*, **11** (1960), 223.
7. Nybom, N., *Nature, Lond.*, **198** (1963), 1219.
8. Bhatnagar, J. K., Kapur, K. K. & Atal, C. K., *Indian J. Pharm.*, **26** (1964), 103.
9. Marcucci, M. & Mussini, E., *J. Chromat.*, **11** (1963), 270.
10. Wasicky, R., *Analyt. Chem.*, **34** (1962), 1346.
11. Hofmann, A. F., *Analyt. Biochem.*, **3** (1962), 145.
12. Pastuska, G., *Z. anal. Chem.*, **179** (1961), 355.
13. Tschesche, R., Freytag, W. & Snatzke, G., *Chem. Ber.*, **92** (1959), 3053.
14. Bekersky, J., *Analyt. Chem.*, **35** (1963), 261.
15. Morita, K. & Haruta, F., *J. Chromat.*, **12** (1963), 412.
16. Peifer, J. J., *Mikrochim. acta*, (1962), 529.
17. Sweely, C. C., *J. Lipid Res.*, **4** (1963), 402.
18. Hörhammer, L., Wagner, H. & Bittner, G., *Der Deutsche Apotheker*, **14** (1962), 148.
19. Bhandari, P. R., Lerch, B. & Wohlleben, G., *Pharm. Ztg., Reichenb*, **107** (1962), 1618; *Chem. Abstr.*, **58** (1963), 7346.
20. Mottier, M. & Potterat, M., *Anal. chim. acta*, **13** (1955), 46.
21. Lábler, L., *Thin layer chromatography*, Proceedings of the symposium held in Rome, 2-3 May 1963, edited by G. B. Marini-Bettolo (Elsevier Publishing Co., Amsterdam), 1964.
22. Černy, V., Joska, J. & Lábler, L., *Coll. Trav. chim. Tchecosl.*, **26** (1961), 1658.
23. Brockmann, H. & Schodder, H., *Chem. Ber.*, **74** (1941), 73.
24. Hermanek, S., Schwarz, V. & Cekan, Z., *Coll. Trav. chim. Tchecosl.*, **26** (1961), 3170.
25. Feltkamp, H., *Dtsch. ApothZtg.*, **102** (1962), 1269; *Chem. Abstr.*, **62** (1965), 2227.
26. Lie, K. B. & Nyc, J. F., *J. Chromat.*, **8** (1962), 75.
27. Musha, S. & Iinoya, H., *Bunseki Kagaku*, **14** (7) (1965), 647.
28. Rosi, D. & Hamilton, P., *J. Chromat.*, **9** (1962), 388.
29. Nybom, N., *Nature, Lond.*, **198** (1963), 1229.
30. Gref, C. G. & Saukkonen, J. J., *Analyt. Biochem.*, **8** (1964), 134.
31. Kabara, J. J., Kabara, G. C. & Wojtalik, R. S., *J. Chromat.*, **15** (1964), 267.
32. Gamp, A., Studer, P., Linde, H. & Meyer, K., *Experientia*, **18** (1962), 292.
33. Koss, F. W. & Jerchel, D., *Naturwissenschaften*, **51** (1964), 382.
34. Przybylowicz, E. P., Staudenmayer, W. J., Perry, E. S., Baitsholts, A. D. & Tischer, T. N., Lecture in Pittsburg Conference on Analytical Chemistry & Applied Spectroscopy, Pittsburg, USA, 1965.
35. Randerath, K., *Angew. Chem.*, **73** (1961), 674.
36. Seiler, H., *Helv. chim. acta*, **44** (1961), 1753.
37. Stahl, E., *Arch. Pharm., Berl.*, **292** (1959), 411.
38. Reichelt, J., *Coll. Trav. chim. Tchecosl.*, **27** (1962), 1709.
39. Nürnberg, E., *Arch. Pharm., Berl.*, **292** (1959), 610.
40. Hunek, S., *J. Chromat.*, **7** (1962), 561.
41. Grasshof, H., *Dtsch. ApothZtg.*, **103** (1963), 1396.
42. Tore, J. P., *J. Chromat.*, **12** (1963), 413.
43. Malis, J., Adamec, O. & Galvanek, M., *Nature, Lond.*, **194** (1962), 477.
44. Kaufmann, H. P. & Khoe, T. H., *Fette Seifen AnstrMittel*, **64** (1962), 81.
45. Hofmann, A. F., *J. Lipid Res.*, **3** (1962), 391.
46. Hofmann, A. F., *Biochem. biophys. acta*, **60** (1962), 458.
47. Mangold, H. K., *J. Amer. Oil Chem. Soc.*, **38** (1961), 708.
48. Brodasky, T. F., *Analyt. Chem.*, **35** (1963), 343.
49. Ritter, F. J., Meyer, G. M. & Geiss, F., *J. Chromat.*, **19** (1965), 304.
50. Morgan, M. E., *J. Chromat.*, **9** (1962), 379.
51. Coleman, M. H., *Lab. Pract.*, **13** (1964), 1200.
52. Farmer, L. B., *J. Chromat.*, **16** (1964), 412.
53. Bacon, M. F., *J. Chromat.*, **16** (1964), 553.
54. Stocker, H. R., *Helv. chim. acta*, **46** (1963), 2050.
55. Tamura, Z., *J. Chromat.*, **19** (1965), 429.
56. Strain, H. H., *Chromatographic adsorption analysis* (Interscience Publishers Inc., New York), 1941.
57. Hesse, G., *Z. anal. Chem.*, **181** (1961), 274.
57a. Wohlleben, G., cited in *Handbuch der Lebensmittelchemie*, Band II, Teil I (Springer Verlag, Berlin), 1965, 584.

58. HÖRHAMMER, L. & WAGNER, H., *Pharm. Ztg.*, **104** (1959), 783.
59. WOHLLEBEN, G., *Angew. Chem.*, **67** (1955), 741.
60. WOHLLEBEN, G., *Angew. Chem.*, **68** (1956), 752.
61. Woelm Publication No. AL 7 (M. Woelm, Eschwege, W. Germany), 1958, 11.
62. BOBBIT, J. M., *Thin layer chromatography* (Chapman & Hall Ltd, London), 1963, 63.
63. STÄHL, E., *Dünnschicht Chromatographie* (Springer Verlag, Berlin), 1962, 20.
64. JÄNCHEN, D., *J. Chromat.*, **14** (1964), 261.
65. GRITTER, R. J. & ALBERS, R. J., *J. Chromat.*, **9** (1962), 392.
66. TSCHESCHE, R., BIERNOTH, G. & WULF, G., *J. Chromat.*, **12** (1963), 342.
67. GÄNSHIRT, H. & MORIANZ, K., *Arch. Pharm., Berl.*, **293** (1960), 1065.
68. BHANDARI, P. R., *Pharm. Ztg.*, **110** (1965), 687.
69. SEIKEL, M. K., MILLET, M. A. & SAEMANN, J. F., *J. Chromat.*, **15** (1964), 115.
70. MISTRYUKOV, E. A., *J. Chromat.*, **9** (1962), 311.
71. STAHL, E., *Parfüm. Kosmet.*, **39** (1958), 564.
72. KAPADIA, G. J. & RAO, G. S., *J. pharm. Sci.*, **53** (1964), 233.
73. RANDERATH, K., *Dünnschicht Chromatographie* (Verlag Chemie GmbH, Weinheim/Bergstrasse, Germany), 1965, 59.
74. KORZUN, B. P. & BRODY, S., *J. pharm. Sci.*, **53** (4) (1964), 454; *Pharm. Ztg.*, **109** (1964), 1305.
75. TRUTER, E. V., *J. Chromat.*, **14** (1964), 57.
76. BRENNER, M. & NIEDERWIESER, A., *Experientia*, **17** (1961), 237.
77. REISERT, P. M. & SCHUMACHER, D., *Experientia*, **19** (1963), 84.
78. TURINA, S., MARJANOVIC-KRAJOVAN, V. & OBRADOVIC, M., *Analyt. Chem.*, **36** (1964), 1905.
79. HAALPAAP, H., *Chem.-Ing.-Tech.*, **35** (1963), 488.
80. HONEGGER, C. G., *Helv. chim. acta*, **45** (1962), 1409.
81. STAHL, E. & SCHORN, P. J., *Naturwissenschaften*, **49** (1962), 14.
82. STAHL, E. & KALTENBACH, U., *J. Chromat.*, **5** (1961), 458.
83. WEICKER, H., *Klin. Wschr.*, **37** (1959), 763.
84. VON ARX, E. & NEHER, R., *J. Chromat.*, **12** (1963), 329.
85. STAHL, E., *Arch. Pharm., Berl.*, **293** (1960), 531.
86. RITTER, F. J. & MEYER, G. M., *Nature, Lond.*, **193** (1962), 941.
87. BAEHLER, B., *Helv. chim. acta*, **45** (1962), 309.
88. SEHER, A., *Nahrungsmittelindustrie*, **4** (1960), 466.
89. OSWALD, N. & FLÜCK, H., *Pharm. acta Helvet.*, **39** (1964), 293.
90. GENEST, K., *J. Chromat.*, **19** (1965), 531.
91. MILLET, M. A., MOORE, W. E. & SAEMANN, J. F., *Analyt. Chem.*, **36** (1964), 491.
92. RABENORT, B., *J. Chromat.*, **17** (1965), 594.
93. POETHKE, W. & KINZE, W., *Arch. Pharm., Berl.*, **297** (1964), 593.
94. OPIÉNSKA-BLAUTH, J., KRACZKOWSKI, H., BRZUSZKIEWICZ, H. & ZAGÓRSKI, Z., *J. Chromat.*, **17** (1965), 288.
95. OELSCHLAEGER, H., VOLKE, J. & LIM, G. T., *Arch. Pharm., Berl.*, **298** (1965), 213.
96. SEILER, N., WERNER, G. & WEICHMANN, M., *Naturwissenschaften*, **50** (1963), 643.
97. KLAUS, R., *J. Chromat.*, **16** (1964), 311.
98. IKRAM, M. & BAKSH, M. K., *Analyt. Chem.*, **36** (1964), 111.
99. DRAWERT, F., BACHMANN, O. & REUTHER, K. H., *J. Chromat.*, **9** (1962), 376.
100. RICHARDSON, G. S., WELIKY, I., BATCHELDER, W., GRIFFITH, M. & ENGEL, L. L., *J. Chromat.*, **12** (1963), 115.
101. CHAMBERLAIN, J., HUGHES, A., ROGERS, A. W. & THOMAS, G. H., *Nature, Lond.*, **201** (1964), 774.
102. STAHL, E., *Chem.-Ing.-Tech.*, **36** (1964), 941.
103. BERGER, J. A., *C.R. Acad. Sci., Paris*, **257** (1963), 1534.
104. ABBOTT, D. C. & THOMSON, J., *Chem. & Ind. (Rev.)*, (1965), 310.
105. DETERMANN, H., WIELAND, T. & LUBEN, G., *Experientia*, **18** (1962), 430.
106. NIEDERWIESER, A. & HONEGGER, C. G., *Helv. chim. acta*, **48** (1965), 893.
107. WIELAND, T. & GEORGOPOULOS, D., *Biochem. Z.*, **340** (1964), 476
108. PASTUSKA, G. & TRINKS, H., *ChemikerZtg.*, **86** (1962), 135.
109. DOBICI, F. & GRASSINI, G., *J. Chromat.*, **10** (1963), 98.
110. SPRENGER, H. E., *Z. anal. Chem.*, **199** (1964), 338.
111. LICHTENBERGER, W., *Z. anal. Chem.*, **185** (1962), 111.
112. BECKETT, A. H. & CHOULIS, N. H., *J. Pharm. Pharmacol.*, **75** (1963), 236T.
113. MORRIS, L. J., HOLMANN, R. T. & FONTELL, K., *J. Lipid Res.*, **2** (1961), 68.
114. MISTRYUKOV, E., *J. Chromat.*, **9** (1962), 314.
115. ROTHER, A., BOBBITT, J. M. & SCHWARTING, A. E., *Chem. & Ind. (Rev.)*, (1962), 654.
116. MANTHEY, J. A. & AMUNSON, M. E., *J. Chromat.*, **19** (1965), 522.
117. SEILER, H. & ROTHWEILER, W., *Helv. chim. acta*, **44** (1961), 941.
118. BROWN, T. L. & BENJAMIN, J., *Analyt. Chem.*, **36** (1964), 446.
119. GEISS, F., KLOSE, A. & COPET, A., *Z. anal. Chem.*, **211** (1965), 37.
120. TOYOSHIMA, S. & NISHIMOTO, Y., *J. pharm. Soc. Japan*, **85** (3) (1965), 235; *Chem. Abstr.*, **63** (1965), 7.
121. HESSE, G., *Z. anal. Chem.*, **211** (1965), 5.
122. TORKAR, K., *Mh. Chem.*, **94** (1963), 110.
123. Woelm Publication No. AL 10 (Eschwege, W. Germany), 1963, 19.
124. SCHREIBER, K. & ADAM, G., *Mh. Chem.*, **92** (1961), 1093.
125. SMITH, L. L. & FOELL, T., *J. Chromat.*, **9** (1962), 339.
126. WEICKER, H. & BROSSMER, R., *Klin. Wschr.*, **39** (1961), 1265.
127. INSCOE, M. N., *Analyt. Chem.*, **36** (1964), 2505.
128. SZEGLEDT-JANKÓ, G., *Z. klin. Chem.*, **3** (1965), 45.
129. BRENNER, M., NIEDERWIESER, A., PATAKI, G. & FAHMY, A., *Experientia*, **18** (1962), 101.
130. PASTUSKA, G. & PETROWITZ, H. J., *ChemikerZtg.*, **86** (1962), 311.
131. BRODASKY, T. F., *Analyt. Chem.*, **35** (1964), 996.
132. PATAKI, G., *Helv. chim. acta*, **47** (1964), 784.
133. PATAKI, G. & KELEMEN, J., *J. Chromat.*, **11** (1963), 50.
134. STARKA, L. & HAMPL, R., *J. Chromat.*, **12** (1963), 347.
135. GEISS, F., SCHLITT, H., RITTER, F. J. & WEIMAR, W. M., *J. Chromat.*, **12** (1963), 469.
136. HESSE, G., ENGELHARDT, H. & KOWALLIK, W., *Z. anal. Chem.*, **214** (1965), 81.
137. STAHL, E., *Pharm. Rdsch., Hamb.*, **1**, Nr. 2 (1959), 1.
138. SHELLARD, E. J., *Lab. Pract.*, **13** (4) (1964), 290.
139. TEICHERT, K., MUTSCHLER, E. & ROCHELMEYER, H., *Dtsch. ApothZtg.*, **100** (1960), 477.
140. LIST, P. H. & HANAFI, S., *Dtsch. ApothZtg.*, **103** (1963), 1314.
141. RAGAZZI, E., VERONESE, G. & GIACOBAZZI, C., *Thin layer chromatography*, Proceedings of the symposium held at Rome, 2-3 May 1963 (Elsevier Publishing Co., Amsterdam), 1964, 149.
142. IKRAM, M., MIANA, G. A. & ISLAM, M., *J. Chromat.*, **11** (1963), 260.
143. GRÖGER, D. & STOLLE, K., *Arch. Pharm., Berl.*, **298** (1965), 246.
144. FARNSWORTH, N. R. & HILINSKI, I. M., *J. Chromat.*, **18** (1965), 184.
145. SUSZKO-PURZYCKA, A. & TRZEBNY, W., *J. Chromat.*, **17** (1965), 114.
146. KORTE, F. & SIEPER, H., *J. Chromat.*, **14** (1964), 178.
147. TSCHECHE, R. & OCKENFELS, H., *Chem. Ber.*, **97** (1964), 2316.
148. SHRIVASTAVA, R. M. & KHARE, M. P., *Chem. Ber.*, **97** (1964), 2732.
149. STEEHLE, J. A., *J. Chromat.*, **19** (1965), 300.
150. SCHUNAK, W., MUTSCHLER, E. & ROCHELMEYER, H., *Dtsch. ApothZtg.*, **105** (1965), 1551.
151. SCHLEMMER, F. & LINK, E., *Pharm. Ztg., Reichenb*, **104** (1959), 1349.
152. PARRISH, J. R., *J. Chromat.*, **18** (1965), 535.
153. TEICHERT, K., MUTSCHLER, E. & ROCHELMEYER, H., *Dtsch. ApothZtg.*, **100** (1960), 283.
154. GRASSHOF, H., *J. Chromat.*, **20** (1965), 165.
155. SEGURA-CARDONA, R. & SOEHRING, K., *Medna Exp.*, **10** (1964), 251.
156. WALDI, D., *Arch. Pharm., Berl.*, **295** (1962), 125.

157. SUBBARAO, R., ROOMI, M. W., SUBBARAM, M. R. & ACHAYA, K. T., *J. Chromat.*, **9** (1962), 295.
158. WEKELL, J. C., HOULE, C. R. & MALINS, D. C., *J. Chromat.*, **14** (1964), 529.
159. KUCERA, J., *Coll. Trav. chim. Tchecosl.*, **28** (1963), 1341.
160. MALINS, D. C., WEKELL, J. C. & HOULE, C. R., *Analyt. Chem.*, **36** (1964), 658.
161. PREY, V., BERBALK, H. & KAUSZ, M., *Mikrochim. acta*, (1962), 449.
162. JEAN PURDY, S. & TRUTER, E. V., *J. Chromat.*, **14** (1964), 62.
163. RUFFINI, G., *J. Chromat.*, **17** (1965), 483.
164. CAMP, B. G. & OBRIEN, F., *J. Chromat.*, **20** (1965), 178.
165. COBB, W. Y., *J. Chromat.*, **14** (1964), 512.
166. KLOUWEN, M. H., TER HEIDE, R. & KOK, J. G., *Fette Seifen AnstrMittel*, **65** (1963), 414.
167. GUDRINIETSE, E. & KREITSBERGA, D., *Latv. PSR Zinat. Akad. Vestrs. Ser. Khim.*, (1963), 515.
168. KUBECZKA, K. H., *Dtsch. ApothZtg.*, **104** (1964), 369.
169. GRANT, D. W., *J. Chromat.*, **10** (1963), 511.
170. BRAUN, D. & GEENEN, H., *J. Chromat.*, **7** (1962), 56.
171. BANCHER, E., SCHERZ, H. & PREY, V., *Mikrochim. acta*, (1963), 712.
172. PASTUSKA, G. & PETROWITZ, H. J., *J. Chromat.*, **10** (1963), 517.
173. PASSERA, C., PEDROTTI, A. & FERRARI, G., *J. Chromat.*, **14** (1964), 291.
174. HILLER, K., *Pharmazie*, **20** (1965), 353.
175. DANCIS, J., HUTZLER, J. & LEVITZ, M., *Biochim. biophys. Acta*, **78** (1963), 85.
176. RINK, M. & HERMANN, S., *J. Chromat.*, **14** (1964), 523.
177. SCHELLENBERG, P., *Angew. Chem.*, **74** (1962), 118.
178. BRENNER, M., NIEDERWIESER, A. & PATAKI, G., *Experientia*, **17** (1961), 145.
179. RANDERATH, K., *Dünnschicht Chromatographie* (Verlag Chemie, Weinheim/Bergstrasse, Germany), 1965, 114.
180. HESSE, G., ENGELHARDT, H. & KLOTZ, D., *Z. anal. Chem.*, **215** (1966), 182.
181. DI BELLO, C. & SIGNOR, A., *J. Chromat.*, **17** (1965), 506.
182. CARISANO, A., *J. Chromat.*, **13** (1964), 83.
183. EHRHARDT, E. & CRAMER, F., *J. Chromat.*, **7** (1962), 405.
184. PATAKI, G., *J. Chromat.*, **16** (1964), 541.
185. DETERMANN, H., *Experientia*, **18** (1962), 430.
186. DETERMANN, H. & MICHEL, W., *Z. anal. Chem.*, **212** (1965), 211.
187. RITSCHARD, W. J., *J. Chromat.*, **16** (1964), 327.
188. WIELAND, T. & DETERMANN, H., *Experientia*, **18** (1962), 431.
189. DETERMANN, H., ZIPP, O. & WIELAND, T., *Liebigs Ann.*, **651** (1962), 172.
190. IKAN, R. & CUDZINOVSKI, M., *J. Chromat.*, **18** (1965), 422.
191. HÄNSEL, R., RIMPLER, H. & SCHOPFLIN, G., *Planta med.*, **12** (1964), 169.
192. YAWATA, M. & GOLD, E. M., *Steroids*, **3** (1964), 435.
193. DUVIER, J., *J. Chromat.*, **19** (1965), 352.
194. LISBOA, B. P. & DICZFALUSI, E., *Acta Endocrin.*, **40** (1962), 60.
195. JACOBSOHN, G. M., *Analyt. Chem.*, **36** (1964), 275.
196. LISBOA, B. P., *J. Chromat.*, **13** (1964), 391.
197. DYER, W. G., GOULD, J. P., MAISTRELLIS, N. A., PENG, T. C. & OFNER, P., *Steroids*, **1** (1963), 271.
198. STARKA, L., SULCOVA, J., RIEDLOVA, J. & ADAMEC, O., *Clinica chim. Acta*, **9** (1964), 168.
199. FREIMUTH, U., ZAWTA, B. & BUECHNER, M., *Acta biol. med. germ.*, **13** (1964), 624.
200. SCHWARZ, V., *Pharmazie*, **18** (1963), 122.
201. TSCHECHE, R., MÖRNER, I. & SNATZKE, G., *Liebigs Ann.*, **670** (1963), 103.
202. HARA, S., TAKEUCHI, M., TACHIBANA, M. & CHIHARA, G., *Chem. pharm. Bull. Tokyo*, **12** (1964), 483.
203. HOFMANN, A. F., cited in *New biochemical separations* by A. T. James & L. J. Morris (D. Van Nostrand Co. Ltd, London), 1964, 26.
204. BLUNDEN, G. & HARDMAN, R., *J. Chromat.*, **15** (1964), 273.
205. KAWASAKI, T. & MIYAHARA, K., *Chem. pharm. Bull. Tokyo*, **11** (1964), 1546.
206. LÁBLER, L. & ČERNY, C., *Thin layer chromatography*, Proceedings of the symposium held in Rome, 2-3 May 1963, 144.
207. WALDI, D., cited in *New biochemical separations* by A. T. James & L. J. Morris (D. Van Nostrand Co. Ltd., London), 1964, 195.
208. ZELNIK, R. & ZITI, L. M., *J. Chromat.*, **9** (1962), 371.
209. FAUCONNET, L. & WALDESBUEHL, M., *Pharm. acta Helvet.*, **38** (1963), 423.
210. STEINEGGER, E. & VAN DER WALT, H. J., *Pharm. acta Helvet.*, **36** (1961), 599.
211. CORONA, G. L. & RAITERI, M., *J. Chromat.*, **19** (1965), 435.
212. ZURKOWSKA, J. & OZAROWSKI, A., *Planta med.*, **12** (1964), 222.
213. NAN-CHUN-SUN & HUI-YING LANG, *Yao Hsueh Hsueh Pao*, **11** (2) (1964), 101; *Chem. Abstr.*, **61** (1964), 2168.
214. MORRIS, L. J., *J. Chromat.*, **12** (1963), 321.
215. SUBBARAO, R. & ACHAYA, K. T., *J. Chromat.*, **16** (1964), 235.
216. KAUFMANN, H. P., MAKUS, Z. & DEICKE, F., *Fette Seifen AnstrMittel*, **63** (1961), 235.
217. KAUFMANN, H. P. & MAKUS, Z., *Fette Seifen Anstr-Mittel*, **62** (1960), 1014.
218. SUBBARAO, R., ROOMI, M. W., SUBBARAM, M. R. & ACHAYA, K. T., *J. Chromat.*, **9** (1962), 295.
219. MANGOLD, H. K. & MORRIS, L. J., Vth ISF Congress, London, 1962.
220. KAUFMANN, H. P., MAKUS, Z. & KHOE, T. H., *Fette Seifen AnstrMittel*, **64** (1962), 1.
221. KAUFMANN, H. P. & KO, Y. S., *Fette Seifen Anstr-Mittel*, **63** (1961), 828.
222. KNAPPE, E. & YEKUNDI, K. G., *Z. anal. Chem.*, **203** (1964), 87.
223. KAUFMANN, H. P. & DAS, B., *Fette Seifen Anstr-Mittel*, **64** (1962), 214.
224. MANGOLD, H. K. & KAMMERECK, R., *Chem. & Ind. (Rev.)*, (1961), 1032.
225. BARRETT, C. B., DALLAS, M. S. J. & PADLEY, F. B., *Chem. & Ind. (Rev.)*, (1962), 1050.
226. DE VRIES, B., Fall meeting of the American Oil Chemists Soc., Toronto, Canada, Oct. 1962.
227. HAAHTI, E., NIKKARI, T. & JUVA, K., *Acta chem. scand.*, **17** (1963), 538.
228. ZINKEL, D. F. & ROWE, J. W., *J. Chromat.*, **13** (1964), 74.
229. SKIPSKI, V. P., PETERSON, R. F., SANDERS, J. & BARCLAY, M., *J. Lipid Res.*, **4** (1963), 227.
230. REDMAN, C. M. & KEENAN, R. W., *J. Chromat.*, **15** (1964), 180.
231. SAMBASIVARAO, K. & MCCLUER, R. H., *J. Lipid Res.*, **4** (1963), 106.
232. YOUNG, O. M. & KAUFER, J. N., *J. Chromat.*, **19** (1965), 611.
233. EL-NOCKRASHY, A. S. & OSMAN, F., *Planta med.*, **13** (1965), 326.
234. DE BOHNER, L. S., SOTO, E. F. & DE COHAN, T., *J. Chromat.*, **17** (1965), 513.
235. SZOKA, K., KRAMER, M. & LINDNER, K., *Fette Seifen AnstrMittel*, **67** (1965), 257.
236. WINTERSTEIN, A., STUDER, A. & RUEGG, R., *Chem. Ber.*, **93** (1960), 2951.
237. ISLER, O. & RUEGG, R., cited in *Dünnschicht Chromatographie* by K. Randerath (Verlag Chemie, Weinheim/Bergstrasse, Germany), 1965, 188.
238. HAGER, A. & BERTENRATH, T., *Planta*, **58** (1962), 564.
239. SCHALTEGGER, K. H., *J. Chromat.*, **19** (1965), 75.
240. EGGER, K., *Planta*, **58** (1962), 664.
241. BACON, M. F., *J. Chromat.*, **17** (1965), 322.
242. IKAN, R., KASHMAN, J. & BERGMANN, E. D., *J. Chromat.*, **14** (1964), 275.
243. GUPTA, A. S. & DEV, S., *J. Chromat.*, **12** (1963), 189.
244. MCSWEENEY, G. P., *J. Chromat.*, **17** (1965), 183.
245. BETTS, T. J., *J. Pharm., Lond.*, **17** (1965), 520.
246. HÖRHAMMER, L. & WAGNER, H., *Dtsch. ApothZtg.*, **103** (1963), 1737.
247. GRÄB, R., *Dtsch. ApothZtg.*, **103** (1963), 1424.
248. PARIS, R. R. & GODON, M., *Ann. pharm. Fr.*, **19** (1961), 86.
249. HERMANEK, S., SCHWARZ, V. & CEKAN, Z., *Pharmazie*, **16** (1961), 566.
250. BECKMANN, S. & VOLKMANN, D., *Naturwissenschaften*, **32** (1965), 208.
251. WANG, K. T., *J. Chinese chem. Soc.*, Ser. II, **8** (1961), 241; *Angew. Chem.*, **75** (1963), 109.

252. STADLER, P. & ENDREAS, H., *J. Chromat.*, **17** (1965), 587.
253. RAMANT, J. L., *Bull. Soc. Chim. Belg.*, **72** (1963), 316.
254. HÖRHAMMER, L., WAGNER, H. & HEIN, K., *J. Chromat.*, **13** (1964), 235.
255. BHANDARI, P. R., *J. Chromat.*, **16** (1964), 130.
256. BHANDARI, P. R., *Naturwissenschaften*, **53** (1966), 82.
257. EGGER, K., *Z. anal. Chem.*, **182** (1961), 161.
258. DAVIDEK, J. & PROCHAZKA, Z., *Coll. Trav. chim. Tchecosl.*, **26** (1961), 2947.
259. BIRKOFER, L., KAISER, C., MEYER-STOLL, H. A. & SUPPAN, F., *Z. Naturf.*, **17b** (1962), 352.
260. PARIS, R. R., PARIS, M. & POITOUX, P., *Bull. Soc. chim. Fr.*, (1963), 1597.
261. HÖRHAMMER, L. & WAGNER, H., *ArzneimittelForsch.*, **12** (1962), 1002.
262. GRISEBACH, H. & PATSCHKE, L., *Chem. Ber.*, **93** (1960), 2326.
263. KAWANO, N., MUIRA, H. & KIKUCHI, H., *J. pharm. Soc. Japan*, **84** (1964), 469.
264. HÄNSEL, R., LANGHAMMER, L., FRENZEL, J. & RANFT, G., *J. Chromat.*, **11** (1963), 369.
265. KORTE, F. & VOGEL, J., *J. Chromat.*, **9** (1962), 381.
266. DANILOVIC, M. & NAUMOVIC-STEVANOVIC, O., *J. Chromat.*, **19** (1965), 613.
267. ABDEL HEY, F. M., ABU-MUSTAFA, E. A., EL-TAWILL, B. A. H. & FAYEZ, M. B. E., *Planta med.*, **13** (1965), 91.
268. BEYRICH, T., *J. Chromat.*, **20** (1965), 173.
269. KUZNETSOVA, G. A. & KUZMINA, L. V., *Rast. Resutsy*, **1** (1965), 149; *Chem. Abstr.*, **63** (1965), 4662.
270. HÄNSEL, R. & RIMPLER, H., *Z. anal. Chem.*, **207** (1965), 270.
271. ATAL, C. K. & SHAH, K. C., *Indian J. Pharm.*, **26** (1964), 265.
272. HAZNAGY, A., SZENDREI, K. & TÓTH, L., *Pharmazie*, **20** (1965), 541, 651.
273. GRASSHOF, H., *J. Chromat.*, **14** (1964), 513.
274. HAAS, H. J. & SEELIGER, A., *J. Chromat.*, **13** (1964), 573.
275. RINK, M. & HERMANN, S., *J. Chromat.*, **12** (1963), 415.
276. WEIDEMANN, G. & FISCHER, W., *Hoppe Seyl. Z.*, **336** (1964), 89.
277. ANET, E. F. L. J., *J. Chromat.*, **9** (1962), 291.
278. GEE, M., *Analyt. Chem.*, **35** (1963), 350.
279. MEYER ZU RECKENDORF, W., *Chem. Ber.*, **97** (1964), 1275.
280. ADACHI, S., *J. Chromat.*, **17** (1965), 295.
281. DAHLBERG, J. & SAMUELSON, O., *Svensk. Kem. Tidskr.*, **75** (1963), 178.
282. DEMAIR, W. & KÖLBEL, R., Z. *LebensmittUntersuch.*, **124** (1964), 157.
283. GUVEN, K. C., *Chem. Abstr.*, **63** (1965), 906.
284. GRIPPO, P., IACCARINO, M., ROSSI, M. & SCARANO, E., *Biochim. biophys. Acta*, **95** (1965), 1.
285. RANDERATH, K., *Nature, Lond.*, **194** (1962), 768.
286. DIETRICH, C. P., DIETRICH, S. M. C. & PONTIS, H. G., *J. Chromat.*, **15** (1964), 277.
287. RANDERATH, K. & NEUHARD, J., *Fed. Proc.*, **24** (1965), 669.
288. RANDERATH, K. & RANDERATH, E., *Angew. Chem.*, **76** (1964), 494.
289. RANDERATH, K. & RANDERATH, E., *Analyt. Biochem.*, **12** (1965), 83.
290. KECK, K. & HAGEN, U., *Biochem. biophys. Acta*, **87** (1964), 685.
291. PETROWITZ, H. J., *ChemikerZtg.*, **88** (1964), 235.
292 SAWICKI, E., STANLEY, T. W. & JOHNSON, H., *Microchim. J.*, **8** (1964), 257.
293. WIELAND, T., LÜBEN, G. & DETERMANN, H., *Experientia*, **18** (1962), 430.
294. BERG, A. & LAM, J., *J. Chromat.*, **16** (1964), 157.
295. HYYRYLAINEN, M., *Farm. Aikakauslehti*, **72** (1963), 161.
296. PREY, V., BERGER, A. & BERBALK, H., *Z. anal. Chem.*, **185** (1962), 113.
297. SALO, T., AIRO, E. & SALMINEN, K., *Z. Lebensmitt-Untersuch.*, **125** (1964), 20.
298. ATTAWAY, J. A., WOLFORD, R. W. & EDWARDS, G. J., *Analyt. Chem.*, **37** (1965), 74.
299. KAHAN, S. & FITELSON, J., *J. Ass. off. agric. Chem., Wash.*, **47** (1964), 551.
300. SALO, T. & SALMINEN, K., *Z. LebensmittUntersuch.*, **124** (1964), 448.
301. COPIUS-PEEREBOOM, J. W. & BEEKES, H. W., *J. Chromat.*, **14** (1964), 417.
302. PINZON, R., MIRIMANOFF, A. & KAPELANIDIS, I., *Pharm. acta Helvet.*, **40** (1965), 141.
303. DILLEY, R. A., *Analyt. Biochem.*, **7** (1964), 240.
304. DAVIDEK, J. & POKORNY, J., *Z. LebensmittUntersuch.*, **115** (1961), 113.
305. PIETSCH, H. P. & MEYER, R., *Nahrungsmittelindustrie*, **9** (1965), 154.
306. SYNODINOS, E., KOTAKIS, G. & KOKKOTIKOTAKI, E., *Hem Hron.*, **28** (1963), 77; *Chem. Abstr.*, **60** (1964), 189.
307. MONTAG, A., *Z. LebensmittUntersuch.*, **116** (1962), 413.
308. COPIUS-PEEREBOOM, J. W. & BEEKES, H. W., *J. Chromat.*, **20** (1965), 43.
309. GÄNSHIRT, H. & MALZACHER, A., *Naturwissenschaften*, **47** (1960), 279.
310. BOLLIGER, H. L., cited in *Dünnschicht Chromatographie* by E. Stahl (Springer Verlag, Berlin), 1962, 244.
311. HAYASHI, M. & KAMIKUBO, T., *Vitamins, Kyoto*, **31** (1965), 362; *Chem. Abstr.*, **63** (1965), 2103.
312. JOHN, K. V., LAKSHMANAN, M. R., JUNGALWALA, F. B. & CAMA, H. R., *J. Chromat.*, **18** (1965), 53.
313. VARMA, T. N. R., PANALAKS, T. & MURRAY, T. K., *Analyt. Chem.*, **36** (1964), 1864.
314. STANLEY, C. W., *J. Chromat.*, **16** (1964), 467.
315. FISCHER, R. & KLINGELHÖLLER, W., *Arch. Toxicol.*, **19** (1961), 119.
316. LUDWIG, E. & FREIMUTH, U., *Nahrungsmittelindustrie*, **8** (1964), 559.
317. WALKER, W. C. & BEROZA, M., *J. Ass. off. agric. Chem., Wash.*, **46** (1963), 250.
318. STAHL, E. & PFEIFLE, J., *Naturwissenschaften*, **52** (1965), 620.
319. HENKEL, H. G., *Chimia*, **19** (1965), 128.
320. NICOLAUS, B. J. R., CORONELLI, C. & BINAGHI, A., *Farmaco*, **16** (1961), 349.
321. SONANINI, D. & ANKER, L., *Pharm. acta Helvet.*, **39** (1964), 518.
322. NUSSBAUER, P. A. & SCHORDERET, M., *Pharm. acta Helvet.*, **40** (1965), 205.
323. CASSANI, G., ALBERTINI, A. & CIFFERI, O., *J. Chromat.*, **13** (1964), 238.
324. ANDERSON, T. T., *J. Chromat.*, **14** (1964), 127.
325. HUTTENRAUCH, R. & SCHULZE, J., *Pharmazie*, **19** (1964), 334.
326. BICON-FISTER, T. & KAJGANOVIC, V., *J. Chromat.*, **16** (1964), 503.
327. NIEDLEIN, R., KRULL, H. & MEYL, M., *Dtsch. ApothZtg.*, **105** (1965), 481.
328. NIEDLEIN, R., KLÜGEL, G. & LEBERT, U., *Pharm. Ztg.*, **110** (1965), 651.
329. NORFALISE, A., *J. Chromat.*, **19** (1965), 68.
330. EIDEN, F. & STACHEL, H. D., *Dtsch. ApothZtg.*, **103** (1963), 121.
331. SCHMIDT, E., HOPPE, E., MEYTHALER (Jr), CHR. & ZICHA, L., *ArzneimittelForsch.*, **13** (1963), 969.
332. SAHLI, M. & OESCH, M., *J. Chromat.*, **14** (1964), 526.
333. MORRISON, J. C. & CHATTAN, L. G., *J. Pharm. Lond.*, **17** (1965), 655.
334. ZARNACK, J. & PFEIFER, S., *Pharmazie*, **19** (1964), 216.
335. BICAN-FISTER, T., *Acta pharm. jugosl.*, **12** (1962), 73.
336. NORFALISE, A., *J. Chromat.*, **20** (1965), 61.
337. BAILEY, R. W., *Analyt. Chem.*, **36** (1964), 2021.
338. REICH, J., BORNFLETH, H. & RHEINBAY, J., *Pharm. Ztg.*, **108** (1963), 1183.
339. SAHLI, M., ZIEGLER, H. & OESCH, M., *Pharm. Ztg.*, **110** (1965), 1542.
340. NONCLERCQ, M. & NYS, C., *J. Pharm. Belg.*, **19** (46) (1964), 421.
341. SARSUNOVA, M., *Pharmazie*, **18** (1963), 748.
342. PITTERA, A., CASSIA, B. & FERLITO, S., *Arch. Stud. Fisiopatol. Clin. Ricamb.*, **27** (1963), 97.
343. FRAHM, M., GOTTESLEBEN, A. & SOEHRING, K., *Pharm. acta Helvet.*, **38** (1963), 785.
344. BURGI, W., *Schweiz. ApothZtg.*, **103** (1965), 351.
345. KÖHLER, P. & BAUFELD, H., *Das Ärztliche Laboratorium*, **10** (1964), 224.
346. RINK, M. & HERMANN, S., *J. Chromat.*, **12** (1963), 249.

347. SANKOFF, I. & SOURKES, T. L., *Canad. J. Biochem., Physiol.*, **41** (1963), 1381.
348. TAUTZ, N. A., VOLTMER, G. & SCHMID, E., *Klin. Wschr.*, **43** (1965), 233.
349. WALDI, D., *Laboratorio Scient.*, **11** (1963), 81; *Klin. Wschr.*, **40** (1962), 827.
350. PETZOLD, J. A., CAMP, W. J. R. & KIRCH, E. R., *J. Pharm. Sci.*, **52** (1963), 1106.
351. MORRIS, L. J., *J. Lipid Res.*, **4** (1963), 354.
352. YAMAMUTO, K. K., *Acta Soc. opthal. jap.*, **68** (11) (1964), 1619; *Chem. Abstr.*, **63** (1965), 4801.
353. PATAKI, G. & KELLER, M., *Z. klin. Chem.*, **1** (1963), 157.
354. GARBUZOV, A. G., PYATBITSKII, N. N. & PISKUNOV, A. K., *Arkh. Patol.*, **27** (1965), 58; *Chem. Abstr.*, **63** (1965), 8807.
355. CZEGLEDI-JANKO, G., *Z. klin. Chem.*, **3** (1965), 14.
356. KAUFMANN, H. P. & VISWANATHAN, C. V., *Fette Seifen AnstrMittel*, **11** (1963), 925.
357. WELLS, M. A. & DITTMAR, J. C., *J. Chromat.*, **18** (1965), 503.
358. FROSCH, B., *ArzneimittelForsch.*, **15** (1965), 178.
359. FIKE, W. W. & SUNSHINE, I., *Analyt. Chem.*, **37** (1965), 126.
360. EBERHARDT, E. & NORDEN, O., *ArzneimittelForsch.*, **14** (1964), 1354.
361. EBERHARDT, H. & DEBACKERE, M., *ArzneimittelForsch.*, **15** (1965), 930.
362. GLOMBITZA, K. W., *J. Chromat.*, **19** (1965), 320.
363. LUISI, M., *Chem. Abstr.*, **60** (1964), 16154.
364. REISERT, P. M. & SCHUMACHER, D., *Experientia*, **19** (1963), 84.
365. MUENZEL, M. & KLINGMUELLER, G., *Arch. klin. exp. Derm.*, **221** (3) (1965), 250; *Chem. Abstr.*, **62** (1965), 13493.
366. KELLER, M. & PATAKI, G., *Helv. chim. acta*, **46** (1963), 1687.
367. SEUTTER, E. & MALI, J. W. H., *Clinica chim. acta*, **12** (1965), 17.
368. VOIGT, S., SOLLE, M. & KONITZER, K., *J. Chromat.*, **17** (1965), 180.
369. SUNSHINE, I., *Amer. J. clin. Pathol.*, **40** (1963), 576.
370. MAROZZI, E. & FALZI, G., *Chem. Abstr.*, **63** (1965), 7331.
371. VERCRUYSSE, A., *J. Pharm. Belg.*, **18** (45) (1963), 569.
372. BAEUMLER, J., BRAULT, A. L. & OBERSTEG, J. I., *Schweiz. Arch. Tierheilk.*, **106** (1964), 346; *Chem. Abstr.*, **61** (1964), 16432d.
373. KLAMBERG, H., cited in *Dünnschicht Chromatographie* by K. Randerath (Verlag Chemie, Weinheim/Bergstr.), 1965, 232.
374. SEILER, H., *Helv. chim. acta*, **46** (1963), 2629.
375. SHERMA, J., *J. Chromat.*, **19** (1965), 458.
376. GOLLER, E. J., *J. chem. Educ.*, **42** (1965), 442.
377. SEILER, H. & SEILER, M., *Helv. chim. acta*, **48** (1965), 117.
378. DANEELS, A., MASSART, D. L. & HOSTE, J., *J. Chromat.*, **18** (1965), 144.
379. CHIH-TE HU & CHENG-LI LIU, K'O HSUEH, *Chem. Abstr.*, **63** (1965), 7.
380. MARKL, P. & HECHT, F., *Chem. Abstr.*, **60** (1964), 6196.
381. MUTO, M., *J. chem. Soc. Japan*, **85** (1964), 782; *Chem. Abstr.*, **63** (1965), 12299.
382. CLESCERI, N. L. & LEE, G. F., *Analyt. Chem.*, **36** (1964), 2207.
383. BANDLER, M. & MENGEL, M., *Z. anal. Chem.*, (1964), 206.
384. SEILER, H. & ERLENMEYER, H., *Helv. chim. acta*, **47** (1964), 264.
385. HRANISAVLJEVIĆ-JAKOVLJEVIC, M. & PEJKOVIĆ-TADIĆ, I., *Thin layer chromatography*, Proceedings of the symposium held in Rome, 2-3 May 1963 (Elsevier Publishing Co. Amsterdam), 1964, 221.
386. SCHLÖGL, K., PELONSEK, H. & MOHAR, A., *Mh. Chem.*, **92** (1961), 533.
387. HÄFELINGER, G. & BAYER, E., *Naturwissenschaften*, **51** (1964), 136.
388. MERKUS, F. W. H. M., *Pharm. Weekbl. Ned.*, **98** (21) (1963), 947; *Chem. Abstr.*, **61** (1964), 950.
389. TAKITANI, S., SUZUKI, M., FUJITA, N. & HOZUMI, K., *Chem. Abstr.*, **63** (1965), 9039.
390. STEPHEN, R. & GORDON, J., *Nature, Lond.*, **203** (1964), 749.
391. MASTRYUKOVA, T. A., SSACHAROWA, T. B. & KABATSCHNIK, M. I., *Izv. Akad. Nauk. SSSR. Ser. Khim.*, (1963), 2211; *Chem. Abstr.*, **60** (1964), 9882.
392. PRINZLER, H. W., PAPE, D. & TEPPKE, M., *J. Chromat.*, **19** (1965), 375.
393. WAGNER, H., HÖRHAMMER, L. & NUFER, H., *ArzneimittelForsch.*, **15** (1965), 453.
394. PETROWITZ, H. J., PASTUSKA, G. & WAGNER, S., *ChemikerZtg.*, **89** (1965), 7.
395. BALEK, R. W. & SZUTKA, A., *J. Chromat.*, **17** (1965), 127.
396. NEALEY, R. H., *J. Chromat.*, **14** (1964), 123.
397. KNAPPE, E. & PETRI, D., *Z. anal. Chem.*, **190** (1962), 386.
398. PETTERSSON, G., *J. Chromat.*, **12** (1963), 352.
399. GRAU, W. & ENDRES, H., *J. Chromat.*, **17** (1965), 587.

Growth & Developmental Hormones as Tools for the Study of Biosynthetic Control Mechanisms

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VIRTUALLY every tissue in multicellular plants and animals depends on hormones for the attainment of its proper mass and degree of maturation. As a rule, growth promoting and developmental hormones do not directly control cell division or initiate differentiation, but help the already differentiated but immature cell to complete its functional specialization or regulate its size. With the knowledge that enzyme synthesis can be induced in animal cells as in microorganisms[1] and the recent advances in experimental study of nucleic acid and protein metabolism, increasing evidence is accumulating that growth and developmental hormones exert a profound influence at various levels on the genetic regulation of protein synthesis. Much of this work has of course been designed with a view to understanding the mechanisms of action of these hormones. But sufficient information has now accumulated, with both animal and plant hormones, to encourage the use of growth and developmental hormones as tools in attacking the much wider problems of cellular mechanisms responsible for regulating development.

It is in this context of using hormones as tools that the effects of some animal hormones on important biosynthetic control mechanisms are discussed in this review. Many of our concepts of control mechanisms in higher organisms are derived from hypotheses originally tailored to fit experiments on microorganisms; the use of developmental hormones may, therefore, offer a way of deciding to what extent development in higher organisms conforms to or deviates from these working hypotheses. But before describing the effects of hormones at the molecular level, let us briefly consider some basic points of hormone action at the physiological level in order to interpret the significance of experimental findings.

Some Basic Considerations

Distinction between Growth Promoting and Metabolic Effects

Many of the earlier hypotheses concerning the mechanism of action of hormones were based on the assumption that the hormone may directly affect the activity of some enzyme or other cellular component and alter the rate of a regulatory metabolic process[2]. Such a hypothesis may still be valid for hormones which strictly control metabolic activity, for example, the action of adrenaline on cellular oxidation via regulation of phosphorylase[3]. However, it now seems that developmental hormones may act by altering the distribution or induction of enzymes or other cellular constituents. Some investigators even go further and explain the action of those hormones with 'metabolic' activity in adult animals, such as insulin and aldosterone, on the basis of a rapid and subtle regulation of RNA or protein synthesis[4,5]. An important point to be noted is that many developmental hormones have multiple biological actions which may be manifested simultaneously. For example, administration of small amounts of thyroid hormone to stimulate growth in young animals will also be accompanied by a stimulation of basal metabolic rate, mitochondrial respiration, and RNA and protein synthesis[6-8].

Selective Response of Tissues to Hormones

There is in most animal tissues a discriminatory mechanism that allows the cells to respond to only those hormones on which their growth and maturation depend. Good examples are the specific responses of endocrine tissues to the specific trophic hormones released from the pituitary in vertebrates, such as adrenocorticotrophic and thyrotrophic hormones, or from the brain in invertebrates as illustrated by the dependence of the pro-thoracic glands on 'brain hormone' in many insects. The accessory sexual tissues like the prostate, seminal vesicles and the uterus will only develop and grow in response to testosterone and oestrogen although other growth promoting hormones may also be localized in these tissues.

Responses of One Tissue to Multiple Hormones

The growth and development of many tissues is known to depend on the action of more than one hormone. Such a combination of hormonal action may have either additive or mutually antagonistic effects. For example, thyroxine and growth hormone will have additive effects on the growth of liver and bone[9], and similarly thyroxine and testosterone or cortisone have a synergistic effect on the growth of salivary gland of rodents[10]. On the other hand, it is known that in insects, ecdysone will enhance pupal molting whereas the secretion of 'juvenile hormone' will prevent developmental changes[11,12].

It is worth noting that, as a rule, where two or more hormones act synergistically or additively to promote development, different hormones control the levels of different proteins or structural components without much overlap.

Different Responses of Different Tissues to the Same Hormone

The opposite phenomenon to the above is best illustrated by the wide variety of functional and structural changes induced by thyroid hormones during metamorphosis in amphibia (Table 1). Thus the same hormone when acting on the tail or intestine initiate and accelerate their resorption but promote growth of limbs when acting on the limb

TABLE 1 — MORPHOLOGICAL AND BIOCHEMICAL CHANGES PROVOKED IN DIFFERENT TISSUES OF THE FROG TADPOLE DURING THYROXINE INDUCED METAMORPHOSIS

Tissue	Change
Liver	Little or no morphological change. Induction or accelerated synthesis of urea cycle enzymes, serum albumin, and perhaps adult haemoglobin
Eye	Shift in pigment from prophyropsin to rhodopsin. Modification of muscular anatomy
Limb buds	Cell division, growth and maturation of bone, skin, nerves, etc.
Tail, intestine gills	Tissue resorption. Activation or induction of hydrolases
Skin (tail)	Collagen breakdown, increase in collagenase
Skin (back, head)	Collagen deposition

buds. In the liver, there is little change in the size or mass of the tissue but an acceleration or induction of the synthesis of a new set of proteins such as the urea cycle and respiratory enzymes, serum albumin and adult haemoglobin[13,14]. Experiments on the effect of thyroid hormones on isolated organ cultures or local application of the hormone to the eye, limb bud or tails have shown that the hormone interacts directly with each tissue[15-17].

Physiological Actions of Hormones

Minute amounts of hormones are required for promoting growth and development in their target cells; if used in large amounts they may even produce an opposite effect. For example, at low doses thyroid hormones promote growth in most vertebrates but have a catabolic action and retard growth at doses above 50-70 μg./100 g. body weight. At the cellular and molecular levels, large doses mask the physiologically relevant sites of action and may provoke irrelevant biochemical effects.

A lag period preceding the physiological effect is observed after the administration of a hormone to an immature animal or to an organism artificially deprived of it. The length of this latent period of action can vary widely according to the hormone, the target tissue and the biological end-point. A period of 80 hr elapses before morphological changes can be detected in bullfrog metamorphosis induced precociously with thyroid hormones[14,18]. On the other hand, the stimulatory effect on the size and maturity of the uterus can be detected within 3 hr after the administration of oestrogen to an ovariectomized rat[16,19,20]. It is this early period preceding the stimulation of growth and development that we shall consider below in discussing the chain of biochemical events associated with biosynthetic control mechanisms that are triggered off by some hormones.

Sequential Biochemical Events during the Early Period of Hormone Action

After administration of many hormones, a change in enzyme activity, or the level of existing constituents, or the induction of a new constituent of the target cells can be detected before the morphological signs of hormonal action. It is not surprising, therefore, that growth and developmental hormones have been found to produce drastic changes in the target cell's protein and RNA synthesizing capacity before other actions become apparent.

Cytoplasmic Protein Synthesis

A number of workers have now demonstrated an increased deposition of total protein or the accelerated synthesis of specific proteins *in vivo* during growth stimulation by hormones in a variety of systems[16,21-24]. In some cases 'growth' does not necessarily ensue, as with the enhanced synthesis of tryptophan pyrrolase and tyrosine-α-ketoglutarate transaminase in mammalian liver by cortisone[25]. In most cases when growth does take place, the administration of the hormone is soon followed by an increased protein synthesizing capacity *in vitro* of cell-free preparations of those tissues that specifically respond to the hormone. Thus liver ribosomes from hypophysectomized or thyroidectomized rats show an enhanced rate of incorporation of amino acids into protein after a single injection of growth hormone[26,27] or thyroid hormone[28]. Similarly, one can cite the higher activity of prostatic, uterine or adrenal ribosomes from animals after stimulation with testosterone, oestrogen or ACTH respectively[16,29-32].

Without extending the above list to other systems, three features of hormonal stimulation of cytoplasmic protein synthesis need to be emphasized. Firstly, the enhancement of the capacity of the tissue to synthesize proteins precedes, or coincides with, the first manifestation of the physiological action of the hormone. Secondly, there is a relatively long lag period after hormone administration *in vivo* and the addition of the hormone in small amounts to the amino acid incorporating system *in vitro* usually has no effect. Thirdly, an alteration in the RNA content of the ribosomal or microsomal particles accompanies the increased protein synthesizing capacity of the cytoplasmic system. For these reasons and because of the regulatory role of nucleic acids in protein synthesis, it is quite obvious why many investigators are now exploring the effects of hormones on the synthesis and turnover of nucleic acids under conditions that promote growth.

Nucleic Acid Synthesis

Some of the sequential biochemical events observed in our laboratories following the administration of a single injection of the thyroid hormone, 3,5,3′-triiodothyronine, to young thyroidectomized rats are summarized in Fig. 1. Under the conditions employed, the thyroidectomized rat which has virtually stopped growing will resume growth for a few days after receiving the hormone. A simultaneous rise in the amino acid incorporating capacity of both mitochondria and microsomes was detected several hours before an increase in basal metabolic rate and before any increase in the weight of the liver. The accelerated rate of protein synthesis by mitochondria, which is distinct from the ribosomal protein synthesizing system[33], is of interest in explaining the well-known property of regulation of basal metabolic rate by thyroid hormones.

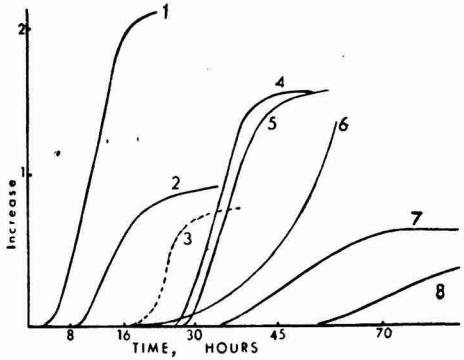

Fig. 1 — Schematic representation of the time course of biochemical and physiological responses of thyroidectomized rats following a single injection of 12-25 μg. of triiodothyronine [Ordinate : relative increase over control values after injection of the hormone. Abscissa : time after the administration of the hormone. Curves : (1) synthesis of rapidly labelled nuclear RNA; (2) Mg^{2+}-activated DNA-dependent RNA polymerase in nuclei; (3) ribosomal RNA recovered per g. liver; (4) mitochondrial and microsomal amino acid incorporation into protein; (5) cytochrome oxidase/mg. mitochondrial protein; (6) microsomal NADPH-cytochrome *c* reductase; (7) basal metabolic rate; and (8) liver weight]

Under physiological or growth promoting conditions thyroid hormones seem to regulate the basal metabolic rate by controlling the levels of respiratory and phosphorylative units in liver and skeletal muscle mitochondria, rather than by a direct interaction with mitochondrial enzymes or structures as had been suggested earlier[6,7]. Electron microscopic examination has indeed revealed a dramatic growth and multiplication of skeletal muscle mitochondria after repeated stimulation with thyroid hormones[34].

Fig. 1 also shows that the abrupt rise in protein synthesizing capacity of cytoplasmic particles does not take place for about 30 hr after triiodothyronine administration. During this time, however, impressive changes occur in the rate of synthesis of RNA by the cell nucleus. This is evident both from the stimulation of the DNA-dependent RNA polymerase in isolated nuclei[35] and of the incorporation of ^{32}P and orotic acid-^{14}C *in vivo* into nuclear RNA[36,37]. The precedence of the accelerated synthesis of rapidly labelled nuclear RNA suggests that the increase in RNA polymerase activity of the nucleus may reflect a secondary response of the nucleus to satisfy a demand created for the supply of RNA. The hormones added directly to nuclei have no effect on the RNA polymerase activity.

Although RNA synthesis in the nucleus is enhanced relatively early after hormone administration, there is a further lag of about 24 hr before a significant stimulation of cytoplasmic protein synthesis can be observed. The latter almost coincides with an abrupt accumulation of ribosomal RNA (Table 2). It appears, therefore, that the enhanced protein synthetic activity in the cytoplasm is due to the newly synthesized RNA molecules, the bulk of which are ribosomal RNA. Isotope incorporation studies of the slowly labelled RNA also show an increased turning over of RNA from the nucleus into the cytoplasm[36,37] as a result of hormonal treatment. In connection with the time lag between the stimulation of rapidly labelled nuclear RNA synthesis and cytoplasmic protein synthetic activity, it is useful to recall the suggestion by Harris *et al.*[38] that only a minute fraction of rapidly labelled RNA found in the nucleus may be transferred to the cytoplasm.

Table 2 — Nuclear and Ribosomal RNA in Livers of Thyroidectomized Rats after a Single Injection of Triiodothyronine (T_3)

Time after T_3* hr	Nuclear RNA mg./mg. DNA	Ribosomal RNA mg./g. liver
0	0·31	3·21
16	0·26	3·36
22	0·36	3·62
42	0·38	5·77
66	0·29	5·85

*T_3 (16 μg.) injected per 100 g. body weight.

Inhibitors of RNA and protein synthesis are also powerful inhibitors of the physiological action of thyroid hormones[39]. It is interesting to note that not only the growth promoting action of these hormones is affected by these inhibitors but also their metabolic action.

Hormonal regulation of RNA synthesis is by no means restricted to thyroid hormones. Administration of testosterone or oestrogen to castrated animals is soon followed by a rise in the DNA-dependent RNA polymerase activity measured in nuclei isolated from the prostate or uterus[20,29,40]. Oestrogen has a growth promoting effect on bird liver and this hormone has also been shown to increase nuclear RNA polymerase activity in chicken liver[41]. The enzyme from the above tissues has not been purified, as is the case with microbial RNA polymerase, and it is, therefore, impossible to say whether the above hormones cause a rapid increase (within 1 hr in the case of the oestrogen-uterus system) in the amount of the enzyme or somehow facilitate the copying of the DNA template. It should be noted again that direct addition of the hormone to the nuclear preparation does not enhance the activity of the polymerase. Information is also available on the synthesis of RNA *in vivo*. It has been well known for some years that the level of RNA is low in the relevant target tissue in the absence of a variety of vertebrate and invertebrate developmental hormones, and quite considerable increases can be observed after the hormone has been administered. With the use of radioactive precursors of RNA and cellular fractionation, the earlier stages of action of many hormones have been characterized by a marked increase in the specific activity of rapidly labelled RNA localized in the nucleus[19,20,27,42,43]. An enhancement of labelling of rapidly labelled nuclear RNA has also been obtained with hormones which induce enzyme synthesis but cannot be

strictly defined as growth promoting or developmental, such as cortisone and insulin[5,44].

It is not surprising that inhibitors of RNA and protein synthesis, such as actinomycin D, 5-fluorouracil and puromycin are potent inhibitors of the biological action of a variety of hormones[19,21,45-51]. In the case of the inhibition by actinomycin D of the pupation of blowfly larvae, which is under the control of ecdysone, Sekeris and Karlson[52] found that the order of and time interval between the administration of the hormone and the inhibitor is very critical in order to obtain an inhibition. The same is generally true for the inhibition of action of other hormones.

Protein and RNA Synthesis at the Onset of Induced Amphibian Metamorphosis

The profound developmental changes occurring in amphibian metamorphosis are obligatorily dependent on thyroid hormones. Administration of exogenous thyroid hormone to frog or toad tadpoles will induce and accelerate metamorphosis; in the American bullfrog, *Rana catesbeiana*, metamorphosis can be induced 18-20 months before its normal term. Below is a brief account of the results we have obtained recently[18].

The results presented in Fig. 2 show that while there is only a small increase in total liver protein for the first few days after induction of metamorphosis, there is a great increase in amount or initial appearance of certain liver proteins. The formation of urea cycle enzymes, adult haemoglobin and serum albumin have been shown earlier to represent *de novo* synthesis following the administration of the hormone[14,53]. There is also a relatively greater increase in the proteins associated with the membranous structures of microsomes and mitochondria. There exists, however, a lag period of 60-80 hr after hormone administration to *Rana catesbeiana* tadpoles before any increase in the levels of enzymes or other proteins or a morphological change can be detected. We have now demonstrated a marked acceleration of the turnover and synthesis of all classes of RNA during this apparent lag period preceding the onset of metamorphosis.

The earliest response is that of the nucleus as judged by the synthesis of rapidly labelled RNA (Fig. 3). If the animals were killed at increasing time intervals after the administration of ^{3}H-labelled uridine, there was a two- to threefold increase in the specific radioactivity of mitochondrial and ribosomal RNA between 35 and 50 hr after the induction of metamorphosis. Finamore and Frieden[54] had also noticed a greater incorporation of ^{32}P into the total RNA of the liver under similar conditions of induction of metamorphosis. There is, however, little or no accumulation of RNA in the cytoplasm for 100-150 hr after induction, thus suggesting an enhanced overall rate of turnover. This interpretation has been supported by the accelerated breakdown of existing ribosomal RNA when metamorphosis was induced in tadpoles in which RNA was prelabelled with tritiated uridine. Much of the radioactivity released from the breakdown of ribosomes is soon recovered as nuclear RNA, suggesting a reutilization of the breakdown products.

In other isotopic experiments, using sucrose density gradient centrifugation to separate the cytoplasmic 78S ribosomes from their polyribosomal aggregates, we have found the appearance of both new messenger and ribosomal RNA 40-60 hr after induction of metamorphosis (Fig. 4). It is of

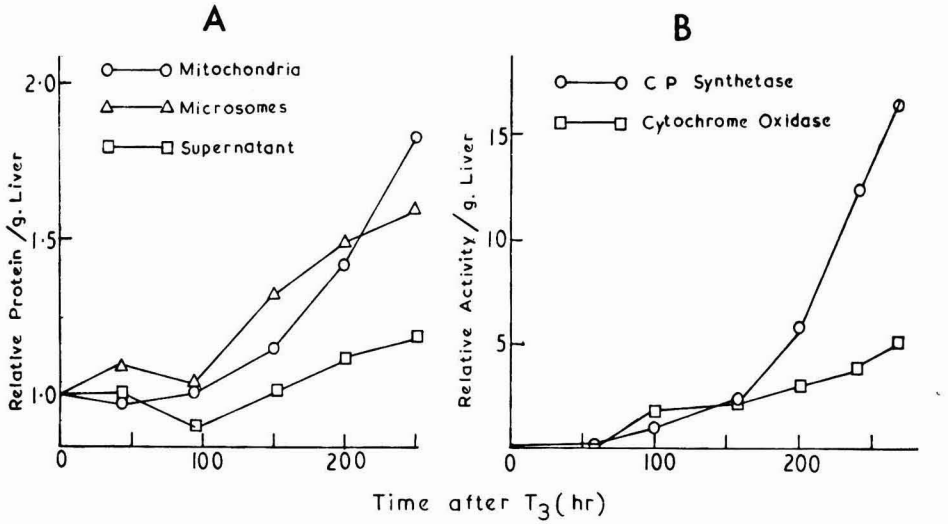

Fig. 2 — Cytoplasmic protein and enzyme formation in the liver as a function of time after the induction of metamorphosis in *Rana catesbeiana* tadpoles with triiodothyronine (T_3) [Ordinate: relative values in induced animals on the basis of a value = 1 for the non-induced controls. (A) protein recovered per g. liver in mitochondria, microsomes and the post-microsomal supernatant; and (B) specific activities of carbamyl phosphate (CP) synthetase and cytochrome oxidase in the liver]

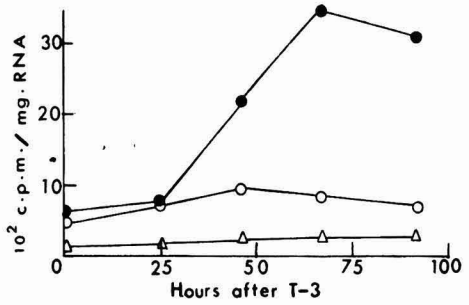

Fig. 3 — Specific radioactivity of nuclear, soluble and microsomal RNA in liver of *Rana catesbeiana* tadpoles killed at different time intervals after the induction of metamorphosis with triiodothyronine (T_3) [^{3}H-labelled uridine (10 μC.) was injected 100 min. before killing the tadpoles. ● — ●, nuclear RNA; Δ — Δ, microsomal RNA; and ○ — ○, soluble RNA]

course assumed that the polysomes are aggregates of messenger RNA with 78S ribosomes[55,56]. The increase in specific radioactivity of the polysomes was evident before that of the 78S monomeric ribosomes but increases in both fractions occurred before the formation of new enzyme or protein could be demonstrated.

Nature of RNA Synthesized under the Influence of Hormones

Much of the work dealing with the influence of hormones on the nature of the new RNA formed has been directed towards establishing the appearance of new messenger RNA in nuclear or cytoplasmic extracts. Several workers have now claimed to have done so by determining: (a) the base composition of sedimentation constants of newly formed nuclear RNA, or (b) the distribution of polyribosomal aggregates, or (c) the response of ribosomes to synthetic messenger RNA.

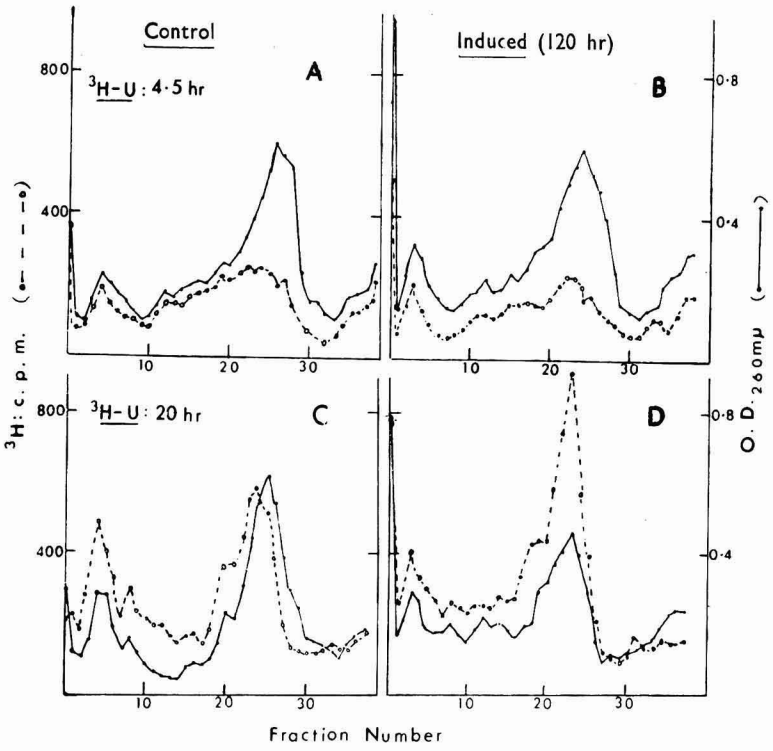

Fig. 4 — Sucrose density gradient centrifugation of liver ribosomes obtained from non-induced and induced *Rana catesbeiana* tadpoles at different times after the administration of 15 μC. of ^{3}H-labelled uridine (^{3}H-U) per animal [Metamorphosis was induced by the injection of 0·5 μg. of the hormone 120 hr before killing the tadpoles. Ribosomes were obtained by treating the mitochondria-free supernatant with 0·4 per cent Na deoxycholate. Ribosomes from 300 to 600 mg. of liver were layered on top of a 15-30 per cent sucrose linear density gradient containing 0·02*M* Tris-HCl buffer (*p*H 7·6), 0·05*M* KCl and 0·0015*M* $MgCl_2$; the samples were centrifuged at 25,000 r.p.m. for 125 min. in a Spinco No. 30 angle-head rotor. A cushion of 3 ml. of 60 per cent buffered sucrose was placed at the bottom of the tube in order to separate the membranes from the membrane-free polysomes (fraction No. 1 contains the membranes dispersed in 1 per cent Na deoxycholate). The direction of sedimentation is from right to left. (A) non-induced, killed 4·5 hr after the administration of tritiated uridine; (B) induced, 4·5 hr after ^{3}H-uridine; (C) control, 20 hr after ^{3}H-uridine; and (D) induced, 20 hr after ^{3}H-uridine. The large peak between fractions 22 and 26 is that of the 78S ribosomes with the dimeric and trimeric forms sedimenting just ahead of them but not quite separated]

Messenger RNA

The specific activity of 'pulse-labelled' RNA extracted from liver nuclei or cytoplasm is much increased by treating rats with growth hormone[27,57], thyroid hormone[37] or cortisone[44,49]. This hormone sensitive component resembles in its sedimentation characteristics the rapidly labelled RNA of rat liver described by Hiatt[58] and Sporn and Dingman[59] and part of it is assumed to be messenger RNA. An accelerated synthesis of messenger RNA-like fraction has also been suggested as an effect of insulin on the isolated rat diaphragm[60]. There is, however, little or no conclusive evidence yet to show that the additional RNA synthesized under hormonal influence has messenger activity in stimulating protein synthesis in a cell-free system. Kenney and Kull[44] in fact failed to find any significant difference in the messenger-like activity between liver nuclear RNA obtained from cortisone treated and control animals, in spite of a very large increase in the specific radioactivity of rapidly labelled RNA of the nucleus in the hormone treated animals. Base composition analysis has also failed to show that growth or thyroid hormone administration causes any specific increase in DNA-like RNA extracted from isolated liver nuclei[37,57]. Wicks and Kenney[61] found that the base composition of the total rapidly labelled RNA of the seminal vesicles of castrated rats was halfway between that of ribosomal RNA and DNA-like RNA; the same base ratios were maintained after testosterone administration which caused a sharp rise in the overall specific radioactivity.

There is, however, other evidence to suggest that growth promoting hormones regulate the level of messenger RNA in the cytoplasm. Ribosomes or microsomes from hormone responsive tissues are known to exhibit a lower or higher amino acid incorporating activity *per unit* RNA according to whether the animals are made deficient in or stimulated with the relevant hormone. Liao and Williams-Ashman[62] found that testosterone lowered the high degree of response shown by prostatic ribosomes from castrated rats to a synthetic messenger (poly AG) in the incorporation of valine into protein. This effect was interpreted as an enhancement by the hormone of the level of endogenous messenger RNA associated with the ribosomes. A similar effect has been demonstrated by Korner[27] with the polyuridylic acid (poly U)-directed stimulation of phenylalanine incorporation into protein, using liver ribosomes from normal and hypophysectomized rats treated with growth hormone. In another study, uterine ribosomes from ovariectomized rats showed a higher amino acid incorporating activity with a lowered response to poly U stimulation, 4 hr after oestrogen treatment *in vivo*[31]. In our laboratory only a very limited effect of thyroid hormones and growth hormone on the response of liver ribosomes or microsomes to poly U could be demonstrated under conditions in which the hormones considerably increased the protein synthesizing capacity of the particles[37]. On the other hand, we have been able to show that the higher amino acid incorporating activity of liver ribosomes from thyroid hormone or growth hormone treated rats is associated with a higher recovery of the particles as polyribosomal aggregates. Korner[27], who has also found an increase in liver polyribosomes after growth hormone treatment, showed that the polyribosomes from hypophysectomized or hormone treated animals had identical protein synthesizing capacity.

Ribosomal RNA and Ribosomes

It is well known that a variety of growth and developmental hormones increase the total RNA content of their respective target tissues, especially under conditions in which the immature tissue is most responsive[6,11,37,63,64]. The bulk of this additional RNA is made up of ribosomal RNA. Recent kinetic studies on the formation of ribosomal RNA have shown that much of the new RNA in cytoplasmic ribosomes appears at the same time or a little before the increase in protein synthetic capacity of the tissue caused by hormone administration. Herein lies the importance of studying ribosomal RNA synthesis during the initial period of hormone action. It raises the possibility that the synthesis of new ribosomal RNA or new ribosomes is as important as the synthesis of messenger RNA for the expression of the necessary developmental change.

Several recent isotopic studies have demonstrated a greatly enhanced synthesis of the 28S and 18S ribosomal RNA components in a relatively short time after the treatment of animals with growth hormone[27,57], insulin[60], testosterone[61] and cortisone[44]. Much of this RNA was found in the nucleus but would not necessarily be all turned over into the cytoplasm[38]. However, in the livers of thyroidectomized rats after triiodothyronine treatment[28,36] or in the uterus of oestrogen treated ovariectomized rats[31,65], it has been possible to show the appearance of ribosomes with newly synthesized RNA. Moore and Hamilton[65] found a very high and sustained rate of formation of new 78S ribosomes by analysing on a sucrose density gradient the cytoplasmic fraction of uteri from ovariectomized rats after the simultaneous administration of oestrogen and ^{3}H-labelled uridine.

In thyroid hormone induced metamorphosis the formation of new ribosomes could be demonstrated during the period when no net accumulation of RNA occurred in the cytoplasm[18]. It will be seen from Fig. 4 that an exposure for 4·5 hr to the labelled precursors led to an almost identical accumulation of radioactivity (mostly in the polysome fractions) in induced and control tadpole liver. With exposures of 18-22 hr, however, much of the 2- to 3-fold increase in radioactivity in preparations from metamorphosing animals was accounted for by a large increase in the specific radioactivity of the 78S ribosomes. It should also be noted that induction of metamorphosis results in a higher fraction of total ribosomal RNA being recovered as polysomes and a higher fraction of ribosomes associated with membranous material resistant to 0·4 per cent deoxycholate. It is quite obvious from these studies that several developmental hormones can serve as valuable tools in studying the regulation of the synthesis of ribosomes.

The Site of Action of Developmental Hormones

The site or sites of action of hormones in the cell are not known. What is known are sequential phenomena associated with basic control mechanisms that anticipate hormone action. As we have seen above, hormones with developmental activity will stimulate, soon after their administration, the synthesis of messenger and ribosomal RNA and the capacity of ribosomes to synthesize proteins in the cells of their target tissues. The question now arises as to whether, when dealing with sites of action, we are dealing with some common, if not identical, features, or whether the effects of different hormones on RNA synthesis and function are mediated via quite different routes. There is no way yet of answering this question directly but recent attempts in our laboratory suggest that the latter possibility is more likely[63,67].

Additive Effects of Hormones on RNA Synthesis

In order to decide whether two hormones act in a competitive or in a synergistic fashion to stimulate RNA synthesis, we have examined tissues that depend on more than one hormone for their growth and development. In the first system studied, the combined action of growth hormone and thyroid hormone on rat liver was investigated. When thyroid hormone and growth hormone are given separately to hypophysectomized rats (which are also functionally 'thyroidectomized') the Mg^{2+}-activated DNA-dependent RNA polymerase in isolated liver nuclei is stimulated by each hormone[66,67]. The dose response of polymerase stimulation with respect to the two hormones is similar to that of the one shown by the growth rate of the liver *in vivo*. As shown in Table 3 an additive stimulation of the RNA polymerase activity was produced soon after a single injection of either triiodothyronine or human growth hormone to a hypophysectomized rat maintained on replacement doses of the other hormone. The dose of the hormone used was such that at higher levels it would have caused no further stimulation of the enzyme activity. The latent period preceding the stimulation of RNA polymerase was very different for the two hormones. As shown in Fig. 1, the enzyme stimulation with triiodothyronine reaches its peak about 36 hr after the injection of the hormone and the latent period is about 10 hr; the corresponding periods for growth are 3 hr and less than 1 hr respectively. The stimulation of RNA polymerase was preceded by an increased formation of the rapidly labelled nuclear RNA in the case of thyroid hormone (Fig. 1); with growth hormone the sequence was reversed. These differences lead us to believe that the two hormones may act at different sites initially and the subsequent chain of events may also be different. It is important to note that the product of RNA polymerase assayed in the absence of a high salt concentration is mainly ribosomal RNA; when the enzyme is assayed in the presence of a high salt concentration the product is more DNA-like but under these conditions the enzyme shows little or no response to the hormones.

TABLE 3 — ADDITIVE EFFECTS OF TRIIODOTHYRONINE (T_3) AND HUMAN GROWTH HORMONE (HGH) ON THE Mg^{2+}-ACTIVATED DNA-DEPENDENT RNA POLYMERASE IN NUCLEI ISOLATED FROM LIVERS OF HYPOPHYSECTOMIZED RATS

(Nuclei were isolated from hypophysectomized rats killed at the appropriate time intervals indicated following a single injection of 20 μg. of T_3 and 50 μg. of HGH, separately or together)

Hormone	Time after hormone administration hr	RNA polymerase μμmoles ^{14}C-ATP incorporated into RNA/mg. DNA
None	—	646
T_3	45	861
HGH	3	955
T_3 and HGH	45 and 3	1245

TABLE 4 — ADDITIVE INCREASES IN SALIVARY GLAND RNA OF THYROIDECTOMIZED-CASTRATED RATS AFTER TREATMENT WITH TRIIODOTHYRONINE AND TESTOSTERONE

[Triiodothyronine (6 μg.) and of testosterone propionate (250 μg.) per 100 g. body weight were injected daily for 5 days prior to killing the rats]

Hormone	Salivary gland mg. RNA/ mg. DNA	Seminal vesicles mg. RNA/ mg. DNA
None	1·55	0·55
Triiodothyronine	2·05	0·42
Testosterone	2·22	2·39
Triiodothyronine + testosterone	3·58	1·98

In the second system we have studied the effect of thyroid hormone and testosterone on the growth and maturation of the salivary glands in rodents has been investigated[10,68]. The data presented in Table 4 show that the two hormones have additive effects on the amount of RNA (the DNA per organ remains constant during the period of the experiment) in the submaxillary glands of young thyroidectomized-castrated rats (Tata, unpublished data). The anabolic effect of testosterone on salivary glands is considered to be different from its androgenic action in promoting growth of the seminal vesicles. The data presented in Table 4 also show that only testosterone elevated the level of RNA in the seminal vesicles while triiodothyronine has no effect, or a slightly antagonistic action, when given simultaneously with testosterone. In young thyroidectomized-castrated rats the androgen also provoked a slight stimulation of RNA polymerase of isolated liver nuclei and this stimulation was superimposed on the maximal stimulation by thyroid hormone.

Some Suggested Sites of Action

The most frequently cited model system to illustrate hormonal control of development (or even differentiation) is the ecdysone induced puffing of polytenic chromosomes of salivary glands in insects[69-71]. Puffing of the chromosomes is considered as direct gene activation by ecdysone and Karlson[72] has generalized gene activation of this

type to explain the action of other hormones. Using inhibitors of RNA and protein synthesis, Clever[71] has shown that in the salivary gland of *Chironomus tentans*, ecdysone initially triggers off puffing in only two loci of the chromosome which in turn bring about, via some cytoplasmic process, the puffing or activation of other genes. The effect of ecdysone on the primary genes is specific and very rapid; quite clearly, whatever be the mechanism, it is a useful model system. However, there are some difficulties in accepting it as the basis for a unified concept of hormone action. It may well be that the interaction between other hormones and genes may be too subtle to detect as a puffing; if this is so, we shall have to look for other manifestations of the interaction of the hormone with its primary site.

The very high degree of specificity shown by developmental hormones can best be explained at the present on the basis of a selective regulation of messenger RNA synthesis. Karlson[72] has suggested that hormones could de-repress genes by combining with the appropriate repressors, for instance, histones or the basic chromatin bound proteins. While it may be reasonable to believe that histones play a fundamental role in regulating RNA synthesis[73], there is as yet no evidence of any hormone being effective as a result of an interaction with histones except for an indirect evidence in the case of action of cortisone on rat liver[74]. Any interaction *in vitro* between hormones and purified histones or other hypothetical repressors should be interpreted with caution in terms of physiological processes since past experience has shown that most developmental hormones will firmly bind to a variety of cell structures, membranes, proteins, etc. Finally, it is difficult to visualize repressor molecules as a primary site of action in those cases in which the same hormone regulates the synthesis of quite different enzymes and proteins in different tissues of the same organism (Table 1).

Caution should also be exercised in interpreting the inhibition of the physiological action of developmental hormones by actinomycin D as indicative of the gene-repressor complex as the site of action. The mechanism of action of inhibitors of RNA and protein synthesis in non-microbial systems has by no means been firmly established. Breuer and Davis[75] have found that cortisone may not act at the same locus as actinomycin D in the synthesis of messenger RNA. An inhibition of ribosome formation should also be considered. Puromycin, besides its inhibitory effect on cytoplasmic ribosomal function, is thought to prevent ribosome maturation in the nucleus[76]. This substance is also a powerful antagonist of many developmental hormones and inhibits the oestrogen induced stimulation of RNA synthesis[21,45,77]. Cycloheximide, which preferentially inhibits the synthesis and methylation of ribosomal RNA[78], is also a potent inhibitor of the action of oestrogen[79].

Most of our attention in looking for the active site for hormone action is now focused on processes immediately connected with RNA synthesis; however, the consideration of other control mechanisms needs no emphasis. There are numerous examples of hormones affecting the structural elements of the cell, such as the endoplasmic reticular membranes. As yet we know very little about how the structural make-up of the cell would affect biosynthetic processes. There is already evidence that the ribosomes attached to membranes are the major sites of protein synthesis[80,81] and it could be conjectured that the type of proteins to be synthesized may be determined by the state or orientation of such structural elements.

All the above suggestions concern intracellular mechanisms and the use of hormones in interactions between cells may prove to be very useful. Interactions between cells have now been recognized as an important feature of the development of multicellular tissues[82]. In this connection, the report that the presence of thyrotropic hormone (TSH) caused thyroid cells in culture to aggregate in a pattern resembling that in the mature thyroid tissue is of significance[83]. Furthermore, the TSH induced orientation of the cells in culture was accompanied by a functional development as well. If such a phenomenon is of general occurrence then it may well be that growth and developmental hormones act at multiple levels of intra- and intercellular control mechanisms.

Concluding Remarks

Regulatory mechanisms in multicellular animal cells appear to conform to the major intracellular patterns found in microorganisms[84]. Some deviations from the principles derived from microbial systems are also becoming apparent, such as the relatively stable messenger RNAs and short-lived enzymes in rat liver[85]. The study of hormone action at the cellular level has revealed both these features in animal cells during growth and maturation. Thus the sequence of biochemical events from the synthesis of nuclear rapidly labelled RNA to the actual manifestation of biological actions following hormonal stimulation of the target cell, described in this article, conforms to the general concept of genetic regulation of protein synthesis in bacteria. At the same time, the synthesis of new ribosomes preceding the stimulation of protein synthesis seems to be a special feature of hormone dependent developmental processes in animal cells. The role of complex intracellular structural elements and the interactions among tissue cells in higher organisms are other areas in which growth and developmental hormones also appear to influence control processes not encountered in microbial systems. It is this multiplicity of levels at which hormones can exert their action and the wide variety of systems that are now available that makes them versatile tools in exploring regulatory mechanisms in higher organisms during development.

Summary

It is becoming increasingly clear that growth promoting and developmental hormones (plant, vertebrate and invertebrate) act through the cellular mechanisms regulating nucleic acid and protein synthesis in their target tissues. More than one hormone may regulate growth and development of a given tissue or the same hormone may produce

different types of changes in different cells. The first feature is illustrated by the effects of growth hormone, thyroid hormones and testosterone on nucleic acid synthesis and growth promotion in mammalian tissues. Although many hormonal effects on the synthesis of nuclear RNA appear to be identical, it nas been shown that they may actually be mediated via different routes for the different hormones. The second feature is illustrated by nucleic acid synthesis and development changes in different tissues during amphibian metamorphosis which is under hormonal control. Emphasis is laid, in discussing the above two features, on how growth and developmental hormones can be valuable tools in investigating regulatory processes in multicellular organisms.

References

1. KNOX, W. E., AUERBACH, V. H. & LIN, E. C. C., *Physiol. Rev.*, **36** (1956), 164.
2. TEPPERMAN, J. & TEPPERMAN, H. M., *Pharmacol. Rev.*, **12** (1960), 301.
3. SUTHERLAND, E. W. & RALL, T. W., *Pharmacol. Rev.*, **12** (1960), 265.
4. EDELMAN, I. S., BOGOROCH, R. & PORTER, G. A., *Proc. nat. Acad. Sci., Wash.*, **50** (1963), 1169.
5. WOOL, I. G., cited in *Actions of hormones on molecular processes* edited by G. Litwack & D. Kritchevsky (John Wiley & Sons Inc., New York), 1964, 422.
6. TATA, J. R., cited in *Actions of hormones on molecular processes* edited by G. Litwack & D. Kritchevsky (John Wiley & Sons Inc., New York), 1964, 58.
7. TATA, J. R., cited in *Advances in metabolic disorders*, Vol. 1, cited by R. Levine & R. Luft (Academic Press Inc., New York), 1964, 153.
8. TATA, J. R., cited in *Mechanisms of hormone action* edited by P. Karlson (Georg Thieme Verlag, Stuttgart), 1965, 173.
9. SIMPSON, M. E., ASLING, C. W. & EVANS, H. M., *Yale J. Biol. Med.*, **23** (1950), 1.
10. SHAFER, W. G. & MUHLER, J. C., *Ann. N.Y. Acad. Sci.*, **85** (1960), 215.
11. GILBERT, L. I., cited in *The hormones*, Vol. 4, edited by G. Pincus, K. V. Thimann & E. B. Astwood (Academic Press Inc., New York), 1964, 67.
12. SCHNEIDERMAN, H. A. & GILBERT, L. I., *Science*, **143** (1964), 325.
13. BROWN (Jr), G. W. & COHEN, P. P., cited in *A symposium on the chemical basis of development* edited by W. D. McElroy & B. Glass (Johns Hopkins Press, Baltimore), 1958, 495.
14. BENNET, T. P. & FRIEDEN, C., cited in *Comparative biochemistry*, Vol. 4, edited by M. Florkin & H. S. Mason (Academic Press Inc., New York), 1964, 484.
15. WEBER, R., cited in *Ciba foundation symposium on lysosomes* edited by A. V. S. de Rueck & M. P. Cameron (J. & A. Churchill Ltd, London), 1963, 282.
16. WILSON, J. D., cited in *Protein metabolism* edited by F. Gross (Springer Verlag, Berlin), 1962, 26.
17. TATA, J. R., *Develop. Biol.*, **13** (1966), 77.
18. TATA, J. R., *Nature, Lond.*, **207** (1965), 378.
19. HAMILTON, T. H., *Proc. nat. Acad. Sci., Wash.*, **51** (1964), 83.
20. HAMILTON, T. H., WIDNELL, C. C. & TATA, J. R., *Biochim. biophys. Acta*, **108** (1965), 168.
21. NOTEBOOM, W. D. & GORSKI, J., *Proc. nat. Acad. Sci., Wash.*, **50** (1963), 250.
22. WAGLE, S. R., *Arch. Biochem. Biophys.*, **102** (1963), 373.
23. MICHELS, R., CASON, J. & SOKOLOFF, L., *Science*, **140** (1965), 1417.
24. FARESE, R. V. & REDDY, W. J., *Biochim. biophys. Acta*, **76** (1963), 145.
25. FEIGELSON, P. & FEIGELSON, M., cited in *Actions of hormones on molecular processes* edited by G. Litwack & D. Kritchevsky (John Wiley & Sons Inc., New York), 1964, 218.
26. KORNER, A., cited in *Protein metabolism* edited by F. Gross (Springer Verlag, Berlin), 1962, 8.
27. KORNER, A., *Biochem. J.*, **92** (1964), 449.
28. TATA, J. R., ERNSTER, L., LINDBERG, O., ARRHENIUS, E., PEDERSEN, S. & HEDMAN, R., *Biochem. J.*, **86** (1963), 408.
29. WILLIAMS-ASHMAN, H. G., LIAO, S., HANCOCK, R. L., JURKOWITZ, L. & SILVERMAN, D. A., *Rec. Progr. Hormone Res.*, **20** (1964), 247.
30. KOCHAKIAN, C. D., *Acta Endocrinol.*, **46**, Suppl. No. 92, (1964).
31. GREENMAN, D. L. & KENNEY, F. T., *Arch. Biochem. Biophys.*, **107** (1964), 1.
32. FARESE, R. V., *Endocrinology*, **74** (1964), 579.
33. ROODYN, D. B., FREEMAN, K. B. & TATA, J. R., *Biochem. J.*, **94** (1965), 483.
34. GUSTAFSSON, R., TATA, J. R., LINDBERG, O. & ERNSTER, L., *J. Cell Biol.*, **26** (1965), 555.
35. WIDNELL, C. C. & TATA, J. R., *Biochem. J.*, **92** (1964), 313.
36. TATA, J. R., *Biochim. biophys. Acta*, **87** (1964), 528.
37. TATA, J. R. & WIDNELL, C. C., *Biochem. J.*, **98** (1966), 604.
38. HARRIS, H., FISHER, H. W., RODGERS, A., SPENCER, T. & WATTS, J. W., *Proc. roy. Soc.*, **157B** (1963), 177.
39. TATA, J. R., *Nature, Lond.*, **197** (1963), 1167.
40. GORSKI, J., *J. biol. Chem.*, **239** (1964), 889.
41. WEILL, J. D., BUSH, S., CHAMBON, P. & MANDEL, P., *Biochem. biophys. Res. Commun.*, **10** (1963), 122.
42. TALWAR, G. P., PANDA, N. C., SARIN, G. S. & TOLANI, A. J., *Biochem. J.*, **82** (1962), 173.
43. GORSKI, J. & NICOLETTE, J. A., *Arch. Biochem. Biophys.*, **103** (1963), 418.
44. KENNEY, F. T. & KULL, F. J., *Proc. nat. Acad. Sci., Wash.*, **50** (1963), 493.
45. MUELLER, G. C., GORSKI, J. & AIZAWA, J., *Proc. nat. Acad. Sci., Wash.*, **47** (1961), 164.
46. HAMILTON, T. H., *Proc. nat. Acad. Sci., Wash.*, **49** (1963), 373.
47. CANTAROW, A. & ZAGERMAN, A. J., *Exp. Biol. Med.*, **115** (1964), 1052.
48. FERGUSON (Jr), J. J., *J. biol. Chem.*, **238** (1963), 2754.
49. JERVELL, KR. F. & OSNES, J. B., *Life Sci.*, **12** (1963), 975.
50. TALWAR, G. P. & SEHGAL, S. J., *Proc. nat. Acad. Sci., Wash.*, **50** (1963), 226.
51. GREENGARD, D., SMITH, M. A. & ACS, G., *J. biol. Chem.*, 238 (1963), 1548.
52. SEKERIS, C. E. & KARLSON, P., *Arch. Biochem. Biophys.*, **105** (1964), 483.
53. PAIK, W. K. & COHEN, P. P., *J. gen. Physiol.*, **43** (1960), 683.
54. FINAMORE, F. J. & FRIEDEN, E., *J. biol. Chem.*, **235** (1960), 1751.
55. NOLL, H., STAEHELIN, T. & WETTSTEIN, O., *Nature, Lond.*, **198** (1963), 632.
56. WARNER, J. R., KNOPF, P. M. & RICH, A., *Proc. nat. Acad. Sci., Wash.*, **49** (1963), 122.
57. TALWAR, G. P., GUPTA, S. G. & GROS, F., *Biochem. J.*, **91** (1964), 565.
58. HIATT, H. H., *J. mol. Biol.*, **5** (1962), 217.
59. SPORN, M. B. & DINGMAN, W., *Biochim. biophys. Acta*, **68** (1963), 389.
60. WOOL, I. G. & MUNRO, A. J., *Proc. nat. Acad. Sci., Wash.*, **50** (1963), 918.
61. WICKS, W. D. & KENNEY, F. T., *Science*, **144** (1964), 1346.
62. LIAO, S. & WILLIAMS-ASHMAN, H. G., *Proc. nat. Acad. Sci., Wash.*, **48** (1962), 1956.
63. WILLIAMS-ASHMAN, H. G., cited in *Cellular control mechanisms and cancer* edited by P. Emmelot & O. Muhlbock (Elsevier Publishing Co., Amsterdam), 1964, 103.
64. SZIRMAI, J. A., cited in *Protein metabolism* edited by F. Gross (Springer Verlag, Berlin), 1962, 45.
65. MOORE, R. J. & HAMILTON, T. H., *Proc. nat. Acad. Sci., Wash.*, **52** (1964), 439.
66. WIDNELL, C. C. & TATA, J. R., *Biochim. biophys. Acta*, **72** (1963), 506.
67. WIDNELL, C. C. & TATA, J. R., *Biochem. J.*, **98** (1966), 621.

68. RAYNAUD, J., cited in *Salivary glands and their secretions* edited by L. Sreebny & J. Mayer (Pergamon Press, London), 1964, 47.
69. BEERMAN, W., *J. Zool.*, **157** (1964), 49.
70. CLEVER, U. & KARLSON, P., *Exp. Cell Res.*, **20** (1960), 623.
71. CLEVER, U., *Science*, **146** (1964), 764.
72. KARLSON, P., *Perspectives in biology & medicine*, **6** (1963), 203.
73. ALFREY, V. G., LITTAU, V. G. & MIRSKY, A. E., *Proc. nat. Acad. Sci., Wash.*, **49** (1963), 414.
74. DAHMUS, M. E. & BONNER, J., *Proc. nat. Acad. Sci., Wash.*, **54** (1965), 1370.
75. BREUER, C. B. & DAVIS, F. F., *Biochem. biophys. Res. Commun.*, **14** (1964), 215.
76. TAMAOKI, T. & MUELLER, G. C., *Biochem. biophys. Res. Commun.*, **11** (1963), 404.
77. UI, H. & MUELLER, G. C., *Proc. nat. Acad. Sci., Wash.*, **50** (1963), 256.
78. FIALA, E. S. & DAVIS, F. F., *Biochem. biophys. Res. Commun.*, **18** (1965), 115.
79. GORSKI, J. & AXMAN, M., *Arch. Biochem. Biophys.*, **105** (1964), 517.
80. HALLBERG, P. A. & HAUGER, J. G., *Biochim. biophys. Acta*, **95** (1965), 80.
81. HENSHAW, E. C., BOJARSKI, T. B. & HIATT, H. H., *J. mol. Biol.*, **7** (1963), 122.
82. MOSCONA, A. A., *J. Cell. Comp. Physiol.*, **60**, Suppl. No. 1, (1964).
83. KERKOFF, P. R., LONG, P. J. & CHAIKOFF, I. L., *Endocrinology*, **74** (1964), 170.
84. UMBARGER, H. E., *Science*, **145** (1964), 674.
85. PITOT, H. C. & PERAINO, C., *J. biol. Chem.*, **239** (1964), 1783.

The Hormones of the Neurohypophysis*

WHEN V. du Vigneaud in 1954 elucidated the structure of oxytocin and vasopressin, he established the occurrence of two variants of the latter hormone by showing that ox pituitary lobes contain 8-arginine vasopressin and pig posterior lobes contain 8-lysine vasopressin. This finding raised the question of the amino acid composition of these hormones in other mammals. However, such work — which by necessity had to include investigations on wild-living and often rare animals — necessitated the elaboration of a method which permitted the characterization of the active peptide from very few or even a single gland. A combination of chromatography and multiple bioassays proved suitable. This method has shown that 8-lysine vasopressin is widely distributed in the pig-like animals, but that the other groups of even-toed ungulates — like indeed all other Eutheria and Marsupialia so far investigated — elaborate 8-arginine vasopressin. However, some wild species of pigs were found to carry both vasopressins in the same gland, suggesting that they were heterozygotes of strains which produce each of the variant hormones.

Further work made it likely that the vasopressins are hormones peculiar to the mammals. Neurohypophysial extracts of non-mammalian vertebrates were found to resemble those of mammals in usually containing two hormones — one with a basic and one with a neutral amino acid in the tripeptide side chain — but the 'basic' octapeptide in the lower vertebrates is usually vasotocin, i.e. a compound with the pentapeptide ring of oxytocin and the side chain of vasopressin. Judging from the fact that vasotocin appears to be the only neurohypophysial hormone of the most primitive surviving vertebrates — the jawless lampreys and hagfishes — it may be also the oldest in evolution. There is some suggestion from the analysis of neurohypophysial extracts of the least advanced Gnathostomata like the lungfishes and the primitive shark *Hexanchus* that the earliest jawed vertebrates developed the 'neutral' peptide oxytocin — perhaps by a single-step mutation from vasotocin. A further step — replacement of leucine in position 8 in oxytocin by isoleucine — may have led to a further hormone which seems to be phyletically almost as widely distributed as oxytocin itself. This hormone — 8-ile oxytocin or mesotocin — seems to occur in the primitive bony fish *Polypterus*, in the lungfishes, in amphibians and probably in reptiles. Mesotocin again appears to have given rise to a further development in the more advanced bony fishes whose main hormones are vasotocin and ichthyotocin (or isotocin), i.e. 4-ser, 8-ile oxytocin. In contrast, it would seem that neurohypophysial hormones of the cartilaginous fishes developed in a highly original manner; while one of their main groups, the Chimaeroidea, probably adhered to the ancestral model by producing vasotocin, oxytocin and mesotocin, pituitary extracts of some higher sharks and rays have been found to contain 4-ser, 8-glu oxytocin (glumitocin) which postulates intermediate derivatives of vasotocin like 4-ser oxytocin or 8-glu oxytocin.

The number of neurohypophysial hormones — all octapeptides with a disulphide bridge — identified during recent years has been steadily increasing, but considering the relatively small number of species investigated, the discovery of further active peptides can confidently be expected.

*Summary of a paper presented by Dr H. Heller, Department of Pharmacology, University of Bristol, at the Symposium on Regulatory Mechanisms organized by the Society of Biological Chemists, India, New Delhi, 28-30 December 1965.

Comfort Properties of Leather

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PRIOR to the Second World War, leather was judged mainly on the basis of its physical characteristics, workability in the factory, durability and aesthetic appeal. But of late the comfort properties of leather have gained considerable prominence, primarily due to the increasing competition from synthetic substitutes for leather. Almost a dozen synthetic materials[1] are now in commercial use as substitutes for leather in a variety of uses. Roughly 20-30 per cent of the leather market has been taken over by synthetics. This situation calls for greater emphasis on maintaining and improving the desirable qualities of leather.

The degree of comfort afforded is the primary consideration in the choice of footwear, handwear, garments, etc. Comfort in footwear is determined by their initial fit and retention during wear, response to the forces involved in walking, the resistance of the outer covering to weather, and the reaction of the materials and other such factors which come up during wear, including colour fastness, resistance to scuffing, response to repeated flexing and ease of polishing[2].

The comfort properties of leathers vary with different products like footwear, handwear and garments, depending upon the environmental conditions and physiological factors. Also the comfort properties of the finished products depend on the physical properties of leathers that go into their production, such as thermal conductivity; dimensional changes; water vapour permeability and absorption; amount and rate of water absorption; resistance to perspiration, stiffening, fatigue, heat, oil, scuff and nail penetration; possibility of rendering them stronger and more flexible with moisture; and ability to withstand lateral compression; reservoir capacity; strength, stretching, frictional, electrical and thermal lasting properties; plasticity; mechanical or foot insulation; colour fastness; insole shrinkage; and lightness[3-7]. Air porosity, colour, thermal expansion and heat of wetting of leather do not significantly influence the comfort of leather products.

Though considerable amount of information is available on the physical properties of different kinds of leather, our knowledge on the comfort properties of leather is still incomplete, since very little work has been done on the quantitative evaluation of the comfort properties of leather used for different purposes under different environmental conditions. The present article reviews the information on comfort properties of leather that has accumulated during the last 40 years. Areas where further work is necessary for a better understanding of the comfort properties of leather are also indicated.

The study of comfort properties of leather falls under three distinct heads: (i) fundamental or physical characteristics affecting comfort properties of leather; (ii) quantitative evaluation of comfort properties; and (iii) modification of leather and leatherwear to get a suitable product with specific characteristics.

Fundamental Properties of Leather Affecting Comfort Properties of Finished Products

Thermal Insulating Property

Besides imparting desirable mechanical properties, the fibrous network of leather makes it a good thermal insulator[7,8] and thereby affords protection. The foot, hand and body have two means of regulating their temperature and maintaining them at a comfortable level : blood circulation and perspiration. Footwear, handwear and garments supplement these ' built-in ' comfort devices and also protect the feet, hands and body from external hazards.

The thermal conductivity of leather is directly related to the amount of air trapped in it; in other words, to its apparent or bulk density and thickness[8]. For example, chamois leather has the lowest density of all leathers and hence is a good insulator. There are also other factors which affect thermal conductivity of leather. For instance, the presence of air in leather lowers its thermal conductivity, while water and grease increase the thermal conductivity. The influence of the nature of a surface like soft and fibrous flesh side of an upper leather on the thermal conductivity needs investigation. When walking, the foot is not only protected by the sole but also by the insole and the filling material between the sole and insole. It is, therefore, necessary to determine the thermal conductivity of these materials in combination. McLachlan *et al.*[7] have determined the thermal conductivity of these components singly and in combination in a shoe. The thermal conductivity of a cork-gum filler is relatively low and the conductivity of the combination sole-plus-filler insole is lower than the usual value for a sole, which is between 4·0 and 4·5. The value for the thermal conductivity of crepe rubber (3·47-3·76) reported by McLachlan *et al.*[7] is very near that of leather. On the other hand, the thermal conductivity of composition rubber soling is much higher. Regarded simply as a thermal insulator between the foot and the ground, crepe rubber soling is a more serious competitor for leather than rubber composition[8]. Apparatus developed by Baxter[9,10] and by Stafford and Hatfield[11] can be used for measuring the thermal conductivity of leather and fibres respectively. With these the thermal conductivity of the raw and variously tanned collagen fibres can be found out. These measurements help in assessing the thermal insulating capacity of different leathers.

Thermostatic Properties

When damp leather loses moisture and attains an equilibrium with air of low relative humidity, it becomes colder. When one steps out in hot weather

this loss of heat from the shoes provides a feeling of comfort by preventing undue increase in foot temperature. Conversely, when dry leather absorbs water vapour, it becomes warm; when the water vapour condenses on the leather substance, it gives up the kinetic energy as heat. Thus the additional heat generated is responsible for the comfort of the wearer when he suddenly enters a cold damp room. Thus the leather sole makes the shoe much more responsive to conditions of wear and doubles the uptake of moisture as well as thermostatic powers of the shoe. Only a limited work[12,13] has been reported on the thermostatic properties of leather and little information is available concerning the changes in the moisture content of shoes during wear. Further work on these lines is necessary to throw more light on this subject.

Fibre Surface

Due to the presence of non-conducting air, leather clothing and uppers protect body against changes in temperature. Fibres entrap air because of their greater surface area in relation to their bulk. It is, therefore, of interest to compare the total surface area of different leathers and find out how it is influenced by the nature of skin, the degree of splitting of the fibres into fibrils and the nature of the tannage.

The surface area of hides and skins can be determined from the air permeabilities according to Mitton[14]. The values in the case of wool and silk can be calculated from the diameters of the fibres[15]. According to Zettlemoyer *et al.*[16] the values for the raw and the variously tanned hides can be determined from the nitrogen absorption value.

Mechanical Protection

The shoe must be sufficiently hard to give protection against mechanical damages, for instance, from stones, pebbles, nails, hard and irregular grounds. Insulation from ground irregularities is obtained in two ways[17] : (i) by the shoe bottom bridging the irregularities where high stiffness is desirable and (ii) by the sinking into the bottom materials where softness is desirable. A bottom, with firm insole, sponge bottom filling and microcellular has very good insulation values, besides being flexible. Persons wearing shoes with rubber soles often complain that they feel the roughness of the ground they walk on and every small stone much more than with shoes with leather soles. This is due to the lower compression resistance of rubber sole. Leather by virtue of its three-dimensional fibre structure spreads locally limited pressure of a small stone over a wider area and in this way renders it ineffective. This property of leather can be studied by the performance test. The test which was followed by SATRA[18] can be used for determining the resistance of leather to nail penetration.

Water Vapour Permeability

Water vapour permeability and water vapour absorption characteristics of leather are more important in the context of comfort properties of leather than air permeability. The enormous surface area of the fibres in leather enables it to take up water vapour rapidly and to convey it to the outside air. When perspiration does not evaporate, the foot becomes uncomfortable and shoes with uppers of synthetic material usually cause discomfort due to internally generated moisture.

Water vapour permeability of the sole leather is relatively low compared to that of upper leathers. Shoes soled with leather seldom pour out sweat. The amount of moisture absorbed by the shoes without becoming wet is considerable. The absorption is very rapid due to the large surface area of the fibres and quicker penetration of water vapour inside the fibres. The amount of water vapour absorbed by leather depends on the relative humidity of the air. Chrome leather has greater water absorption capacity compared to vegetable tanned leather. Mitton's method[19] is usually employed for determining water vapour permeability of leather.

Water vapour permeability depends partially on the ability of leather to draw moist air into the more dry, centre layers and to deliver the moisture from there to the outer surface, from whence it is lost to the air[20]. Water vapour mainly moves through the capillaries of the leather and diffusion through the solid fibres is insignificant except perhaps at very high humidities[21].

Water vapour permeability of leather, which is inherently very high, depends upon a number of factors[5,22] like thickness of the sample, grease content, relative humidity and temperature of the atmosphere. The use of fats and greases to improve water resistance of leather may lower water vapour transmission. The grain layer of leather appears to be the first stratum to become saturated with fat and consequently a highly sensitive stratum with respect to water vapour permeability. The finish that clogs or keeps open the pores on the grain of leather influences the water vapour transmission either adversely or favourably. Flexing of the specimen has no influence on the water vapour permeability of degreased leathers; however, in the case of leathers containing grease, there is an increase in water vapour permeability on flexing. It has been reported[5,20,22] that in addition to gaseous diffusion, water is transmitted through leather by conduction over the surface or by some form of activated diffusion. Studies on the influence of special treatments and materials on this property are desirable for the development of more comfortable and serviceable leathers.

Water Resistance, Penetration and Rate of Absorption

Leather for functional footwear, handwear and clothing is expected to keep the feet, hands and the body dry by resisting the moisture from the outside, and by absorbing moisture from perspiration from the inside and allowing the same to evaporate. Shoes that cannot brush off external water obviously rot and deteriorate quicker than the water resistant ones. If sole and upper leathers absorb water, the shape of the shoe is spoilt while walking. Fungus growth occurs and the shoes give unpleasant appearance. But the waterproofed insole and upper leathers do not absorb perspiration from the

inside and allow to evaporate. Hence, they should be water resistant.

Many static and dynamic methods have been developed for measuring water resistance of leather[23-43]. Static water absorption techniques have been used to determine water resistance characteristics of almost all types of leather. These tests involve immersion of the leather in any suitable container of water for a given time, noting the weight increase in a closed system to prevent loss of water by evaporation. The Kubelka device which measures water absorption by measuring the volume of water absorbed by the leather could be used for determining the water resistance of leather under static conditions. The static water penetration apparatus designed by Muthiah[42a] can be used for measuring the water resistance of leather. Although static water absorption simulates the end use conditions of some types of leather (particularly mechanical leather), it is seldom that leather is not subjected to some type of dynamic stress. Testing of leather should be done with the end use of the material in mind. It is for this reason that the leather must be subjected to dynamic tests. Dynamic tests flex the leather under water. In this test, leather is flexed in a particular fashion intended to simulate the flexing of the vamp of the shoe in actual wear. The method of holding the sample and the degree of severity of the flex vary, however, from tester to tester. Initial water penetration is determined either electrically or visually. After a known period of time, the increase in weight of the leather gives the water absorption under dynamic conditions. Refined forms of the dynamic tests include the 'walking machine' where the shoes are flexed in water under controlled conditions. Of the several test methods developed for determining the dynamic water absorption, the one devised by Maeser[30] is considered to be most satisfactory. The Maeser machine is reported to give results similar to those obtained with the 'walking machine'. Roddy and Gapuz[32] have also demonstrated that a positive correlation exists between the values obtained with Maeser and walking machines and those obtained under field conditions. Therefore, the Maeser machine can be used for evaluating the shoe upper and glove leather. For sole leathers, the Bally penetrometer can be used. The dynamic water resistance of garment leather can be determined by a simple tumbling test.

From the wearer's point of view, waterproofness of leather is a function of the time required for water to pass through the leather under wet wearing conditions and to wet his feet. Thus waterproofing is related to the rate of water absorption and the absorbing capacity of leather. Therefore, the rate of water absorption must be determined for different types of leathers. The above-mentioned equipment can be used for this purpose also.

In addition to the above-mentioned laboratory methods, water absorption by leather can be measured by wear trials in which socks and shoes worn by test subjects are weighed before and after walking, marching or light or heavy exercise. Values obtained have to be corrected for changes in the relative humidity of the atmosphere during the experiments.

Rate of Drying

The ability of leathers to dry rapidly is a desirable quality. The rate of drying can be determined as follows. The treated and untreated specimens are dipped in a beaker of water and removed when they had absorbed about the same amount of water. The samples are weighed, hung and allowed to dry naturally at room temperature. Periodic weighings are made and then the percentage water evaporated can be plotted against drying time. Alum-treated vegetable leather has been found to lose about 50 per cent of its moisture in 30 min., while the untreated sample loses only 20 per cent. As the shoes are likely to become wet especially in winter season or in the cold countries, the rate of drying of leather assumes great importance.

Effect of Wetting and Drying

In field use, leather in contact with water and mud will sometimes crack and stiffen. Water containing large amounts of salts and other materials has an adverse effect on leather shoes. During the process of wetting and drying, poor leathers lose more water solubles, become flabby, and wear out quickly. A study of the physical properties of leather before and after wetting is, therefore, necessary to improve the comfort properties of leather.

Resistance to Perspiration

Considerable amount of work has been done on the deterioration of leather by perspiration or the degradation of leather articles by prolonged contact with the human body[44-56]. However, perspiration continues to be one of the most prominent factors in the premature failure of footwear. In considering the action of perspiration on leather, the effect of sweat solutes has to be studied separately from the effect of water. Water produced by sweating acts no differently than water from other sources with which leathers come in contact, resulting successively in wetting and drying of the leather. The action of water solubles in sweat, on the other hand, is cumulative, i.e. the concentration of the components is built up in the leather. Perspiration-damaged insoles have a blackened, cracked and distorted appearance while the uppers get a salt spued, stiffened, stained and cracked appearance. In addition, the shoes usually carry the pungent sour smell associated with excessive perspiration. Several testing devices and methods[45,48,53,54,57-61] have been reported for predicting the durability of insole leather. The method which has been approved by the Commissioner, Federal Supply Service[62], can be used for this purpose. This method is used for determining the effect of perspiration on leather. The leather is subjected to treatment with artificial perspiration and the cracking measured after bending the material through 180 degrees.

Perspiration resistant leather footwear should have an insole of low initial water-soluble content and high water absorption capacity, and the ability to dry out rapidly between periods of wear. The uppers should be highly vapour permeable but water repellent. Both insole and upper leathers should be as stable as possible in the presence of active sweat ingredients.

Insole Shrinkage

Insoles of men's shoes often shrink in wear by as much as 0 5 in. or 5 per cent in length, and this is a contributory factor in impairing both the comfort and shape of shoes. Insole shrinkage[63] is generally regarded as a perspiration effect, but it is by no means certain that it is wholly or even mainly due to the chemical action of sweat. Thus when insole leathers are wetted in water and then redried, there is an appreciable shrinkage in area, accompanied by the leaching out of water solubles. Therefore, it is necessary to study the effect of repeated wetting and drying on insole leather. A method which has been developed at SATRA[63] can be used for quantitative evaluation of insole shrinkage.

Dimensional Stability

This is one of the important factors which imparts comfort in footwear. Extremes of humidity may cause chrome leathers to gain or lose as much as 10 per cent in area. Vegetable leather rarely expands more than 3 per cent[64]. Nevertheless, dimensional changes in chrome upper leather have a bearing on comfort. In the course of a day, while absorbing up to 50 per cent of its weight in perspiration, chrome leather expands sufficiently to compensate for the 4-7 per cent increase in foot volume. Theoretically, increased humidity may add one whole size to shoes across the vamp[65]. In the sole area, this is not serious because of the greater area stability of vegetable tanned leather.

High temperatures also cause leather to expand but to a much smaller degree[5]. The amount of expansion due to high temperature is too small to affect comfort appreciably. If the dimensional changes are too pronounced, the shoe begins to lose its shape. The loss of shape is more in synthetics as compared to leather. As this property influences the comfort properties of the shoe, a study of the shape retention property of different leathers and synthetics is important.

Resistance to Stiffening

Mellowness and temper are important qualities of leather as far as comfort of footwear is concerned. Some of the most effective impregnating materials, such as polysulphide and polyurethanes, have a distinct stiffening effect. In a wear test[66], boots with polyurethane-treated uppers were found to be too stiff to be comfortable. The stiffening of leather at low temperature is due to hardening of the oils and greases used for its lubrication. These oils and greases comprise 10-33·3 per cent of the weight of the chrome leather used. The stiffness factor can be determined by the Tinius-Olsen stiffness tester and Method 4211 of Federal Specification KKL-311[67].

A stiffness tester for shoe and insole back parts has been devised by SATRA[68]. High stiffness being an important requirement, especially in women's shoes with high heels, this apparatus is able to provide valuable information not only about different insole materials but also about the way a shoe has been put together since many factors combine to determine the stiffness of the finished shoe.

Flexibility

Flexibility of upper leather is very important for shoe comfort[69]. It is a measure of the ability of leather to bend to the shape of the foot without forming stiff creases which might cause blisters on the foot. Although softness and flexibility are desirable, the leather should not be too soft. If the leather is too soft and flexible, the upper will sag down around the ankle after a short period of wear; this is both unsightly and uncomfortable.

Three test methods based on tensile, flexure and torsion strength determination are employed for quantitative measurement of the degree of flexibility (stiffness) of leather, rubber and plastics[69]. Flexibility of uppers and soles is measured using the Tinius-Olsen stiffness tester. Stiffness values of light leathers at room temperature range from those of rubber and plasticized polyvinyl chloride (300 lb./sq. in.) to those of polyethylenes (10,000 lb./sq. in.). Sole leathers at room temperature range in stiffness from 8000 to 40,000 lb./sq. in. Synthetic sole compositions have much lower stiffness values except neolite (10,000-18,000 lb./sq. in.). The effect of temperature variations on the stiffness of leather and synthetics has not been investigated.

The flexibility of the shoes can be determined using the SATRA[70] shoe flexometer which can also be used to find out the effect on flexibility of modifications in construction or materials.

Effect of Repeated Flexing on Uppers and Soles

On repeated flexing, upper leather forms wrinkles, resulting in an unpleasant appearance and the strength of leather is also reduced. On flexing, the grain and finishes of the upper leather crack, and water vapour and air permeabilities increase. The effect of flexing on the various properties of leather could be determined by Bally flexometer[71]. The used shoes are more comfortable than the new ones due to their 'breaking-in'[71a]. 'Breaking-in' increases the softness of the shoe materials as a result of repeated flexing and stress during walking. Therefore, studies on the influence of breaking-in property of the various materials on the comfort properties of footwear are linked with performance tests. Repeated flexing on leather and its effects have an important bearing on the comfort properties of leather.

Cushioning Comfort

The cushioning property of leathers and synthetics enhances the comfort property of footwear. It can be determined using a compression and resiliency tester[72] in which the change in thickness of the specimen, on applying a specific load per unit area on the leather specimen, is measured by means of a thickness gauge.

Distribution of Body Weight

Footwear comfort requires not only lightness but also balance and sufficient elasticity to permit conformity to the shape of the foot and its normal pressure points. Foot pressure generally decreases after footwear has been broken in[73]. Pressures are more evenly distributed in a shod foot than

in an unshod one; it is this shift to an even distribution that is important in footwear comfort. A Japanese study[74] recorded pressures as high as 30 lb./sq. in. using 20-35 mm. high heels. Obviously the higher the heel, the higher the metatarsal and great toe pressures, and pressure increases with loads to be carried. Any dynamic study of the interplay between foot and insole should take into account this alternate pressure and relaxation. That the shape of most shoes should not change too much under repeated light pressures is also important.

Apparent Density

Shoe leather density has been reported to be directly related to the amount of strain felt by a man walking in the heat[74a]. A reduction in the weight of shoes facilitates better walking, for even small differences in weight are of significance. For example, the addition of 1 lb. to the weight of a man's shoes produces the same increase in his metabolic rate as the addition of 4 lb. to his back[74a]. Hence, in order to improve the comfort factors, leathers used for various purposes should be as light as possible. Studies conducted at the Central Leather Research Institute[75] have indicated that the weight of sole leather can be reduced by 60 per cent without any adverse effect on the other properties. Similar studies on other leathers are very essential.

Frictional Properties

In order to avoid slipperiness, the frictional properties of sole should be high. The slipperiness of footwear depends on the friction between the surfaces of the outer sole and the ground. To avoid slipperiness, while working on wet and oily metal surfaces, the friction should be fairly high. It has been observed that a better grip is also possible in case of leather than in synthetics. The friction between the foot and the surrounding lining leather or cloth should be low, for greater friction forms blisters on the foot. According to Mitton and Morgan[76], frictional properties of sole leather on metal surfaces are generally superior to those of synthetic soling materials. On oily wood, concrete and linoleum leather soles are likely to be as slippery as rubber soles. The determination of optimum frictional properties of sole and lining leathers used for various purposes calls for further studies in this direction.

Durability

Foot comfort and health are dependent to a considerable extent on durability of the material. Shoe leather shields the foot without interfering in foot's normal function and protecting foot against mechanical damages. The degree of protection with comfort is related with factors like tensile strength, bursting strength, stretch and resilience. The uniformity of shoe size and shape largely depends upon the tight stretchability of leather over the last. Several methods have been devised to evaluate the strength and stretch of leather under various conditions. Strength can be measured under tension applied either in one or many directions simultaneously as in breaking and burst tests respectively. In the burst test, force is applied perpendicular to the flesh surface of a specimen. A motor-driven tensile strength machine[77] can be used for finding out the tensile strength and Mullen tester[77] for bursting strength.

Elasticity

A shoe provides comfort only if it conforms to foot shape which, in turn, depends on the elastic property of leather. Every time a shoe is bent or flexed, it should immediately recover its shape. The 'give' around the child's growing feet is an important comfort factor in children's footwear. Glove leather should conform to the shape of hand without bagginess which gives discomfort. Welts should be pliable and be in conformity with the outline of shoe bottom for accelerating the footwear comfort. The elastic properties of leather can be determined using a compression and resiliency tester. Even though elastic properties of various leathers were reported by Mitton[78], further work on this line is necessary.

Plasticity

This property imparts leather stretchiness and compressibility to retain the shape of upper without allowing its stretching indefinitely during wear. Plasticity in leathers depends on the tensile, flexural and compressional forces which tend to deform it. Flexural plasticity is the main force that acts on shoe leathers in wear. The plastic components of various leathers can be estimated by bending the specimen in the stiffness tester. Linear plasticity was determined by Wilson[79] and recently by Popplewell and Ward[80]. Butlin[81] has studied in detail the elongation of leather at different stages for various time intervals using Dome plasticity apparatus.

Health

The materials used for footwear and leather goods should not cause any skin or foot disease. If the soling materials used are impermeable to air, water vapour, etc., maceration between the toes are extremely common[82]. When mildly macerated, skin is moist, soft and blanched; when the condition is more severe, the skin breaks open and peels away, sometimes leaving a raw and tender area. This may cause discomfort or infection leading to more serious trouble.

Relative Comfort Properties of Leather and Synthetics

Rubber[83] and Corfam[81-87] are the widely used substitute materials. While rubber is more flexible, water resistant and resistant to abrasion, it lacks the breathing property of leather. Rubber soles are very slippery and give an uncomfortable feeling of coldness in winter and of warmth in summer due to their high thermal conductivity. Rubber yields very easily to external stresses and, therefore, the contact with any hard object of the shoe is easily transmitted to the foot of the wearer, whereas leather acts as a mechanical cushion due to its natural three-dimensional structure. Strength values of rubber

soles are considerably lower than those of leather soles.

The most advantageous property of Corfam is its lightness (density 0·53), high water and perspiration resistance, scuff resistance and high resistance to flexing. But water vapour permeability and weathering air permeability are not as high as in leather. Shoes made out of Corfam tend to lose their shape with time and become stiff upon repeated wetting and drying when flexed. In general, most of the upper substitute materials possess poor (three times less) strength, 'breaking-in' and plasticity characteristics compared to leather. It has been reported that the skin ailments of the feet increase with the use of leather substitute[88]. Plastics and other synthetics lead to many skin diseases whereas leather does not cause any irritation or disease. Valuable data in respect of more specific and best suited uses of leather and different substitutes can be obtained from field trials and performance tests.

Performance Test

A satisfactory performance test[89,90] to obtain an idea on the performance of goods made from leather or substitute materials is necessary to understand the comfort properties of leather more comprehensively. However, accurate evaluation of the comfort properties in wear is very difficult. An abrasion test can be expected to give a satisfactory evaluation of sole leather in wear. Many types of apparatus now in use for testing abrasion resistance of leather give divergent results with the same material. Also, tests for other properties give equally disappointing results. The only reliable test under these circumstances would be to watch the performance of both sole and upper leathers on the feet of selected groups of people belonging to different walks of life, viz. postmen, policemen, soldiers, nurses, labourers and school children. Longevity of shoes used on fairly uniform surface, effect of rough and hard use in long and short periods, and the effect of continuous or drastic use could easily be studied in the above groups. This method of testing should give satisfactory results. Actual wear test covers abrasion, ability to withstand frequent flexing and to hold stitches under strain at different humidities. This test will also enable one to understand the behaviour of leather in actual service and provide reliable information on the relative merits of different types of treatments under service conditions. A comparative study of the performance of leather and synthetics is necessary to gain an insight regarding the service properties of different materials for footwear.

Physical Properties of Fibres

The physical properties of leather cannot be fully evaluated unless the properties of the constituent collagen fibres are determined. The data on the relationship between fibres and leather project to some extent the properties of leather. The general fibre structure is interrelated with certain physical properties of fibres.

The most important methods of fibre study include the use of X-rays, determinations of moisture regain, densities and refractive indices. These techniques can be successfully applied to get a clear picture of the collagen fibre structure and influence of various tanning materials. The shrinkage temperature and moisture content of fibres can be determined using methods of Nutting and Borasky[91] and Mohanaradhakrishnan *et al.*[92] respectively. Weight, density, refractive index, birefringence and torsional rigidity of fibres can be determined by using microbalance[93], density gradient tube[94], half-shodow method[95], polarizing method[96] and Peirce method[97] respectively. The strength and elongation can be worked out in a hydraulic type strength testing machine. A relatively simple test similar to the one suggested by Lefferdink[98] can be developed to measure the resistance of single fibres to fatigue in flexure.

Modifications of Leather and Leatherwear for Improving Comfort Properties

Thermal insulating property of leather can be suitably altered by proper tanning, splitting and currying and that of the footwear by using suitable materials and adopting various constructions. Water vapour permeability can be adjusted by the selection of proper raw materials, tanning, splitting, fat liquoring and finishing processes, or by retanning the vegetable tanned leather with suitable tanning materials. Water resistance of leather can be increased by increasing the degree of tannage, rolling, decrease in water-soluble content, impregnation with oils, fats, waxes, polymers and water repellents, and by retanning with various materials. The processes developed for waterproofing of leather use silicones, polymers or monomers, basic aluminium sulphate and impregnation with molten substances. The water resistance of footwear can be increased using water resistant uppers, soles, threads and seam sealing compounds.

Flexibility of the leather and footwear can be changed by suitably altering the tanning process and construction. Area stability and perspiration resistance can be improved employing suitable tannages like wattle, quebracho, chrome-vegetable, aluminium-vegetable, chrome-aluminium, vegetable-cationic syntans or resins such as melamine or dicyandiamide or formaldehyde. Lightness of leather improves with modification of the beam house operations, tanning and post-tanning operations, e.g. swelling of the pelt before tanning, use of lightweight tanning or impregnating materials, freeze-drying method, and splitting the leather to the required thickness. The weight of the footwear can be reduced using suitable materials and construction or design.

Thus the physical properties of leather could be correlated with the comfort feelings in quantitative terms. Such correlation can be used even in predicting how a particular leather or a substitute is going to behave in actual wear.

Conclusion

Attempts were and are being made to modify the tanning and finishing processes to improve the quality of leather to suit various requirements of finished products such as footwear, gloves, garments, etc. Now leather has to meet the challenge of

substitutes and this has emphasized the need for developing quality leathers possessing comfort properties. A systematic study on the lines suggested in this review would throw more light on the comfort properties of leather.

Summary

The need for evaluating the comfort properties of leather is discussed and the importance of the study of the physical properties of different materials that go into the production of footwear, handwear, garments, etc., in relation to their comfort properties has been emphasized. The physical testing methods and field tests employed in evaluating comfort properties of leather are described indicating the scope of new ones. The purpose of investigations on the comfort properties of leather is to (i) obtain an insight into the fundamental properties of leather affecting its desirable qualities, (ii) develop test procedures for evaluating the comfort properties of leather quantitatively and devise methods for their measurement, and (iii) produce suitable leathers for use under different environmental conditions. These aspects have been reviewed critically. The importance of studies on the fibre structure and characteristics of leather in relation to its comfort properties as well as the optimum requirements of leather for use under different environmental conditions are discussed.

References

1. RENCE O'CONLON, T., *Leather & Shoes*, **145** (1963) (No. 12), 22.
2. WARD, A. G., *New Scient.*, **19** (1963), 500.
3. MANN, C. W., cited in *Research and development report, Footwear and leather series — No.* 9 (US Department of the Army Office of the Quartermaster General), 1954.
4. SELIGSBERGER, L., cited in *Footwear and leather series — Report No.* 17, US Army Natick Laboratories, 1963.
5. KANAGY, J. R. & VICKERS, B. A., *J. Amer. Leath. Chem. Ass.*, **45** (1950), 211.
6. TURRELL, E. S. & ROBINSON, S., cited in *Research and development report, Footwear and leather series — No.* 9 (US Department of the Army Office of the Quartermaster General), 1954.
7. MCLACHLAN, N. W., GOOD FELLOW, H. & CUSHINE, H. C., *J. Soc. Leath. Tr. Chem.*, **26** (1942), 23.
8. PHILLIPS, H., *J. Amer. Leath. Chem. Ass.*, **49** (1954), 574.
9. BAXTER, S., *Proc. phys. Soc. Lond.*, **58** (1946), 105.
10. HERCUS, E. O. & LABY, T. H., *Proc. roy. Soc.*, **95** (1919), 190.
11. STAFFORD-HATFIELD, *J. sci. Instrum.*, **30** (1953), 460.
12. LYKOF, A. W., *Kolloidzscher*, **71** (1935), 353.
13. PHILLIPS, H., *J. Amer. Leath. Chem. Ass.*, **49** (1954), 575.
14. MITTON, R. G., *J. Soc. Leath. Tr. Chem.*, **29** (1945), 255.
15. SASSIE, A. B. D., *J. Text. Inst.*, **40** (1949), 444.
16. ZETTLEMOYER, A. C., SCHWEITZER, E. D. & WALKER, W. C., *J. Amer. Leath. Chem. Ass.*, **41** (1946), 253.
17. CHAWADE, D., *S.A.T.R.A. Bull.*, (March 1959), 126.
18. BOOTH, W. E. & LUCOCK, L. J., *S.A.T.R.A Bull.*, (October 1962), 129.
19. MITTON, R. G., *J. Soc. Leath. Tr. Chem.*, **44** (1960), 502.
20. MAESER, M., *J. Amer. Leath. Chem. Ass.*, **53** (1958), 132.
21. MITTON, R. G., *J. Soc. Leath. Tr. Chem.*, **45** (1961), 415.
22. ZACHARIAS, W. B., MANN, C. W., STEINER, E. T. & HERKOWITZ, F. C., *J. Amer. Leath. Chem. Ass.*, **48** (1953), 460.
23. KUBELKA, V. & NEMEC, V., *Collegium, Haltingen*, **758** (1933), 311.
24. KUBELKA, V., KUBELKA (Jr), V. & KOTASCK, Z., *Tech. Hlid. Kozel.*, **23** (1948), 149.
25. American Leather Chemists Association, Physical Tests Committee, *J. Amer. Leath. Chem. Ass.*, **32** (1937), 516.
26. STATHLER, F. & HERFELD, H., *Collegium, Haltingen*, **777** (1935), 13.
27. American Leather Chemists Association, Physical Tests Committee, *J. Amer. Leath. Chem. Ass.*, **38** (1943), 2.
28. OTTO, G., *Collegium, Haltingen*, **854** (1941), 1158.
29. SPIERS, C. H., *J. Soc. Leath. Tr. Chem.*, **26** (1942), 134.
30. MAESER, M., *J. Amer. Leath. Chem. Ass.*, **42** (1947), 390.
31. WEIR, C. E., CARTER, J., NEWMAN, S. & KANAGY, J. R., *J. Amer. Leath. Chem. Ass.*, **43** (1948), 69.
32. RODDY, W. T. & GAPUZ, D. P., *J. Amer. Leath. Chem. Ass.*, **43** (1948), 690.
33. HOPTON, A. W., *J. Amer. Leath. Chem. Ass.*, **53** (1958), 436.
34. MANN, C. W., *J. Amer. Leath. Chem. Ass.*, **51** (1956), 634.
35. BAUMANN, E., *Leder*, **8** (1957), 185.
36. ROSSITER, W. T., *Leather & Shoes*, **128** (1954), 44.
37. MITTON, R. G. & HYDE, G. R., *J. Soc. Leath. Tr. Chem.*, **33** (1949), 300.
38. RODDY, W. T., *J. Amer. Leath. Chem. Ass.*, **43** (1948), 419.
39. OEHLER, R., KILDUFF, T. J. & DAHL, S., *J. Amer. Leath. Chem. Ass.*, **45** (1950), 349.
40. Dow Corning Corporation Sylflex Sole Leather Report — 1956, cited in *The chemistry and technology of leather*, Vol. 3, by F. O'Flaherty, W. T. Roddy & R. M. Lollar (Reinhold Publishing Corp., New York), 1962, 108.
41. CHEROMIS, N. D., cited in *The chemistry and technology of leather*, Vol. 3, by F. O'Flaherty, W. T. Roddy & R. M. Lollar (Reinhold Publishing Corp., New York), 1962, 108.
42. *Dupont Magazine*, December 1953-January 1954, p. 12, cited in *The chemistry and technology of leather*, Vol. 3, by F. O'Flaherty, W. T. Roddy & R. M. Lollar (Reinhold Publishing Co., New York), 1962, 126.
43. KRENUN, S. S., *Shoe Leath. Reptr*, **265** (1952) (No. 7), 16.
43a. MUTHIAH, P. L., *Tanner*, **20** (1965) (No. 5), 141.
44. PETTIT, D., *J. Soc. Leath. Tr. Chem.*, **45** (1961), 415.
45. MANN, C. W. & STEINER, E. T., *J. Amer. Leath. Chem. Ass.*, **51** (1956), 304.
46. BEEBE, C. W., HAPPICH, W. F., KIP, W. S. & ROGERS, J. S., *J. Amer. Leath. Chem. Ass.*, **49** (1954), 630.
47. VAGO, G. & FEKETE, K., *Leder*, **8** (1957), 137.
48. GRASSMANN, W. & STADLER, P., *Leder*, **7** (1956), 8.
49. WHITEMORE, L. M., DOWING, G. V. & SHERRAD, S. S., *J. Amer. Leath. Chem. Ass.*, **43** (1948), 634.
50. HOLMES, N. L. & WOLLENBERG, H. G., *J. Soc. Leath. Tr. Chem.*, **42** (1958), 278.
51. COLIN-RUSS, A., *J. Soc. Leath. Tr. Chem.*, **31** (1947), 329.
52. GUSTAVSON, K. H., *J. Amer. Leath. Chem. Ass.*, **50** (1955), 414.
53. GALLAY, W. & TAPP, S. J., *J. Amer. Leath. Chem. Ass.*, **36** (1941), 513.
54. RODDY, W. T. & LOLLAR, R. M., *J. Amer. Leath. Chem. Ass.*, **50** (1955), 180.
55. BOWES, J. H. & MOSS, J. A., *J. Soc. Leath. Tr. Chem.*, **44** (1960), 419.
56. MUTHIAH, P. L. & RAMANATHAN, N., *Leath. Sci.*, **12** (1965), 221.
57. RODDY, W. T. & O'FLAHERTY, F., *J. Amer. Leath. Chem. Ass.*, **41** (1946), 506.
58. HIGHBERGER, J. H., *Industr. Engng Chem. Anal.*, **8** (1936), 227.
59. KREMEN, S. S., cited in *Shoe Leath. Reptr*, **265** (1952) (No. 7), 15.
60. CLARKE, I. D. & FLAHERTY, R. M., *J. Amer. Leath. Chem. Ass.*, **49** (1954), 624.
61. NEUMAN, R. E. & LOGAN, M. A., *J. biol. Chem.*, **184** (1950), 299.
62. Federal Specification, Leather, *Methods of sampling and testing, KKL*-311a, 19 January 1953, 3211.
63. *S.A.T.R.A. Bull.*, **6** (1955), 179.
64. WILSON, J. A., *Modern practice in leather manufacture* (Reinhold Publishing Corp., New York), 1941, 674.
65. *S.A.T.R.A. Reprint* SA 2026 (1930).

66. MANGUM, E. W., cited in *Technical report* T-235 (QM Field Evaluation Agency, Fort Lee, Va, US), 1962.
67. Federal Specification, Leather, *Methods of sampling and testing, KKL*-311a, 19 January 1953, 4211.
68. *S.A.T.R.A. Bull.*, **11** (1964) (No. 9), 142.
69. WITNAUER, L. P. & PALM, W. E., *J. Amer. Leath. Chem. Ass.*, **60** (1964), 246.
70. *S.A.T.R.A. Bull.*, **8** (1959) (No. 17), 144.
71. *J. Soc. Leath. Tr. Chem.*, **47** (1963), 126.
71a. MAESER, M., *Leath. Mfr.*, **79** (1962) (No. 1), 53.
72. BOOTH, W. E., *J. Soc. Leath. Tr. Chem.*, **43** (1959), 347.
73. TOTH, G. & ELTER, J., *Leder*, **13** (1962), 59.
74. MIURA, T., *Shoes and foot health* (Institute of Science of Labour, Tokyo), 1957, 58.
74a. NEWBURG, L. H., *Physiology of heat regulation and the science of clothing* (W. B. Saunders Co., Philadelphia), 1949, 349.
75. MUTHIAH, P. L., SELVARANGAN, R. & NAYUDAMMA, Y., *J. Amer. Leath. Chem. Ass.* (1966).
76. MITTON, R. G. & MORGAN, F. R., *J. Soc. Leath. Tr. Chem.*, **48** (1964), 54.
77. RAMANATHAN, N. & SUBBALAKSHMI, D. V., *Proceedings of the ISI convention*, Session T-7, Document A (1957) (Indian Standards Institution, New Delhi), 19.
78. MITTON, R. G., *Progress in leather science* (British Leather Manufacturers' Association, London), 1946, 48.
79. WILSON, J. A., *Industr. Engng Chem.*, **17** (1925), 829.
80. POPPLEWELL, D. & WARD, A. G., *J. Soc. Leath. Tr. Chem.*, **47** (1963), 502.
81. BUTLIN, J. G., *J. Soc. Leath. Tr. Chem.*, **47** (1963), 3.
82. BUNTEN, J. & COOPER, D. S., *S.A.T.R.A. Bull.*, **6** (1954), 25.
83. HERFELD, H. & KONIGFELD, G., cited in *Gerbereiwiss. Prax.*, (No. 10) (1963(, 1.
84. *Tanner*, **19** (1964) (No. 4), 117.
85. MYER, R. G., *Leather & Shoes*, **148** (1964) (No. 18), 49.
86. LAWRENCE, L., *Life*, **37** (11) (1964), 75.
87. GRAY, B. E., *Leather & Shoes*, **148** (1964) (No. 18), 43.
88. *Leath. Tr. Rev.*, **149** (1963), 544.
89. MORGAN, F. R., *J. Soc. Leath. Tr. Chem.*, **46** (1962), 190.
90. SALT, H., *A study of leather substitutes for leather* (His Majesty's Stationery Office, London), 1946.
91. NUTTING, G. C. & BORASKY, R., *J. Amer. Leath. Chem. Ass.*, **44** (1949), 831.
92. MOHANARADHAKRISHNAN, V. & RAMANATHAN, N., *Leath. Sci.*, **12** (1965), 291.
93. ANATHANARAYANAN, S. & RAMANATHAN, N., *Bull. cent. Leath. Res. Inst., Madras*, **8** (1962) (No. 10), 480.
94. MOHANARADHAKRISHNAN, V. & RAMANATHAN, N., *Leath. Sci.*, **11** (1964), 260.
95. MOHANARADHAKRISHNAN, V. & RAMANATHAN, N., *Leath. Sci.*, **11** (1964), 267.
96. MOHANARADHAKRISHNAN, V. & RAMANATHAN, N., *Leath. Sci.*, **13** (1966).
97. MOHANARADHAKRISHNAN, V. & RAMANATHAN, N., *Leath. Sci.*, **12** (1965), 150.
98. CARLINE, P. W., *J. Text. Inst.*, **38** (1947), 38.

REVIEWS

QUANTUM MECHANICS AND PATH INTEGRALS by Richard P. Feynman & Albert R. Hibbs (McGraw-Hill Book Co. Inc., New York), 1965. Pp. xiv+365. Price $ 12.50

Path integral approach to problems in quantum mechanics has been developed by Feynman since he was a graduate student. It is a picturesque way of looking at things and it lends itself readily to intuition. Even for the most abstract theories some kind of a picture is very helpful for the thinking process. However, no picture is without a fault and hence it is necessary to be aware of this.

The difficulty with the path integral approach is that there is no problem that it alone can solve. Sometimes a little elegance or brevity may be realized but that is all. On the other hand, the conventional approach of operator calculus is more powerful and it solves more problems.

Clearly, the path integral approach is not the right medium for students to learn quantum mechanics. The study of this approach should be confined to those who have already learnt the conventional quantum mechanics but have a special interest in this alternative version. In the reviewer's opinion this book should not be a text-book for students learning quantum mechanics.

However, the numerous problems appearing throughout the text are extremely instructive and interesting. In fact a good part of the subject matter has been reinforced through problems in a very creditable way. From such a point of view, there are few books to match this one.

GYAN MOHAN

QUANTUM THEORY OF MOLECULES AND SOLIDS: Vol. 2—SYMMETRY AND ENERGY BANDS IN CRYSTALS by John C. Slater (McGraw-Hill Book Co. Inc., New York), 1965. Pp. xii+563. Price $ 12.50

Prof. Slater has contributed quite a few important books on various aspects of quantum theory in the international series in pure and applied physics under the consulting editorship of L. I. Schiff. He has recently started a multi-volume series covering the entire field of modern solid state theory. The first volume in this series which appeared last year is on the electronic structure of molecules. The volume under review is the second volume under the same series. This volume deals with the theory of symmetry and energy bands in crystals. There is at present no other text-book in the field which deals with the theory of energy bands as thoroughly as Prof. Slater has tried to do in this volume. Energy band calculations of a hitherto unattainable accuracy are being released in the last year or two. Not only all the published data on this subject but also some of the unpublished data have been obtained by the author from his colleagues working in this field, for inclusion in the volume under review. Experimental results on the electronic energy levels of solids are accumulating so fast that detailed correlation between experimental and theoretical results would soon be possible. For this purpose the publication of the present book is very welcome.

The book is divided into ten chapters dealing respectively with (1) Crystals and their symmetry properties, (2) Space groups for structures of the elements, (3) Space group for structures of compounds, (4) Atomic radii and the chemical bond, (5) The symmetry of electronic wave functions in crystals, (6) Plane-wave expansions of wave functions in crystals, the one-dimensional case, (7) Plane-wave expansion in the two- and three-dimensional cases, (8) The tight-binding and the orthogonal plane-wave methods, (9) The cellular and augmented plane-wave methods, and (10) Calculations of energy bands in crystals. The last chapter also contains an extensive bibliography of not less than 184 principal papers dealing with detailed energy band calculations. The references are arranged chronologically under each type of crystal.

The regular chapters are followed by a list of nine appendices dealing respectively with the data on the following topics: (1) Interatomic distances and crystal structures, (2) Crystal parameters, (3) Symmetry properties and projection operators for 20 space groups, (4) The momentum eigenfunction and Fourier expansion of the potential, (5) Power series expansions of energy bands near symmetry points, (6) Matrix elements for the augmented plane-wave method, (7) The free electron approximation, (8) Binding energy diamond by the method of Schmid, and (9) Spin orbit and relativistic effects in energy bands.

There are certain specific comments about the contents of the book which make it very useful for workers in the field of solid state physics. The book contains complete description of many types of crystal structure and many individual compounds whose energy bands have yet to be computed. Reference has been made to a large variety of important crystalline solids to which the theory could be extended profitably by the workers in the field. Because of their special magnetic and optical properties many complicated compounds are coming into prominence more and more. Such compounds have also been included in the present volume in anticipation of future work. The treatment of space groups and their implications regarding symmetry of wave functions has been carried out in greater detail in this book than is found in other books. The method adopted for handling the symmetry of wave functions demands complete information regarding the symmetry elements of the irreducible representation of the space groups. Using the material provided here, it is easy for the reader to extend the same to other cases. The group theoretical methods used by the author differ in many respects from the more conventional treatment adopted by Seitz and other writers. The author claims that his methods and procedures

are much more adoptable for actual use than the conventional methods. Many would prefer to have the same conventional treatment in all the books on the same subject. There is no doubt that the present book would appeal to a much larger audience than some of the earlier publications.

The author has included in the book a very extensive bibliography containing references to more than 950 original papers bearing on the subject. Not only the names of authors and journals, but the titles of the papers are also included in the bibliography. As such the usefulness of the bibliography is very much increased.

R. S. KRISHNAN

FUNDAMENTAL ANALOGUE TECHNIQUES by R. J. A. Paul (Blackie & Sons Ltd, London), 1965. Pp. x+216. Price 35*s*.

This book is one of Electronic User Series; the series " is intended for qualified scientists and engineers for whom electronics is a fringe activity ".

In Chapter 1, an introduction to computing aids, and, in particular, to electronic digital and analogue computers is made. Here, the analogue computer is referred to as a member of a class of simulators, and its complementary role to digital computers is indicated.

Chapter 2 describes the principles of electronic differential analyser and introduces the reader to basic operations of integration, summation, sign reversal and multiplication, that are performed using analogue units.

In Chapter 3, simplifying techniques for the choice of time scale factor and amplitude scale factor are illustrated through examples and final computing arrangements are shown for the solution of chosen differential equations.

Chapter 4 concerns analogies and the construction of analogous networks. A noteworthy part of this chapter is a section on the direct simulation on an analogue computer which includes an instructive example of a simulated electrical network consisting of lumped elements.

Chapter 5 discusses the iterative operation of analogue computers. With such an operation it is possible to utilize the inherent storage capabilities of integrators to solve wider classes of problems especially when the storage of value one problem variable is required until the other variables reach a certain value. This approach also makes it possible to work an analogue computer in situations where the independent variable is other than time. All these points are fully discussed with relevant examples from diverse fields.

The last chapter studies simulation of rational transfer functions using analogue units like adders and integrators.

There are three appendices; one on unilateral s-multiplied Laplace transform, another on generalized two-port network, and the third on symmetrical lattice network.

The book does not contain either any computing circuits (of operation amplifier, etc.) or description of any working analogue computer system. There is a brief mention of static check (on page 79) but no details are given on error check of solutions.

The book can be recommended to those interested in the application of differential analysers to network problems.

D. S. KAMAT

ELECTRON OPTICS by P. Grivet (Pergamon Press Ltd, Oxford), 1965. Pp. x+781. Price £10

This book, although entitled 'Electron Optics', deals for the most part with the commonly used electron/ion optical instruments, viz. the cathode ray tube, the emission and the electron microscopes, and the mass and the β-ray spectrographs. Of these, the electron microscope and related techniques have been dealt with in considerable detail. In addition, image converters, diffraction cameras and electron probe devices are also included, though in relatively less detail.

The above forms Part II of the book. In Part I, the principles of electron optics, pertinent to the general design, understanding and use of the instruments described in Part II, are presented. Different types of electron lenses, including strong focusing lenses, and lens defects have been discussed in some detail. In many cases, analogies have been drawn from geometrical optics, making it relatively easy to understand the behaviour of complex electron lenses.

Besides giving the basic physical theory, the book also provides useful information on many practical aspects and techniques related to the design as well as use of the various instruments. It would, thus, be of considerable interest not only to advanced students of electron optics and designers of electron optical instruments but also to users of the latter.

There is an extensive bibliography provided at the end of the book. It appears to be fairly complete up to about 1960, though a few references up to 1963 have also been cited. The subject index is, on the other hand, somewhat sketchy, and could have been more exhaustive.

The book is, however, to be highly commended for the fact that so many related topics have been presented, in good detail, under one cover.

S. S. S. AGARWALA

APPLIED MAGNETISM — A STUDY OF QUANTITIES by E. Olsen (Philips Technical Library, Eindhoven), 1965. Pp. x+144. Price Rs 33.66

The fundamental study of magnetism has been given new impetus because of recent applications, e.g. in the field of microwaves, computers and physical acoustics. This English edition (1966) is a translation from the original Dutch edition (1964). The MKSA system, frequently called Giorgi system, is employed and also, what the author calls, the p-system which in the main consists of CGS, V and A. The price as mentioned in a technical bulletin is 37*s*. 6*d*.

The author deals briefly with several types of hysteresis loops and definitions of a number of permeabilities. One could wish that the former had been a little more exhaustive and descriptive. Chapter 5 deals with complex permeability expressed in series and parallel terms. Readers accustomed to American and British technical literature may

find some of the explanations and discussions difficult to follow. Chapter 6 deals with magnetic circuit consisting partially of ferromagnetic material and the effect of air gap upon hysteresis loop and incremental permeability. Eddy current losses in conducting materials and in ferrites where the dielectric constant has also to be considered are dealt with in an interesting way. There is a brief mathematical treatment of harmonics caused by the hysteresis loop.

In the chapter on some conceptions from the microwave region, the author discusses gyromagnetic ratio, tensor permeability, etc., and in Chapter 10, terms from the field of application of square loop materials. The dynamic character of square loop materials and their temperature dependence are briefly discussed. The chapter on magnetostriction first deals with some terms from the theory of elasticity and then with general magnetostrictive terms.

The printing is good and the diagrams very clear and attractive. This book is recommended for all scientists interested in this branch of science.

T. V. SREENIVASAN

INTRODUCTION TO QUANTITATIVE ULTRAMICROANALYSIS by I. M. Korenman (Academic Press Inc., New York), 1965. Pp. ix+234. Price $ 9.50

This book is the English translation of the original book in Russian compiled on the basis of reports of numerous authors by Prof. Korenman, Profe sor of Analytical Chemistry, Gorkiy State University, USSR. The translation has been very ably done and makes a pleasant and easy reading.

As science and technology advance, the amount of matter capable of being analysed tends to diminish. Ultramicroanalysis is a technique of dealing with exceedingly small quantities of the magnitude of microlitres. It should not be misunderstood as just a replica of microanalysis or microanalysis on a greatly reduced scale. Though the basic chemistry remains the same, depending on the amount handled such process in ultramicroanalysis develops its own peculiarities, advantages and sources of error. The book describes with many illustrations details of equipment and processes. Thus details of principle operations like sampling, addition and measurement of solution, reaction of fibres, paper and gelatin, microcrystalloscopic tests, reaction in capillary test tubes and cones, use of gaseous reactions, mixing, heating, evaporation, ignition, isolation of precipitates from solutions, electrolysis, extraction, chromatography and microscopic determination of size are fully described.

Enough details have been given of the fabrication of microbalances and other apparatus used in gravimetric and volumetric analysis, particular attention being paid to discussion on errors involved. A number of practical applications have been discussed.

The physico-chemical methods of analysis described relate to potentiometry, spectrophotometry, photocolorimetry, photographic methods and kinetic methods of analysis. Gas analysis has been discussed in a separate chapter. An extensive bibliography has also been given.

Ultramicroanalysis is relatively a new branch of analysis and those who are interested in its development would find the thorough treatment contained in this book very valuable.

V. T. ATHAVALE

RADIOACTIVITY AND ITS MEASUREMENT by W. B. Mann & S. B. Garfinkel (D. Van Nostrand Co. Inc., New York), 1966. Pp. 168. Price $ 1.75

Radioactivity is a fascinating subject, both historically and in its role in modern science and technology. The layman tends to associate it with atomic power, atomic weapons and fallout. The science student is anxious to know more about it. Therefore, inclusion in the Van Nostrand momentum book series for commission on college physics of a book under the title 'Radioactivity and Its Measurement' seems a very good choice. This series is conceived to serve scientist, engineer, teacher, student and the inquisitive layman.

The fact that a lot is written about radioactivity and its measurement in most of the standard textbooks on nuclear and radiation physics raises the question as to how this book has added to the subject. This book is a moderately priced paperback edition and has the advantage of explicitness of style with least possible number of mathematical equations. These advantages would induce wider readership of the subject, and especially so because the authors of the book are persons handling important assignments on the subject in the National Bureau of Standards (USA).

The book starts with a historical account of the discovery of radioactivity and the progress made thereafter, with special mention of pioneering workers like Rutherford and Curie and their experiments. Chapter 2 describes the sources of natural radioactivity and basic equations of radioactive decay. Chapters 3 and 4 are devoted to the phenomena of interaction of radiation with matter. Chapter 5 explains the basis of nuclear reactions and artificial radioactivity with special emphasis on the energetics of nuclear change. The last three chapters deal with various types of radiation detectors, nuclear instruments and techniques commonly used in radioactivity measurements.

The striking feature of the book is the simplicity of presentation of the subject. This feature is particularly advantageous to the student to enable him to form a firm background before he takes up advanced studies in the field. However, the book is limited in its scope as regards the practical applications of the methods of measurement of radioactivity. Special problems of detection and measurements are encountered in the application of radioactive substances in physics, chemistry, biology, medicine and industry. The inclusion of important techniques in low level activity measurement and energy analyses would have been within the scope of the title of the book. The treatment of detectors and instruments is merely introductory.

Any attempt at writing a small size book on a subject of enormous scope and magnitude is bound to leave gaps in the presentation of the subject. The book tends to give more about the historical

aspect of the subject, and the modern trends in the measurement and analysis of radiation have not been fully described.

To sum up, the book under review is an excellent introductory book on the subject and will be useful not only to the college student for whom it is intended but also for the general science reader and even the research worker intending to use radioactivity as a tool for his study.

K. G. VOHRA

PLANT GROWTH AND DEVELOPMENT by A. Carl Leopold (McGraw-Hill Book Co. Inc., New York), 1964. Pp. xii+466

This is the first book in the series in physiology of the McGraw-Hill publications in the biological sciences. In this age of multi-authorship volumes, Prof. Leopold has written an admirable book " centred about the workings of the growing plant, without organized coverage of biochemistry and nutrition. This assumes a logical pedagogical division of the subject of plant physiology into a section on growth and development and another section on nutrition and metabolism. This is also done with the hope of maintaining an appropriate level of interest in the living plant, in the face of a current tendency toward preoccupation with grindates, supernates, and simulated life activities in test tubes without sufficiently clear relationship to the whole plant " (author's preface).

A brief introduction on ' The cell and its habitat ' is followed by Part I — Assimilation, containing four chapters on Photosynthesis, Organic translocation, Inorganic translocation and Mobilization; Part II — Growth, containing five chapters on Auxins, Gibberellins, Kinins, Inhibitors and Differential growth; Part III — Development, containing nine chapters on Juvenility, Senescence, Flowering, Flower physiology, Fruit set, Fruit growth, Fruit ripening, Tuber and bulb formation and Dormancy; Part IV — Environmental physiology, containing four chapters on Light, Radiation, Temperature and Water; and Part V — Chemical modification of plants, containing a single chapter on Applications of chemicals to plants — a grand total of twenty-four chapters. There is a list of abbreviations (rather incomplete) and a fairly comprehensive author and subject index. The printing and the get-up speak for the high standards or quality for which the publishers are always known.

The book contains a balanced and well-integrated account of *Plant growth and development* with all relevant facts critically discussed. Certain sections like Part I are very uneven, perhaps included only for the sake of completeness and could have been omitted. On page 17, although Arnon's work was mentioned briefly, no mention was made of Calvin and all his work is wound up in just two sentences ! This is only a minor criticism and Leopold's book is likely to be used extensively not only by the students of plant physiology in general but also by the new generation of specialists who are likely to view the problems of growth and development in the light of the recent advances in molecular biology.

E. R. S. TALPASAYI

NEWER METHODS OF NUTRITIONAL BIOCHEMISTRY WITH APPLICATIONS AND INTERPRETATION: Vol. 2, edited by Anthony A. Albanese (Academic Press Inc., New York), 1965. Pp. xiii+558. Price $ 18.50

It is only during the last decade that attempts have been initiated at interpreting nutritional problems and disorders at the molecular level, especially in terms of enzymic changes. This does not, however, take away the importance of the approach of the classical nutritionist, who was more concerned with gross changes in body composition and energy metabolism. While studies at the molecular level can definitely lead to a better understanding of the metabolic processes involved, the overall picture of the metabolic status of the animal must not be lost sight of.

In this context, Vol. 2 under review of the book *Newer methods of nutritional biochemistry* edited by Anthony A. Albanese truly represents this changing line of approach towards nutritional problems. Appropriately enough, the first two chapters have been devoted to problems relating to changes in body composition and energy metabolism and a very clear and concise description of the methodology involved in the determination of these factors has been given.

The protein component of the diet has received considerable importance in nutritional studies. The necessity for a proper balance among the essential amino acids and the requirement in relation to age, sex, species and others have been discussed in the chapter on the utilization of essential amino acids by man. The following chapter on the abnormal urinary metabolites of amino acid origin provides a basis for detecting several inborn errors of metabolism. Such defects are essentially due to changes in the enzymic make-up and a striking illustration of this point is found in the study on the conversion of tryptophan to nicotinic acid. The changes in the levels of the enzymes involved in this conversion have been studied under a wide variety of pathological conditions, both experimental and natural. The influence of nutritional deficiencies, age and sex has also been included here, which emphasizes the great care one should exercise in the interpretation of results.

The chapter on pantothenic acid, biotin and folic acid is not only devoted to the methods involved in the assay of these vitamins, but also includes their deficiency effects and functional role. The treatment on the vitamins A, D, E and K is confined to the methods involved in their extraction and estimation.

The nutritional aspects of endocrinology have been discussed in the chapters on growth and pituitary hormones, anabolic steriods and calcium and phosphorus metabolism. Especially the treatment on growth and pituitary hormones covers both the gross changes produced in the body due to the hormones and the changes at the enzyme level.

This volume, as the title itself suggests, is primarily devoted to the methodology involved in the estimation of cellular components that have been discussed. But it is clearly more than a mere catalogue of the methods involved, and is a solid

attempt to place nutritional problems on a firmer biochemical basis. The hope of the editor to use enzyme assay for diagnostic purposes is modest, when attempts are already on way to make enzyme therapy a clinical practice. This volume should prove very useful to all biochemists and clinical investigators.

P. S. SARMA

MOLECULAR BIOPHYSICS edited by Bernard Pullman & M. Weissbluth (Academic Press Inc., New York), 1965. Pp. x+452. Price $ 19.50

This book consists of a series of articles (fifteen) written by different authors. The first of these, by F. Gros on the cell machinery, contains an excellent discussion of, particularly ribosomes, transfer of genetic information and the regulation of protein synthesis. The Pullmans have contributed the next two articles on description of molecules by the molecular orbital method and on the electronic structures of nucleic acid. B. Pullman deals with, among other things, properties of base pairs in nucleic acids, molecular associations, and the structural basis of carcinogenicity of aromatic hydrocarbons. This latter article also contains a forceful plea for extension of the quantum mechanical approach to problems of biophysics and biochemistry. J. S. Griffith deals with electron spin resonance (ESR) in biological iron compounds and discusses the possible origins of fine structure in spectra. M. Weissbluth covers ESR studies of triplet states in organic molecules, and P. Douzou gives an interesting account of studies of transient molecular configurations in biophysical problems.

The absorption and rotation of polarized light by polymers is dealt with by Tinocco (Jr). The study of electron distribution in molecules by nuclear quadrupolar resonance (NQR) spectroscopy is covered by E. Scrocco. A treatment of magnetic excitations in crystals is given by H. M. McConnell, and of intermolecular forces by J. O. Hirschfelder. J. Jehle and his colleagues consider, in two articles, the problem of structures of single-strand nucleic acids and the replication of double-strand nucleic acids, with illustrations of models. An excellent article on the binding of small molecules to proteins is contributed by G. Weber. The mechanism of muscle contraction is covered by M. F. Moraeles. The final article on information theory and memory, by J. S. Griffith, poses this problem in perspective.

Several minor errors were noted in the first article, and expressions such as 'results itself', 'X-ray diffusion', etc. Legends in one figure, elsewhere in the text, were found to be incorrect.

On the whole, this is a worthwhile book which should serve to extend the horizons for the reader interested in chemistry, physics, biochemistry or biology.

L. K. RAMACHANDRAN

LIBRARIES IN THE MODERN WORLD by G. Chandler (Pergamon Press Ltd, Oxford), 1965. Pp. vii+164. Price 17*s*. 6*d*.

The author attempts to give a bird's-eye view of the different types of libraries, in an international setting. The scope and purpose of these libraries are given under broad groups such as local libraries, academic libraries, private libraries, industrial libraries, national libraries, national special libraries and international libraries. Under each of these groups, special libraries are arranged according to their subject fields by decimal classification. The examples are mostly from Britain and USA with an occasional mention of libraries from other regions. A brief account of the role of Unesco in furthering library programmes at international level and in the newly developing countries is given. The book is primarily intended for British library authorities and as such the appropriate recommendations of the British Public Libraries and Museum Act of 1964 are given under each type of library. The 'modern world' pertains mainly to Great Britain, USA, USSR and a few other European countries. The treatment of other regions is sketchy. The statement by the author that the book aims at "several levels of readership and is not satisfactory on any single level" is not an understatement. Though the information is collected from various sources, the book does not give any bibliography.

S. PARTHASARATHY

PUBLICATIONS RECEIVED

DOCUMENTATION RESEARCH AND TRAINING CENTRE ANNUAL SEMINAR 3: DEPTH CLASSIFICATION SUBJECT HEADING (DRTC, Bangalore), 1965. Pp. 569. Price Rs 20.00

METALLURGICAL SOCIETY CONFERENCE: Vol. 28 — PRECIPITATION FROM IRON BASE ALLOYS edited by G. R. Speich & J. B. Clark (Gordon & Breach Science Publishers, New York), 1965. Pp. vii +412. Price $ 8.50 (paper); $ 21.00 (cloth)

THE PEPTIDES: Vol. 1 — METHODS OF PEPTIDE SYNTHESIS by Eberhard Schröder & Klaus Lübke (Academic Press Inc., New York), 1965. Pp. xxix+481. Price $ 20.00

ADVANCES IN THEORETICAL PHYSICS: Vol. 1, edited by Keith A. Brueckner (Academic Press Inc., New York), 1965. Pp. x+323. Price $ 12.00

QUANTUM STATISTICS AND COOPERATIVE PHENOMENA by John G. Kirkwood (Gordon & Breach Science Publishers, New York), 1965. Pp. x+182. Price $ 4.95 (paper); $ 8.00 (cloth)

DIELECTRICS — INTERMOLECULAR FORCES — OPTICAL ROTATION by John G. Kirkwood (Gordon & Breach Science Publishers, New York), 1965. Pp. x+271. Price $ 4.95 (paper); $ 8.50 (cloth)

ENERGETICS IN METALLURGICAL PHENOMENA: Vol. II, edited by William M. Mueller (Gordon & Breach Science Publishers, New York), 1965. Pp. lx +203. Price $ 11.00 (cloth); $ 5.50 (paper)

ACCÉLÉRATEURS CIRCULAIRES DE PARTICULES by Henri Bruck (Institut National des Sciences et Technique Nucléaires, Saclay), 1966. Pp. xii +358

KINETIC METHODS OF ANALYSIS by K. B. Yatsimirskii (Pergamon Press Ltd, Oxford), 1966. Pp. xvi+155. Price 50*s*.

THE STATE AND MOVEMENT OF WATER IN LIVING ORGANISMS edited by G. E. Fogg (Cambridge University Press, London), 1965. Pp. vii+432. Price 75*s*.

NOTES & NEWS

Explosion-built magnetic fields

The idea conceived in 1951 by the Soviet physicist Andrei Sakharov for conversion of explosive energy into magnetic energy has been turned into practical achievement with the recent first trials of new machines in Soviet Russia, for the production of superstrong magnetic fields. The necessity for the production of ultrahigh magnetic fields has been felt in recent years because of the importance of the study of the properties of material in such fields and this achievement will open up unexplored fields of research in fundamental properties of matter. In one of these installations called MK-1, magnetic fields of intensities never so far reached and having magnetic pressures of tens of millions of atmospheres are produced. The installation consists of a metallic tube surrounded by an explosive charge and having a strip of aluminium foil wound round the tube. When a bank of capacitors is discharged through the strip, a strong magnetic field is set up inside the tube with the lines of force oriented along the axis of the tube. An explosion quickly compresses the tube towards the axis. As a result of such symmetrical and rapid squeezing, the strength of the field inside the tube steeply rises to nearly 25 times the original value.

The second type of installation (MK-2) consists of a central metallic tube and a spiral. On the one end the spiral terminates into a metallic cap, the base of which is connected to the tube. An explosive charge is placed in the tube and the blast is triggered off from one end of the tube. The bank of the capacitors is discharged across an electric circuit formed by the tube, the spiral and the can. Under the explosion the tube gets stretched at one end in the form of a cone. When the discharge current reaches a maximum, the walls of the internal tube approach the beginning of the spiral in an abrupt jump. The movement is analogous to the rapid insertion of a metallic cone into a spiral. The moving cone short-circuits the coils of the spiral, which reduces the inductance of the electric circuit and results in a rapid rise of the current and increase of magnetic energy. The installation MK-2 produced within a volume of several litres, magnetic fields of 1,500,000 oersteds and currents up to 100,000,000 amp.

Generators which could accelerate charged particles to tremendous energy have also been designed on this principle [*Soviet Features*, **4** (No. 103) (11 May 1966), 2].

New relationship between the critical field and energy gap of a superconductor

A new empirical relationship between the critical field of a superconductor and its energy gap has been reported from the IBM Watson Research Centre, New York. The value of this relationship in the determination of energy gaps is obvious, since the critical field measurements can be much more easily determined as compared to the tunnelling measurements. The relationship may be stated as follows: The magnitude of the slope of the reduced critical field of a superconductor at the critical temperature is equal to the energy gap of the superconductor at 0°K., measured in units of kT_c. This is mathematically expressed as

$$|dh/dt|_{t=1} = \Delta(0)/kT_c \quad ...(1)$$

where h is the reduced critical field, H_c/H_0; t, the reduced temperature, T/T_c; $\Delta(0)$, the gap at 0°K.; and k, the Boltzmann constant. Relation (1) is found to be true in the case of a number of materials, the two exceptions so far noticed being niobium and mercury. Relation (1) is also observed to satisfy the weak coupling limit of the BCS [Bardeen, J., Cooper, L. & Schrieffer, R., *Phys. Rev.*, **108** (1957), 1175] theory. It is significant that relation (1) holds well for the entire range of superconductors, weak as well as strong coupling. Thus, it is seen that although both $\Delta(0)/kT_c$ and $(dh/dt)_{t=1}$ depart from the values predicted by the weak coupling theory, their ratio does not [*Phys. Rev. Lett.*, **15** (1965), 462].

Production of two-coloured pictures from holograms

A two-colour picture has been produced without the aid of lenses, etc., for the first time using holographic techniques at the Electron Tube and Microwave Circuit Department of Bell Telephone Laboratories. Although hologram pictures using black and a single colour have been made before, this is the first time two-colour images are produced and this has given indications regarding the possibility of producing multicoloured images also. The two-coloured image is achieved by using two different lasers (a helium-neon laser giving a red beam and an argon laser giving a blue beam) as the sources of the coherent light required to make a hologram. The two beams are combined into a single beam of bluish-pink and then split into two parts — one part scattered directly from the object to be photographed on to a photographic plate, the other part reflected from a mirror to the same photographic plate. The two beams striking the plate form interference patterns throughout the emulsion. When the original red and blue beams are shone on the completed holograms, a single, two-coloured image emerges.

Besides their potential for three-dimensional photography, holograms, because of their unusual storage capacity, have potential use in memory systems for data processing and in X-ray microscopy because of their great ability to magnify and because X-rays cannot be focused with lenses [*Bell Lab. Rec.*, **43** (1965), 416].

New photographic film for use in solar radiation warning system

A new and specialized black and white photographic film known as Kodak special solar recording film, Type SO 375, has been

developed by Messrs Kodak Ltd for use in the radiation warning system for manned spacecraft. It is fine grain, high resolution film with an extended red sensitivity and unusual contrast characteristics that are valuable in detecting subtle differences in the structure of the sun and is being used to photograph the sun in order to record solar flare activity. From a study of the structure of the sun obtained with the new film, scientists can follow solar radiation activity more closely and thereby aid in predicting future solar patterns.

The Manned Spacecraft Programme of the US National Aeronautics and Space Administration is establishing seven stations around the world to photograph the sun at 10 sec. intervals in a series, to continue for a period of 11-15 years.

The film is used in special telescope cameras to photograph the entire disc of the sun through a filter that excludes the entire spectrum except the red hydrogen alpha radiation (6563 A.). The use of this filter makes it possible to single out solar flare activity on the sun's surface.

It is hoped that from a study of the solar flares it will eventually be possible to more accurately forecast weather patterns and radio transmission disturbances [*Information from Kodak Ltd, Bombay*, 3 *June* 1960].

Direct current transformer

A breakthrough concept in stepping up and stepping down a direct current, hitherto considered impossible, has been outlined by Dr Ivar Giaver of the General Electric Research and Development Centre, New York, using thin films of tin deposits on a glass slide as the primary and secondary of the a.c. transformer. A model of the 'd.c. transformer' has been built by him recently. Though the development does not offer any immediate commercial applications, it ranks in its epoch-making significance with the a.c. transformer which has revolutionized power generation and its transport. The d.c. transformer has been operated at very low voltages and currents with an efficiency of about 10 per cent. The primary and secondary 'turns' of the new device are made of thin tin films belonging to a family of type II superconductors, characterized by allowing a magnetic field to be penetrated but only in the so-called 'flux spots'. When a direct current is passed through the tin primary, the magnetic flux spots begin to move in one direction relative to the film. In the d.c. transformer these flux spots also penetrate and move through the adjacent secondary film. As a result of this movement — and hence changing magnetic field — a direct current is induced in the secondary film. The magnetic field fluctuations produced by the primary exist only very close to the film. Thus the secondary film must be placed no more than about a millionth of an inch (250 A.) away from the primary film. By placing a number of secondary films in series in the d.c. transformer it is possible to develop a secondary voltage many times higher than the primary voltage. Similarly, by placing a number of films in series, the output voltage can be stepped down below the input voltage [*Sci. J.*, **2** (No. 4) (1966), 19].

New dielectric tape camera for space use

A novel camera that uses a dielectric tape in place of the ordinary film for recording and storing images which lends itself for effective use in space applications has been made by Radio Corporation of America's (RCA) Astro Electronics Division recently. The main features of the camera include a reusable 'film', electronic processing, extremely high radiation resistance and high density information storage, low power consumption and comparative lightness. It provides image resolutions approaching photographic systems used with electronic readout.

In the dielectric camera, image sensor and storage medium are both in the camera head. When required, the stored information is read out as an electrical signal and is immediately amenable for transmission over a communications system. The tape is made up of a flexible base of cronar tape coated essentially with a thin photoconducting layer on top of which is an insulating layer. The optical image is 'written' into the insulator and stored there until readout or erasure. When the system is operating, the tape unwinds from a storage reel and is first hit by a flood of electrons to erase past images and establish a uniform known potential. The target is then imaged optically on the tape, which is electron flooded at the same time to create a charge pattern on the insulator proportional to the amount of illumination falling on the tape. The image then would be stored or readout. For readout a finely focused, high velocity electron beam would measure the differing potential on the dielectric tape. It is expected that the camera's resolution may be as good as 600 television lines per in. at 50 per cent response which compares well with that for other camera systems in vogue for space work [*Missiles & Rockets*, **18** (No. 16) (1966), 28].

Surgery with laser light

Dr Thomas E. Brown assisted by Dr Leon Goldman and Dr Bruce Henderson of the Children's Hospital in Cincinnati, Ohio, has used the intense light of laser as a scalpel to remove diseased tumour from a man's thigh. This operation lasted 15 min. and was quite bloodless as the heat from the laser scalpel sealed the numerous blood vessels around the tumour as it cut through them.

The 50-year old patient had skin cancer. A local anaesthetic was used to deaden the patient's leg before making an incision with ordinary scalpel into the diseased area. The tumour, about 1 in. and ½ in. diam., was locked ½ in. below the skin. Dr Brown used an argon gas laser developed at the Bell Telephone Laboratories, New Jersey, which emits a steady stream of green light that can be easily controlled. A curved mirror was used to direct the beam into the tissue surrounding the tumour. On the operation, by virtue of the green colour of the laser beam the red blood cells absorbed it, thus enabling laser's energy to be transferred to the surrounding tissue.

Although laser's use as a surgical tool other than in eye was eclipsed by the other equally effective and less dangerous techniques, the Cincinnati operation gives new hope for its future use in delicate surgery especially in which bleeding is a critical factor. It is believed to be useful in brain, spleen and liver operation. Another prospective application of laser beam is in cauterizing the wounds of patients suffering from haemophilia [*Sci. J.*, **2** (No. 5) (1966), 19]. — D. S. R. MURTY

A convenient synthesis of cubane system

Cubane has been synthesized through a series of reactions in which the cyclobutadiene transfer reaction plays a key role. Decomposition of cyclobutadieneiron tricarbonyl (I) in the presence of 2,5-dibromobenzoquinone yields a Diels-Alder adduct (II), in 80 per cent yield, as fine yellow needles; m.p. 127-8°. This has been shown to have *endo* configuration. Irradiation of the Diels-Alder adduct in benzene with a mercury lamp affords a colourless isomer (III) in 80 per cent yield; m.p. 152-4° (from methanol) containing one molecule of solvent. Treatment of compound (III) with aq. KOH at 100° affords cubane-1,3-dicarboxylic acid. The decarboxylation is effected by the thermal degradation of the di-*tert*-butyl perester. The hydrocarbon obtained in this manner shows an identical NMR with that synthesized earlier [*J. Amer. chem. Soc.*, **88** (1966), 1328].

Preparation of Grignard compound in hydrocarbon solution

Preparation of Grignard compounds soluble in benzene and toluene, of academic and commerical value, has been reported. The method involves the reaction of an alkyl or aryl halide and magnesium turnings in benzene in the presence of an equimolar amount of a tertiary amine. The use of tertiary amine as the complexing agent appears to be successful owing to the non-disproportionation tendency of RMg X species when complexed with a tertiary amine.

The Grignard compounds of aliphatic and aromatic halides are obtained in good yields. The reactions are slightly more difficult to initiate than the corresponding reactions in diethylether and the reaction normally starts at room temperature to 50° and is maintained at 50° during reaction.

The tertiary amine of choice is triethylamine, although other tertiary amines, viz. tri-*n*-propylamine, tri-*n*-butylamine should also work well. Trimethylamine is not preferred due to the low boiling point which makes the reaction difficult to start. Dimethylaniline has the disadvantage that not all Grignard compounds complexed with this amine are soluble in benzene. This new method eliminates the use of the more expensive and hazardous diethylether [*J. org. Chem.*, **31** (1966), 971].

Lubricants based on iodine

Iodine is finding increasing use as lubricant and cutting oil additive. As little as 0·6 per cent iodine additive in common hydrocarbon oils allows titanium and stainless steel bearings to operate without seizing. The largest use of iodine additives is expected to be in the metalworking field where alloys of titanium, stainless steel, nickel and cobalt are being increasingly used, such as in jet aircraft turbine blades, nuclear reactor components, heat exchangers and other process equipment, such as pumps and vessels.

The use of iodine as lubricant is based on its reaction with clean titanium and iron, under favourable conditions, to form diiodides. The crystal structure of both titanium diiodide and iron diiodide is lamellar like that of graphite and, therefore, when subjected to a stress, the crystal layers slide easily in one direction. The actual use as lubricant involves the generation of diiodides *in situ*, at the wearing surface between two sliding surfaces. Elemental iodine being only slightly soluble in hydrocarbon oils cannot be used as such. When iodine is combined with aromatic compounds, such as *n*-butylbenzene, a charge-transfer complex is formed which makes iodine available at the wearing surfaces, causing marked increase in lubricating properties. The iodine complexes formed can operate effectively at interfacial temperatures in excess of 400°C., almost twice as high as the best long-chain hydrocarbon oils [*Chem. Engng*, **73** (2) (1966), 102].

Nuclear batteries

Two simple devices are under study at the University of Illinois for converting nuclear energy directly into electric power. In the first device, the fission electric cell, one electrode is coated with a thin layer of a fissionable fuel, such as uranium. Fission occurs in the fuel layer when it is bombarded by neutrons, and some of the fission fragments escape the layer, cross the vacuum gap and strike the other electrode. Since each fragment is positively charged, a voltage potential (sometimes as high as millions of volts) builds up between the two electrodes. This high voltage, low amperage power is suitable for operating space engines, microwave radio equipment, etc.

The other device, the gamma cell, is simpler in construction and produces electric energy from gamma radiation. The cell consists of a gamma ray source, two metallic electrodes, and a separating insulator. Gamma rays emitted from the source scatter electrons in the insulators, pushing them to the collector electrode, which accumulates a negative potential relative to the emitter electrode. The emitter and collector thus become the electrodes of a high voltage power supply. Because of its relatively low efficiency as a power converter, its potential field of application is as a power-salvaging shield for space reactors [*Mech. Engng*, **87** (12) (1965), 48].

β-Carotene by fermentation

There are three methods known so far for the production of β-carotene. (i) The classical method involves the production of β-carotene and other carotenoids by solvent extraction from plant sources such as carrots, palm oil and alfalfa. (ii) The synthetic method employs acetone and acetylene in ammonia as starting materials. These are reacted to

give methylbutynol which is converted to methylheptenone and eventually to pseudoionone by partial hydrogenation, condensation (with diketene) and pyrolysis. Pseudoionone is converted directly to vitamin E or to β-ionone. β-carotene and vitamin E are made from β-ionone. (iii) The third, also a synthetic method, consists in coupling two molecules of retinal. Retinal reacts with hydrogen sulphide to form thiapyrane, which desulphurizes to β-carotene when heated with amalgamated zinc.

Recently Pfizer International has studied the production of β-carotene by fermentation using mated strains of *Blakeslea trispora* in the inoculation step. These microorganisms are added to a medium containing (on percentage basis) cotton seed embryo meal, 4; corn meal, 2; white grease oil or vegetable oil, 3; deodorized kerosene, 3; citrus molasses, 5; and thiamine hydrochloride, *c.* 0·002. An inoculum of 5 per cent (by volume) of the medium to be fermented is used; incubation temperature is 28°C. and the time of fermentation is 72 hr. The solids would be removed by filtering the product through a continuous rotary filter and drying the filter cake on an atmospheric double-drum drier. The product yield would be 5·06 lb. of moisture-free solids per 100 lb. medium. Yield of β-carotene would be 1 lb. per 59 lb. of solids; 1 g. of β-carotene is equivalent to 1,666,666 U.S.P. of vitamin A and the price of β-carotene is related to units of vitamin A [*Chem. Engng News*, **44** (8) (1966), 44].

Preparative starch-gel electrophoresis

A convenient method for the recovery of protein components in high yield by continuous elution during starch-gel electrophoresis has been reported [*Biochim. biophys. Acta*, **115** (1965), 81]. A standard starch-gel mould is modified by drilling two tapered elution channels (holes) through the walls of the mould to allow the flow of buffer at right angles to the direction of electrophoretic migration. After an initial period of electrophoresis, the section of starch-gel between the holes is excised and the anodal wall of the trough formed covered with a plastic sheet. Electrophoresis is then continued with a transverse flow of buffer which removes the proteins in the order of their electrophoretic mobility. The method has been successfully employed in the isolation of human thyroxine-binding prealbumin and in the preparation of electrophoretically homogeneous ^{131}I-labelled thyroxine-binding prealbumin and human serum albumin for turnover studies in man.

Progress Reports

National Research Council, Canada

The National Research Council Canada Review for 1965 records the research activities of the various science and engineering laboratories, Medical Research Council and different Associate Committees during 1964-65.

During the year under review, a new Churchill Research Range Branch was set up to administer the space research programmes at the rocket launching site at Fort Churchill, Man, to be conducted in collaboration with the US Aeronautics and Space Administration.

New chromatographic methods, that give essentially complete recovery, have been developed for the separation of lipovitellin and phosvitin in the amphibian yolk platelet, and α-lipovitellin, β-lipovitellin and phosvitin from hen's egg yolk.

Research in the field of carbohydrate chemistry has resulted in the isolation of four polysaccharides from the capsular layer of *Serratia marcescens* cells grown on a sucrose medium. Analytical data indicate that the polysaccharides are an acidic glucomannan, a rhamnoglucan, a glucoheptan and a rhamnoheptoglucan. New methods for the analysis of hexuronic acids and aldoses have been developed.

^{14}C incorporation studies in rapeseed proteins have led to the isolation of two well-defined protein components which together contain about 40 per cent of the protein nitrogen. It has been shown that the rapeseed proteins, unlike those of wheat kernel, are extractable in cold water and these extracts can be chromatographed directly avoiding denaturation.

Work done on organic crystal semiconductors has established that photocurrents are generated in anthracene crystals by excitation of the triplet level at 14,750 cm.$^{-1}$. It has been discovered that by using a solution of anthracene negative ions as the negative electrode on an anthracene crystal, electron injection into the crystal occurs with high efficiency.

The structures of a number of positive and negative aromatic ions, prepared for the first time, have been established by proton magnetic resonance measurements. Single crystals of several aromatic radical ions have been obtained for the first time. These crystals which are completely ionic in the ground state, include alkali salts of aromatic anions and the 1:1 salt of tetracyanoquinodimethane with tetramethyl-*p*-phenylenediamine.

The structures of two new diterpenoid alkaloids, chasmanine and homochasmanine, have been determined. A convenient method of preparing di-(1,1-dideuteroethyl)-amine in 70 per cent yield from diacetamide and lithium aluminium deuteride was discovered.

It has been shown that the seed oil of *Helenium bigelwii* (Compositae) contains *trans*-3-hexadecenoic acid, previously unknown in seed oils, as a major component. Seed oil of *Doxantha unguis* (Bignoniaceae) has been shown to contain 64 per cent of *cis*-9-hexadecenoic acid in its glycerides.

High flow porous cellulose acetate membranes capable of yielding potable water from sea water at the rate of 27 gal./day/sq. ft at operating pressure of 1500 lb./sq. in. have been developed.

A new method of measuring the virial coefficients of the vapours of liquids has been devised; its main advantage is that the volume of the vessel containing the vapour is not measured independently of the equation of state. This would be most useful in high temperature studies.

Van Allen radiation belts have been studied by the satellite 'Alouette' which is in a nearly polar orbit. New information on its variation with time, with geomagnetic or solar activity, and lifetimes of trapped particles in the outer layer has been obtained.

Plasma physics studies have demonstrated that a value for

the axial magnetic field in a θ-pinch can be obtained from the rotation of the plane of polarization of a He-Ne laser beam passing through the plasma. Theoretical studies have shown that the (P, Pα) and (α, 2α) reactions can provide information about alpha-particle clustering of nucleons in the nucleus. A formalism has been developed for these reactions at medium energy, and the resulting theory has been applied to the ^{12}C (P, Pα) and ^{9}Be (α, 2α) reactions.

X-ray diffraction study of pascoite has revealed the structure of decavanadate ion $V_{10}O_{28}^{6-}$, which has a rock-salt type structure with *mmm* symmetry.

A new cesium resonator has been constructed and compared with travelling standards of frequency and time; it enables to relate time as kept in the Division of Applied Physics with scales of 'atomic' time in other laboratories of NRC to within a few microseconds.

A hybrid simulation of an orbiting earth satellite has been developed. An analogue simulation of the dynamic behaviour of various passenger vehicles has been developed with a view to predicting fuel economy and performance under various situations.

A cheap and safe method of quick wear assessment of cylinder liners and piston rings in diesel engines has been developed. It comprises sampling the cylinder oil through small holes in the liner and analysing it spectrographically.

On the basis of experimental investigation carried out in the Low Speed Aerodynamics Section, a simple theory has been developed to predict the burst point of a streaming vortex from the edge of a narrow wing or fuselage, in terms of the pressure field traversed by the vortex. A fluid amplifier anemometer for the measurement of very low airspeeds has also been developed. Analytical methods have been developed for determining the effects of viscosity on the static and dynamic stability derivatives of simple two- and three-dimensional bodies.

Studies carried out to determine the effect of sorbed water on the mechanical properties of Portland cement pastes and compacts have shown that there is a definite relation between Young's modulus and porosity and also between strength and porosity. An apparatus has been fabricated to determine the translucency of gravels, marble, air-cooled slag, and expanded slag. A study of the mortar-making qualities of sands indicated higher water requirements resulting in mortars with lower strengths and higher drying shrinkage for the traditionally used black sands, as compared to a well-graded pit sand. Higher air contents occurred when these fine black sands were combined with masonry cements.

New Periodicals

Journal of Computational Research

The Academic Press has commenced this new quarterly. It is devoted to the computational aspects of physical problems, the techniques involved in the numerical solution of mathematical equations and in automatical data reduction. The annual subscription rates are $ 10.00 for individuals and $ 25.00 for institutions.

Siberian Mathematical Journal of the Academy of Sciences of the USSR, Novosibirsk

Starting from Volume 7 (1966), an English translation of this bimonthly, originally issued by the Siberian Section of the USSR Academy of Sciences, Akademgorod, is being published by Consultants Bureau, New York 7. The annual subscription of the English version of the periodical is $ 150.00.

Earth and Planetary Science Letters

This bimonthly journal being started by the North-Holland Publishing Co., Amsterdam, will publish articles on various subjects connected with the development of the earth in time and its relation to the planetary system, viz. geology, geophysics, marine science, meteorites, planetary system, etc. Annual subscription for the journal is $ 16.00.

Announcements

▪ *A Symposium on Electrode Processes*, sponsored by the University Grants Commission, will be held at the University of Jodhpur during 1-5 November 1966. The intending participants should send a short summary of their papers to Prof. R. C. Kapoor, Head of the Department of Chemistry and Director of the Symposium on Electrode Processes, University of Jodhpur, Jodhpur, so as to reach him before 31 August 1966.

▪ *The Twelfth International Congress of Refrigeration* will be held at Madrid, during 30 August-6 September 1967, under the auspices of the International Institute of Refrigeration. The following subjects will be covered in the three plenary sessions: (i) Low temperatures in the generation and transmission of electric power; (ii) Latest developments in insulating materials and techniques; (iii) Liquefaction, storage and transport of natural gas; (iv) Refrigeration as applied to the desalination of sea water and brackish water; and (v) Aids to refrigeration for the preservation of perishable foodstuffs.

Intending participants should submit a brief review of 200 words in English, French or Spanish so as to reach the General Secretariat of the Congress at Madrid, before 1 January 1967.

▪ *The Lady Tata Memorial Trust* have announced the annual awards of scholarships and grants for the years 1966-67. The international awards, totalling £ 9000, for research in diseases of the blood with special reference to leukaemias have been given to Dr A. Agostoni, Dr D. Quaglino, Dr F. Squartini, Dr G. Tridente and Dr L. Mazzarella (all of Italy), Prof. L. Israel, Dr J. Reviron and Dr D. Viza (all of France), Dr V. Balazs and Dr M. M. Frohlich (Holland), Dr M. Matsuyama (Japan), Dr R. Hancock (Switzerland) and Dr R. P. Hill (Sweden).

The Indian scholarships for investigations having a bearing on the alleviation of human suffering from disease are awarded to Dr P. G. R. Pillai, Dr Jagdish Chandra Sharma, Dr A. P. Mehrotra, Dr Anjan Chakraborty, Dr (Miss) Usha C. Parekh, Shri Selvarajan, Shri Deshpande, Miss D. K. Manghani, Miss R. V. Prabhu and Miss Dipti Chaudhuri.

RESEARCH ELECTROMAGNET

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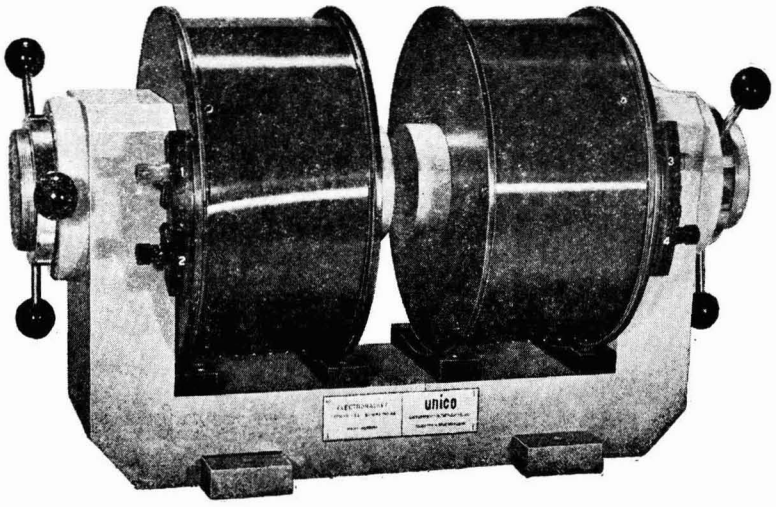

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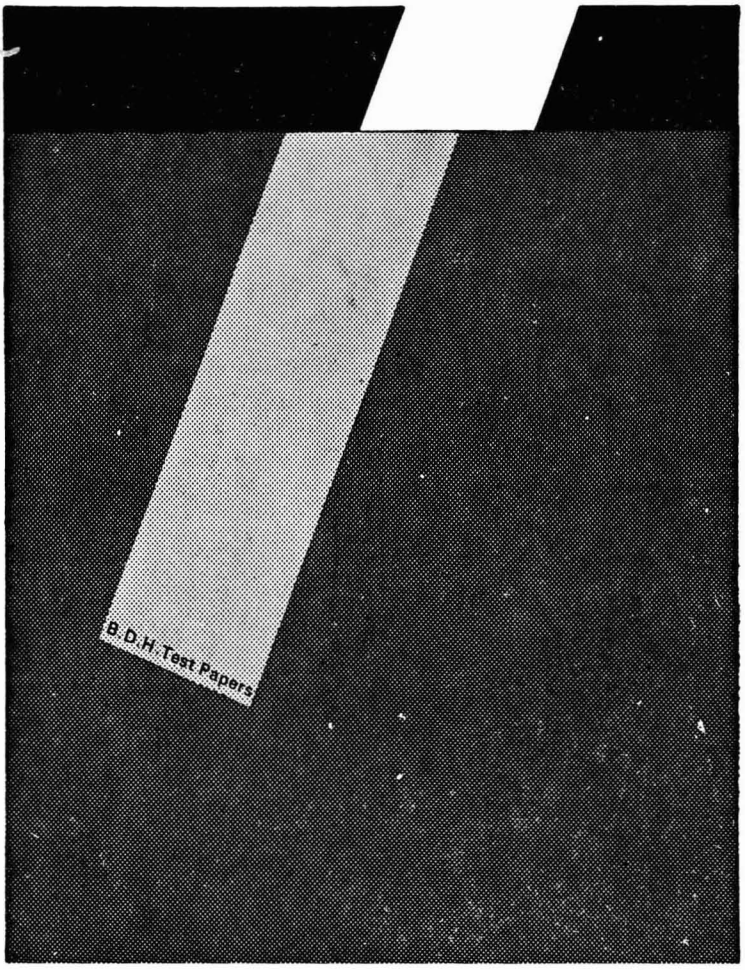

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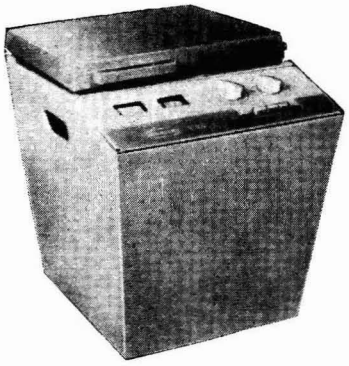

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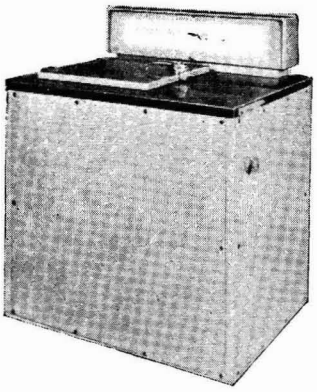
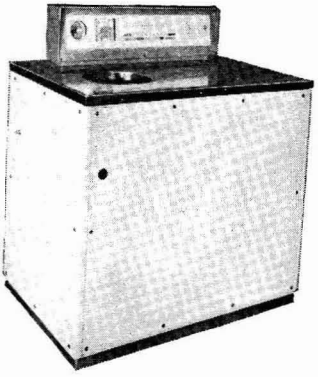


TYPE S 60



TYPE VAC 60

JANETZKI CENTRIFUGES

a synonym for quality!

for medical, chemical and biological laboratories

Laboratory sugar centrifuges Type TZ 1

Rotating speed	2900 r.p.m.
Field of gravitation	520 g

Micro-haematocrite centrifuges Type TH 1

Rotating speed of	
Haematocrite rotor	max. 16,000 r.p.m.
Microrotor	max. 20,000 r.p.m.
Field of gravitation	
Haematocrite rotor	max. 18,500 g
Microrotor	max. 18,000 g

Laboratory table centrifuge Type T 4

Rotating speed	max. 3200 r.p.m.
Field of gravitation	max. 1200 g

Laboratory table centrifuge Type T 20

Rotating speed	max. 6400 r.p.m.
Field of gravitation	max. 4500 g

Laboratory table centrifuge Type T 22

Rotor freely swinging	max. 3400 r.p.m.
Angle rotor	max. 4800 r.p.m.
Field of gravitation	
Rotor freely swinging	max. 2000 g
Angle rotor	max. 3250 g

Laboratory table centrifuge Type T 23

Rotating speed	
(4×100 ml)	max. 6000 r.p.m.
(6×100 ml)	max. 6500 r.p.m.
Field of gravitation	
(4×100 ml)	max. 5600 g
(6×100 ml)	max. 5800 g

Laboratory table centrifuge Type T 24

Rotating speed	max. 16,000 r.p.m.
Field of gravitation	max. 21,500 g

Stand centrifuge Type S 60

Rotating speed	
Rotor freely swinging	max. 3500 r.p.m.
Angle rotor	max. 4500 r.p.m.
High-speed attachment	max. 16,000 r.p.m.
Field of gravitation	
Rotor freely swinging	max. 2600 g
Angle rotor	max. 4000 g
High-speed attachment	max. 21,500 g

Cooling centrifuge Types K 60 and K 60 S (similar to Type S 60)

Type K 60 with cooling aggregate	500 cal/h	
Type K 60 S with cooling aggregate	1250 cal/h	
Minimum temperatures	K 60	K 60 S
Rotor freely swinging		
4×1000 ml	±0°C	−15°C
4×250 ml	−2°C	−22°C
Angle rotor		
8×250 ml and 6×500 ml	−5°C	−25°C
High-speed attachment		
Rotor 6×20 and 12×10 ml	±0°C	−8°C
Rotating speed		
Rotor freely swinging	max. 3500 r.p.m.	
Angle rotor	max. 4500 r.p.m.	
High-speed attachment	max. 16,000 r.p.m.	
Field of gravitation		
Rotor freely swinging	max. 2600 g	
Angle rotor	max. 4000 g	
High-speed attachment	max. 21,500 g	

Preparative ultracentrifuge Type VAC 60

Rotating speed	max. 60,000 r.p.m.
Field of gravitation	max. 300,000 g
Vacuum in the rotor chamber	5×10^{-2} Torr
Minimum temperature in the rotor chamber	−20°C

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