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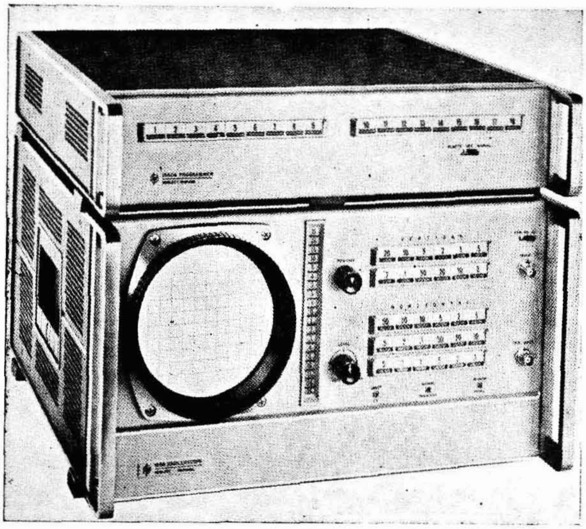

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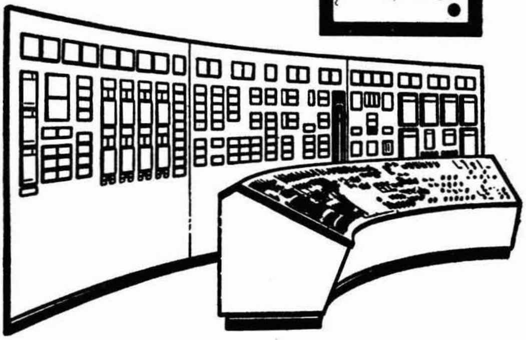

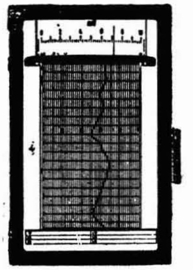

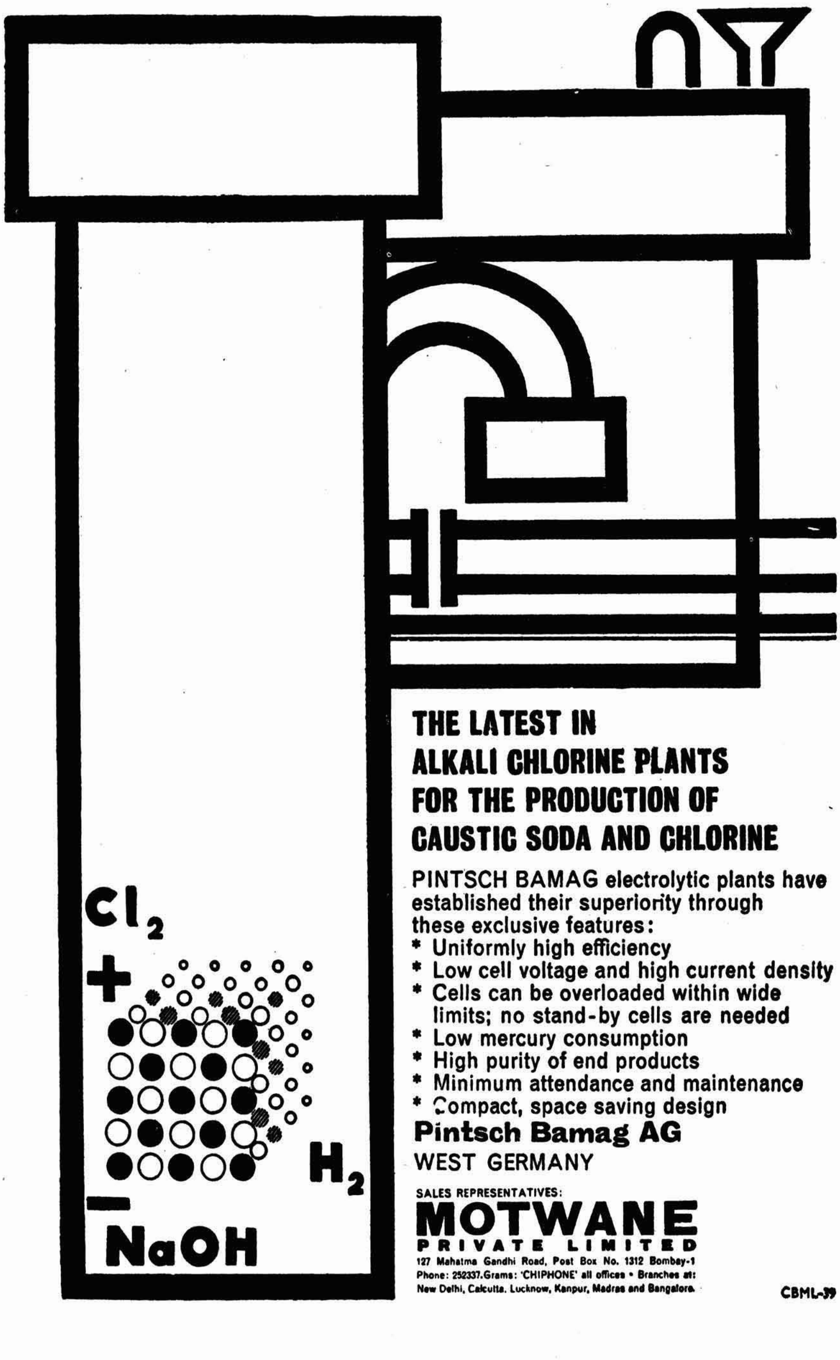
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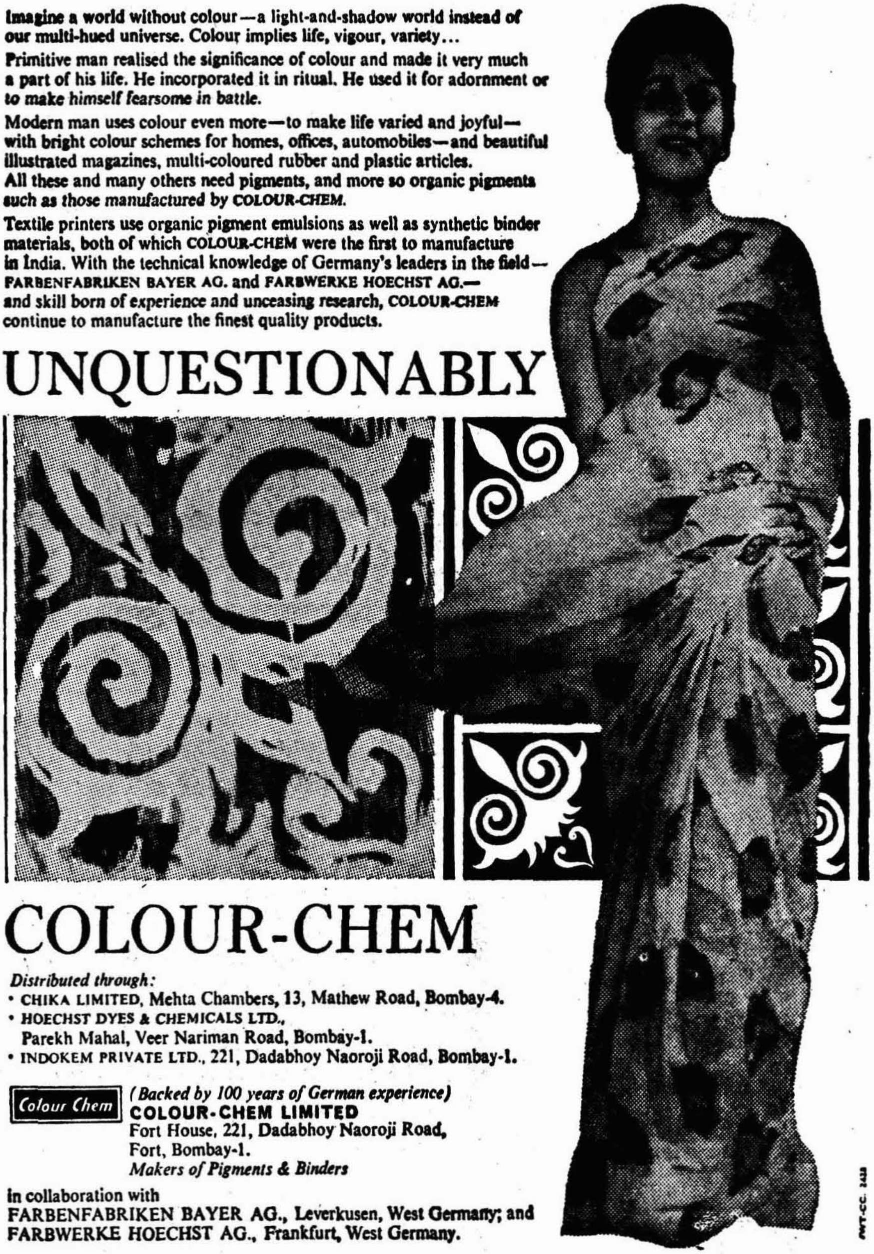

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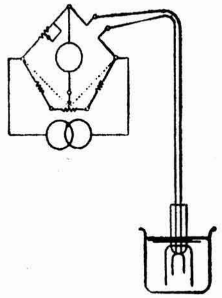

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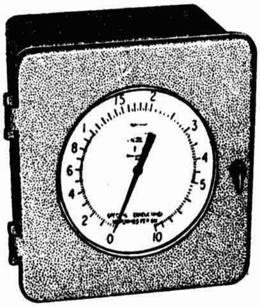

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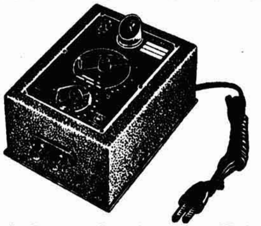

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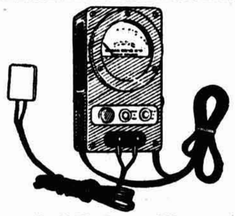

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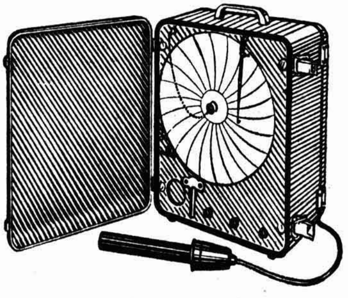

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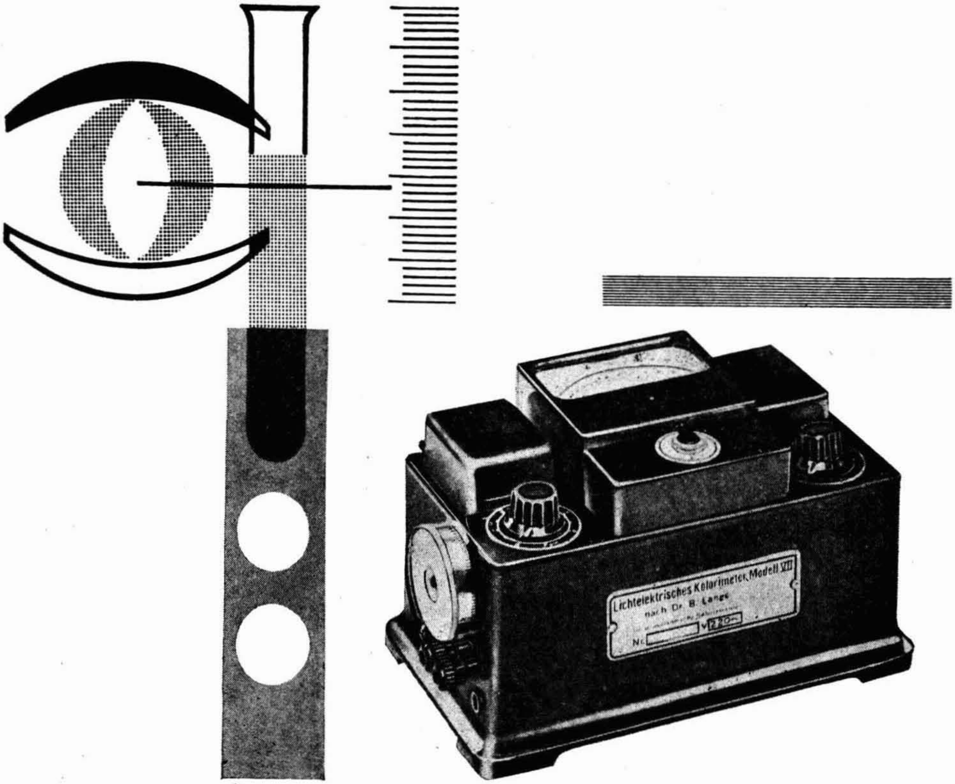
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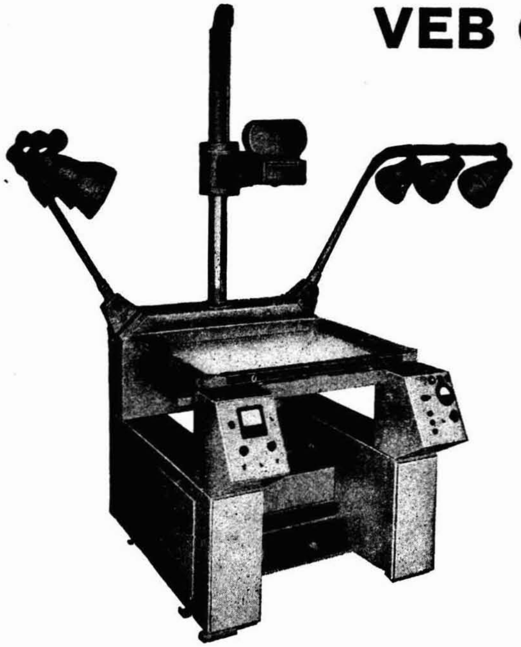

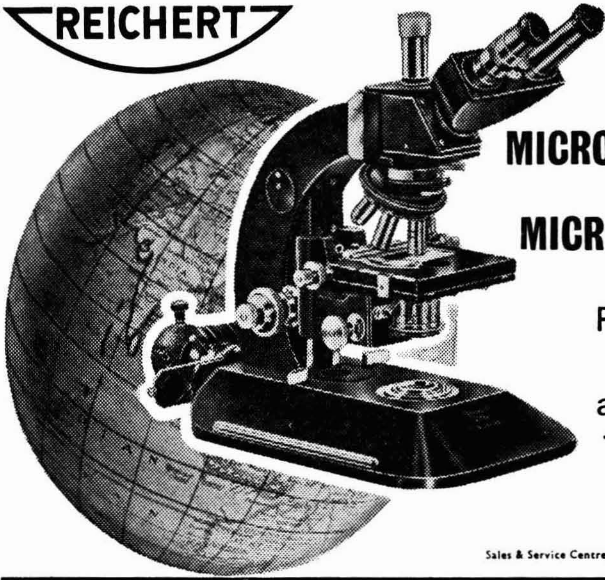

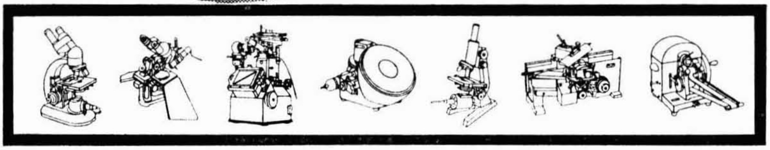

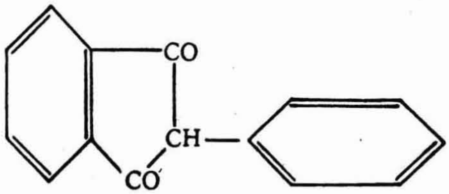
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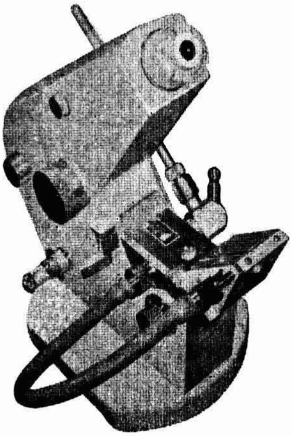

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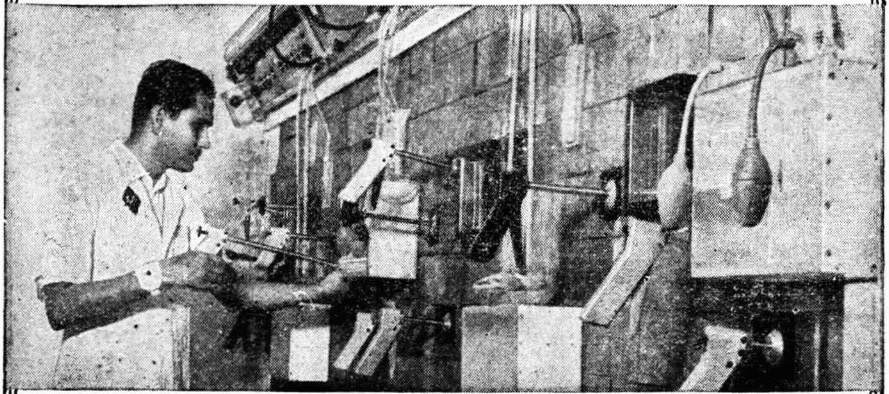

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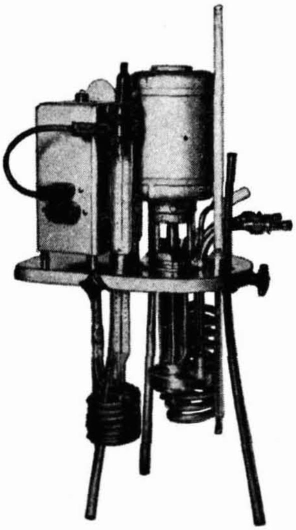

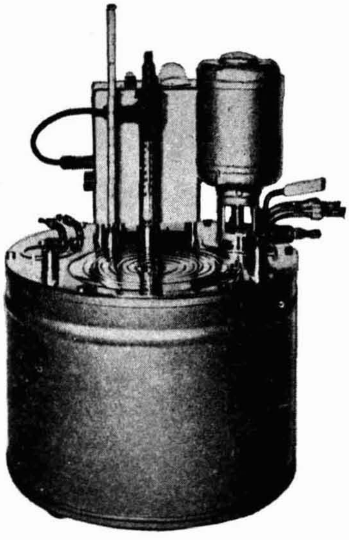

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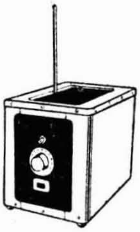
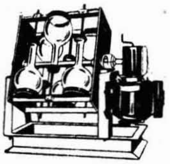




Dr Atma Ram

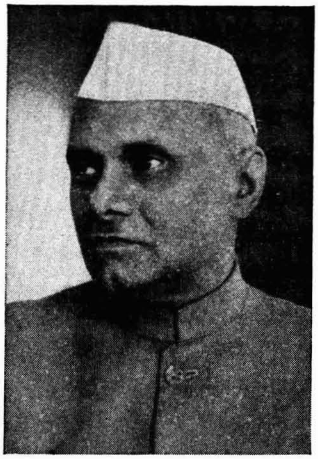
DR ATMA RAM, Director, Central Glass & Ceramic Research Institute, Calcutta, has been appointed Director-General, Scientific & Industrial Research. He took charge on 22 August 1966.

Born on 12 October 1908 at Pilana, District Bijnor, UP, Dr Atma Ram had a brilliant academic career at the University of Allahabad. He was awarded the degree of Doctor of Science in 1936 by the Allahabad University for his fundamental researches on photochemical reactions.

Dr Atma Ram started his career as a member of the research team, headed by the late Dr S. S. Bhatnagar, in the Industrial Research Bureau and subsequently joined the Council of Scientific & Industrial Research (CSIR). In 1945, the Governing Body of CSIR entrusted to him the responsibility of organizing the newly started Central Glass & Ceramic Research Institute at Calcutta and he was appointed Director of the Institute in 1952. Under his inspiring leadership, the technology of producing optical glass has been developed solely by indigenous effort and the Central Glass & Ceramic Research Institute, Calcutta, has been producing optical glass since 1960 in quantities sufficient to meet the entire requirements of the country. Of the many contributions which the Institute has made under his initiative and guidance, mention may be made of the production of foam glass and selenium-free red glass, utilization of waste mica for the manufacture of insulating bricks, and a technique for wet grinding of mica, many of which have given rise to new industries in the country. On the fundamental side, investigations on the constitution of glass and the origin of colour in copper-red glass are noteworthy.

Dr Atma Ram is the author of about a hundred scientific and technical papers in the field of physical chemistry, photochemistry, glass and ceramics. He has also a number of patents to his credit. He is the author of a book in Hindi on *History of Chemistry*.

Dr Atma Ram was honoured with Padma Shri in 1959 by the President of India for his distinguished services to the country. He has been the first recipient of the Shanti Swarup Bhatnagar Medal (1959) of the National Institute of Sciences of India for his outstanding contributions to the development of technology and creating science consciousness in the glass and ceramic industry in the country. Dr Atma Ram is also the first recipient of the Uttar Pradesh Scientific Research Committee Gold Medal and Cash Award, and the K. G. Naik Gold Medal of the M.S. University of Baroda. In recognition of his distinguished services to the cause of the industry, the All-India Glass Manufacturers' Federation awarded a Plaque of Honour to him in 1964.



Dr Atma Ram

Dr Atma Ram is a fellow of several learned societies and scientific institutions in the country and abroad. Recently, he was elected Honorary Fellow of the Society of Glass Technology, Sheffield, one of 6 such fellows from UK, USA and Sweden. He is a Fellow of the National Institute of Sciences of India, Institution of Chemists (India) and the Indian Chemical Society. He was elected member of the International Commission on Glass in 1948, and Honorary Member of the International Academy of Ceramics, Geneva. He was nominated member from India on the Commission on Chemistry of High Temperatures of the International Union of Pure & Applied Chemistry. He was elected President of the Indian Ceramic Society for 1952-53. Dr Atma Ram was President of the Indian Science News Association for 1965-66; he has been re-elected President for 1966-67. He is the General Secretary of the Indian Science Congress Association and Vice-President of the National Institute of Sciences of India. Dr Atma Ram has been associated with the work of the Publications & Information Directorate as a member of the Editorial Board of the *Indian Journal of Technology* since its inception.

Cosmological Theories*

J. V. NARLIKAR

A FEW years ago a discussion of cosmological theories would have been almost entirely theoretical. Today, thanks to the improvement in the observational situation, we can also consider a number of possible checks on the theories. Although there is no clear-cut distinction between the two it is convenient to divide the discussion into two classes, viz. theoretical and observational.

Theoretical Considerations

Singularity — The equations of classical general relativity have so far failed to yield nonsingular cosmological models. The homogeneous-isotropic models have a singular origin. The so-called big bang models oscillating between radii have not been possible to obtain. Dropping isotropy leads to shear and rotation. Raychaudhuri's equation shows that shear helps and rotation prevents the singularity. However, no nonsingular model has been obtained. An investigation by Penrose for finite objects shows that dropping homogeneity and isotropy is not likely to prevent singularity. This has been extended to the universe (which may be infinite) by Hawking, Ellis and Carter. One of the requirements is that $T_{ik}U^iU^k$ be positive definite for all time like vectors U^i. That singularity can be prevented if this condition is violated is shown by the explicit solution of Hoyle and Narlikar using the C-field. If the C-field is creating matter it leads to the nonsingular steady state universe. If no matter is being created the universe (or a finite massive object) oscillates between two finite radii. Thus an oscillating model is possible if one is willing to use a negative energy field like the C-field.

The age of the universe — Associated with the problem of singularity is the problem of age. Assuming that a big bang universe came into existence at $t=0$, and that we confine physical discussion only to $t>0$, what is the ' age ' of the universe at present? The answer in most cases is not more than H^{-1}, where H is the Hubble constant. The observations show $H^{-1}\sim 10^{10}$ years. This is much too small, for the estimated age of our galaxy is $\sim 1\cdot 5\times 10^{10}$ years and galaxies older than our own are also likely to exist. Unless the galactic ages are wrongly computed or H is wrongly measured or both, there is a serious discrepancy.

The discrepancy is present in the steady state model also, although not in an acute form. Although the universe is infinitely old and can have arbitrarily old galaxies in it, the average age is $\frac{1}{3}H^{-1}$ for all galaxies. Why do we observe galaxies much older than this in our neighbourhood? We may answer this only when a reasonable theory of galaxy formation is available.

Dicke has attempted to reduce the discrepancy in the big bang case by having a theory of gravitation in which the gravitational constant changes with the epoch.

The arrow of time—The Wheeler-Feynmen theory has been applied by Hogarth and by Hoyle and Narlikar to cosmological models. It turns out that retarded solutions are consistent in the steady state model and advanced solutions in the big bang Einstein-de Sitter model. Thus the electrodynamic arrow of time agrees with the cosmological arrow in the former but not in the latter model. Similar results have been obtained by other methods by Penrose for fields with spin $>1/2$, for neutrino by Narlikar, and for gravitational radiation by J. Krishna Rao and P. C. Vaidya.

Mach's principle — The observational result behind Mach's principle is explained in the big bang theories by choosing suitable initial conditions at $t=0$. The need for this choice may not arise in the steady state model because creation of matter would tend to bring about homogeneity and isotropy. Hoyle and Narlikar have demonstrated this conjecture to be true in the case where the departure from homogeneity and isotropy is small.

Observational Considerations

Magnitude of the red shift—The magnitude of the red shift for distant galaxies would, in principle, measure the Hubble constant and the deceleration parameter:

$$H=\left(\frac{\dot{S}}{S}\right)_{t=t_0} \qquad qH^2=-\left(\frac{\ddot{S}}{S}\right)_{t=t_0}$$

where t_0 is the present epoch. Sandage's observations indicate $q\simeq 1$. The steady state value is $q\simeq -1$. Thus $q=1$ would favour a universe which is slowing down and which would contract later. In practice, there are many uncertainties of observations and it is not yet possible to draw a definitive conclusion from this test.

Counting of radio sources — The Cambridge survey gives a slope of log N—log S curve (here N is the number of extragalactic sources brighter than flux level S) to be $\sim -1\cdot 8$. The steady state value is $-1\cdot 5$ for large S, and diminishing in magnitude as S decreases. The data on their face value indicate a larger source density in the past than now — against the steady state prediction.

Veron has found that the slope is made up of two different components. For radio galaxies the slope is $-1\cdot 56$, and this would not contradict the steady state theory within observational errors. For quasi-stellar radio sources the slope is $-2\cdot 2$. If the quasi-stellars are distant objects this is evidence heavily against the steady state theory.

Neutral hydrogen density — The continuum of the source 3C-9 with large red shift $Z=2\cdot 01$ is only partially absorbed on the blueward side of Lyman-α. This means that if the source is distant the

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*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.

intergalactic medium contains very little neutral hydrogen—about $\sim 10^{-34}$ g./cc. The cosmological models predict a density of matter about 10^5 times this value. Ionized hydrogen might exist at this density value if the intergalactic medium has electron temperature as high as 10^5-10^6°K.

The three tests detailed above will have valuable cosmological significance if the quasi-stellar objects are distant. They have very little importance if these objects are local, being thrown out of our galaxy or a nearby source. This issue has not yet been resolved.

Background radiation — Observations by Penzies and Wilson at Bell Telephone Laboratories show the universe to have a radiation temperature ~ 3°K. at 7 cm. Similar results are indicated in the observations made at Princeton. This radiation could be accounted for if the universe had a high temperature-density phase when radiation was predominant. The steady state model cannot account for such a temperature yet.

Helium abundance — The abundance of elements can be explained in terms of synthesis in stars as shown by Burbidge, Burbidge, Fowler and Hoyle. However, the He/H ratio on this basis turns out to be only 0·01 as compared to the observed value in the range 0·09-0·18. The high helium abundance can be explained by imagining the matter in the universe to have passed through a high temperature ($\sim 10^{10}$°K.) phase when radiation was most important. This is possible in a radiation universe as pointed out by Tayler and Hoyle. They also account for this ratio in a steady state universe by cooking helium inside massive superstars. But this requires a large number of such massive objects in the universe.

Conclusion

One of the advantages of the assumption that the universe was more dense and hotter in the past than now is that the formation of galaxies can be explained very satisfactorily, as pointed out by Hoyle. Indeed the theory of galaxy formation works out so satisfactorily that Hoyle was tempted to give up the simple steady state theory he had put forward in 1948.

What are the alternatives? An oscillating universe would be a possibility but it has thermodynamic difficulties. What happens to the arrow of time? Why are the oscillations not damped already, if the universe is infinitely old? These questions have not been answered.

We can overcome these difficulties if the oscillations are not over the whole universe but over finite regions only. Hoyle and Narlikar have considered such a model which behaves like a dense steady state universe in which creation of matter takes place in pockets of strong gravitational field (e.g. near massive objects). In regions where there are fewer pockets, creation is ultimately cut off and such regions expand and contract like bubbles. We may be living inside such a bubble. What lies outside such bubbles is indicated by elliptical galaxies which are bubbles that did not expand fully owing to the attraction of masses in the nuclei of the galaxies.

Symposium on Comparative Endocrinology

L. S. RAMASWAMI

Department of Zoology, University of Rajasthan, Jaipur

A SYMPOSIUM on Comparative Endocrinology, the first of its kind to be held in India so as to serve as a common forum for the large number of workers in the field, was organized under the joint auspices of the National Institute of Sciences of India and the University of Rajasthan, Jaipur, during 23-30 December 1965. Thirty-four papers were read and discussed during the symposium.

Effect of Adrenalectomy

P. Seth and M. R. N. Prasad (University of Delhi) reported in their paper that adrenalectomized squirrels (*Funambulus*) develop adrenocortical-like tissue in the extra-ovarian region close to the tunica, and the cortex also develops cells of this type in the atretic follicles. The effect of ACTH on this new tissue was discussed.

In another paper, A. H. Reddi, M. R. N. Prasad and S. Duraiswami (University of Delhi) discussed the formative sites of fructose and citric acid in the male accessory glands of *Funambulus*; the seminal vesicle produces only fructose while the compact prostate produces both citric acid and fructose.

Lipogenesis in the kidney of *Hemidactylus* after testosterone administration was discussed by M. N. Sanyal and M. R. N. Prasad (University of Delhi) with particular reference to the sex segment. Choline containing phospholipids are present in the sex segment in large amounts; the various constituents have been confirmed by ^{32}P turn over studies. The phosphatides in the testosterone induced sex segment in both sexes are similar to those that the male sex segment normally develops. Sperm survival depends on the sex segment secretions. Androgens are also found to be lipogenic.

Clomiphene Studies

The effect of clomiphene, a blastotoxic agent, on the male rats was discussed by S. P. Kalra and M. R. N.

Prasad (University of Delhi). Spermatogenesis is inhibited when clomiphene is administered in doses of 250 μg. a day or higher doses for long periods and then stopped. Leydig cells atrophy and the accessories are thus put out of action. Withdrawal of treatment causes the gland to resume its activity within 30 days. The sperms are fertile. The effect of clomiphene as a temporary suppressor of spermatogenesis may be due to its estrogenic activity suppressing the flow of gonadotropins.

Clomiphene on Gerbil

The effect of clomiphene (MRL 41) has been studied on the reproductive glands of male and female gerbils (*Meriones*). D. K. Kaul and L. S. Ramaswami (University of Rajasthan, Jaipur) reported a reduction in normal ovarian weights after clomiphene treatment in unilaterally ovariectomized female gerbils receiving a total dose of 0·75 mg. either subcutaneously or intratesticularly. Though compensatory hypertrophy of the ovary is checked in the animals, an increase in uterine weight is observed. The males also show a reduction in weight of the testes and spermatogenesis is inhibited variably while the accessories are unaffected.

Portal Circulation in Fish

The hypothalamo-hypophysial vascular system of the minnow, *Phoxinus*, has been critically examined by H. N. Bhargava (University of Saugar). Since there is no nervous contact between the adeno- and neuro-hypophysis, the influence of the latter appears to be hormonal. In the minnow, there is no portal system and the blood system is therefore systemic. There is a rich vascularization of the stalk region and also above it. The author felt that the region above the stalk containing Gomori positive bodies could not be homologized with the median eminence of higher vertebrates. There is no direct arterial blood supply to the adenohypophysis, and there is only a tiny venous drainage. Since the blood vessels lie within the neurohypophysis, a ramification of the latter in the adenohypophysis is considered necessary. The central longitudinal sinus is enlarged during prespawning and spawning periods.

Thyroid Gland

The functions of the thyroid cell are not clearly understood, particularly with reference to the synthesis of the colloid. The colloid is held to be the exclusive site of its synthesis. E. Raghupathy (Madras Medical College) reported that dispersed cultures of thyroid cells obtained by continuous flow trypsinization show most of the characteristics of the thyroid gland. They concentrate iodide, form protein-bound iodotyrosines and iodothyronines, and deiodinate iodotyrosines and not iodothyronines. When cultivated as monolayers, they reorganize and show structures similar to the thyroid gland. The results show that protein iodination occurs in the follicular cells and that if iodide secretion into extracellular colloid does occur, it is not an essential aspect of iodine metabolism. He also summarized the results obtained from iodine metabolic studies in *Branchiostoma*, *Eptatretus* (hag-fish), *Taricha*, *Amphiuma*, *Necturus* and *Pituophis* (snake).

Thyroid-gonad Axis in Birds

The relationship between the thyroid gland and gonads in the birds has been receiving considerable attention these days. It is known that wild and domestic birds vary in their reactions. D. K. Vyas and L. S. Ramaswami (University of Rajasthan, Jaipur) using the crow and pigeon noted that both surgical and radiochemical thyroidectomy during the non-breeding season retain the gonads in a regressed condition and if done during the sexually active season, the gonads regress and continue to remain regressed. They pointed out that this relationship is different when compared with the findings in wild birds.

Hypothyroidism in Lower Vertebrates

It is known that hypothyroidism considerably interferes with the reproductive physiology in the lower vertebrates. H. B. Sarkar and M. A. Rao (University of Mysore) discussed results of *in vitro*, *in vivo* and transplantation experiments on *Rana*. The results indicate that thiourea treatment or thyroxine feeding to normal control frogs has an inhibitory effect on ovulatory and spawning response both *in vivo* and *in vitro*. Transplantation studies indicate that thiourea affects the body system and thyroxine affects the ovaries. But thyroxine feeding to thiourea treated frogs has a normalizing effect both *in vivo* and *in vitro*. The possibilities of both hypothyroidism and hyperthyroidism interfering with production and release of pituitary hormones and thus affecting the reproductive capacities through pituitary-adrenal gonad axis were discussed.

Gonadotropins

The presence of a FSH inhibitor in the monkey (*Macaca*) has been brought out in a convincing way by N. R. Moudgal (Indian Institute of Science, Bangalore) by an examination of refrigerated urine from the animal. Several investigators have reported the presence of such FSH inhibition in human urine. All age groups, of both sexes and even castrates excrete the inhibitor which has been found to be a non-dialysable, heat labile protein fraction. This does not inhibit the LH activity as tested by ovarian ascorbic acid depletion and ventral prostate tests. The inhibitor does not produce any weight loss in rats tested. The author discussed the effects of the inhibitor in rats. In another paper, H. G. Madhawa Raj and N. R. Moudgal (Indian Institute of Science, Bangalore) reported the presence of a luteinizing hormone inhibitor in human urine, using the ovarian ascorbic acid depletion test of Parlow.

Avian Body-weight Cycle

There is very little reliable information regarding the body-weight cycles of the non-migratory birds. The physiological factors regulating the cycles are still not understood clearly. To fill this lacuna, J. P. Thapliyal (Banaras Hindu University, Varanasi) discussed the body-weight cycle of the spotted *munia*, *Uroloncha*. In local non-migratory spotted *munia*, both sexes experience annual body-weight cycles that closely correspond with the gonadal cycles. Although castration experiments suggested the absence of a relationship between gonadal activity and weight gain, the average weight of a female is lower than a male. The average weights of the sexes, however, overlap when the birds are thyroidectomized. In the absence of thyroids, rapid increase in the gonad size and body weight or of the body weight in the case of castrates occurs which after reaching a maximum, unlike in the normal birds, does not regress, but follows a plateau. To explain this phenomenon, the author suggested that the thyroid activity is inversely related to the output of the trophic and metabolic hormones of the adenohypophysis, which regulate the body weight and gonadal cycles.

Spawning of Catfish

Heteropneustes has been found to be an ideal catfish for studies on induction of spawning. B. I. Sundararaj and S. V. Goswami (University of Delhi) described the use of various hormones that cause ovulation in hypophysectomized catfish and metopiron. Three days after hypophysectomy, the response of the ovary sharply declines probably due to the disintegration of ova. The female pituitary is found to be 3-4 times more potent than that of male. Administration of metopiron for 3 days to hypophysectomized catfish fails to induce ovulation with LH whereas corticoid injection causes ovulation.

B. I. Sundararaj and S. K. Nayyar (University of Delhi) discussed the effects of testosterone propionate and estrogen on the testes and seminal vesicles of hypophysectomized male catfish. Testosterone propionate (2·5 mg. or 0·5 mg./day for 30 days) increases the weight of testes and seminal vesicles, the latter gland being secretory. In the testes, spermatogenesis is qualitatively restored and the sperm are present in most tubules. Estrogen 20 μg./day for 30 days) did not activate the testes or seminal vesicles.

Immunological Methods

Immunological methods are increasingly used in comparing gonadotropins from various sources. Shanta S. Rao, K. Sadri, S. R. Munshi and S. K. Shahani (Indian Cancer Research Centre, Bombay) described their findings with regard to human, ovine, bovine and porcine gonadotropins. How exactly the common antigens worked between these gonadotropic hormones from these sources were well illustrated.

Experimental Cryptorchism

The desert gerbil, *Meriones*, has been the target of extensive reproductive biological studies. Experimentally these rodents were made cryptorchid and then their hydrolytic enzymes were studied for a period ranging over 10-22 days. R. S. Mathur and K. Singh (University of Rajasthan, Jaipur) brought out that in 10-15 days' cryptorchid testes, mitotic activity in the primary spermatocyte became suppressed but the Leydig cells were normal. Testes of the animal sacrificed after 21 days revealed a progressive damage resulting in the complete cessation of the spermatogenesis. At 32 days, there was marked sloughing off of the germinal epithelium in the majority of the tubules including the primary spermatocytes. The Leydig cells showed hyperplasia commonly noted in the cryptorchid testes. In the 32 days' cryptorchid testes, the atrophied seminiferous tubules indicated a decrease in alkaline phosphatase, acid phosphatase and ATPase activities. The histochemical extraction of hyaluronidase revealed a marked fall in the enzymatic activity from 10 to 21 days. The non-specific esterase activity in the Leydig cells showed an increase. These observations suggest an increased hormone production in the Leydig cells of the cryptorchid testes and the consequent effects on the enzyme activities. The decreased enzymatic activities in the atrophied seminiferous tubules may be either due to the thermal effect when the testes are anchored in the abdomen or to a combined thermal effect and FSH inhibitory factor.

Reproductive Biology of Bird

Ploceus is the common Indian weaver bird exhibiting a number of reproductive peculiarities. One aspect, viz. gonadal inhibition of hypophysis, was discussed by R. N. Saxena (University of Delhi). It is generally believed that sex hormones bring about the inhibition of the adenohypophysis in birds, but recently these are not credited with any such important role and it is supposed that the cycle ends due to 'self-exhaustion' of hypophysis and it becomes refractory when it cannot react to any environmental stimuli. But working with the Indian weaver birds, the results obtained run contrary to the current thesis that seasonal exhaustion of the hypophysis is voluntary and independent of gonads. It is found out that in this bird sex hormone(s) at the peak of the reproductive activity interacts with the hypophysis causing it to stop the secretion of gonadotropins. The absence of satisfactory environmental stimuli and essential day length at that time of the year helps in the process. Thus the reproductive cycle of this species of bird is under dual control of stimulatory effects of increased day length and inhibitory influence of gonadal hormones. These results were secured through various experimental approaches, viz. castration, transplantation of male gonads and sex hormone injections.

Radiation Effects

Use of radioactive chemicals in the study of reproductive biology is a fairly recent development. It is known that certain chemicals bring about necrosis of the testes, causing chemical sterilization of the testes, when administered subcutaneously or intratesticularly. Using the desert gerbil, *Meriones hurrianae*, P. N. Srivastava and A. R. Rao (University of Rajasthan, Jaipur) discussed that ^{45}Ca injected intratesticularly bring about radiopathological changes in the testes and the accessory organs. They showed that statistically significant reduction in accessory male organs was indicative of structural and functional degradation of the interstitium considered radioresistant. These authors also discussed the effect of beta irradiation on the function of FSH in the gerbil.

P. N. Srivastava and P. Umadevi (University of Rajasthan, Jaipur) brought out interesting results on the effect of thyrotropic hormone (TSH) on radiophosphorus and radiocalcium utilization in the catfish, *Heteropneustes*. As the fish lacks a parathyroid, the thyroid appears to take over this function. The effect of TSH on the uptake of ^{32}P and ^{45}Ca in the ovary, kidney, heart, brain, muscle and bone have been determined with a counter. The authors reported that TSH favours the utilization of ^{32}P which supports the role played by thyroid phosphorus metabolism.

Estrogen has been shown to have radioprotective effect provided it is given prior to irradiation. P. N. Srivastava and S. K. Rathi (University of Rajasthan, Jaipur) described that *Heteropneustes fossilis* were given high doses of estrogen and some of them were subsequently injected with ^{60}Co and ^{32}P. Estrogen produced deleterious effects on the mature oocytes but irradiation brought about neutralization of the estrogen effect to some extent. This is possible either because of the increased androgen production after irradiation or because of the conversion of estradiol-17β into estradiol-17α which is devoid of any estrogenic activity.

Radiosensitivity and thyroid activity in two varieties of goldfish, *Carassius auratus*, was discussed by H. Etoh (National Institute of Radiological Sciences, Japan), P. N. Srivastava (University of Rajasthan, Jaipur) and N. Egami (National Institute of Radiological Sciences, Japan). They demonstrated thyroid function by the thyroid uptake factor (per cent) and plasma protein-bound ^{131}I conversion ratio. Demekin variety which shows greater thyroid activity is found to be more radiosensitive than the Wakin variety.

B. K. Batra and Kochar (Indian Cancer Research Centre, Bombay) discussed the X-ray induced responses in the ovaries and progeny of C-57 (BI) ICRC mice. The reproductive performance of C-57 female mice following single total body-irradiation was discussed. The observations made were coordinated with observations on the histology of the ovaries. It has been reported that the dose of 300 r brings about partial sterility in the animals which do not produce more than one litter each. The ability to produce fertilizable egg is lost about 21 days after the treatment. No appreciable difference was observed in the litter size of irradiated and control animals. The immediate effect on the ovaries is the degeneration of oocytes. About a month after irradiation, few normal follicles remain in the gland but there is an increase in anovular follicles. This is followed by the appearance of follicular nests till eventually the ovarian stroma occupies the entire gland. The gland either is completely hyalinized or presented an adenomatous condition. In the progeny, observations on mortality have shown that at 13 days *in utero*, day of birth and at 21 day, or weaning age, a large percentage of young are eliminated. Besides this, in the irradiated young there is a significant incidence of various types of morphological abnormalities. The effects of radiation in the ovaries was discussed with the view to understanding the action on the ova and the response and role of the tissue components of the gland, other than the ova.

Invertebrate Neurosecretion

It is well known that in the central nervous system of *Mollusca*, a number of neurosecretory cells are present. In discussing the same in the oyster, *Crassostrea*, R. Nagabhushanam (University of Marathwada, Aurangabad) brought out two types of cells: pyriform with Gomori positive cytoplasm and oval with Gomori negative cytoplasm. The author brought out that secretory products from both types of neurosecretory cells have been found within the axons and in the neuropile of the ganglia. Pyriform cells show a distinct annual cycle of activity which is correlated with the reproductive cycle. These cells may exert an influence on the gonads. Cytochemical tests on these neurosecretory materials revealed that Nissl bodies (RNA) and Golgi substance are involved in the granular appearance of the secretory substance. Some general effects caused by extirpation of cerebral and visceral ganglia in the *Crassostrea* are also observed. Mortality is highest in the oysters from which the visceral ganglia are extirpated. Ablation of the cerebral ganglia seems to hasten the spawning reaction in the female oysters. Visceralectomy results in considerable increase in body weight. Injection of fresh visceral homogenate in visceralectomized animals causes a rapid decrease in weight. The rate of water filtration and heart beat are considerably reduced in visceralectomized animals.

Chromatophorotropins in Crustacea

R. Nagabhushanam (University of Marathwada, Aurangabad) also discussed the occurrence of chromatophorotropins in the central nervous system of some crustacea. He described five types of chromatophorotropins in the extracts of the central nervous organs of *Gelasimus*. A high concentration of black pigment dispersing hormone is present in the optic ganglia, sinus gland and supraoesophageal ganglion while thoracic ganglia and circumoesophageal connectives have a low concentration of this hormone. Maximum black pigment dispersing activity is noticed in the alcohol soluble fraction of various nerve extracts. He described the dosage response curve for the sinus gland and reported that the black dispersing activity increases to a peak beyond which the activity decreases. The activity of black dispersing hormone from the eyestalk is unaffected by drying but increases somewhat by boiling; it is destroyed when treated with alkali while its activity is enhanced by boiling with acid. The black pigment dispersing principle is adsorbed by charcoal and is inactivated by trypsin suggesting that it might be a polypeptide.

The red chromatophores of *Gelasimus* are controlled by two hormones, one that disperses and another that concentrates the red pigment. The red concentrating factor was highest in the thoracic ganglion while maximum red dispersing hormone was present in the optic ganglia. R. Nagabhushanam reported that the white chromatophores of *Gelasimus* are under the control of two hormones. Sea water extracts of the central nervous organs exhibit the presence of white chromatophore dispersing hormone in the total eyestalk, sinus gland, optic ganglia and supraoesophageal ganglion while the eyestalks, optic ganglia, circumoesophageal connectives and thoracic ganglion show white chromatophore concentrating hormone. He also reported that the eyestalks of

Alpheus produce appreciable quantities of red pigment concentrating hormone and a small quantity of red pigment dispersing hormone.

Insect Neurosecretion

Corpora allata of insects represent the endocrine glands and form a part of the neuroendocrine system. K. K. Nayar and V. K. K. Prabhu (University of Kerala, Trivandrum) discussed that in *Periplaneta* spurts of neurosecretory discharge occurs into the corpora allata when they are exerting the gonadotropic effects on ovary for the growth of the eggs. The corpora allata themselves enlarge and shrink, concomitant with the beginning of egg growth and its termination, and throughout this period of 3-4 days there is regulated supply of neurosecretory material. When the egg is released from the ovary into the ootheca, which corresponds to ovulation, neurosecretory depletion starts conspicuously, and towards the middle of ootheca-bearing period, it is maximal. Towards the latter part of ootheca-bearing period, the neurosecretory material tends to accumulate in the tract. The corpus allatum by now gets distended with its secretion. These are suggestive of the neurosecretory stimulation of corpora allata.

Corpora allata are presumed to secrete one hormone, neotenin or juvenile hormone, producing juvenilizing effects on the one hand and gonadotropic effects on the other. That the two functions are carried out by two separate hormones has also been suggested, but the consensus of opinion today is that it is not so. However, it is found that in *Iphita* the gonadotropic effects of the corpora allata are not very pronounced. In *Periplaneta* the corpora allata of adults have a better defined gonadotropic function. The corpora allata from adults have been found to stimulate and accelerate the growth of the gonads of the crab *Paratelphusa*. This effect is seen better defined in female crabs. The nymphal corpus allatum, however, does not possess this ability.

Hormones and Neoplasia

Pathology also figured among the discussions and the relation of hormones to neoplasia formed an interesting topic discussed by S. M. Sirsat (Indian Cancer Research Centre, Bombay). Hormones influence malignant tissue as well, particularly with reference to deoxycorticosterone acetate. The author discussed the changes brought about in the biological behaviour of cancer by adrenal steroids.

S. M. Sirsat also discussed the localization of adrenal steroids in connective tissue fibroblasts. Both gluco- and mineralo-corticoids affect animal connective tissue. Electron microscopic studies on hormone treated rats and mice have brought out the involvement of fibroblasts in the production of this response. Autoradiographic technique has been utilized for studying the exact intracellular deposition of adrenal hormones.

Androgens and Blood Proteins

Exogenous steroids have great effect upon the synthesis of blood proteins in the lower vertebrates. C. P. A. Rao, G. F. X. David and L. S. Ramaswami (University of Rajasthan, Jaipur) discussed the anabolic changes brought about by injecting a large number of weak androgens into *Uromastix* (Lacertilia). 19-Nortestosterone and methyl testosterone increase alpha globulin content but decrease gamma globulin content. Widening of blood sinuses in the adrenal is noticed with the majority of the hormones; sudanophila is also commonly noticed in all.

Pituitary Inhibitors

S. R. Kankaraj and N. S. Gangadhara (University of Mysore) using ICI 33,828, a well-known non-steroidal pituitary inhibitor, on frogs for 8 days reported their response to spawning. There is reduction of spawning and the eggs lack jelly coat and no change is seen in the ovarian ascorbic acid content but there is an increase in cholesterol content.

Metopiron has been largely used in assessing pituitary ACTH reserve and excess secretion of certain mineral corticoids. M. E. A. D'Souza and M. A. Rao (University of Mysore) investigated the compensatory hypertrophy of the ovary employing hemispayed and bilaterally adrenalectomized rats. Metopiron (total dose 9 mg.) injection into intact rats causes a significant increase in the weight of both the ovaries. But in hemispayed rats the ovarian growth is just the same as in the saline treated controls. But in adrenalectomized hemispayed rats there is reduction in the ovarian weight, thereby indicating that metopiron treatment is not so effective in increasing the ovarian response to gonadotrophins in the absence of adrenals. Histological observations indicate that metopiron treatment of hemispayed rats increases the sensitivity of the ovary to gonadotrophins causing the formation of cystic Graafian follicles, but in adrenal ablated rats it inhibits the development of the ovarian follicles. Similar weight changes have been noticed in the uterus.

The Interaction of Nucleic Acids with Proteins & Other Molecules as Models of Regulatory Mechanisms

G. FELSENFELD

National Institute of Arthritis & Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

THERE is a class of models of biological regulatory mechanisms in which it is assumed that there exist molecules capable of interacting in a specific manner with DNA to alter its ability to serve as template in the production of messenger RNA. With these models in mind, we have been examining in our laboratories, during the past several years, the interactions between DNA and a number of molecules of biological interest. In each case we have attempted to learn something about the strength and specificity of the interaction, and about the nature of the forces which stabilize the interaction. In some cases preferred interaction with certain bases or sequences of bases can be shown.

Ribonuclease

The first results which I shall discuss are concerned with the effects of the protein, bovine pancreatic ribonuclease (RNase), upon DNA. This work has been presented elsewhere in some detail[1]. It should be made clear at once that the effects described here do not arise from any hydrolytic activity of RNase samples toward DNA; very thorough tests have shown that there is no deoxyribonuclease (DNase) activity associated with our preparations.

It is well known that DNA can be denatured by heating, and that the process is accompanied by an increase in ultraviolet optical absorbance and of optical rotation which may be used to follow the reaction. In Figs. 1 and 2 are shown the results of a typical experiment with calf thymus DNA, with and without the addition of RNase. It is apparent that RNase has a profound effect upon the course of denaturation, causing the first part of the denaturation process to occur at temperatures markedly lower than in the control. At higher temperatures there is a reversal of the effect, so that the DNA appears to renature, and then to undergo final denaturation at a temperature higher than that of the control. The explanation of this phenomenon is that RNase itself undergoes a thermal transition to an inactive form at about 58°C. The native form of RNase destabilizes the helical DNA structure, causing premature denaturation. The 'denatured' form of RNase, on the other hand, behaves like most other polycations in stabilizing the DNA helical structure. Thus, the transition of RNase is accompanied by a reversal of the destabilizing effect. The destabilization reaction is reversible: if the denaturation is carried as far as the first 'peak' in the curve, it is possible to regain all hypochromism by cooling to room temperature.

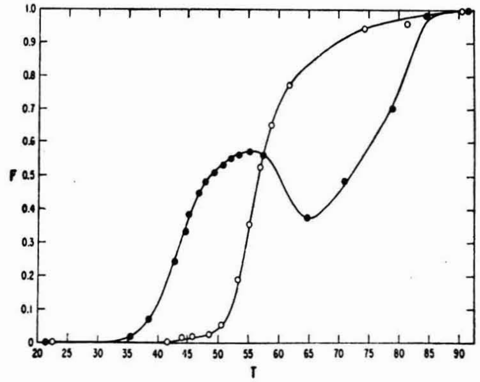

Fig. 1 — Absorbance increase of a calf thymus DNA-RNase mixture at 260 mμ (plotted as fraction of maximum increase) as a function of temperature in 0·002M NaCl-0·001M phosphate buffer (pH 7·1) [The small absorbance contribution from RNase has been subtracted. ○—○, DNA control; starting absorbance = 0·624 = A_0; fractional absorbance increase = 0·43 ($\Delta A/A_0$); and ●—●, 0·17 mg. RNase per ml.; A_0 = 0·647; $\Delta A/A_0$ = 0·43 (Felsenfeld *et al.*[1])]

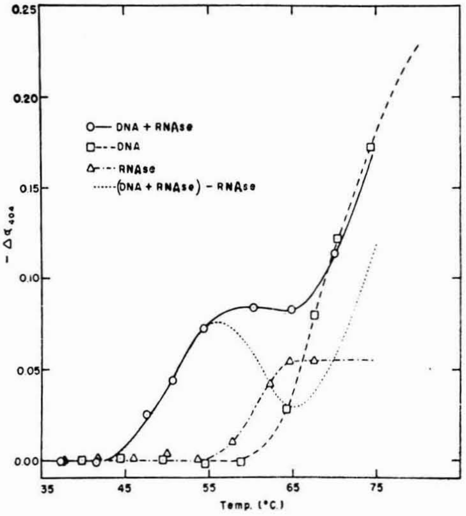

Fig. 2 — Optical rotatory change at 404 mμ as a function of temperature for RNase, DNA and RNase+DNA [All measurements made at pH 8·8 in 0·01M NaCl-0·005M tris-0·001M phosphate buffer. DNA concentration = 0·8 mg./ml.; RNase concentration = 2·3 mg./ml. The conditions appear to be near the solubility limit of the complex. The values of optical rotation at the lowest temperature have been set at the same point on the scale, though there is a very small deviation from additivity of components in the DNA-RNase complex. $\alpha_{404}^{35°}$ (for DNA) = +0·25° (Felsenfeld *et al.*[1])]

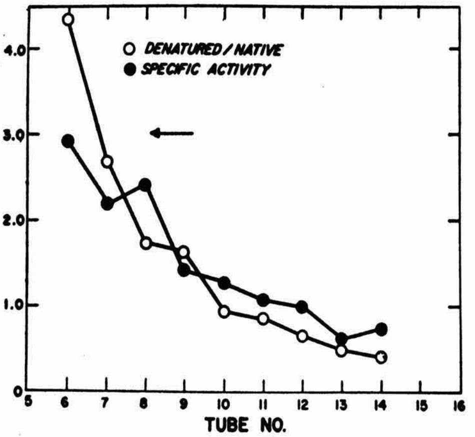

Fig. 3 — Sucrose gradient sedimentation of a mixture of whole denatured DNA and sonicated native DNA in the presence of RNase [Details of this and the complementary experiment are given in Fig. 4]

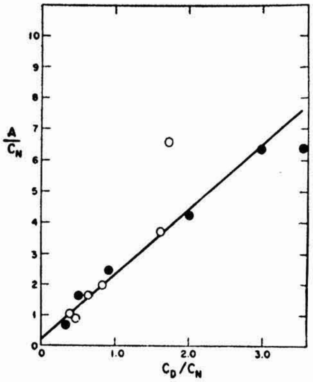

Fig. 4 — Results of two sucrose gradient centrifugation experiments in which native DNA and denatured DNA of different molecular weights are mixed and sedimented in the presence of RNase [Gradient: 4·6 ml. of 5-20 per cent sucrose in 0·01*M* NaCl. The sample in each case was 0·25 ml. of a solution containing 0·67 mg./ml. each of whole and sonicated calf thymus DNA and 0·06 mg./ml. of RNase in 0·01*M* NaCl-0·001*M* phosphate buffer (*p*H 7·1). Centrifugation time was 16 hr at 28000 r.p.m. A = RNase activity in arbitrary units; C_N = concentration of native DNA; and C_D = concentration of denatured DNA. o, whole denatured DNA+sonicated native DNA; and ●, whole native DNA+sonicated denatured DNA (Felsenfeld *et al.*[1])]

If the interaction of native RNase with DNA is reversible, then simple thermodynamic reasoning tells us that native RNase must bind more strongly to denatured DNA than to native DNA. The results of experiments summarized in Figs. 3 and 4 demonstrate that this is indeed the case. A sample of DNA is sonicated, and portions of both the whole and sonicated material are denatured by heat. Two experiments are performed: in one, whole native and sonicated denatured DNA's are mixed, while in the other, whole denatured and sonicated native DNA's are mixed. RNase is added to each mixture, and zone centrifugations are performed through sucrose gradients. Fractions are collected and analysed for native and denatured DNA and RNase activity. Results of one of the experiments are shown in Fig. 3. When plotted as shown in Fig. 4, the data fall on a straight line, a result consistent with a simple equilibrium in which active RNase has an affinity for denatured DNA about 4-10 times its affinity for native DNA.

We may speculate that preference for denatured DNA arises from the ability of RNase to interact preferentially at its enzymic site with the opened-out bases of denatured DNA. Unfolded RNase presumably lacks this site, and behaves like a normal polycation. It seems possible that many enzymes of nucleic acid metabolism that have sites of this sort would tend to destabilize helical structure. Thus the ' unzipping ' step, which is presumably essential to such functions as DNA replication, might be a spontaneous one which accompanies enzyme binding; input of energy would be required, of course, to pry the enzyme loose. It is also possible that there exist classes of proteins which serve as destabilizing agents of DNA helical structure, and which are involved in the regulation of DNA function. No such proteins have yet been found, and I do not wish to suggest that RNase has any such function, but it seems reasonable to propose it as a prototype of molecules with destabilizing activity.

Actinomycin

The next molecule which I shall discuss is quite different both in its effects upon DNA and in the nature of the forces which are associated with its interaction. Actinomycin D may be thought of as a ' regulatory ' molecule in the sense that it is capable of completely inhibiting the activity of RNA polymerase. Actinomycin D has been the subject of a large number of recent physico-chemical studies, notably by Reich *et al.*[2], by Haselkorn[3] and by Gellert *et al.*[4]. The work of Reich *et al.*[2] established that actinomycin is strongly bound only to helical regions of DNA: it is not bound strongly to RNA, or to synthetic helical polydeoxynucleotides containing no guanine-cytosine base pairs. Earlier work (Kersten[5]) had shown that actinomycin interacts weakly with nucleosides and nucleotides, with a marked preference for deoxyguanosine and deoxyguanylic acid. On the basis of the above evidence, Reich proposed that actinomycin D is bound to DNA through guanine residues.

The experiments which I shall describe were the result of a collaboration with Dr Martin Gellert, and with Dr Carl Smith and Dr David Neville. We have attempted to make more quantitative measurements of the interaction between actinomycin and DNA. The principal technique used was spectrophotometric titration; the results of a typical titration are shown in Fig. 5. The method is based upon the change which occurs in the absorption spectrum of actinomycin when it interacts

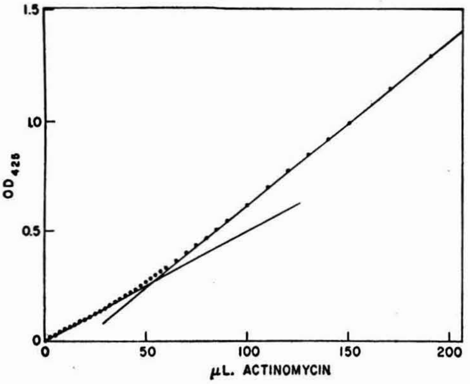

Fig. 5 — Spectrophotometric titration of *M. lysodeikticus* DNA with actinomycin at 425 mμ [DNA concentration, $1 \cdot 91 \times 10^{-5}M$. For each 10 μl. of actinomycin added, the actinomycin concentration was increased by $3 \cdot 36 \times 10^{-7}M$. Solvent, 0·01$M$ NaCl-0·001M phosphate buffer (*p*H 7·0) (Gellert *et al.*[4])]

with DNA. From data such as these it is possible to deduce the number of binding sites and the equilibrium constant for the reaction. In Table 1 are presented the results obtained with DNA samples from a number of sources. One important observation which we have made is that there is more than one class of sites. At higher concentrations of DNA and actinomycin, additional interaction is observed with a class of sites possessing smaller binding constants. By working at quite low reagent concentrations we are able to eliminate contributions from this more weakly binding class. Since actinomycin inhibits RNA polymerase at extremely low concentrations, it appears that the more strongly binding sites are the biologically relevant ones. Our measurements have, therefore, been concerned entirely with this class of sites.

The data given in Table 1 suggest that the number of strongly binding sites varies with base composition. The results are consistent with a site composed of guanine-cytosine pairs, with the restriction that no two actinomycin molecules can be located closer than about five base pairs from one another, a restriction which is reasonable in view of the large size of the actinomycin molecule.

It is informative to examine the thermodynamic parameters of binding of actinomycin to DNA and to deoxyguanosine. These parameters have been obtained by studying the temperature dependence of binding. It is apparent from the results presented in Table 2 that all of the stabilization energy of binding to DNA arises from a large increase in entropy. Such large entropy changes immediately suggest the involvement of solvent, and this is confirmed by a measurement of the heat of solution of actinomycin in pure solvent. We find that at 24°C. the solution of a mole of actinomycin is accompanied by an entropy decrease of −103 units. Thus, we may suppose that dissolving actinomycin in water immobilizes a large number of water molecules, and that some of these are liberated by interaction with DNA. The associated entropy increase provides the driving force for the interaction.

TABLE 1 — NUMBER OF SITES AND EQUILIBRIUM CONSTANTS FOR DNA-ACTINOMYCIN INTERACTION

[All experiments carried out in 0·01M NaCl-0·001M sodium phosphate buffer (*p*H 7·0). Temperature was 22°C. unless otherwise specified]

DNA source	AT %	Nucleotides/site	K mole^{-1} litre
SPECTROPHOTOMETRIC TITRATION			
dAT	100	>100	
Cl. welchii	74	30	3×10^6
Phage T4 D	65	14	9×10^6
Calf thymus	57	15	6×10^6
Calf thymus (0°C.)		17	4×10^6
Esch. coli (Albany B)	50	14	6×10^6
M. lysodeikticus	27	11	2×10^7
dGdC*	0	22	5×10^6
EQUILIBRIUM DIALYSIS			
Calf thymus (4°C.)	57	18	4×10^6

*Base composition about 65 per cent guanine (G) and 35 per cent cytosine (C).

TABLE 2 — THERMODYNAMIC CONSTANTS OF ACTINOMYCIN BINDING

	ΔH°	ΔS°
Deoxyguanosine (25°C.)	−9100 cal./mole	−15·1 e.u./mole
DNA (calf thymus) (22°C.)	~0	~+31 e.u./mole

The binding of actinomycin to DNA provides an example both of specificity with regard to site, and of the involvement of 'hydrophobic' contributions to stability of binding.

Polyamines

The interactions of RNase and actinomycin with DNA bring into play forces which are not electrostatic in nature. However, the most frequently encountered stabilization forces in DNA interactions with other ions and molecules are certainly electrostatic forces. Divalent cations, basic proteins, polyamines, streptomycin and many other molecules owe a large part of their binding energy to their positive charge. Recently, it has been suggested that polyamines such as spermine are able to interact preferentially with adenine-thymine (A-T) rich DNA (Mandell[6]). In view of the potential relevance of this finding to regulatory mechanisms, we have investigated the interaction of spermine with DNA more closely (Hirschman, Leng & Felsenfeld, unpublished data).

The finding of Mandell[6] is that under fixed salt condition the ability of a fixed concentration of spermine to elevate the denaturation temperature of DNA is directly related to the base composition of DNA: the higher the A-T content of DNA, the larger the effect of spermine. It is implied by Mandell[6] that A-T rich DNA binds spermine more

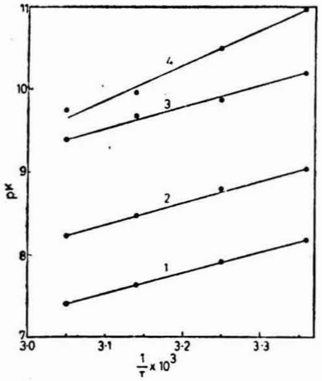

Fig. 6 — The values of the four ionization constants of spermine as a function of the reciprocal of absolute temperature (Hirschman, Leng & Felsenfeld, unpublished data)

strongly (or at a larger number of sites) than does G-C rich DNA.

Results obtained in our laboratories suggest that thermal denaturation experiments with molecules such as spermine must be interpreted with caution. This is because spermine is capable of losing some of its positive charge by dissociation of protons, and the extent of the dissociation is a function of temperature. The effect is summarized in Fig. 6, which shows the variation with temperature of the ionization constants of spermine. It is apparent that in the range of temperatures at which DNA denatures, solutions maintained at *p*H 7 will permit considerable dissociation of protons from spermine, with increasing effect as the temperature is raised. The ability of spermine to raise the denaturation temperature depends upon its charge. Since the charge decreases with temperature, and the temperature of measurement increases with the G-C content of the DNA being studied, we would predict a decreasing stabilizing effect of spermine with increasing temperature, of the kind which is observed.

Though this argument does not constitute a proof that the observed behaviour of spermine has been misinterpreted, it at least suggests that deductions about strength of binding to DNA derived solely from thermal denaturation studies must be interpreted with caution. A further demonstration of this fact is given by an experiment in which the apparent elevation of T_m by spermine is studied as a function of *p*H. It is found that the magnitude of the base composition dependence of ΔT_m can be changed simply by changing the *p*H of the solution, a result entirely consistent with the analysis presented above.

We conclude that there is no valid evidence at present to support the proposition that spermine interacts preferentially with A-T rich DNA.

Synthetic Polypeptides

As a final illustration of the problem of DNA interaction with potentially site-specific molecules, I wish to discuss some recent work done in collaboration with Dr Marc Leng (Leng & Felsenfeld, unpublished data). This work is concerned with the interaction of DNA with the basic synthetic polypeptides, polylysine and polyarginine. In Fig. 7 are presented the results of the titration of calf thymus DNA with increasing amounts of polylysine, at fixed salt concentration. The results are essentially independent of the DNA concentration used, depending only upon the lysine-nucleotide ratio. The salt concentration has been chosen so as to minimize gross electrostatic interactions. Under these conditions polylysine forms a precipitate with all the DNA with which it interacts. The reaction is reversible for short time periods after mixing and it is also ' cooperative '; the precipitated complex contains all of the bound polylysine. The relatively small amount of polylysine left in solution is completely dissociated from the DNA left in solution. Of greatest interest is the base composition of the precipitate. If the DNA precipitated at very low lysine-DNA ratios is compared to that remaining in solution at high lysine-DNA ratios, it is found that the former is considerably richer in adenine-thymine pairs than is the latter. This is demonstrated both by thermal denaturation and cesium chloride density gradient banding studies of the DNA fractions. The effect of polylysine can be contrasted with that of polyarginine, which under similar solvent conditions does not have the selectivity of polylysine with regard to base composition. There is very little difference between the base compositions of the most and least precipitable fractions; occasionally we have detected what appears to be a slight preference for precipitation of material richer in guanine and cytosine.

This behaviour leads us to conclude that there are specific properties of polylysine which permit it to interact selectively with A-T rich regions of DNA. Findings similar to these have been reported some years ago by Chargaff and his collaborators[7], but this is the first evidence to suggest the existence of a reversible reaction. The specific mechanism of selection is as yet undetermined. While it is attractive to suppose that this specificity

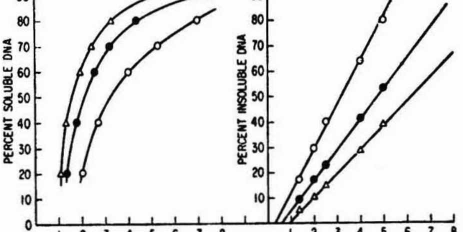

Fig. 7 — Precipitation of DNA by polylysine [Polylysine S, degree of polymerization = 7. Polylysine F, degree of polymerization ~100. The X axis in each case represents monomolar ratio (Leng & Felsenfeld, unpublished data)]

is of biological importance, only certain kinds of mechanisms would be reasonably consistent with biological activity.

Conclusion

The description which I have given of studies of interaction between DNA and other biologically important molecules will, I hope, serve to illustrate the variety of forces which can come into play in these reactions. It is apparent that in some cases at least the potential for specificity of interaction with certain bases or sequences of bases exists. Whether that specificity is in any of the present cases related to biological regulatory mechanisms remains to be determined.

Summary

A summary is given of a series of studies concerning DNA interaction with molecules of biological interest. The molecules are pancreatic ribonuclease, actinomycin, spermine, and the basic polypeptides, polylysine and polyarginine. Each molecule presents a different kind of measurement problem, and involves a different kind of binding force. Each molecule is examined with regard to its possible role as a prototype of molecules which might exert biological regulatory action. Some of the molecules appear to possess properties suiting them to such a role.

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Chemical Nature & Mode of Action of Ribonuclease T_1*

FUJIO EGAMI

Department of Biophysics & Biochemistry, Faculty of Science, University of Tokyo, Tokyo, Japan

EIGHT years ago we isolated RNase T_1 [EC 2.7.7.26, ribonucleate guaninenucleotido-2′-transferase (cyclizing)] together with RNase T_2 [EC 2.7.7.17, ribonucleate nucleotido-2′-transferase (cyclizing)] from Taka diastase, a commercial product of *Aspergillus oryzae*[1]. Since then we have been studying RNase T_1 from two points of view[2], viz. (1) its contribution in the elucidation of nucleotide sequence of RNA and (2) as the most suitable enzyme for the elucidation of the relationship between the structure and function of enzymes. I would like to discuss in this review the second point, namely the chemical nature and mode of action of the enzyme. However, I should like to discuss briefly the first point also.

We found[1] that the base specificity of the enzyme is quite different from that of the well-known pancreatic RNase [RNase I-A, EC 2.7.7.16, ribonucleate pyrimidinenucleotido-2′-transferase (cyclizing)]. Soon after the discovery of the enzyme, it was suggested[3,4] that it would become a useful tool for the elucidation of nucleotide sequence in RNA just as various proteases with different specificity have contributed to the elucidation of amino acid sequence of proteins. Indeed, since 1962, several workers in USA (R. W. Holley, G. L. Cantoni, V. M. Ingram and others), UK (F. Sanger and others), Germany (H. G. Zachau and others), Switzerland (M. Staehelin and others) and Japan (S. Takemura and others) have tried to use RNase T_1 for the nucleotide sequence analysis of RNA, especially in highly purified specific s-RNAs. Finally, the brilliant research of Holley at the Cornell University resulted in the elucidation of the complete nucleotide sequence of alanine specific yeast s-RNA using RNase T_1 as the main tool[5].

RNase T_1 is an acidic protein. In this respect it is quite different from the familiar pancreatic RNase (RNase I-A), which is a basic protein. However, RNase T_1 is as thermostable as the latter. The most remarkable characteristic of RNase T_1 is its substrate specificity. Unlike RNase I-A, it splits the internucleotide bonds between 3′-guanylic acid groups and the 5′-hydroxy groups of the adjacent nucleotides in RNA.

Purification

By heat treatment, ammonium sulphate fractionation, acid clay treatment and DEAE-cellulose column chromatography[6], RNase T_1 can be obtained as crystals which are homogeneous in regard to electrophoresis and ultracentrifugation.

Physical and Chemical Properties

Physical and chemical characteristics of RNase T_1 are summarized in Table 1. RNase T_1 has a molecular weight of 11000 which is less than that of RNase I-A. It is a simple protein with alanine as N-terminal and threonine as C-terminal amino acids.

Amino Acid Composition

The amino acid composition of RNase T_1 is given in Table 2 and compared with RNase I-A. RNase T_1 is characterized by a lower content of basic amino

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*Lecture delivered at the Fifty-third Session of the Indian Science Congress, Chandigarh, 5 January 1966.

TABLE 1 — PHYSICAL AND CHEMICAL PROPERTIES OF RNASE T_1

	RNase T_1	RNase I-A
Molecular weight		
Sedimentation diffusion	11000	12700
Sedimentation equilibrium	—	14000
Amino acid analysis	11085*	13683
$S_{20,w}$	1·62S	1·85S (S_{25})
$D_{20,w}$	$12{\cdot}0 \times 10^{-7}$ cm.^{2} sec.$^{-1}$	$13{\cdot}6 \times 10^{-7}$ cm.^{2} sec.$^{-1}$ (D_{25})
f/f_0	1·21	—
Electrophoretic mobility ($\mu = 0{\cdot}1$)	$-2{\cdot}82 \times 10^{-4}$ cm.^{2} V.$^{-1}$ sec.$^{-1}$ (*p*H 7·0)	—
	$-0{\cdot}71_3 \times 10^{-4}$ cm.^{2} V.$^{-1}$ sec.$^{-1}$ (*p*H 4·0)	—
	$+0{\cdot}25_1 \times 10^{-4}$ cm.^{2} V.$^{-1}$ sec.$^{-1}$ (*p*H 2·5)	—
Isoelectric point	*p*H 2·9	*p*H 7·8
Absorption maximum	277·5 mμ	277·5 mμ
Absorption minimum	251-252 mμ	—
OD max./OD min.	$3{\cdot}0_1$	—
$OD^{0{\cdot}1\%}_{max.\ 10\ m.}$	$1{\cdot}9_1$	$0{\cdot}71_8$
$[\alpha]^D$	−24°	−71·7°
Nitrogen content	16·5%	16·5%
Sugar content	$0 \sim < 0{\cdot}5\%$	—
N-terminal amino acid	Ala	Lys
C-terminal amino acid	Thr	Val
*p*H optimum for RNA digestion	7·4	7·0-7·5
Specific enzyme activity	14×10^3 units/mg.	—

*Based on the complete amino acid sequence.

TABLE 2 — AMINO ACID COMPOSITION OF RNASE T_1

Amino acid	RNase T_1	RNase I-A
Aspartic acid	15	15
Threonine	6	10
Serine	15	15
Glutamic acid	9	12
Proline	4	4
Glycine	12	3
Alanine	7	12
Half-cystine	4	8
Valine	8	9
Methionine	0	4
Isoleucine	2	3
Leucine	3	2
Tyrosine	9	6
Phenylalanine	4	3
Lysine	1	10
Histidine	3	4
Arginine	1	4
Tryptophan	1	0
Amide ammonia	(12)	(17)
Total	104	124

acids (only one residue of arginine and lysine), and a higher content of glycine (12 residues), the presence of one residue of tryptophan and the absence of methionine. These results indicate that RNase T_1 is fairly different from pancreatic RNase in the primary structure.

The remarkable high acidity of the enzyme can be attributed to the lower content of cationic groups (6 residues) and relatively high content of anionic groups (15 residues). Despite the lower content of lysine and arginine, the enzyme contains three histidine residues per molecule. This is comparable to that of RNase I-A. This fact is noteworthy, for histidine residues might be involved in the catalytic function of RNase T_1 as in the case of RNase I-A. RNase T_1 contains no free SH group like RNase I-A. So RNase T_1 may be regarded to have two cystine residues.

Substrate Specificity

RNase T_1 splits the internucleotide bonds between 3′-guanylic acid groups and the 5′-hydroxyl groups of the adjacent nucleotides with the intermediary formation of guanosine 2′,3′-cyclic phosphate. Hence it may be regarded as a guanylic acid-specific endoribonuclease. This mechanism (Chart 1) was deduced from the following experimental evidence: (1) RNase T_1 digests yeast RNA and produces only 3′-guanylic acid as the mononucleotide; (2) the terminal residue of the oligonucleotides produced is exclusively guanylic acid; and (3) guanosine-2′,3′-cyclic phosphate is obtained as an intermediate of the reaction in good yield and it is further hydrolysed to 3′-guanylic acid.

As RNase T_1 does not split the secondary phosphate ester bonds of adenosine 3′-phosphate, its specificity is considered to depend on the substituents in the purine ring. This consideration led us to study the effect of RNase T_1 on deaminated RNA. The experiments revealed that RNase T_1 can digest deaminated RNA, hydrolysing the secondary phosphate ester bonds of both inosine 3′-phosphate and xanthosine 3′-phosphate, the latter far more slowly. In both cases, nucleoside 2′,3′-cyclic phosphates were formed as intermediates.

These results suggest that the essential requirement in the structure of the base for susceptibility to RNase T_1 may be the oxo (or hydroxy) group at the 6-position of the purine base. Other substituents in the purine base affect more or less the susceptibility to RNase T_1. The 2-oxo group in xanthylic acid residues decreases the susceptibility.

Further studies on the base specificity have been carried out by Whitfeld and Witzel with several dinucleotides and related compounds as substrates, and by Cantoni, Holley, Staehelin and their coworkers with s-RNA as substrates. The results are summarized in Chart 2. The results further support the absolute requirement of the oxo group

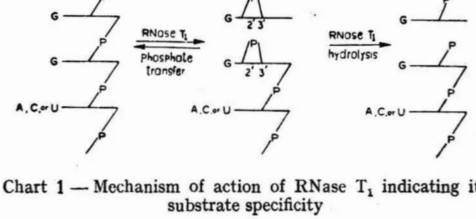


Chart 1 — Mechanism of action of RNase T_1 indicating its substrate specificity

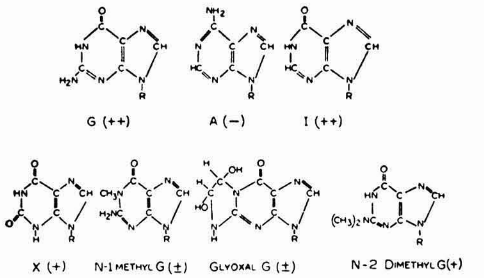
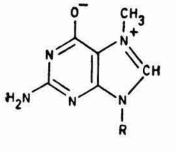


Chart 2 — Base specificity of RNase T_1 [(++) easily attacked; (+) attacked; (±) difficultly attacked; and (−) not attacked]

at the 6-position of the purine base. The loss of proton at N-1 of the purine base remarkably decreases the susceptibility.

We have recently found that RNA methylated with dimethyl sulphate is quite resistant to RNase T_1 at the optimum *p*H (7·4) of the enzyme. This may be explained by the consideration that the methylation products, 7-methylguanine residues, in RNA are dissociated at the optimum *p*H as shown in (I).



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Inhibitors and Activators

The effects of inhibitors and activators on RNase T_1 are summarized in Table 3. It is inhibited by heavy metals and divalent cations. It is strongly inhibited by Zn^{2+}. It is not inhibited by SH reagents, DFP (diisopropylphosphorofluoridate) and EDTA (ethylenediaminetetraacetate), suggesting that it is neither a SH-enzyme nor a serine-enzyme. In this respect, it is similar to RNase I-A. No divalent cations such as Ca^{2+} or Mg^{2+} are required for the activity of RNase T_1.

Action of Proteases

The effect of the various proteases on RNase T_1 are summarized in Table 4. RNase T_1 is resistant to the action of trypsin, chymotrypsin, and leucine aminopeptidase, but sensitive to pepsin. RNase T_1 is sensitive to carboxypeptidase A, suggesting that a sequence near the C-terminal participates in some way in the enzymatic activity. However, the terminal threonine is removed without loss of activity. The effect of various proteases on RNase T_1 is somewhat similar to that of proteases on RNase I-A.

Primary Structure

The primary structure of RNase T_1 has been elucidated by Takahashi[7]. The terminal groups were analysed by Sanger's DNFB method and Edman's PTH method for the amino terminal and by Akabori's hydrazinolysis method and the carboxypeptidase method for the carboxyl terminal.

Although native RNase T_1 is resistant to trypsin and chymotrypsin, it is easily digested after heat denaturation or performic acid oxidation. As the primary step in the elucidation of the primary structure of RNase T_1, performate-oxidized RNase T_1 was digested with trypsin and chymotrypsin and the resulting peptides were separated and analysed.

TABLE 3 — INHIBITORS AND ACTIVATORS

Reagents	Final conc. (−log *M*)	Activity remaining (%)
NaCl	0	75
	1	100-115
NaF	1	93-100
NaN_3	2	95-100
Na_2S	2	10
$AgNO_3$	3	0
$MgCl_2$	1	60
$CaCl_2$	2	70-75
$HgCl_2$	3	10
$MnSO_4$	2	45
$ZnSO_4$	3	0
$CuSO_4$	3	20-50
$FeSO_4$	2	20
ICH_2COOH	2	100
$BrCH_2COOH$	4	100
DFP	6	100
Histidine	2	82
	3	150
EDTA	2	125-150

TABLE 4 — ENZYMATIC INACTIVATION OF RNASE T_1

Enzyme	RNase T_1
Trypsin	Resistant, fully active (in 0−6*M* urea)
Chymotrypsin	Resistant, fully active (in the absence of urea); slightly sensitive, 12% inactivation (in 6*M* urea, 20 hr)
Pepsin	Sensitive, 70% inactivation (22 hr)
Carboxypeptidase A	Sensitive, 65% inactivation (44 hr)
Leucine aminopeptidase	Resistant, almost fully active

By putting together the information on the amino acid sequence of the peptides, the tentative structure for RNase T_1 was deduced. Peptic digestion of the heat-denatured RNase T_1 and papain digestion and Nagarse (subtilisin) digestion of native enzyme were carried out to further confirm the results and determine the localization of the disulphide bonds. Thus the complete amino acid sequence was determined. Moreover, from the Nagarse digestion products, two peptides each containing one cystine residue and a peptide containing two cystine residues were isolated and identified (Chart 3).

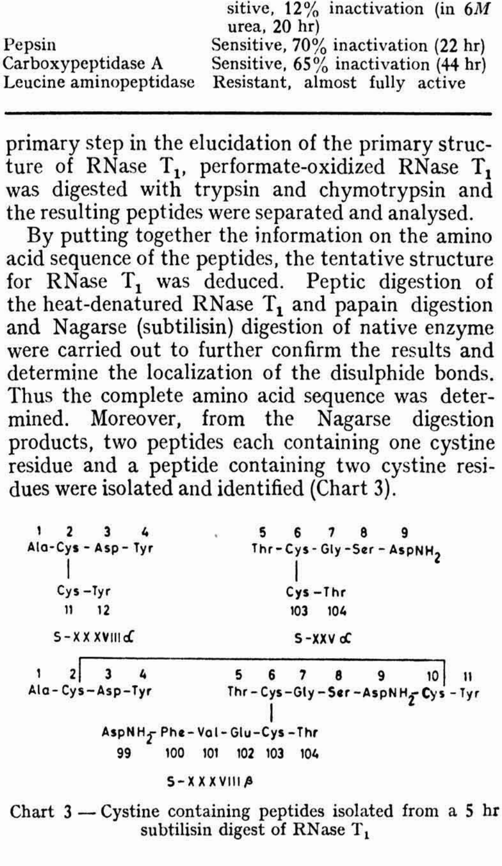


Chart 3 — Cystine containing peptides isolated from a 5 hr subtilisin digest of RNase T_1

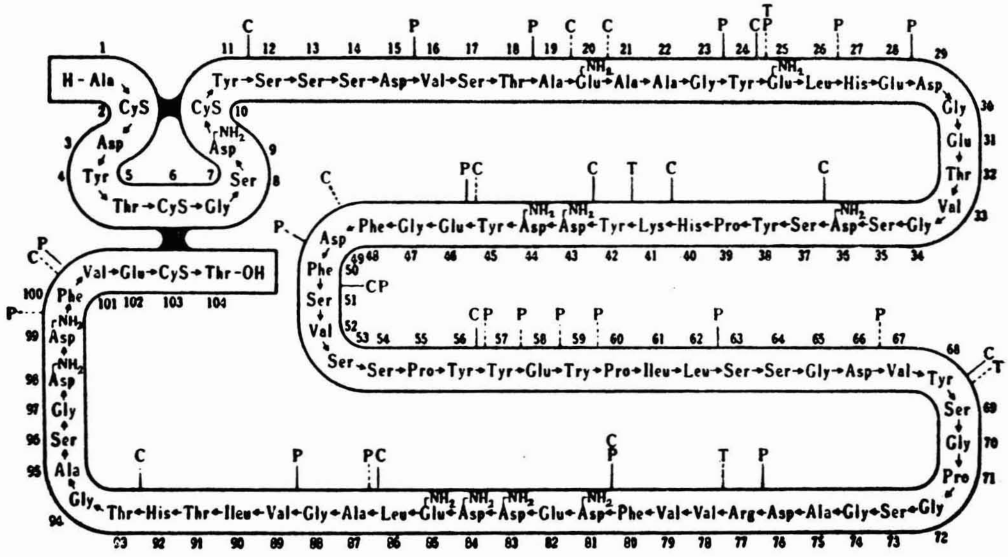


Chart 4 — Amino acid sequence of RNase T$_1$ [The points of hydrolysis by trypsin and chymotrypsin in the performic acid-oxidized protein and by pepsin in the heat-denatured protein are marked by T, C and P respectively. The solid lines represent extensive or rapid hydrolysis, and the dashed lines, incomplete or slower hydrolysis]

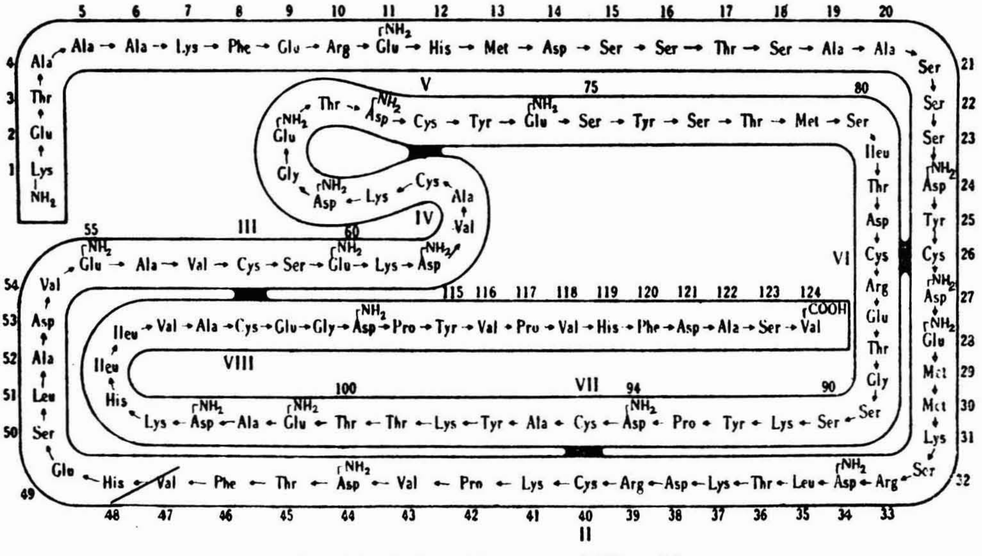


Chart 5 — Amino acid sequence of RNase I-A

From these results the complete primary structure of RNase T$_1$ was determined as shown in Chart 4.

The most remarkable characteristic of the structure of RNase T$_1$ is the positions of the two disulphide bonds. As they are located at the vicinity of N- and C-terminals, the total structure resembles a rope of which two ends are bound.

This primary structure of RNase T$_1$ may be compared with that of RNase I-A (Chart 5) elucidated by Stein and Moore, and Anfinsen *et al.* If we assume the existence of a catalytic site and a specific binding site at the active centre, one can expect to deduce from the comparison some similarity in the catalytic site and some difference in the specific binding site. Although no definite conclusion can be reached, a few speculations can be made.

One of the disulphide bonds in RNase T$_1$ forms a ring structure consisting of 9 amino acid residues with alanine and tyrosine adjacent to the disulphide bonds. There is a similar ring structure consisting of 8 residues in RNase I-A.

It remains to be elucidated if such an apparent similarity may have some significance in the activity of the enzymes. However, there seems to be little similarity between RNase I-A and RNase T_1. This is to be expected as they are derived from quite different sources, one from a mammalian gland and the other from a mould, and have different substrate specificities.

As will be discussed later, one or two histidine residues seem to be essential for the activity of RNase T_1 and resembling RNase I-A. The disulphide bonds are essential for the maintenance of the active structure of both the enzymes.

In order to elucidate the relationship between the activity and the structure of the enzyme, several chemical modifications of RNase T_1 have been attempted.

Photo-oxidation

RNase T_1 was inactivated by methylene blue-catalysed photo-oxidation. Photo-oxidative decomposition of one of the three histidine residues in RNase T_1 resulted in almost 90 per cent inactivation (Fig. 1), suggesting the importance of at least one of the histidine residues. Besides histidine, tryptophan was also decomposed though to a lesser extent. Thus an active role of tryptophan in the enzyme activity cannot be ruled out.

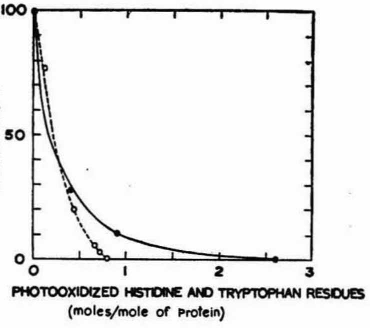


Fig. 1 — Relationship between the photo-oxidation of histidine and tryptophan residues and the changes in the enzyme activity of RNase T_1 [●—●, histidine; and ○- - -○, tryptophan]

Deamination

As mentioned earlier, RNase T_1 is a very acidic protein, containing only two free amino groups, the α-amino group of N-terminal alanine and ε-amino group of one lysine residue in the molecule. The two amino groups in RNase T_1 are deaminated with nitrous acid at 2°C. Deamination of the α-amino group of the amino terminal alanine occurs rapidly without any effect on the enzyme activity. Deamination of the ε-amino group of the sole lysine residue occurs slowly together with modifications of some of the tyrosine residues. Even after the complete deamination of both amino groups, appreciable activity remains. These results indicate that neither of the two amino groups are directly involved in the expression of the enzyme activity. Recently, it was further confirmed by the finding that RNase T_1 trinitrophenylated at both amino groups retained the full activity[8]. It is rather remarkable that a protein molecule deprived of free amino group is enzymatically active.

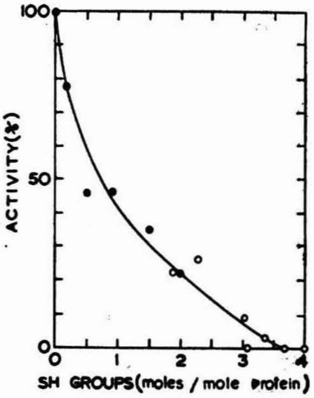


Fig. 2 — Relationship between the degree of disulphide cleavage and the changes in the enzyme activity of RNase T_1 [Reduction was carried out in the presence (○) or absence (●) of 7·2*M* urea]

Reductive Cleavage

Reductive cleavage of the disulphide bonds by thioglycolate or mercaptoethanol inactivated RNase T_1 (Fig. 2).

Reoxidation

However, the enzyme activity was regenerated by air oxidation (Fig. 3). These results demonstrate the importance of disulphide bonds for the maintenance of the enzymatically active structure.

Rotatory Dispersion

The rotatory dispersion was estimated with native and reduced RNase T_1 in 8*M* urea solution, and native and reduced-reoxidized RNase T_1 in the absence of urea. The reduced-reoxidized RNase T_1 may be regarded as identical with the native enzyme (Fig. 4, Table 5).

Carboxymethylation

RNase T_1 was inactivated by carboxymethylation with bromoacetate in weak acidic medium. In

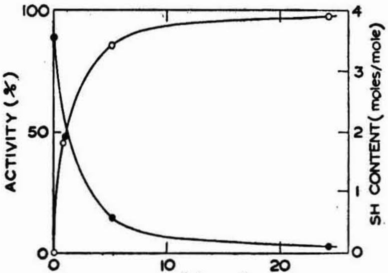


Fig. 3 — Regeneration of enzymatic activity and disappearance of free SH groups on oxidation of reduced RNase T_1 in air [The solution of reduced RNase T_1 (1·7 mg. per ml.) in 0·05*M* tris HCl (*p*H 8·0) was stored at room temperature. ○—○, enzymatic activity; and ●—●, free SH groups]

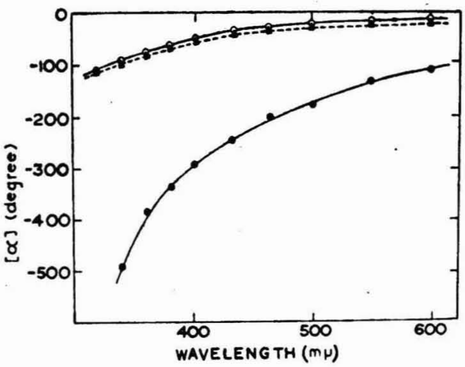


Fig. 4 — Optical rotatory dispersion curves of RNase T_1 [○—○, native RNase T_1 (5·9 mg./ml.); ●—●, RNase T_1 in 8*M* urea (8·0 mg./ml.) in the presence or absence of β-mercaptoethanol; and ▲- - -▲, reoxidized RNase T_1 (7·2 mg./ml.)]

TABLE 5 — COMPARISON OF SOME PROPERTIES OF NATIVE, REDUCED AND REDUCED-REOXIDIZED RNASE T_1

Property	Native RNase T_1	Reduced RNaseT_1	Reduced-reoxidized RNase T_1
Enzyme activity (%)	100	<0·3	100
Free SH (moles/mole)	0	~3·3	0
$-[\alpha]_{400}$	51·0°	293°*	54·3°
λc (mμ)	243	212*	244
UV absorption max. (mμ)	278-279	276-277	278-279

*In 8*M* urea.
λc, rotatory dispersion constant.

the presence of 2′-guanylic acid, which inhibits competitively RNase T_1, the enzyme was scarcely inactivated by bromoacetate. By analogy to RNase I-A, we have the working hypothesis that 2′-guanylic acid binds the histidine residue in the active centre and protects the enzyme from inactivation by bromoacetate. In accordance with the base specificity of RNase T_1, 2′-cytidylic acid did not protect the enzyme from inactivation by bromoacetate. The finding[9] that 5′-guanylic acid hardly protected RNase T_1 from inactivation by bromoacetate suggests the importance of the position of the phosphate group in the nucleotides in the binding of nucleotides to the enzyme (Fig. 5).

Binding with 2′-GMP

The ultraviolet absorbance of equimolecular mixture of RNase T_1 and 2′-guanylate was not a simple sum of the absorbance of the constituents. As shown in Fig. 6, the absorbancy decreases in the vicinity of 250 mμ and increases in the vicinity of 290 mμ. The more or less decreased absorbancy in the vicinity of 250 mμ was observed in the equimolecular mixture of RNase T_1 and 3′-guanylate or 5′-guanylate too, but the increased absorbancy in the vicinity of 290 mμ was observed only with 2′- and 3′-guanylate. It should be pointed out that such difference spectrum could not be observed with guanosine, 2′-cytidylate or 2′-adenylate (Fig. 7).

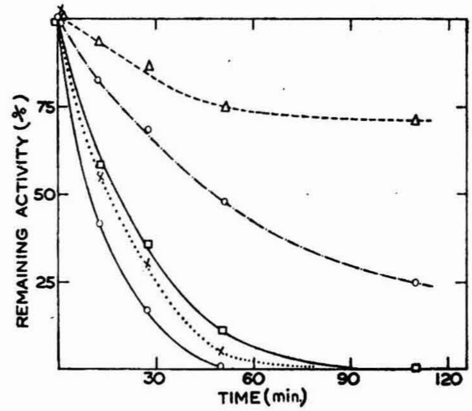


Fig. 5 — Effect of nucleotides on the reaction of RNase T_1 with bromoacetate in 0·02*M* acetate buffer (*p*H 5·5), at 27°C. [Final concentration of RNase T_1 was 7·5 μ*M* and that of bromoacetate was 0·25*M*. ○—○, no addition; △- - -△, in the presence of 2′-GMP (0·28 m*M*); ○— · —○, in the presence of 3′-GMP (0·28 m*M*); □ — □, in the presence of 5′-GMP (0·28 m*M*); and ×......×, in the presence of 2′-CMP (0·52 m*M*)]

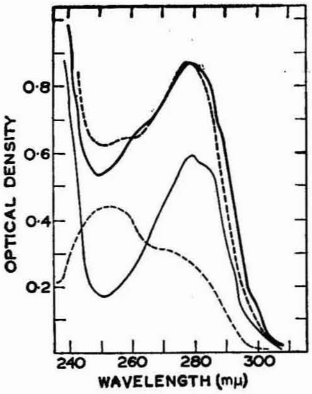


Fig. 6 — Ultraviolet absorbancy of RNase T_1 (light solid line) and 2′-GMP (light dashed line) alone and in combination (heavy solid line) [Heavy dashed line shows the calculated absorbancy of the RNase T_1-2′-GMP mixture as if interaction had not occurred. Final concentrations of RNase T_1 and 2′-GMP were respectively 0·028 m*M* and 0·033 m*M* in 0·025*M* acetate buffer (*p*H 5·0)]

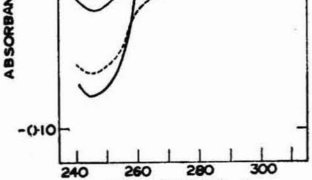


Fig. 7 — Difference spectra upon mixing 2′-GMP (heavy solid line), 3′-GMP (dashed line), 5′-GMP (light solid line) and 2′-AMP (dotted line) with RNase T_1 in 0·025*M* acetate buffer (*p*H 5·0) [The concentrations were as follows: 2′-GMP, 0·025 m*M*; 3′-GMP, 0·026 m*M*; 5′-GMP, 0·025 m*M*; 2′-AMP, 0·029 m*M*; and RNase T_1, 0·029 m*M*]

Taking into consideration the specificity of RNase T_1 and the observation on the protective effect of 2′-guanylate for carboxymethylation of the enzyme, it may be concluded that 2′-guanylate binds RNase T_1 at the active site, probably at one or two of the histidine residues.

In Fig. 8 are shown the absorbancy difference at 290 mμ and the molecular ratio of 2′-GMP to RNase T_1. A break is observed at the point where the ratio is about 1. The 1: 1 binding of RNase T_1 with 2′-GMP was further confirmed by Sephadex gel filtration. An equimolecular mixture of 2′-GMP and RNase T_1 passed through the Sephadex as a single component. However, when a mixture of 2′-GMP and RNase T_1 in a molecular ratio 2: 1 was passed through a Sephadex column, one-half of the 2′-GMP was eluted after the RNase T_1-GMP complex.

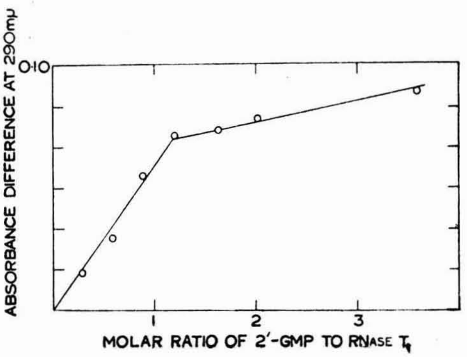


Fig. 8 — The change in absorbancy at 290 mμ upon addition of increasing amounts of 2′-GMP to 0·025 m*M* RNase T_1 at *p*H 5·0 in 0·025*M* acetate buffer

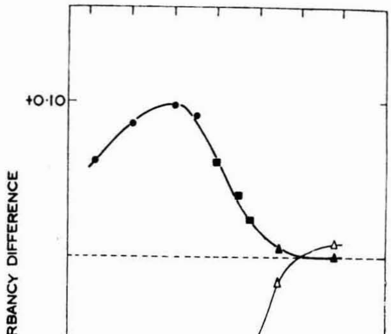
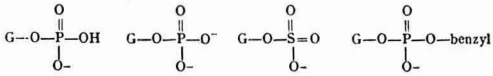


Fig. 9 — Effect of *p*H on the absorbancy difference at 290 mμ (heavy solid line) and 247 mμ (light solid line) in 0·025*M* acetate buffer (●, ○), 0·025*M* imidazole buffer (■, □) and 0·025*M* tris buffer (▲, △) [Final concentrations of RNase T_1 and 2′-GMP were 0·028 m*M* and 0·033 m*M* respectively]

In order to elucidate the mode of binding of RNase T_1 with 2′-guanylate, the effect of *p*H on the binding was investigated. The spectral differences were maximal at *p*H 5·0-5·5 (Fig. 9). The differences decreased steeply as *p*H was raised and disappeared at *p*H 7·5. This finding suggested that the phenomenon might be related to the dissociation of the secondary phosphate group or/and that of imidazole group of histidine residues.

The probable participation of the undissociated secondary phosphate group was further supported by the finding that guanosine 2′-sulphate and 2′-GMP benzyl ester, both compounds without undissociated secondary hydroxy group, could not bind RNase T_1 as shown below:



$$\mathrm{G{-}O{-}\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}{-}OH} \qquad \mathrm{G{-}O{-}\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}{-}O^-} \qquad \mathrm{G{-}O{-}\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{S}}{=}O} \qquad \mathrm{G{-}O{-}\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}{-}O{-}benzyl}$$

	2′-GMP at *p*H 5·0	2′-GMP at *p*H 7·5	2′-GMS	2′-GMP benzyl ester
Difference spectrum	+	—	—	—

From these observations and base specificity of RNase T_1, we postulate the working hypothesis that 2′-GMP and 3′-GMP bind RNase T_1 at at least three sites: A, B and C as shown in Chart 6. We speculate that the binding at site B and C might be as shown in Chart 7.

This hypothesis is consistent with the finding that carboxymethylated RNase T_1 reveals no increase in absorbancy at 290 mμ when mixed with 2′-GMP (Fig. 10).

However, it should be pointed out here that at the optimum *p*H (7·4) for RNA digestion by RNase T_1, no such difference spectrum could be observed in a mixture of 2′-guanylate and RNase T_1. This suggests that there may be some difference between

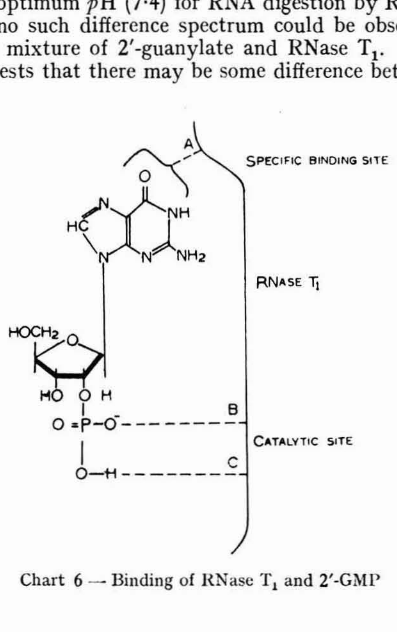


Chart 6 — Binding of RNase T_1 and 2′-GMP

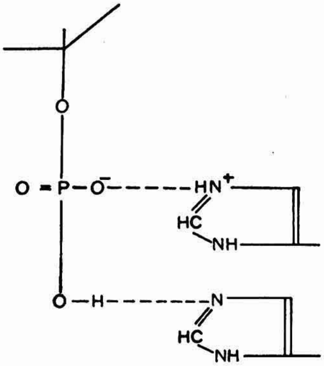


Chart 7 — Probable binding sites of phosphate group and histidine residues

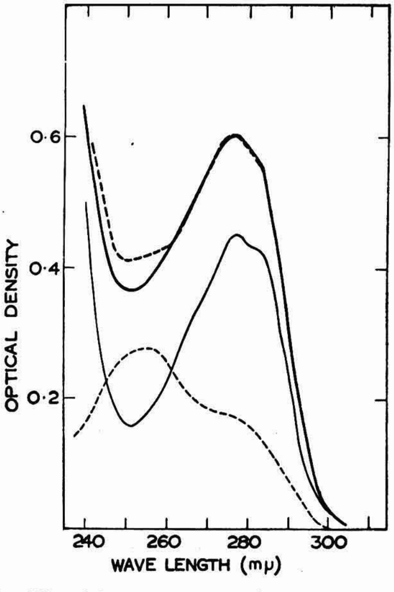


Fig. 10 — Ultraviolet absorbancy of carboxymethylated RNase T_1 (light solid line) and 2′-GMP (light dashed line) alone and in combination (heavy solid line) [Heavy dashed line shows the absorbancy calculated for the sum of the components. Final concentrations of CM-RNase T_1 and 2′-GMP were respectively 0·022 m*M* and 0·024 m*M* in 0·025*M* acetate buffer (*p*H 5·0)]

the binding of RNA to RNase T_1 and the binding of 2′-guanylate to the enzyme.

Conclusion

Though the primary structure of RNase T_1 has been elucidated, we cannot yet deduce any definite evidence which might explain the similarity in the mode of action and the difference in the base specificity between RNase T_1 and RNase I-A. To do this, studies on the secondary and tertiary structures of RNase T_1 must be carried out. As for the active centre, histidine may be regarded as a common component of the active centre in both RNase T_1 and RNase I-A.

Summary

Studies on RNase T_1 are summarized and the structure and properties of RNase T_1 and pancreatic RNase (RNase I-A) are compared and discussed. RNase T_1 is a simple protein consisting of a single polypeptide chain with following characteristics: mol. wt, 11000; isoelectric point, 2·9; no free SH group; and two disulphide bonds. The complete amino acid sequence has been elucidated. RNase T_1 is inactivated by methylene blue-catalysed photo-oxidation and by the action of bromoacetate. These findings suggest the participation of histidine residues in the active centre. The enzyme is inactivated by reductive cleavage of disulphide bonds and reactivated by air oxidation. This means that the disulphide bonds participate in the maintenance of the enzymatically active conformation. From spectroscopic study of the interaction of 2′-guanylate, a competitive inhibitor, and RNase T_1, a possible mode of binding of 2′-guanylate to the active centre of the enzyme is suggested.

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Enrichment Culture Technique

J. V. BHAT

Fermentation Technology Labcratory, Indian Institute of Science, Bangalore 12

EVEN though enrichment culture technique is almost as old as the subject of microbiology itself, it is not infrequently that a microbiologist is faced with the questions " What is this enrichment culture technique, anyway? Has it served to any extent the cause of microbiology? " put to him by even those who employ microorganisms as tools in their scientific pursuit inasmuch as they often employ only the direct method for the isolation of organisms. Rather than refer to the multitude of reports wherein are discussed enrichment cultures, this review will concern itself to (1) tracing the origin and development of the technique, (2) indicating the possible uses to which it may be advantageously put to, and (3) bringing out the promises it holds to those who resort to it for a better understanding of the nature of organisms and their activities. An extensive account of its applicability may be found elsewhere[1-3].

Origin of the Technique

The origin of the technique may be traced to the early research work of Pasteur[4] on fermentation though the conscious use of the principle involved therein was made much later by Schloesing and Muntz[5] in their classic experiments on nitrification. It may be recalled here that their study was very simple in that it involved a column of soil over which was poured sewage daily. What was interesting was the fact that the ammonia contained in the sewage was completely transformed into nitrate at the end of the experiment. Intriguingly, the oxidation of ammonia to nitrate was inhibited by antiseptics like chloroform, but the activity was restored after thorough washing of the column with water and addition therein of washings from the garden soil. This observation led them to regard nitrification as a process brought about by biological agency and herein may be found the first experimental design of enrichment culture technique in that the ammonia of the sewage was comparable to the enriching substrate in the medium and sewage itself or the garden soil washings serving as the inoculum.

The technique had its modest beginning in the laboratory in the success achieved by Winogradsky[6] in the isolation of nitrifying bacteria, an event which not only helped to solve the riddle of nitrification but was the cornerstone on which he advanced his brilliant hypothesis of chemosynthetic mode of life. The exploitation of the technique on a wide scale was achieved early this century by Beijerinck[1] and it was he who made it applicable as a direct approach to microbial ecology by forging it into what it is today, a mighty tool in microbiological and biochemical investigations.

Principle Involved

Briefly stated, enrichment culture technique implies an application, on the microscale, of the well-known principles of natural selection — the survival and/or persistence of the fittest — as postulated by Darwin over a century ago. That the mechanism operates in the microbial world has since been fully documented with this difference that whereas in nature selection occurs under natural environments, in the laboratory, the living organisms are allowed to undergo the struggle for existence under predetermined conditions. In other words, the technique envisages free competition among different organisms in what is ordinarily referred to as *elective*, *selective* or *enrichment* medium, so named because the investigator deliberately encourages therein the growth of one (or group) of the organism at the expense of the rest obtaining in any inoculum. For bringing this about, the investigator chooses the physical and chemical conditions under which the desired organism, if present in the inoculum, is allowed to proliferate in the chosen liquid medium. " In an experimental sense the ecological approach to microbiology consists of two complementary phases which give rise to an endless number of experiments. On the one hand, it leads to investigating the conditions for the development of organisms that have for some reason or other, perhaps fortuitously, come to our attention; on the other hand, to the discovery of living organisms that appear under predetermined conditions, either because they alone can develop, or because they are the more fit and win out over their competitors. Especially, this latter method, in reality nothing but the broadest application of the elective culture method, is fruitful and truly scientific, and it is no exaggeration to claim that the rapid and surprising advances in general microbiology are due to this methodology " (ref. 7). Indeed, we have today a vast body of literature about microorganisms, their ecology and their chemical activities because, more recently than ever before, investigators have resorted to this technique for the isolation of any organism expected to perform a definite function in nature.

Environmental Factors to be Considered while Designing Enrichments

For the enrichment cultures to be successful, certain environmental factors, which markedly influence the development of the organism to be enriched, have to be provided during the incubation period. Obviously, different organisms would demand different cultural conditions which would offer them selective advantages for their growth. In general, these factors may be termed as physical and chemical, though undoubtedly certain biological factors may also determine the outcome. The important factors which allow easy manipulation are: (1) aerobiosis (anaerobiosis), (2) temperature, (3) illumination (or otherwise), (4) *p*H, (5) chemical composition of the medium, and (6) osmotic pressure and/or surface tension of the medium, and all these in combination of two or more can lead to designing virtually ' an endless number of experiments '. Besides these, environmental changes resulting from the growth of organisms themselves may create conditions selective for the proliferation of one group over the rest, e.g. lactic acid bacteria growing in rich complex media both under natural and laboratory conditions would produce enough lactic acid to prevent development of other bacteria[8].

Practical Applications

In practice, an inorganic salt solution containing a particular substrate, if non-nitrogenous supplemented with a source of nitrogen and if nitrogenous with a source for energy and/or carbon is inoculated with a mixed culture of microorganisms derived from any habitat wherein the desired organisms are likely to exist. Since most substances and microorganisms eventually reach either the soil, rivers, lakes or oceans, the common materials which serve as good inocula are soils and muds, though foods, body fluids and other materials may be used with equal success. Sewage, in itself, an enrichment medium for several species of bacteria for reason of its origin and character serves as an excellent inoculum for the isolation of many interesting bacterial species[9,10]. As a matter of fact, the biological methods of sewage disposal represent practical application of the theory of natural selection or the principle of enrichment culture technique.

In the laboratory, for example, if one desires to discover bacteria that can attack oxalate — a carbon source which is toxic to this group of microorganisms by virtue of its chelating action on calcium — all that one has to do is to prepare a mineral solution containing essential inorganic elements, incorporate into it a suitable source of nitrogen, e.g. ammonium sulphate or chloride, and restrict the carbon source to oxalate only. Such a medium, obviously, will favour only the development of organisms capable of attacking oxalate inasmuch as the medium is devoid of any other source of carbon and energy. Naturally, therefore, the medium, if adjusted to around *p*H 7·0 and inoculated with soil, would permit the development of oxalate-decomposing bacteria preventing, at the same time, the growth of the rest of microorganisms which a mixed culture like soil may carry. Needless to mention that the medium after inoculation should be incubated under desired set of physicochemical conditions as temperature, aeration and light. For the growth of bacteria in general incubation in the absence of light is preferable, as in its presence photosynthetic cells as those of algae might develop. In the present case, incubation at the room temperature (about 25°C.) on reciprocal shaker is adequate and conducive to the development of aerobic bacteria attacking oxalate. When growth and total or partial decomposition of oxalate in the primary culture have been ascertained, further cultures are carried out twice serially in the same medium and each time the ability of the growing culture to decompose the substrate (in this case of oxalate by permanganate titrations) is established. Finally, from the liquid culture the isolation of the oxalate-decomposing strains can be achieved with ease by resorting to the technique specially designed for that purpose[11]. Oxalate-decomposing microorganisms so far discovered were, in fact, isolated in this manner from enrichment cultures grown under aerobic conditions and incubated at room temperature, using soil, plant tissues, and other materials like earthworm intestinal contents as inocula[11-22]. However, attempts to isolate bacteria decomposing oxalate at 37°C. or under anaerobic conditions were unsuccessful[14,15,21] until recently when an anaerobic Clostridium was isolated in this laboratory[23]. None the less, the information so far gathered on the enzymes, carbon assimilation and other biochemical aspects of the aerobic species has not only been of unusual interest but has contributed substantially to our present knowledge of bacterial physiology in particular and biochemistry in general[11,24-30].

Promises and Potentialities

Once the outcome of an enrichment culture is known, it can be employed to isolate the same or similar organisms at will from any inoculum wherein the species may be expected to occur; for instance, nitrifying bacteria are both difficult to obtain and/or to maintain in pure culture and in consequence their periodic isolation has been attempted by different investigators, including Winogradsky himself, for various reasons and in every case the enrichment culture technique alone had to be resorted to[31-36]. Even the soil perfusion technique developed and perfected recently by Quastel and Scholenfield[37] is, in a sense, an interesting example of application of the principle of enrichment culture methodology.

Isolation of Microbial Population in Soils

In no other instance has this principle been more perfectly exploited with richer harvests than in the isolation of or determining the population in soils, etc., of nitrogen-fixing bacteria. The circumstance that these bacteria only are endowed with the ability to utilize elemental nitrogen has rendered this method at once typical and successful in that the medium, for their enrichment is free of combined nitrogen and need only contain, besides an otherwise common mineral base, one organic source of carbon like mannitol, glucose, saccharose, dextrine or glycerine. The absence in the medium of a combined source of nitrogen renders it unsuitable for the proliferation of cells which cannot do without it. Naturally, the organism that gets enriched in the medium will depend on the aerobic or anaerobic state under which the enrichments are set, not to emphasize the presence or absence of light. In the absence of light and under anaerobic conditions, the Clostridia capable of fixing nitrogen usually proliferate. In the presence of light and under aerobic conditions, on the other hand, the nitrogen-fixing blue-green algae would develop whereas in the absence of any source of illumination the *Azotobacter* species are the most likely ones to be enriched. Indeed, by the exploitation of this principle, Winogradsky and Beijerinck at the end of last century and several others during this century have not only helped to unearth newer species capable of fixing nitrogen but advanced the knowledge of biological nitrogen fixation.

Isolation of Microorganisms of Low Viability

Not infrequently microbiologists encounter in nature organisms which do not live under laboratory conditions beyond a few hours, or for a day or two, although their presence can be established by enrichment culture technique. As microbiologists improve their techniques and know more about the nutritional requirements of microbial life, their urge to study such cultures is more often in evidence. The recent reports on *Sarcina ventriculi* is a case in point inasmuch as the presence of this organism was demonstrated by Beijerinck[38] as early as 1905, but its study was rendered difficult by its very low viability (2 days) and by the absence of satisfactory stock culturing methods. The recent reports on this organism[39-41], whose isolation could not have been achieved by methods other than the enrichment culture, have not only explained the cause of its poor viability but have defined methods for maintenance of cultures for as long a period as two months. Why, the ready availability of cultures of all kinds of organisms with the individual investigators or from the national collections can be explained by the ease with which the cultures can be enriched for isolation purposes.

Detection and Enumeration of Pathogenic Bacteria

During recent years an increased tempo for evolving enrichment media for easy detection, enumeration, or recovery of pathogenic bacteria has much been in evidence as these would aid a great deal the epidemiologists and food scientists in the control of the harmful species. The simple elective media proposed for the detection and isolation of *Staphylococcus* and *Salmonella* species typify some of the attempts. Such media, it may be pointed out, offer certain advantages as their high degree of selectivity allows larger samples to be examined rather rapidly without the danger of overgrowth by the associated flora. In other words, a more rapid presumptive and definitive identification of organisms becomes possible. For example, incorporation of 7·5 per cent NaCl to solid cultural media[42] render them selective for the isolation, pigmentation and preservation of pathogenic staphylococci. Even more selective media containing tellurite and lithium chloride[43] or polymyxin B[44] have also been proposed for the purpose.

In view of the significance attached to the presence of *Salmonella* in eggs, poultry and meat, continued efforts have been and are being made to evolve enrichment media for the *Salmonella* species. Eggs giving previously negative reactions have been shown to yield positive results when the selenite F broth is enriched with 10 μg./ml. of cysteine[45]. Stokes and Osborne[46,47] have devised a method which permits isolation of the species even if the contaminated egg material contained only 1 cell in as large a quantity as 100 g.! Techniques which allow speciation in the genus have also been achieved[48]. Likewise, concerted attempts are being made to evolve enrichment media[49,50] for the isolation even of such hardy and easily culturable bacterium as *Bacillus anthracis*, the first of the pathogens to be detected in blood and isolated in a routine laboratory medium a century ago! In fact, if one were to think of it in proper perspective, isolation of certain pathogenic bacteria by animal inoculation is in effect an application of enrichment culture technique, the animals serving in those instances as the medium of natural selection.

Contributions to Biochemistry and Physiology

It is easy to see that a good deal of information on the biochemistry and physiology of bacteria is derived from a study of cultures made from enrichments. All the knowledge we have on some genera, e.g. *Acetobacter, Azotobacter, Cytophaga, Desulfovibrio, Methanobacterium, Nitrobacter, Propionibacterium, Rhodospirillum* and *Thiopedia*, has indeed been gained through the application of this technique without which their isolation would have been impossible. Though it may be argued that this has been a matter of necessity, it cannot be denied that the information obtained from such studies, with suitable precautions, could be applied to other organisms not necessarily obtained from enrichments. The formulation of metabolic pathway is a case in point. The conversion of benzoate to catechol was postulated to proceed without touching salicylate as the intermediary[51], because the pseudomonads (grown on the routine media capable of attacking benzoate) failed to utilize salicylate. The pseudomonads from hippurate and benzoate enrichments, on the other hand, not only utilized both these substrates but also salicylate with equal facility[52] to give rise to catechol. The conversion of salicylate to catechol has been demonstrated in the cultures of *Azotobacter*[53] (enrichments) as well as in *Mycobacterium*[54] (routine media) suggesting thus the utility, nay advantage, of the method. The theory of simultaneous adaptation[55], which has contributed in so small measure to our understanding of microbial metabolism, has indeed been the result of studies based on cultures grown under conditions not dissimilar to those obtaining under enrichments.

For ascertaining the fate of any substrate, e.g. creatine, a herbicide or a pesticide, in soils or other habitats, or even to assay the relative activity in certain habitats of the microflora to decompose any substrate, e.g. cellulose, the principle of enrichment culture is usually resorted to. A few organisms have been shown to use creatine as a substrate for growth, but all these seem to do so rather adaptively[56-58]; none of them seems to possess the degree of specificity as does *Pseudomonas ovalis* isolated from enrichments[59] set with creatine and soils. In other words, the fate of creatine in soils is to undergo degradation, presumably via the pathway suggested by Appleyard and Woods[60], to urea, CO_2 and NH_3, and it would be possible for anyone, almost anywhere in the world, to isolate the organism and study it further as its isolation is very much an easy matter. In a like manner, microbiologists can explain the disappearance in soils of residual insecticides or herbicides used for spraying by proving the existence in soils enriched with the spray material, of organisms[61-64] capable of decomposing such materials even as they often seek to assay the relative efficiency of soils to decompose cellulose by studying, at regular intervals, the degree of attack on filter paper and/or cellophane by the microflora of those soils. As a matter of fact, but for this technique it would not have been possible for microbiologists to follow the destructive abilities of microorganisms to break down such substances in nature as keratin, fats, chitin, lipoid, sterols and hydrocarbons or to explain the total disappearance in soils of remains of carcases, corpses, plant tissues, and other organic materials. The scavenging activities of microorganisms, it would appear, take care of everything; one cannot do better than quote Gale[65] who said: " it is probably not unscientific to suggest that somewhere or other some organism exists which can, under suitable conditions, oxidize any substance which is theoretically capable of being oxidized ". Recent reports from this laboratory have indeed shown some of the possibilities[66-75].

Other Applications

Ingenious application of the enrichment technique has successfully enabled investigators to discover (1) bacteria in soil[76] capable of removing the capsule of *Pneumococcus*, a process whereby it becomes more amenable to host-resistant mechanisms of the body, and (2) an antibiotic, tyrothricin, from *Bacillus brevis*[77,78], which can kill a few pathogens both *in vivo* and *in vitro*. The isolation of *Streptomyces antibioticus* from soils by repeatedly feeding it with living *Esch. coli* cells is yet another example wherein the principle has been exploited in a novel manner[79]. The principle of enrichment is very much in evidence even in the study designed to understand the mechanisms involved in the synthesis of adaptive enzyme in that the substrate itself[80] or the products[81] of enzyme action themselves act as inducers for the enzyme.

From what has been stated above it does not follow that enrichment cultures would always yield successful results or that all substrates can be used for the enrichments. Setting enrichments with certain compounds, e.g. pectin, have not yielded desired results[82] because the ' purest ' pectin samples contain fairly significant amounts of arabans and galactans and would, on autoclaving, get degraded to units of lower molecular weight with the consequence that enrichment cultures resulting therefrom would comprise not only of pectin utilizers but also those capable of attacking some of the products of pectin. Enrichments made with filtered pectin media in one instance, however, have led successfully to the isolation and characterization of *Corynebacterium barkeri* sp. nov. from sewage[83].

Perhaps no other microbiological technique has contributed as much to the advancement of microbiology as enrichment culture methodology, for it allows the isolation of any organism at will from any source known to contain it, no matter how sparse the population therein might be. Furthermore, organisms isolated by this technique from one source would furnish information applicable to other organisms isolated elsewhere. Thus enrichment culture technique has not only been extremely useful but it is more than likely that it will prove itself even more valuable for enhancing our knowledge of microorganisms and their activities as there exist in nature many species whose ecology and functions remain as yet unknown.

Summary

The origin of the enrichment culture technique, the principle involved therein, and the environmental factors to be kept in mind while designing enrichment cultures are outlined. Some of its practical applications, e.g. in the isolation of physiologically interesting species of oxalate-decomposing and nitrogen-fixing bacteria, are discussed in detail. The potentialities of the technique for the detection, enumeration, isolation and re-isolation of specific bacteria and the promise it holds for providing information on their ecology, metabolic pattern and function are indicated. Few examples involving ingenious applications of the technique employed for the isolation of some organisms are cited. The technique has been pointed out to be the only successful one available for the isolation and/or maintenance of some of the important bacterial genera, even as it has been shown to be the only one applicable for the study of organisms decomposing certain metabolites or poisonous substances in soils or other natural habitats.

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Purification of Organic Semiconductors

S. C. DATT*, J. K. D. VERMA & B. D. NAG
Saha Institute of Nuclear Physics, Calcutta 9

IN recent years, organic semiconductors have been subjected to physical studies of ever-increasing order and sensitivity. In most of the cases the purity of the specimen always comes into question. Many phenomena, for example, electrical and thermal conductivity, magnetic resonance measurements, etc., can be interpreted meaningfully only if the purity of the test sample is known or is at least known to be high. Also, in order to obtain the necessary properties of organic semiconductors, purification techniques as extensive as those applied to inorganic semiconductors must be applied. It was the hope of early workers that useful semiconducting properties might be discovered among organic semiconductors without such careful processing. But no method is available for the detection of some impurities, even at very high impurity levels. This leaves us with two alternatives: either to develop better methods of purification and analysis, or to ignore the effect of impurities and find some other way to achieve the desired electrical and allied properties. Physicists and physical chemists would agree to the first alternative, because the literature of chemical physics contains many observations of phenomena that can be explained by elegant theories invoking new concepts — or simply by the presence of an impurity!

Purity and Ultrapurity

The classic definition of purity is that a sample of chemical compound is pure when it contains molecules of only one kind. Another more useful definition which is a practical one is that a sample is pure when the physical properties of the compound prepared in different ways are the same, or when a given physical property is not changed by repetition of a purification process.

Ultrapurity, as distinguished from purity, may be considered as an extension of anyone or combination of these points of view into the microregion of impurities.

It is very difficult to agree with any of these definitions because none of them is rigorous. In the first instance, the question of what constitutes 'one kind of molecule' is left open. In the ordinary chemical sense, molecules of differing isotopic composition are the same (except the isotopes of hydrogen, as their chemical properties are different because of large percentage difference in masses), but for many purposes, even for some chemical studies, isotopic variants constitute impurities. For example, a mixture of keto-enol tautomers may be considered as a 'pure compound', if in some reaction all of the material reacts in one form or the other. But from a physical viewpoint, such a system clearly contains more than one molecular species and is certainly not 'pure'. The same considerations apply to optically active isomers. It is even conceivable that the apportionment of an organic molecule among its nuclear spin states might be altered by a suitable catalyst[1]. If this were possible, a nuclear spin isomer would have to be considered an impurity. Polymers, the most promising organic semiconductors of the future, present unique problems: molecules of differing molecular weight, branching, or steric configuration may be considered as impurities or not depends on viewpoints[2,3] held. The second definition says nothing about how small a change is perceptible to the analysing system chosen. As the sensitivity and subtlety of analytical techniques increase, the level of impurity tolerable in a 'pure' specimen decreases. Thus, there cannot be universal and unambiguous definition of purity. Purity of a specimen can be specified only in terms of the most sensitive applicable analytical technique and in terms of the use to which it will be put. These points have been discussed in some excellent reviews[4-7].

Determination of Purity

There are two types of analytical methods for determining purity—physical and chemical. Friedenberg[8] has suggested a division of the methods into the 'absolute' and 'relative' methods. A 'relative analytical method' depends on the comparison of the data of the sample regarding any special characteristic with that of a known primary standard. Since the ultrapurity of the primary standard itself is not known definitely, such methods are not of great use in the microregion. An 'absolute analytical method' is based upon some physical property being measured of an 'internal' standard. These methods also have their limitations depending upon the refinement of the experimental technique in the measurement of the chosen physical property. The main relative methods are spectrophotometry, scintillation analysis, fluorimetry and chemical analysis, while the absolute methods are thermal analysis, chromatography, phase stability determinations, mass spectrometry, radioactive tracer studies and electrical conductivity measurements.

It is evident that purity of the sample depends upon the sensitivity of the method used for detection. For ultrapurity determinations, melting points are not considered as valid tests for testing purity[9,10]. Similarly, other such elemental analysis may be completely deceiving since isomers and closely related materials may yield similar values.

Thermal analysis and differential thermal analysis are known as rigorous methods and have yielded the most reliable data[11,12]. Chromatography gives only total organic impurities. Phase solubility methods[8] have been recently used for zone refined material. The radioactive tracer technique has a disadvantage that for rigorous results the major component should be labelled[13-17].

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*On leave of absence from Education Department, Government of Madhya Pradesh, Bhopal.

One of the most common method is the electrical conductivity measurements[18-20]. Beynon[21] has used the mass spectrometry method to a great advantage in identifying the total impurity concentrations as well as in identifying individual impurities.

Unfortunately, many fundamental chemical techniques, such as elementary analysis, functional group analysis, etc., are not precise enough to be useful in establishing the purity of a compound beyond 0·1 mole per cent contamination. According to Skau[22], the calorimetric method is one of a very few techniques that offer 'non-comparative criteria' of overall purity, without regard to the number and identity of impurities. To measure total impurity levels below 0·1 mole per cent, it is usually necessary to determine each impurity or class of impurities, and to arrive at the purity of the principal compound by difference.

Another difficulty is the use of several systems of units to measure impurities in the microregion, with the result it is difficult to assess the validity of such ultrapurity studies. For example, thermal analysis and other cryoscopic methods yield quantities in terms of mole per cent of total impurities. If the identity of impurities is unknown and the mole fraction depends upon the molecular weights of the impurities and the major component, the conversion to weight per cent or parts per million of impurities is not possible. Thus, it can be readily seen that if impurities have proportionately large molecular weights, small mole per cents might represent large weight per cent of impurities. Since mole per cent is the standard measure for impurities the utility of the other methods of representing impurity content is questionable.

The question of what constitutes the limits of ultrapurity has been rarely studied. The samples of organic semiconductors purer than 99·9 mole per cent are uncommon. For convenience a special nomenclature has been developed dealing with such quantities. For example, 3*N* purity indicates not less than 99·9 per cent pure while 4*N* indicates 99·99 per cent, and so on.

The manipulation of materials during and after purification is a problem of some magnitude. Moisture, airborne particles, and even atmospheric oxygen can vitiate the results of long painstaking work. Some operations can be carried out in evacuated systems or in gloved boxes, but it is desirable to maintain 'clean room' facilities for convenient handling of pure materials[23-25].

Various Methods for Laboratory Purification

A number of methods are available for the purification of organic semiconductors. Some of the important methods are: (i) recrystallization, (ii) distillation, (iii) sublimation, (iv) chromatography, (v) combination of different methods, and (vi) zone refining and allied techniques.

Obviously, it is not possible to treat all these methods in detail here. Out of the chemical methods, more attention has been paid to chromatography. Zone refining and allied techniques have been discussed in some detail as these are being used more and more in the purification of organic semiconductors. These techniques are the best amongst the methods available.

Recrystallization

Recrystallization[26,27] from a solution is one of the most popular methods of purifying organic semiconductors. It involves dissolving a substance or mixture in a hot solvent and allowing the material to crystallize as the solvent cools. Unlike distillation, this is an absolute method of purification and the purified material would contain no impurities if they were not occluded by the crystals as they were formed, if all the mother liquid could be washed from the precipitate, and if the solubility of the impurity was not exceeded as the mixture was cooled. As an example, consider the recrystallization of a mixture of 10 g. of compound A and 1 g. of compound B from a solvent in which each compound had a solubility of 1·5 g. per 100 ml. at room temperature and 10 g. per 100 ml. at the boiling point. 100 ml. of solvent would be required to dissolve the mixture, and upon cooling, the solution would precipitate 8·5 g. of A and nothing of B, because the solubility of B was not exceeded. Only if there were more than 1·5 g. of B in 10 g. of A there would be crystallization of B and even then two recrystallizations would remove all of B if there were less than 2·5 g. of B per 10 g. of A.

If a separation of A and B was tried by using distillation and if their vapour pressures were the same (the solubilities were the same as stated before), no separation could be effected. Even if the vapour pressures were considerably different, a column with an exceedingly large number of theoretical plates would be required to effect a separation approaching that of recrystallization. These considerations lead one to expect that relatively pure materials are best purified by recrystallization and that relatively impure materials are usually best purified by distillation, since recrystallization is often tedious and wasteful for grossly impure materials. With distillation, the degree of separation which may be effected is usually greatest with relatively impure materials, for the mole fractions of the components are then more nearly equal.

The most important factor in obtaining a good separation by recrystallization is the proper choice of a solvent in which the desired compound will have a large temperature coefficient of solubility (in order to have a minimum loss of material) and in which the impurity will be quite soluble (in order to minimize the number of recrystallizations required for complete purification). The method is most suited to the treatment of a relatively large quantity of material.

The main solvents for organic semiconductors having molecules of small or moderate sizes are chloroform, chlorobenzene, dibromoethylene, nitrobenzene, toluene, benzene, acetone, carbon tetrachloride, dichloroethylene, carbon disulphide, aniline, hexane, butyl alcohol, acetic acid, ethanol and methanol.

However, in the case of the organic semiconductors having large molecular size, a relatively great amount of solvent would be required because of the low solubility of the materials in ordinary organic solvents. Aromatic solvents such as xylene, durene, α-methylnaphthalene, quinoline and dichlorobenzene are the most suitable ones because of their relatively high solvent power for high molecular aromatics. There are a number of commercial solvents produced from the cracking of petroleum which are also applicable to the same purpose. It should be remembered that the incorporation of a small amount of the solvent into the purified crystal often has a strong effect on the electrical properties.

Distillation

If the compound is relatively impure, crystallization usually entails considerable loss of material and several recrystallizations are required to effect complete purification. The procedure often may be considerably facilitated by a prior distillation which is a process concerned with the vaporizing of a liquid at its boiling point. Separation of impurities is obtained if the impurities have a different vapour pressure from the host material. The process is extremely common for liquids but not so much for organic semiconductors in laboratory. For details of the process, many excellent reviews[28-33] are available.

Sublimation

Sublimation, as a procedure for purification, is an old technique[33,34] and is limited to compounds which have reasonably high vapour pressure and which pass directly from the solid state to the vapour. However, the process involving initial melting followed by vaporization and deposition on the condenser may be carried out in the same apparatus and is often loosely called sublimation.

The sublimation method more generally applied than the recrystallization method has the advantage of being free from solvent. Because of the minimum number of transfer operations, there is little loss of material (thus applicable to the treatment of quantities as small as 1 mg.) and saving of time compared to recrystallization. The main disadvantage of this method is that separation being based on vapour pressures two components having similar vapour pressures will sublime together and hence a poorer separation is obtained compared to recrystallization. The method is particularly valuable in separating volatile from non-volatile compounds such as salts or other inorganic compounds; bicyclic and other rather symmetrical molecules which usually have rather high vapour pressures may also be efficiently separated from other less volatile reaction products.

When the sublimation procedure is applied to an admixture, the lighter molecules condense far from the heavier ones. Thus the two are effectively separated. One can obtain a series of condensed layers on a glass surface which usually exhibit a successive change in colours. The temperature of sublimation can be greatly decreased by employing a high vacuum, from 10^{-5} to 10^{-6} mm. of Hg. Moreover, the separation of impurities from the desired compound is carried out effectively. A schematic diagram for multiple vacuum sublimation apparatus is shown in Fig. 1. The variation of the sublimation temperature with the vapour pressure as observed by Inokuchi *et al.*[35] is presented in Fig. 2.

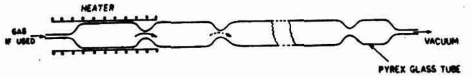

Fig. 1 — Schematic diagram for multiple vacuum sublimation apparatus

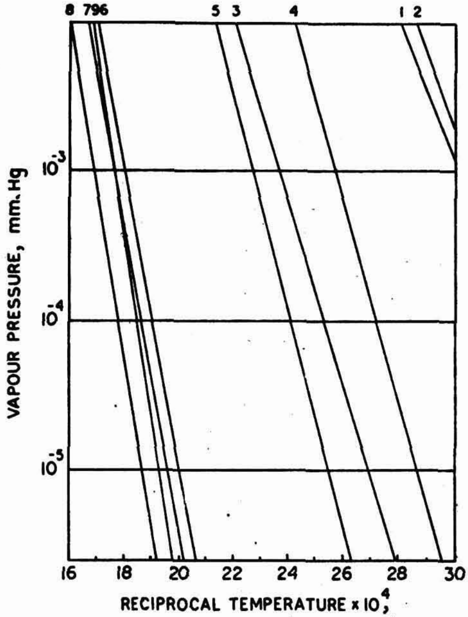

Fig. 2 — Variation of sublimation temperature with the vapour pressure of the polycyclic aromatic hydrocarbons [1, anthracene; 2, pyrene; 3, naphthalene; 4, perylene; 5, anthanthrene; 6, meso-naphthodianthrene; 7, pyranthrene; 8, ovalene; and 9, violanthrene]

It is believed that the decomposition and carbonization of aromatic compounds may lead to the appearance of unpaired electrons. It has been found by means of the method of electron spin resonance that the polycyclic aromatic compounds may be sublimed without decomposition when the temperature is not over 350°C.[36]. As shown in Fig. 2, the application of high vacuum in the sublimation procedure may prevent the contamination resulting from the presence of a minute amount of decomposed products, because the sublimation temperature is kept below that for decomposition.

Recently, however, a number of new 'twists' have been introduced that greatly increase its effectiveness.

Gradient Sublimation

In most sublimation processes, the less volatile constituents of a mixture do not sublime until the bulk of the mixture has been sublimed; this leads to entrainment and poor separation.

An improved method which may be called gradient sublimation has been introduced by Melhuish[37]. A glass tube (4 mm. internal diam. and about 25 cm. long) is placed in an insulated, close-fitting copper tube, one end of which is heated electrically. A small amount of sample (about 15 mg.) is placed behind a plug of glass wool in the heated end of the tube, and a slow stream of inert gas (about 10 ml./min.) is passed through the tube, from hot end to cold. Each constituent sublimes and resublimes until it reaches a point in the temperature gradient at which its vapour pressure is negligible. The more volatile constituents move further down the tube, and complete separations can be effected. In a number of experiments with mixtures of aromatic hydrocarbons and their derivatives, it was found that fluorescent substance could be detected in the tubes in quantities of a few micrograms[37]. This technique may be used to advantage with larger tubes, either in inert gas streams or *in vacuo*.

Microfractor

Another new vacuum sublimation technique has been introduced in which a mixture is sublimed repeatedly between two travelling bands of stainless steel[38]. The apparatus is known as microfractor and has been used to separate mixtures of dyes, alkaloids and sterols into fractions of 80-90 per cent purity. Refractionation would presumably give higher purity. The entire unit is enclosed in an evacuated bell jar during the operation.

Chromatography

The chromatographic methods[39,40] originally used for separating number of mixtures of natural products[41,42] have now been well developed and the recent developments have been summarized in literature[42-45].

Definition, methods and analysis of chromatography — Chromatography is a physical method of separation in which the materials to be separated are partitioned between two phases. One of these phases is stationary, the other is mobile and percolates through the stationary phase. Obviously, only solids or liquids may serve as stationary phases, while the mobile phases may be gaseous or liquid. Therefore, four fundamental combinations can be envisaged as shown in Table 1, all of them have been used in practice.

Chromatography, in its varied developments, is now applied to many aspects of the problem of purifying organic semiconductors. Liquid chromatography is often used for the isolation of pure samples weighing many grams and it can also be used for the concentration of small amounts of impurity. Paper chromatography has been used primarily for the qualitative and quantitative analysis of microgram quantities. Gas chromatography is largely an analytical tool, but it is rapidly becoming a purification procedure as well.

TABLE 1 — FUNDAMENTAL COMBINATION OF MOBILE AND STATIONARY PHASES IN CHROMATOGRAPHY

Mobile phase	Stationary phase	
	Solid (adsorption)	Liquid (partition)
Gas (gas chromatography)	Gas-solid chromatography	Gas-liquid chromatography
Liquid	Liquid-solid chromatography (adsorption chromatography)	Liquid-liquid chromatography (paper chromatography)

All these methods involve the passage of a fluid through a column of a rigid medium — usually with large surface area — which retards selectively certain constituents of the fluid. Lederer and Lederer[44] have classified chromatography into three types on the basis of the mechanism of selective retardation as follows: (1) adsorption, in which an equilibrium is established between the fluid phase and an adsorbed phase consisting of all the material retained in the pores of the adsorbent; (2) ion exchange, in which the stationary phase is the surface of a finely divided polyelectrolyte; and (3) partition, in which a solute is distributed between two liquid phases or between a liquid and a gas phase. In this case, the stationary phase is distributed in a thin film of large area, on a finely divided, inert support.

Each of these three types of retardation can in principle be pursued experimentally in three ways. (1) Elution analysis (flowing chromatogram), in which the column is washed with liquids of successively greater eluting power, and the elute is collected in fractions. Eluants function by being themselves absorbed[46]. A number of instruments are available commercially for the continuous collection of successive fractions. (2) Frontal analysis, in which a solution of the mixture to be separated is forced through a column and the effluent is analysed continuously. (3) Displacement analysis, in which the chromatogram is developed by addition of a solution of a substance more strongly absorbed than any of the materials to be separated.

Liquid Chromatography

The chromatographic method most relevant to the preparation of sizeable samples of pure organic semiconductors is the flowing-absorption chromatogram. This method is very useful for lower condensed aromatics, in spite of the labour involved, because they are highly soluble in organic solvents. The essential requirements are simple: a glass or plastic tube, whose length is about 30 times its diameter, with a means of supporting the charge of adsorbent. A number of papers[47-49] describing the apparatus used have been published.

In the apparatus shown in Fig. 3 (taken from ref. 49) the solvent in the upper vessel seeps through the crude material and extracts the more soluble components to form a solution which then enters the multiple or single adsorption column. The more highly adsorbed impurities are retained in the column so that a purified solution enters the bottom reservoir. The solvent is returned from the bottom reservoir into the upper vessel by means of distillation to complete the solvent cycle. The purified material concentrates in the bottom vessel. This apparatus may be used to carry out extraction,

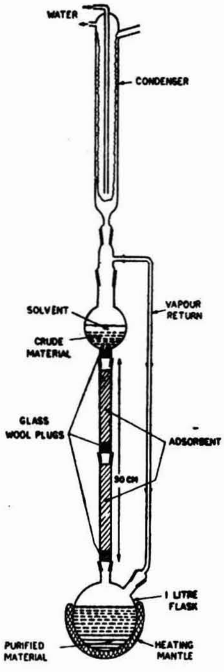

Fig. 3 — Chromatographical purification apparatus

filtration, adsorption and further recrystallization simultaneously.

In this procedure, a few adsorbents such as activated alumina, silica-gel and sodium carbonate, are appropriate for the purification of organic semiconductors of aromatic type. Although alumina generally possesses the greatest adsorbing power and the suitable chromatographic effect on the lower condensed aromatics, there are some cases in which silica-gel and/or Na_2CO_3 is a much more adequate medium for the higher polycyclic aromatics because of their higher absorbing powers.

Many variations have been introduced in the above apparatus to deal with specific problems. For example, some slightly soluble aromatic hydrocarbons have been chromatographed in heated columns or in systems allowing continuous addition of sample to the column[49,50].

Some substances require special precaution while being purified by chromatography. For example, the oxidation products of anthracene (anthraquinone and hydroxyanthraquinones) are much more strongly adsorbed on alumina than is anthracene, but they are not completely removed by chromatography. This is because anthracene is photochemically oxidized on alumina and silica, even in a nitrogen atmosphere, apparently by adsorbed oxygen[51,52]. Oxidation is probably not the only process that can be caused by chromatographic adsorbents, in view of their capacity to convert aromatic hydrocarbons to the corresponding radical cations, which have been detected by electron spin resonance measurements[53,54]. The best possible ways of avoiding photo-oxidation and photo-decomposition of materials is to carry out purification in dark.

Gas Chromatography

Since 1952 there has been a greater application of partition chromatography with elution analysis and a gaseous mobile phase[55]. The technique which is now known as gas chromatography[56-58] was not initially applicable to organic solids. However, operating temperatures have been pushed upwards gradually and now not only it is possible to analyse but also to purify solids of high melting point with this technique.

The experimental requirements are quite simple and in Fig. 4 is shown a block diagram of a typical gas chromatographic unit[59].

The stationary phase must be non-volatile and stable at the operating temperature and should also have fair solvent power for the mixture to be purified[60]. The detector should be sensitive to all vapours, quick in response and for qualitative analysis, the response should be a linear function of the number of vapour molecules. Further, the output of the detector should be capable of operating a recorder. Thermal conductivity, gas density, flame ionization and infrared detectors have been used and even more sensitive detectors are contemplated[60-62].

A sample is introduced into a gas chromatography column by injecting it into the inert gas stream through a membrane, by means of a hypodermic syringe. Solids should not be injected in solution for two reasons: firstly, the solvent may contain impurities and, secondly, if the solubility of the solid is low, the amount of a trace impurity that can be introduced may become too small to be detected. A new method for introducing a sample has been developed which is equally suitable for both liquids and solids. The sample is sealed in a capillary made of Wood's metal and is placed near the column inlet. After this the system is flushed with inert carrier gas and then a heated gas stream melts the capillary and carries the sample into the column[63].

Samples of a few tenths of a milligram of organic solids, dissolved in a volatile solvent, have been carried out into gas chromatography columns for analysis by injecting into a flash vaporizer at 375°C. The columns were packed with glass microbeads as support for the stationary phase and operated at temperatures as much as 250°C. below the boiling points of the solids[64]. Baxter and Keen[65] have designed instruments and have made some studies for high temperature gas chromatography of aromatic hydrocarbons at temperatures up to 430°C.

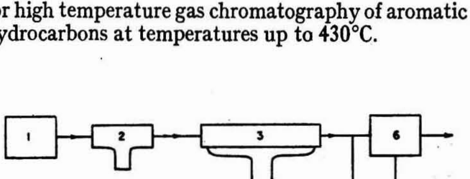

Fig. 4 — Block diagram of a unit for gas chromatography [1, source of inert carrier gas at known constant rate of flow; 2, sample inlet; 3, column containing a stationary phase on an inert support; 4, manometer, to indicate pressure drop across column; 5, absolute manometer; 6, detector; and 7, recorder]

The F and M scientific corporation has reported[66] a striking gas chromatographic analysis of a mixture of 17 aromatic compounds ranging from xylene (boiling point 139°C.) to chrysene (boiling point 448°C., melting point 255°C.). This analytical separation was effected by a linearly programmed chromatograph, in which column temperature was raised continuously during the analysis[67].

Another approach to the gas chromatographic analysis of non-volatile substance is to decompose them thermally and analyse the characteristic ' spectrum ' of products. This technique has been applied to polymeric systems[68].

Combination of Different Methods

As a matter of fact, it has not been conclusively proved that anyone of the methods already discussed possesses advantages over another in all cases. Thus, one must carry out the purification by a combination of more than one of the methods until self-consistent results are obtained.

Okamoto *et al.*[69] have purified some organic semiconductors of hydrocarbon type in a very novel way and achieved high purity. Saturated solutions of the various hydrocarbons in electronic grade benzene were prepared and then refluxed with 10 per cent aqueous hydrochloric (for anthracene) or sulphuric acid (for naphthalene) for several hours. After cooling to room temperature, the benzene layer was decanted and thoroughly washed repeatedly with distilled water. The remaining solvent was then removed by distillation. The solids were dried under vacuum at room temperature. Further purification was effected by two successive sublimations under reduced pressure (5-20 mm. of Hg).

Aftergut and Brown[70] have purified various substances by combination method. Phenazine was chromatographed and then either sublimed at 125-130°C. (pressure 2 mm. of Hg) or subjeced to zone refining. Imidazole was recrystallized three times from benzene and zone refined. Benzimidazole was recrystallized twice from aqueous methanol, sublimed twice at 125-130°C. (pressure 0·01-2 mm. of Hg), and then zone refined. Phenothiazine was purified by chromatography, multiple vacuum sublimations and zone refining.

Aftergut and Brown's results on phenazine show that the resistivity over the measured temperature range is higher for zone refined material than the sublimed material. While the process of zone refining in itself does not constitute evidence of ' ultrapurity ', the results obtained lend support to the assumption that this method of purification furnished phenazine of greater purity than that afforded by multiple sublimations. This assumption is based on the observation that, whereas at the lowest measured temperature the order of magnitude of the resistivity of the samples is the same, the sublimed materials have significantly lower resistivities at the higher temperatures. This yields higher energy gaps for the sublimed materials and can be accounted for by postulating the presence of impurities. This is further supported by the poorer fit of the experimental data of the sublimed samples to a single straight line.

Kepler[71] obtained ultrapure anthracene by first chromatographing high purity commercial anthracene in dark on alumina. This material was sublimed in vacuum and then zone refined. The crystals grown from this material contained less than 2 p.p.m. impurities as estimated by emission spectroscopy and mass spectrometer measurements. It was possible with these crystals to measure the drift mobilities of both holes and electrons with pulsed photoconductivity technique.

Zone Refining and Allied Techniques

A much more powerful method of purifying solids than by fractional crystallization has been developed by Pfann[72] who termed this process as zone refining which, in principle, is a general separation technique comparable to distillation.

Though there are fundamental differences between the behaviour of inorganic and organic materials, yet the application of the zone refining results obtained on inorganic materials has already produced many experimental successes in the ultrapurification of organic semiconductors.

Initially, the development of the theory was much behind the experimetal development in the zone refining of organic materials. But now at least in some areas, the theoretical work has surpassed empirical work as, for example, in obtaining ' ideal thermodynamic equilibrium conditions '.

The outstanding advantage of zone refining technique is that there is no contact between the compound being purified and any other solvent or chemical. The other advantages are (a) wide applicability of the technique, (b) sensitivity, (c) freedom from contamination, (d) efficiency, and (e) possibility of theoretical deduction of optimum conditions.

Zone refining and allied techniques have scope of much wider applications. For example, they are capable of concentrating microquantities of impurities for identification, controlling impurities which may require uniform impurity concentrations (zone levelling) within a crystal at levels ranging over many decades down to a few parts per billion, resolving polymers into fractions by molecular weight, isolating individual isomers, and separating and purifying enzymes and other heat-labile biological materials.

Theory of Zone Refining

DEFINITIONS

The redistribution of components in a mixture during a phase change is well known and a common example is a solution of water and salt in a beaker placed in freezing mixture. As the outer portions approach the freezing temperature, pure ice crystals start forming on the inner surface of the beaker and gradually grow towards the centre, while the salt concentrates in the remaining water. This example is often used to illustrate the phenomenon of ' segregation ' which is common to almost all the purification processes. The above process is known as ' progressive or directional or normal freezing '. Thus ' segregation ' may be defined as the redistribution of a component or components of a mixture at an interface during a phase change.

Whereas ' normal freezing ' makes use of the slow freezing of an entire solution, ' zone refining ' utilizes a relatively narrow zone of solution in a charge, moving slowly through its entire length (Fig. 5). The redistribution of impurities occurs at the freezing interface, while the melting interface continuously adds liquid to the zone. If at room temperature the charge is solid and a heating unit is used for creating a molten zone, the process is known as ' zone melting ' (Fig. 5). The term zone melting as such is also used for other allied techniques. For liquids, the correct term is ' zone freezing ', because a freezing system is used for creating a frozen zone.

Pfann[72] borrowed the terminology for zone melting from solution chemistry, where it is more common to use components or solutes instead of impurities. Due to this, the segregation phenomenon is usually described as a solubility difference of impurities in the solid and liquid phases. When a part of the impurity remains with the solid phase as the interphase moves into the melt, a ' segregation coefficient ' or ' distribution coefficient ' arises [Fig. 6(a)]. This coefficient (*k*) can be defined as the ratio of the concentration of impurities in the solid to that of the liquid at the interface. These definitions are based on the concept of ' solid solution ' developed by Bruni[73]. For an understanding of segregation phenomena in zone melting, it is essential to consider first the behaviour of the major component and its impurities as described by their phase diagrams, and secondly, the conditions under which zone melting is carried out.

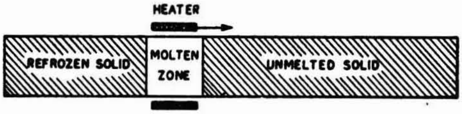

Fig. 5 — Procedure adopted in zone melting (zone freezing for liquids) operation

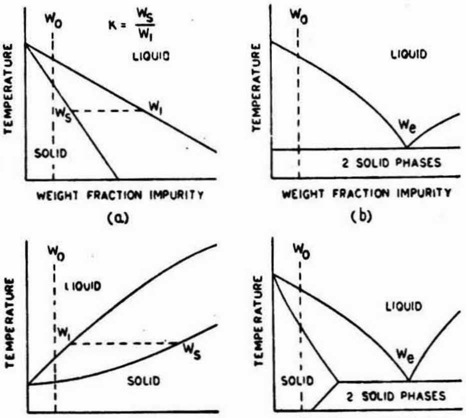

Fig. 6 — Typical binary solid-liquid equilibrium phase behaviour [(a) Solid solution with constant distribution coefficient *k*; (b) simple eutectic system; (c) solid solution with variable distribution coefficient; and (d) partial solid solution with eutectic point]

PHASE DIAGRAMS

The theoretical principles of phase diagrams are almost the same as given by White[74]. Findlay[75] divided them into two basic types: one type describes eutectic mixtures [Fig. 6(b)] while the other the solid solutions [Figs. 6(a) and 6(c)]. Almost all the binary organic compounds are of the eutectic type. All other phase diagrams can be constructed by modifications or combinations of these two types[75] as shown in Fig. 6(d). Much of the theoretical treatment on zone melting starts with an analysis of these diagrams[24].

In a eutectic mixture [Fig. 6(b)] as the temperature falls, the major component will crystallize out in the pure state while the impurity moves towards the remaining liquid. This continues till the eutectic composition reaches the freezing interface, when the major component as well as the impurity crystallize out together. Wilcox[76-78] has derived theoretically the exact mechanism by which a eutectic-forming mixture is zone melted. If the ' ideal conditions ' are available, the major component would crystallize out 100 per cent pure during the initial stages of the zone melting. Friedenberg[8] has derived the necessary variables required for ' ideal conditions '. It is shown that the ' ideal conditions ' require a temperature gradient of 0·100 to 0·010°C./mm. with a zone travel rate of the order of 5×10^{-1} to 1×10^{-3} cm./hr. With the boundary temperature sharp and the growth rate slow (due to the similarly slow movement of the zone), the impurities have sufficient time to move into the bulk of the molten zone without occlusion. This mass transfer of impurity could be facilitated by mechanical stirring of the zone. Simonelli and Higuchi[79,80] have confirmed this observation experimentally. With the eutectic type of phase diagram, the impurity always lowers the melting point[75].

A few organic systems with solid solution type of phase diagram have also been studied. Here the impurity may either raise or lower the melting point. The impurity distribution at the interface can only be described if segregation coefficients are known. If the phase diagram is known, the segregation coefficient can be obtained directly from the slopes of the solidus and liquidus curves. However, one should apply such information carefully to zone melting process, because sometimes the conditions during zone melting may alter the constancy of the distribution coefficient considerably. It must also be noted that for concentrated mixtures the equilibrium segregation coefficient is a function of concentration.

The thermodynamic aspects of these two types of phase diagrams have been rarely studied. Therefore, no thermodynamical explanation is available even for ideal substances under equilibrium conditions, for the difference in formation of a eutectic mixture or solid solution. But some qualitative explanations[75,81-84] lacking a rigorous treatment are available.

Theoretical studies dealing with phase diagrams in zone melting rarely discuss the conditions and methods used for determining these diagrams[24,72,85]. It is expected that with the availability of refined instruments for thermal analytical measurements[8,79,80], it might be possible to show that many hitherto considered solid solutions behave as eutectics under more rigorously controlled conditions.

MASS TRANSFER

Role of mass transfer in zone melting — It is seen that the phase diagram determines only the relationship between the solid concentration and liquid concentration at the interface. However, the degree of separation attained in zone melting is determined by the relationship between the solid concentration and the concentration of the bulk zone. Therefore, the success of a zone melting operation depends strongly on the effectiveness of mass transfer between liquid at the interface and in the bulk zone as well as on the phase diagram of the mixture. Mixing in the zone helps the mass transfer while the net flow of material towards the interface is a hindrance. The flow of material through the zone is responsible for the separation, depending on the zone travel rate.

Impurity segregation problem — The ultimate goal of the theoretical analysis of the zone refining process is to predict from basic principles or empirical correlations the solute concentrations resulting from any number of passes. All mathematical solutions obtained so far have been for binary mixtures and an analysis[86] of the literature where such solutions to this problem have been obtained is given in Table 2.

The first step for obtaining the solute concentration is to calculate the distribution resulting from a single zone pass of an infinitely long charge. This is connected to the problem of infinite normal freezing where the zone is of an infinite size. But the charge is always finite, hence corrections should be applied for the end conditions. It is rather easier to calculate the distribution after one pass or after an infinite number of passes than the one resulting from any number of passes. The ultimate distribution is the one which is not at all changed by another pass. Details of the theoretical work can be obtained from the references cited in Table 2.

Assumptions—The following assumptions are made for solving the above problems: (a) constant zone length, (b) constant zone travel rate, (c) constant and equal cross-sectional area in zone and solid, (d) constant density in solid and in liquid (some have taken equal solid and liquid densities while some have not), (e) uniform initial concentration, (f) planar freezing interface, (g) equilibrium (according to phase diagram) at solid-liquid interface, (h) constant distribution coefficient in melt, and (i) no diffusion in the solid.

TABLE 2 — ANALYSIS OF THE LITERATURE SURVEY GIVING THEORETICAL SOLUTIONS OF IMPURITY REDISTRIBUTION IN ZONE MELTING

(Numbers refer to references where solutions are derived and numbers in parentheses refer to references in which solutions are merely given)

	Phase diagrams											
	Constant distribution coefficient [Fig. 6(a)]			Simple eutectic-forming system [Fig. 6(b)]			Variable distribution coefficient [Fig. 6(c)]			Limited solid solubility [Fig. 6(d)]		
	Liquid mixing			Liquid mixing			Liquid mixing			Liquid mixing		
	Complete	Incomplete	None	Complete	Incomplete	None	Complete	Incomplete	None	Complete	Incomplete	None
Single pass, semi-infinite rod	(72),76, 87,88, (109)	(72),76, (109), 90	76,77	78	78	76,77, 111	78,87, (109), 91	78		78,87	78	
Semi-infinite zone (progressive freezing, infinite charge)	(72)	*	92,93, 94,95, 96,97	78	78	98	78	78		78	78	
End condition (progressive freezing, finite charge)	(72),87, (109)	*	96	78	78		78,87	78		78,87	78	
Multipass, semi-infinite rod	(72),87, 99,100, 101,102, 103	*										
Multipass, finite rod	(72),104, 105	*										
Ultimate distribution, finite rod	(72),87, 100,105, 106,107, 108	*		87			87					87

*The solutions for incomplete mixing are found by substituting the effective distribution coefficient for the distribution coefficient appearing in the results for complete mixing.

Attempts have already been made to obtain a more rigorous solution of the problem by having a lesser number of assumptions. Pfann[89] has recently discussed the situations in which zone and solid have different cross-sectional areas.

Types of liquid mixing — The degree of segregation in zone melting depends on transport conditions in the liquid. Three cases can be distinguished. In the first case, complete mixing occurs in the liquid (concentration in the zone is uniform). This occurs either with very slow zone travel rates or with large amounts of forced convection. Complete mixing gives the optimum separation that can be attained. No dependence on zone travel rate has been predicted. Theoretical solutions to segregation problems for this type of mixing are simpler.

In the second case, there is no mixing at all — the transport being only by diffusion. This can happen only in microzone refining, i.e. in very small capillaries. This class gives the least separation and is predicted to depend on zone travel rate.

Partial mixing, the third case, which is the most common and corresponds to the usual experimental conditions in zone melting of organic materials, falls in between the above two extremes. Here the zone is insufficiently mixed, the concentration in the zone is not uniform and the separation depends on the zone travel rate. Burton *et al.*[90] have developed a boundary-layer approach for this case. It is assumed that the bulk of the zone is well mixed and uniform, but near the interface a stagnant boundary layer is present in which no mixing occurs. The thickness of this layer then naturally becomes a basic parameter for theoretical discussions. Wilcox[76,110] has developed techniques for estimating the boundary-layer thickness for free convection mixing conditions.

An objection to the boundary-layer approach is that its thickness most probably varies slightly with the zone travel rate. This might be due to a uniform flux of liquid through the boundary layer in zone melting. At present, it is not possible to predict by any means the quantitative effect of this flux on the boundary-layer thickness. But the importance of boundary-layer treatment lies in the fact that it has successfully explained many experimental results.

Complete solid solutions — A survey of the literature as presented in Table 2 shows that most of the theoretical work has been done by assuming a constant distribution coefficient. There are two reasons for this. Firstly, it is easier to find such solutions and, secondly, much of the early zone melting work was done on high purity inorganic semiconductors and metals, which usually have constant distribution coefficient. Comparatively, less work has been done for the more complex case of variable distribution coefficient.

Simple eutectic-forming systems — The impurity redistribution for complete mixing resulting from zone melting a eutectic-forming mixture can be obtained easily. It is clear from Fig. 6(b) that material will come out completely pure, if the zone concentration builds up to the eutectic composition. When the eutectic point is reached, the original composition solidifies giving a step function concentration profile. The length L_p of purified material is given by

$$L_p = L\left[\frac{W_e}{W_o}-1\right]\left(\frac{\rho_l}{\rho_s}\right) \qquad \ldots(1)$$

where L is the length of the zone, W_e and W_o are the eutectic and original compositions respectively in weight fraction, and ρ_l and ρ_s are the liquid and solid densities respectively. For multiple passes of a long charge each pass will leave behind an additional length L_p of the purified material. Thus, the ultimate concentration profile for a finite charge would be a region at the head end of pure material and a region at the tail end of material of eutectic composition. The relative sizes of the two regions would be given by the requirement that the impurities removed from the head end must be found in the tail end. The ultimate distribution is independent of zone size, contrary to the results for materials with constant distribution coefficient where smaller zones give higher separation. But the rate to ultimate distribution is affected by zone size — the rate is larger for larger zones. Therefore, if all the assumptions are valid, then normal freezing should give the ultimate distribution in one step only.

Calculation of the impurity redistribution resulting from zone melting with no mixing in the melt is much more complicated. It is not possible to specify with some certainty the boundary condition at the freezing interface. This is because a solid consisting of two insoluble phases consists of intermixed but discrete microcrystals of the two phases. As the exact effect of this secondary diffusion on the primary diffusion from freezing interface to bulk zone cannot be calculated easily, its effect is neglected in calculating the mass transfer in zone.

Due to non-mixing of the melt, the change from pure product to initial composition is not a step function, but is an approximately exponential increase to the initial composition.

After a zone has moved far down a long charge, the concentration profile in the zone itself is given by[76,77]

$$W = W_o\left[1+\left(\frac{W_e}{W_o}-1\right)\exp\left(-Z\frac{V\rho_s}{D\rho_l}\right)\right] \qquad \ldots(2)$$

where W is the weight fraction at a distance Z from the freezing interface; D, the primary diffusion; and V, the zone travel rate.

Integration of Eq. (2) over the zone gives an average zone concentration W_l given by

$$W_l = \frac{\int_0^L W dZ}{L}$$

$$= W_o\left[1+\frac{D\rho_l}{LV\rho_s}\left(\frac{W_e}{W_o}-1\right)\left\{1-\exp\left(-\frac{LV\rho_s}{D\rho_l}\right)\right\}\right] \qquad \ldots(3)$$

This shows that each zone moving along a semi-infinite charge will carry along with it

$$Q \simeq L\rho_l\ (W_l-W_o)$$

$$= \frac{D}{V\rho_s}(W_l-W_o)\left[1-\exp\left(-\frac{LV\rho_s}{D\rho_l}\right)\right] \qquad \ldots(4)$$

amount of impurity per zone pass per unit cross-sectional area. This is similar to that found for complete mixing, except that the amount moved is less. The ultimate concentration in a finite charge for no mixing in the zone can be approximated. The same method as for complete mixing is used, except that W_l as given by Eq. (3) is used for the tail concentration instead of W_e. The transition from pure material to W is not sharp as with complete mixing, but would smear out. The relative sharpness, however, increases as the parameter LV/D decreases, approaching complete mixing for $LV/D<0{\cdot}1$.

An identical treatment is from boundary-layer approach. The results are in between the above two cases. The average zone concentration after moving through a long charge is[76,111]

$$W_l = W_o\left[1+\left(\frac{W_e}{W_o}-1\right)\exp\left(-\frac{\delta V\rho_s}{D\rho_l}\right)\right] \qquad \ldots(5)$$

where δ is the boundary-layer thickness. Therefore, the impurity per unit cross-sectional area moved with the zone is

$$Q=L\rho_l\ (W_e-W_o)\exp\left(-\frac{\delta V\rho_s}{D\rho_l}\right) \qquad \ldots(6)$$

The ultimate distribution is similar to the case corresponding to no mixing, but with the concentration given by Eq. (5).

Constitutional supercooling — The above solutions in the case of simple eutectic-forming systems are obtained on the assumption that a planar freezing interface advances into the melt. But often the interface is not smooth; it is rough and even consists of needles and dendrites. Thomas and Westwater[112-114] have observed this fact from a study of a motion picture of the freezing phenomenon. The rough interface is produced by the redistribution of solute which occurs at the interface, and is due to changes in composition in the melt. The phenomenon responsible for this is known as 'constitution supercooling'[112-123]. Near the freezing interface the temperature falls nearly linearly with the distance. The concentration of the solute rejected at the freezing interface falls approximately exponentially with distance. This decreases the freezing point of the material towards interface as shown in Fig. 7. Under certain conditions the actual temperature will fall below the melting point in a region near the freezing interface as in Fig. 7. The melt in this region is said to be constitutionally supercooled and is unstable with respect to perturbations of the shape of the interface. The phenomenon has been analysed theoretically for mixtures with a constant distribution coefficient[119,124,125] and the results have been confirmed by experimental work on inorganic[115-118,120,122,123,126-129] materials. The comparative work on organic materials has not yet been done, but the results obtained for constant distribution coefficient could be adopted. For low supercoolings a cellular interface is formed. At higher supercoolings the interface breaks down to dendrites or needles. As the interface becomes rougher, the mass transfer is hindered and thereby more impurities solidify. Portions of the melt can even be occluded if the interface is dendritic. Constitutional supercooling increases as (1) the zone travel rate increases; (2) the general impurity level rises; (3) the actual temperature gradient decreases; (4) the degree of separation decreases according to phase diagram; and (5) the dependence of melting point on composition increases.

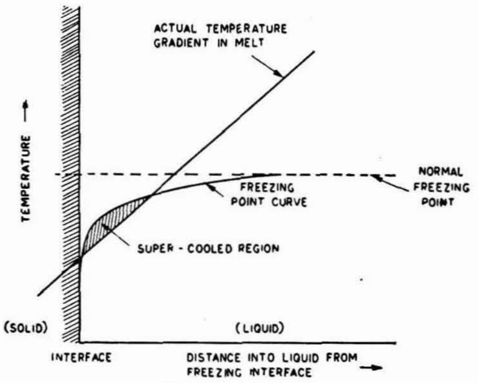

Fig. 7 — Phase diagram corresponding to constitutional supercooling

The quantitative effect of constitutional supercooling on mass transfer in the zone cannot be predicted as yet. Tiller[130] and Kramer[131], however, have made a beginning in this direction.

Experimental segregation data — Few measurements[13,14,76,110,133,134] of concentration profiles of zone refined organic materials have been made. The results[13,14,76,112,133] on binary eutectic-forming systems may be summarized as follows. Although the overall separation can be correlated on the basis of the boundary-layer treatment, the concentration profiles are very much different from any of those expected for a eutectic-forming mixture. The break from pure material occurs quite soon and is more gradual than predicted. Constitutional supercooling is supposed to be responsible for this.

As many passes are essential for ultrapurification of organic materials, instead of the ideal single pass predicted for simple eutectic-forming systems, use of an effective distribution coefficient has sometimes been proposed[87,135]. This is justified because the quantity of impurity trapping is expected to increase with the impurity content of the melt. If the increase is assumed proportional, then the proportionality constant would be an effective distribution coefficient. But this approach has two flaws. Firstly, the experimental concentration profiles generally do not correspond to a constant effective distribution coefficient. Secondly, the effective distribution coefficient is not predictable and should be a function of all those things on which constitutional supercooling depends and perhaps some more. Dependence on the overall impurity level would make any prediction of an ultimate distribution impossible. More experimental and theoretical work is needed for the better understanding of impurity segregation in zone melting of eutectic-forming organic mixtures.

Some General Considerations

It is an accepted rule that the ultrapurification should start from relatively pure materials[136]. Its advantage is that various impurities can be easily removed *in toto* early in the purification process rather than applying ultrapurification techniques directly to the crude material. But there are exceptions to this also. Hines *et al.*[137] have ultrapurified natural sea water by zone freezing and achieved 100 per cent purity with one zone pass only. The reason is obvious; sea water forms eutectic combinations with its salts. Some results[8,76,136-140] are available on organic materials where success have been achieved in directly ultrapurifying crude materials.

Herington[87] has pointed out that the two critical factors, the amount and the nature of impurities present, must be given due consideration before zone refining any sample.

If one decides to zone refine a crude material, there is always the danger that the eutectic composition will be reached in the fractionation process. Also ultrapurification would involve many kinds of difficulties[111]. However, if one assumes the classical approach, and an initially pure sample is zone refined, then only one[8] zone pass is required under ideal conditions. But it is not yet possible to achieve these ideal conditions in practice. Hence, a distribution coefficient arises as a function of the conditions and is not due to the nature of the material or its phase diagram. Therefore, multiple zone passes are required for ultrapurification. With a eutectic mixture, the ultrapure fraction is always found at the beginning of the charge.

If solid solutions are formed with impurities, a distribution coefficient arises as a function of the nature of the materials. Here the methods of zone refining inorganic materials are applicable and multiple zone passes are essential. As impurities may either raise or lower the melting point[75], the ultrapure fraction may be found at either end of the charge. Hence the safest is to take the middle portion of the charge as the ultrapure material, if one is unable to analyse the sample.

Much of the experimental evidence indicates that there is sufficient grounds for believing that most organic materials do form eutectics at both high and low concentrations with their impurities.

Allied Techniques

Normal freezing — A number of organic solids have been ultrapurified by normal freezing. Compounds are normally frozen by lowering a tube through a sharp temperature gradient. The techniques[24,141,142] used for the preparation of organic single crystals are applicable. Schwab and Wichers[143] have described a device in which a sample tube is supported in a vertical furnace on a float resting on a cylinder of water; the float is lowered by draining the water slowly from the cylinder.

It has been seen experimentally and theoretically that normal freezing is a more efficient purification process than zone refining for a single pass. As already seen, normal freezing is very useful as the initial step of a multistep zone refining process. The normal freezing has some disadvantages also such as: (1) automation is difficult since portions of charge must be removed periodically for a multistep process; (2) contamination is a problem in removing unfrozen sections; (3) in normal freezing, the material is liquid for a greater time than in zone refining; thus the risk of thermal decomposition is greater here; and (4) a comparison of multistep zone refining with cropping and multiple-normal freezing indicates that the yield of purified material is less for normal freezing.

Dickinson and Eaborn[144] and Doede[145] have developed refined equipment and operating procedures with the result that techniques of stirring, temperature control, rate of movement of the freezing interface, and the mechanics of operation have made the normal freezing process yield better purity material as compared to even zone refining.

Vapour zone refining—Some organic semiconductors cannot easily be zone refined, either because they decompose at or near their melting point or because they sublime too readily. Weisberg and Rosi[146] have developed a technique known as ' vapour zone refining ' for such compounds. In this, the temperature control along the heated zone allows the compound to sublime across a gap where it is collected on previously sublimed material. The movement of the charge by Teflon plugs allows for successive sublimations with a minimum of contamination. The difference in sublimation rates of impurities and major component allows for a separation at both ends of the charge. Sloan[147] has refined tetracene by this technique. Although vapour zone refining is gradually coming into use, its theoretical aspects are still not clear.

Laboratory Considerations of These Techniques

The techniques used in zone refining of inorganic materials may not be directly applied to the organic materials. The low rates of crystallization, low thermal conductivities, widely differing densities of solid and liquid phases, and the reactivity of the organic compounds require special apparatus and techniques. However, the essential requirement is to pass molten zones through a sample. The following factors have to be considered in designing an apparatus.

Parameters of Importance

Zone travel rate and direction of movement — The purification per zone pass increases as the zone travel rate decreases, which means that the time required to complete a pass increases. Pfann[72] has analysed the selection of an optimum travel rate. Generally, unstirred organic compounds may be zone refined at rates of 10-50 mm./hr[8,133]. From the available data it seems that 20 mm./hr is the most common rate.

It has already been shown that constitutional supercooling, which lowers the separation, increases as impurity content increases. Due to this, it is often advantageous to have a lower zone travel rate at the beginning of purification than in later passes, where the purity is higher. Presence of constitutional supercooling may be detected by examining the interface with a magnifying glass for surface irregularities.

In organic compounds, vertical zone melting is more efficient. For example, an attempt to refine naphthalene in a horizontal tube did not give good results because the melt flowed back under the solid that had just been refined. With charge in a vertical tube the zone must be started from the top and moved down. If it is required to remove gases from the product, an upward zone pass should also be included, preferably initially.

Zone size — The capacity of a zone to carry impurities increases with its size: But a large zone has more inter-mixing with the impure tail end of a charge which reduces the ultimate purification attained. Therefore, some compromise is essential between small and large zones. The best result from a single zone passed repeatedly through a charge can be obtained as follows. The first pass should be normal freezing. After this, zone passes should be made with the zones initially about one-third of the total charge length and then decreasing the length as small as possible in latter zones. The smallest zone length should be equal to the diameter of the container.

This analysis is not so easy where many zones are passed simultaneously through a charge. To a first approximation, the rate of purification at the beginning of operation is proportional to the number of freezing interfaces present and to the total volume of melt in the charge. Hence, the product of the number of zones and the total melt volume should be made as large as possible in order to have the maximum purification. Since the total volume of melt is proportional to the product of the number of zones and the zone length, the purification rate increases with the square of the number of zones times the zone length. Hence, the maximum purification rate is achieved when the zones are spaced as close as possible, which means a small zone size.

Temperature gradient — The success of zone melting depends a lot on the temperatures and the temperature gradients. For free convection, a high temperature gradient at the freezing interface usually gives better mixing in the zone. Physically, this means a large temperature difference between the heater and the ambient. It also means a heater of the same diameter as the zone, so that the line of demarcation between heated and cooled material is sharp.

As already shown, a high temperature gradient is of help in reducing constitutional supercooling, which can reduce the separation drastically. But a high temperature gradient is disadvantageous also, if the zone motion is not smooth because it would increase any jerk in zone motion, while a low gradient, due to latent heat effects, tends to smooth them out. One should also remember the thermal stability requirements before adjusting the temperatures and gradients.

Temperature control of the heater is also very important because fluctuations in temperature lead to fluctuations in the rate of movement of the freezing interface and can greatly reduce the separation. The simplest way of controlling temperature is to use a constant voltage transformer for supplying input to heater. Control of room temperature is also essential. Long-term fluctuations (hours) should be kept below about 0·2°C. while short-term fluctuations (minutes) be below 0·01°C.

Container diameter and length — Most of the organic semiconductors in the molten state are highly volatile; therefore, open boats are usually not used as containers. Pyrex tubes are most common, although vycor and quartz are also used. As glass tubes break, Teflon[13] as well as stainless steel[134] tubes have also been used. The metal tubes have the disadvantage that the zone melting operation and the possibility of contamination cannot be seen.

The tubes should be as long as practical, but at least ten times the diameter of the tube. The chance of back-mixing of impurity from the tail of the charge is less, if the tube is long. Hence, the ultimate purification of the head of the charge is more.

Under free convection conditions, large diameter tubes give greater stirring and greater separation. But the size is limited by the shape of the solid-liquid interfaces, which become increasingly curved as tube diameter increases. The tubes of 10-20 mm. inner diameter are most convenient and common.

A serious problem with glass tubing is their breakage. Most organic semiconductors adhere to glass and expand on melting; the resultant force on the tube is sufficient to shatter it. This often happens after the first pass, especially if the zone is moved up. The problem is more severe with larger tubes, and apparently with high purity materials which seem to adhere better to the glass. Walter[148] has suggested the use of a close-fitting glass plug with a rubber policeman at the end of the tube, thus permitting expansion on melting. The best method for avoiding breakage as well as facilitating removal of the purified sample from the tube is to treat the glass with a chlorosilane (for example, Dri-Film SC-87 made by the General Electric Co., Silicon Products Division, Waterford, New York). After application of the chlorosilane, the glass should be rinsed thoroughly with reagent grade hydrocarbon solvent or with reagent grade ether, and then baked *in vacuo* at 400°C. The resulting film adheres very strongly and is removed only by treatment with hydrofluoric acid or with the very effective cleaning solution suggested by Crawley[149]: consisting of 5 per cent hydrofluoric acid, 33 per cent nitric acid, 2 per cent Teepol (an alkyl sulphate wetting agent) and 60 per cent water. For horizontal zone refining, O'Neal[150,151] devised a hot finger to keep a space open above the charge. Lacey and Lyons[152] used a heater at the top of a vertical tube of anthracene to prevent sublimation from the top.

Mixing of the zone — The rate of purification can be increased by stirring the zone. Ultrasonic mixing has been used in growing organic single crystals, but its use in zone refining organics has to be investigated.

Hardly anyone has used forced convection, but a sort of stirring is achieved by free convection in the zone, if the tube is not a capillary. Free convection is fluid motion caused by density variations resulting from temperature and/or concentration variations within the fluid. In zone refining both temperature and concentration in the melt are non-homogeneous. Variation in temperature results from heat transfer to and from the melt and to the freezing and melting solid, while variation in concentration is due to segregation at the freezing interface.

In the usual equipment, the zone is heated by conduction from a resistance heater. Therefore, bulk mixing of the zone results primarily from the free convection currents generated at the tube walls by transfer of heat from the heater, through the tube walls to the zone; under these conditions, the bulk of the zone is actually well stirred. But these free convection currents are dead near the solid-liquid interfaces, leaving a boundary layer of nearly stagnant fluid adjacent to the interfaces. As already shown, the purification in zone refining is strongly dependent on the boundary-layer thickness at the freezing interface. This boundary-layer thickness, in turn, depends primarily on the free convection generated at the freezing interface itself by the density difference between fluid at the freezing interface and in the bulk zone. The larger the density difference, the smaller the boundary-layer thickness, and the greater the purification attained. This indicates that with high purity materials a higher temperature gradient will normally give a larger density difference and hence a larger purification.

Stirring is also much greater when the fluid of higher density (between the bulk zone and the freezing interface) is at the top rather than at the bottom. For a vertical tube, this normally means that the zone is moving down. Another simple way of increasing the free convection at the freezing interface is to introduce a thin axial rod of high thermal conductivity — a metal — into the centre of the charge. Wilcox[76] has also suggested high speed rotation of the zone melting tube as a method of increasing free convection stirring.

Gas liberation — Usually gases are more soluble in liquids than in solids. Because of this, gases are trapped among the solid crystallites formed during the normal tube-filling operation. Unless special precautions are taken, gas will be dissolved at the melting interface and liberated at the freezing interface in zone refining. If a horizontal tube is used, this would give rise to a channel along the top of the material. In a vertical tube, if the zone is going down, the bubble collects at the freezing interface. In larger tubes, this normally forms a gas tube inside the frozen solid, which would somewhat avoid breakage in multiple passes — a definite advantage. In small tubes, however, it can block the tube completely and may stop the purification. If the zone is moved up instead, the bubbles collect at the melting interface in larger tubes and so are swept out, while in smaller tubes blockage again occurs. Many a times the bubbles can be moved up by periodically tapping the tube or vibrating it.

Container charging — Organic semiconductors are often available as fluffy, crystalline powders which are not easily loaded into zone refining tubes[59]. To overcome this difficulty, tubes of the design shown in Fig. 8 (taken from ref. 59) are convenient. The material to be purified is placed in the upper section, the tube is evacuated, and then is filled with an inert gas, argon or nitrogen. The material is melted by a tubular heater and is allowed to flow into the lower section and solidify. The tube is again evacuated, and the constriction between the sections is heated gently to remove adhering solid. Inert gas is admitted until the pressure is about 30 cm. Hg and the tube is then sealed at the constriction. By carrying out zone refining in an inert atmosphere rather than *in vacuo*, sublimation from the top of the charge is reduced.

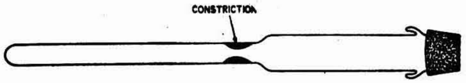

Fig. 8 — Zone refining tube

If oxygen causes decomposition of a substance at its melting point, the sample may be sublimed *in vacuo* and the evacuated sublimator may be transferred to a gloved box for loading of the zone refining tube. The tube may be closed in the box by an adapter, through which the evacuation and gas filling described above may be carried out. This time-consuming procedure may be avoided, in many instances, by using a portion of the sample as its own scavenger. Anthracene, for example, reacts with oxygen to form anthraquinone, and the latter is rapidly segregated by zone refining.

Heater design — Simple resistance heaters are usually adequate. They should be concentric to the tube containing the material to be purified. For most materials, special coolers are not necessary because the ambient temperature is often low. A number of heater designs have been discussed by Lawson and Neilsen[23] and by Joncich and Bailey[14].

Heaters have been made by wrapping Nichrome wire toroidally around sections of pyrex or vycor tubing with notch ends. The outside surfaces of these easily made heaters are insulated; they are found to give high efficiency and sharp zones[153].

A focused light source has also been used as zone refining heater. This method[154,155] cannot easily be adopted to the formation of many zones, and it can cause the decomposition of many organic compounds unless the light is filtered.

Several other designs[136,156-159] are also reported in the literature.

Zone travel mechanism — The sample tube may be raised through a set of heaters or the heaters may be lowered over the sample tube, the results being identical. Motion is usually provided either by a rotating threaded shaft or by winding cord on a rotating spool and hanging the tube by the cord. A motor attached to an electric clock is very convenient for effecting this motion. Potential energy change in the level of a liquid[143] or of a falling weight has also been used successfully[24,136,160,161].

Passage of *n* zones through a charge may be effected in the following three ways: (a) *n* passes

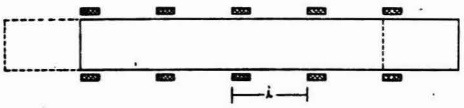

Fig. 9 — Schematic representation of a five-heater unit for reciprocating motion in zone refining

through one heater; (b) one pass through *n* heaters; (c) $(n+m-1)$ reciprocating strokes through a battery of *m* heaters at intervals of *i* cm. with charge of *mi* cm. long, as shown in Fig. 9. The tube is slowly moved from its starting position (solid line) through *i* cm. (dashed line); it is then quickly returned to the starting position. Method (c) is by far the best since it requires fewer heaters, less heat and much less space than method (b) and it requires less time and space than method (a). The apparatus[158,160,162] becomes complicated when automatic multipass arrangement is made*.

Summary

Various methods in use for purifying organic semiconductors which are applicable *in toto* to other organic solids as well have been briefly outlined. A critical survey of the literature concerned with the zone refining and allied techniques has been made in view of their importance in the purification of semiconductors. It is shown that zone refining and allied techniques have in common a partial or complete segregation of the components of a mixture at a solid-liquid interface during crystallization. The segregation depends on the mixture's phase diagram. Most of the organic semiconductors have eutectic-type phase diagrams. Therefore, their behaviour in zone refining is quite different as compared to inorganic semiconductors which have considerable solid solubility. It has also been shown that the degree of separation in zone melting depends not only on the phase diagrams but also on the mass transfer conditions in the zone. The effect of mass transfer conditions on separation are discussed in detail. The difference between theoretical predictions and experimental results are attributed to 'constitutional supercooling'. It is pointed out that the achievement of ideal thermodynamic equilibrium between freezing, solid and bulk zones is advantageous in the ultrapurification of organic semiconductors.

Considerations in the design of zone refining apparatus have been discussed in detail.

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*An interesting work [Pfann, W. G., Miller, C. E. & Hunt, J. D., *Rev. sci. Instrum.*, **37** (1966), 649] has been reported since this review was written. An improved zone refining apparatus has been developed which simultaneously utilizes natural convection in the liquid and slow rotation of a horizontal charge tube to achieve unusually short zone lengths in tubes of modest diameter. A new cooling technique is used to achieve short interzone spacing and planar liquid-solid interfaces. The method is specially suitable for organic solids of low thermal conductivity.

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REVIEWS

COMPLEX ANALYSIS by Lars V. Ahlfors (McGraw-Hill Book Co. Inc., New York), Second Edition, 1966. Pp. xiii+317. Price $ 8.95

Conformal mapping, contour integration, infinite sequences and complex integration are but a few topics of complex analysis, which find useful application in the fields of physics and engineering. Prof. L. V. Ahlfors' well-written book on complex analysis fulfils the need of a useful text-book and guide on the subject. The presentation is forceful, logical, lucid and analytical.

The book under review is the second revised edition of the book first published in 1953. Besides containing about 70 additional pages, the revised edition includes an extra chapter on elliptic functions. The other chapters have been revised, rewritten or remodelled to some extent.

The first two chapters deal with the algebraic properties of complex numbers and the properties of power series. It has been clearly brought out that in the theory of complex analytic functions all elementary transcendental functions can be expressed through e^z and its inverse log Z. The author has introduced with advantage in the section on 'point set topology' discussion about metric spaces and compactness, which should be of interest to students of pure mathematics.

In Chapters 3 and 6, analytical functions as mappings, conformal mapping, canonical mappings of multiply connected regions and Dirichelet's problem have been discussed at great length. It would have added to the usefulness of the book if applications to physical problems had been indicated.

Cauchy's contribution in the field of complex analysis has been well recognized. In recent years quantum mechanical theories of dispersion have been attempted on the basis of Cauchy's results. Prof. Ahlfors has discussed Cauchy's investigations in Chapter 4 of his book. The chapter on complex integration also includes a brief discussion on harmonic functions.

Chapter 5 which deals with infinite sequences has been given the new title, 'Series and product developments'. The treatment of the last two chapters on 'Elliptic functions' and 'Global analytic functions' exhibits great mathematical rigour. A number of exercises have been included at the end of each section to illustrate the principles discussed. A few more exercises on applied subjects like hydrodynamics, aerodynamics and physics would have added to the utility of the book.

The arrangement of topics is original and the printing and get-up of the book bear the excellence of McGraw-Hill books. The book will continue to be popular with teachers and students of pure and applied mathematics.

K. VIJAYARAGHAVAN

INFRARED RADIATION by Ivan Simon (D. Van Nostrand Co. Inc., New York), 1966. Pp. 119. Price $ 1.50

The book under review is published for the Commission on College Physics and obviously intended to give a short and precise account of the various aspects of infrared radiation. The subject matter is dealt with in a simple and lucid style, and follows the pattern of the text-books that are generally used in our intermediate classes.

The first chapter deals with the nature of the infrared radiation presented in a historical and descriptive pattern. The well-known laws of radiation are also included in this chapter.

The second chapter discusses various sources of infrared radiation both thermal and non-thermal. A sub-heading like non-thermal sources for infrared radiation is apparently unorthodox; the laser is described under this. The limits of detection of radiation, particularly with reference to the signal noise ratio, are also discussed.

The fourth chapter deals with materials and optics. The optical part includes a concise account of optical systems as well. In Chapter 5 elements of molecular spectroscopy are discussed in relation to infrared spectroscopy. The applications of infrared radiation are described in Chapter 6. Typical examples like infrared viewing and imaging devices, evaporograph and infrared photography are described.

In all the above chapters the stress is mainly on the basic principles and typical equipment. Neat diagrams are generously provided in the descriptive portions to make the subject matter very readable. Each chapter is followed by adequate references and bibliography.

If this book is representative of the others in the series, they can all be recommended for the libraries.

V. RAMAKRISHNA RAO

DOCUMENTS ON MODERN PHYSICS — QUANTUM STATISTICS AND COOPERATIVE PHENOMENA by John Gamble Kirkwood, Collected Works, Vol. 1, edited by F. H. Stillinger, Jr (Gordon & Breach Science Publishers, New York). Pp. x+182. Price $ 4.95 (paper); $ 8.00 (cloth)

This volume contains Kirkwood's papers on quantum statistics and cooperative phenomena. This collection is very valuable, because it contains original and basic ideas put forward in the early days of the development of quantum statistics which even today is an important field in theoretical physics.

Many of Kirkwood's later publications are in the field of classical statistics and, therefore, it is interesting to note that he wrote the two papers 'Quantum statistics of almost classical assemblies' and 'Statistical theory of low frequency intermolecular forces'. These two papers in a way verify that for most applications of interest in physical chemistry the quantum corrections to predictions of classical statistical mechanics are indeed small. In some of his papers he has used the electrolyte solution theory technique in the application of finite temperature Thomas Fermi theory to correlation effects in systems with a degenerate electron gas. This work, however, did not give good agreement in the high density region with the known electron gas energy limit, particularly because it neglected exchange energy and the fact that electrons are not static point charges but have a finite velocity. The phenomenon of order-disorder transformation in binary alloys has been the subject studied by Kirkwood in some of his papers. Anybody who has gone through these papers will agree that they contain the very comprehensive and systematic theoretical analysis of this broad class of problems in classical statistical mechanics. Some of the ideas of Kirkwood as given in the papers collected here when utilized to recalculate his results with new data and modern computational methods are likely to give better and interesting results.

It would have been better if a complete list of Kirkwood's publications was included in this volume. The name and reference of the journal should have been given along with each paper rather than listed in the beginning. The volume is very important for those who want to understand the nature and characteristics of cooperative phenomena, because the basic ideas and approach of Kirkwood is still an important tool in this field.

KRISHNAJI

DOCUMENTS ON MODERN PHYSICS — DIELECTRICS, INTERMOLECULAR FORCES AND OPTICAL ROTATION by John Gamble Kirkwood, Collected Works, Vol. 2, edited by R. H. Cole (Gordon & Breach Science Publishers, New York). Pp. x+271. Price $ 4.95 (paper); $ 8.50 (cloth)

This volume contains Kirkwood's papers in the fields of dielectric constant measurements, dielectric theory, intermolecular forces and optical rotatory power of molecules. Some of the papers in this volume have long been classics and essential reading for anyone actively interested in the subjects they treat. The set of papers dealing with dielectric properties of polar liquids are very important, particularly the extension of Onsager's theory and the study of the influence of hindered molecular rotation. Besides clearly showing how and when Onsager's theory could be more useful, the papers have been able to throw some light on hydrogen bonding and its utility in drawing semi-quantitative inferences from dielectric data about the form of local correlation of molecules. Recent developments in microwave spectroscopy have revived interest in intermolecular forces greatly and it is interesting to find that the volume includes the 1931 paper 'The van der Waals' forces in gases' by Slater and Kirkwood. This paper uses the variational method to calculate the polarizability and mutual polarization energy of helium atom. Both the formula for the attractive energy of helium and a generalization for the energy of closed shell atoms in terms of their polarizabilities have been widely used and frequently quoted. I think the readers will be careful in using the quantitative results obtained in the various papers because quite a number of them have been revised by later works.

Recently, areas common to physicists and chemists have been expanding rapidly and today persons who understand the relation between molecular and macroscopic phenomena are needed most. It is interesting to note that Kirkwood understood the relation of molecular and macroscopic fields clearly at a time when much of the discussion in the literature was very confused. The editors need to be congratulated for bringing out this volume for the benefit of specialists in molecular physics as well as those non-specialists who wish to understand basic concepts in the theory of dielectrics and related fields.

KRISHNAJI

WET COMBUSTION AND CATALYTIC METHODS IN MICROANALYSIS: Methods in Microanalysis Series: Vol. 2, edited by J. A. Kuck; translated by K. Gingold (Gordon & Breach Science Publishers, New York), 1965. Pp. xx+412. Price $ 21.00

This book is the second volume in the series Methods in Microanalysis and presents translated papers on wet combustion and catalytic methods. The objective is to familiarize the microchemists with the research work carried out in this branch of analytical chemistry, mainly in Russia and Czechoslovakia. The methods described, both qualitative and quantitative, seem to have advantage over the classical methods in that the degradation of the compound is achieved more satisfactorily. Although some of the papers elaborate the use of the catalysts for macro and semimicro methods, the observations on their activity in many cases show a good promise for the possible transposition of the methods to micro scale.

Some chapters are devoted to simultaneous identification and estimation of elements; however, some of the procedures, though of sufficient theoretical interest, are not quite convincing for the necessary ease in manipulation.

Of the total forty-two chapters, twenty are due to the publications of the noted Russian academician, Prof. A. P. Terentev, and ten are the contributions of the famous Czech microanalyst, Körbl, who has dealt with the investigations relating to the properties and analytical applications of the decomposition product of silver permanganate.

Three chapters describe sensitive qualitative tests for elements in organic and organometallic compounds which include the metals As, Sb, Bi, P and Hg. The traditional sodium fusion has been replaced by fusion with Ca, Mg, magnesium nitride and decomposition product of silver permanganate. Eleven chapters describe simultaneous determination of elements. For this purpose, wet combustion methods employing chromic acid-sulphuric acid mixture have been successfully used for a variety of compounds. Estimation of Si with many other elements seems to have been of particular interest in organosilicon compounds and also in Si-Cu alloys. Four chapters cover the methods for simultaneous determination of C, N, S and halogens. In addition, the use of acrylonitrile for the determination of monohydric alcohols, thioalcohols and thiophenols, microhydrogenation and determination of active hydrogen are also described in some chapters. Semimicro and micro methods for carbon and hydrogen with chromium sesquioxide as catalyst are reported in two chapters. An account of the preparation, properties and uses of the decomposition product of silver permanganate as a powerful oxidation catalyst is given in full detail in ten chapters. The remarkable properties of this reagent permit smooth analysis of compounds covering a wider range of elements, comparatively at lower temperatures.

This volume thus provides valuable information on some surface combustion catalysts and wet combustion processes. It should be very much useful as a ready reference book for the analysis of difficultly combustible compounds.

V. S. PANSARE

ENGINEERING THERMODYNAMICS by Munir R. El-Saden (D. Van Nostrand Co. Inc., New York), 1965. Pp. xv+216. Price $ 5.95

This is an interesting little book for a one-semester introductory course in the subject. The book approaches the subject matter in terms of probability and uncertainty, as was done by the early pioneering book of Dr Myron Tribus, *Thermostatics and thermodynamics*. The language is simpler and explanations using numerical examples abound in the early section. The concepts of heat and increasing entropy principle are shown to follow logically from the basic postulate of statistical inference. The student gets the strong impression that a microscopic approach to the subject is equally possible, once the averaging methods are logically applied. In Chapters I and II the author introduces the students to probability, uncertainty entropy and Jayne's formalism based on information theory. This is a potent approach to the concept of entropy which is usually a bugbear to the normal undergraduate students. Soon after, the properties of gases and a detailed treatment of the Maxwell-Boltzmann distribution are introduced. This will prove extremely useful to an engineering student. Following this the author presents the concepts of thermal equilibrium in a general system, and of increasing entropy. These chapters and the problems given therein are well worth the study of both students and teachers, as they contain a clear delineation of the basic tenets of thermodynamics.

It is of interest to note that these concepts, particularly the increasing entropy principle, are presented before the formal statement and application of the first two laws of thermodynamics.

In the next three chapters the author introduces the laws of thermodynamics for closed and open systems as a logical sequence of the formalism introduced in the early sections of the book. The last chapter discusses the fundamentals of energy conversion and refrigeration — of particular importance to engineering students.

The layout of the subject matter is extremely well thought out, the language and examples used are clear and simple, such as to sustain the interest of the student. The problems given at the end of the chapters are of varying degree of complexity — some of them being indeed too simple to serve as anything better than review questions. The appendices contain some data for conversion factors and properties of steam and a few refrigerants. Surprisingly, there are no serious printing mistakes. The units used in the text are FPS units and temperature is given in degrees Rankine. Indian students may find these units strange, but nevertheless a careful study of this book is recommended for those who wish to take up advanced work in this field. Some adventurous schools may wish to try this delightful book as an introductory text for the subject of engineering thermodynamics which is so often presented as a dreary subject to the students.

V. RAMAKRISHNA

VACUUM SCIENCE AND ENGINEERING: PROPERTIES OF GASES AT LOW PRESSURE, VACUUM MEASUREMENTS, DESIGN AND OPERATING FEATURES OF VACUUM PUMPS AND SYSTEMS by C. M. Van Atta (McGraw-Hill Book Co. Inc., New York), 1965. Pp. xiv+459. Price $ 15.00

This is a highly commendable book in a new and rapidly developing field. The vast experience of Dr Van Atta in the fields of vacuum science and engineering is clearly visible in this comprehensive and thorough volume.

The book has been aptly titled, since it emphasizes both the fundamentals and the engineering applications of vacuum science. The first two chapters are devoted to developing the basic ideas concerned with the nature and behaviour of gases and gas flow. In the second chapter, a very useful treatment of gas flow in the transition pressure range has been included. The next two chapters deal with pressure measurement and vacuum analysers and leak detectors. The fifth and the sixth chapters are particularly informative regarding mechanical vacuum pumps and vapour-jet vacuum pumps. The seventh and the eighth chapters deal with the measurement of pumping speeds and the design of vacuum systems. The last chapter describes the latest developments in the field of ultra-high vacuum, such as getter-ion pumps and cryogenic pumping. Detailed references and useful appendices have been given. Ample illustrations have been provided. Special care has been taken to facilitate practical applications of formulae, by choosing appropriate forms and units, and incorporating conversion factors in Appendix IV.

This book will be of great interest and use to research scientists, industrial engineers and students who are interested in the field of vacuum science.

A. S. DIVATIA

ENERGETICS IN METALLURGICAL PHENOMENA: Vol. I, edited by William M. Mueller (Gordon & Breach Science Publishers, New York), 1965. Pp. xiv+425. Price $ 19.50 (reference edition); $ 9.50 (paperback edition)

One of the earliest seminars on 'Thermodynamics in Physical Metallurgy' was organized by the American Society of Metals in 1949. The late Dr Bridgman who participated in this seminar, had, in his introductory talk emphasizing the value of the thermodynamic approach, observed, "The methods of thermodynamics are endowed with greater certainty than any others, and conclusions reached by a rigorous thermodynamic argument are absolutely secure. This is partly due to the freedom of thermodynamics from any assumption about the detailed mechanism of the system under analysis, assumptions which are always in danger of being controverted by new knowledge. It is also partly due to the ostensible generality of thermodynamics." Over the years, there has been a growing awareness of the value of the thermodynamic and the kinetic approach to the elucidation of metallurgical phenomena. For the physical metallurgist, 'energetics' have been the means for organizing available information, and gaining insight to the driving forces in phenomena, such as phase transformations, nucleation and growth, recovery and recrystallization, and oxidation and corrosion.

The compilation under review — which presents the proceedings of a seminar on 'Energetics in Metallurgical Phenomena' held at the University of Denver in the summer of 1962 — is, in the words of the editor, "an attempt to consolidate up-to-date thinking on the subject and to provide the means by which science faculty members and other researchers may keep abreast of this fast-moving field". The subject matter of this lecture series covers the following major areas of physical metallurgy: (i) 'Intermetallic diffusion' by David Lazarus, discussing the temperature and pressure dependence of diffusion, atomic models for diffusion, experimental techniques, and non-equilibrium diffusion; (ii) 'Solid solution' by Rudolph Speiser, presenting a thermodynamic treatment of binary phase diagrams, interstitial solid solution, and strain energy; (iii) 'Nucleation processes' by Michael Bever, discussing the classical as well as the more recent theories of nucleation from fluid and solid phases (and including a set of problems for the student); (iv) 'Transformation' by Earl Roberts, discussing the thermodynamics and kinetics of phase transformations; (v) 'Metastable phases obtained by rapid solidification' by Pol Duwez, describing experimental techniques and results, in this relatively new field; (vi) 'Annealing mechanisms in deformed metals' by Paul Gordon, discussing the nature of the cold-worked state, the mechanism of recovery below and at room temperature, and the energetics of boundary migration; (vii) 'Energetics in dislocation mechanics' by John Dorn, discussing the structure and nature of dislocation, and some thermally activated dislocation mechanisms; and (viii) 'Oxidation of metals' by Kenneth Lawless, discussing imperfections in crystals, oxidation theory, and epitaxy. The individual treatments are critical and authoritative, and each section carries a bibliography, bringing the literature survey up to 1962.

In an era of fastly expanding frontiers in materials science, such a compendium is invaluable to the teacher and to the researcher, for gaining an understanding of materials behaviour. The metallurgical community owes a great debt of gratitude to the principal lecturers for the enlightening résumés and the discussions.

C.V.S.

THE FLORA OF DELHI by J. K. Maheshwari (Council of Scientific & Industrial Research, New Delhi). 1963. Pp. viii+447. Price Rs 28.00; 56*s*.; $ 8.00

ILLUSTRATIONS TO THE FLORA OF DELHI by J. K. Maheshwari (Council of Scientific & Industrial Research, New Delhi), 1966. Introduction Pp. 20+278 illustrations. Price Rs 28.00; 56*s*.; $ 8 00

The first 50 pages of the *Flora of Delhi* deal with climate, geology, vegetation types, introduced flora, and notes on the flora and classification. The text is well written but is possible of much condensation with benefit. For example, the two paragraphs on page 11 which mention that the plant exploration work was difficult because of the occurrence of plants that cause very intense irritation of the skin, and the presence of snakes might be all right in the journal of a junior student after his first botanical excursion, but not in a book of this kind. Such difficulties are in the nature of things and of the profession, and are encountered by every botanist almost in every excursion.

The families are arranged according to the system of Bentham and Hooker. The keys given are artificial and much can be said against artificial keys as for them. One of the main objections to the usage of an artificial key by a student is that he would come to place a wrong emphasis on single characters instead of combinations of characters in differentiating taxa.

The descriptions of the species are written in a good, concise manner without sacrificing clarity or usefulness. The nomenclature used is that considered correct by many at the time of publication of the volume. Another good feature is that synonymy is kept at the minimum, giving only those synonyms that are essential. The author has taken pains also to emphasize the correct identity of a species, as in the case of *Avena sterilis* Linn.

In the volume of Illustrations, there are 278 figures relating to some of the species described in the Flora. (Since the volume of Illustrations is published about 3 years after the appearance of the Flora, the latter does not have the desirable feature of references to the figures, but the legend of each illustration carries a reference to the page number of the Flora for the description.) It appears as though the author did not have enough time to devote to the figures, which could definitely have been much better, with greater clarity. Details of the floral parts are illustrated only for some plants but they are omitted even in those cases where it is desirable to give them. It is of course agreed that for some plants they can be dispensed with, but there are also many for which the details of the floral parts have to be illustrated for the understanding of the student.

A few errors of nomenclature and identity of plants noticed by the author after the publication of the Flora are corrected in the introduction to the volume of Illustrations. Some additions to the Flora are also given.

The *Flora of Delhi*, along with its companion volume of Illustrations, will be definitely useful, not only to students at Delhi but also to those at other places because most of the Delhi plants occur elsewhere in India. An index is given for each volume which facilitates easy reference. The printing and get-up of both the volumes maintain the high standard of CSIR publications.

V. S. RAO

AN INTRODUCTION TO STATISTICAL MODELS IN GEOLOGY by W. C. Krumbein & F. A. Graybill (McGraw-Hill Book Co. Inc., New York), 1965. Pp. xi+475. Price $ 13.00

Earth scientists usually not accustomed to quantitative thinking are passing through a state of semi-quantitative thinking through a period of data collecting and their final synthesis; thus geologists are preparing themselves for the computer age. The book under review will prepare the earth scientists for such a task. Application of statistics in geology has been changing in its emphasis from purely descriptive aspects to analytical statistics through the use of statistical models.

The book is well thought out and has been in preparation since the authors offered summer conference in 1961. Although the material in several chapters of this book has been previously published as scientific papers, they have been much revised and arranged systematically to provide continuity and unity. The book is divided into 15 chapters. Appendix, author index, subject index, and more than 600 references increase the value and usefulness of the book.

The reader should bear in mind that this book is not a book on statistics and one who wants to follow it should have had a course in statistics or must have read Dison and Massey's book *An introduction to statistical analysis* and Miller and Khan's *Statistical analysis in the geological sciences*. Krumbein and Graybill, in Chapters 1-4, provide the geological foundation necessary for statistical approach. The rest of the 11 chapters are concerned with statistical analysis on a univariate level and multivariate models by the help of matrix algebra. Thus the book has mainly three objectives: (a) To set up geological models — this is concerned with objects, events, measurements to be made and properties of such measurements. Geological analysis of a problem is necessary before the application of statistics. (b) Geological observations are related to statistical models. Chapters 5-8 consider geological population, its densities, the problem of geological sampling and methods to test the exactness of population models in geological setting. Chapters 9 and 10 discuss analysis of variance and simple linear model. (c) In the last five chapters are included multivariate methods of analysis; this necessitates the use of computer. General linear and advanced models are provided with the background of matrix algebra. A good discussion on the empirical use of statistical models for data evaluation is provided.

Numerous examples and references to geological literature are provided so as to enable the reader to understand and appreciate the significance of statistical models in geology. This is a valuable book for teachers, students and research workers in the field of earth sciences.

B.L.S.

PUBLICATIONS RECEIVED

GRAPHIC HANDBOOK OF CHEMISTRY AND METALLURGY by Pai Yen Loung (Chemical Publishing Co. Inc., New York), 1965. Pp. 144. Price $ 10.00

THE INDIAN EPHEMERIS AND NAUTICAL ALMANAC FOR THE YEAR 1967 (The Manager of Publications, Delhi), 1965. Pp. xviii+464. Price Rs 14.00; 32*s*. 8*d*.; $ 5.4¢

INSECT SEX ATTRACTANTS by Martin Jacobson (John Wiley & Sons Inc., New York), 1965. Pp. xi+154. Price $ 7.75

THE ROLE AND APPLICATION OF ELECTRIC POWER IN THE INDUSTRIALIZATION OF ASIA AND THE FAR EAST (United Nations, New York), 1965. Pp. vii+98. Price $ 1.50

BASIC CONCEPTS IN QUANTUM MECHANICS by Alexander Kompaneyets (Reinhold Publishing Corp., New York), 1966. Pp. vi+152. Price $ 3.95

ESSENTIALS OF PALYNOLOGY by P. K. K. Nair (Asia Publishing House, Bombay), 1966. Pp. vii+96. Price Rs 9.00

PROGRESS IN BIOPHYSICS AND MOLECULAR BIOLOGY: Vol. 16, edited by J. A. V. Butler & H. E. Huxley (Pergamon Press Ltd, Oxford), 1966. Pp. 276. Price 84*s*.

INTRODUCTION TO THE THEORY OF DIFFERENTIAL EQUATIONS WITH DEVIATING ARGUMENTS by L. E. El'sgol'ts (Holden Day Inc., San Francisco), 1966. Pp. 109. Price $ 7.65

OBJECTIVITY OF SPACE-TIME AND PURE WAVE APPROACH TO ATOMIC PROCESSES AND DYNAMICS by A. G. Lahiri (Burdwan Press, Burdwan), 1966. Pp. 44

THE MANY-ELECTRON PROBLEM by K. S. Viswanathan (Asia Publishing House, Bombay), 1966. Pp. viii+244. Price Rs 22.00

BULLETIN OF STATISTICS ON WORLD TRADE IN ENGINEERING PRODUCTS— 1963 (United Nations, New York), 1966. Pp. xxxi+163. Price $ 1.50

NEWER REDOX TITRANTS by A. Berka, J. Vulterin & J. Zýka (Pergamon Press Ltd, Oxford), 1965. Pp. ix+246. Price 60*s*.

KURZES LEHRBUCH DER PHYSIKALISCHEN CHEMIE by Hermann Ulich & Wilhelm Jost (Dietrich Steinkopff Verlag, Darmstadt), 1966. Pp. xviii+482. Price LN DM 29,40

EXPERIMENTAL METHODS FOR ENGINEERS by J. P. Holman (McGraw-Hill Book Co. Inc., New York), 1966. Pp. xii+412. Price $ 9.95

ORGANIC CHEMISTRY by L. O. Smith & S. J. Cristol (Reinhold Publishing Corp., New York), 1966. Pp. xv+966. Price $ 12.50

NOTES & NEWS

Multiple-beam interferometry by wavefront reconstruction

The successful achievement of two-beam as well as multiple-beam interferometry from the surfaces of standard engineering components using hologram techniques has been reported from the National Physical Laboratory, Teddington, Middlesex, UK. These interferometric techniques could find applications in industry for material testing and study of dilatations of a structure. The method consists in recording the hologram of an object using coherent laser light, and afterwards replacing the processed hologram plate in its original position in the same apparatus. One then sees the reconstructed image of the object superimposed on the object itself. Any slight change in the surface contours produced, for example, by mechanical strain or thermal expansion will give rise to interference effects between corresponding points on the object and its holographic image, and fringes will be seen to cross the surface. In the experimental arrangement used for producing holographic recording of a piece of steel channel section girder and for demonstrating the interference effect on distorting it, light from a gas laser is directed on the girder through a microscopic objective which diverges the beam slightly so that the object is illuminated obliquely. A photographic plate receives an undisturbed spherical wave, together with light scattered from the surface of the girder. The resultant hologram on the photoplate contains sufficient information to reconstruct a three-dimensional image of the girder in exactly the same position as the original. When viewed through the replaced hologram of the girder after it has been clamped across its webs, two-beam interference fringes are formed over the surface; the contrast between the fringes is governed by the relative brightness of the object and the reconstructed image, while the number and position of the fringes are governed by the degree of distortion.

For permanent record of the fringes, it is unnecessary to form 'live' fringes between the object and its recorded image. Instead, a double hologram may be made by exposing the photographic plate twice, once with the girder unclamped, and a second time with it clamped. The fringes are now 'frozen' into the photographic recording. The object together with the fringes covering it may now be reconstructed without special re-positioning in the apparatus. In fact, it is no longer necessary to use highly coherent light for the purpose; a divergent beam of monochromatic light from a filtered mercury lamp is sufficient.

An extension of the method of 'frozen' fringes makes it possible to observe multiple-beam interference, since a multiple exposure hologram can be made with equal increments of strain between exposures. Sharp multiple-beam effects are obtained only when each individual point on the surface of the object is displaced by equal amounts between exposures. However, if due to overstress, Hooke's law is not obeyed, the fringes would not be sharp. It is, therefore, possible that this method could be used to detect weak spots in an engineering component [*Nature, Lond.*, **209** (1966), 1015].

Radar observations of Mars

Radar studies of Mars made at the Jet Propulsion Laboratory, California Institute of Technology, Pasadena, using radiations of 12·5 cm. wavelength during its recent opposition, have indicated that certain areas of Mars are quite smooth, though rough and strongly as well as poorly reflecting regions have also been found. Mars as a whole is found to be significantly smoother to radiations of 12·5 cm. wavelength as compared to Venus. The experimental procedure consisted of transmitting to Mars for a period of 11 min. (the round trip time of electromagnetic waves) and then receiving for the next 11 min. The frequency spectrum of the signal was then measured with an instrument of 3700 c/s. bandwidth and 84 c/s. revolution. The limb-to-limb Doppler broadening which Mars produces in the signal is 7670 c/s.; thus the small amount of power which is returned outside the 3700 c/s. bandwidth is not detected. Altogether 1300 such 11 min. runs were made and all the runs were assorted into 36 sets, according to the 10° interval of Martian longitude which was facing earth at that time. The runs in each set were then averaged and 36 spectrograms, each corresponding to a different point of view of Mars, were made. Most of the spectrograms show a small amount of power reflected diffusely by the disk. Some of them, however, show relatively strong narrow band reflections which originate from an area less than 2° in extent about the sub-earth point. An echo of a very narrow band was received from the region of Trivium charontis (longitude 180-200°); the reception ended abruptly when the neighbouring area of Elysium was the target. One may conclude that there is a very smooth, strongly reflecting area, extending 20-30° in longitude and of unknown latitudinal extent, in the region of Trivium charontis. A similar sequence of echoes, of wider band, was received from the region of Nodus Laocoontis (240-250°). Surprisingly, the northern tip of Syrtis Major did not show a strong echo and the dark markings, Ascraeus Lacus and Albis Lacus, returned the weakest echoes. The large 'desert' region Amazonis was also a poor reflector [*Science*, **150** (1965), 1715].

Direct synthesis of phenylacetylenes

Direct synthesis of phenylacetylenes has been achieved for the first time by oxidation of the monohydrazones. The reaction involves dropwise addition of a substituted benzyl ketone hydrazone solution to a stirred slurry of the mercurous trifluoroacetate in the presence of diethyl ether or *p*-dioxane, which prevents the addition of trifluoroacetic acid to the acetylenes by forming addition complexes with the acid, at 40-50°C.

The solvent and the reactants used should be perfectly dry in order to prevent hydrolysis of the hydrazone. With phenyl or alkyl as the substituted groups of the hydrazones, the yields are up to 60 ± 10 per cent. Hydrazones without benzyl moiety do not yield acetylene products [*J. org. Chem.*, **31** (1966), 624].

Anion-selective membrane electrodes for titrimetric determination of iodide and sulphate

Two anion-selective membranes have been successfully used as indicator electrodes in the potentiometric titration of iodide and sulphate in solution. The membranes in these electrodes consist of sparingly soluble salts embedded in a silicone rubber matrix. The electrode used for iodide determination contains silver iodide, whereas for sulphate determination the insoluble salt used is barium sulphate.

Iodide can be titrated with silver ion and sulphate can be titrated with barium ion. Titration curves with sharp breaks for both the titrations have been obtained. The break in the titration curve for $10^{-3}M$ KI is about 300 mV. in magnitude. It has also been found that voltages obtained with both iodide and sulphate membrane electrodes lie in nearly a straight-line relationship to the log of the anion concentration at $0{\cdot}1\text{-}10^{-5}M$. The iodide membrane electrode can be used for iodide determination in the presence of fairly large excess concentrations of other anions, viz. Cl^-, SO^{2-}, PO_4^{3-}, ClO_4^{3-}, etc.

The membrane electrodes are suitable for use in automatic titrators because of their rapid response. The uncertainty for e.m.f. measurement of the membrane electrodes is between 1 and 2 mV., giving a precision not quite as satisfactory as glass electrode [*Chem. Engng News*, **44** (No. 5) (1966), 24].

The mechanism of suppression of nonsense mutations

The 'nonsense mutations', occurring in the gene-specifying proteins which are as diverse as the head protein of the f_2 virus and the alkaline phosphatase of *Esch. coli*, are characterized by the fact that while present in certain 'non-permissive' (Su^-) host strains, the particular phenotype involved is not expressed. On the other hand, certain other 'permissive' strains permit the expression of the particular phenotype. For example, in these Su^+ strains the phage T_4 harbouring a nonsense mutation in the head protein gene will grow and lyse the host, whereas in the Su^- strain it does not multiply since it cannot synthesize the head protein.

The mechanism underlying the action of the 'amber' mutants and their suppression by certain bacterial host strains was for a long time shrouded in mystery. The nonsense mutations were later found to be of two types — 'amber' and 'ochre' mutations in the case of the phage or N_1 and N_2 class of mutations with respect to alkaline phosphatase gene of *Esch. coli*. The work of M. G. Weigert and A. Garen [*Nature, Lond.*, **206** (1965), 992] has shown that the nonsense codon involved in the N_1 class of phosphatase nonsense mutations and the amber mutations is the RNA triplet UAG. Subsequently, UAA has been identified as the nonsense codon in the case of the N_2 class or ochre mutations. The suppressor genes which occur at different, though closely linked, genetic loci such as Su-1, Su-2, Su-3, Su-4 and Su-5 also fall into two groups. The first three of them suppress only N_1 or amber mutations. The Su-4 and Su-5 suppressors permit the expression of both N_1 and N_2 class of mutations.

How does the presence of the nonsense mutation result in loss of activity of the gene? The nonsense codons, UAG and UAA, have been implicated in chain termination [Takanami, M. & Yan, Y., *Proc. nat. Acad. Sci., Wash.*, **54** (1965), 1450]. Hence it is possible they have the same role here and may lead to premature chain termination. A. O. W. Stretton and S. Brenner [*J. mol. Biol.*, **12** (1965), 456] have shown that amber mutants of the head protein of the bacteriophage T_4D produce N-terminal fragments of the polypeptide chain when grown in non-suppressing bacteria. These peptides varied in length depending upon the position at which the nonsense mutation was mapped. When these mutants were grown in suppressor strains the complete polypeptide chain was produced with one change; a new amino acid appeared at the site of the nonsense mutation. Another interesting feature was that the same suppressor always introduced the same amino acid irrespective of the position of the amber triplet and whatever might have been the amino acid in the wild type protein. Thus Su-1 class always inserted serine, Su-2 glutamine and Su-3 tyrosine. These results were found to be applicable in the case of the alkaline phosphatase gene [Weigert, M. G. & Garen, A., *J. mol. Biol.*, **12** (1965), 448] and in an amber mutant of f_2 virus [Notani, G. W., Engelhardt, D. L., Konigsber, W. & Zinder, N. D., *J. mol. Biol.*, **12** (1965), 439].

As a result of these studies, it has become clear that the presence of the nonsense codon brings about abrupt termination of the protein and that in permissive strains the nonsense codon is 'wrongly' read as an amino acid, thus permitting complete peptide chain production. The effect of suppression may be at the transcription or translation step. The fact that changing the phase of reading abolishes the nonsense codon indicates that the translation step is involved. Thus it is possible to imagine that the insertion of the 'wrong' amino acid at the nonsense triplet may be the result of a defective amino-acyl-s-RNA recognizing the nonsense codon on m-RNA. It is also possible that the specific amino acid activating enzyme may have got mutated in such a way as to have an altered specificity.

S. Brenner [*Nature, Lond.*, **206** (1965), 994] has postulated an s-RNA which does not bind any amino acid to be involved in chain termination. Its anticodon will be complementary to UAG. If by a mutation anyone of the several aminoacyl-s-RNAs were to be altered such that its anticodon is complementary to UAG, an ambiguity would result and at the nonsense codon a competition between chain terminating s-RNA and the aminoacyl-s-RNA would result. It is interesting to note that the amino acids inserted by suppression have codons related to the nonsense triplet UAG by single base changes. In addition, the different suppressors do not completely suppress the nonsense mutations, and permit chain propagation in varying degrees of efficiency. Thus both the N-terminal fragment and the complete chain are made. These results appear to be in agreement with the thesis that the amino-acyl-s-RNA competes with the chain terminating s-RNA and the varying efficiency may be directly related to the relative amounts of the two s-RNAs.

M. Cappechi and G. Gussin [*Science*, **149** (1965), 417] using a phase assay system for *in vitro* suppression have recently shown that the carrier of suppressor activity in an Su-1 strain is a serine-s-RNA molecule. More recently, R. E. Engelhardt, Webster, R. C. Wilhelm and N. D. Zinder [*Proc. nat. Acad. Sci., Wash.*, **54** (1965), 1791] have done experiments in an *in vitro* system pinpointing the role of s-RNA. Their results indicated that addition of s-RNA from Su^+ bacteria to Su^- extract containing mutant phage RNA markedly increases the synthesis of the coat protein similar to that in Su^+, whereas addition of s-RNA from wild type or Su^- bacteria does not give this response. Again the completed polypeptide synthesis was increased as more and more of the Su^+ s-RNA was added, showing its limited availability even in Su^+ strains.

Thus it seems certain that the nonsense mutation which leads to chain termination is corrected in suppressor strains by the ambiguity introduced by a mutation in one of the several s-RNAs whose anticodons are related to the anticodon of the chain terminating s-RNA, thus competing with the latter, and achieving insertion of a new amino acid at the nonsense triplet, permitting completed protein production.— S. MUTHU KRISHNAN

Vitamin A and the sulphate activating enzymes

The role of vitamin A on an enzymatic level in mucopolysaccharide biosynthesis was brought to light when it was shown that vitamin A is involved in the reactions leading to the activation of sulphate ion [Varandani, P. T., Wolf, G. & Johnson, B. C., *Biochem. biophys. Res. Commun.*, **3** (1960), 97].

G. Wolf, J. G. Bergan and P. R. Sundaresan [*Biochim. biophys. Acta*, **96** (1963), 524] demonstrated that a particular fraction resembling mitochondria and obtained from rat colon had the property of synthesizing mucopolysaccharides. They further showed that this capability was almost completely abolished in vitamin A deficiency and could be restored to normal by addition to the particles incubated of a metabolite of vitamin A acid. If the interruption of mucopolysaccharide synthesis in vitamin A-deficient colon particles is caused by loss of the ability to form APS, then (i) such particles should activate sulphate to APS and (ii) they should have reduced sulphate activation in condition of vitamin A deficiency.

P. R. Sundaresan demonstrated that the activity of ATP-sulphurylase was depressed to about one-half to one-third its normal value in liver supernatant fractions from deficient rats [*Biochim. biophys. Acta*, **113** (1966), 95]. Vitamin A acid as well as vitamin A when fed to deficient rats for 10-15 days (100 g./day) caused restoration of enzymatic activity.

If a lipid extract is made of the normal enzyme by means of butanol and added to deficient liver supernatant fraction its lowered activity is greatly raised. The active substance in this extract is an ether-soluble acidic compound. The lipid extract was radioactive if the rats had received radioactive vitamin A orally or radioactive vitamin A acid by intraperitoneal injection for 10 days. The explanation, therefore, was that the ATP-sulphurylase had an attached acidic lipid compound which was derived from vitamin A or vitamin A acid. That the enzyme bound lipid is not vitamin A acid was shown by the fact that (i) neither vitamin A acid nor vitamin A itself can restore the deficient liver supernatant fraction to normal activity, and (ii) the radioactive lipid factor which can activate the deficient enzyme, when purified by thin layer chromatography on silica gel in the solvent system benzene-chloroform-methanol (4:1:1 vol./vol.), showed an R_f 0·32-0·34, whereas vitamin A acid has an R_f 0·82 in that system.

The activating effect of the lipid factor on the enzyme was achieved by showing an increased rate of reaction with deficient enzyme, with increasing concentrations of lipid factor to a saturation level.

The acid treatment of the normal enzyme prior to extraction of the lipid factor inactivates the normal enzyme. It can be reactivated by addition of lipid factor from normal but not from deficient enzyme. In other words,

$$\text{N-enzyme} + \text{N-lipid factor} \longrightarrow \text{Activity}$$
$$\text{N-enzyme} + \text{D-lipid factor} \longrightarrow \text{No activity}$$
$$\text{D-enzyme} + \text{N-lipid factor} \longrightarrow \text{Activity}$$
$$\text{D-enzyme} + \text{D-lipid factor} \longrightarrow \text{No activity}$$

where N stands for normal and D for deficient.

Thus it is clear that a lipid factor attached to ATP-sulphurylase and possibly derived from vitamin A or vitamin A acid can activate the enzyme. However, the coenzyme status for a derivative of vitamin A for ATP-sulphurylase cannot be claimed. Identity and relationship of the lipid factor to vitamin A and its mode of interaction with the enzyme have not yet been clarified. The lipid factor might possibly be the coenzymatic form of vitamin A.— A. KRISHNA MALLIA

Biochemical individuality

Voluntary consumption of alcohol (4 per cent) by members of same group of chicks over a 4-day period varied from 1·5 to 35·0 ml., a 23-fold range. Highly inbred rats showed distinctive individualities in their eating, drinking and exercise behaviour. With respect to excretion patterns and voluntary alcohol consumption, substantial individual differences were observed in rats which had resulted from brother-sister mating from as many as 101 generations [Reed, J. G., *University of*

Texas publication No. 5009, 1951, 144].

Recently, R. J. Williams, R. B. Pelton and F. L. Siegel [*Proc. nat. Acad. Sci., Wash.*, **48** (1962), 1461] have reported observations on the weights of 17 different organs including heart, kidneys, brain, spleen and liver of 645 male rabbits. These weights were adjusted to take into account the fact that the rabbits were not all of the same size and were expressed in terms of the weights of the organs net body weight. The least variation (2- to 3-fold) was found to be in heart weight and the maximum (80-fold) variation in spleen weight; the average variation of organ size was over 14-fold.

Abundant evidence is available that there is substantial variation in the weights of organs in 'normal' humans. It was found more than fifty years ago that the livers varied 2·8-fold in weight, kidneys 3·7-fold, brains 1·6-fold and heart 3·3-fold when as few as 69 cases were studied [Greenwood, L. & Brown, F., *Biometrica*, **2** (1908), 473]. More recent data bear out the fact that each individual has a distinctive set of organ weights, organ activities, hormone levels, enzyme levels, etc. [Anson, B. J., in *Atlas of human anatomy* (W. B. Saunders Co., Philadelphia), 1951; Williams, R. J., *Biochemical individuality* (John Wiley & Sons Inc., New York), 1956].

The nutritional needs of an organism are determined by the characteristics of the metabolic machinery it has inherited. This is valid for different species like rats, guinea-pigs, fruit flies, maize, etc., and it requires no further proof. The differences in the nutritional needs of individuals within a species of animals and human beings was postulated by R. J. Williams. To test this hypothesis in the light of the above observations, the effect of vitamin A deficient and other deficient diets on different strains of inbred rats and inbred mice was studied [Williams, R. J. & Pelton, R. B., *Proc. nat. Acad. Sci., Wash.*, **55** (1966), 126]. Four different strains of male rats were given white enriched bread and their weight gain, water consumption and life span were observed. The weight gains ranged from 2 to 212 g., life span from 6 to 144 days and water consumption (per day) from 10·9 to 44·9 ml. The deaths of the animals were associated with different symptoms. Similar inter-individual differences were observed in mice. However, mice as a group showed only 40 per cent mortality after 147 days in contrast to rats. These and other experiments showed that although there is some similarity between groups, the behaviour of a group of animals does not reflect in any case how individuals within the group would respond. Even among the relatively small number of animals observed were those which thrived unusually well on a diet, while others on the same diet showed unusual impairment. Different groups of rats were fed on vitamin A for six months. Mortality was very much higher in rats fed on deficient diets than other groups. However, enormous inter-individual differences were observed in weight gain. Animals used in these experiments were tested for fertility. Similar number of male rats given different diets for 54 days sired litters when mated with proven fertile females — 6 out of 21 on deficient diet, 9 out of 23 and 6 out of 23 on diets supplemented with 0·4 and 2·0 units of vitamin A per g. of diet respectively. These results reveal the uncertainty of the amount of vitamin A requirement per individual animal. The human need for vitamin A also appears fully uncertain. Mead, Johnson & Co. offered for 13 years (1932-45) a $ 15,000 award to anyone who would determine this need. The offer went unclaimed.

In conjunction with individuality in anatomy and physiology, individuality in nutrition has been established. Possessing about the same genetic pool (being highly inbred) is by no means the equivalent of being identical. There are unknown factors which control differentiation, and the extent to which various differentiated cells proliferate. These may make possible, for example, the development of large or small thyroid and comparable differences with respect to the entire gamut of hormone-producing and other cells, and probably play an important part in producing other cells, and probably in producing biochemical individuality.— P. A. KUMAR

Progress Reports

Central Mining Research Station, Dhanbad

The annual report of the station for the year 1963-64 covers in brief the research activities of its four divisions, viz. Mining, Ventilation, Health and Engineering. In realization of its aim of solving problems connected with mine working, explosion, health and ventilation, a number of research projects of industrial and fundamental importance were taken up during the year. Testing facilities created at the station have been instrumental in the growth of flame-proof machinery industry and mining equipment industry in India, thereby saving foreign exchange worth Rs 57 lakhs during 1962.

Investigations carried out by the Mining Division on hydraulic stowing have been responsible for improving the rate of sand stowing in a coal mine from about 30 tons/hr to over 80 tons/hr which, in turn, increased the coal production of the colliery by over double its original production. During studies on mine working and mining methods, three new convergence recorders which are versatile and facilitate more accurate recording of convergence data than the existing ones have been developed. Further work to perfect these instruments is in progress. With a view to finding the effective working time for each operation on a standard basis, a time study of the operations of (i) coal cutting by picks, (ii) loading of coal into tubs, and (iii) hauling out of the tubs from the face up to the depot has been made. For standardization of the different mining operations further work is in progress.

Investigations on the strata control survey at a colliery indicated that for 1 mm. of convergence, approximately 1 tonne of prop load increases at the mid-position of the face and the rate of roof convergence is maximum during cutting and blasting.

The Ventilation Division which comprises (i) air flow laboratory,

(ii) flame-proof laboratory, (iii) mine fire laboratory, (iv) gas analysis laboratory, (v) analytical laboratory, and (vi) physics laboratory was mainly engaged in the testing of flame-proof and other mining equipments and in the development and modification of instruments in order to improve ventilation and facilitate mine air analysis. The carbon monoxide detector tubes manufactured according to the improved process worked out at the gas analysis laboratory can detect carbon monoxide from 10 to 100 p.p.m. by volume.

An extensive study of the mode of action of CO disappearance from the sealed off districts and the effect of various parameters, such as *p*H, wetted mass of coal bed, surface area exposed and the nature of superincumbent atmosphere have been made. Investigations to analyse a gas sample containing any amount of combustibles without fractional combustion have resulted in the development of a new Universal Gas Analysis apparatus which can analyse CO iodometrically with an accuracy of 0·008 per cent and carry out combustion of hydrogen and methane. The combustion pipette is so designed as to allow the analysis of even explosive mixtures of gases without any hazard of explosion.

Ventilation surveys consisting of (i) pressure survey, (ii) quantity survey, (iii) temperature survey, and (iv) testing of surface and underground fans were undertaken in six coal mines and it has been shown that only one-third to one-sixth of the ventilation pressure is available for the ventilation of mine workers.

A study of the flow characteristics through ducts has indicated that for developing flow through the duct, the duct length should not be less than 30 times the diameter after bends or restrictions, thereby suggesting the use of orifice plates instead of Pitot tubes, for the measurement of air quantity flowing through the duct.

Graham's apparatus, normally used for pressure surveys of shallow mines, has been modified to enable measurement of pressure difference of 10 in. Hg with an accuracy of 0·001 in. Hg. In the modified instrument, murcury, instead of liquid paraffin, and a Vernier scale are provided.

FORTHCOMING INTERNATIONAL SCIENTIFIC CONFERENCES

Date	*Conference*	*Place*
3-6 October	International Soil Water Symposium	Prague
3-7 October	Fifth International Coal Preparation Congress	Pittsburgh
3-7 October	Symposium on Solid State and Chemical Radiation Dosimetry in Medicine and Biology	Vienna
3-12 October	Symposium on Mines: Health and Safety	Turkey
8-11 October	Symposium on the Physics and Chemistry of the Crystal Surface	Halle an der Saale (GDR)
10-14 October	Symposium on the Standardization of Radionuclides	Vienna
10-15 October	Symposium on Chemical Methods in Freshwater Productivity Studies	Amsterdam
12-13 October	Symposium on Limited Conditions of Temperature and Pressure in Industrial Chemistry	Milan
12-14 October	International Symposium on Phosphorylated Glucides	Milan
13-15 October	International Conference on Magnetism	Dresden
17-19 October	International Plastics Congress	Amsterdam
17-21 October	International Conference on Hyperfine Nuclear Spectroscopy	Wellington
16-19 November	International Colloquium on the Physiology of Reproduction	Paris
22-25 November	International Conference on Metal Finishing	Basel
23-25 November	Fifth International Reinforced Plastics Conference	London
28 November to 3 December	Second International Conference on Methods of Preparing and Storing Labelled Molecules	Brussels
4-9 December	Thirty-third International Foundry Congress	New Delhi
December 1966	International Colloquium on Oceanography	Monaco
December 1966	International Symposium on Fuel Elements	Dresden
9-18 Jan. 1967	International Conference on Spectroscopy	Bombay

With a view to finding acceptable standards of dustiness in mines, over one thousand workers have been examined radiologically and clinically for pneumoconiosis. The qualitative and quantitative assessment of dustiness in Indian mines was also carried out side by side. The incidence of pneumoconiosis and a safe limit of dustiness in Indian mines are being assessed.

Photoelastic patches with suitable photosensitive materials to indicate approximately in tons the amount of settlement pressure due to the roof on the wooden props have been developed by the Engineering Division. These and the reflecting cement so far imported could be made in the laboratory from indigenous materials.

The effect of size on the crushing strength of coal is under study. A project on the study of the keeping qualities of a certain type of detonators under conditions of storage at elevated temperature (120°F.) is in hand.

Announcement

▪ *The Fifth Symposium on Comparative Endocrinology* will be held in New Delhi during 23-28 November 1967. The topics to be discussed at the symposium are: (i) Endocrinology of the Agnatha and Protochordata; (ii) Endocrine control of molting and pigmentation; (iii) Endocrine control of central nervous function; (iv) Comparative endocrinology of reproduction; (v) Adaptive mechanisms; (vi) Thyroid; (vii) Comparative physiology of prolactin; and (viii) Comparative endocrinology of the invertebrata. The main papers will be by invited speakers. Further details can be had from Dr M. R. N. Prasad, Department of Zoology, University of Delhi, Delhi 7.

FIRST GET-TOGETHER OF RESEARCH AND INDUSTRY PUBLICATIONS

The Council of Scientific & Industrial Research (CSIR) organized a two-day First Get-Together of Research and Industry in New Delhi during 20-21 December 1965.

The gist of speeches delivered and the key papers prepared are presented in one volume entitled *Addresses and Key Papers.* Out of the papers received, some selected papers having a direct bearing on the objectives of the Get-Together are being published separately under the 15 Working Groups embracing related groups of industries.

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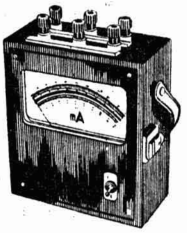
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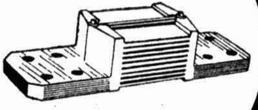

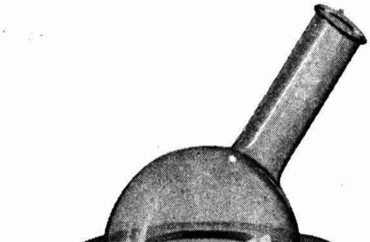

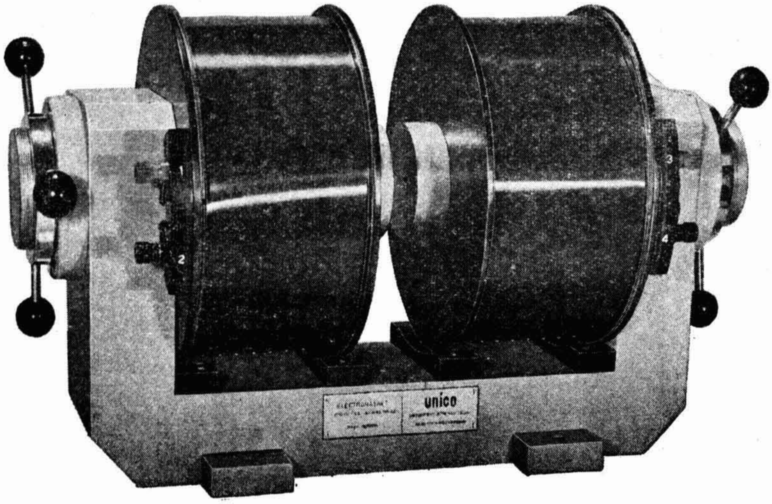

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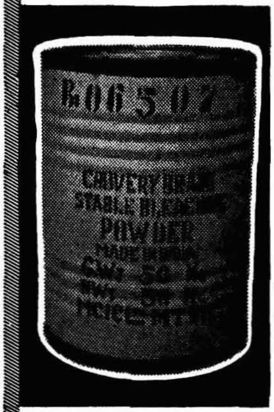

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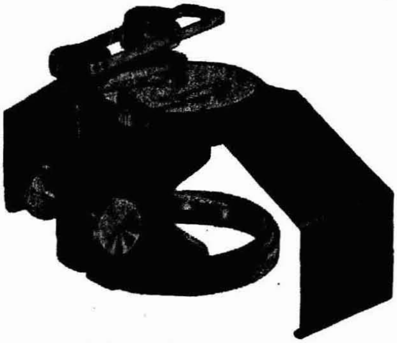

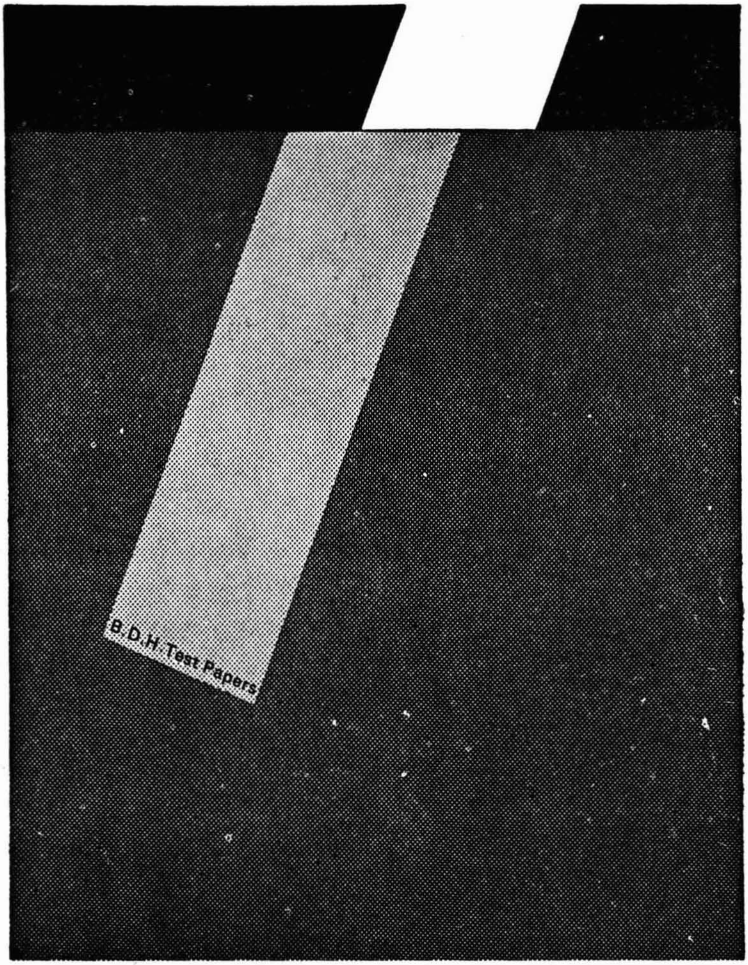
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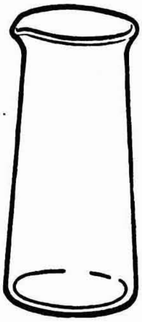

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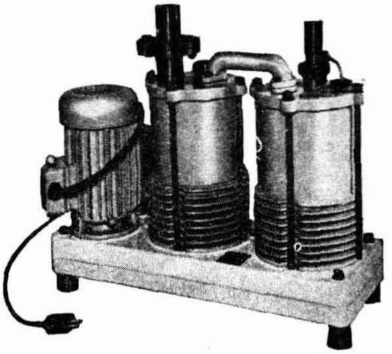

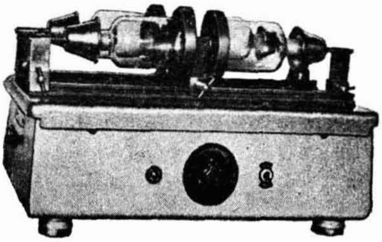

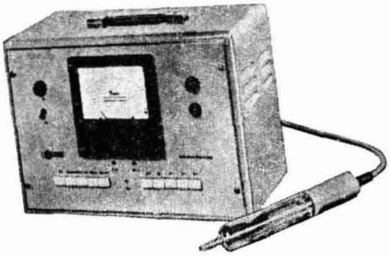

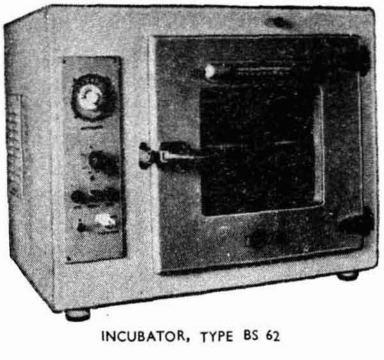

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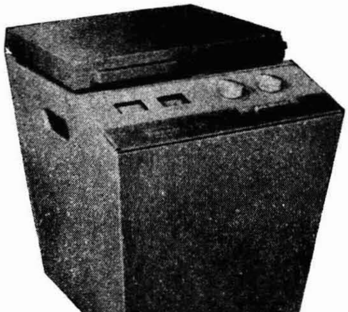

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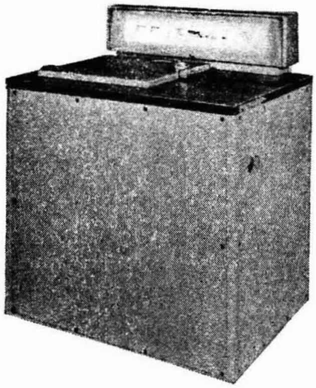

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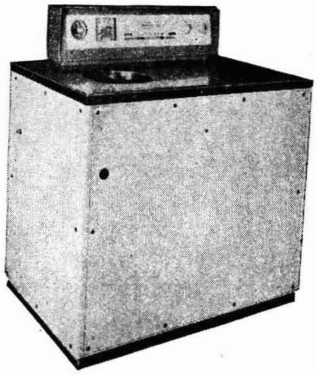

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Microrotor	max. 20,000 r.p.m.
Field of gravitation	
Haematocrite rotor	max. 18,500 g
Microrotor	max. 18,000 g

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	

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Rotating speed	max. 60,000 r.p.m.
Field of gravitation	max. 300,000 g
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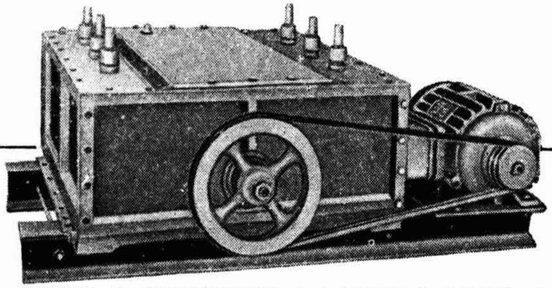

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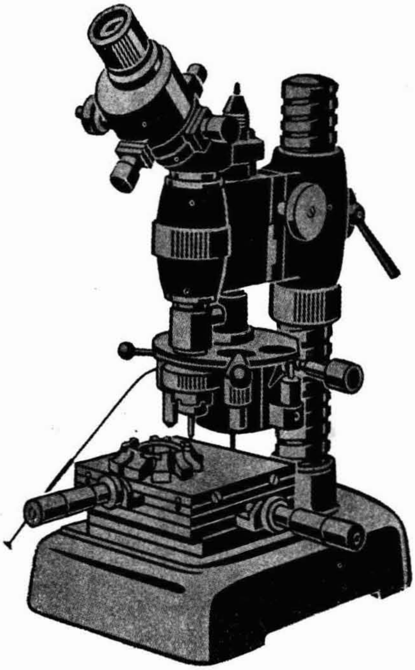

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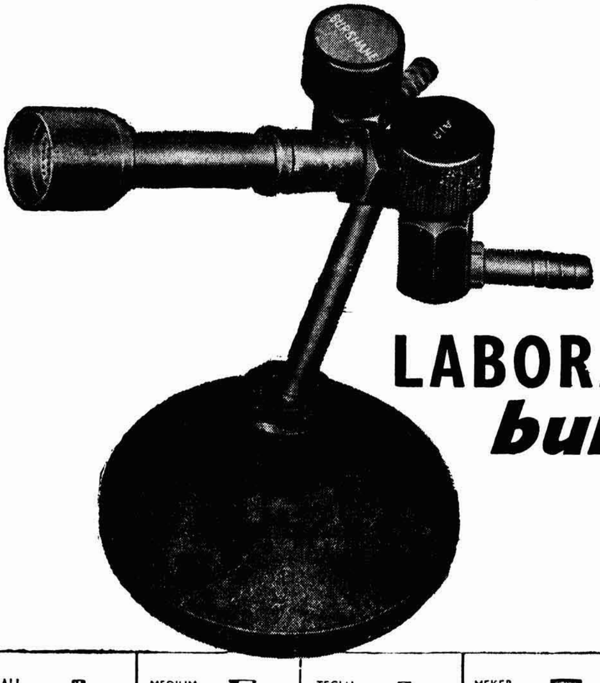

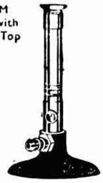

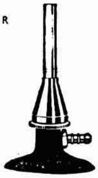

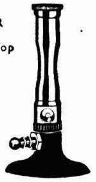

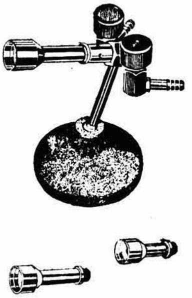

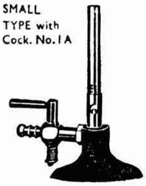

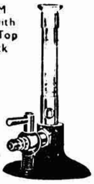

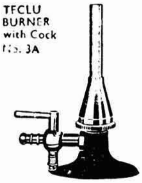

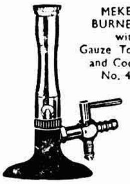

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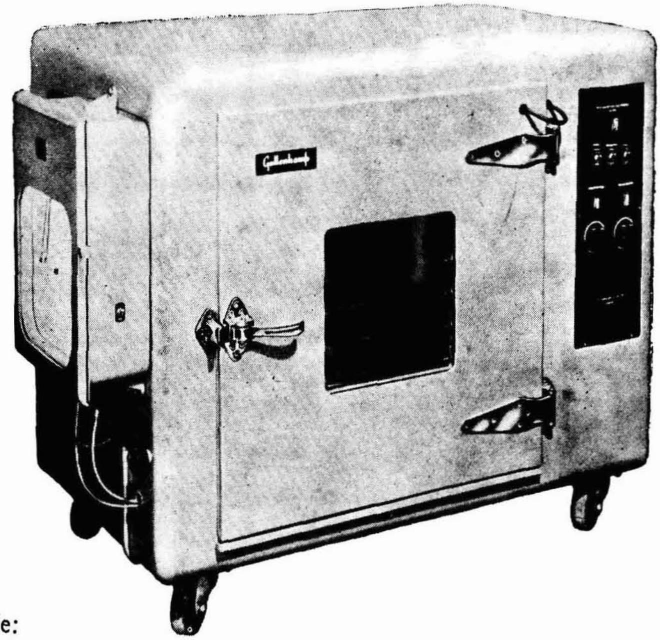

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