Meetings

Cytochromes: Chemical and Structural Aspects

The Symposium on Cytochromes—Chemical and Structural Aspects is the fifth in a series of meetings emphasizing the mechanisms of biological oxidation. The series began with the Symposium on Enzyme Chemistry in Tokyo 10 years ago, and was followed by the Canberra Symposium on Haematin Enzymes in 1959, the Amherst Meeting on Oxidases and Related Redox Systems in 1964, and the Johnson Foundation Colloquium on Hemes and Hemoproteins in 1966. The most recent meeting was organized by Kazuo Okunuki and Martin D. Kamen; it was held at the UNESCO Hall near the top of Mount Rokko in Kobe, Japan, 16–18 August 1967. Approximately 150 scientists registered for the meeting, of whom approximately 70 were from outside Japan. The well-organized program focused upon structural rather than functional aspects of the hemoproteins.

The first program dealt with cytochrome oxidase. Q. H. Gibson summarized the kinetic approach to the function of cytochrome oxidase; he emphasizes using the flash photolysis method for the determination of the sequence of rate constants for the oxidation reactions of the two hemes (a and a_3) and of the copper moiety of cytochrome oxidase. Gibson's experiments set definite limits on possible mechanisms. He detected no oxygenated compound of a lifetime longer than approximately 10 μsec, and found the remainder of the kinetics to be consistent with two heme intermediates and one copper intermediate.

So-called oxygenated compounds, studied in detail by Okunuki, Lemberg, and Wainio, are detected when the dithionite-reduced oxidase is treated with oxygen. Although neither Gibson nor Chance could detect the existence of the compounds in the initial portions (10 μsec) of the oxygen kinetics, there is great interest in their ultimate function.

Beinert reported new electron-spin-resonance (ESR) data on cytochrome oxidase, with detailed titrations of the reduction of the components of a purified cytochrome oxidase preparation. His experimental data apparently require the consideration of three ESR signals (the copper signal, heme signals where $g = 3$ and $g = 6$, respectively) in oxidized cytochrome oxidase. Interestingly enough, the ESR signals corresponding to the "oxygenated" compound were observed to be no different from those of oxidized cytochrome oxidase, at least under the experimental conditions employed by Beinert. Ehrenberg reported preliminary results based on an increased intensity of the copper ESR signal. The increase was attributed to a change in the spin state of one of the hemes. Mason described some properties of a copper-deficient cytochrome oxidase and its reconstitution.

Horio has made an extensive study of the oxidation-reduction potentials of cytochrome oxidase; he has identified three heme a components of $E_0' <$ 0.338, 0.300, and 0.208 volts.

The finding of Ishimura that the cyanide and CO-binding constants for tryptophan pyrrolase could be altered by two orders of magnitude by binding tryptophan now focuses special attention upon the allosteric control of reactivity in this enzyme. Binding constants for cyanide to the pyrrolase of $10^{-4}M$ in the absence of tryptophan are converted to $10^{-6}M$ in the presence of tryptophan, a value which is characteristic of most peroxidases as well. In this sense, tryptophan addition acts almost as if it compensated for a deletion in the structure of the pyrrolase.

The chemical structure of the heme of cytochrome oxidase was discussed by Caughey, Lynen, and Lemberg. The hexose sugar moiety, previously suggested by Caughey, has not been substantiated by subsequent studies in his laboratory nor in those of Morrison or Lemberg. However, the presence of two types of heme a derived from cytochrome oxidase, as first proposed by Morrison, has been confirmed by Caughey. Lynen has extended his definitive studies on the structure of cytohemin of yeast with the positive identification of the C-17 side chain; farnesyl pyrophosphate seems to be the exclusive precursor of cytohemin. Thus, greater agreement now seems to exist concerning the structure of heme a. The only question, raised by Caughey, is whether the free hydroxyl group exists in the compound in its state in nature.

I. Sekuzu, representing Okunuki's group, discussed the relation of various polymeric forms of cytochrome oxidase to its function. He concluded that the dimer form was at least necessary for optimal activity; since the activity of the dimeric form is approximately threefold higher than that of the usual tetramer preparation. A similar study has been made by Morrison, in which he suggests that the enzyme consists of two to three heme a and three coppers. Sekuzu also showed that the mode of binding of copper with the enzyme is strictly related to its specific sulfhydryl group, and that the conformation change to the monomer enables the ready removal of the copper by chelators, with accompanying loss of activity. Sekuzu also noted that variable ratios of cytochromes a and a_3 can be obtained by comparing properties of a highly purified yeast cytochrome oxidase.

Horie described a cytochrome oxidase preparation in which the heme a of cytochrome a_3 had been partially destroyed. Chemically, there seems to be a difference in the heme or the heme-protein linkage of the oxidase, although the question of whether the heme a is associated with a single protein or exists as separate hemoproteins remains unresolved.

Nicholls and Slater discussed the azide compound of cytochrome oxidase in detail; the former emphasized the change of redox potential caused by azide binding, and the latter indicated the existence of two types of azide compound. The apparent discrepancy between the concentration of azide required to cause a spectroscopic alteration of the absorption of cytochrome oxidase and the concentration required for inhibition of enzymatic activity has now been resolved by the detailed studies reported by Slater.

T. E. King and D. W. Urry discussed optical rotatory dispersion and circular dichroism measurements of cytochrome

oxidase. King's discussion was in terms both of hematin *a* and cytochrome oxidase, with particular emphasis on the optically active center associated with the disputed side chain hydroxyl group. Urry interprets his results on the assumption that the two hemes of cytochrome oxidase are closer to one another in the ferrous than in the ferric state.

L. Smith reported on experimental methods for obtaining maximal activity of a particular cytochrome oxidase preparation toward cytochrome *c* and obtained turnover numbers of cytochrome a_3 as high as 300 per second.

Papers by Dickerson and Kraut dealt with the 4-Å structure of horse heart ferri-cytochrome *c* and the 5-Å structure of photosynthetic bacterial cytochrome c_2, respectively. The x-ray crystallographic data show the heme of cytochrome *c* to be inserted sideways into a deep cleft in the protein, leaving an edge nearly flush with the surface. The agreement of the crystallographic data with the known amino acid sequence points to a satisfactory completion of the high resolution structure in the near future. At the present time, the structure allows some interpretation of the nature of the folding process in the synthesis of the protein. In this connection, Lemberg noted that porphyrinogen readily recombines with apo-cytochrome *c*, as originally shown by Sano. Heme binding is well-established for histidine-18 and highly probable for methionine-80 although the possible role of tryptophan-59 cannot be excluded as yet. The structure also affords possibilities for a cytochrome oxidase binding site, and for interaction with lipid membranes by means of the "hydrophobic patches" on the surface of the structure. Less progress has been made in the lower-resolution structure for bacterial cytochrome c_2. Nevertheless, the available data indicate the approximate locations for the two cysteine and histidine linkages, with possibilities for a methionine linkage.

There are several challenges ahead for the crystallographer. First, the interaction with the heme group of groups that lie at the back of the heme crevice (for example, tryptophan-59) must be more clearly resolved. Second, the question of whether significant conformation changes occur in oxidation and reduction needs further study, although it seems to have been partly resolved by the study of bacterial cytochrome c_2. In this case, the oxidized and reduced forms are isomorphous, indicating that any conformational differences between the two structures may be restricted to small structural changes such as those found in $MbFe^{3+}CN$. Thus, it seems likely that large conformation changes are not essential to the electron transfer process in cytochrome *c*, although small changes may be of significance in both electron transport and energy conservation. The crystallographic structure does provide points of immediate interest in this respect. If, indeed, the conformation change involves the movement of one of the heme linkages, it is more likely to involve the methionine-80 than the histidine-18 in both cytochromes studied so far.

E. L. Smith, in discussing the cytochrome *c* structure, focused considerable attention on tryptophan-59 as an invariant component of the available sequences. T. Yonetani showed that reaction of this group with bromo-succinimide had a remarkable effect on the spin state of the iron, though reaction of methionine residues was not excluded as a possible cause of the observed effect. In synthetic heme peptides, S. Sano showed that methionine linkage to the iron gave a heme peptide with a 552-mμ absorption band, while a tryptophan linkage to the iron gave an absorption band at 550 mμ. Sano has also made a very good start on the synthesis of cytochrome *c*, having shown, among other things, that the histidine-18 cannot be substituted by lysine.

The remarkably exhaustive studies of E. Margoliash and E. Smith on the primary sequence of cytochrome *c* from various sources affords a firm basis for considerations of evolutionary phenomena (Smith) as well as genetic and immunological characterizations (Margoliash). The latter are of special interest in the sense that one of the groups responsible for antigenicity appears to have been pinpointed as isoleucine-58. Conformation changes associated with oxidation and reduction are also identified with the immunological technique. The chicken protein which has so far shown no effect of reduction on its immunological response is concluded, therefore, to show very little conformation change on reduction in this particular case. This was confirmed by direct observations of crystals undergoing reduction with sodium dithionite.

Dus reported the primary structure of cytochrome c_2 and K. Wada presented the amino acid composition of heme peptide purified from beef heart cytochrome c_1.

After the morning session of formal presentation Kamen initiated an experiment in the presentation and discussion of many contributed papers. Arranging five or six papers into topic-oriented groups, the authors were asked to give 2- to 3-minute summaries of new data not in the preprint, and then respond to questions from the audience as a group. Unfortunately, the assumption that all participants had read and digested the 400 pages of preprints was wishful thinking, and as a consequence, many interesting topics which might have been discussed failed to receive the full attention and consideration they deserved.

Another aspect which receives considerable attention concerned the optical rotatory dispersion (ORD) and circular dichroism studies of cytochrome *c* presented by Urry and by Hamaguchi. The inability of the ORD method to distinguish between changes of helicity and parallel or antiparallel β-structures affords a reconciliation of previous estimates of appreciable helicity of the cytochrome *c* molecule with the finding of almost no helicity in the crystallographic structure. Flatmark had attributed the error in interpretation of the ORD data to symmetrical interactions of the side chains of the aromatic amino acids with the polypeptide chains. The ellipticity centered around 263 mμ appeared to be one of the more prominent and constant features of CD studies discussed by Flatmark, Hamaguchi, and Urry.

A second approach to the structural changes which occur was summarized in several papers which described studies on the nature of the 695-mμ absorption band: this absorption band is taken to identify the "native" form of cytochrome *c*. Chance and Wilson showed that cytochrome *c in situ* in mitochondria of pigeon heart and beef heart exhibit this "native" absorption band. However, in accordance with the unreactivity of bound cytochrome *c* toward cyanide, the cytochrome *c* bound to mitochondria lacks the relative thermolability of the isolated material. C. Greenwood and G. R. Williams both supported the proposal that the intensity of the 695-mμ chromophore is related to a specific conformation change closely associated with the iron-imidazole structure. Williams pointed to the

possibility that this chromophore may not, however, be functionally involved in the reactions of the hemoprotein with ascorbic acid. Of further interest were the responses of the 695-mμ chromophore to tetranitromethane, which differed depending on whether the cytochrome was initially oxidized or reduced.

Many modifications of cytochrome *c* were reported, among them Morrison's addition product of cytochrome *c* and quinone that shows ascorbic acid oxidase activity in which, interestingly enough, the cytochrome *c* valency state of the heme of the iron appears to be unaltered.

T. Yamanaka and T. Haneishi both described exhaustive studies on the oxidizability of cytochromes of type *c* isolated from an extremely wide variety of organisms including many species of fungi. The reactivity of cytochrome *c* with *Pseudomonas* cytochrome oxidase appeared to reflect the evolutionary relationships of the organisms from which the cytochrome *c* had been isolated. Yamanaka has proposed a model for evolutionary relationships on this basis.

The value of those studies of comparative biochemistry involving the relative activities of various types of cytochrome *c*'s was tempered by the questionable validity and variability of the assay methods for cytochrome *c* oxidase.

T. Masuda studied the reaction of hydrated electrons with cytochrome *c*, and concluded that the reaction was diffusion-controlled, although he had some difficulty in explaining the details of the molecular statistics.

Bartsch reported the binding of flavin to two cytochromes from photosynthetic bacteria, *Chlorobium* c_{553} and *Chromatium* c_{552}. The nature of flavin moiety is as yet unknown, but the possibility that a flavin peptide is involved is not ruled out, although electron transfer between the flavin and the heme has not yet been clearly demonstrated. The possibility that *Chromatium* c_{552} has two closely interacting hemes is suggested by the relatively low extinction coefficient for its CO-compound and by the circular dichroism data which suggest that two hemes are close enough together to permit interaction between their electron systems. *Chromatium* c_{552} is the cytochrome whose light-activated oxidation is observed to persist even at extremely low temperatures.

E. Yakushiji has continued his classical studies of algal cytochromes *c*, in which the crystallization and characterization of many types of cytochrome *c* obtained from red, green, and brown algae allow the tracing of the nature of the photosynthetic cytochrome from the *c*-type of the algae to the *f*-type of higher plants. Mitsui reported on the crystallization of *Euglena* cytochrome c_{522}; so far, his preliminary attempts to find histidine in the heme peptide of either the *Euglena* c_{522} or the *Porphyra* c_{553} have been unsuccessful. However, Dus recalled that histidine is seldom recovered under the conditions of assay described, and no significance can be attached to these observations as yet.

C. T. Gray reported the changes of cytochrome patterns in *Escherichia coli* and Enterobacteriaceae, respectively. The paradoxical finding that mutant cells, incapable of reducing nitrate to nitrite, showed the highest levels of the *c*-type cytochrome raised some questions as to the functionality of this cytochrome in nitrate reduction, and on the relationship of the intracellular enzyme concentration to enzyme activity as well.

The relationship between tertiary structure and electron transport function in cytochromes was studied from the kinetic point of view by Chance. He concluded, on the basis of similarity of oxidation and reduction rate constants in the sequence a-c-c_1, that the structural asymmetry of cytochrome *c* had been "averaged out." This could be accomplished by thermal rotation of the cytochrome about its point of attachment or by mechanisms that allowed nearly equally effective electron transfer from c_1 to *c*, and from *c* to *a* either by proximity of the three hemes to one another, or by electron tunneling processes involving barriers of different widths and heights. Some hypotheses for the control of electron flow in membrane systems were based upon analogy with the effects of lattice constraints upon the reactivity of crystalline hemoproteins, or of London force ligands such as xenon. No xenon binding to cytochrome *c* was found by Dickerson. Thus xenon is specific in its interactions with hemoprotein.

DeVault based a novel hypothesis of oxidative phosphorylation upon the idea of Cusanovitch that the multiplicity of cytochromes in bacteria may in part be due to multiple forms of the same cytochrome in different energy and redox states.

Two papers explored the nature of the mitochondrial membrane. Penniston described the segments of the respiratory chain obtained by disruption, with particular emphasis on the cytochrome *b*-*c* complex. Oda discussed the possibilities of reconstituting functional electron transport of phosphorylating particles associated with the reformation of membrane-like structures. While some progress has been made, incisive tests which might evaluate whether the reconstruction involves artifactual electron transport and phosphorylative pathways are lacking. Penniston suggested that the sequence from cytochrome *b* to cytochrome *c* contains five individual electron-transporting components, which are believed to act in a concerted fashion to achieve the necessary free energy difference of 22 millivolts. Slater, however, emphasized that a free energy gap of 16 kilocalories is required to achieve the high ratios of ATP to ADP-P_i observed experimentally in oxidative phosphorylation and ion movement.

The discussion on peroxidase began with Mössbauer studies by T. Moss and Y. Morita, employing horseradish prepared from European and Japanese varieties of the plant. The difference in their values for quadrupole splitting of the ferric enzyme at 77°K (1.83 and 2.47 mm/sec, respectively) are probably attributable to the different distribution of high and low spin states in the two enzymes. On the other hand, the ethyl hydrogen peroxide derivative, compound II, was found to have very similar values for quadrupole splitting and isomer shift, the values at 77°K being approximately 1.4 mm/sec for quadrupole splitting in both species, and −0.24 and −0.35 for the isomer shift when corrected to the same reference standard. The similarity between these negative isomer shifts in the ethyl hydrogen peroxide compound and those observed in the $d^{4.7}$ ferrocyanide provides confirmation that the iron in the peroxide intermediate has moved from the Fe^{3+} state of HRP to the Fe^{4+} state. A substantial part of the charge associated with the formal 4+ valency state is localized on the iron.

Morita observed that peroxidase, when over 80 percent in the form of its primary intermediate, compound I, shows quadrupole splitting and isomer shifts at 77°K almost identical to those of compound II (1.33 mm/sec and −0.34, on the basis given above). These very important results (more recently confirmed by Moss in a personal communication) indicate that approximately the same valency charges are associated with the iron atom in each com-

pound. This is in agreement with suggestions put forward that the additional oxidizing equivalent of the primary intermediate is associated with the methene bridges of the porphyrin ring, as indicated by the unique spectra of these primary complexes.

These results on valency states of the peroxidase intermediates can be considered in connection with Moss's interpretation of Mössbauer data indicating a formal 3+ charge on the iron atom of oxyhemoglobin. Such an assignment is in agreement with Wittenberg's suggestion for the structure of the oxygenated compounds of horseradish peroxidase and myoglobin based upon spectroscopic studies at temperatures of liquid helium. A similar configuration is adopted by Yamazaki, who studied the sequence of one-step reductions: $Fe^{3+}O_2$ — FeO_2H_2 — FeOH using dimethylparaphenylenediamine. It is important, however, to note that these oxy- and peroxy-compounds exhibit the chemical characteristics of the lower valency state. From the chemical standpoint, the characteristics of the higher valency state may be no more apparent than are those of ferrocyanide.

The 37-year-old question of the nature of the para-peroxidase and wheat germ peroxidase seems to have been resolved to a considerable extent by I. Yamazaki's identification of a remarkably high binding constant for cyanide in these aberrant peroxidases: $10^{-9}M$ in the ferric form and $10^{-7}M$ in the ferrous form. The question of whether the liganded form affords these peroxidases a special redox function poses an interesting problem for the future.

Yonetani's Mössbauer data on cytochrome *c* from yeast has verified its low spin nature and further demonstrated the decoupling of spins by an imposed magnetic field. Wittenberg, on the basis of ESR spectra taken at 4°K, put forward an objection to the exclusive assignment of higher oxidation state to the iron atom in the peroxide compounds of peroxidase. Chance's rapid flow studies showing that an absorbancy change at 403 mμ occurs slightly in advance of that at 424 mμ provided kinetic evidence for the very rapid reduction of the initial peroxide complex in the yeast peroxidase in an intramolecular reduction with a half-time of 50 μsec. Morrel has studied the sulfhemoglobin compounds in connection with his work on the chromophores of myelo- and lacto-peroxidase. He concludes that a free radical mechanism is involved in the formation of sulfhemoglobin, one of the intermediates being a ferrous H_2O_2-hemoglobin compound. He also reported that in sulfhemoglobin, as in myeloperoxidase, the prosthetic group is a new Cl-S compound rather than hydrogen added to a β-pyrrole double bond.

Baudras and Iwatsubo presented their recent experimental data on the successful reconstitution of cytochrome b_2 and on its fluorescence and ORD properties. The possibility that there may be direct intramolecular transfer of electrons from the flavin to the heme of cytochrome b_2 remains unconfirmed but kinetic experiments seem to exclude such a possibility.

There followed two most interesting papers on the primary structure of cytochrome b_5 from P. Strittmatter and B. Hagihara, who employed calf and rabbit liver, respectively. Although a number of similarities exist, it is possible that Hagihara's preparation method has extracted a somewhat larger molecule from rabbit microsomes than Strittmatter has from calf liver: a molecular weight of 11,500 versus 11,000. Hagihara stated that he could prepare cytochrome b_5 containing methionine as well, and R. Sato believes that the molecular weight of the native b_5 molecule may approach 30,000. However, the definition of a native cytochrome *b* appears to be rendered meaningless by the fact that the microsomes themselves contain considerable cathepsin activity. Ehrenberg described EPR studies of a cytochrome b_5 preparation from pig liver with a molecular weight of 12,800. The ESR data at 88°K show mainly low spin resonances; however, reversible increases of the high spin signal and changes in the character of the low spin signal are obtained at *p*H 12. Volume magnetic susceptibility measurements indicate the oxidized and reduced forms to be low spin. As measured directly in the microsome, cytochrome b_5 shows some trace of the low spin form, although this is difficult to observe because of its width and low intensity relative to the large ESR contribution of cytochrome P450 (FeX).

A discussion of microsomal cytochrome P450 began with a study of model compounds, particularly the reaction of ethyl isocyanide with protohemin which, according to R. Sato, showed many properties similar to those of the natural pigment. His general conclusion was that two interacting hemes are present in P450. each, depending upon the strength of their interaction, giving the various spectral forms at P450. However, the identification of two hemes in native P450 remains unclear.

T. Yamano studied the effect of a number of reagents in demonstrating the spectroscopic and EPR conversion states of P450. He postulated only one heme in this unusual hemoprotein, but emphasized hydrophobic bonding in the natural state. Estabrook provided kinetic and titrametric data on the reaction of P450 with oxygen, giving a dissociation constant in the range of 5 μM, with a half-time of 100 msec for the reaction with 15 μM oxygen. It was apparent that the kinetic relationships and functional activity of P450 are such that it may be totally oxidized in the steady state. Appleby has greatly extended the range of occurrence of P450 by finding it in bacteroids from soy bean nodules. The pigment was 60-fold purified. Spectroscopic and other data indicate it has properties remarkably similar to those of the mammalian pigment. In common with Sato, Appleby postulated two hemes, and a copper moiety as well, and proposed various interactions between these hemes to explain the various structural moieties. The possible functional role of P450 in yeast or bacteroids remains undetermined. Mori described cytochrome *b* (β-574); although he has accomplished partial isolation of this unusual pigment from a haloterant *Micrococcus*, he was unable to identify its function in these organisms.

J. Keilin presented new results on helicorubin and cytochrome *h* of the snail, giving revised molecular weights of the order of 12,500 and preliminary data on the amino acid composition. Of considerable interest was the apparent absence of methionine, and the high content of proline in each hemoprotein. Whereas the prosthetic group in helicorubin is protoheme, in cytochrome *h* it is more likely to be hematoheme. Since clearcut structural dissimilarities between cytochrome *h* and helicorubin are now established, the function of helicorubin and the relationship of the two pigments become of great interest, bearing in mind that both react well with a bacterial oxidase, but poorly with mammalian cytochrome *c* oxidase.

In the final presentation, Wainio described cytochrome *b* of the respiratory chain from a partially purified, cholate-treated preparation. This cytochrome *b* showed differences in the

equilibrium established by a variety of reducing agents, which led Wainio to conclude that different forms of this cytochrome exist, at least in his preparation.

Kamen ended the symposium with a short discourse, in which he stressed the importance of the structural and immunological implications of the recent elaboration of the tertiary structure of cytochrome *c* and c_2. He expressed the hope that primary sequence determinations of cytochrome *c* will lead eventually to the elucidation of its structure, or to its chemical synthesis, and that the chemical synthesis of other cytochromes would be achieved as well. Kamen reminded the gathering that the difficulty in interpreting the results from various preparations of the oxidase extracted from cells and tissues still remains.

A full publication of the Symposium will be edited by Kazuo Okunuki and Martin D. Kamen. It will be published by the University of Tokyo Press.

BRITTON CHANCE
School of Medicine,
University of Pennsylvania,
Philadelphia 19104

Phenotype: Postnatal Development

An international symposium on the Postnatal Development of Phenotype was held 18–22 September 1967 at the castle of Liblice, Czechoslovakia, under the auspices of the Czechoslovakian Academy of Science. The organizer of the conference was Jiri Krecek (Institute of Physiology, Prague) and the general chairman was R. A. McCance (Infantile Malnutrition Research Unit, Uganda) who was awarded the Purkyne Medal by the Czechoslovakian Academy of Science during the meeting.

In one of the opening papers, Krecek described his Institute's research program on the effects of early weaning in rats; he stated the thesis that the period of weaning in mammals is a critical one inasmuch as several basic physiological processes are being reorganized at this time, particularly those involving salt balance, general nutrition, and fat intake. Other workers described the results of early weaning (defined as weaning at 16 days of age). V. Novakova reported that rats who were weaned early elaborated a conditioned reflex less rapidly than those weaned at 30 days of age and also that the adult brains of these animals showed deficiencies of RNA content in certain neurons. M. Kraus reported that aldosterone regulation was affected by early weaning, and V. Palaty reported that androgenic activity in males is adversely affected. Thus there is objective evidence that the period around 16 days of age in the rat is a critical one for several processes. Not all effects are as definite as the above, as P. Wiener found with studies of gastric erosion. S. Kazda described a pilot study of human adults indicating that reproduction and certain kinds of pathology may be affected by early weaning.

An earlier period of development, namely the first few days after birth, appears to be a critical one in rats for the development of normal sexual behavior and reproduction. Papers by C. A. Barraclough, N. Takasugi, G. Mayer, and I. Ostadalova demonstrated that females can be sterilized by injections of either estrogens or androgens at this period, and that males are sensitive to estrogen which causes involution of the seminiferous tubules and inhibition of growth later in life. J. M. Tanner presented a series of diagnostic stages for measuring skeletal maturation in rats as a technique for measuring some of the effects of sex hormones. H. Peters reported that the mouse ovary is unusually sensitive to x-rays between 14 and 21 days of life and that a single dose of 20 roentgens at this time will cause the development of ovarian tumors and consequent sterility in adult life.

The physiological responses of the adrenal cortical stress mechanism in response to infantile stimulation were discussed in detail in papers by Levine, Denenberg, Zarrow, Ader, Angermeier, and Kincl. S. Levine showed that the release but not the synthesis of steroid by the adrenal is refractory to corticotropin from 3 to 15 days of age. M. X. Zarrow and V. H. Denenberg emphasized the ability of the adrenal gland of the 2-day-old rat to respond to ACTH and to systemic stressors with increased corticoid release. Thus the impact of early handling could be mediated by means of the corticoid release of the neonatal rat. These authors emphasized that the ability of the gland to respond was a continuation of the embryological development seen in the fetus. Another significant paper was that by I. A. Arshavski, who found that growth can be stimulated both before and after birth by appropriate and moderate stress conditions which result in increased physiological activity and hence better nourishment. This suggests a physical mechanism through which the effects of early stimulation on growth could act.

Another group of papers by E. M. Widdowson, G. C. Kennedy, J. Cravioto, J. MysPivecek, H. A. Waisman, J. Lat, and K. V. Shuleikina dealt with undernourishment and various nutritional factors on the development of the young organism. Of particular interest was the study of undernourished children by Cravioto. He reported that children whose height had been seriously affected by undernutrition also showed a deficiency in tests involving geometric form recognition. Widdowson (in pigs) and Waismann (in an unusual human case) gave evidence that considerable recovery of growth can follow early starvation. If there is a critical period for this effect, it is quite long. Shuleikina reported success with recording EEG's of kittens during feeding and other normal activities, an important technical advance in the recording of developmental changes in neurophysiology.

Another series of papers dealt with the development of the heart and problems of hypertension. K. Rakusan and O. Poupa reported that exercise plays a critical role in early development of the capillary circulation of the heart. Exercise in adults results only in an increase in size of fibers. J. Jelinek reported that the process of organization of the functional system regulating the homeostasis of water and electrolytes is active only before sexual maturation and therefore that there is a critical period for the development of salt hypertension. A. Grollman reported that administration of a variety of toxic agents during certain critical periods in pregnancy causes inhibition of development of the embryonic kidney and consequent gradual development of hypertension in adult life. H. Musilova noted that adult hypertension could be developed by treating young rats with DCA plus a high salt diet.

J. P. Scott summarized experimental evidence on the critical period for primary socialization in the dog and commented on the general theory of critical periods and its implications. This theory, that the time when an organizational process is proceeding most rapidly constitutes a period when it is most easily modified, will apply to organizational processes on any level. It follows that the analysis of a critical-