18 Mitochondrial Structure as a Controlling Factor of Monoamine-oxidase Activity and the Action of Amine-oxidase Inhibitors. H. AEBI (Switzerland).

The rate limiting factor was studied in suspensions of isolated mitochondria from rat liver, as well as in purified monoamine oxidase (MAO) preparations. Under a variety of experimental conditions the following activities were measured: (1) NH_3-production (Seligson's method); (2) total-O_2-consumption (Warburg's direct method); (3) rate of H_2O_2-production, using the coupled oxidation of [^{14}C] formate by catalase as an indicator reaction; and (4) the aldehyde formation by semicarbazide-trapping.

Mitochondrial suspensions behave quite differently according to their functional state. Compared with lysed mitochondria—as used usually—those suspended in isotonic saccharose and incubated in presence of pyruvate and tyramine exert a much smaller NH_3-production and H_2O_2-formation effect (about $\frac{1}{4}$), whereas their O_2-consumption is 4–5 times higher. Similar differences can be observed if pyruvate is omitted in the suspending medium, if the mitochondrial suspension is frozen before incubation, or if a detergent, i.e. 0·1 per cent Triton-X-100 is added. In all these instances there is reciprocity between the rate of pyruvate oxidation (requiring intact mitochondrial structure) and MAO-activity as well as coupled formate oxidation (either activity being limited by structural factors).

In presence of increasing amounts of MAO-inhibitors all the activities mentioned above are reduced to the same extent, except coupled formate oxidation. Thus, the I_{50}-value of $^{14}CO_2$ formation in isotonic suspensions of rat liver mitochondria is about twice. In presence of *cis*-2-phenyl-cyclopropylamine, added simultaneously with the substrate (0·02 M tyramine) the I_{50}-values for O_2 consumption and NH_3-production are $8{\cdot}0 \times 10^{-6}$ M; for coupled formate oxidation it is $1{\cdot}6 \times 10^{-5}$ M. Furthermore, these I_{50}-values largely depend on the functional state of the suspended mitochondria. In this respect Iproniazid behaves as follows: O_2-consumption and NH_3-production of liver mitochondria, lysed in distilled H_2O, are inhibited 50 per cent by $2{\cdot}5 \times 10^{-4}$ M Iproniazid, on the other hand, an approximate threefold inhibitor concentration (i.e. $7{\cdot}0 \times 10^{-4}$ M) is required if the mitochondrial suspension is incubated under conditions that essentially conserve structural integrity. The significance of these observations is discussed in view of the competitive behaviour of MAO-substrates and inhibitors in mitochondria and in the cell in general.

19 Action of Chlorethoxybutamoxane on Respiratory Enzymes of Rat's Brain in Traumatic Shock. H. NINOMIYA, R. W. BUXTON and M. MICHAELIS (U.S.A.).

Several dehydrogenases of rat brain including some which participate in the tricarboxylic acid cycle are depressed in Noble–Collip drum shock (500 turns). The degree of inhibition is in keeping with the severity of the shock, i.e. whether or not the rats' paws were taped or whether they were left unfettered to avoid or diminish impact of the drum. Rats tranquillized with chlorethoxybutamoxane (5-chloro-ethoxy-2-butylamino-methyl, 1-4-benzodioxane) prior to drumming show better resistance to shock. The dehydrogenases of such animals' brain are not significantly changed from controls, or from controls which had not been sedated. There is further differentation between the Pasteur effect in non-sedated shock cases, when repression occurs, and sedated ones, where the effect is lowered but remains at the control level after exposure to drumming. The protein-N contents of brain homogenates of non-sedated and drummed, and sedated, and drummed cases shows statistically highly significant differences.

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20 Drug-induced Alterations in the Intra-cellular Distribution of 5-Hydroxytryptamine in Rat's Brain. N. J. GIARMAN and S. M. SCHANBERG (U.S.A.).

Differential centrifugation of rat's brain in isotonic sucrose has shown that approximately 30 per cent of the endogenous 5-hydroxytryptamine (5-HT) is present in the non-particulate fraction, while about 70 per cent resides in granules. A number of neuro-active drugs have been found to alter this "normal" distribution. These drugs fall into two broad categories: (1) those which failed to change, increased, or decreased the level of 5-HT in the whole brain and, simultaneously, tended to shift the particulate to supernatant (^{P}/S; "bound"/"free") ratio from 2·5 toward unity (e.g. chlorpromazine, phenobarbital, reserpine, and α-methyl-DOPA); and (2) those which elevated the whole brain level of 5-HT and tended, at the same time, to maintain a normal ^{P}/S ratio or to raise it beyond 2·5 (e.g. iproniazid, phenylisopropylhydrazine, LSD-25, and certain adrenocortical steroids).

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21 Modern Concepts in Relationship between Structure and Biological Activity. Introductory Remarks. K. J. BRUNINGS (U.S.A.).

22 Size and Shape of Organic Molecules and Biological Activity. A. H. BECKETT (United Kingdom).

Biological processes involve three dimensions. Increasing attention is therefore being focused upon the size, shape and surface characteristics of enzymes, proteins, antigen–antibody reactions, drug–drug receptor interactions. Any chemically mediated, biological action involves interaction between suitably orientated surfaces.

Our knowledge of biological receptors is mainly derived from a knowledge of the size, shape and other characteristics of molecules producing a particular biological response and the characteristics of the activated state are ignored. Examples of accumulation of evidence to help to define several biological receptors are described.

Antibodies may be considered as model biological receptor sites, and hapten–antibody association used to study the importance of size and shape of molecules in the surface interactions. Stereoselective adsorbents prepared from silica may also be used as simple drug–receptor models. Physicoorganic measurements and modern conformational principles facilitate a representation of size and shape of molecules under biological conditions. Effective molecular size may be altered by hydration, aggregation and micelle formation and thus the biological action of a particular type of molecule affected.

Many enzymatic processes are stereo-selective. Penetration of certain membranes is under stereoselective control. The action of many drugs is partly dependent upon their geometry. Side effects of drugs may be largely attributed to the molecules being adsorbed at "undesirable" as well as at "desirable" receptor sites of slightly differing size and shape requirements. One approach to the search for clinically more selective drugs should therefore involve minor modifications on a three dimensional plan of molecules with known biological actions.

23 Chemical Structure, Physical Properties, and Biological Activity. A. BURGER (U.S.A.).

This lecture will explore the possibilities that medicinal chemists now have to influence the activity of different chemicals during their paths to their receptor sites and their duration and selectivity of action at these sites. The first problem is to study structural variations or temporary alterations of compounds known to possess a given biological activity if their potency or specificity is inadequate. Protective alterations may keep a reactive compound from being absorbed randomly and prematurely and from being excreted, or they may activate an unreactive molecule so that it will be properly absorbed and distributed. The methods available in these areas will be reviewed.

Analogous considerations are applied to improve the actual reactions or to block alternate metabolic reactions at receptor sites. Following a "lead", the molecule can be changed by increasing or decreasing those binding forces which affect the attachment and maintenance of the drug at the receptor. Apart from the few successful methods of producing irreversible inhibitors by covalent binding, one has to rely on minute and mostly empirical methods of balancing reversible polar, electronic and short-range forces in the molecule. A summary of contemporary work in this field will be given.

Drug activity at receptor sites can also be affected by altering metabolic and blocking reactions. This leads to the intellectually most challenging aspect of medicinal chemistry, the rational design of new biologically active structures. At present, most of the pertinent working hypotheses are still in formative stages, and there are too many exceptions to make them really satisfactory. Theories of metabolite antagonism are overlayed with stereochemical ideas to accommodate various advanced forms of the lock and key fit explanations of drug mechanisms. Since several stereochemical observations have been reviewed by Beckett in the preceding paper, other aspects of reactivity, that is, of various chemical and physical properties, will be dealt with here. How far have we come since the early days of isosterism? How can we get out of the shackles of minor alterations imposed by small molecules as the only acceptable classical procedure? How much should one adhere to biochemical analogy, and how can unorthodox structures be reconciled with metabolite analogues? An attempt will be made to illustrate such cases with established observations as a basis of predictability of novel drug structures.

24 Discussion of Previous Papers. P. JANSSEN (Belgium) and G. B. MARINI-BETTOLO (Italy).

25a Inhibiteurs de la Monoaminoxydase et Potentialisation du Sommeil Expérimental. P. LECHAT et M. LEMEIGNAN (France).

Les effets sur le sommeil expérimental de l'administration préalable d'inhibiteurs plus ou moins puissants de la mono-amino-oxydase ont été étudiés chez la souris et chez le rat. Les trois hypnotiques utilisés ont été choisis de structure chimique différente: chloral, hexobarbital et méthyl-4-β-chloréthyl-5-thiazole (Hémineurine). Les trois inhibiteurs ont été: isoniazide (100 mg/kg), iproniazide (100 mg/kg) et JL 1314 (10 mg/kg). A ces doses, seuls les deux derniers exercent un pouvoir inhibiteur puissant et sensiblement égal sur la MAO. Ils ont été administrés par voie péritonéale, 30 min avants les hypnotiques, ceux-ci étant injectés par voie veineuse.

En considérant comme seule significative d'une potentialisation, une multiplication par deux au moins de la durée du sommeil témoin, on constate dans ces conditions expérimentales, que: (1) l'hexobarbital est potentialisé d'une façon sensiblement identique par les trois substances chez la souris et chez le rat; (2) le choral n'est potentialisé que par le JL 1314 chez la souris; (3) le méthyl-4-β-chloréthyl-5-thiazole n'est potentialisé que par l'iproniazide chez la souris.

On peut on conclure qu'inhibition de la MAO et augmentation de l'effet des hypnotiques ne vont pas systématiquement de pair, puisque suivant leur nature chimique, les hypnotiques se trouvent ou non potentialisés par des substances dont le pouvoir inhibiteur sur la MAO est très différent.