**25b Monoaminoxydase Inhibitors and the Potentiation of Experimental Sleep.** P. LECHAT and M. LEMEIGNAN (France).

The effect of preliminary administration of inhibitors of mono-amine-oxydase of varying degrees of effectiveness on experimental sleep was studied on the mouse and on the rat. The three hypnotics used were of different chemical structure: chloral, hexobarbital and methyl-4-β-chlorethyl-5-thiazole (Hemineurine). The three inhibitors were: isoniazide (100 mg/kg), iproniazide (100 mg/kg) and JL 1314 (10 mg/kg). In these doses only the latter two had a marked inhibitory effect on MAO which was approximately equal. They were administered intraperitoneally 30 min before the hypnotics which were injected intravenously.

Considering a duration of at least twice that of control sleep to be a significant sign of potentiation, we found the following results: (1) hexobarbital is potentiated to practically the same extent by all three substances in the mouse and rat; (2) chloral was potentiated by only JL 1314 in the mouse; (3) methyl-4-β-chlorethyl-5-thiazole was only potentiated by iproniazid in the mouse.

It can be concluded that the inhibition of MAO and the augmentation of the effect of hypnotics do not systematically go hand in hand as, depending on their chemical composition, the hypnotics are either potentiated or not by substances which show very different potency as MAO inhibitors.

**26 Further Studies on Monoamine Oxidase Inhibitors.** S. S. PARMAR and M. NICKERSON (Canada).

We previously reported that several hydrazine derivatives, including iproniazid (Marsilid) and pheneprazine (Catron), react relatively irreversibly with the active sites on the enzyme monoamine oxidase (MAO) to produce a "non-equilibrium antagonism". In the present study the antagonism between hydrazine MAO inhibitors and amphetamine and two of its derivatives (P-1726, *p*-trifluoromethyl, and P-1882, *p*-S-methyl) was investigated using rat liver mitochondria as a source of MAO. Enzyme activity was determined on the basis of both oxygen uptake and substrate (tyramine) utilization, which were found to give equivalent results. Preincubation for 15 min with subinhibitory concentrations of amphetamine or of P-1882 protected MAO against inactivation by pheneprazine. Greater protection by higher, inhibitory, concentrations of these agents was demonstrated by determinations of enzyme activity after dialysis. With respect to both inhibition of MAO and protection against pheneprazine inhibition, D-amphetamine was more active than the L-isomer. P-1726 was found to be a more potent and considerably more persistent inhibitor of MAO than either amphetamine or P-1882. It was not possible to demonstrate antagonism between pheneprazine and P-1726. However, the action of the latter was antagonized by amphetamine. The above results indicate that amphetamine, P-1726, P-1882, and pheneprazine all combine with the same active sites on the MAO molecule.

**27 A New Test for MAOI Detecting Effects.** A. LEHMANN and R. G. BUSNEL (France).

Specific effects of substances related to the metabolism of biogenic amines led us to study the effect of monoamine oxidase inhibitors (MAOI).

The clinical anti-depressing effect of these substances can hardly be studied on animals or only indirectly by using their antagonism against reserpine.

As reserpine potentializes lethal audiogenic convulsions in most animals an antagonism between reserpine and MAOI can be demonstrated. The test is quite clear: (a) total protection (suppression of seizure); (b) partial protection (clonic seizure only or tonic non-lethal seizures); and (c) death after convulsions.

The experimental drugs are: Tranylcypramine, nialamide, iproniazide, harmaline and feeble MAOI's like benactysine and D-amphetamine. Injections intraperitoneally to mice of the sub-line Rb.

Only tranylcypramine (30 mg/kg), nialamide (500 mg/kg), benactysine (80 mg/kg), and D-amphetamine (40 mg/kg) give a 100 per cent protection against audiogenic seizures.

On the other hand, all these drugs injected at threshold, non-protective doses (tranylcypramine (25 mg/kg), nialamide (20 mg/kg), iproniazide (100 mg/kg), harmaline (30 mg/kg), benactysine (60 mg/kg), D-amphetamine (10 mg/kg) and associated to reserpine (1 mg/kg) prevent the mortality during seizure generally provoked by reserpine alone and give complete or partial protection.

This antagonism is quite different from that obtained when associating tranquilizing drug like carbamate of methyl pentynol, and reserpine; in this case one only obtains a protection with a dose of tranquilizer high enough to have a protecting action by itself (50 mg/kg). It seems worthwhile to draw attention to this test for detecting MAOI effects. One objection can be raised: imipramine (25 mg/kg) and azetazolamid (50 mg/kg) have a similar action as the classical MAOI's but at present we have no biochemical proof that they act like MAOI's. This must still be investigated.

**28 Depletion of Catecholamines by Shigella Shigae Toxin in the Mouse Brain.** K. MAŠEK, R. SMETANA and H. RAŠKOVÁ (Czechoslovakia).

The influence of *Shigella shigae* toxin on the content of serotonin, adrenaline and noradrenaline in mouse brain was investigated.

In preliminary experiments the content of these compounds in the brain was measured every 12 hr. It was found that maximal changes develop 48 hr

after the toxin administration. On an average, the content of serotonin and noradrenaline in pooled brains was about 50 per cent of that of the controls. *Shigella shigae* thus causes a considerable depletion of catecholamines in the brain.

**29 Excretion of Vanilmandelic Acid by Psychiatric Patients as Related to Drug Therapy.** I. MUNKVAD and A. RANDRUP (Denmark).

The demonstration that several psychopharmaca affect brain amines, seems to furnish a clue for further studies on the modes of action of these drugs. Measurement of the influence of the drugs upon the excretion of the amines and their metabolites in urine may thus be of interest, as this could give some information on the direct or indirect influence of the drugs upon the production and the ways of metabolism of the amines. As it is also possible to measure the urinary excretion products in the clinical situation, we have chosen this procedure for studying the mode of action of some drugs in psychiatric patients.

In earlier publications from this hospital the effect of reserpine and of iproniazid upon adrenaline and noradrenaline excretion was reported. This work has now been extended by measurements of vanilmandelic acid, the oxidized excretion product of these two amines. The vanilmandelic acid is isolated by high voltage electrophoresis at pH 3 and measured colorimetrically. The effects of chlorpromazine (in varying doses), reserpine and tetrabenazine is studied.

**30 Suppression by Iproniazide of the Antagonistic Action of Reserpine on Amphetamine "Group Toxicity".** B. N. HALPERN and C. BARACCO-DRUDI (France).

Reserpine is known to reduce the "group toxicity" of amphetamine in mice, as does chlorpromazine. It has been found in this laboratory that treatment with iproniazide suppresses the effect of reserpine and raises the amphetamine "group toxicity" in reserpine-treated animals to the level of the controls.

The time interval between the injection of iproniazide and reserpine is an essential factor. In our experiments, iproniazide is injected first, followed by the injection of reserpine, while DL benzedrine is administered, in all cases, 4 hr after reserpine. No inversion of the action of reserpine is observed, if the interval iproniazide-reserpine is less than 4 hr. The inversion is regularly observed during the time interval 4–36 hr.

The effect of chlorpromazine is not affected by pretreatment with iproniazide. The mechanism of the inversion by iproniazide of the action of reserpine on the "group toxicity" of amphetamine in relation with the cerebral metabolism of aromatic amines will be discussed.

**31 Studies on the Mechanism of Uptake of Catecholamines by Isolated Brain Tissues.** E. O. TITUS and H. J. DENGLER (U.S.A.).

Norepinephrine-$^3$H (NE) in slices of cat cerebral cortex incubated with 5 mμg/ml of the labelled amine in Krebs bicarbonate approaches a steady state concentration approximately 4 times that in the medium after 45–60 min. The uptake mechanism, which operates against a concentration gradient and becomes saturated at levels near 100 mμg/ml may be an active transport. It does not function at 0°C and like active transport of 5-hydroxytryptamine in platelets is inhibited by reserpine and cardiac glycosides. Since administration of 3 mg/kg of phenyl α-methylprophylhydrazine (JB 516) 24 hr before the experiment or preincubation of brain slices in $10^{-6}$ M JB 516 is without effect on uptake, this amine oxidase inhibitor is used to minimize NE breakdown during transport studies. After 45 min incubations, 70 per cent of the isotope in the medium is NE (determined chromotographically). Isotope emerging from the slice in the steady state is accounted for by the following percentages: NE, 42; epinephrine, 2; normetanephrine 35; acidic products of amine oxidase, 19. Corresponding percentages from slices without JB 516 are: 13·6, 10·7, 6·2 and 70·2, respectively. Since 80 to 90 per cent of endogenous NE does not exchange with isotopic NE in 2 hr, NE taken up by the transport mechanism must equilibrate very slowly or not at all with the stored NE in isolated slices. The data suggest existence of at least two intracellular pools of NE.

**32a Teneur en Nor-Adrenaline du Tissu Cerebral d'Animaux soumis a l'action de l'Aminodipropionitrile.** M. BEAUVALLET et J. FUGAZZA (France).

En 1952, Delay *et al.* ont constaté que l'aminodipropionitrile:HN = $(CH_2\text{-}CH_2\text{-}CN)_2$ (I.D.P.N.) provoque chez la souris une agitation motrice permanente; l'animal présente une activité généralisée avec forte tendance à tourner en rond.

Les rats soumis à l'injection du même produit réagissent de façon voisine, présentant des troubles de la coordination motrice avec perte d'équilibre.

Dans ce travail, nous avons recherché la teneur en nor-adrénaline du tissu cérébral du rat et de la souris avant et après l'injection d'I.D.P.N.

Les premières expériences ont été faites sur des rats de race Wistar de 60–100 g; 3 groupes de 6 femelles et 1 groupe de 6 mâles reçoivent deux injections intrapéritonéales d'I.D.P.N. à 48 heures d'intervalle. Le tissu cérébral est prélevé dès l'apparition du syndrome excito-moteur. En même temps on prélève le cerveau d'un animal normal de même portée, de même sexe et de même poids.

Les résultats que nous avons obtenus montrent que la teneur en nor-adrénaline du tissu cérébral du rat mâle ou femelle soumis à l'action de l'I.D.P.N. est très voisine de celle de l'animal normal.