Further experiments confirm that acetylcholine (in injections of 0·1 ml of 10^{-12}M, 10^{-10}M, 10^{-8}M, 10^{-6}M and 10^{-4}M) is a more potent dilator of the ear vessels constricted by noradrenaline (10^{-7} g/ml) at 20°C than at 38°C. A Peripheral Resistance Unit (PRU) is determined by dividing the perfusion pressure (in mm Hg) by the flow (in ml/min) if C=PRU under control conditions and R=PRU during the response to the drug, $\Delta\,\%\,\text{PRU}=\frac{C-R}{C\times 100}$. The difference between Δ % PRU (Green, Lewis, Nickerson & Heller, 1944) at 20°C and 38°C is significant ($P<0{\cdot}001$). Physostigmine (10^{-6}M) and ecothiopate (10^{-6}M) potentiate the dilator actions of acetylcholine; this potentiation is greater at 38°C than 20°C ($P<0{\cdot}001$) for both drugs and more marked when the agonist concentration is low (ACh 10^{-12}M) rather than high (ACh 10^{-4}M).

Injections of atropine sulphate (0·1 ml of 10^{-10}M, 10^{-8}M, 10^{-6}M and 10^{-4}M), homatropine hydrobromide (0·1 ml of 10^{-10}M, 10^{-8}M, 10^{-6}M and 10^{-4}M) and hyoscine hydrobromide (0·1 ml of 10^{-10}M, 10^{-8}M, 10^{-6}M and 10^{-4}M) dilate the noradrenaline-constricted vessels of the rabbit ear, in that order of potency; these effects are dose dependent. In six of ten rabbits tested and at a temperature of 23°C physostigmine salicylate (10^{-6}M) potentiated this dilator action of homatropine more than that of atropine. The effects of hyoscine were not potentiated.

Nicotine tartrate (10^{-3}M and 10^{-4}M of the base), when injected into the ear vessels perfused without noradrenaline and therefore fully dilated, causes a constriction; when injected into the noradrenaline-constricted ear vessels nicotine (10^{-4}–10^{-10}M) causes a dilatation, as does ACh. The constriction due to nicotine is abolished by prior addition of hexamethonium (10^{-6}M) and the dilatation due to nicotine is greatly reduced. The dilatation due to ACh is not affected.

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Acute tolerance to a sedative in man.

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When daily doses of phenobarbitone are given as an anti-convulsant or anti-anxiety agent, plasma levels of drug have been shown to rise as the drug accumulates, but drowsiness decreases after the first few days. Such tolerance to sedatives is well known in patients and experimental animals, but little is known of either the time course or mechanism of the development of tolerance.

A preliminary study has been made of the relationship of sedative effect to the concentration in plasma of glutethimide (Doriden) in three volunteer subjects. The suppression of smooth tracking eye movement was measured (Norris, 1968), and subjects rated their feelings of drowsiness, etc. Drug concentration in plasma was assayed by gas chromatography (Grieveson & Gordon, 1969). Drugs were taken orally and doses of 500 mg (normal hypnotic dose) and 250 mg were used. Eye movement recordings were made every 10 min and venous blood samples taken, usually every 30 min.

The time course of drug absorption and effect were different for each subject, but certain relationships between the two variables were common to all three.

(*a*) With the larger dose, drug concentration rose more steeply to roughly double the concentration given by the smaller dose. The effect of the larger dose was upwards of 4 times that of the smaller. (*b*) For any drug concentration, the effect generated was always greater when drug concentration was rising than when falling. (*c*) With the smaller dose, the effect was pronounced only when drug concentration was rising; at the highest drug level there was little or no effect. (*d*) Subjective feelings of sedation followed a course roughly similar to that of eye movement suppression but provided a less sensitive measure.

The phenomenon observed could well be called "tolerance" but the most striking feature was the dependence of the effect on the change in drug concentration. In one subject a second 250 mg dose was given 2 hr after the first. At that time the plasma drug concentration had fallen a little from its peak level, but the effect had passed off entirely. The second dose generated an effect rather greater than the first, so there was no "tolerance" to fresh drug.

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The effect of acute and chronic morphine administration on brain acetylcholine levels in the rat.

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In 1964, Hano, Kaneto, Kakunaga & Moribayashi showed that an injection of morphine hydrochloride (100 mg/kg), which caused an increase in brain concentration of acetylcholine when given in acute doses, did not cause this increase in mice that had been chronically treated with morphine. They were, however, not able to detect any changes in acetylcholine metabolism during subsequent withdrawal of the drug. Previously Hano, Kaneto & Kakunaga (1963) had demonstrated that mice do not exhibit certain symptoms of physical dependence when compared with the rat, so it was decided to study concentrations of acetylcholine in the brains of rats during various phases of the tolerance cycle.

Rats were made tolerant to morphine sulphate by giving an initial dose of 20 mg/kg intraperitoneally twice daily and gradually increasing the dose to 100 mg/kg twice daily over a period of 16 days; the rats were maintained at this dose level until the end of the experiment (6–10 weeks). Control animals were injected twice daily with an