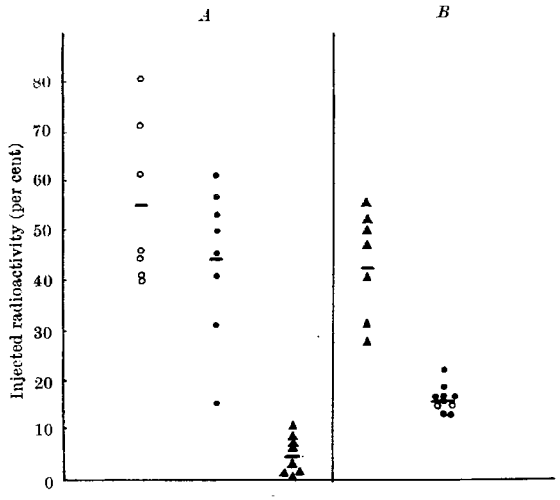


Fig. 1. (*A*) Uptake of iron-59 into erythrocytes. ○, Normal dogs 72 hr. after injection of iron-59; ●, dogs with ligated ureters 72 hr. after injection of iron-59; ▲, maximal uptake in nephrectomized dogs; —, mean. (*B*) Uptake of iron-59 into liver. ○, Normal dogs; ●, dogs with ligated ureters; ▲, nephrectomized dogs; —, mean

(0·09–0·49 mgm./kgm. day; mean, 0·24); the differences observed are statistically significant ($P < 0·01$). It is slightly lower than in normal animals (0·25–0·92 mgm./kgm./day; mean 0·64) but these differences are not significant ($0·1 < P < 0·2$).

The incorporation of iron-59 into red cells three days after injection of the isotope is almost the same in dogs with ligated ureters (15·6–60·9 per cent; mean, 44·1) as in normal animals (40–80·8 per cent; mean, 55·3), while it is less than 12 per cent in the nephrectomized dogs, 8 and even 10 days after the isotope administration. Maximal incorporation averages about 70 per cent in the normal and 61 per cent in the five dogs with ligated ureters (Figs. 1 and 2).

Our observations included an estimation of erythropoiesis by post-mortem measurements of radioactivity in the liver (Fig. 2). This radioactivity varies from 27 to 55 per cent (mean, 43·3 per cent) of the injected tracer dose in the group of seven bilaterally nephrectomized dogs and from 13 to 22 per cent (mean, 16·1 per cent) in the control group of dogs with ligated ureters and in two normal dogs. This suggests that in the controls iron-59 is chiefly deposited in erythropoietic organs. We also regard as noteworthy the persistence of erythroblasts in the bone-marrow of dogs with ligated ureters, since these elements disappear three days after bilateral nephrectomy.

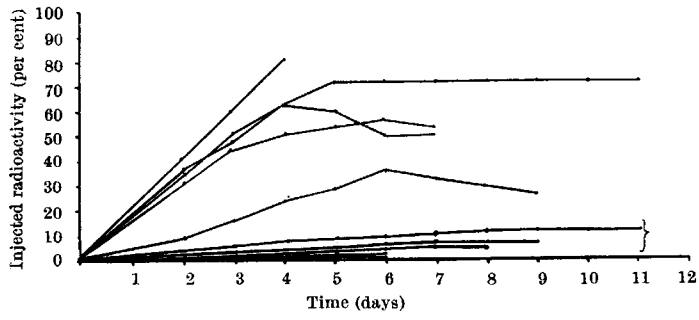


Fig. 2. Uptake of iron-59 into erythrocytes. The curves in the bracket are for nephrectomized dogs, the others for dogs with ligated ureters

These observations lead to the conclusion that the kidney produces an erythropoietic-stimulating factor, or transforms some precursor into an active agent. Besides this, it was shown that retention of toxic products does not impair erythropoiesis.

Our results agree with those of Jacobson[3] working on rats, but diverge from the observations of Mirand and Prentice, who noted an erythropoietic response in anoxic nephrectomized rats[4].

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## Effect of Drugs on Conditioning and Habituation to Arousal Stimuli in Animals

Previous studies[1,2] using the feline *encéphale isolé* of Bremer have shown differential effects of certain drugs on the thresholds for electrocortical activation and behavioural arousal from sleep produced by auditory stimulation. In order to confirm and extend these results further experiments were carried out in conscious, chronic cat preparations carrying permanently implanted cortical recording electrodes[3]. For recording purposes these animals were placed in a constant-environment chamber fitted with a one-way glass window. Auditory stimuli were presented from a loudspeaker fitted in a side wall of the chamber, and the threshold for arousal was taken as the intensity of stimulation necessary to bring about abrupt desynchronization of the electrocorticogram and behavioural arousal.

It is clear that in any comparative study of the changes produced by drugs in the behavioural and electrocortical arousal reaction evoked by repeated sensory stimulation, we must take into consideration the normal processes which occur simultaneously. For example, in the present study the thresholds for arousal produced by a given auditory stimulus were not the same in every animal, and in any one animal they tended to fluctuate from day to day. In addition, the thresholds for a particular stimulus, although remaining constant when determined two or three times over a 4-hr. period, tended to increase when the stimulus was continually repeated over a similar period of time.

Habituation of the arousal reaction therefore developed very rapidly in these preparations where minimal intensities of auditory stimulation were employed, and it was found that the thresholds for auditory-induced arousal responses were only constant from approximately the first to the fifth presentation. This does not present a very stable base-line on which to test the effects of incremental doses of drugs. Sharpless and Jasper[4], however, have shown that habituation to a stimulus may be overcome by positive conditioning, and

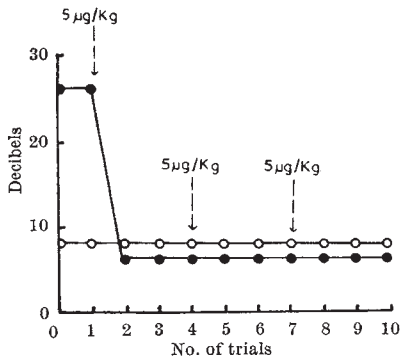


Fig. 1A

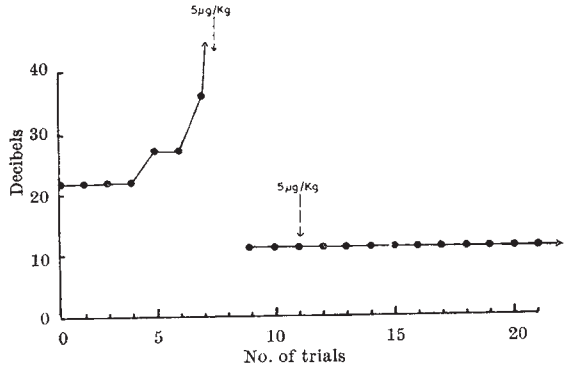


Fig. 1B

Fig. 1. *A*, The effect of lysergic acid diethylamide on conditioned and unconditioned auditory-induced arousal responses. *B*, The effect of lysergic acid diethylamide on habituation of the arousal reaction. ●, Threshold for unconditioned arousal; ○, threshold for conditioned arousal

therefore all preparations used in this study were conditioned to arouse upon the presentation of a specific tone. The conditioned response, consisting of both electrocortical desynchronization and behavioural arousal from sleep, was obtained by pairing an electric shock with the particular tone chosen. In doing this it was found that the arousal thresholds remained stable from day to day and the stimulus could be repeatedly presented at short intervals for something like 30–40 trials before extinction took place. It is interesting to note that during the conditioning trials the thresholds for electroencephalographic and behavioural arousal produced by the conditioned stimulus progressively decreased in every animal to a level approaching the threshold of hearing.

In all the experiments carried out with chronic unrestrained preparations the drugs were injected by the intraperitoneal route. Chlorpromazine in doses of 5–10 mgm./kgm. produced a rise in the thresholds for conditioned and unconditioned auditory-induced arousal responses. Following a total dose of 15–20 mgm./kgm., the conditioned as well as the unconditioned arousal response was blocked completely even when the intensity of the stimulus was considerably increased above control levels. At this point visual and tactile stimuli which normally evoked arousal responses failed to do so.

Reserpine also produced an increase in the arousal thresholds. Since this drug appears to have a delayed action incremental doses were not given. Instead, a single dose of 200 or 250 μgm./kgm. was injected and the thresholds then checked every half-hour over a 5-hr. period. The effects of reserpine were very slow to appear, and no significant effect was observed until 1–1½ hr. after the injection, when the thresholds for conditioned and unconditioned auditory-induced arousal showed a slight rise. After 2–3 hr., however, a dissociation of the threshold/time response curves became apparent. The thresholds for the unconditioned response rose progressively until 3½ hr. after the injection arousal produced by the neutral stimulus was blocked completely. On the other hand, the threshold for the conditioned response remained constant or rose a further step but in no case was the conditioned response blocked by reserpine. Moreover, the animal still responded to novel stimuli, which suggests that the blocking of the unconditioned response might be due to habituation.

The effect of lysergic acid diethylamide was tested on conditioned and unconditioned arousal responses. In addition, the animal was also habituated to an auditory stimulus by continued presentation over a short period of time. After an initial dose of 5 μgm./kgm. of lysergic acid diethylamide conditioned arousal responses remained unaffected but the threshold for the unconditioned response fell sharply (Fig. 1*A*). Similarly, the stimulus to which the animal had become habituated now evoked a marked response. The intensity of the stimulus needed to produce this effect was also much lower than the original level (Fig. 1*B*). Following a total dose of 10–15 μgm./kgm. of lysergic acid diethylamide the animal became more responsive to sensory stimulation and remained alert for long periods. The thresholds for arousal, however, did not show any further change.

The arousal value of a sensory stimulus depends upon the significance which is attached to it by the animal. Positive conditioning increases the significance, whereas during habituation there is a loss of significance. In these experiments chlorpromazine appeared to simulate the effects of habituation for a wide range of sensory stimuli, the animal becoming indifferent to stimuli which previously had evoked electrocortical activation and behavioural arousal. In contrast, lysergic acid diethylamide appeared to increase the significance of most sensory stimuli to a level similar to that achieved by positive conditioning for any given stimulus. The fact that both lysergic acid diethylamide and chlorpromazine produced no marked effect on the thresholds for arousal evoked by direct stimulation of the brain stem reticular formation[2], and yet have an effect on auditory-induced arousal responses, emphasizes the importance of the neural mechanisms regulating the inflow of sensory information in relation to the action of these drugs.

The blocking of the unconditioned arousal responses following reserpine can, as already stated, be explained on the basis of habituation, since novel stimuli were still capable of producing arousal. Thus in contrast to chlorpromazine, it would appear that the sedation produced by reserpine does not seem to be entirely dependent upon the action related to the inflow of impulses into the brain stem from the classical sensory pathways. The site of action of reserpine cannot be defined at this stage, but it is interesting to note that reserpine did not block the conditioned arousal responses, whereas numerous workers have reported that this drug severely depresses conditioned avoidance behaviour[5]. The use of

conditioned arousal responses eliminates a task involving muscular co-ordination, and it may well be that responses of this sort may help to distinguish more clearly the selective effects of some drugs from a more diffuse and non-specific action of others.

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## Lenticular Effect in Mice of Some Morphine-like Drugs

DRUG-INDUCED opacities of the lens of the eye have been recorded by Busche[1] using nitrophenol compounds in chicken. He was unable to produce this in any other small animals except in one particular strain of mice. While investigating the acute toxic effects of a series of reversed esters of pethidine, we observed that a number of these also produced an opacity. This was completely reversible and has been obtained by using other analgesic drugs in several strains of mice.

Fig. 1 shows the typical reaction seen in the eyes of mice after subcutaneous injection of 2 mgm. of (DL)-methadone hydrochloride. All doses mentioned are expressed as mgm./100 gm. of body-weight, and the mice used (both sexes) weighed 18–22 gm. The cloudiness begins to appear at 15 min., reaching a maximum in 50 min., when it slowly disappears. 3 hr. later, the eye is clear again. Smaller doses produce less effect. The opacity develops more slowly with morphine, reaching a maximum in 75–90 min. The effect can be produced in rats but less readily. This species is more sensitive to other toxic actions of these drugs so that the blood concentration to produce an opacity would usually be lethal.

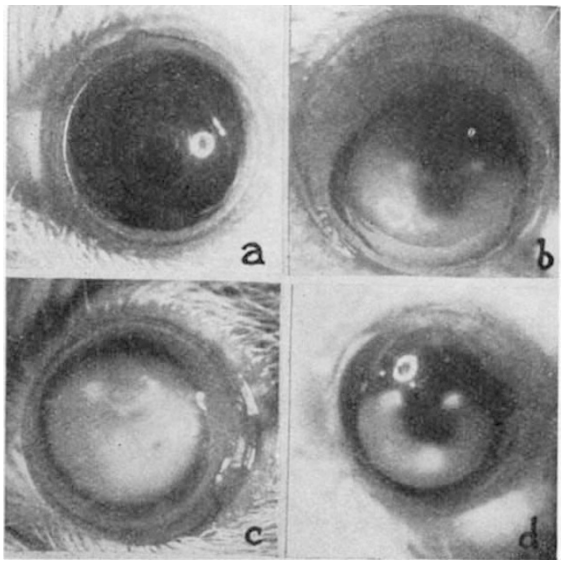


Fig. 1. Various degrees of eye opacity developing in the lens after subcutaneous injection (2 mgm./100 gm.) of (DL)-methadone hydrochloride: (*a*) normal eye; (*b*) 30 min.; (*c*) 50 min.; (*d*) 75 min.

The cloudiness developed in the lens while the cornea remained clear when the eye was viewed through a slit-lamp microscope. Removal of the lens confirmed the site of the opacity. Further investigation has shown that in the intact animal clouding takes place on the anterior surface of the capsule while the lens itself remains transparent. In isolated normal lenses exposed to the same drugs, similar changes occur, but the whole capsule is involved.

A range of drugs has been tested to determine whether the production of this opacity is confined to a certain class of compound. This was found to be the case with narcotic drugs, and particularly with the potent analgesic members of this group. The exceptions were cocaine and papaverine, which had only one-tenth to one-twentieth of the activity of methadone. This effect was produced in at least 20 per cent of the mice by subcutaneous injection, in doses less than 5 mgm., of pethidine hydrochloride and three of its reversed esters (to be published in detail elsewhere); of morphine sulphate and some of its structural analogues, and of (DL)-methadone hydrochloride. Of those tested, the more active the drug was as an analgesic the more readily it produced an opacity.

No effect could be obtained by sublethal doses of the following drugs:

| | |
|---|---|
| Codeine phosphate | Pentobarbitone sodium |
| Apomorphine hydrochloride | Cyclobarbitone sodium |
| Mepyramine maleate | Phenobarbitone sodium |
| Diphenhydramine hydrochloride | Acetyl-salicylic acid |
| Histamine acid phosphate | N-acetyl-*para*-amino phenol |
| Atropine sulphate | Phenacetin |
| Physostigmine salicylate | Lignocaine hydrochloride |
| Quinidine sulphate | Amethocaine hydrochloride |
| Amiphenazole hydrochloride | Procaine hydrochloride |

This additional activity of these analgesic drugs has been studied in more detail. In Table 1 a comparison is made of the dose which will produce analgesia in 50 per cent of the mice (*ED*50) as determined by the 'tail clip' method[2], the dose which will kill 50 per cent of the mice (*LD*50) and the dose which will cause cloudiness in 20 per cent of the mice (minimum opacity dose). In each case the dose required to produce the opacity is greater than the corresponding *ED*50, but less than the *LD*50. By changing from the subcutaneous to the intravenous route, clouding of the lens, analgesia and toxicity are increased by all three drugs. When any of these are given intraperitoneally they become less active both as analgesics and in their effect on the eye, yet they are more toxic. Thus it appears that the production of opacity follows the degree of analgesia more closely than the lethal action of these drugs.

The lenticular opacity produced by analgesic drugs can be prevented by the simultaneous injection of

Table 1. MINIMUM DOSES TO PRODUCE OPACITY AND ANALGESIA COMPARED WITH THOSE FOR LETHAL EFFECTS BY DIFFERENT ROUTES

| Analgesic drugs | Route | *ED*50 (mgm./100 gm.) | Minimum opacity dose (mgm./100 gm.) | *LD*50 (mgm./100 gm.) |
|---|---|---|---|---|
| Morphine | s.c. | 0·5 | 4·0 | 50 |
| | i.p. | 0·8 | 6·0 | 31 |
| | i.v. | 0·4 | 2·5 | 15 |
| Pethidine | s.c. | 1·4 | 5·0 | 17 |
| | i.p. | 3·1 | 7·5 | 12 |
| | i.v. | 1·2 | 2·0 | 4 |
| Methadone | s.c. | 0·3 | 1·0 | 4·0 |
| | i.p. | 0·4 | 1·5 | 2·8 |
| | i.v. | 0·3 | 0·75 | 1·5 |