An improved implantation pellet for rapid induction of morphine dependence in mice

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A new type of morphine implantation pellet for the rapid induction of physical dependence in mice can be prepared by absorbing 7 mg morphine sulphate onto molecular sieves Type 4A (BDH). The small cylindrical pellets can be implanted subcutaneously without trauma and the need for anaesthesia, and are easily removed at any time from the animals. The peak of physical dependence is reached 24 h after implantation, and mortality is negligible. Withdrawal symptoms can be precipitated by intraperitoneal injection of naloxone, without removal of the pellet, and up to 70% of a group of mice show the characteristic urge to jump off a raised platform. This type of pellet has definite advantages over some other sustained-release preparations used in studies on morphine addiction in small animals.

Several methods have been devised to induce morphine dependence in small animals which avoid the tedious routine of frequent injections of increasing doses of the drug. For example, Collier, Francis & Schneider (1972) used a single injection of 150 mg/10 ml kg^{-1} of an oily sustained-release preparation of morphine in rats. The animals became morphine-dependent within 24 h. Other workers have employed implanted tableted pellets which slowly release their content of morphine (Maggiolo & Huidobro, 1961; Way, Loh & Shen, 1969; Gibson & Tingstad, 1970). Such pellets have been widely adopted in studies on the mechanisms of morphine addiction in rodents. Although they produce marked physical dependence, they possess disadvantages. The pellets of Gibson & Tingstad are 3 mm thick and 7 mm in diameter. They are bulky (volume: 0·12 cm^3), and have to be implanted surgically, generally under anaesthesia. The pellets become surrounded by a layer of connective tissue, their volume increases due to absorption of tissue fluid and their subsequent removal is difficult, any remaining will release small amounts of morphine which may vitiate further experimentation. The technique also causes some mortality in mice.

The present technique utilizes pellets prepared by absorbing morphine onto molecular sieves (aluminium sodium silicate), and these have been shown to possess distinct advantages over the pellets of Gibson & Tingstad (1970).

MATERIALS AND METHODS

Animals. Mice (WHT/Ht strain), 27–32 g (female) 30–35 g (male), were used.

Chemicals and drugs

Molecular sieve Type 4A (aluminium sodium silicate),as pellets 1/8th inch (3·18 mm) in diameter (BDH); liquid paraffin S.G. 0·839–0·870 (BDH); morphine sulphate (Macfarlan Smith Ltd., Edinburgh); Arlacel A (mannide monooleate; Sigma); naloxone hydrochloride (Endo Labs Inc., N.Y.). All chemicals were of reagent grade. Implantation pellets prepared according to the method of Gibson & Tingstad (1970)

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(morphine base, 75 mg; microcrystalline cellulose, 75 mg; fumed silicon dioxide, 0·75 mg; calcium stearate, 1·5 mg; pellet weight, 0·152 g; hardness 15 Strong-Cobb Units; thickness 3 mm) were obtained through the kindness of Dr. E. Leong Way.

Preparation of molecular sieve pellets

The cylindrical molecular sieves were cut to 7 mm in length (volume: 0·06 cm³), and those weighing between 65 and 70 mg were used. They were washed with distilled water and dried thoroughly at 100° for 3 h. After immersion in morphine sulphate solution at 100° for 15 min, the pellets were removed, blotted on filter paper and then dried at 100° for 3 h. The amount of morphine taken up depended on the concentration of the solution; from a solution containing 580 mg ml^{-1} each pellet took up 6·97 ± 0·06 mg morphine sulphate.

Determination of morphine in the pellet

The molecular sieve pellets were broken up, and ground in a glass mortar and pestle to a powder which was then dissolved in 10 ml 0·1 N sodium hydroxide. The solution was centrifuged at 7500 g for 20 min and an aliquot of the supernatant read directly for morphine at 250 nm in a spectrophotometer. The standard curve for morphine was plotted by adding known amounts of the sulphate to the crushed blank pellets. Interference from the components of the blank pellet was negligible.

Implantation technique

The Capette Implanter for poultry (Chas. Pfizer and Co., U.S.A.) was modified by replacing the needle with another of sufficient internal diameter to take the molecular sieve pellet. Using unanaesthetized mice, the needle was inserted at the back of the neck 2 cm behind the ears in the midline, and the pellet pushed out towards the head by the plunger to lie s.c. 1 cm from the point of insertion of the needle. The trauma to the skin was negligible and needed no treatment. The pellets of Gibson & Tingstad (1970) were implanted according to Dr. E. L. Way, (personal communication). The slow release preparation of Collier & others (1972) was made by suspending 150 mg morphine base in 0·75 ml Arlacel A and 4·25 ml light liquid paraffin, and emulsifying with 5 ml 0·9% saline. Administration was according to the published method.

Estimation of physical dependence

The withdrawal syndrome was precipitated either by naloxone or by abrupt removal of the pellet. The naloxone (10 mg kg^{-1}, i.p.) was administered without removing the pellet. This dose is similar to that used by Goldstein & Schulz (1973) to estimate the degree of physical dependence in the guinea-pig. For dose-response studies, various doses of naloxone were given to mice 3½ h after the removal of the molecular sieve morphine pellet. The most striking symptom in mice is an uncontrollable urge to jump (Huidobro & Maggiolo, 1961). After the naloxone injection, the mice were placed in groups of five on a cylindrical platform 75 cm high and 35 cm in diameter. The percentage of mice leaping off the platform within 15 min was taken as a measure of the degree of physical dependence (Way & others, 1969).

Removal of pellets

After implantation, the pellets prepared from molecular sieves soon became surrounded by a thin layer of connective tissue, but could be readily removed by reopening the wound and squeezing them out.

Statistical analysis

The data were analysed by Student's *t*-test, using, where possible, the method of comparing correlated means, i.e. each animal in a group acting as its own control.

RESULTS

Uptake of morphine by molecular sieve pellets

The uptake of morphine, calculated as morphine sulphate, by the molecular sieve pellets from morphine sulphate solutions of different concentrations was approximately a rectangular hyperbola. From the solution of maximum strength (580 mg ml^{-1}), 6·98 mg $\pm$ 0·06 of morphine sulphate was taken up. These pellets containing about 7 mg of morphine sulphate were used in all subsequent experiments.

Release of morphine from molecular sieve pellets

The pellets were removed from the mice after differing periods of implantation ranging from 3 to 72 h, and the residual morphine estimated. Fig. 1 shows results from pellets which contained originally 7 mg morphine sulphate. The release of drug is roughly exponential, and after 24 h less than half remains in the pellet.
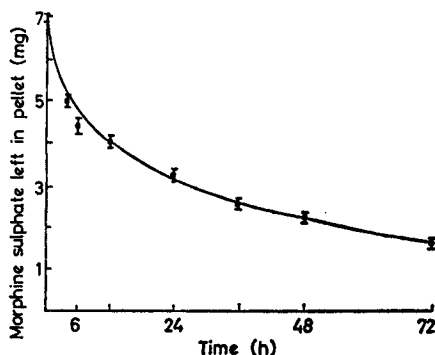

FIG. 1. Morphine sulphate remaining in the molecular sieve pellets (each containing 7 mg morphine sulphate at implantation) at various times after implantation in mice. (Each point represents the mean of 10 determinations $\pm$ s.e.m.).

Physical condition of the mice during administration and after removal of the pellets

Five min after the implantation of the molecular sieve pellets, acute morphine effects were observed, i.e. the Straub effect, hyperactivity and respiratory depression. These effects slowly subsided and after 12 h the animals showed normal behaviour. Their physical condition was good, and the slight loss of body weight was not significant at any time following implantation as compared with controls. Tables 1 and 2 record some results using the paraffin suspension technique and the tableted pellet; both produced markedly greater body weight loss.

If molecular sieve pellets were removed 24 h after implantation, withdrawal symptoms appeared 3 h later. The mice showed diarrhoea, head-shaking, tremor of the forelimbs, and began to walk round the edge of the platform. The diarrhoea, hypodipsia and anorexia led to a significant fall in body weight at 3 and 6 h after removing the pellets. Afterwards their weight began to increase steadily, and by 24 h the weight and behaviour of the animals were back to normal. Blank pellets (containing no morphine) caused no significant changes in body weight after removal.

Mortality following implantation of the molecular sieve pellets was less than 0·6%. Table 1 shows a high mortality after using Gibson and Tingstad's pellet, 6 out of 15 in

Table 1. *Loss of body weight in mice at various times after administration of a morphine-paraffin suspension (150 mg morphine base/10 ml kg⁻¹).*

Mean body weight (g) at time of injection (n = 10)	27·0 ±0·4	26·1 ±1·1	29·0 ±0·6	26·5 ±0·8	28·5 ±0·8
Time after injection (h)	12	24	48	72	96
Mean body weight (g) at various times after injection (n = 10)	26·1** ±0·5	25·0* ±1·0	27·4* ±0·7	24·2** ±0·7	27·8 ±0·8
Body weight loss (%)	3·3	4·2	5·5	8·7	2·5

n = number of animals; weights are expressed as mean values ± s.e.m.
* $P < 0.01$; ** $P < 0.001$, compared with controls (0 h), using the method of comparing correlated means.

Table 2. *Mortality and changes in body weight of mice 3 days after implantation of Gibson and Tingstad's pellet.*

	5 male	5 female	10 female
Number of mice and sex	5 male	5 female	10 female
Mortality after 3 days (%)	100	40	40
Mean body weight (g) at time of implantation		29·0 ±0·6	28·8 ±0·6
Mean body weight (g) after 3 days		25·0 ±1·3	27·4 ±1·6
Body weight loss (%)		13·8	4·9

Weights are expressed as mean values ± s.e.m.

female mice, and 100% with 5 male mice. It should be pointed out, however, that this pellet was devised for a different strain of mice (Swiss-Webster strain). The paraffin suspension method elicited no mortality in 50 mice, but it caused the skin of the mice to become wet and oily. This might be due to the passage of the paraffin through the skin, or, more likely, leak-back down the needle track.

Physical dependence

Withdrawal symptoms precipitated by naloxone injection are much more prominent than those produced by simple removal of the pellet. An uncontrollable urge to jump was observed as early as 3 h after implantation (Fig. 2), with 50% leaping off the platform. After 24 h of implantation, 70% jumped off, but subsequent to this the % fell and reached about 20% after 72 h. The maximal degree of physical dependence is
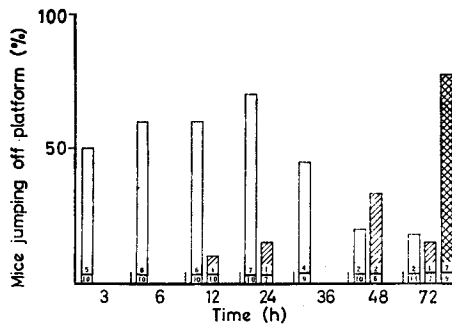

FIG. 2. Naloxone-precipitated withdrawal symptoms (jumping off a raised platform) in mice at various times after implantation of molecular sieve pellets containing 7 mg morphine sulphate, open columns; Gibson and Tingstad's pellet, cross-hatched columns, and s.c. administration of Collier's morphine-paraffin suspension (150 mg morphine base 10 ml kg⁻¹), hatched columns. The pellets and suspension remained *in situ* during naloxone treatment.   Naloxone: 10 mg kg⁻¹, i.p.

reached, therefore, 24 h after implantation of the molecular sieve pellets. Parallel experiments with tableted pellets showed that approximately the same degree of physical dependence was attained only after 3 days implantation. Maximum effect with the paraffin suspension occurred at 48 h, but only one third of the mice showed the jumping behaviour. At 96 h, the jumping behaviour was no longer observed.

$3\frac{1}{2}$ h after the removal of the molecular sieve morphine pellet, mice in groups of five were challenged with various doses of naloxone. The jumping effect shown by the mice was related to the log dose of naloxone in the usual sigmoid fashion. In the mice implanted with placebo pellets (blank pellets without morphine), no jumping was seen even with a dose of 100 mg kg^{-1} of naloxone HCl.

DISCUSSION

The new molecular sieve morphine pellets possess a number of important advantages over other methods of inducing physical dependence in animals. These pellets can be made to take up different amounts of morphine according to the concentration of the drug solution in which they are placed. This enables one to adjust the content according to the sensitivity of different species, or strains of the same species, of animal to the drug. Implantation of the small cylindrical molecular sieves can be performed with speed and minimal trauma by use of the special implanter. After implantation, the pellet does not soften, disintegrate or increase in size, and, although it becomes surrounded by a connective tissue capsule, subsequent complete removal from the animal is easy. The new pellet does not produce significant mortality or body weight loss in our strain of mice, and there is no need for previous priming injections of morphine. The expected loss of body weight after removing the new pellet reaches its maximum earlier than with the tableted pellet of Gibson & Tingstad (Ho, Loh & Way, 1973; Friedler, Bhargava & others, 1972). This may be due to the difficulty of ensuring complete removal of the latter pellets. In general, the present method of implantation and removal of the pellets is simpler and less disturbing to the animals.

The peak of physical dependence with the molecular sieve pellets is reached after only 24 h. With the paraffin suspension 48 h is required, and with the tableted pellet, 72 h. The degree of physical dependence, as estimated by the % of mice jumping off a platform, is similar in molecular sieve and tableted pellets, both being much higher than with the paraffin suspension. Apart from the advantage of saving time, there is also a considerable saving in the amount of morphine used (approximately one thirteenth). The absorption of drugs onto molecular sieves in this way might be of general application for the preparation of slow-release preparations for animal studies.

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