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ANIMAL PHARMACOLOGY AND TOXICOLOGY

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SUMMARY

1. During toxicity studies of long-acting contraceptives, where repeated high doses have to be administered, cumulation causes problems, for which so far no solution has been found. A few suggestions for a practical experimental design are presented.
2. For the pharmacological assessment of long-acting contraceptives, methods for the testing of short-acting preparations can be adapted.
3. Although it is easy to prove in animals that a preparation has long-acting properties, it is not possible to predict how long such a preparation will act in the human.

In this paper I shall limit myself to a discussion of injectable long-acting steroids, since Shearman has already described the various types of depot contraceptives and van der Vies, in another symposium at this congress, discussed the pharmacokinetic and pharmacodynamic differences between these various release forms. In fact the same problems of testing which I intend to discuss also occur with these other dosage forms, but they are most pronounced when testing the injectable long-acting steroids.

The first question I have been asked to deal with is: "What is it about the depot property that calls for special pharmacological and toxicological assessment?" The answer is simple. With short-acting preparations the dose determines mainly the magnitude of the effect, and a higher dose causes only a marginally longer duration of effect than the lower one. Using long-acting preparations the opposite occurs and although the higher dose augments the effect, prolongation is the most obvious result of the elevation of the dose.

This is shown in Fig. 1, which has not been obtained with a contraceptive, since the effects are difficult to quantify with such preparations, but demonstrates the same principle by comparing the effect of the short-acting acetylcholine and the longer-acting methacholine on the blood pressure of the rat. The figure to the left shows that a 20-fold increase in the i.v. dose of acetylcholine causes a fall in blood pressure to about zero, but that for both doses, blood pressure returns to the same level after about 60 s. The figure to the right shows the result of a 20-fold increase of the i.v. dose of the longer-acting methacholine. At the high dose the fall in blood pressure is definitely less than with acetylcholine, although there is hardly any difference between the effects of the low doses. However, the duration of the effect has increased much more, and even at the end of the observation period (after 115 s) the blood pressure after the high dose is still lowered. This means that next to dose and effect a third dimension is introduced; the factor time, which has to be taken into account when studying animal (and human) pharmacology and toxicology.

Once this is realized, it is relatively easy to take into account when single administration is practiced, as we shall see later, when discussing the pharmacological evaluation of such compounds. However, it may

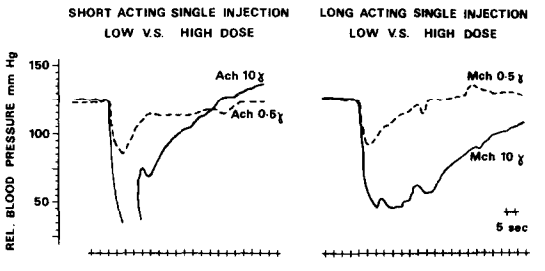

Fig. 1. Effects on blood pressure of the rat, using a single low ---- and a single high —— dose of the short acting acetylcholine and the long acting methacholine.

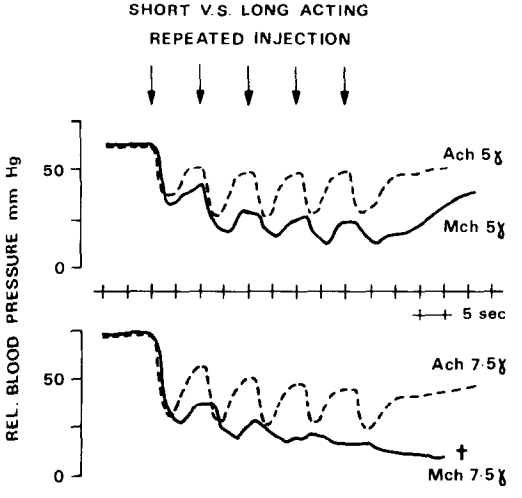

Fig. 2. Effects on blood pressure of the rat, using repeated low and high doses of the short acting acetylcholine ---- and the long acting methacholine ——.

create tremendous problems when long-acting compounds have to be repeatedly administered, in particular when high doses must be administered, as in all long-term toxicological studies.

Again, a comparison of acetylcholine and methacholine on the blood pressure of the rat may demonstrate this point. The upper part of Fig. 2 shows: (a) Five doses of 5 μg acetylcholine, administered at intervals of 10 s, had substantially the same effects on blood pressure; and (b) five doses of 5 μg methacholine, administered with the same time intervals, caused an increasingly lower blood pressure. The lower part of the figure shows the result of five higher doses of 7·5 μg, again administered with an interval of 10 s. Here, even the later doses of acetylcholine caused a slightly greater lowering of the blood pressure. However, this is so much more pronounced with methacholine that after the fifth dose blood pressure did not return to normal and the animal died. This could obviously have been avoided by giving the doses of methacholine at 100-s instead of at 10-s intervals; the consequences will have to be discussed in respect of toxicological studies of long-acting compounds. Returning to contraceptives, I quote some illuminating calculations that Kolb[1] has made with respect to the long-acting steroid norethisterone oenanthate. He showed that after five weekly i.m. injections in rats only 10% of the dose administered had been eliminated. In a toxicity study where, for this type of study, a not unusual weekly dose of 100 mg/kg was administered for a period of 6 months, more than 1 g/kg was present in the body of the rat at the end of that period instead of the 100 mg/kg which should be present immediately after the injection. This brings us to the concept of biological "half-life" (or "half-time") of substances, which is of particular importance for the evaluation of long-acting compounds, but which may easily be misused or interpreted erroneously.

"Half-life" is determined by elimination and indicates the time at which 50% of the dose administered has disappeared from the body, from the blood, from a depot and so on.

The half-life in the body, provided elimination can be represented by an exponential curve, is independent of the absolute dose administered. It is a *constant* for a particular substance, dosage form and animal, but this does not mean that the absolute magnitude and the absolute duration of the effect are independent of the dose. On the contrary, it means that when comparing the duration of effect of a dose $2y$ with a dose y, it takes a "half-life" before the dose $2y$ is reduced to y as a consequence of elimination and metabolism. Hence the former dose requires a "half-life" longer before the threshold level in the body is reached below which an effect no longer occurs. Thus the effect of the higher dose will be longer and obviously stronger than that of the lower one, as the example of methacholine has shown. In view of this, what are the consequences of cumulation, as described by Kolb[1] for norethisterone oenanthate? This is impossible to predict, for not only should we know the half-life of the compound under the experimental conditions, but also the threshold level at the site of action, and not at one site only, but at all sites of action (endometrium, cervix, ovary, pituitary, etc.), and these may all be different. So here we face the problem to its full extent. In medical practice we can find out by experience how long a certain dose of long-acting preparation exerts the desired effect, and we can space the injections in order to avoid (or make use of) cumulation.

But in a toxicological study we are supposed to administer a compound repeatedly at various dose levels over considerable periods of time. How then could the problems caused by cumulation be avoided?

A first possibility would be a constant check of the plasma levels, and studying not the effect of different doses administered but the effect of different plasma levels. Quite apart from the fact that, although undoubtedly plasma levels are related to effective levels at the site of action and we do not know the mathematical function relating both levels, this also would mean a new approach to toxicity studies. The acceptability of this would have to be studied and discussed.

A second possibility would be to study *not* the original long-acting compound, but its active short-acting metabolite. Sometimes there is such a metabolite, as van der Vies[2] has shown to be the case with the long-acting esters of nandrolone. He proved that the substance acting at the sites of action is nandrolone and not the ester, and it could be argued that for such a compound a toxicity study of the free nandrolone, possibly completed by a study of the acid with which it was esterified, might yield more relevant information than a toxicity study of the ester following the classical design. But apart from the fact that this suggestion could only be carried out with hydrolysable esters and not with long-acting compounds which are not changed in the body (e.g. 16-α-ethylprogesterone, a long-acting progestogen [3], this would mean a basic change of the principle that a compound should be tested in a form as similar as possible to its therapeutic one. A third possibility could be to space the intervals of the injections differently for the different dose levels involved. The next injection would only be given when the effect of the previous one had worn off (Fig. 3). This procedure would have the advantage that it would nicely simulate clinical practice. On the other hand it is often difficult to assess accurately the end of the effect.

The latter problem is avoided when proceeding according to a fourth suggestion. First the half-life for disappearance from the body is to be determined for the species and the mode of administration. The study is started by injecting the doses to be studied and subsequent injections of half of these doses are injected after an interval equal to the previously determined half-life. As explained before this half-life will be the same for all dose levels and the amount present in the body will at no time exceed the amount

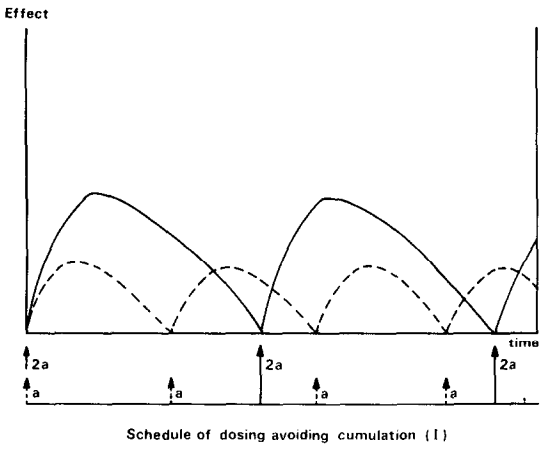

Fig. 3. Schedule of dosing avoiding cumulation (I). Effects after administration of doses a ---- and $2a$ ——.

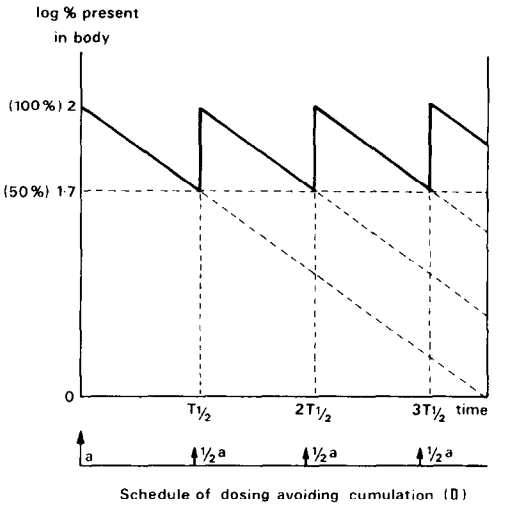

Fig. 4. Schedule of dosing avoiding cumulation (II). —— Amount of a drug present in the body after administration of a starting dose a, followed by doses $\frac{1}{2}a$ with intervals of $T_{1/2}$.

present after the first injection. This principle* is demonstrated in Fig. 4.

It is not surprising that Health Authorities in various countries have avoided giving guidelines for handling the toxicity studies of long-acting compounds in general and of long-acting contraceptives in particular (see [4] and the ensuing discussion at the W.H.O. meeting on "Pharmacological models to assess toxicity and side effects of fertility regulating agents", 1973, following this lecture, p. 352). Nevertheless these problems require attention and a practical solution should be agreed upon. Personally I should prefer to accept the last suggestion (intervals based on half-times), which seems theoretically sound and reasonably practicable.

The discussion of the first question posed by the organizers of this symposium has taken a considerable time, but fortunately most of the remaining questions can now be dealt with relatively briefly.

The period of proposed depot activity in the human will obviously be related to the duration of action in the animal, but the intended spacing of the doses in the human bear no direct relation to that in the experimental animals (see below). This means that before starting a long-term study in animals, either the duration of action of each dose that will be injected into the animal to be used, or the half-life of disappearance from the body, should be determined.

Special toxicity requirements for depot formulations do not seem to be necessary, although special schedules for dosing should be envisaged. There are no reasons for using more or less animals per dose group than used for the study of short-acting compounds. With regard to animal species it should be realized that the duration of action may be different in various species and that this should be studied before starting a long-term study. With some long-acting compounds like esters and ethers, which are transformed in the body into an active short-acting compound, useful information may be obtained by studying the rate of hydrolysis *in vitro* after incubation with plasma or liver mash from that particular species and comparing the result with those from similar studies with human material.

Dose range and duration of study should be related to human use, as with short-acting compounds, again with the provision that the schedule of dosing prevents excessive cumulation. Obviously this is of particular importance when performing long-lasting carcinogenic and tumourigenic studies. When properly performed such studies are just as relevant or irrelevant as similar studies with short-acting compounds. With regard to the demonstration of reversibility of the anti-fertility effect the usual design (Fig. 5) can be followed [5] although the withdrawal period may have to be prolonged. In most cases single administration will suffice, but if desirable multiple application when correctly spaced can be applied. For teratogenicity studies in rats and rabbits a single injection will usually suffice. When a long-acting progestational compound is studied spontaneous delivery may not occur, and either caesarian section will have to be performed or the mother will have to be sacrificed. When the compound is oestrogenic pregnancy will be interrupted, as with short-acting oestrogenic compounds, making the teratogenic study impossible unless either extremely low doses are used or an animal species is found which is less sensitive to oestrogens. The hamster may be a suitable animal [6] but unfortunately very little is known about the incidence of spontaneous malformations and of the type of malformations in this species. Again the relevance

* Obviously the same principle could be adapted for use in humans, although it may be an advantage to base the interval of the injections not on 50%, but perhaps on 80% disappearance. This would depend on the amount that should still be present to allow full contraceptive efficacy.

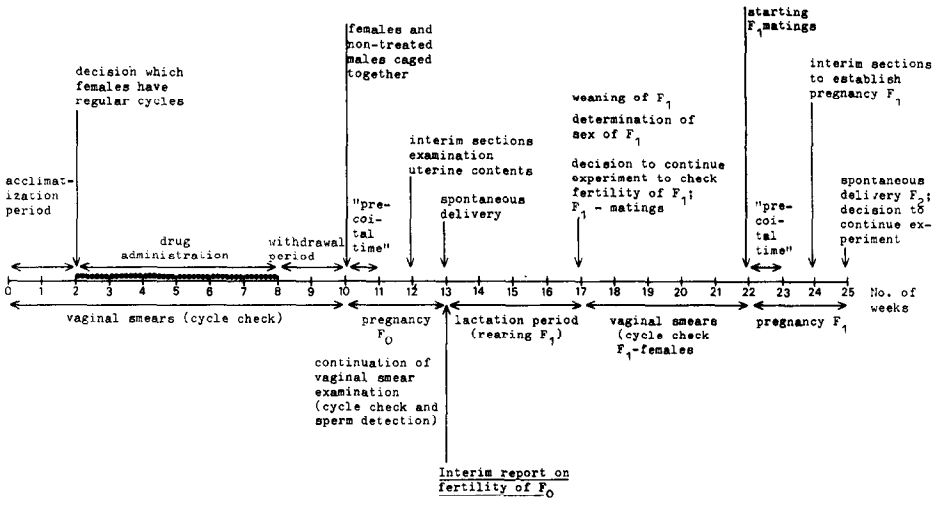

Fig. 5. Schedule of a fertility experiment in sexually mature female rats. To determine whether the effect of a contraceptive compound—when administered for 6 weeks to non-pregnant sexually mature rats—is reversible and has an effect on mating behaviour ("precoital time") and on the fertility of the offspring.

of these studies with respect to prediction of malformations in the human is not different from that of teratogenic studies in other animals.

The final item for discussion is the pharmacological assessment. Here all usual tests can be applied, substituting a single dose for the daily administration. In order to test for progestational effect the following tests are used in our department of endocrinology:

1. The Clauberg–Junkmann test for the effect on the endometrium in rabbits as described by Junkmann[7].
2. The deciduoma test in mice, giving a single injection one day before making the endometrial lesion and performing autopsy on the desired day (Madjerek[8]).
3. Maintenance of pregnancy in the rat spayed on day 11 of pregnancy and injected on day 9. When the activity has not yet worn off on the 21st day, spontaneous delivery will not occur, making caesarian section necessary.
4. Suppression of oestrous cycle in the normal rat.

In all four tests one injection should substitute for the repeated oral or parenteral treatment with a short-acting preparation.

When the compound to be tested has oestrogenic activity one single injection is given to spayed female rats and the vaginal smears are inspected daily (adapted Allen–Doisy test). This test can also be used to study progestational or anti-oestrogenic effects, provided the animals are constantly treated with an oestrogenic preparation.

In the same way tests for inhibition of ovulation, effect on ovum transport, and sperm transport can be adapted (de Visser[9]).

Clearly, it is not difficult to prove by means of animal tests that a compound is long-acting. However, such tests hardly allow a prediction about the duration of action in man, since this depends on the dose administered and on a number of factors which can and do differ in animals and man. Among the latter, pharmacokinetic factors are important, such as the rates of absorption and metabolism.

As shown by Overbeek *et al.*[10], the slower the rates of absorption and hydrolysis the longer *steroid esters* act.

Hence when in one species these rates are lower than in the other, the same compound will act longer in the former than in the latter species. Sometimes these rates may become so low that no effect at all can be detected, as is shown by the following example. An ester of nandrolone was shown to have long-acting anabolic activity in the rat, but proved to be inactive in the human. This could be explained by the fact that the rat easily hydrolyzed the ester, whereas in the human the hydrolysis was too slow to reach levels of nandrolone required for activity. This example also shows that the use of reference preparations in the animal studies is of limited help in predicting the duration of activity in the human.

Pharmacokinetic studies cannot solve this problem, since they suffer from the limitations just mentioned. An additional problem is that radioactive long-acting substances cannot be used in humans because of the hazards of the long-term presence of concentrated radioactive material at the site of injection. When the dose administered remains within an acceptable radioactive level this would require urine or blood to be considerably concentrated before measurements would be possible.

Hence the animal studies do not allow more than a rather wild guess as to the duration of action in the human, and clinical pharmacology has to provide the answer.

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