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DRUG-DELIVERY SYSTEMS AND THE BIOLOGICAL ACTIVITY OF STEROIDS

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SUMMARY

The release of steroids and steroid esters from various drug-delivery systems, including water, vegetable oils and polymer implants, was studied *in vivo* and *in vitro*. It was found that for lipophylic compounds the process of release both *in vivo* and *in vitro* is similar for different systems, and that in equal time intervals a constant proportion of the amount present is released. This suggests that the underlying physical process of the absorption is the partition of the compound between the material with which it is administered and the extra-cellular fluid.

Evidence was obtained that the transport of lipophylic steroids in the plasma takes place after incorporation of the compounds into the lipoproteins.

It could be demonstrated that the way in which a steroid is delivered to the body greatly influences its biological effect.

INTRODUCTION

The administration of medicaments usually involves a vehicle or carrier, in which the active principle is dispersed. Although in the past such a carrier was chosen with the aim of facilitating dosing and was sometimes determined by the manner in which the medicament was prepared, *e.g.* an extract, it was later realized that the vehicle could play an important role in the overall activity of the drug. Today, therefore, vehicles for drugs are sometimes referred to as "drug-delivery systems", clearly indicating and emphasizing that the carrier is involved in the way in which the drug is absorbed by the body.

Systems from which the active principle is slowly released prolong the pharmacological action, but it has also been shown by van der Vies that the rate of release affects different receptors in the body to a different extent [1].

In the present paper the release characteristics both *in vivo* and *in vitro* of a number of frequently used drug-delivery systems for steroids and steroid esters, including water, vegetable oils and polymers are discussed.

The discussion will deal mainly with systems that are administered parenterally. For information on tablets for oral administration the reader is referred to the review of Hänselmann and Voigt[2].

EXPERIMENTAL

1. *Release of steroids from drug-delivery systems* in vivo

The simplest vehicle which can be used for the administration of a drug is water, made isotonic with the extracellular fluid by addition of salts, glycerol, glucose etc.

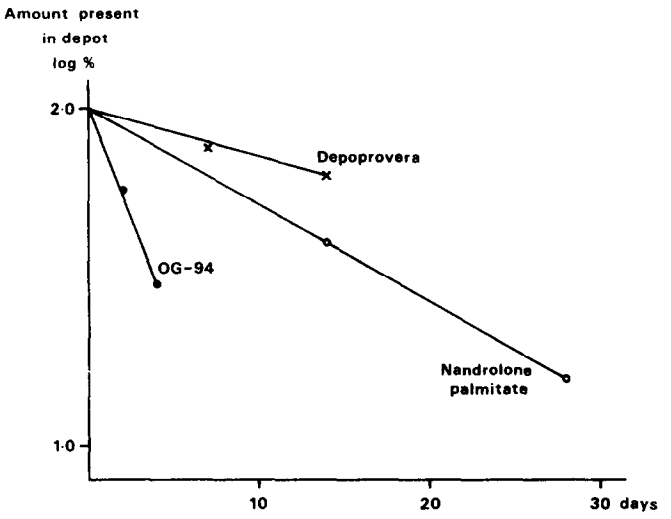
If the drug does not dissolve in the water, a suspension of solid material results. The absorption of such microcrystals is quite different from that of a drug which is in solution. For the latter Bederka, Takemori and Miller[3] have made interesting observations. These investigators injected intramuscularly into rats a number of radioactively labelled substances with molecular weights ranging from 20 (tritium-labelled water) to 585 (ouabain) and found that the rates of disappearance from the intramuscular depots were very similar for all compounds. The absorption appeared to be a logarithmic process and the mean half-life ($T^{1/2}$) of the substances in the depot was 4·4 min. This figure was in agreement with a predicted value calculated from the rate of blood flow through a muscle.

These observations strongly suggest that the administered compound and the water in which it is dissolved are absorbed simultaneously and that the nature of the compound is of little importance, provided it is soluble in water.

A totally different situation is found for compounds which are insoluble in water, but soluble in lipids. Lipid-soluble drugs are often parenterally administered as a solution in a vegetable oil, *e.g.* olive oil or arachis oil.

It has been shown [4] that absorption of a compound dissolved in oil from an intramuscular depot is slow and that it is not absorbed together with the oil, but is released from it. Absorption rates of lipid-soluble compounds vary widely. The release of compounds from a solution in oil is also a logarithmic process; in equal time intervals a constant proportion of the amount present leaves the depot, which suggests that the underlying physical process of the absorption is the partition of the compound between the oil and the transporting extracellular fluid.

Half-lives found for lipid-soluble steroids ranged from 0·6 h for nandrolone to 504 h for nandrolone oleate, which are obviously much longer than those for the absorption of aqueous solutions.



OG-94 = 16α-ethyl-21-hydroxy-19-nor-4-pregnene-3,20-dione-21-(3'-phenyl propionate)

Fig. 1. Absorption of steroid microcrystals from an intramuscular depot in the M. gastrocnemius of rats.

The addition of an organic solvent, *e.g.* benzyl alcohol, to the oil in order to increase the solubility of the steroid can effect $T^{1/2}$ and alter the rate of absorption.

Absorption of microcrystal suspensions is slower than from a solution in oil. Although the process of absorption is probably complex, it can be described again as a logarithmic process and the half-lives are expressed in days rather than in hours (see Fig. 1). A similar situation holds for the release of steroids from solid drug-delivery systems. The polymer Silastic® is the best-known representative of this class, but other polymers can be used as well.

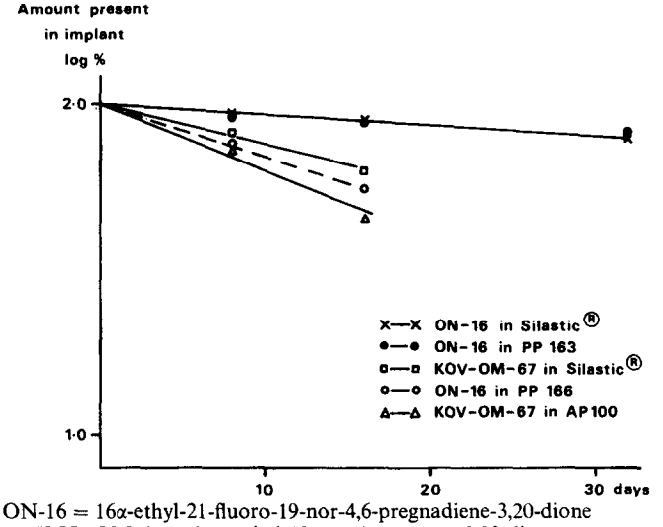
Kincl and Rudel[5] described the release characteristics of steroids from implants in which steroids in solid form were encapsulated in a polymer membrane, *i.e.* a tube filled with crystalline material. If, however, the steroid is dissolved in the polymer the data found in absorption studies are in agreement with the assumption of a logarithmic decrease of the compound in the implant (see Fig. 2). The half-lives are very long [6].

2. *Release of steroids from drug-delivery systems* in vitro

From the foregoing discussion it is clear, that the study of the release of a drug from a depot can help to elucidate its pharmacological activity and to explain differences in activity of structurally related compounds.

For this reason models have been sought which could help to obtain such information.

For solutions of steroids in oil, a model has been described by Van Der Vies[4]. This consists of a strip of filter paper on which a small volume of the solution of the steroid in oil is applied. The oil spot is eluted with a stream of hog plasma in the same way as paper chromatography is done.



ON-16 = 16α-ethyl-21-fluoro-19-nor-4,6-pregnadiene-3,20-dione
KOV-OM-67 = 6α-methyl-19-nor-4-pregnene-3,20-dione

Fig. 2. Absorption of steroids from subcutaneous polymer implants in sheep.

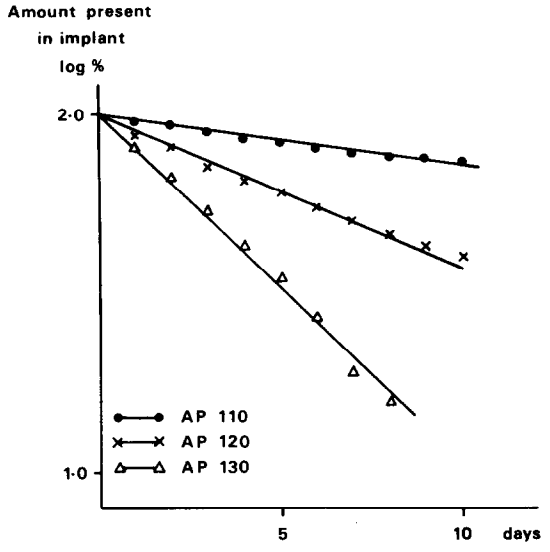


Fig. 3. Elution of 6α-methyl-19-nor-4-pregnene-3,20-dione *in vitro* with methanol–water (1:3, v/v) from polymer implants.

Using this model, results were obtained which correlated with those of *in vivo* absorption studies, indicating that a similar physical process is involved in both.

Another, less elaborate, method is the measurement of the partition of steroids between arachis oil and methanol. It was found that the results obtained with this method, again correlated with the *in vivo* studies and with the results of the paper strip technique. On the other hand substitution of the arachis oil by liquid paraffin greatly reduced the correlation, and in a more extensive study with 17 different steroids, a correlation coefficient of only 0·36 was found between the partition of the compounds in arachis oil–methanol on the one hand and liquid paraffin–methanol on the other. This shows that the former system is to be preferred because it allows a better prediction of the absorption *in vivo* of the compound than the latter when the drug is to be administered in arachis oil.

The release of steroids from polymers *in vitro* has been studied by Huntjens *et al.*[7] using subsequent extractions of implants with methanol–water (1:3, v/v). In Fig. 3 a selection of the results is given.

The logarithmic decrease of the amount of steroid in the implant as found in the *in vivo* studies is duplicated in this model, making it suitable for comparing various compositions of polymers with different steroids in order to predict the release and absorption of the steroid *in vivo*.

3 *Transport of steroids by the plasma from drug-delivery systems*

So far, the main emphasis of the discussion has been concerned with the delivery system from which the drug is released in the body. Another, just as important, factor is the extracellular fluid or the plasma which accepts the drug and transports it from the depot to other sites in the organism. Water-soluble compounds seem to pose the least transportation problems because their aqueous solutions simply mix with the plasma. For non-polar, less water-soluble compounds, it is generally accepted that hydrogen bonds between the drug and components of the plasma, *i.e.* proteins, aid in solubilizing the compound and facilitating the transport.

In our laboratory Hobbelen *et al.*[8] found that after oral administration of a [^{3}H]-labelled non-polar steroid ester, testosterone undecanoate, to rats the compound is present in the chylomicrons and the lipoproteins of the plasma.

The evidence for this is given in Fig. 4, in which the radioactivity of the sample coincides with the location of the lipoproteins on the electropherogram. This finding indicates that incorporation of non-polar, lipid-soluble compounds into lipoproteins permits the plasma to transport such substances.

The authors also found that incorporation of non-polar compounds into plasma lipoproteins can also

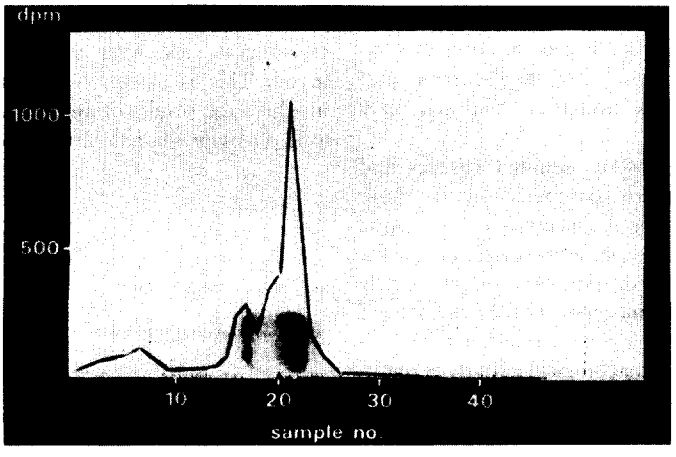


Fig. 4. Distribution of radio-activity over the electropherogram of plasma lipoproteins after oral administration of ^{3}H-testosterone undecanoate to rats.

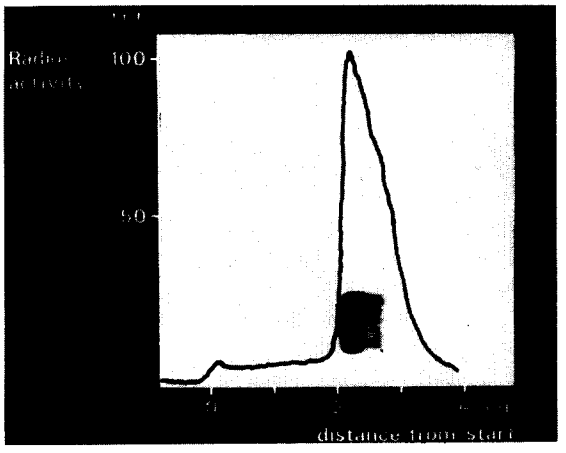


Fig. 5. Distribution of radio-activity over the electropherogram of hog's serum lipoproteins after addition of ^{3}H-testosterone undecanoate.

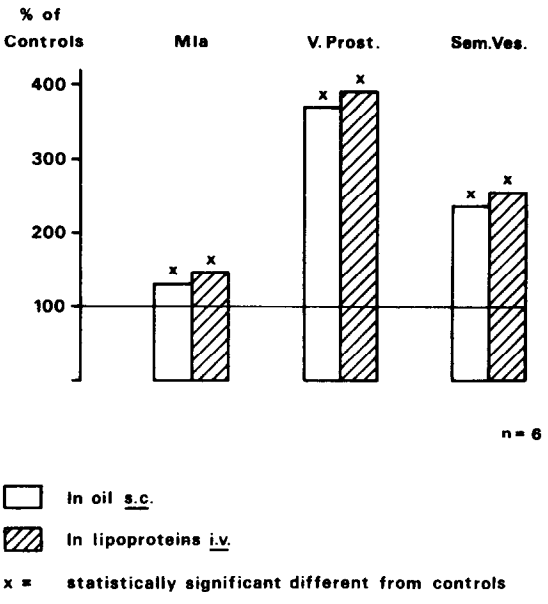


Fig. 6. Androgenic effects in immature castrated male rats of testosterone undecanoate after subcutaneous and intravenous administration.

be achieved *in vitro* by addition of the compound dissolved in a very small volume of a suitable organic solvent, *e.g.* ethanol or acetone, to a solution of isolated lipoproteins. An example of this is given in Fig. 5 and the similarity of this figure with the previous one is obvious.

It may be remarked here, that this is a way of bringing lipid-soluble drugs into an aqueous solution which can be used for pharmacological investigations and *in vitro* experiments where aqueous solutions are essential.

It was not surprising to find that the extent of incorporation of a compound into lipoproteins is inversely related to its polarity. The polarity of 29 compounds, mostly steroids, was measured as R_F on silica thin layer plates with the system acetone–water, (7:3, v/v). The most polar compound, dexamethasone, had an R_F value of 0·95 and the least polar compound, α-tocopherol, an R_F of 0·04.

Spearman's rank correlation coefficient between polarity and capacity for forming stable complexes with lipoproteins was $-0{\cdot}92$ ($P < 0{\cdot}001$). The question arose as to what the consequences were for the pharmacological activity of a steroid incorporated into lipoproteins. This was studied using testosterone undecanoate as an example. The compound was incorporated into lipoproteins from hog's serum and injected intravenously into immature castrated male rats [9].

The effects on the prostate, seminal vesicles and levator ani muscle were compared with those observed after subcutaneous injection of the same dose of testosterone undecanoate dissolved in arachis oil. Both routes of administration gave similar results (see Fig. 6), proving that incorporation of the steroid into lipoproteins does not seriously interfere with bioavailability and that pharmacological activity is maintained.

4. *Applications*

It has already been mentioned that information on the release characteristics of a drug-delivery system sometimes aids in the understanding of the pharmacological properties of a drug. This is illustrated by the following examples:

A. It was found, that the compound 6216, 11β-hydroxy-16α,17α,21-trimethyl-1,4-pregnadiene-3,20-dione, a steroid with anti-inflammatory activity and low systemic corticosteroid activity [10], did not increase liver glycogen in adrenalectomized rats after subcutaneous administration as a microcrystal suspension. After solubilization of the compound in plasma the same dose was active (see Fig. 7). This indicated that the lack of systemic activity of the compound is, at least in part, due to insufficient dissolution at the site of injection.

B. The steroid ester 2762, 3,5-estradiene-3,17β-diol-3,17β-diphenylpropionate, a very lipid-soluble compound, which is the 3-enol phenylpropionate of nandrolone phenylpropionate, was surprisingly found to have no androgenic and anabolic activity in the Hershberger test after subcutaneous administration in oil. This is shown in the upper part of Fig. 8, in which the results of an equivalent dose of nandrolone phenylpropionate are given for comparison. The

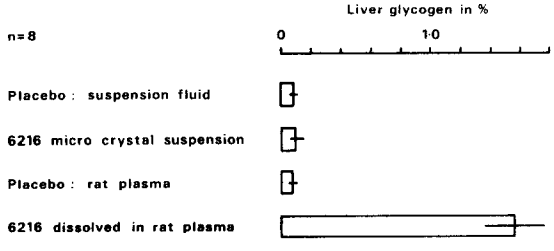


Fig. 7. The effect of dissolution on the corticosteroid activity of 11β-hydroxy-16α,17α,21-trimethyl-1,4-pregnadiene-3,20-dione in the liverglycogen test in rats.

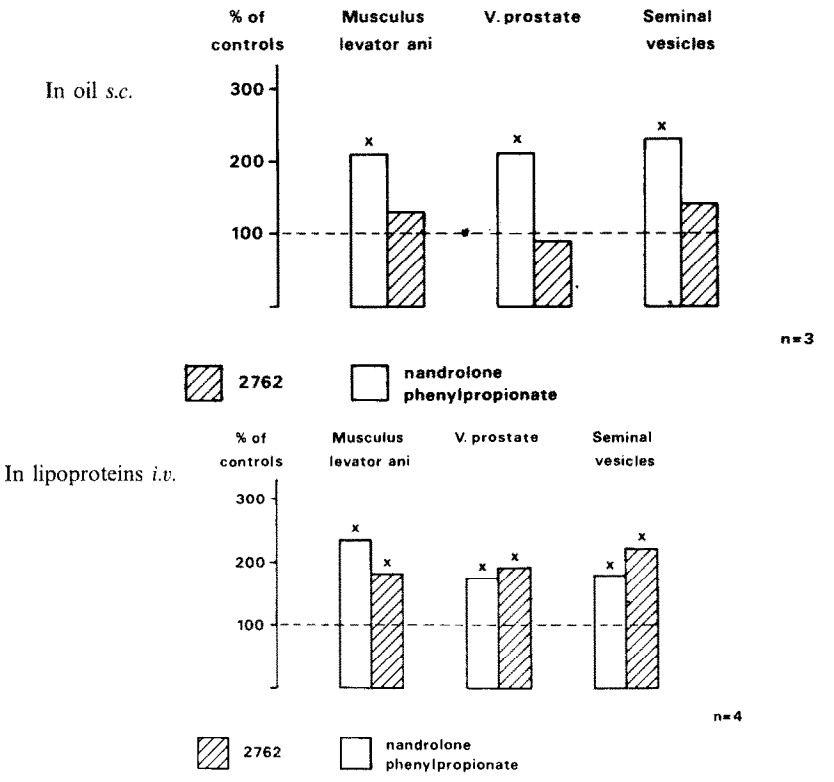


Fig. 8. The effect of dissolution on the androgenic and anabolic activity of 3,5-estradiene-3,17β-diol 3,17β-diphenylpropionate in immature castrated male rats.
X means statistically significantly different from controls.

half-life of the latter compound in an intramuscular depot in oil is known to be 25 h [1, 4]. After dissolution of the steroid esters in a solution of lipoproteins, equivalent doses were continuously infused intravenously into conscious rats during seven days with the help of a gradient diluting device which simulated an entrance of the drug into the body with a half-life of 25 h. From the lower part of Fig. 8 it is clear that under these conditions both esters are active and give similar results. This indicates that the high lipid-solubility of 2762 restricts absorption from the oil, thus preventing entrance of adequate amounts of the drug into the body; release from the depot appears to be the limiting factor for the pharmacological activity of this ester.

C. The last example deals with a compound already mentioned above, testosterone undecanoate. The presence of this drug in plasma lipoproteins after oral administration to rats has been mentioned (see Fig. 4).

It could be shown by Coert *et al.*[11] that part of the orally administered testosterone undecanoate is metabolized by the cells of the intestinal wall during the process of absorption, giving rise to the formation of a number of polar metabolites which are transported to the liver *via* the portal vein. Another part of the steroid is converted to dihydro-testosterone undecanoate and this, together with unaltered ester, is incorporated into chylomicrons which are released into the intestinal lymph. Because this lymph does not pass to the liver but to the peripheral circulation, the esters escape metabolism in the liver and can easily reach their target organs.

This explains why testosterone undecanoate is an androgen which is active after oral administration. If during absorption of the drug more fat were present more chylomicrons would be formed and this would facilitate absorption of the ester *via* the intestinal lymphatics. In agreement with this prediction it was found, that after oral administration of testosterone undecanoate dissolved in arachis oil, it was 1·85 (1·36–2·63) times as active as when the ester was administered in tablets not containing fat.

The results of these experiments in rats were recently complemented by studies in humans by Nieschlag *et al.*[12] who showed that in men the oral administration of testosterone undecanoate in oil increases the level of free testosterone in the plasma.

CONCLUSION

From the foregoing discussion it is clear, that the release of a drug from a delivery system and its transport by the plasma to the site of action are determined by the physicochemical properties of the drug. These properties are determined by the molecular configuration of the drug, as is the specific interaction

between drug and receptor, and are just as important for the resultant biological action.

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REFERENCES

1. Van Der Vies J.: *Acta endocr., Copenh.* **49** (1965) 271–282.
2. Hänselmann B. and Voigt R.: *Die Pharmazie* **26** (1971) 57–77.
3. Bederka J., Takamori A. E. and Miller J. W.: *Eur. J. Pharmac.* **15** (1971) 132–136.
4. Van Der Vies J.: *Acta endocr., Copenh.* **64** (1970) 656–669.
5. Kincl Fred S. and Rudel Harry W.: *Acta endocr., Copenh.* (1971) suppl. 151.
6. de Vries H.: unpublished results.
7. Huntjens F. J., van Aalten H. A. A. and Oldenhof H. B.: unpublished results.
8. Hobbelen P. M. J., Coert A., Geelen J. A. A. and Van Der Vies J.: *Biochem. Pharmac.* **24** (1975) 165–172.
9. Hershberger J. G., Shipley E. G. and Meyer R. K.: *Proc. Soc. exp. Biol. Med., N.Y.* **83** (1953) 175–180.
10. Woods G. F. *et al.* (1974) to be published.
11. Coert A., Geelen J. A. A., de Visser J. and Van Der Vies.: *Acta endocr., Copenh.* in the press.
12. Nieschlag E., Mauss J., Coert A. and Kićović T.: *Acta endocr., Copenh.* in the press.