SUMMARY.

The central theme of this thesis is the question whether the effects of the tetracyclines on animals and animal cells can be explained by their inhibitory effects on mitochondrial protein synthesis.

Chapter 1 gives a review of the main biological and chemical properties of the tetracyclines. These antibiotics interfere with protein synthesis in bacteria. Also mitochondrial protein synthesis is inhibited at low concentrations of these drugs. From the more than 25 derivatives known to date, we have been using those six which are most widely used in medicine: tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), demethylchlortetracycline (DMCT), methacycline (MC) and doxycycline (DC). From a pharmacological point of view the main differences concern the degree of lipid solubility of the derivatives and their affinity to divalent cations.

Chapter 1 further summarizes the present stage of our knowledge of the translation products of mitochondrial protein synthesis. Inhibition of this process leads to a decrease in the concentrations of the cytochromes *b*, *c*1 and *aa*3 and to the disappearance of a membrane factor of the respiratory chain-linked ATPase complex. Loss of this factor renders the ATPase oligomycin-insensitive.

A disturbance of mitochondrial biogenesis will provoke a decreased energy-generating capacity of the mitochondria, especially in rapidly growing tissues. Unless glycolysis can sufficiently compensate an energy-crisis will occur in the cells and tissues. This energy deficiency may well lead to a variety of secondary effects at the level of active transport or nucleic acid-, protein- en lipid biosynthesis.

Besides inhibition of mitochondrial protein synthesis, direct inhibition of cytoplasmic protein synthesis has been reported in the litterature. The concentrations neccessary to obtain the latter, however, are one to two orders of magnitude higher than those at which mitochondrial protein synthesis is blocked completely. At these high concentrations (> 25 µg/ml) it is very difficult to rule out other targets for the action of tetracyclines or their chelates.

Side effects of the tetracyclines are not often seen and rarely severe or alarming. Among the effects gastro-intestinal disturbances are the most frequent. In contrast to chloramphenicol, also an inhibitor of mitochondrial but not of cytoplasmic protein synthesis, the tetracyclines have no effect on bloodcell proliferation, they do not suppress bone marrow function.

Inhibition of mitochondrial protein synthesis will manifest itself in an altered composition of the mitochondria primarily in rapidly growing tissues. An energy deficiency at the cellular level may, therefore, become apparent especially in these tissues, as a decrease in all sorts of energy-dependent activities. If so, these effects have to be considered secondary.

The work described in this thesis was started as a continuation of earlier experiments (Kroon and de Vries, 1970). The aims of the investigation were to find a convenient target tissue and to test if *in vivo* sensitivities can be correlated to the inhibitions of either mitochondrial or cytoplasmic protein synthesis as determined *in vitro*.

Intestinal epithelium, a rapid proliferating tissue notably also under physiological conditions, seemed to fulfil the demands. In chapter 2 the main characteristics of this tissue are described. In this chapter attention is also paid to the available method for isolation of intestinal cell fractions by differential vibration. In chapter 3 the methods used are dealt with in detail. In chapter 4 the experiments carried out to characterize and optimalize the [^{14}C]leucine incorporation into protein by isolated intestinal cells are reviewed. From a morphological study it appeared that the isolation and incubation procedures have slightly destructive effects on the structure of the cells, but the cells incorporate leucine very actively.

In chapter 5 the *in vitro* inhibition of mitochondrial and cytoplasmic protein synthesis by tetracyclines is described. For the studies of mitochondrial protein synthesis rat-liver mitochondria were used. Cytoplasmic protein synthesis was studied in isolated intestinal cells, rabbit reticulocytes and reticulocyte lysates.

Mitochondrial protein synthesis is inhibited by low concentrations of the tetracyclines. Differences between the derivatives are not detectible, they all inhibit the incorporation for 50 % at about 3 µg/ml. An accumulation of the tetracyclines within the mitochondria, as has been described for bacteria, could not be detected. The inhibition is concentration-dependent and does not vary as a function of the tetracycline/protein ratio. The Mg^{2+}-concentration in the incubation medium does hardly affect the percentage of inhibition.

These data permit the following conclusions:

1. Differences in the rate of membrane permeation, possibly related to differences in lipid solubility of the derivatives do either not influence the inhibition or are abolished by differences in the affinity to ribosomes;
2. The affinity of tetracyclines for mitochondrial ribosomes is many times higher than the affinity for magnesium ions in solution;
3. Non-specific binding to cell components other than ribosomes must be weak or quantitatively unimportant.

Accordingly, the total intracellular concentration of the tetracyclines determines the inhibition of mitochondrial protein synthesis and not the "free" concentration. This leads to the conclusion that all tissues mitochondrial protein synthesis will be inhibited to the same extent if at least the intracellular concentration is equal to the plasma concentration (during a treatment with normal doses this concentration is 5 - 20 µg/ml).

With regard to cytoplasmic protein synthesis amino acid incorporation by reticulocyte lysates confirmed that this process is indeed inhibited by tetracyclines. The inhibition by DC is stronger than by OTC and the least by CTC. With intact reticulocytes DMCT inhibits slightly more than CTC, DC less, MC still a little less, and TC and OTC only very weak. The 50 % inhibitory concentration of DC is in both systems about 250 µg/ml (1,5 mM Mg^{2+}). CTC inhibits the leucine incorporation by intact reticulocytes much stronger than DC. These differences are most likely due to the fact that the plasma membrane of the reticulocytes acts as a barrier for some of the derivatives under the experimental conditions used. A comparison with intestinal cells might help explaining this point. With the epithelial cells

CTC inhibits as strong as DC. This shows that the plasma membrane is selective; a selectively which is not identical for all tissue. With intestinal epithelium DC and CTC inhibit to the same extent, DMCT less and TC, MC and OTC the least. Therefore, intestinal cells do differ from reticulocytes in sensitivity not only for CTC but also for DMCT and MC.

An important observation is further that the inhibition decreases if the Mg^{2+} concentration in the incubation medium is raised. This effect is most dramatic for DC and MC with reticulocytes. It is concluded that the decrease of the inhibition is caused by the inability of the tetracycline chelates to pass the plasma membrane. Extrapolating, this phenomenon may explain why effects of the tetracyclines on the rapidly proliferating cells in the bone marrow, as observed with chloramphenicol, have not been described, because of the strong binding of the tetracyclines to bone and to the extracellular divalent and trivalent cations the antibiotics do not enter the cells. The intracellular tetracycline concentration remains therefore far below the plasma concentration. Under these conditions mitochondrial protein synthesis will hardly be affected and cytoplasmic protein synthesis not at all.

From *in vivo* experiments we conclude that in intestinal epithelium mitochondrial protein synthesis is strongly inhibited but cytoplasmic protein synthesis is not during a OTC treatment (chapter 6). With regard to the other derivatives the following can be said in the light of the pharmacological data and of the results of chapter 5. TC will behave like OTC; both will inhibit cytoplasmic protein synthesis only slightly even if the concentration is raised far above 25 µg/ml. CTC and DMCT are more potent inhibitors and may affect cytoplasmic protein synthesis if the tissue concentration becomes higher than the therapeutic values. Also DC is *in vitro* a more potent inhibitor of cytoplasmic protein synthesis than OTC, but during a standard DC therapy the plasma and tissue concentrations remain relatively low.
In conclusion:

1. To prevent a direct inhibition of cytoplasmic protein synthesis in case of a therapy with tetracyclines, the plasma concentration should not exceed 25 µg/ml. This limit is not as stringent with TC and OTC as with DMCT, CTC, DC en MC. The limit should be adapted, however, in further search for the relation between plasma and tissue concentrations and the relation between the total and the "free" concentration of the antibiotics.
2. Since mitochondrial protein synthesis is very sensitive to inhibition by the tetracyclines, it is clear that *in vivo* also at low tissue concentrations mitochondrial protein synthesis will be affected. If a treatment with a tetracycline lasts longer than 5 days a diminished functioning of successively intestine and kidney will arise. The risks of such a diminished function should be considered at the start of a therapy.