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ANTIESTROGENS

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1. INTRODUCTION

During the past two decades considerable insight has been gained into the manner in which steroid hormones act on responsive target tissues. The availability of sophisticated *in vitro* and *in vivo* techniques for detection and measurement of minute amounts of steroids has made it possible to track hormone molecules within the target cell and thus unravel their mechanism of action. The discovery of intracellular receptor proteins for steroid hormones has led to the understanding of the mechanism of action of hormones as well as of their agonists and antagonists. An important related event is the emergence of substances known as "antiestrogens" which have great potential as therapeutic agents in diverse fields such as cancer and infertility.

2. DEFINITION

In a broad sense all agents capable of blocking estrogenic effects may be called antiestrogens. The expression of antagonism is specifically at molecular or biological level. There are no compounds which antagonise all the actions of estrogens. Almost all known 'antiestrogens' are at least weakly estrogenic in conventional tests when given alone and to understand fully the mechanism of action of antiestrogens one must explain why they are weak estrogens on the one hand, and on the other, why they prevent the action of estrogens.

The 'impeded estrogens' as typified by estriol behave as antagonists of the stronger and more physiological estrogens like estradiol, a phenomenon which suggests that a relatively weak estrogen may very likely interfere with the action of a stronger one by occupying receptor sites.

3. CHEMISTRY

All agents capable of blocking estrogens are antiestrogens. Progestogens, androgens, corticoids and even some weaker natural estrogens are antiestrogenic in that they inhibit uterotropic activity in experimental animals (Lerner, 1964). Of the newer steroids, those related to 19-nortestosterone are particularly potent as determined by a variety of tests including vaginal cornification. For example, 17-ethinyl-19-nortestosterone (Nilevar) is 70 times as potent as testosterone propionate as an antagonist of estrone induced uterine growth. Discussion of these steroids has not been included in this review, since they are more likely to be used in therapeutic practice for properties other than their antiestrogenic activity.

In the continuing search for an orally effective non-steroidal contraceptive, several compounds have been investigated for their potential as antiestrogens. One such compound, clomiphene citrate, is now marketed as an agent for induction of ovulation.

These potential compounds cover a wide range of structural series namely ethanes (MER-25), ethylenes (clomiphene ON 55945-27), butenes (ICI 46474, 47699) indenes

(U-11555A), dihydronaphthalenes (U-10520, U-11100A), tetrahydronaphthalenes and stilbenes (DMS). Of these, Tamoxifen (ICI 46474), Nafoxidine (U-11100A) Centchroman (67/20 chroman) and Clomiphene (MRL-41) have been dealt with in some detail in the subsequent sections of this review.

4. METHODS OF STUDY AND EVALUATION

Various *in vivo* and *in vitro* techniques have been proposed for study of the different estrogen sensitive biochemical parameters in target tissues for assessment of estrogenicity or antiestrogenicity of test compounds (Sankaran and Prasad, 1972). The results of these studies show variations according to the type of compound, the species of animals used and the particular parameters studied (Dorfman, 1962; Emmens, Cox and Martin, 1962; Lerner, 1964; Emmens, 1970).

Emmens *et al.* (1962) reviewed the techniques in experimental animals including tests using mitosis and epithelial thickness after intravaginal application, tests of tetrazolium reduction, deciduoma formation in rats and effects on uterus and pregnancy in mated animals. The availability of radioactive labeled hormones and substrates of high specific activity and automated instrumentation for measurements of minute quantities of hormones have made possible the measurement of receptor levels in different tissues and the purification of receptor proteins (Clark *et al.*, 1973; Jensen and DeSombre, 1972; O'Malley and Means, 1974). The biochemical changes which occur at the molecular level in different tissues under the influence of hormones or their antagonists can thus be studied either *in vitro* or *in vivo*. Significant species variation has been observed in target tissue response to estrogens and antiestrogens making it hazardous to predict therapeutic activity in the human by extrapolation of effects in experimental animals to humans.

Development of suitable *in vitro* techniques has made a significant contribution to advancement of knowledge in this field particularly as regards physiological and pathological processes in the human. For example Jensen *et al.* (1975) reported that the estrogen receptor content of excised specimens of human breast cancers could be used to predict response to endocrine ablation. Since then studies of estrogen receptors and more recently of progesterone receptors in breast cancer are being carried out in many laboratories with a view to predict responses to chemical and hormonal agents.

5. MODE OF ACTION

Theoretically, the biological response of a specific tissue to estrogens can be altered in several ways. The antagonist when administered *in vivo* or *in vitro* may compete with estrogen for reactive sites, inactivate estrogen for a substrate or a specific nutrient (Lerner, 1964).

Considerable evidence has accumulated in recent years to suggest that the binding of steroid hormone to a specific receptor protein is an early step in a series of biochemical events. Thus, the steroid enters the cell by diffusion, binds spontaneously to the cytoplasmic receptor and then this hormone receptor complex is transported to the cell nucleus. After the entry of the steroid receptor complex into the nuclear compartment the molecular interaction of the steroid receptor complex with chromatin is followed by steroid mediated alterations which allow RNA polymerase to transcribe certain previously repressed gene sites which then leads to cytoplasmic protein synthesis (Leung *et al.*, 1973; O'Malley and Means, 1974).

The action of non-steroidal antiestrogens is dependent on their ability to compete for cytoplasmic estrogen binding sites or receptors thus reducing the formation of receptor estrogen complexes leading to a decreased physiological response to estrogen. Recent developments suggest that the antiestrogenic action is often based on depletion or failure of replenishment of cytoplasmic receptors (Clark *et al.*, 1976; Jordan and Koerner, 1975). It has been stated earlier that these compounds are both agonistic and antagonistic. They are agonists because they stimulate the metabolic and regulatory pathways that cause

uterine growth. It is noted that antiestrogens promote uterotrophic activity by stimulating cell growth as well as hyperplasia since the increase in uterine protein, DNA and weight induced by the antagonists equal those induced by estradiol. A review by Segal and Koide (1979) may be consulted for more details and references regarding the biochemical pharmacology of estrogens.

6. CLINICAL USE

A number of antiestrogens have been used for clinical trials in the human in the last decade. It is proposed to discuss in this presentation four of these compounds about which a substantial number of reports have appeared. Several large multicentred trials on these drugs are in progress and hence the precise scope for the use of these agents is still to be delineated. Though originally many of them were developed for exploring their use as an antifertility agent and currently a very important ovulation inducing agent and tamoxifene, now undergoing clinical trials for palliation of advanced breast cancer.

6.1. Centchroman

(67/20 CDRI) is a chroman derivative which is estrogenic at low doses and is an effective antifertility agent (Kamboj *et al.*, 1971) and antiestrogenic in high doses (Kamboj *et al.*, 1973). Despite the reported uterotrophic activity of this compound it has failed to support the process of nidation (Singh *et al.*, 1973). This anti-implantation effect cannot be prevented by concomitant administration of progesterone (Kamboj *et al.*, 1977; Steinetz *et al.*, 1976). Studies on the relative binding affinity of chromans and chromones to rat uterine cytosol estrogen receptors have thrown light on their mode of action. Their relative binding ability seems to be dependent on their molecular configuration and shows a reasonable correlation with their anti-implantation activity. Such a relationship between the estrogen receptor binding affinity and the anti-implantation activity has also been demonstrated with certain phenolic steroids (Muller and Wotiz, 1977).

Roy *et al.* (1979) reported the results of systematic studies in the human carried out for further elucidation of its possible gonadotropin modulating, ovulation inducing and luteolytic effects. This compound has been extensively studied for contraceptive efficacy in the sub-human primates by Kamboj and co-workers (1971 and 1973).

Limited trials in the human using post coital administration of 60 mgm and a once a week schedule of 45–125 mgm have been carried out for evaluation of its contraceptive efficacy. Though contraceptive efficacy was reported to be satisfactory menstrual irregularities were common (Kamboj, 1979). The drug has properties similar to clomiphene since it releases FSH and LH from the pituitary by an effect similar action both in the male and the female (Vaidya *et al.*, 1976, 1977).

6.2. Clomiphene Citrate

At the present time this is the most successful single agent available for the induction of ovulation. It is an analogue of chlorotrianisine and is structurally related to the synthetic estrogen stilbestrol. The commercially marketed preparation consists of a 1:1 mixture of the *cis* and *trans* forms. The *cis* isomer is more potent.

Clomiphene has been studied in a number of biological systems in order to define its action as an estrogen antagonist. Schulz *et al.* (1973) showed that 1 hr after the injection of labeled clomiphene, radioactivity could be detected in the hypothalamus, pituitary, ovaries and uterus of newborn guinea pigs. When the injection of radioactive clomiphene was followed by administration of tritiated 17-beta estradiol within 1 hr the incorporation of estradiol into the uterus was markedly decreased showing thereby that clomiphene competes with natural estrogen at receptor sites. This effect is dose dependent but

at extremely high doses the inherent estrogenicity is such that the overall biological effect may resemble that of natural estrogen.

In the human, when used for the induction of ovulation, it competes for estrogen receptor sites in the hypothalamus leading to release of gonadotrophin releasing hormones. Secretion of FSH and LH follows. This stimulates the growth of ovarian follicles. The binding to the estrogen receptors is a time limited process as shown by elimination of 94% orally administered labeled clomiphene within 5 days (Kistner, 1968).

Available data on endocrinologic profiles of women in whom clomiphene has been used for induction of ovulation supports the view that the effect of clomiphene is mediated through the hypothalamo-pituitary axis (Greenblatt, 1966; Pennington, 1969; Charles *et al.*, 1969). There is a significant elevation of FSH and/or LH following the administration of clomiphene in responsive patients and this is followed by an increase in estrogen levels (Newton and Dixon, 1971).

6.2.1. *Dose and Duration of Treatment*

Clomiphene citrate is administered orally as 50 mgm tablets in short courses of 5–7 days, the daily dose being 50–150 mgms. Combined therapy with HCG or estrogen is recommended if ovulation is not induced at doses ranging from 100 to 150 mgms per day. Treatment is given generally for six cycles and since conception is reported to occur after discontinuation of therapy in a significant proportion of patients treatment can be given on alternate cycles (Murray and Osmond Clarke, 1971). Details of combined therapy and monitoring during treatment are discussed by Taymor *et al.*, 1973; Rabau *et al.*, 1971; Greenblatt and Dalla Pria, 1971; Zourias, 1973; Insler and Lunenfeld (1974).

6.2.2. *Results of Treatment*

Several investigators have reported the results of treatment with clomiphene for induction of ovulation. The efficacy is usually estimated in terms of ovulation and pregnancy rates. Lunenfeld and Insler (1978) analysed the results obtained from a total of 7817 patients compiled from eleven reports and observed ovulation rates ranging from 60 to 96%. Most authors reported a 70% induction of ovulation. The highest pregnancy rate was only 45.9%. Several explanations have been put forward for this discrepancy i.e. cervical mucus hostility (Insler *et al.*, 1973; Figuerora Casas *et al.*, 1970), luteal phase deficiency (Seegar Jones *et al.*, 1970) or abnormal tubal transport (Whitelaw *et al.*, 1970). Pregnancy rates have been improved by combining clomiphene with estrogens or HCG or both. Proper selection of patients, good monitoring and judicious use of combined therapy can improve pregnancy rates up to 60 to 70%.

6.2.3. *Side Effects*

Hot flushes not ameliorated by concomitant use of estrogens are seen in 10% of patients (Greenblatt, 1966). Nausea, vomiting, breast discomfort, mild visual disturbances and mild abdominal or pelvic discomfort are observed in 1–2% of women and these are reversible on cessation of therapy. The problems related to induction of ovulation are hyperstimulation, increased abortion rates and higher incidence of multiple pregnancies. Severe degrees of hyperstimulation are rarely observed and the length of therapy as well as the dosage are important (Kistner, 1968). An abortion rate of 20–25% in clomiphene induced pregnancies has been reported by most workers (MacGregor *et al.*, 1968; Rust *et al.*, 1974; Rabau *et al.*, 1967). The use of progestational agents following confirmation of pregnancy to reduce abortion rates is recommended. The multiple pregnancy rate in clomiphene induced pregnancies is about eight times higher than the normal incidence (Kistner, 1968; Hack *et al.*, 1972; Greenblatt and Dalla Pria, 1971).

6.2.4. *Other Indications*

Other indications for clomiphene therapy are the treatment of metropathia haemorrhagica (Murray and Osmond Clarke, 1971), evaluation of pituitary reserve (Newton and Dixon, 1971) and oligospermia due to hypothalamic failure (Lunenfeld and Insler, 1978). Clomiphene has been used at doses of 100–300 mgms per day for sixty days for the treatment of advanced breast cancer. Legha and Carter (1976) reported a response of 28% (47 out of 167 cases) in subjects with breast cancer. The prolonged use of the drug results in increased side effects in these patients i.e. blurring of vision in 7% of cases. Hence it would appear that agents with less side effects would be preferred.

6.3. Tamoxifene

This is a trans isomer of 1-(p-dimethyl-amino-eltroxy-phenyl)-1,-2-diphenyl-2-ethyl-ethylene (ICI 46474) synthesised in 1963). The *cis* isomer acts like a conventional estrogen. In experimental animals it was observed that tamoxifene inhibited or reversed the growth of some chemically induced tumors in rats and decreased the frequency of tumor development when administered concomitantly with the carcinogenic agent DMBA (Harper and Walpole, 1967; Latsetwar, 1970). Tamoxifene inhibited cellular reproduction when added to the culture medium of tumors containing estrogen receptors in *in vitro* studies but had little or no effect in the absence of estrogen receptors. The mechanism of its antiestrogenic as well as its antitumor activity is probably related to competitive attachment to receptor proteins.

6.3.1. *Dosage and Duration of Treatment*

Tamoxifene when used for the treatment of cancer is administered initially at doses of 10 mgm twice daily for a month. The dose is increased to 20 mgm twice daily if no response is observed (Heel *et al.*, 1978).

6.3.2. *Clinical Studies*

The first clinical studies with Tamoxifene were reported in the early part of this decade (Klopper and Heel, 1971; Williamson and Ellis, 1973). Cole *et al.* (1971) presented the first report on its use in advanced breast cancer and currently the major use of this drug is for this indication. Most trials report evidence of regression in one third of the patients treated (Legha and Carter, 1976; Tormey *et al.*, 1976; Lerner *et al.*, 1976; Kiang and Kennedy, 1977; Willis *et al.*, 1977). Postmenopausal patients fare better although the response in patients up to five years after menopause appears to be the same as in patients treated further beyond menopause (Morgan *et al.*, 1976). In a comparative study with a cytotoxic regimen, comparable effective response rates were seen in postmenopausal women with predominantly soft tissue involvement. As with other hormonal treatment visceral metastases responded less frequently than soft tissues, skin or bone involvement. The estrogen receptor assay has proved of value in the selection of patients and as a predictor of response to treatment (McGuire *et al.*, 1975). Though the correlation is not absolute less than 10% of receptor negative patients respond to any form of endocrine therapy.

6.3.3. *Side Effects*

The drug is usually well tolerated and overall incidence of withdrawal from treatment due to adverse effects is less than 3% (Heel *et al.*, 1978). Side effects are mild and are in the form of hot flushes, pruritus, nausea or vomiting. Transient haematological changes like thrombocytopenia, mild leucopenia and hypercalcaemia have been occasionally noted. Retinopathy is observed as a side effect in patients given more than 60 mgms of tamoxifene per square meter for over one year (Kaiser-Kupfer and Lippman, 1978).

If the duration of remission is satisfactory, this agent may prove to be superior to other forms of chemotherapy for advanced breast cancer because of the remarkable absence of serious side effects. There seems to be scope for investigating this agent in combination with other chemotherapeutic or cytotoxic drugs and also as an adjuvant with surgical treatment.

6.4. NAFOXIDINE HYDROCHLORIDE

1-(2-(p-(3,4-Dihydro-6-methoxy-3-phenyl-1-naphthyl)phenoxy)-ethyl)-Pyrrolidine or U-11100A, a representative of a series of dihydronaphthalenes which showed antifertility effects in the female rat, rabbit and guinea pig (Duncan *et al.*, 1962, 1965) but did not have the desired efficacy in monkeys or man (Morris *et al.*, 1967). Nafoxidine is an antiestrogen since it competes for estrogen receptors in the cytoplasma of target tissues (Rochefort *et al.*, 1972). Further, Clark and co-workers (1973) after carrying out studies *in vivo* found that nafoxidine treatment results in both the translocation and atypical long term retention of the estrogen receptor by the nuclear fraction. Thus the antiestrogenic effect of nafoxidine is the result of failure to stimulate the replenishment of cytoplasmic receptor with subsequent reduction in the ability of the tissue to bind estrogen.

6.4.1. *Dosage and Duration of Treatment*

The drug is given in doses of 60 mgms. three times daily for at least six weeks and continued indefinitely in responsive patients.

6.4.2. *Clinical Studies*

Clinical trials of nafoxidine for the treatment of breast cancer were initiated in 1969 by the European Organisation for Research on Treatment of Cancer (EORTC). Heuson *et al.* (1975) reported, as a result of these studies that objective remissions occurred in 30 out of 108 cases indicating 28% response rate which was distributed equally among patients with visceral, soft tissue and osseous lesions. Most patients were post menopausal and in good general health. The cumulative data for all published clinical trials of nafoxidine show a response rate of 31% (Bloom and Boesen, 1974; Heuson *et al.*, 1972). When studies correlating response to nafoxidine with estrogen receptor values (ER) were analysed, there was a positive response in 70% of the patients in 17 ER positive cases. None of the 16 ER negative cases responded (Engelsman *et al.*, 1973).

6.4.3. *Side Effects*

The most common side effects are dermatologic; photosensitivity or ichthyosis of varying degrees have been observed in most patients after 4 to 8 weeks of treatment (Bloom and Boesen, 1974).

7. CONCLUSIONS

Antiestrogens are steroidal or nonsteroidal agents which antagonise the action of estrogens on target tissues by their action at the molecular or biological level. Several nonsteroidal compounds with predominantly antiestrogenic and weak estrogenic action have been investigated in the past decade. Four of these which have been investigated in humans are centchroman, clomiphene, nafoxidine and tamoxifene. These compounds act by binding to cytoplasmic estrogen receptor proteins. The receptor protein complex on entry into the nuclear compartment of target cells induces changes in the chromatin that allows RNA polymerase to transcribe certain previously repressed gene sites for protein synthesis. Biological studies with the compounds show wide species variations in their effect on various reproductive processes. These agents have been used in experimental

work to uncover certain fundamental features of estrogen action at molecular level. Though originally developed as antifertility agents these compounds have shown promising application in such areas as ovulation induction, promotion of spermatogenesis and palliation of estrogen dependent cancers.

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BREAKDOWN AND FATE OF ACTH AND MSH

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1. INTRODUCTION

It is well established that adrenocorticotropic hormone (ACTH) and melanocyte stimulating hormone (MSH) have short biological half-lives. However, little is known about the sites and mechanisms responsible for the rapid inactivation of these hormones. Interest in their metabolism has been stimulated recently by numerous studies showing that hypothalamic and pituitary peptides may have direct action on the central nervous system. Thus, in addition to the classical role of ACTH in steroidogenesis and that of MSH in pigmentation, evidence has accumulated that these hormones can act on the brain to produce specific behavioral responses (De Wied, 1977a; De Wied and Gispen, 1977; De Wied, 1977b; Kastin *et al.*, 1979).

In view of the presence in brain of immunoreactive ACTH (Krieger *et al.*, 1977; Moldow and Yalow, 1978; Watson *et al.*, 1978; Pelletier and Leclerc, 1979) and MSH (Barnea *et al.*, 1977; Oliver and Porter, 1978; Parker and Porter, 1979), and the potent CNS activities of various ACTH and MSH fragments, the metabolism of ACTH/MSH peptides in brain is of special interest.

This article is aimed at reviewing the present knowledge of the fate and degradation of ACTH/MSH peptides. The available information on breakdown of MSH is very limited. Although the literature on ACTH degradation is more extensive, it still reflects the paucity of information that exists, in general, concerning the mechanism of protein and peptide degradation and its regulation, in contrast to our understanding of processes involved in protein synthesis.

2. THE HALF-LIFE OF ACTH IN THE CIRCULATION

Studies on the circulatory half-life ($t_{\frac{1}{2}}$) of ACTH have been concerned with both exogenous and endogenous hormone. Clearance of exogenous ACTH has been studied by intravenously injecting or infusing ACTH and measuring the decay of the radioactivity, bioactivity or immunoreactivity of the hormone in the blood. In order to measure the rate of disappearance of endogenous ACTH from the blood, it is necessary to raise ACTH levels by stress, adrenalectomy or drugs, and subsequently to abolish or suppress pituitary ACTH secretion by hypophysectomy or corticosteroid administration.

2.1. STUDIES ON THE EXOGENOUSLY ADMINISTERED HORMONE

When ACTH is administered by intravenous injection or infusion its disappearance from the plasma is rapid. Inactivation of ACTH also occurs in blood *in vitro*, but at a much slower rate than *in vivo* (Richard and Sayers, 1951; Snydor and Sayers, 1953; Besser *et al.*, 1971; McMartin and Peters, 1975). Imura *et al.* (1967) showed that native ACTH and ACTH-(1–39) are stable in fresh plasma, but inactivated slowly by stored plasma. From the order of decreasing stability, ACTH-(1–26)>-(1–19)>-(1–18) and ACTH-(1–18)NH_2>-(1–18), they concluded that the chain length of the C-terminal portion of the ACTH molecule is an important factor in protecting ACTH from inactivation in plasma.

These findings indicate that ACTH degradation takes place in one or more tissue compartments rather than in plasma itself. Such a mechanism would be in agreement with the biphasic circulatory decay rates reported by a number of investigators (Cats and Kassenaar, 1957a; Meakin *et al.*, 1959; Murphy *et al.*, 1969; Matsuyama *et al.*, 1972; McMartin and Peters, 1975; Liotta *et al.*, 1978; Normand and Lalonde, 1979). The initial decline in ACTH levels would then be associated with the equilibrium between plasma and various tissue spaces, with subsequent metabolism taking place in the latter compartments. From experiments on the tissue distribution of radioactive ACTH, kidney, liver, muscle and adipose tissue have been suggested as sites of ACTH penetration (Nicholson *et al.*, 1978). Kidney seems to be especially active in the removal of the hormone from plasma (Richard and Sayers, 1951). Later, Cats and Kassenaar (1957b) also showed that the largest percentage of an injected dose of ^{131}I labeled ACTH becomes localized in this organ. Skeletal muscle also may provide a site for ACTH accumulation (Hudson and McMartin, 1978).

The finding that ACTH initially enters a space larger than the plasma volume indicates (Cats and Kassenaar, 1957b) that half-life times from observations done some minutes after injection (Greenspan *et al.*, 1950; Gemzell *et al.*, 1951; Richard and Sayers, 1951) may be inaccurate since ACTH has not been fully distributed at that time. The fall in the level of ACTH in the first minutes can be ascribed to the quantity accumulated in the 'hormone space', in particular the kidneys (see also Nicholson *et al.*, 1978).

Approximate equilibration of distribution can be obtained by infusing the hormone for a period of time (McMartin and Peters, 1975) as opposed to a single injection. In rats, the rapid phase of decay of bioactivity after a 20 min infusion of ACTH-(1–24) and -(1–39) had a $t_{\frac{1}{2}}$ of a few min (McMartin and Peters, 1975). Longer half-lives in the order of 4–18 min have been found for the rapid phase of clearance of bioactive ACTH after intravenous infusion for periods of 2–8 hr in humans (Meakin *et al.*, 1959). It has been suggested that the slower phase of clearance after infusion results from slow release of peptide from binding sites which act as a depot (McMartin and Peters, 1975). A mathematical model of the two compartment system has recently been presented in which the size of initial hormone space was estimated for the rat (Normand and Leland, 1979). This was about 60 per cent larger than the plasma value and was approximately equal to the total blood volume.

When corrections are made for the initial distribution of the injected ACTH, some estimate of the rate of degradation can be obtained. However, half-lives are still short when the second, slower decay component of the disappearance is used. Thus, Matsuyama *et al.* (1972) found a biological $t_{\frac{1}{2}}$ of 2.9 min for rats, and Murphy *et al.* (1969) reported a $t_{\frac{1}{2}}$ of 13 min for the disappearance of immunoprecipitable ^{131}I-labeled ACTH-(1–39) from pig plasma. In humans, a plasma $t_{\frac{1}{2}}$ of 7 min has been reported for ^{131}I-labeled ACTH-(1–24) assayed by paper electrophoresis (Wolf *et al.*, 1965). In a more recent study in humans, half-lives of 17–31 min have been calculated from the plasma volume and metabolic clearance rate of immunoassayable ACTH (Liotta *et al.*, 1978). A similar calculation for the clearance of immunoprecipitable [^{125}I]ACTH in pregnant sheep gave a $t_{\frac{1}{2}}$ of only 1 min (Jones *et al.*, 1975). It is difficult to compare these reported half-lives since variables are involved such as species, nature of injected peptides, methods used for assaying ACTH, and procedures for calculating half-lives from disappearance curves. For instance, simultaneous measurements of bioactivity and immunoactivity showed relatively longer half-times for the decay of radioimmunoassayable ACTH, suggesting that during the metabolism of ACTH, fragments arise in circulating plasma which are biologically inert but immunologically active (Matsuyama *et al.*, 1972; Nicholson *et al.*, 1978).

2.2. ENDOGENOUS ACTH

A number of studies have been conducted on the inactivation of endogenous ACTH. Sydnor and Sayers (1953) studied the decline in ACTH levels in plasma following hypo-

physectomy in rats in which the plasma levels had been raised by bilateral adrenalectomy and found biological half-times of 0.95 and 1.25 min 1 and 2 weeks after adrenalectomy, respectively. In a similar experiment, using both adrenalectomy and stress to raise ACTH levels in rats, Matsuyama *et al.* (1972) found half-times of 1.7 min and 3.6 min for bioassayable and radioimmunoassayable ACTH, respectively. In stressed intact rats, ACTH levels were still measurable by radioimmunoassay, indicating a $t_{\frac{1}{2}}$ of 4.1 min (Matsuyama *et al.*, 1972).

Slower rates have been found after suppressing the secretion of pituitary ACTH by corticosteroids. However, these rates are likely to be underestimated, since suppression of ACTH secretion is not always complete. Thus, following the abrupt termination of the hypoglycemic stimulus to ACTH secretion in pigs by the intravenous injection of glucose and dexamethasone, Murphy *et al.* (1969) observed a $t_{\frac{1}{2}}$ of 7 min using a radioimmunoassay. This is in agreement with half-lives of 10–15 min reported for endogenous ACTH in adrenalectomized humans with Cushing's disease during suppression by hydrocortisone (Yalow *et al.*, 1964). In an adrenalectomized subject, suppressed by cortisol, and in a normal subject in whom initially elevated plasma ACTH was lowered by dexamethasone, half-times of 22 and 30 min respectively were found (Berson and Yalow, 1968). In Addison's patients with adrenal insufficiency, Tanaka *et al.* (1978) reported an average $t_{\frac{1}{2}}$ of 40 min for immunoassayable ACTH and 83 min for β-MSH. These lower rates in humans may be explained in part by differences in metabolism between large and small animals. Similar differences were found in comparable infusion experiments in humans (Meakin *et al.*, 1959) and rats (McMartin and Peters, 1975).

3. METABOLIC FATE OF ADMINISTERED ACTH

Questions about its disposition are intimately connected with considerations about the physiology of the hormone. Degradation clearly is not merely a process of inactivation. Especially in connection with ACTH, where many partial structures are known to have biological activity, specific pathways of degradation could modulate the various activities of the hormone and selectively influence sites of activity.

Some examples of differential effects of ACTH-fragments have been reported. Although *in vivo* ACTH-(1–24) and -(1–39) have a similar steroidogenic potency, -(1–24) is more potent than -(1–39) in isolated adrenal cells (Bennett *et al.*, 1974). Indeed, after a 20 min infusion of ACTH-(1–24) lower levels and a more rapid decline of blood levels of bioactive ACTH were found than after infusion with -(1–39) (McMartin and Peters, 1975), suggesting that -(1–24) may also be more potent at the *in vivo* receptor than is the larger fragment. The high potency and prolonged action *in vivo* of (D-Ser^1, Lys^{17}, Lys^{18}) ACTH-(1–18)-octadecapeptideamide (intermediate potency in the isolated cell assay) are also in agreement with the higher levels and longer $t_{\frac{1}{2}}$ after infusion. The shorter plasma $t_{\frac{1}{2}}$ of ACTH-(1–24) as compared with -(1–39) may be explained by the finding that a large range of circulating products have been found in plasma after intravenous injection of tritiated ACTH-(1–24) indicating extensive cleavage at the N- and C-terminus (Hudson *et al.*, 1977), whereas the cleavage of the larger peptide is confined to the N-terminus resulting in ACTH-(3–39) as the major metabolite in plasma (Hudson *et al.*, 1979). These results with ACTH-(1–39) are in agreement with evidence presented by Nicholson *et al.* (1978) suggesting that the great reduction in biological activity of circulating ACTH with no significant loss of immunoreactivity observed after intravenous injection of C^3H_3-methylated ACTH-(1–39) could be due to the removal of either one, or a few, N-terminal residues.

After ACTH administration the various metabolic products of degradation also appear to be unequally distributed among various tissue spaces. Analysis of peripheral tissue using high pressure liquid chromatography showed that 1 min after injection of tritiated ACTH-(1–24), the peptide in the liver and kidneys consisted almost entirely of intact-(1–24); many fragments which appear in the circulation after 2 min are present in skeletal muscle at earlier times suggesting that peripheral tissues such as muscle may be

responsible for the generation of the fragments which circulate (Hudson and McMartin, 1978). At longer times after injection of tritiated ACTH-(1–24), Baker *et al.* (1976) found labeled peptide fragments also in the kidneys. The level of radioactive peptides in the kidney was higher after injection of [^{3}H-Phe^7]ACTH-(1–24) than after that of [^{3}H-Tyr^2]ACTH-(1–24) or [^{3}H-Tyr^{23}]ACTH-(1–24) indicating a rapid cleavage of the N- and C-terminus in agreement with results from analysis of circulating fragments (Hudson *et al.*, 1977). The absence of appreciable C-terminal cleavage of ACTH-(1–39) may indicate a conformational protective effect of the 25–39 sequence.

4. METABOLISM WITHIN TISSUES

Although it is evident that ACTH has a short biological half-life (Section 2), no particular organ or tissue seems to be responsible for its inactivation. Everson and Dobson (1968) showed that the rapid inactivation of physiologically active ACTH cannot be accounted for by degradation in any of the following systems alone: adrenals, liver, kidney, intestine and blood. Inactivation, rather, seems to occur in many tissues in such a way that significant arterial–venous differences in any single organ are not apparent. Metabolism of ACTH within tissues has been studied from various angles depending on the aims of the different investigations. The intestine is of particular interest in view of the high oral dosages of ACTH required to cause steroidogenesis in humans. Obviously, the pituitary, where ACTH is produced and stored, and the adrenals, the classical target organ for ACTH, have been studied in greater detail. Finally, various studies have been concerned with the degradation of ACTH within the brain, since the action of brain peptidases may lead to the formation and inactivation of a variety of ACTH fragments, many of which have been shown to exert potent behavioral activities (De Wied, 1977a).

4.1. INTESTINE

A detailed study on ACTH metabolism in rat intestine was carried out by Lowry and McMartin (1974). Amino acid release and the formation of peptide intermediates were measured using gel filtration and ion-exchange chromatography. When ACTH-(1–24) was administered by stomach tube, no breakdown was found to occur in the stomach, however ACTH-(1–24) was not detected in the contents of the small intestine. This, and the rapid hydrolysis by gut segments and intestinal juice *in vitro* suggest that ACTH-(1–24) is rapidly broken down as soon as it leaves the stomach. Upon incubation of ACTH-(1–24) with intestinal juice, large amounts of free Phe and Arg were found. The absence of peptide fragments containing intact Phe^7-Arg^8 suggests that this is the first bond to break followed by the cleavage of Arg^8-Trp^9 and His^6-Phe^7 bonds to release free amino acids. Although some hydrolysis occurred at the C-terminus and in Lys and Arg containing regions (15–17, 20–22), the extensive breakdown expected from known intestinal peptidases (trypsin, chymotrypsin, carboxypeptidases) did not occur. Digestion with washed everted small intestine resulted in appreciable attack at the C-terminus, in addition to the cleavage of the Phe^7-Arg^8 bond. The N-terminal degradation was greatly inhibited by the introduction of D-Ser at position 1 in (D-Ser^1, Lys^{17}, Lys^{18})ACTH-(1–18)-amide. Thus, both ACTH-(1–24) and the D serine containing analog are cleaved rapidly in the small intestine at a few specific sites, which may explain the high oral dosages required to produce steroidogenesis compared with subcutaneous or intravenous administration.

4.2. ADRENALS

Studies on ACTH breakdown by adrenal cell suspensions were prompted by the question as to whether potency differences between ACTH analogs in producing steroidogenic response are related to their degradation rates in this assay system (Giordano and Sayers, 1971). Bennett *et al.* (1974) separated peptide fragments from incubations of