conditions reported in Figure 1. Washout experiments (after 60 min preincubation with the labelled compound tissues were transferred to fresh medium) showed a relatively slow rate of loss of either total radioactivity and [^{3}H]-NA (the latter declined linearly with a $T_{½}$ of about 4 h from tissue preincubated with the catecholamine at 25 ng/ml), while 95% of the radioactivity was lost within 5 min from tissues preincubated with [^{14}C]-sorbitol. The effect of 5C3MP pretreatment in washout experiments was of questionable significance.

It is concluded that hypersensitivity following pretreatment with antilipolytic agents does not depend on major changes in the disposition of noradrenaline by the isolated fat pad.

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The effect of cyproterone acetate on enzymic steps in the biosynthesis of steroid hormones

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Testosterone exerts its action upon the testis, on the male secondary sex characteristics, on the trophism of the male accessory sexual glands, prostate and seminal vesicles, and by a negative feedback on the hypothalamo-pituitary axis. These androgenic effects have been shown experimentally to be antagonized by the administration of cyproterone acetate (CA) to the intact animal.

Goslar, Mehring & Neumann (1970) reported that CA enlarges the interstitial cell complex of the testis and increases 3β-hydroxysteroid dehydrogenase activity in the rat, suggesting increased androgen production. However, decreased testosterone formation from rat testicular incubates containing CA was reported by Sommerville, Bottiglioni, Collins & Neumann (1969). Similarly, experiments by Engel & Karsznia (1971) showed that, when rat testicular tissue was incubated in the presence of CA, the production rate of testosterone from pregnenolone was lowered. Recent work in our laboratories (Grants & Stitch, 1972) has shown that CA may mediate at least some of its anti-androgenic effects by blockage of enzymatic steps in the biosynthesis of testosterone.

Samples for analysis were taken every 20 min during a 3 h incubation of tritium-labelled androgen precursors of high specific activity with testicular minces from adult rabbits. Intermediates in the biosynthesis of androgen were characterized after the addition of ^{14}C-labelled compounds and purification to constant isotopic ratios. These experiments demonstrated that CA impairs the Δ^4 pathway of androgen biosynthesis in the rabbit testis. The transformation of [^{3}H]-pregnenolone to [^{3}H]-testosterone and [^{3}H]-androstenedione is almost completely blocked in the presence of CA (25 μg/100 ml incubation medium). The cumulative yield of testosterone formed from pregnenolone at 130 min reached a maximum in the presence of CA of 0.005% of the precursor, whereas in control incubations without CA the conversion amounted to 0.035%. Further examination of the Δ^4 pathway of steroid biosynthesis has shown that CA at a concentration of 0.1 μg/100 ml of incubation medium markedly impairs 3β-ol dehydrogenase activity required for the transformation of [^{3}H]-pregnenolone to [^{3}H]-progesterone. The conversion of [^{3}H]-pregnenolone to [^{3}H]-17-α-hydroxyprogesterone and the side-chain cleavage of 17-α-hydroxyprogesterone to androstenedione appear to be inhibited in the presence of CA. When [^{3}H]-pregnenolone is incubated with rabbit testicular minces in the presence of CA, the formation of dehydroepiandrosterone is enhanced compared with that obtained from control incubations without CA. This would suggest that CA acts by impairing the conversion of pregnenolone to progesterone on the Δ^4 pathway of steroid biosynthesis, causing a shunt to 17-α-hydroxypregnenolone and dehydroepiandrosterone on the Δ^5 pathway of steroid biosynthesis. This result would suggest further that CA does not impair side-chain cleavage in the Δ^5 pathway of steroid biosynthesis to the same extent that it does in the Δ^4 pathway.

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Inhibition of human platelet aggregation by aspirin *in vitro* and *ex vivo*

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Aspirin (acetylsalicylic acid; ASA) has been tested clinically as an antithrombotic, but with disappointing results (M.R.C. Steering Committee, 1973). The rationale for clinical trial was the previous observation that platelet aggregation was inhibited by ASA *in vitro* and *ex vivo* (i.e. *in vitro* testing after oral administration) (Weiss, Aledort & Kochwa, 1968). Using pig platelets, we recently showed that the inhibitory potency of ASA was much greater in citrated platelet-rich plasma (CPRP) than in heparinized platelet-rich plasma (HPRP) (Gordon & MacIntyre, 1974). We have now extended these studies to human platelets.

Collagen-induced platelet aggregation was measured photometrically (Born, 1962; Gordon & Drummond, 1974) in CPRP (3.8 mg/ml trisodium citrate), in HPRP (5 iu/ml sodium heparin), and in washed platelet suspensions (Walsh, 1972). Inhibition by ASA was calculated as described previously (Gordon & MacIntyre, 1974).

Collagen was 2-3 times more potent as an aggregating agent in CPRP than in HPRP. The IC_{50} value for ASA *in vitro* was 17.6 ± 2.5 μg/ml in CPRP and 144.1 ± 13.2 μg/ml in HPRP (mean ± s.e.; $n = 18$). This difference was not due to the higher collagen concentration used in HPRP. We previously reported in studies with pig platelets a similar difference in the inhibitory potency of ASA, although collagen was a more potent aggregating agent in pig HPRP than in CPRP. The IC_{50} in suspensions of washed human platelets was 21.0 ± 3.5 s.e. μg/ml—similar to that in CPRP. The inhibitory potency of ASA was increased by preincubation in PRP, and the time-course was similar in CPRP and HPRP.

After ingestion of 900 mg ASA, aggregation responses in CPRP, HPRP and washed platelet suspensions were similarly inhibited. This was unexpected and we therefore repeated the studies *in vitro* in CPRP with added heparin. The effectiveness of ASA was greatly reduced by the prior addition of heparin, but heparin added after ASA had only a slight antagonistic effect. Control aggregation responses were not significantly reduced by adding heparin to CPRP.

Our earlier results (Gordon & MacIntyre, 1974) indicated that the antithrombotic value of ASA might have been over-estimated because the potency of ASA in CPRP was exaggerated compared with HPRP. The present experiments show, however, that the potency of ASA is in fact reduced by prior addition of heparin, and no discrepancy between HPRP and CPRP exists in tests *ex vivo*. If platelet aggregation is mediated by activation of coagulation factors at the platelet membrane (Ardlie & Han, 1974) ASA could act by acetylating one or more of these factors. Heparin might interfere with this process by masking the site of acetylation. The present study does not explain the discrepancy between the effectiveness of ASA *in vitro* and *in vivo*, but provides a possible clue to the site of action of ASA on platelets.

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